20TH ANNUAL

SYMPOSIUM

OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

SCANDIC STAR

LUND, SWEDEN

JULY 23 - 27, 2010

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PROGRAMME AND ABSTRACTS

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The 2010 ICRS Symposium on the Cannabinoids
is dedicated in loving memory of
Dr. Ester Fride – 1953-2010

Registration: July 23rd, 2010 (16.00 – 19.00)

SCANDIC STAR LOBBY

Welcome Reception: 18.30 – 20.00

LOBBY

Day 1
Saturday, July 24th

6.00	**Breakfast**		
8.00	Welcome and Opening Remarks		
Symposium – Cannabinoid Receptors: Candidates Under Consideration			
8.10	Introduction by IUPHAR Nomenclature Committee *Chairs:* Roger Pertwee and Allyn Howlett *Discussant:* Nephi Stella		Page #
8.20	Peter Greasley Astra-Zeneca, Sweden	DISCOVERY OF CANNABINOID AGONISTS FOR GPR55: THERAPEUTIC IMPLICATIONS	1
8.55	Mary Abood. Temple University, USA	GPR55: PHARMACOLOGY AND SIGNAL TRANSDUCTION	2
9.30	Harald Hansen University of Copenhagen, Denmark	N-ACEYLETHANOLAMINES AND DEORPHANIZED GPCRs	3
10.05	**Coffee Break**		
10.35	Vincenzo Di Marzo Institute of Biomolecular Chemistry, Italy	CANNABINOID INTERACTIONS WITH TRP CHANNELS	4

11.10	Steve Alexander University of Nottingham, UK	ENDOCANNABINOID INTERACTIONS WITH PEROXISOMAL PROLIFERATOR-ACTIVATED RECEPTORS	5
11.45	Maurice Elphick Queen Mary University, London, UK	PHYLOGENETIC RELATIONSHIPS OF CANNABINOID RECEPTORS AND RECEPTORS FOR OTHER LIPID MEDIATORS	6
12.20	COMMENTARY AND DISCUSSION		
12.45	**LUNCH**		
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14.00	Concepción García, Cristina Palomo, Moisés García-Arencibia, José A. Ramos, Roger G. Pertwee and Javier Fernández-Ruiz	Δ^9-THCV, A PHYTOCANNABINOID ABLE TO REDUCE MOTOR INHIBITION BUT ALSO TO DELAY DISEASE PROGRESSION IN PARKINSON'S DISEASE	7
14.15	Stanislav I. Svetlov, Ronald L. Hayes and Kevin K. W. Wang	CANNABINOID RECEPTORS REMODELING IN NEURAL PROGENITORS AND AFTER TRAUMATIC BRAIN INJURY (TBI): POTENTIAL IMPLICATION FOR TBI BIOMARKERS	8
14.30	Eva de Lago, Miguel Moreno-Martet, Carmen Rodríguez-Cueto, María Gómez-Cañas, María Gómez-Ruiz and Javier Fernández-Ruiz	THE TREATMENT WITH WIN55,512-2 AMELIORATES DISEASE PROGRESSION IN A MODEL OF MULTIPLE SCLEROSIS IN MICE, ACTING THROUGH ANTI-INFLAMMATORY AND ANTI-GLUTAMATERGIC MECHANISMS	9
14.45	María-Paz Viveros, Ana Belén López Rodríguez, Beatriz Mateos, Silvana Y. Romero-Zerbo, Noé Rodriguez- Rodriguez, María José Bellini, Fernando Rodriguez de Fonseca, Francisco J Bermudez-Silva, Iñigo Azcoitia and Luis M. Garcia-Segura	ESTRADIOL DECREASES REACTIVE ASTROGLIOSIS IN A RAT MODEL OF BRAIN INJURY BY A MECHANISM INVOLVING THE ENDOCANNABINOID SYSTEM	10

15.00	Ryan McLaughlin, Matthew Hill, Christopher Roberts, Bruce McEwen, Qing-song Liu, Boris Gorzalka and Cecilia Hillard	ENDOCANNABINOID SIGNALING IN THE MEDIAL PREFRONTAL CORTEX PROMOTES TERMINATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS FOLLOWING ACUTE STRESS	11
15.15	Mariateresa Cipriano, Daniele De Filippis, Livio Luongo, Enza Palazzo, Maria Pia Cinelli, Vito de Novellis, Sabatino Maione and Teresa Iuvone	PALMITOYLETHANOLAMIDE CONTROLS HYPERALGESIA BY MODULATION OF MAST CELL ACTIVATION IN AN IN VIVO MODEL OF CHRONIC INFLAMMATION	12
15.30	Bright Okine, Andrew J Bennett and Victoria Chapman	THE ROLE OF PPARα IN MODULATING INFLAMMATORY PAIN RESPONSES	13
15.45	Gemma K. Ford, Orla Moriarty, Brendan Harhen, Eoin Tully, Adrian Mulcahy and David P. Finn	INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN ATTENTIONAL MODULATION OF NOCICEPTION IN RATS	14
16.00 - 17.30	**POSTER SESSION 1**: TOPICS A-D AFTERNOON SNACK BREAK		P1
ORAL SESSION 2. METABOLISM AND FEEDING BEHAVIOR *CHAIRS*: PAL PACHER AND SAOIRSE O'SULLIVAN			
17.30	Luigia Cristino, Giuseppe Busetto, Roberta Imperatore, Stefania Petrosino, Ida Ferrandino and Vincenzo Di Marzo	CB1 CONTROL OF OREXINERGIC NEURONS IN THE LATERAL HYPOTHALAMUS SHIFTS FROM INHIBITION TO DISINHIBITION IN OBESE MICE	15
17.45	Linda A. Parker, Erin M. Rock, Cheryl L. Limebeer, Katharine Tuerke, John Chambers, Paul J. Fletcher and Raphael Mechoulam	SUPPRESSION OF SEROTONIN RELEASE IN THE INSULAR CORTEX: A POTENTIAL CANDIDATE FOR THE ANTI-NAUSEA EFFECTS OF CANNABIDIOL	16
18.00	Giacomo Mancini, Carmelo Quarta, Raj Kamal Srivastava, Susanne Klaus, Uberto Pagotto and Beat Lutz	ADIPOCYTE-SPECIFIC CB1 CONDITIONAL KNOCK-OUT MICE: NEW INSIGHTS IN THE STUDY OF OBESITY AND METABOLIC SYNDROME	17

18.15	Matthew N. Hill, Nicole M. Bowles, Sarah M. Bhagat, Ilia N. Karatsoreos and Bruce S. McEwen	CANNABINOID CB1 RECEPTOR SIGNALING IS REQUIRED FOR GLUCOCORTICOID-MEDIATED METABOLIC SYNDROME	18
18.30	Vincenzo Di Marzo, Alexander Bartelt, Concetta Mele, Pierangelo Orlando, Stefania Petrosino, Alessia Ligresti, Klaus Toedter, Ludger Scheja and Joerg Heeren	LIVER AND ADIPOSE TISSUE ENDOCANNABINOID TONE IN DIET-INDUCED OBESITY IS DEPENDENT ON APOLIPOPROTEIN E EXPRESSION AND ASSOCIATED WITH HEPATIC STEATOSIS AND INSULIN RESISTANCE	19
18.45	James Burston, Aron Lichtman, Jonathan Long, Benjamin Cravatt and Jenny Wiley	SELECTIVE ELEVATION OF AEA OR 2-AG, BUT NOT BOTH, ALTERS CALORIC INTAKE IN MICE	20
19.00	Stefan Engeli, Nadine Friggemann, Jürgen Janke, Kerstin Gorzelniak, Mario Ost, Frauke Adams and Jens Jordan	BIOLOGICAL ROLE OF CB1-RECEPTORS IN HUMAN ADIPOCYTES	21
19.15	Miriam Schneider, Nicole Bausbacher, Matthias Schreckenberger, Beat Lutz and Rainer Spanagel	INVOLVEMENT OF THE CNR1 GENE IN RISK TAKING BEHAVIOR AND REWARD SEEKING: ENHANCED CB1 RECEPTOR ACTIVITY INDUCES A LASTING PUBERTAL PHENOTYPE IN ADULT RATS	22
19.30	**Evening Buffet (BBQ) – Scandic Star**		

NOTES:

Day 2
Sunday, July 25th

6.00	**Breakfast**		
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8.00	Miralkumar Parmar, Caroline Brennan and Maurice Elphick	THE ZEBRAFISH *DANIO RERIO*: A MODEL SYSTEM FOR MECHANISTIC ANALYSIS OF THE BEHAVIOURAL CONSEQUENCES OF EMBRYONIC EXPOSURE TO CANNABINOIDS	23
8.15	Christopher Roberts, Amy Sasman and Cecilia Hillard	SEXUAL DIMORPHISM IN CB1R-MEDIATED BEHAVIORAL COPING STRATEGY IN THE FORCED SWIM TEST	24
8.30	Francis Chaouloff, Sarah Dubreucq, Isabelle Matias and Giovanni Marsicano	ROLE OF CB1 RECEPTORS IN THE PSYCHONEUROENDOCRINE CONSEQUENCES OF REPEATED SOCIAL STRESS	25
8.45	Shimon Rabichev, Sharon Anavi-Goffer, Ruth A.Ross and Ester Fride	POSTNATAL INHIBITION OF THE ENDOCANNABINOID SYSTEM IS ASSOCIATED WITH ADHD-LIKE SYMPTOMS IN ADULTHOOD	26
9.00	Ken Soderstrom	ACUTE ALTERATION OF ANTI-CB1 CANNABINOID RECEPTOR IMMUNOREACTIVITY IN ZEBRA FINCH SONG CONTROL REGIONS: DIFFERENTIAL RESPONSIVENESS FOLLOWING DEVELOPMENTAL CANNABINOID EXPOSURE	27
9.15	Naoki Amada, Akihito Watanabe, Yuki Yamasaki, Serena Deiana, Tetsuro Kikuchi and Gernot Riedel	EFFECTS OF PHYTOCANNABINOIDS ON THE ELEVATED PLUS MAZE IN MICE	28
9.30	Linda Klumpers, Amita Sandhu, Roelof Soeter, Marieke de Kam, Serge Rombouts and Joop van Gerven	SEX DIFFERENCES OF THC EFFECTS IN HUMANS	29

9.45	Erica Zamberletti, Daniela Viganò, Cinzia Guidali, Tiziana Rubino and Daniela Parolaro	CHRONIC BUT NOT ACUTE ADMINISTRATION OF AM251 REDUCES PSYCHOTIC-LIKE SYMPTOMS IN ISOLATION-REARED RATS	30
10.00	**COFFEE BREAK**		
ORAL SESSION 4. DRUG ADDICTION *CHAIRS*: DANIELA PAROLARO AND GINGER SEYBOLD			
10.15	Amaia M Erdozain, Steve PH Alexander, David A Kendall, Elsa M Valdizan, J Javier Meana and Luis F Callado	EVALUATION OF THE ENDOCANNABINOID SYSTEM IN POSTMORTEM HUMAN PREFRONTAL CORTEX OF ALCOHOLIC SUBJECTS	31
10.30	Zheng-Xiong Xi, Xiao-Qing Peng, Xia Li, Haiying Zhang, Jie Li, and Eliot L. Gardner	BRAIN CANNABINOID CB2 RECEPTORS INHIBIT COCAINE SELF-ADMINISTRATION AND COCAINE-ENHANCED EXTRACELLULAR DOPAMINE IN MICE	32
10.45	Tiziana Rubino, Natalia Realini, Pamela Prini, Erica Zamberletti, Daniela Viganò and Daniela Parolaro	ADOLESCENT EXPOSURE TO THC INDUCED AN INCREASED SENSIBILITY TO PHENCYCLIDINE IN ADULT ANIMALS	33
11.00	Divya Ramesh, Joel Schlosburg, Gracious Ross, Rehab Abdullah, Steven Kinsey, Jonathan Long, Benjamin Cravatt, Hamid Akbarali, Laura Sim-Selley and Aron Lichtman	TARGETING ENDOCANNABINOID CATABOLIC ENZYMES FOR THE TREATMENT OF OPIOID WITHDRAWAL	34
11.15	Eliot L. Gardner, Krista Spiller and Zheng-Xiong Xi	CANNABINOID CB1 AND CB2 RECEPTORS MODULATE BRAIN REWARD FUNCTION IN OPPOSITE DIRECTIONS IN RATS	35

11.30	**PRESIDENTIAL PLENARY SPEAKER** ON THE EXISTENCE OF CB1 CONTAINING RECEPTOR HETEROMERS IN THE BRAIN: MOLECULAR INTEGRATION VIA ALLOSTERIC MECHANISMS **KJELL FUXE, M.D.** *Department of Neuroscience, Karolinska Institutet*	PS1
12.30	**LUNCH**	

ORAL SESSION 5. DRUG DESIGN AND DISCOVERY
CHAIRS: HERB SELTZMAN AND DIDIER LAMBERT

14.00	Herbert Seltzman, Patricia Reggio, Lauren Chun, Zheng-Xiong Xi and Eliot Gardner	PIMSR1 AS A NON-DYSPHORIC NEUTRAL CB1 ANTAGONIST	36
14.15	Thierry Groblewski, Xiao Hong Yu, Etienne Lessard, Stéphane St-Onge, Hua Yang, Rosemarie Panetta, Chang Qing Cao, Michael Swedberg, Gvido Cebers, Svante Nyberg, Magnus Schou, Christer Halldin, Balazs Gulyas, Katarina Varnäs, Chistopher Walpole, Kemal Payza, Martin Perkins and Julie Ducharme	PRE-CLINICAL PHARMACOLOGICAL PROPERTIES OF NOVEL PERIPHERALLY-ACTING CB1-CB2 AGONISTS	37
14.30	Thierry Groblewski, Rolf Karlsten, Märta Segerdhal, Jarkko Kalliomäki, Bror Jonzon, Margareta Bielenstein, Gvido Cebers, Michael Swedberg, Anita Annas, Greg Christoph, Pernilla Tellefors, Lars Ståhle, René Bouw, Urban Fagerholm, Agneta Berg, Stephen Butler, Michael O'Malley and Gudrun Anstrén	PERIPHERALLY-ACTING CB1-CB2 AGONISTS FOR PAIN: DO THEY STILL HOLD PROMISE?	38
14.45	Pia K. Noerregaard, Marianne Fridberg and Christian E. Elling	TM38837 – A NOVEL SECOND GENERATION PERIPHERAL SELECTIVE CB1 RECEPTOR ANTAGONIST WITH EFFICACY AND POTENCY IN RODENT OBESITY MODELS EQUAL TO BRAIN-PENETRANT CB1 ANTAGONIST RIMONABANT	39

15.00	Didier M. Lambert, Jamal El Bakali, Giulio G. Muccioli, Nicolas Renault, Delphine Pradal, Mathilde Body-Malapel, Madjid Djouina, Virginie Andrzejak, Pierre Desreumaux, Philippe Chavatte and Régis Millet	DESIGN OF NOVEL SELECTIVE CB2 RECEPTOR INVERSE AGONISTS BASED ON A 4-OXO-1,4-DIHYDROPYRIDINE SCAFFOLD	40
15.15	Patricia Reggio, Dow Hurst, Hadley Iliff, Teresa Barber, Gemma Baillie and Ruth Ross	IDENTIFICATION OF ORG27569 BINDING SITE AT THE CB1 RECEPTOR	41
15.30	Lital Magid, Catherine E. Goodfellow, Morris Srebnik, Michelle Glass, Alexander Alexandrovich, Lumir Hanuš, Esther Shohami and Raphael Mechoulam	DESIGN, SYNTHESIS AND NEUROPROTECTIVE POTENTIAL OF NOVEL CB2 RECEPTOR SELECTIVE AGONISTS	42
15.45	John M. McPartland and Geoffrey W. Guy	THC SYNTHASE IN CANNABIS HAS UNDERGONE ACCELERATED EVOLUTION AND POSITIVE SELECTION PRESSURE	43
16.00 - 17.30	**POSTER SESSION 2**: TOPICS E-H AFTERNOON SNACK BREAK		P2
ORAL SESSION 6. RECEPTORS AND SIGNALING *CHAIRS*: TIZIANA RUBINO AND ANDREW IRVING			
17.30	Brian Hudson and Melanie Kelly	WIN 55,212-2 REDUCES INTRAOCULAR PRESSURE IN MICE THROUGH A MECHANISM DEPENDANT ON β-ADRENERGIC RECEPTORS	44
17.45	Takayuki Sugiura, Ryo Ota, Mitsuru Shima, Atsushi Yamashita and Saori Oka	GPR55 AND GPR35, PUTATIVE Δ^9-TETRAHYDROCANNABINOL RECEPTORS, AND THEIR ENDOGENOUS LIGANDS	45
18.00	Sharon Anavi-Goffer, Andy J. Irving, Roger G. Pertwee and Ruth A. Ross	MODULATION OF GPR55 SIGNALLING BY PHYTOCANNABINOIDS USING THE ALPHASCREEN ASSAY	46

18.15	Yanan Zhang, David Perrey, Brian P. Gilmour, Tiffany Lanston, Keith Warner and Brian F. Thomas	SYNTHESIS AND EVALUATION OF BIVALENT LIGANDS FOR CB1-OX1 RECEPTOR HETERODIMERS	47
18.30	Bani Mukhopadhyay, Jie Jie Liu, Douglas Osei-Hyiama, Grzegorz Godlewski, Partha Mukhopadhyay, Lei Wang, Won-Il Jeong, Bin Gao, Ken Mackie, Soichi Kojima and George Kunos	TRANSCRIPTIONAL REGULATION OF CANNABINOID RECEPTOR-1 EXPRESSION IN THE LIVER BY RETINOIC ACID ACTING VIA RETINOIC ACID RECEPTOR-γ	48
18.45	Yosef Sarne, Fadi Asaf, Miriam Fishbein, Ora Keren and Mikhal Gafni	LONG-LASTING EFFECTS OF AN ULTRA-LOW DOSE OF THC ON COGNITIVE FUNCTIONING IN MICE	49
19.00	Sam Deadwyler and Robert Hampson	ENDOCANNABINOID MODULATION OF INTRACELLULAR CALCIUM IN RAT HIPPOCAMPAL NEURONS: IMPLICATIONS FOR ENDOCANNABINOID REGULATION OF NEURAL ACTIVITY AND BEHAVIOR	50
19.15	Ken Mackie, Jim Wager-Miller and Alex Straiker	DIFFERENTIAL SIGNALING IN HUMAN CANNABINOID CB1 RECEPTORS AND THEIR SPLICE VARIANTS IN AUTAPTIC HIPPOCAMPAL NEURONS	51
19.30	**EVENING BUFFET (SWEDISH) – SCANDIC STAR**		

NOTES:

Day 3
Monday, July 26th

6.00	**Breakfast**		
	Oral Session 7. Endocannabinoid System *Chairs*: Dale Deutsch and Lumir Hanus		Page #
8.00	Nephi Stella	ABHD6: A NEW COMPONENT OF THE ENDOCANNABINOID SIGNALING SYSTEM	52
8.15	Jacqueline Blankman, Joel Schlosburg, Jonathan Long, Daniel Nomura, Aron Lichtman and Benjamin Cravatt	SUSTAINED INACTIVATION OF MONOACYLGLYCEROL LIPASE PRODUCES FUNCTIONAL ANTAGONISM OF THE BRAIN ENDOCANNABINOID SYSTEM	53
8.30	Alex Straiker, Jim Wager-Miller and Ken Mackie	COX2 AND FAAH CAN REGULATE THE TIME COURSE OF DEPOLARIZATION INDUCED SUPPRESSION OF EXCITATION	54
8.45	Bela Szabo and Flora E. Kovacs	PURINERGIC RECEPTOR-MEDIATED ENDOCANNABINOID PRODUCTION AND INHIBITION OF GABAERGIC SYNAPTIC TRANSMISSION IN THE CEREBELLAR CORTEX	55
9.00	Dale G. Deutsch, Lindsay D Nelson, Michele K McKinney, Benjamin F. Cravatt, Erwin London and Martin Kaczocha	CARRIER-INDEPENDENT TRANSPORT OF ANANDAMIDE THROUGH SYNTHETIC VESICLES WITH INTERNALIZED FAAH	56
9.15	Nadine M. Ulloa and Dale G. Deutsch	CLONING MAGL ISOFORMS FROM PUTATIVE TRANSLATION INITIATION SITES	57
9.30	**Coffee Break**		

SYMPOSIUM – Cannabinoids, Bone Remodeling and Osteoporosis *Chair*: Itai Bab, D.M.D., Hebrew University of Jerusalem, Israel *Discussant*: Mary E. Abood, Ph.D., Temple University, USA			
10.00	Introduction by Vishnudutt Purohit, D.V.M., Ph.D. National Institute on Drug Abuse, NIH, USA		58
10.10	Yossef Tam, National Institute on Alcohol Abuse and Alcoholism/NIH, USA	ROLE OF CB1 RECEPTOR IN REGULATION OF BONE FORMATION	59
10.40	Itai Bab, Hebrew University of Jerusalem, Israel	CB2 REGULATION OF BONE METABOLISM IN HEALTH AND DISEASE	60
11.10	Ruth A. Ross, University of Aberdeen, Scotland, UK	GPR55: A NOVEL ROLE IN BONE PHYSIOLOGY	61
11.40	Discussion		
12.00	**Lunch**		
13.00 – 13.30	**Sativex: The Story of the Successful Development of the First Phytocannabinoid Medicine** Presented by Geoffrey Guy and Stephen Wright, GW Pharmaceuticals		
13.30 – 15.00	**Poster Session 3**: Topics I&J		P3
15.00 – 16.00	**DEBATE: *"CB or NOT CB"*** ***CB***: Maurice Elphick, Cecilia Hillard, Aron Lichtman and Brian Thomas *vs* ***NOT CB***: Heather Bradshaw, Vincenzo Di Marzo, Chris Fowler and Raphael Mechoulam *Moderators*: Allyn Howlett and Roger Pertwee		
16.00 – 20.00	**Outing and Dinner** Exploring Skåne		

Day 4
Tuesday, July 27th

6.00	**Breakfast**		
Oral Session 8. Cell Survival and Proliferation: Translational Impact *Chairs*: Mauro Maccarrone and John McPartland			Page #
8.30	Christopher Fowler, Lina Thors and Anders Bergh	FATTY ACID AMIDE HYDROLASE IMMUNOREACTIVITY IN PROSTATE CANCER – ASSOCIATION WITH DISEASE SEVERITY AND OUTCOME	62
8.45	Filippo Molica, Fabienne Burger, Christian M. Matter, Andreas Zimmer, Pal Pacher and Sabine Steffens	THE CANNABINOID RECEPTOR CB2 PROTECTS AGAINST BALLOON-INDUCED NEOINTIMAL PROLIFERATION AND INFLAMMATION IN ATHEROSCLEROSIS-PRONE MICE	63
9.00	Marta Solinas, Paola Massi, Marta Valenti, Daniele Bolognini and Daniela Parolaro	ANTIANGIOGENIC PROPERTIES OF THE NON PSYCHOACTIVE CANNABINOID COMPOUND CANNABIDIOL: IN VITRO AND IN VIVO STUDIES	64
9.15	Lesley Ann Ford-Taylor, Selina Chiu, Jasmeer Renoo, Roger Pertwee and Ruth Ross	PHYTOCANNABINOIDS IN THEIR ACID FORMS ARE POTENT INHIBITORS OF HUMAN BREAST CANCER CELL VIABILITY	65
9.30	Gabriella Aviello, Barbara Romano, Francesca Borrelli, Fabiana Piscitelli, Vincenzo Di Marzo and Angelo A. Izzo	PROTECTIVE EFFECT OF CANNABIDIOL AND CANNABIS EXTRACT IN COLON CARCINOGENESIS	66
9.45	Cristoforo Silvestri, Fabiana Piscitelli, Andrea Martella and Vincenzo Di Marzo	REGULATION AND POSSIBLE FUNCTION OF THE ENDOCANNABINOID SYSTEM IN MYOGENESIS	67
10.00	**Coffee Break + Poster Session**		

10.00 – 11.30	**POSTER SESSION 4:** TOPICS K-M		P4
11.30	**KANG TSOU MEMORIAL LECTURE** MULTIPLE MESSENGER SYSTEMS: FOCUS ON NEUROPEPTIDES IN DEPRESSION **THOMAS HÖKFELT, M.D., PH.D.** *Department of Neuroscience, Karolinska Institutet*		PS2
12.30	**LUNCH**		
13.30 – 14.00	NIDA CAREER INFOLUNCHEON **"SUPPORTING YOUR LAB: GRANTS VS. CONTRACTS"** *PANELISTS*: PATRICIA REGGIO, RUTH ROSS, ETHAN RUSSO AND BRIAN THOMAS *MODERATOR*: ROBERT HAMPSON		
ORAL SESSION 9. IMMUNE SYSTEM *CHAIRS*: BELA SZABO AND RONALD TUMA			
14.00	Alexander A. Zoerner, Dirk O. Stichtenoth, Sandor Batkai, Stefan Engeli, Frank Schaumann, Norbert Krug, Dimitrios Tsikas, Jens Jordan and Jens Hohlfeld	ALLERGEN CHALLENGE INDUCES ANANDAMIDE IN BRONCHOALVEOLAR FLUID OF ALLERGIC ASTHMA PATIENTS	68
14.15	Pal Pacher, Mohanraj Rajesh, Partha Mukhopadhyay, Sándor Bátkai, Vivek Patel, Keita Saito, Shingo Matsumoto, Bani Mukhopadhyay, Lauren Becker, György Haskó, Lucas Liaudet, Wilmarie Flores-Santana, David A Wink, Murali C Krishna, Aristidis Veves and Raphael Mechoulam	CANNABIDIOL ATTENUATES CARDIAC DYSFUNCTION, OXIDATIVE STRESS, FIBROSIS, INFLAMMATORY AND CELL DEATH SIGNALING PATHWAYS IN DIABETIC CARDIOMYOPATHY	69

14.30	Michelle Roche, Daniel Kerr, Nikita Burke, Weredeselam Olango, Brandan Harhen, Gemma Ford and David Finn	INHIBITION OF MONOACYLGLYCEROL LIPASE AND FATTY ACID AMIDE HYDROLYSE *IN VIVO* MODULATES LPS-INDUCED INCREASES IN CYTOKINE EXPRESSION IN THE RAT PREFRONTAL CORTEX	70
14.45	François Chouinard, Samuel Poirier, Claudine Ferland, Julie S. Lefebvre and Nicolas Flamand	THE ENDOCANNABINOID 2-ARACHIDONOYL-GLYCEROL ACTIVATES HUMAN NEUTROPHILS FUNCTIONS: CRITICAL ROLE OF LEUKOTRIENE B_4	71
15.00	Rangan Maitra and Huaqin Pan	THE CANNABINOID RECEPTOR AGONIST CP 55,940 INDUCES A RECIPROCAL PATTERN OF GENE EXPRESSION IN HEPATIC STELLATE CELLS ENGINEERED TO EXPRESS CB1 OR CB2 RECEPTORS	72
15.15	Janis Noonan, Allan Klompas and Veronica Campbell	ANANDAMIDE STABILISES LYSOSOMES AGAINST AMYLOID-β TOXICITY BY ACTING ON INTRACELLULAR CB_1 RECEPTORS	73
15.30	Partha Mukhopadhyay, Mohanraj Rajesh, Hao Pan, Sándor Bátkai, Vivek Patel, György Haskó, Judith Harvey-White, Bani Mukhopadhyay, Ken Mackie, Bin Gao and Pal Pacher	OPPOSING EFFECTS OF CANNABINOID-1 RECEPTOR INHIBITION AND CANNABINOID-2 RECEPTOR ACTIVATION ON INFLAMMATION, OXIDATIVE/NITROSATIVE STRESS, AND CELL DEATH IN NEPROPATHY	74
15.45	Marco Koch, Susanne Kreutz, Charlotte Böttger, Urszula Grabiec, Chalid Ghadban, Horst-Werner Korf and Faramarz Dheghani	THE CANNABINOID WIN 55,212-2-MEDIATED NEUROPROTECTION IS DRIVEN BY CB1 RECEPTORS AND MODULATED BY TRPA1 CHANNELS	75
16.00	Mauro Maccarrone, Monica Bari, Marianna Tedesco, Nicoletta Pasquariello, Mariangela Pucci, Valeria Gasperi, Maria L. Scaldaferri, Donatella Farini and Massimo De Felici	CHARACTERIZATION OF THE ENDOCANNABINOID SYSTEM IN MOUSE EMBRYONIC STEM CELLS	76

16.15	**ICRS BUSINESS MEETING**
17.30 – 23.00	**ICRS BANQUET** AT THE CASTLE AT LUND UNIVERSITY

DEPARTURE: JULY 28TH, 2010

BUSES AND TAXIES TO TRAIN STATION FOR
RETURN TO KASTRUP AIRPORT OR COPENHAGEN

POSTER SESSION 1: TOPICS A-D
DAY 1, SATURDAY JULY 24TH: 16:00 – 17:30

TOPIC A. *CARDIOVASCULAR*

Marta Baranowska–Kuczko, Hanna Kozlowska, Miroslaw Kozlowski, Eberhard Schlicker, Margaret R. MacLean and Barbara Malinowska	AN ENDOTHELIAL MECHANISM OF ANANDAMIDE-INDUCED VASORELAXATION IN THE HUMAN AND RAT PULMONARY ARTERY	P1-1
Barbara Malinowska, Radosław Rudź, Sebastian Łukasz Łupiński and Eberhard Schlicker	MYOCARDIAL INFARCTION AFFECTS RESPONSES MEDIATED VIA CARDIAC TRPV1 AND CB1 RECEPTORS IN RATS	P1-2
W. -S. Vanessa Ho	INFLUENCE OF FEMALE AND MALE SEX HORMONES ON VASORELAXATION TO ENDOCANNABINOIDS	P1-3
Mauro Maccarrone, Maria V. Catani, Valeria Gasperi, Giuseppina Catanzaro, Samantha Baldassarri, Alessandra Bertoni, Fabiola Sinigaglia and Luciana Avigliano	EVIDENCE THAT HUMAN PLATELETS EXPRESS AUTHENTIC CB1 AND CB2 RECEPTORS	P1-4
Kara L. Stuhr, Andrea Dlugos, Cecilia J. Hillard and Harriet de Wit	2-ARACHIDONOYLGLYCEROL CONCENTRATION IN HUMAN SERUM IS INCREASED BY BLOOD WITHDRAWAL	P1-5

TOPIC B. *GI AND METABOLISM*

Scott D. Smid and Lauren Nicotra	CANNABINOIDS AND PROSTAMIDES ATTENUATE INFLAMMATORY DAMAGE IN A HUMAN EXPLANT COLITIS	P1-6
Mandana Kianian, Juan Zhou, Mel Kelly, Sara Whynot, Orlando Hung, Michael Murphy and Christian Lehmann	EFFECTS OF CB2 RECEPTOR MODULATION ON THE INTESTINAL MICROCIRCULATION IN EXPERIMENTAL SEPSIS	P1-7
Bruno A. Cotrim, Jesús Joglar, Maria Jesus L. Rojas, Juan Manuel Decara, Francisco Javier Bermudez-Silva, Manuel Macias-Gonzalez, Montserrat Fito, Miguel Romero, Magí Farré, Maribel Covas, Fernando Rodriguez de Fonseca and Rafael de la Torrea	SYNTHESIS OF FATTY ACID AMIDES OF CATECHOL METABOLITES WITH ANTIOXIDANT AND ANTIOBESITY PROPERTIES	P1-8

Ming-Shiu Hung, Kak-Shan Shia, Yi-Ting Wang, Yinchiu Lin, Chien-Huang Wu Yen-Nan Yeh, Jen-Shin Song, Wenchi Hsiao and Yu-Sheng Chao	BPR0912, A NOVEL PERIPHERAL CB1 INVERSE AGONIST AND ITS METABOLIC EFFECTS	P1-9
Jemma C. Cable, Garry D. Tan, Stephen P. H. Alexander and Saoirse E. O'Sullivan	FATTY ACID AMIDE HYDROLASE ACTIVITY IN HUMAN ADIPOCYTES DOES NOT CORRELATE WITH METABOLIC MARKERS OR ANTHROPOMETRIC MEASUREMENTS	P1-10
Holiday A. Durham, Alexandros Makriyannis, Jodi T. Wood, John S. Williams and Carol J. Lammi-Keefe	ENDOCANNABINOIDS AND BODY MASS INDEX DURING PREGNANCY - IS THERE A RELATIONSHIP? A LONGITUDINAL STUDY	P1-11
Barbara Romano, Raffaele Capasso, Gabriella Aviello, Luciano De Petrocellis, Antonio Pescatore, Angelo A. Izzo and Vincenzo Di Marzo	INHIBITORY EFFECT OF CANNABICHROMENE ON INTESTINAL MOTILITY IN MICE	P1-12
Edgar Soria-Gómez, Federico Massa, Pavel E. Rueda-Orozco, Giovanni Marsicano and Oscar Prospéro-García	CENTRAL CB1 RECEPTORS DIFFERENTIALLY MODULATE FASTING-INDUCED HYPERPHAGIA IN RATS	P1-13

TOPIC C. *NEURODEGENERATION AND PAIN*

Eric A. Horne, William Marrs, Jonathon Coy, Xiaocui Sun and Nephi Stella	CHRONIC ADMINISTRATION OF SR141716 EXACERBATES SEIZURES IN R6/2 MICE, A MODEL OF HUNTINGTON'S DISEASE	P1-14
Onintza Sagredo, María Ruth Pazos, Tiziana Bisogno, Fabiana Piscitelli, Stefania Petrosino, Mariluz Hernández, Vincenzo Di Marzo and Javier Fernández-Ruiz	POTENTIAL OXIDATION OF 2-AG BY COX-2 ENHANCES MALONATE TOXICITY IN THE STRIATUM: RELEVANCE FOR CANNABINOID TREATMENTS IN HUNTINGTON'S DISEASE	P1-15
William Marrs, Eric Horne, Karen Sun, Weiwei Li, Ben Cravatt and Nephi Stella	THERAPEUTIC EFFECTS OF ABHD6 INHIBITION IN R6/2 MICE, A MODEL OF HUNTINGTON'S DISEASE	P1-16
Antonio Rodríguez-Gaztelumendi, Stig O.P. Jacobsson and Christopher J. Fowler	METABOLISM OF ANANDAMIDE AND 2-OLEOYLGLYCEROL BY GPNT RAT BRAIN ENDOTHELIAL CELLS	P1-17
Riffat Tanveer and Veronica A. Campbell	NEUROPROTECTIVE CONCENTRATION OF ANANDAMIDE INDUCES NICASTRIN EXPRESSION IN PRIMARY NEURONAL CULTURES	P1-18
I.A. Khasabova, M.A, Holman, D.A. Simone and V.S. Seybold	INHIBITION OF ANANDAMIDE UPTAKE BY DORSAL ROOT GANGLION NEURONS REDUCES MECHANICAL HYPERALGESIA IN TUMOR-BEARING MICE	P1-19
Joanna E Slusar, Anna-Maria Szczesniak and Melanie EM Kelly	EXAMINATION OF THE NEUROPROTECTIVE EFFECTS OF URB597 IN YOUNG AND AGED RAT RETINA	P1-20

Basavaraj S. Balapal and Ralph A. Nixon	ROLE OF THE ENDOCANNABINOID-CB1 RECEPTOR PATHWAY IN SYNAPTIC DYSFUNCTION OBSERVED BY BETA-AMYLOID 1-42 AND IN AD MICE	P1-21
Enza Palazzo, Livio Luongo, Luigia Cristino, Vito de Novellis, Francesco Rossi, Vincenzo Di Marzo and Sabatino Maione	FUNCTIONAL INTERACTION BETWEEN PERIAQUEDUCTAL GREY CANNABINOID SUBTYPE 1 AND METABOTROPIC GLUTAMATE SUBTYPE 1 AND 5 RECEPTORS IN NEUROPATHIC RATS	P1-22
Lamont Booker, Steven G. Kinsey, Rehab Abdullah, Jonathan Z. Long, Dale Boger, Benjamin F. Cravatt and Aron H. Lichtman	NEURONAL FAAH INHIBITION VIA PF-3845 REVERSES LPS INDUCED TACTILE ALLODYNIA	P1-23
Nazdar Ghafouri, Bijar Ghafouri, Maria Turkina, Britt Larsson, Bo AG Jönsson, Christopher J Fowler and Björn Gerdle	IDENTIFICATION OF N-ACYLETHANOLAMINES AND CANNABINOID 1 RECEPTORS IN HUMAN TRAPEZIUS MYALGIA	P1-24
Katarzyna Starowicz, Wioletta Makuch, Fabiana Piscitelli, Stefania Petrosino, Vincenzo Di Marzo and Barbara Przewłocka	ACTIVATION OF SPINAL TRPV1 OR CB1 RECEPTORS IN NEUROPATHIC RATS DEPENDS ON ANANDAMIDE CONCENTRATION	P1-25
Weredeselam M.Olango, Michelle Roche and David P. Finn	MODULATION OF NOCICEPTION AND FEAR-CONDITIONED ANALGESIA BY THE ENDOCANNABINOID SYSTEM IN THE RAT DORSOLATERAL PERIAQUEDUCTAL GREY	P1-26

TOPIC D. *PHYTOCANNABINOIDS*

Gemma L. Baillie, Ruth A. Ross and Roger G. Pertwee	THE ABILITY OF NABILONE TO INTERACT WITH CANNABINOID CB1 AND CB2 RECEPTORS	P1-27
Felipe V. Gomes, Leonardo Resstel and Francisco S. Guimarães	CANNABIDIOL INJECTED INTO THE BED NUCLEUS OF THE STRIA TERMINALIS INDUCES ANXIOLYTIC-LIKE EFFECTS IN THE ELEVATED PLUS MAZE VIA 5-HT1A RECEPTOR-DEPENDENT MECHANISMS	P1-28
Jonathan A. Farrimond, Benjamin J. Whalley and Claire M. Williams	NON-Δ^9THC PHYTOCANNABINOID-INDUCED MODULATION OF RAT FEEDING PATTERNS	P1-29
Nicholas A. Jones, Samantha E. Weston, Andrew J. Hill, Gary J. Stephens, Benjamin J. Whalley and Claire M. Williams	CANNABIDIOL EXERTS ANTI-CONVULSANT EFFECTS IN ANIMAL MODELS OF TEMPORAL LOBE AND PARTIAL SEIZURES	P1-30
Carolyn Tanner, Maria-Grazia Cascio, Lesley Stevenson, Ruth A. Ross and Roger G. Pertwee	EFFECT OF SEVEN PHYTOCANNABINOIDS ON CATECHOLAMINE TRANSPORTERS AND RECEPTORS	P1-31

A.C. Campos, V. P. Soares, H. Zangrossi Jr., A. W. Zuardi and F. S. Guimarães	REPEATED ADMINISTRATION OF CANNABIDIOL PRODUCES PANICOLYTIC RESPONSE BY ACTIVATING 5HT1A RECEPTORS IN THE DORSAL PERIAQUEDUCTAL GRAY	P1-32
Gabriella Aviello, Raffaele Capasso, Beniamino Vaccaro, Vincenzo Altieri, Vittorino Montanaro and Angelo A. Izzo	EFFECT OF A CANNABIS EXTRACT ON RAT AND HUMAN BLADDER CONTRACTILITY IN VITRO	P1-33
Linda E. Klumpers, Roelof P. Soeter, Naj Khalili-Mahani, Serge A.R.B. Rombouts, Mark A. van Buchem and Joop M.A. van Gerven	THC-EFFECTS MEASURED IN HUMAN BY RESTING STATE-FMRI	P1-34

NOTES:

POSTER SESSION 2: TOPICS E-H
DAY 2, SUNDAY JULY 25TH: 16:00 – 17:30

TOPIC E. *DRUG ADDICTION*

Chris M. Friemel, Rainer Spanagel and Miriam Schneider	THE EFFECTS OF CHRONIC CANNABINOID TREATMENT ON BEER CONSUMPTION IN PUBERTAL AND ADULT RAT	P2-1
Ryan Vandrey, Una McCann, Michael Smith and Alan Budney	SLEEP DISRUPTION FOLLOWING DAILY CANNABIS USE	P2-2
Barbara J. Mason, Rebecca Crean and Vivian Goodell	PRECIPITATED VERSUS NATURAL WITHDRAWAL IN OUTPATIENTS WITH CANNABIS DEPENDENCE: IMPLICATIONS FOR TREATMENT	P2-3
Joshua A. Lile, Thomas H. Kelly and Lon R. Hays	THE DISCRIMINATIVE STIMULUS EFFECTS OF THE CANNABINOID AGONIST NABILONE ALONE AND IN COMBINATION WITH Δ^9-THC IN HUMANS	P2-4
Kevin M. Gray, Steven D. LaRowe, Noreen L. Watson and Matthew J. Carpenter	REACTIVITY TO IN VIVO MARIJUANA CUES AMONG TREATMENT-SEEKING CANNABIS DEPENDENT ADOLESCENTS	P2-5
Laura Cutando, Emma Puighermanal, Arnau Busquets-Garcia, Rafael Maldonado and Andrés Ozaita	CHRONIC DELTA9-TETRAHYDROCANNABINOL EXPOSURE RESULTS IN A SUBTLE MOTOR COORDINATION DEFICIT THROUGH CEREBELLAR MICROGLIA ACTIVATION	P2-6
Joost Wiskerke, Dustin Schetters, Nicky Stoop, Anton N.M. Schoffelmeer and Tommy Patti	AN IMPORTANT ROLE FOR CANNABINOID CB1 RECEPTORS IN AMPHETAMINE-INDUCED IMPULSIVITY	P2-7
Amanda Reiman	THE RELATIONSHIP BETWEEN CHRONIC ILLNESS AND CANNABIS USE	P2-8
Paul D. Kennedy, William R. Collin and Thomas Buddenbor	AUTOMATED ION TRAP SCREENING METHOD FOR THE DETECTION OF SYNTHETIC CANNABINOIDS IN COMMERCIAL INCENSE PRODUCTS	P2-9
Kristen N. Peskuski	FRESH CANNABIS: A NON-PSYCHOACTIVE THERAPEUTIC MODALITY	P2-10

Mateus M. Bergamaschi, Marcos H. N. Chagas, Regina H. C. Queiroz, José A. B. Martinez, Glício R. Oliveira, Jaime E. C. Hallak, José A. S. Crippa and Antonio W. Zuardi	CANNABIDIOL REDUCED MARIJUANA WITHDRAW SYMPTOMS	P2-11
Francis Rodriguez Bambico, Tommaso Cassano, Noam Katz, Sergio Dominguez Lopez, Jean-Philippe Garant, Daniele Piomelli and Gabriella Gobbi	GENETIC DEACTIVATION OF FATTY ACID AMIDE HYDROLASE PRODUCES ANXIOLYTIC-LIKE AND ANTIDEPRESSANT-LIKE BEHAVIOURS AND MODIFIES SEROTONERGIC TRANSMISSION IN THE DORSAL RAPHE, PREFRONTAL CORTEX AND HIPPOCAMPUS	P2-12

TOPIC F. *LEARNING AND MEMORY / COGNITION*

Alejandro Aparisi, Maria Paz Viveros and Beat Lutz	THE DUAL ROLE OF THE ENDOCANNABINOID SYSTEM AS A REGULATOR OF ANXIETY RESPONSES	P2-13
Áine M. Kelly, Andreea Petrasca, Ita Shanahan and Veronica Campbell	ENDOCANNABINOIDS MODULATE OBJECT RECOGNITION MEMORY IN THE RAT	P2-14
Jean-Ha Baek, Yiwen Zheng, Cynthia L. Darlington and Paul F. Smith	CHANGES IN THE ENDOCANNABINOID SYSTEM FOLLOWING BILATERAL VESTIBULAR DEAFFERENTATION	P2-15
John Sesay, Anushka V.Goonawardena and Robert E. Hampson	EFFECTS OF ENDOCANNABINOID MODULATORS ON SPONTANEOUS FIRING RATE, BURSTING AND CELL SYNCHRONY OF HIPPOCAMPAL PRINCIPAL CELLS IN RATS	P2-16
M. Jerry Wright Jr., Renee Croce and Michael A. Taffe	ACUTE TREATMENT WITH DELTA-9-TETRAHYDROCANNABINOL IMPAIRS COGNITIVE FUNCTION IN RHESUS MACAQUES	P2-17
Anushka V Goonawardena, Andrea Plano, Matt Elverman, Bettina Platt, Robert E Hampson and Gernot Riedel	ACUTE EFFECTS OF CANNABINOID AGONISTS, ANTAGONISTS AND NEUTRAL ANTAGONISTS ON SLEEP-WAKE CYCLE IN MICE	P2-18
Isaac Karimi and Lora A. Becker	MATERNAL INTAKE OF HEMPSEED AS A "JUNK NUT" DID NOT ALTER STRESS, DEPRESSION, AND PSYCHOMOTOR PROFILES IN RAT OFFSPRING AT POST-WEANING	P2-19
Fabricio A. Moreira, Plinio C. Casarotto, Daniele C. Aguiar, Ana Terzian, Helio Zangrossi, Francisco S. Guimaraes and Carsten T. Wotjak	INTERACTION BETWEEN CB1 AND TRPV1 RECEPTORS IN THE MODULATION OF PANIC-LIKE REACTIONS IN RATS	P2-20
Michal Schechter, Albert Pinhasov, Aron Weller and Ester Fride	BLOCKING DAM'S CANNABINOID CB1 RECEPTOR AFFECTS PUP'S SOCIAL BEHAVIOR	P2-21

TOPIC G. *DRUG DESIGN*

Emmelie Björklund and Christopher J. Fowler	INHIBITION OF MONOACYLGLYCEROL LIPASE: ASSAY COMPARISON	P2-22
Robert J. Doerksen, Ronak Y. Patel and Pankaj R. Daga	PREDICTIVE MODELS OF ACTIVITY AND SELECTIVITY FOR CB1 ANTAGONISTS AND CB2 AGONISTS	P2-23
Christeene Haj, Ruth Gallily, Aviva Breuer and Raphael Mechoulam	NOVEL CANNABIDIOL DERIVATIVES AND THEIR USE AS ANTI-INFLAMMATORY AGENTS	P2-24
Reem Smoum, Aviva Breuer, Lumir Hanuš, Naama Mussai, Orr Ofek, Malka Attar-Namdar, Raphael Mechoulam and Itai Bab	HU-433, ENANTIOMER OF THE CB2 AGONIST HU-308, IS A HIGHLY POTENT REGULATOR OF BONE MASS	P2-25
M Fridberg , PK Noerregaard, PB Little, NO Jensen and CE Elling	A SAFETY AND TOLERABILITY STUDY OF SINGLE ASCENDING DOSES OF TM38837 - A NOVEL SECOND GENERATION PERIPHERAL SELECTIVE CB1 RECEPTOR ANTAGONIST IN HEALTHY MALE SUBJECTS	P2-26

TOPIC H. *REPRODUCTION*

Akwasi A. Amoako, Timothy H. Marczylo, Patricia M.W. Lam, Amanda Derry, Jonathon Willets, Janine Elson and Justin C. Konje	SEMINAL PLASMA LEVELS OF N-ACYLETHANOLAMIDES ARE DECREASED IN MEN WITH ABNORMAL SPERM MOTILITY	P2-27
Delphine Psychoyos-Diakakis, K. Yaragudri Vinod, Jin Cao, Richard L. Hyson, Weimin He, Thomas B. Cooper, Basalingappa L. Hungund and Richard H. Finnell	ENDOCANNABINOID SIGNALING IN GASTRULATION AND EARLY NEURODEVELOPMENT OF CHICK AND MOUSE EMBRYOS	P2-28
Douglas McHugh, Emily J. Dunn and Heather B. Bradshaw	N-ARACHIDONOYL GLYCINE POTENTLY INDUCES HUMAN ENDOMETRIAL CELL MIGRATION	P2-29
Evangelia Bakali, Ruth A. Elliott, Jonathon M. Willetts, Anthony H. Taylor, Justin C. Konje and Douglas G. Tincello	DISTRIBUTION OF THE ENDOCANNABINOID SYSTEM IN THE RAT AND HUMAN BLADDER	P2-30
Nathalie Percie du Sert, W.-S. Vanessa Ho, John A. Rudd and Paul L. R. Andrews	THE CANNABINOID, WIN55,212, REDUCES PACEMAKER FREQUENCY IN THE GASTRIC ANTRUM OF CONSCIOUS FERRET; A POTENTIAL MECHANISM FOR CANNABINOID-INDUCED GASTROPARESIS	P2-31

POSTER SESSION 3: TOPICS I&J
DAY 3, MONDAY JULY 26TH: 13:30 – 15:00

TOPIC I. *ENDOCANNABINOID SYSTEM*

Lina Thors, Johanna Ranberg and Christopher J. Fowler	CELLULAR TARGETS FOR ANANDAMIDE REUPTAKE INHIBITORS	P3-1
Toru Uyama, Xing-Hua Jin, Naoki Shinohara, Kazuhito Tsuboi, Takeharu Tonai and Natsuo Ueda	HUMAN TUMOR SUPPRESSORS HAVE A NAPE-FORMING N-ACYLTRANSFERASE ACTIVITY	P3-2
J.L. Martos, D.F. Woodward, J.W. Wang, R.A. Ross, C. Cornell, S.N. Pettit, R.W. Carling and H. Fliri	DESIGN OF ANTAGONISTS FOR PGF2α-ETHANOLAMIDE AND PGE2-GLYCERYL ESTER BY USING A SINGLE OXABICYCLOHEPTANE SCAFFOLD	P3-3
Stefania Petrosino, Pilar Brazis, Anna Puigdemont, Mariella Fusco, Francesca Comelli, Barbara Costa and Vincenzo Di Marzo	POSSIBLE "ENTOURAGE" EFFECT OF PALMITOYLETHANOLAMIDE IN DOG AND HUMAN PLASMA	P3-4
Bela Szabo and Sophia Linder	ANALYSIS OF THE STIMULATORY EFFECT OF RIMONABANT ON THE GABAERGIC SYNAPTIC TRANSMISSION IN THE CEREBELLAR CORTEX	P3-5
Jemma C. Cable, Saoirse E. O'Sullivan and Christopher J. Fowler	INSULIN INCREASES ANANDAMIDE UPTAKE IN CULTURED HUMAN ADIPOCYTES	P3-6
Coco N. Kapanda, Geoffray Labar, Jacques H. Poupaert and Didier M.Lambert	SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF ARYLDITHIOCARBAMATES AS SELECTIVE MONOACYLGLYCEROL LIPASE INHIBITORS	P3-7
Michiel GJ Balvers, Michel M Joosten, Kitty CM Verhoeckx, Heleen M Wortelboer, Jocelijn Meijerink and Renger F Witkamp	FREE FATTY ACID LEVELS IN HUMAN PLASMA CORRELATE WITH N-ACYLETHANOLAMINES	P3-8
Giuseppina Catanzaro, Sergio Oddi, Filomena Fezza, Chiara De Simone, Mariangela Pucci, Daniele Piomelli, Alessandro Finazzi-Agrò and Mauro Maccarrone	PITFALLS AND SOLUTIONS IN ASSAYING ANANDAMIDE TRANSPORT IN CELLS	P3-9
Tiffany T.-Y. Lee, Matthew N. Hill, Silvain Dang, Cecilia J. Hillard and Boris B. Gorzalka	TEMPORAL SPECIFIC CHANGES THAT OCCUR IN ADOLESCENT LIMBIC ENDOCANNABINOID SIGNALING: RELEVANCE TO EMOTIONAL BEHAVIOR IN ADULTHOOD	P3-10

M. de Wit, L Saddik and S.F.A.J. Horsten	CONCENTRATIONS OF Δ^9-TETRAHYDROCANNABOLIC ACID DURING GROWTH AND DEVELOPMENT OF MEDICINAL GRADE CANNABIS PLANTS	P3-11
Szczesniak AM, Slusar JE and Kelly MEM	EFFECT OF TOPICAL AND SYSTEMIC ADMINISTRATION OF ABNORMAL CANNABIDIOL ON INTRAOCULAR PRESSURE IN BROWN NORWAY RATS	P3-12
Josée Guindon and Andrea G. Hohmann	PERIPHERAL ANTINOCICEPTIVE EFFECTS OF INHIBITORS OF MONOACYLGLYCEROL LIPASE IN A RAT MODEL OF INFLAMMATORY PAIN: A COMPARATIVE ANALYSIS	P3-13
Joel Musee and Lawrence J. Marnett	CYCLOOXYGENASE-2-CATALYZED OXYGENATION OF 2-ARACHIDONOYLGLYCEROL IS MORE SENSITIVE TO PEROXIDE TONE THAN OXYGENATION OF ARACHIDONIC ACID	P3-14
Jonathan Z. Long, Xin Jin and Benjamin F. Cravatt	TISSUE-SPECIFIC DIFFERENCES IN THE REGULATION OF N-ACYL ETHANOLAMINES AND N-ACYL TAURINES BY FATTY ACID AMIDE HYDROLASE	P3-15
Arnau Busquets-Garcia, Emma Puighermanal, Rafael Maldonado and Andrés Ozaita	DIFFERENTIAL ROLE OF ENDOCANNABINOIDS IN MEMORY CONSOLIDATION, ANALGESIA AND ANXIETY	P3-16
Luciano De Petrocellis, Alessia Ligresti, Aniello Schiano Moriello, Marco Allarà, Tiziana Bisogno, Stefania Petrosino, Colin Stott and Vincenzo Di Marzo	INTERACTIONS OF NON-THC PHYTOCANNABINOIDS AND CANNABIS EXTRACTS WITH TRP CHANNELS AND ENDOCANNABINOID METABOLIC ENZYMES	P3-17
Lalita D. Shrestha and Cecilia J. Hillard	GLUCOCORTICOID EFFECTS ON NEURONAL MONOACYLGLYCEROL LIPASE ACTIVITY	P3-18
Aoife Gowran, Siobhan Hussey, Katey K McKayed and Veronica A Campbell	THE ROLE OF THE ENDOCANNABINOID SYSTEM IN MESENCHYMAL STEM CELL DIFFERENTIATION, SURVIVAL AND MIGRATION	P3-19
Akwasi A. Amoako, Timothy H. Marczylo, Patricia M.W. Lam, Amanda Derry, Jonathon Willets, Janine Elson and Justin C. Konje	VALIDATION OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF AEA, OEA AND PEA IN HUMAN SEMINAL PLASMA BY UPLC-MS/MS	P3-20

TOPIC J. *RECEPTOR SIGNALING*

Caitlin J. Riebe and Carsten T. Wotjak	DIFFERENTIAL MODULATION OF PTSD SYMPTOMATOLOGY VIA GENETIC MODULATION OF ENDOCANNABINOID RECEPTORS	P3-21
Christopher J. Roberts, Amy L. Sasman and Cecilia J. Hillard	SEXUAL DIMORPHISM IN CB1R-MEDIATED BEHAVIORAL COPING STRATEGY IN THE FORCED SWIM TEST	P3-22
K. Yaragudri Vinod, Shanaz M. Tejani-Butt, Basalingappa L. Hungund and Thomas B. Cooper	REGION-SPECIFIC AND SELECTIVE ABNORMALITES IN THE ENDOCANNABINOID SYSTEM IN WISTAR KYOTO RAT: A GENETIC MODEL OF DEPRESSION	P3-23

Maria Luisa Rojo and Christopher J. Fowler	QUANTIFICATION OF LYSOPHOSPHATIDYLINOSITOL-STIMULATED [35S]GTPγS AUTORADIOGRAPHY IN THE RAT BRAIN. COMPARISON WITH RESPONSE TO CP55,940	P3-24
Heather B. Bradshaw and Siham Raboune	NOVEL ENDOGENOUS TRPV1 AND TRPV4 AGONISTS ARE STRUCTURAL ANALOGS TO ANANDAMIDE	P3-25
Saori Oka, Shinji Kimura, Mitsuru Shima, Ryo Ota, Atsushi Yamashita and Takayuki Sugiura	BIOLOGICAL ACTIVITIES OF LYSOPHOSPHATIDYLINOSITOL AND SEVERAL CANNABINOID RECEPTOR LIGANDS AND RELATED MOLECULES AS GPR55 LIGANDS	P3-26
P. Marini and Ruth A. Ross	CHARACTERIZATION OF WELL-KNOWN LIGANDS ON TISSUES NATURALLY EXPRESSING CB2 RECEPTORS	P3-27
June Penman, Christopher M. Henstridge and Andrew J. Irving	THE ENDOCANNABINOID VIRODHAMINE ACTIVATES G-PROTEIN COUPLED RECEPTOR 55	P3-28
Amy Alexander, Natalia Reglinska, June Penman and Andrew J. Irving	N-ACYL AMIDES PROMOTE GPR119-MEDIATED CALCIUM SIGNALING	P3-29
Lawrence C. Blume, Caroline E. Bass, George D. Dalton, Dana E. Selley and Allyn C. Howlett	CANNABINOID RECEPTOR INTERACTING PROTEIN (CRIP) 1A: SIGNAL TRANSDUCTION AND EPIGENETIC PHENOMENA	P3-30
Badr M. Ibrahim and Abdel A. Abdel-Rahman	DOWN-REGULATION OF ROSTRAL VENTROLATERAL MEDULLA PI3K/AKT SIGNALING UNDERLIES THE CENTRAL CB1R-EVOKED SYMPATHOEXCITATION IN CONSCIOUS RATS	P3-31
D. Piwnica, W.D. Stamer, A.B. Kharlamb, J.W. Wang and D.F. Woodward	FIRST INVESTIGATIONS OF ENDOCANNABINOID FUNCTION IN HUMAN OCULAR CELLS USING CELLULAR DIELECTRIC SPECTROSCOPY	P3-32

NOTES:

POSTER SESSION 4: TOPICS K-M
DAY 4, TUESDAY JULY 27TH: 10:00 – 11:30

TOPIC K. *IMMUNE RESPONSE*

Khalil Eldeeb, Stephen Alexander, David Pritchard and David Kendall	EFFECT OF CANNABINOIDS AND GPR55 AGONISTS ON MICROGLIAL CELLS MIGRATION IN CULTURE	P4-1
Man Hee Rhee, Mehari Endale and Parth Vyas	ANTI-INFLAMMATORY ACTIVITY OF ANANDAMIDE IN MURINE MACROPHAGE RAW264.7 CELLS: INVOLVEMENT OF HEME OXYGENASE-1 EXPRESSION	P4-2
Marcel de Wit	ANTI-INFLAMMATORY CANNABINOIDS WITHOUT PSYCHO-ACTIVE EFFECTS	P4-3
Eric J. Downer, Eileen Clifford, Bruno Gran, Hendrik J. Nel, Padraic G. Fallon and Paul N. Moynagh	R(+)WIN55,212-2 REGULATES TLR3 AND TLR4 SIGNALLING: THERAPEUTIC IMPLICATIONS FOR MULTIPLE SCLEROSIS	P4-4
Sumner Burstein, Catherine McQuain and Rebecca Salmonsen	NOVEL ANTI-INFLAMMATORY ANALOGS OF ANANDAMIDE	P4-5
Daniele De Filippis, Giuseppe Esposito, Carla Cirillo Mariateresa Cipriano, Benedicte De Winter, Caterina Scuderi, Rosario Cuomo, Luca Steardo, Joris De Man and Teresa Iuvone	EFFECT OF CANNABIDIOL ON LPS ACTIVATE GLIAL CELL	P4-6
Camille B Carroll, Marie-Louise Zeissler, Virginia Stone, C Oliver Hanemann and John P Zajicek	CANNABINOIDS ARE NEUROPROTECTIVE IN A HUMAN CELL CULTURE MODEL OF PARKINSON'S DISEASE	P4-7
Urszula Grabiec, Marco Koch, Sonja Kallendrusch, Robert Kraft and Faramarz Dehghani	THE NOVEL ENDOCANNABINOID N-ARACHIDONOYL-DOPAMINE EXERTS NEUROPROTECTIVE EFFECTS AFTER EXCITOTOXIC NEURONAL DAMAGE	P4-8
Sean Emery, Xu Di, David A. Gewirtz and Aron H. Lichtman	CELL LINE SPECIFIC ENHANCEMENT OF SENSITIVITY TO ADRIAMYCIN BY PHYTOCANNABINOIDS IN BREAST CANCER	P4-9
Cecilia J. Hillard and Kara L. Stuhr	ANANDAMIDE SYNTHESIS IS ALTERED BY DEXAMETHASONE	P4-10

TOPIC L. *CELL REGULATION*

TOPIC M. *TRANSLATIONAL RESEARCH*

Samir A. Ross, Mohamed M. Radwan, Abir T. El-Alfy, Susan, P. Manly, Safwat A. Ahmed, Desmond Slade, Anwen Eslinger, Lisa Wilson, Suzanna Seale, Olivia Dale, Stephen Cutler, Ikhlas A. Khan and Mahmoud A. ElSohly	ISOLATION AND PHARMACOLOGICAL CHARACTERIZATION OF MINOR CANNABIS CONSTITUENTS	P4-21
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PRESIDENTIAL PLENARY SPEAKER

ON THE EXISTENCE OF CB1 CONTAINING RECEPTOR HETEROMERS IN THE BRAIN: MOLECULAR INTEGRATION VIA ALLOSTERIC MECHANISMS

KJELL FUXE, M.D.

Department of Neuroscience, Karolinska Institutet, Sweden

K. Fuxe[1], M. Canals[2], M. Frankowska[1,3], M. Filip[3], L. F. Agnati[4], G. Milligan[5] and D. Marcellino[1]

[1]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; [2]Leiden/Amsterdam Center for Drug Research Division of Medicinal Chemistry Faculty of Sciences, Division of Chemistry, Department of Pharmacochemistry, Amsterdam, The Netherlands; [3]Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland; [6]Department of Biochemical Sciences, University of Modena, Modena, Italy and [5]Molecular Pharmacology Group, Neuroscience and Molecular Pharmacology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

The GPCR superfamily of receptors was discovered by Dr. Lefkowitz in 1986 in which the largest family is represented by the class A rhodopsin-like receptors. Based on indications of direct physical interactions between neuropeptide and monoamine receptors from the early 1980s, the term *receptor-receptor* interactions was introduced and later on the term *receptor heteromerization* in the early 1990s. Allosteric mechanisms allow an integrative activity to emerge either intramolecularly among GPCR monomers or intermolecularly via receptor-receptor interactions in GPCR homodimers, heterodimers and receptor mosaics. Receptor-receptor interactions markedly increase the repertoire of GPCR recognition, signaling and trafficking in receptor heteromers (Lefkowitz, 2000).

In vitro results show the ability of the CB_1 receptor agonist CP 55,940 to reduce the affinity of dopamine D_2 receptor agonist binding sites in the basal ganglia. In addition, the FRET technique performed in living cells demonstrated the formation of CB_1-D_2 receptor heteromers independent of receptor occupancy (Marcellino et al., 2008). Antagonistic CB_1-D_2 interactions were also discovered at the behavioral level through an analysis of quinpirole-induced locomotor hyperactivity in rats. The CB_1 receptor agonist CP 55,940 at a dose that did not change basal locomotion was able to block quinpirole-induced increases in locomotor activity. In addition, not only the CB_1 receptor antagonist rimonobant, but also the specific A_{2A} receptor antagonist MSX-3 blocked the inhibitory effect of CB_1 receptor agonist on D_2-like receptor agonist-induced hyperlocomotion. Taken together, these results give evidence for the existence of antagonistic CB_1-D_2 receptor-receptor interactions within CB_1-D_2 heteromers in which A_{2A} receptors also participate.

A_{2A}-CB_1-D_2 receptor-receptor interactions in A_{2A}-CB_1-D_2 receptor mosaics may play a large role in the alterations in dopamine signaling in Parkinson's disease (PD). It has been shown that in models of PD, indirect-pathway endocannabinoid-mediated long-term depression (eCB-LTD) is absent but can be rescued by a D_2 receptor agonist or by inhibitors of endocannabinoid degradation that likely involves the release of endocannabinoids and activation of CB_1 homomers on the striatal glutamate terminals. Co-administration of these drugs *in vivo* reduces Parkinsonian motor deficits suggesting that endocannabinoid-mediated depression of indirect-pathway glutamate synapses involving CB_1 homomers has a critical role in the control of movement in the acute phase. In concert with these findings, endocannabinoid signaling is involved in mediating psychomotor activation of adenosine A_{2A} antagonists (Lerner et al., 2010).

A complete understanding of A_{2A}-CB_1-D_2 interactions at the level of agonist recognition and G-protein coupling of the corresponding receptors will therefore advance understanding of the indirect-pathway's critical role in the control of movement. Using the 6-OHDA hemi-Parkinsonian rat model we have begun to unravel the molecular changes that occur within this receptor mosaic when dopamine signaling is reduced. The major observations are a rise of the density of CB_1 receptors in the dorsal striatum on the lesioned side 13 months post lesion and the appearance of a high affinity CB_1 agonist binding site on the lesioned side at 2 and 13 months post lesion. This high affinity binding site of the CB_1 receptor appears to be G protein coupled, functional and able to reduce D_2 recognition (Marcellino et al., unpublished data). The loss of D_2 signaling in hemi-parkinsonian rats leads to allosteric changes in dorsal striatal CB_1-D_2

heterodimers/receptor mosaics with the appearance of a high affinity CB_1 agonist binding site that upon activation may inhibit D_2 signaling in the CB_1-D_2 heteromer. Such an antagonistic interaction may increase progressively with time in view of the rise of CB_1 receptor density seen 13 months after lesion. It is of interest that *in vitro*, addition of D_2 agonists, but not antagonists, in the dorsal striatal membrane preparations causes the disappearance of the high affinity CB_1 agonist binding site that may be produced by a postulated reciprocal D_2-CB_1 inhibitory receptor interaction in this heteromer in the lesioned striatum of hemi-Parkinsonian rats. These results suggest that CB_1 receptor antagonists, by targeting the CB_1 receptors of CB_1-D_2 receptor heteromers, may be considered as a new strategy for treatment of PD. They may have unique pharmacology versus CB_1 homomers since they may undergo conformational changes in the CB_1-D_2 heteromers that affects the recognition and also the G protein coupling. The available evidence for the operation of the putative A_{2A}-CB_1-D_2 receptor mosaic in the brain states that the antagonistic CB_1-D_2 interaction activated by the D_2 receptor induced formation of endocannabinoids likely involving release in microvesicles into the extracellular fluid (roamer type of volume transmission; Agnati et al., 2010; Fuxe et al., 2010) removes the D_2 receptor brake on A_{2A} receptor signaling to adenylate cyclase. It represents an inhibitory allosteric feedback mechanism in putative A_{2A}-CB_1-D_2 receptor mosaics mainly in the ventral striato-pallidal GABAergic neurons and in cortico-striatal glutamate terminals. This CB_1-D_2 inhibitory feedback mechanism develops to reduce an exaggerated and prolonged activation of D_2 receptors that will produce a maintained silencing of the striato-pallidal GABAergic neurons. The release of the D_2 receptor brake through the antagonistic allosteric CB_1-D_2 receptor-receptor interaction on A_{2A} receptor-induced activation of adenylate cyclase probably plays a major role in making this negative feedback possible. The negative feedback may dominate in the late phase upon prolonged D_2 activation and involves the activation of CB_1 receptors in CB_1-D_2 heterodimers or A_{2A}-CB_1-D_2 receptor mosaics underlining the use of CB_1 antagonists in combination with L-DOPA and D_2 agonist treatment in Parkinson's disease.

Other CB_1 receptor containing heteromers also exist. In 2003 the CB_1 receptor was found to hypersensitize the orexin 1 receptor in cell lines in terms of MAPK signaling, an effect blocked by a CB_1 receptor antagonist, but not in formation of inositol phosphate. Electronmicroscopy showed that they were closely opposed at the plasma membrane indicating the formation of heteromers (Hilairet et al., 2003). Subsequent studies (Ellis et al., 2006) demonstrated CB_1-orexin 1 receptor heteromerization with FRET techniques. The presence of CB_1 receptors brought many of the orexin 1 receptors into an endosomal position from the plasma membrane where the two receptors existed as heteromers. Upon blockade of either one of the receptors the heteromers returned to the plasma membrane. Thus, the two receptors can traffic between the plasma membrane and cytosol as heteromers in which the individual protomers modulate the trafficking and signaling of one another. Thus, this may be one of the mechanisms for the ability of CB_1 antagonists to reduce food intake with potential influence also on the reward arousal produced by orexin receptor activation.

Kang Tsou Memorial Lecture

Multiple Messenger Systems: Focus on Neuropeptides in Depression

Thomas Hökfelt, M.D., Ph.D.

Department of Neuroscience, Karolinska Institutet, Sweden

The last decades have seen an expansion of molecules which are involved in chemical transmission in the brain. Some four decades ago the role of neuropeptides started to be explored, and today we know that they represent the largest group of messenger molecules in the nervous system. More recently unconventional transmitters such as nitric oxide and the endocannabinoids have captured the attention. The latter had an unusually fast journey, from discovery to a clinical drug on the market in ten years or so. It is now clear that all these messengers are not synthesized in separate systems, but can occur in various combinations in single neurons, the one neuron – multiple transmitter concept. Many of these transmitters, including neuropeptides and endocannabinoids, have been associated with depression and their receptors proposed as possible targets for antidepressant therapy. We have focused, in particular, on the neuropeptide galanin which in rats is expressed in noradrenaline neurons in the locus coeruleus and in serotonin neurons in the dorsal raphe, that is targets for current treatment of major depression. Evidence from animal experiments suggests involvement of the galanin system in depression-like behaviour. We are now exploring to what extent expression of galanin and its receptors in the human brain is similar to that found in rodents, with the aim to provide a better basis for developing novel antidepressants. We will discuss these findings also in the light of recent studies showing a close relation of cannabinoid receptors with noradrenaline and serotonin neurons, and with the involvement of the cannabinoid system in mood disorders.

DISCOVERY OF CANNABINOID AGONISTS FOR GPR55: THERAPEUTIC IMPLICATIONS

Peter Greasley

Science & Strategy Manager, Lead Generation Department
AstraZeneca R&D Mölndal, SE 431 83. SWEDEN

Over a number of years a body of evidence has been assembled that suggests that some ligands active at CB1 and CB2 receptors are also active via other mechanisms. This has led to an extensive search to identify and describe these molecular targets and to establish what relationship they have to the endocannabinoid system. Since the initial publication of GPR55 as a putative cannabinoid receptor a number of reports have appeared that vary in their findings in respect to cannabinoid ligand pharmacology at this receptor. In the main part these studies have utilised recombinant expression of GPR55 in different cell backgrounds with a variety of assay techniques making it difficult to understand the true pharmacology of the receptor and reasons for the inconsistencies.

To better understand what the true pharmacology of cannabinoid ligands at GPR55 is, we have sought to investigate ligand activities at endogenous GPR55 in tissues and cells. In brain tissue from wild type and CB1 knock-out mice we found that some cannabinoid ligands bind in the absence of CB1 receptors and mediate GTPγS binding. Using brain tissue from GPR55 knock-out mice we have found evidence that GPR55 is responsible for the non-CB1 binding measured in these studies. A RT-PCR approach was used to screen cell lines for CB1, CB2, TRPV1 and GPR55 mRNA expression. By this method we determined that GPR55 was expressed in a U87 glioma cell line that was then used for subsequent studies. Using dynamic mass redistribution, migration and GTPγS assays we have demonstrated a role for cannabinoid ligands and using siRNA provide evidence that GPR55 is a mediator of the observed effects.

These findings show that cannabinoid agonists may in part mediate some of their effect through GPR55 in vivo. In vivo studies suggest a role for GPR55 in cardiovascular function, an observation supported by data generated in GPR55 knockout mice. A role for GPR55 in other physiological processes will also be discussed.

GPR55: PHARMACOLOGY AND SIGNAL TRANSDUCTION

Mary E. Abood

Temple University, Philadelphia, PA, USA

GPR55 has recently attracted much attention as another member of the cannabinoid family, potentially explaining physiological effects that are non-CB1/CB2 mediated. However, the data gathered so far are conflicting with respect to its pharmacology. I will briefly summarize GPR55 pharmacology, signal transduction and potential physiological functions. Despite the varied pharmacological reports on this receptor, some conclusions may be drawn. The CB1 receptor antagonist/inverse agonist AM251 has been shown to be a GPR55 agonist in all reports in which it was evaluated, as has the lysophospholipid, lysophosphatidylinositol (LPI). Another consensus among reports is that WIN55,212-2 is not an agonist at GPR55. Whether GPR55 responds to the endocannabinoid ligands anandamide and 2-arachidonylglycerol and the phytocannabinoids, delta-9-tetrahydrocannabidiol and cannabidiol, is cell-type and tissue-dependent. GPR55 has been shown to utilize G_q, G_{12}, or G_{13} for signal transduction; RhoA and phospholipase C are activated. Due to a large body of conflicting pharmacological data, no conclusive decision can yet be reached about whether or not GPR55 should be classified as a novel cannabinoid receptor. Nonetheless, it is a receptor of great interest. Experiments with mice in which GPR55 has been inactivated reveal a role for this receptor in neuropathic and inflammatory pain as well as in bone physiology. Thus delineating the pharmacology of this receptor and the discovery of selective agonists and antagonists merits further study and could lead to new therapeutics.

N-ACYLETHANOLAMINES AND DEORPHANIZED GPCRS

Harald S. Hansen

Department of Pharmacology & Pharmacotherapy, Faculty of Pharmaceutical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark

For the cannabinoid research community, anandamide is the most interesting *N*-acylethanolamine, although it is generally found in much smaller amounts than most other *N*-acylethanolamines, e.g. *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine (OEA), *N*-linoleoylethanolamine (LEA), and *N*-steraoylethanolamine (SEA). All of these four non-cannabinoid lipid molecules seem to be formed *in vivo* more or less simultaneously with anandamide, and when exogenously applied they all have individual biological activities. It is generally recognized that they can activate vanilloid receptor (except SEA), PPARalpha (except SEA) and probably also GPR119 (reported for OEA only). Furthermore, they can inhibit some K+-channels. Thus, they (including anandamide) seem to be promiscuous ligands affecting many signalling proteins/receptors.

The presentation will also report on a novel lipid that via an orphan GPCR can stimulate insulin release in humans.

CANNABINOID INTERACTIONS WITH TRP CHANNELS

Vincenzo Di Marzo

Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, 80078, Via Campi Flegrei 34, Pozzuoli (NA), Italy

The transient receptor potential (TRP) superfamily of non-selective cation channels includes 6 subfamilies: TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin'), and TRPA ('Ankyrin') channels. TRP channels are six transmembrane (TM) domain integral membrane proteins, with cytosolic C- and N-terminal domains, and a non-selective cation-permeable pore region between TM5 and TM6. The various subfamilies differ particularly for the number of ankirin repeats present in their N-terminus, which is null in TRPM and very high in TRPA channels. Over 50 members of the TRP family have been characterized in yeast, worms, insects, fish, and so far 28 in mammals. They are involved in the trasduction of a remarkable range of stimuli including temperature, mechanical and osmotic stimuli, electrical charge, light, olfactive and taste stimuli, hypotonic cell swelling, xenobiotic substances and endogenous lipids. Importantly, mutations in different TRPs have been linked to human diseases, and their expression in tissues affected by pathological conditions is often increased.

TRP channels of the vanilloid-type 1-4 (TRPV1-4), ankyrin type-1 (TRPA1) or melastatin type-8 (TRPM8) are involved in thermosensation, pain transduction and inflammation. They are expressed in sensory fibers of Ad and C-type, in dorsal root (DRG) and trigeminal ganglia as well as in perivascular neurons, with TRPV1 (the "capsaicin receptor") and TRPA1 (the "mustard receptor") being often co-expressed in the same neurons. Whilst TRPV1-4 are activated by high temperatures, TRPA1 and TRPM8 (the "menthol receptor") are activated by cold. TRPV1 is also activated by low pH, such as during certain inflammatory conditions, as well as by several pro-inflammatory mediators, and this leads to release of algogenic peptides (substance P, CGRP) from sensory neurons, thus contributing to neurogenic inflammation. TRPA1, instead, is activated by numerous irritants.

TRPV1 is also expressed in central neurons. It is abundant in the periaqueductal grey (PAG) and rostral ventrolateral medulla (RVM), where it modulates the descending pathway of antinociception. Contrary to its role in the spinal cord and sensory afferents, TRPV1 in the PAG-RVM contributes to descending antinociception, and it does so by enhancing both glutamatergic signalling/OFF neuron activity in the RVM and m-opioid receptor-mediated analgesia. TRPV1 is expressed in the hippocampus, where it participates in the control of synaptic plasticity; in the prefrontal cortex, amygdala and nucleus accumbens, where it plays a role in the control of anxiety and reward; in the basal ganglia, where its activation causes inhibition of locomotor activity; and in the hypothalamus.

In both central and sensory neurons, TRPV1 is very often co-expressed with cannabinoid CB_1 receptors, with which this channel shares two endogenous agonists, anandamide and *N*-arachidonoyl-dopamine (NADA). TRPV1 and CB_1 can either act in concert or oppose each other at modulating neurotransmitter release, and this cross-talk might occur through several mechanisms. Furthermore, several cannabinoids from *Cannabis sativa*, such as THC, cannabidiol or cannabichromene, activate at least one among TRPV1, TRPV2 and TRPA1 channels, and/or potently antagonize TRPM8 channels. Importantly, CBD can both activate and desensitise TRPV1, TRPV2 and TRPA1 channels, and this property endows the compound with the capability of potentially influencing nociception and inflammation in several ways, also at the supraspinal level. These interactions have pharmacological relevance and, together with the finding of an increasing number of physiopathological functions that anandamide exerts via TRPV1 channels, justify the proposition that TRP channels be considered as "ionotropic cannabinoid receptors".

ENDOCANNABINOID INTERACTIONS WITH PEROXISOMAL PROLIFERATOR-ACTIVATED RECEPTORS

Stephen PH Alexander, David A Barrett, Andrew J Bennett, Victoria Chapman, David A Kendall, Saoirse O'Sullivan and Michael D Randall

University of Nottingham Medical School, Nottingham NG7 2UH, ENGLAND

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, which form part of the nuclear receptor family. Activated PPARs form functional units as heterodimers with retinoid X receptors. 'Classical' agonists at PPARs are fatty acids and their derivatives, ranging from oleic acid and arachidonic acid, to leukotriene B_4 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. It is a widespread view that PPARs are not activated by a single endogenous ligand, but are generalised lipid sensors, monitoring local changes in metabolism. There are three PPAR isoforms: PPARα (the target of the clinically-employed anti-hyperlipidemic fibrates, including gemfibrozil and fenofibrate); PPARγ (a therapeutic target in type II diabetes using pioglitazone, rosiglitazone or troglitazone); and PPARβ (also known as PPARδ), which has yet to be targetted effectively in the clinic. Signal transduction at PPARs is primarily directed through alterations in gene transcription.

In model cells, THC and ajulemic acid are submicromolar potency PPARγ agonists, while CBD, AEA and 2AG are lower potency agonists (5-10 μM). Many cannabinoid ligands exhibit similar potency (~10 μM) at PPARα, although THC appears to be inactive. In contrast, OEA exhibits reasonable potency at PPARα (0.1 μM). The CB_1 receptor antagonists rimonabant and AM251 can activate both PPARγ and PPARα. In vascular preparations, the endocannabinoids AEA and NADA, as well as THC and CBD, cause relaxation through a PPARγ-dependent mechanism, at least in part. In adipose tissue, THC, CBD, HU210, AEA and 2AG, as well as rimonabant and AM251, all evoke adipogenesis through an apparent PPARγ-mediated mechanism.

Overall, the potency of endocannabinoids and their metabolites as PPAR agonists or antagonists are relatively low compared to their potency as agonists of canonical cannabinoid CB_1/CB_2 receptors. This might be taken as evidence that endocannabinoids are poor candidates as PPAR ligands *in vivo*. However, this fails to take into account background levels of PPAR agonists. One estimate puts intracellular levels of long chain fatty acids at 20 μM, which is at a level sufficient to occupy PPARs in cell-free systems. While this background level may vary depending on the cell type and the active state of the cell, fluctuations in intracellular endocannabinoid levels may well prove sufficient to activate PPARs *in vivo*. Currently, the best evidence that endocannabinoids are endogenous agonists at PPARs *in vivo* derives from the use of a model of inflammatory pain. More specifically, local administration of a FAAH inhibitor, which resulted in local accumulation of both AEA and 2AG (but not OEA or PEA) at a time when behavioural responses to the FAAH inhibitor were blocked by local administration of a PPARα, but not PPARγ, antagonist (Jhaveri, 2008, Neuropharmacology **55**: 85-93)

PHYLOGENETIC RELATIONSHIPS OF CANNABINOID RECEPTORS AND RECEPTORS FOR OTHER LIPID MEDIATORS

Maurice R. Elphick

School of Biological & Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom.

The G-protein coupled cannabinoid receptors CB_1 and CB_2 originated by duplication of an ancestral CB_1/CB_2-type receptor in a common ancestor of extant vertebrates. Furthermore, a genome-duplication in a common ancestor of actinopterygian fish is thought to account for the occurrence of duplicate CB_1 receptors or duplicate CB_2 receptors in the puffer fish *Fugu rubripes* and the zebrafish *Danio rerio*, respectively (see abstract by Parmar et al.). Discovery of CB_1/CB_2-type receptors in the urochordate *Ciona intestinalis* (CiCBR) and in the cephalochordate *Branchiostoma floridae* (BfCBR) and absence of CB_1/CB_2-type receptors in non-chordate animals indicates that CB_1/CB_2-type cannabinoid receptors originated in the common ancestor of the chordates. CB_1 and CB_2 belong to a branch of the rhodopsin-type (α group) G-protein coupled receptors (GPCRs) that include: lysophospholipid receptors ($S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, $S1P_5$, LPA_1, LPA_2 and LPA_3); melanocortin receptors (MC_1-MC_5); adenosine receptors (A_1, A_{2A}, A_{2B}, A_3) and the orphan receptors GPR3, GPR6 and GPR12. However, the only receptors in this group that have orthologs in non-chordates are adenosine receptors. Therefore, the common ancestor of CB_1 and CB_2 receptors and other members of this group of related GPCRs may have been an adenosine receptor.

GPR55 has been proposed as a candidate cannabinoid receptor, but there is also evidence that its endogenous ligand is lysophosphatidylinositol. GPR55 is only distantly related to CB_1 and CB_2 and belongs to the δ group of rhodopsin-type GPCRs. Interestingly, amongst other receptors that share high levels of sequence similarity with GPR55 are receptors that have recently been identified as lysophospholipid receptors. These include GPR23 and GPR92, which are activated by LPA and that are now designated LPA_4 and LPA_5, respectively, to distinguish them LPA_1-LPA_3 (see above). Thus, it appears that LPA receptors have evolved independently in both the α and δ branches of the rhodopsin family of GPCRs. GPR55, LPA_4 (GPR23) and LPA_5 (GPR92) belong to a group of closely related receptors that include P2-type purine receptors (e.g. $P2Y_1$ and $P2Y_2$) and a putative P2-like receptor originally designated $P2Y_5$ is in fact also activated by LPA and therefore has recently been designated as LPA_6. It is interesting that GPR55 and the lysophospholipid receptors LPA_4, LPA_5 and LPA_6 are closely related to P2-type purine receptors because, as discussed above, CB_1/CB_2-type cannabinoid receptors and LPA_1-$_3$ receptors are closely related to P1-type (adenosine) purine receptors. Thus, in different branches of the rhodopsin family of GPCRs (α and δ), lipid receptors that are activated by lysophospholipids or cannabinoids may have evolved independently from receptors that are activated by purines.

Δ^9-THCV, A PHYTOCANNABINOID ABLE TO REDUCE MOTOR INHIBITION BUT ALSO TO DELAY DISEASE PROGRESSION IN PARKINSON'S DISEASE

Concepción García[1], Cristina Palomo[1], Moisés García-Arencibia[1], José A. Ramos[1], Roger G. Pertwee[2] and Javier Fernández-Ruiz[1]

[1]*Departamento de Bioquímica y Biología Molecular and CIBERNED, Facultad de Medicina, Universidad Complutense, 28040-Madrid, Spain, and* [2]*School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, UK*

Certain phytocannabinoids, including Δ^9-THC and cannabidiol (CBD), can protect nigral neurons from death in rat models of Parkinson's disease (PD) (Lastres-Becker et al., Neurobiol. Dis., 2005; García-Arencibia et al., Brain Res., 2007). This effect seems to depend on the antioxidant properties of these compounds, and possibly also on a CB_2 receptor-mediated modulation of glial influences on neurons (García-Arencibia et al., Brain Res., 2007; Price et al., Eur. J. Neurosci., 2009). There is also evidence that blockade of CB_1 receptors can alleviate motor inhibition in parkinsonian rats (Fernández-Espejo et al., Neurobiol. Dis., 2005; González et al., Brain Res., 2006). This previous evidence raises the possibility that a cannabinoid having antioxidant properties and the ability to activate CB_2 receptors but block CB_1 receptors, might be a promising therapy for simultaneously alleviating parkinsonian symptoms (e.g. bradykinesia and rigidity) and arresting/delaying neurodegeneration in PD. The phytocannabinoid, Δ^9-THCV, possesses such pharmacological actions (Bolognini et al., Br. J. Pharmacol., 2010), raising the possibility that it might provide symptom relief by blocking CB_1 receptors, and afford neuroprotection because of its phenolic structure (antioxidant effect) and its ability to activate CB_2 receptors. This hypothesis has been examined in the present study by using an experimental model of parkinsonism in which rats receive an i.c.v. injection of 6-hydroxydopamine, a model that may be used to monitor symptoms and neuroprotective effects simultaneously. In these animals, acute administration of Δ^9-THCV (2 mg/kg, i.p.) attenuated the motor inhibition induced by 6-hydroxydopamine (measured using a computer-aided actimeter) with the same potency as rimonabant, a compound that was evaluated in previous studies (Fernández-Espejo et al., Neurobiol. Dis., 2005; González et al., Brain Res., 2006) and used here as a positive control. This effect seems to be related to an action of Δ^9-THCV on glutamatergic transmission (enhancement of glutamate content in the striatum and reduction of glutamate content in the substantia nigra). Also in these animals, chronic administration of Δ^9-THCV (2 mg/kg, i.p.; 14 days) partially attenuated the loss of nigrostriatal dopaminergic neurons caused by 6-hydroxydopamine and monitored by tyrosine hydroxylase immunostaining in the substantia nigra. Additionally, it significantly attenuated 6-hydroxydopamine-induced microglial activation, monitored using OX-42 immunohistochemistry. Both these effects were also induced by CBD (used as positive control), which had been evaluated in previous studies (Lastres-Becker et al., Neurobiol. Dis., 2005; García-Arencibia et al., Brain Res., 2007). CBD was used in these experiments in the form of CBD-enriched botanical extract. In summary, Δ^9-THCV, given its antioxidant properties and its ability to activate CB_2 but block CB_1 receptors at a dose of 2 mg/kg, seems to have an interesting therapeutic profile for the treatment of disease progression in PD, providing at the same time symptomatic relief, effects not induced to the same extent by other phytocannabinoids.

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CANNABINOID RECEPTORS REMODELING IN NEURAL PROGENITORS AND AFTER TRAUMATIC BRAIN INJURY (TBI): POTENTIAL IMPLICATION FOR TBI BIOMARKERS

Stanislav I. Svetlov[1,2], Ronald L. Hayes[1] and Kevin K. W. Wang[1,3]

Center of Innovative Research, Banyan Biomarkers, Inc., Alachua, FL and Departments of Physiological Sciences[2] and Psychiatry[3], University of Florida, Gainesville, FL, USA

The endocannabinoid (EC) system plays an important role in driving neural progenitors in normal development and after injury. A strong expression of CB1 receptors was detected in CD133-positive immature progenitors inside the core of developing clonal neurospheres. In neurospheres differentiating on an adherent matrix, CB1 expression was found in cells of neuronal lineage, in residual CD133 positive cell clusters, and, partially, GFAP-positive cells, suggesting a possible role of CB1 in astrocyte differentiation. Synthetic endocannabinoids sustained growth of neurospheres from stem/progenitor cells of mouse and rat postnatal forebrain, while AM251 abolished the generation of clonal neurospheres.

Given the significance of stem/progenitor cells in tissue repair, we proposed that the EC system is a major player in tune-up responses following brain damage, particularly traumatic brain injury (TBI), and examined whether CB1 and CB2 receptors can serve as biochemical markers in the course of TBI. In rats subjected to controlled cortical impact (CCI), the N-terminal of the CB1 receptor exhibited a rapid and profound down-regulation in the ipsilateral cortex and hippocampus within an hour after trauma. N-terminal-positive CB1 immunoreactivity gradually recovered at day 5, followed by up-regulation within 5 to 14 days post-injury. A similar expression pattern was found in the C-terminal of CB1, with a delay in down-regulation followed by a gradual restoration without a significant up-regulation. It is important to note that a concomitant increase of C-terminal CB1 reactivity was detected in proteins associated with dimeric and oligomeric forms of the CB1 receptor. Both C-terminal and N-terminal parts accumulated in CSF as monomeric and oligomeric forms, with a characteristic time course after TBI. While CB1 receptors are abundantly expressed in CNS and undergo remodeling after trauma, CB2 receptors are barely detectable in the normal brain and were up-regulated after TBI, suggesting involvement of inflammatory cells presenting the CB2 receptor.

The time-dependent redistribution of CB1 and CB2 in the injured brain provides a rationale for correct timing of CB1/CB2-directed pharmacological intervention of TBI when the target is present and may be responsive to therapy. Appropriate monitoring of CB1 status by measuring its level in circulation may provide a useful method for early diagnostics of TBI, as well as control of therapy.

THE TREATMENT WITH WIN55,512-2 AMELIORATES DISEASE PROGRESSION IN A MODEL OF MULTIPLE SCLEROSIS IN MICE, ACTING THROUGH ANTI-INFLAMMATORY AND ANTI-GLUTAMATERGIC MECHANISMS

Eva de Lago, Miguel Moreno-Martet, Carmen Rodríguez-Cueto, María Gómez-Cañas, María Gómez-Ruiz and Javier Fernández-Ruiz

Departamento de Bioquímica y Biología Molecular and CIBERNED, Facultad de Medicina, Universidad Complutense, 28040-Madrid, Spain

Multiple sclerosis (MS) is an autoimmune disease that affects the CNS and it is characterized by inflammation, demyelination, remyelination, gliosis and axonal damage. Cannabinoids have been proposed as promising therapeutic agents in MS given their capability to alleviate specific MS symptoms (e.g., spasticity, pain). Although MS has been considered mainly as an inflammatory disorder, recent evidence, however, revealed the importance of neurodegenerative events, opening the possibility that cannabinoid agonists, given their cytoprotective properties, may also serve to reduce oligodendrocyte death and axonal damage in MS. Thus, the treatment with WIN55,512-2, a potent CB_1 and CB_2 agonist, was reported to be effective to ameliorate tremor and spasticity in mice with chronic relapsing experimental autoimmune encephalomyelitis (CREAE), a murine model of MS (Baker et al., 2000; Pryce et al., 2007), but also to delay disease progression in this (Croxford et al., 2003) and other murine models of MS (Arévalo-Martín et al., 2003). The purpose of this investigation was to further explore the mechanism(s) underlying the amelioration in the progression of the disease caused by WIN55,212-2, particularly we have paid emphasis in anti-glutamatergic and anti-inflammatory effects of this cannabinoid agonist. In this study, we used mice treated with myelin oligodendrocyte glycoprotein (MOG) that generates a progressive pattern of EAE induction and conducted the pharmacological experiments in an early stage of the disease. As expected, the administration of WIN55,512-2 (5mg/kg, i.p) had a positive effect in reducing neurological disability and improving motor coordination of EAE mice. Levels of glutamate and GABA in the spinal medulla, cerebellum and brainstem of EAE mice were similar to control animals, and, accordingly, they were not altered by the treatment with WIN55,212-2. However, EAE mice showed alterations in mRNA levels for the glutamate transporter GLT1 and, to a lesser extent, GLAST too, in the spinal medulla, alterations that were reversed by the treatment with WIN55,212-2. As regards to inflammatory responses, EAE mice showed a marked up-regulation in mRNA levels for COX-2, inducible NOS and TNF-α, responses that were attenuated after the treatment with WIN55,212-2, particularly in the spinal medulla but also, to a lower extent, in the brainstem and cerebral cortex. Lastly, experiments conducted with selective antagonists for the CB_1 (e.g. rimonabant) or CB_2 (e.g. AM630) antagonists revealed that WIN55,212-2 effects seem to be mediated predominantly by the activation of CB_1 receptors. In summary, the treatment of EAE mice with the cannabinoid agonist WIN55,512-2 reduced their neurological disability and the progression of the disease, acting by mechanisms that combine both anti-inflammatory and anti-glutamatergic events and that are predominantly related to the activation of CB_1 receptors. These observations suggest that cannabinoid agonists may be useful not only to treat symptoms resulting from the disease but also for ameliorating the progression of MS by controlling two important events in the pathogenesis of this disease.

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ESTRADIOL DECREASES REACTIVE ASTROGLIOSIS IN A RAT MODEL OF BRAIN INJURY BY A MECHANISM INVOLVING THE ENDOCANNABINOID SYSTEM

María-Paz Viveros[1], Ana Belén López Rodríguez[1], Beatriz Mateos[1], Silvana Y. Romero-Zerbo [2], Noé Rodriguez-Rodriguez[1], María José Bellini[4], Fernando Rodriguez de Fonseca[2], Francisco J Bermudez-Silva[2], Iñigo Azcoitia[3], Luis M. Garcia-Segura[4].

Departments of [1]Physiology (Animal Phisiology II) and [3]Cell Biology, Faculty of Biology, Complutense University of Madrid, E-28040 Madrid, Spain. [2]Laboratorio de Medicina Regenerativa, Fundación IMABIS, E-29010 Málaga, Spain. [4]Instituto Cajal, CSIC, E-28002 Madrid Spain;

The neuroactive steroid estradiol reduces reactive astroglia after brain injury by mechanisms similar to those involved in the regulation of reactive gliosis by endocannabinoids. In this study we have explored whether cannabinoid receptors are involved in the effects of estradiol on reactive astroglia. To test this hypothesis, the effects of estradiol, the cannabinoid CB_1 antagonist /inverse agonist AM251 and the cannabinoid CB_2 antagonist / inverse agonist AM630 were assessed in the cerebral cortex of male rats after a stab wound brain injury. Estradiol reduced the number of vimentin immunoreactive astrocytes and the number of glial fibrillary acidic protein immunoreactive astrocytes in the proximity of the wound. The effect of estradiol was significantly inhibited by the administration of either CB1 or CB2 receptor antagonists. The effect of estradiol may be in part mediated by alterations in endocannabinoid signaling, because the hormone restored the mRNA levels of some of the enzymes involved in the synthesis and metabolism of endocannabinoids that were reduced in the injured cerebral cortex. These findings suggest that estradiol may decrease reactive astroglia in the injured brain by regulating the activity of the endocannabinoid system.

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ENDOCANNABINOID SIGNALING IN THE MEDIAL PREFRONTAL CORTEX PROMOTES TERMINATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS FOLLOWING ACUTE STRESS

McLaughlin, R.J.[1], Hill, M.N.[2], Roberts, C.J.[3],
McEwen, B.S.[2], Liu, Q.S.[3], Gorzalka, B.B.[1], Hillard, C.J.[3]

[1]Department of Psychology, University of British Columbia, Vancouver, Canada,
[2]Laboratory of Neuroendocrinology, Rockefeller University, New York, United States,
[3]Department of Pharmacology & Toxicology, Medical College of Wisconsin, Milwaukee, United States

Endocannabinoid signaling is known to negatively regulate activation of the HPA axis; however, the role of this system in the terminating the HPA axis response during recovery from stress has not been well characterized. We demonstrate that mice deficient in the cannabinoid CB_1 receptor exhibit prolonged secretion of corticosterone following exposure to acute stress, a phenomenon that was recapitulated following systemic administration of a CB_1 receptor antagonist. Given the importance of the prefrontal cortex (PFC) in termination of the stress response, and the similarity between the effects of CB_1 receptor disruption and prefrontal cortical lesions on stress recovery, we then examined if these effects were driven by endocannabinoid signaling within the prefrontal cortex. Local administration of a CB_1 receptor antagonist into the medial PFC delayed recovery of corticosterone secretion following exposure to an acute stressor. Consistent with this, exposure to an acute stressor resulted in a sustained increase in the tissue content of the endocannabinoid ligand 2-arachidonoylglycerol (2-AG) within the medial PFC. Furthermore, in vitro studies demonstrated that bath application of stress levels of corticosterone to slices of the medial PFC resulted in a suppression of GABA release onto pyramidal neurons within the prelimbic region of the medial PFC which was reversible by application of a CB_1 receptor antagonist. Collectively, these data create a compelling argument that stress-induced mobilization of endocannabinoid signaling within the medial PFC decreases inhibitory drive on pyramidal neurons within the prelimbic region of the PFC, which ultimately results in a dampening of HPA axis activity and termination of the stress response.

PALMITOYLETHANOLAMIDE CONTROLS HYPERALGESIA BY MODULATION OF MAST CELL ACTIVATION IN AN *IN VIVO* MODEL OF CHRONIC INFLAMMATION

Mariateresa Cipriano°+, Daniele De Filippis°+, Livio Luongo§+, Enza Palazzo§+, Maria Pia Cinelli#+, Vito de Novellis§+, Sabatino Maione§+ and Teresa Iuvone°+

+Endocannabinoid Research Group; °Department of Experimental Pharmacology, University of Naples FEDERICO II; §Department of Experimental Medicine, Second University of Naples; #Department of Biomorphological and Functional Sciences, University of Naples FEDERICO II.

Introduction: Mast cells (MCs) are immune-competent cells mainly localized in sites directly interfacing with external environment, where they also orchestrate the inflammatory reaction. Recently, several evidences indicate a bidirectional cross-talk between MCs and sensory nerves (SNs) suggesting that MCs and SNs may be functionally and anatomically assembled within certain tissues as the skin, where MCs are frequently co-localized, not only with vessels but with nerve fibres, too.
In parallel, the discovery of drugs able to control MC activation, among which Palmitoylethanolamide (PEA), is nowadays of great interest. PEA belongs to the family of ALIAmides (*Autacoid Local Injury Antagonism Amide*), since previous evidences show its capability to control MC activation and to reduce the progression of chronic inflammation, too. Starting from the assumption that MC granules contain also pro-algogenic mediators, primarily Nerve Growth Factor (NGF), the present study addresses its attention to investigate whether PEA is able also to control granuloma-associated hyperalgesia.

Materials and Methods: Granuloma, a typical chronic inflammation, was induced by subcutaneous implantation of two λ-carrageenin (1%)-soaked sponges on the back of male Wistar rats. PEA was injected into each sponge at the concentration of 200, 400, 800 μg/mL. The mechanical allodynia was evaluated by using the Von Frey filaments with calibrated bending forces, that were used to deliver punctuate mechanical stimuli of varyious intensity, in the middle of and around the granulomatous tissue; the frequency of withdrawals induced by consecutive applications of the same filament was evaluated, too. After 96 hours to the implantation, rats were sacrificed and the new nerves formation was evaluated in the granulomatous tissue by histological analysis. Western blot analysis for NGF and Protein Gene Product 9.5 (PGP 9.5) was conducted. In parallel, rat Dorsal Root Ganglia (DRG) were excised and transverse sections treated to perform immunohistochemical analysis, to evaluate citotypes involved in the granuloma-induced DRG sensitization. Obtained slides were incubated with primary antibody solutions for the pro-inflammatory markers TNF-α, NGF and COX-2, co-labeled with TRPV1 and satellite cells marker.

Results: Our results show the analgesic properties of PEA, evaluated by its efficacy in reducing mechanical allodynia, by using the Von Frey filaments. Moreover, the histological image of granulomatous tissues evidenced that the massive presence of degranulated MCs in tight contact with nerve fibres were both significantly reduced by PEA. These data were consequently confirmed by the reduction of pro-algogenic mediators expression from MCs, primarily NGF, in granuloma. **Finally,** we found that granuloma-induced mechanical allodynia was associated with an increased expression of the main pro-inflammatory mediators in the DRG, that were significantly reduced by PEA treatment.

Conclusions: The present data support the evidence of an analgesic role played by PEA in several model of pain, identifying in MCs the most important cell-type susceptible to PEA action, in this model of chronic inflammation-dependent pain. Thus, according to our results it is conceivable to hypothesize the use of PEA and its congeners in the treatment of all those painful conditions sustained by MCs production and activation, such as visceral inflammatory pain or neuropathic pain.

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THE ROLE OF PPARα IN MODULATING INFLAMMATORY PAIN RESPONSES

Bright Okine, Andrew J Bennett and Victoria Chapman

School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.

The peroxisome proliferator activated receptor alpha (PPARα) is a ligand dependent nuclear transcription factor, which has anti-inflammatory and analgesic effects. Here, we investigated the effect of intraplantar injection of the endogenous PPAR α ligand N-palmitoylethanolamide (PEA) on the expansion of hindpaw receptive fields of spinal neurones in the carrageenan model of inflammatory pain. Protein levels of PPARα, and the pro-inflammatory mediators, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) were also determined in the inflamed hindpaw.

Method: Effects of intraplantar injection of PEA or vehicle, on neuronal receptive field expansion of wide dynamic range dorsal horn neurones were determined in carrageenan-inflamed rats. Protein levels of PPARα, COX-2 and iNOS in the hindpaw at three hours following injection of carrageenan were determined by Western blotting and quantified using the LiCOR odyssey infrared imaging system.

Results: Intraplantar injection of PEA (50µg/50µl) significantly attenuated the expansion of neuronal receptive fields on the hindpaw of carrageenan-treated rats ($p<0.05$, $n=6$), but did not alter hindpaw oedema formation induced by intraplantar injection of carrageenan.The inhibitory effect of PEA were blocked by the PPARα antagonist GW6471 (30µg/50µl). PPARα, COX-2 and iNOS were present in the ipsilateral hindpaw of carrageenan-treated rats. Protein levels were significantly higher ($p<0.0001$) compared to the contralateral hindpaw. Intra-plantar injection of PEA significantly ($p<0.05$) increased levels of PPARα in the hindpaw, compared to the effects of vehicle in carrageenan-treated rats. PEA significantly decreased levels of COX-2 ($p<0.0001$) and iNOS ($p<0.001$) in the carrageenan-treated hindpaw. The inhibitory effects of PEA on iNOS expression were partly blocked by the PPARα antagonist. By contrast, the effects of PEA on COX-2 protein were not altered by the PPARα antagonist.

Summary and Conclusion: PEA activation of PPARα attenuated receptive field expansion, a neuronal correlate of central sensitization. This functional effect was associated with a marked decrease in the levels of COX-2 and iNOS in the carrageenan-inflamed hindpaw, however only the effects of PEA on iNOS appeared to be sensitive to the PPARα antagonist.

INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN ATTENTIONAL MODULATION OF NOCICEPTION IN RATS

Gemma K. Ford, Orla Moriarty, Brendan Harhen, Eoin Tully,
Adrian Mulcahy and David P. Finn

Pharmacology and Therapeutics, Centre for Pain Research and NCBES Neuroscience Cluster, National University of Ireland, Galway, Ireland

Distraction interventions are used clinically to relieve pain. Attention is a limited capacity and exposure to another attention-demanding stimulus causes withdrawal of attention away from a painful stimulus and thus reduces perceived pain (Wismeijer & Vingerhoets, 2005 Ann Behav Med 30:268–78). We have recently established and validated a rat model of distraction-induced analgesia where exposure to a novel, non-aversive object resulted in a suppression of formalin-evoked nociceptive behaviour (Ford et al., 2008 Eur J Pain 12:970-979). Given its well-described role in modulation of pain and attentional processing, we investigated the hypothesis that the endocannabinoid (EC) system is involved in mediating distraction-induced antinociception. Male Lister-Hooded rats received intra-plantar injection of formalin into the right hind paw to evoke nociceptive behaviour which was scored 30-60min post-formalin in the presence or absence of a novel object. Immediately following formalin injection, animals received an intra-peritoneal injection of either the fatty acid amide hydrolase inhibitor URB597 (0.3mg/kg), the CB_1 receptor antagonist/inverse agonist rimonabant (1mg/kg), URB597 (0.3mg/kg)+rimonabant (1mg/kg) or vehicle. LC-MS/MS was used to determine post mortem concentrations of endocannabinoids and related fatty acid amides (FAA) in brain tissue punches (Palkovits' punch) of the ventral hippocampus (vHip), bilateral insular cortex (RAIC), ventral prefrontal cortex, bilateral somatosensory area 1 and 2 (S1+S2) and rhinal cortex. Exposure to the novel object reduced formalin-evoked nociceptive behaviour and was associated with increased anandamide (AEA), 2-arachidonoyl glycerol (2-AG), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) in the vHip, and decreased 2-AG in the bilateral RAIC. Rimonabant prevented distraction-induced antinociception, an effect accompanied by reduced levels of AEA and PEA in the vHip, reduced AEA in bilateral S1+S2 and right RAIC, and increased 2-AG in the bilateral RAIC. In rats not exposed to distractor, URB597 significantly reduced nociceptive behaviour, an effect associated with increased OEA and PEA levels in all brain regions, increased AEA in the left S1+S2, and decreased 2-AG in the rhinal cortex. Interestingly, behavioural and biochemical effects of URB597 were blocked by co-administration with rimonabant. These data suggest that FAAH inhibition is antinociceptive in a rat model of tonic persistent pain and is associated with increased levels of FAAs in brain regions involved in pain and visual integration and contextualisation. Pharmacological blockade of CB_1 attenuated the expression of URB597-induced antinociception, prevented distraction-induced antinociception and reversed alterations in EC and FAA concentrations associated with novel object exposure. These results provide evidence that the endocannabinoid system may be an important neural substrate subserving attentional modulation of pain.

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CB_1 CONTROL OF OREXINERGIC NEURONS IN THE LATERAL HYPOTHALAMUS SHIFTS FROM INHIBITION TO DISINHIBITION IN OBESE MICE

Luigia Cristino[1,2], Giuseppe Busetto[3], Roberta Imperatore[1,2,3], Stefania Petrosino[1,5], Ida Ferrandino[4] and Vincenzo Di Marzo[1,5]

[1]Endocannabinoid Research Group, [2]Inst. of Cibernetica and [5]Inst. of Chimica Biomolecolare, CNR, Pozzuoli, 80078, Italy; [3]Dep.of Scienze Neurologiche, Università di Verona, 30134, Italy; [4]Dep. of Scienze Biologiche, Università Federico II, Napoli, 80134, Italy;

The lateral hypothalamus (LH) is the region of the brain previously denoted as the "hunger center". Orexin A and B (OX-A and OX-B) are both orexigenic neuropeptides synthesized by perifornical neurons of the LH. OX-A is a short-term modulator of appetite since it increases food intake in pre-fed rats. Pretreatment with subeffective doses of Rimonabant blocks this effect, suggesting that hypothalamic orexinergic circuits cross-talk with cannabinoid CB_1 receptors in the regulation of appetite (Crespo et al., *Neuropharmacology* 2008). Both orexins interact with other modulators of feeding behaviour and leptin, since orexinergic neurons express the leptin receptor. Endocannabinoids exert a bimodal control of stimulated-food intake, as shown by studies with CB_1 conditional mutant mice (Bellocchio et al., *Nat Neurosci.* 2010), and can inhibit orexinergic neurons in lean rodents (Huang et al., *J. Neurosci.* 2007). However, it remains to be clarified if this latter phenomenon also occurs in obesity, during which high hypothalamic neural plasticity is required for both adequate regulation of energy balance (Horvart & Gao, *Cell Metab*. 2005) and leptin mophoregulatory effects (Valerio et al., *JBC* 2006). With this purpose, we have investigated endocannabinoid/orexin-A interactions in *ob/ob,* leptin-knockout, mice.

Immunohistochemical studies were performed in the LH for OX-A, CB_1, DAGL-α, NAPE-PLD, MAGL, synaptophisin and vescicular GABA (VGAT) or glutamate (VGluT) transporters. Hypothalamic endocannabinoid levels were measured by LC-MS. Whole cell-patch clamp recordings was performed to measure the effect of 5 μM of WIN55,212 on the frequency of spontaneous IPSC (cell clamped at -70mV; 10 μM of CPP and NBQX in the bath) in orexinergic neurons of acute hypothalamic brain slices from p27-33 *ob/ob vs*. littermate mice. DAGL-α colocalized on the membrane of OX-A-ir neurons post-synaptically to CB_1-expressing axon terminals and, unlike NAPE-PLD-ir, was up-regulated both in the LH and arcuate nucleus of *ob/ob* mice *vs.* littermates. Optical and electronic microscopy data showed a prevalent colocalization of CB_1 and MAGL with GABAergic rather than glutamatergic afferents onto OX-A-ir neurons in *ob/ob vs.* littermates mice. WIN55,212 more strongly reduced the sIPSC frequency in *ob/ob* (70% of reduction, n=6) than wild-type mice (40%, n=8).

These findings support the existence of a shift of CB_1 from excitatory to inhibitory inputs onto orexinergic neurons in *ob/ob* mice and, hence, suggest that inhibitory inputs on these neurons may be functionally depressed by endocannabinoids more in obese than in normal mice, thus potentially contributing to hyperphagia.

SUPPRESSION OF SEROTONIN RELEASE IN THE INSULAR CORTEX: A POTENTIAL CANDIDATE FOR THE ANTI-NAUSEA EFFECTS OF CANNABIDIOL

[1]Linda A. Parker, [1]Erin M. Rock, [1]Cheryl L. Limebeer, [1] Katharine Tuerke, [2]John Chambers, [2]Paul J. Fletcher, [3]Raphael Mechoulam

[1]Department of Psychology and Neuroscience Graduate Program, University of Guelph, Guelph, Ontario, Canada
[2]Centre for Addiction and Mental Health and Department of Psychology, University of Toronto, Toronto, Ontario, Canada
[3]Institute of Drug Research, Hebrew University Medical Faculty, Jerusalem, Israel

Conditioned gaping reactions in rats are only produced by emetic treatments and anti-emetic drugs (including ondansetron and cannabinoids) consistently attenuate these reactions. Gaping, therefore serves as a selective rodent model of nausea. At low (e.g., 5 mg/kg, ip) but not high (e.g., 20 mg/kg) doses, Cannabidiol (CBD) also attenuates Lithium Chloride (LiCl)-induced vomiting in shrews and conditioned gaping in rats. The anti-emetic and anti-nausea effects of CBD appear to be mediated by its action at 5-Hydroxytryptamine 1A (5-HT_{1A}) receptors, because these effects are reversed by systemic pretreatment with the 5-HT_{1A} antagonists, WAY100135, WAY100635 and cannabigerol (reported here). Manipulations that reduce forebrain serotonin availability reduce LiCl-induced conditioned gaping in rats and toxin-induced vomiting in other species. Here we report the anti-nausea effect of CBD is produced by its action as a somatodendritic 5-HT_{1A} autoreceptor agonist, because intracranial delivery of WAY100635 to the Dorsal Raphe Nucleus (DRN) also attenuated the anti-nausea effects of CBD. These findings suggest that the suppression of nausea by CBD may be mediated by a reduction of forebrain 5-HT in terminal target regions of projections from the DRN.

Considerable evidence implicates the insular cortex as part of a network of forebrain regions responsible for the generation of disgust, conditioned disgust and nausea. Ablations of the insular cortex prevent LiCl-induced conditioned gaping in rats. Here we report that a 76% reduction of 5-HT in the insular cortex by 5,7-DHT lesions dramatically attenuated lithium-induced conditioned gaping in rats. These data suggest that nausea may be mediated by serotonin release in the insular cortex – a potential site for 5-HT/CB_1 interactions in the regulation of nausea.

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ADIPOCYTE-SPECIFIC CB1 CONDITIONAL KNOCK-OUT MICE: NEW INSIGHTS IN THE STUDY OF OBESITY AND METABOLIC SYNDROME

Giacomo Mancini[1], Carmelo Quarta[2], Raj Kamal Srivastava[1], Susanne Klaus[3], Uberto Pagotto[2] and Beat Lutz[1]

[1]Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany
[2]Endocrinology Unit and Centro Unificato di Ricerca BioMedica Applicata, Dept. of Clinical Medicine, University of Bologna, Bologna 40138 Italy
[3]German Institute of Human Nutrition Potsdam-Rehbruecke, Dept. of Pharmacology Nuthetal 14558, Germany

Obesity is a result of an imbalance between food intake and energy expenditure. Increased intra abdominal/visceral fat promotes a high risk of metabolic disorders and cardiovascular diseases. The endocannabinoid system (ECS) plays a relevant role in obesity development and it has been shown that the pharmacological blockade of cannabinoid receptor type 1 (CB_1) is able to reduce body weight and to alleviate obesity-related metabolic dysregulation. Visceral fat (VF) is the main peripheral source of endocannabinoids (EC) and the CB_1 receptors expression result increased during obesity. An unsolved question is whether or not the effects of CB_1 blockade on food intake and energy balance are caused by modulation of peripheral or only central mechanisms. We generated a new mouse line lacking the CB_1 receptor expression in adipocytes (Ati-CB_1-KO). Ati-CB_1-KO mice are protected from diet-induced obesity (DIO), glucose/insulin impairment and show normal plasma profile. Ati-CB_1-KO mice have a reduced feed efficiency and a reduced excreted energy, showing a negative metabolic balance. These results indicate that the blockade of the CB_1 receptor in the visceral fat is sufficient to prevent the development and maintenance of obesity and metabolic syndrome.

CANNABINOID CB1 RECEPTOR SIGNALING IS REQUIRED FOR GLUCOCORTICOID-MEDIATED METABOLIC SYNDROME

Matthew N. Hill, Nicole M. Bowles, Sarah M. Bhagat,
Ilia N. Karatsoreos and Bruce S. McEwen

Laboratory of Neuroendocrinology, The Rockefeller University
New York, NY 10065 USA

Pervasive exposure to stress, and the concomitant increase in circulating glucocorticoids, is accepted as one of the mediators of the ever-growing epidemic of obesity and metabolic syndrome. Glucocorticoids are known to promote the development of metabolic syndrome through a multitude of pathways including the regulation of both feeding pathways and peptidergic signals. Recent evidence has demonstrated that glucocorticoids possess the ability to increase the production and release of endocannabinoid molecules. Endocannabinoids are potent regulators of appetite, energy balance and metabolic processes through both central and peripheral regulation of feeding and metabolism. Pharmacological or genetic disruption of cannabinoid CB_1 receptor signaling can mitigate diet-induced obesity and metabolic dysregulation. Our laboratory has recently developed a non-invasive model of glucocorticoid administration through drinking water in mice which results in dramatic elevations in adipose and body weight, increased feeding behaviour, metabolic syndrome and central leptin resistance. The aim of the current study was to determine the role of the endocannabinoid signaling in glucocorticoid-mediated obesity and metabolic syndrome. Both male and female wild type and CB_1 receptor deficient mice were given free access to 100 μg/ml corticosterone, or vehicle solution, for 4 weeks, at the end of which markers of obesity and metabolic markers were examined. Glucocorticoid administration resulted in an increase in total body weight, the weight of the abdominal/gonadal fat pads, hepatic steatosis (as indicated by elevated Oil Red O staining) and dramatic elevations in the circulating levels of triglycerides, insulin and leptin. All of these effects were either substantially attenuated or abolished in CB_1 receptor deficient mice, in both males and females, indicating that endocannabinoid signaling is involved in the ability of glucocorticoids to modulate metabolism. Taken with previous research, these data suggest that glucocorticoid exposure produces an elevation of tonic endocannabinoid signaling which promotes the development of metabolic syndrome, either through central changes in feeding behavior or peripheral metabolic processes, or both. Further, these data demonstrate that endocannabinoid signaling is involved in the development of obesity in response to hormonal signals, in addition to previously demonstrated role in diet-induced obesity.

LIVER AND ADIPOSE TISSUE ENDOCANNABINOID TONE IN DIET-INDUCED OBESITY IS DEPENDENT ON APOLIPOPROTEIN E EXPRESSION AND ASSOCIATED WITH HEPATIC STEATOSIS AND INSULIN RESISTANCE

Vincenzo Di Marzo[1], Alexander Bartelt[2], Concetta Mele[1], Pierangelo Orlando[3], Stefania Petrosino[1], Alessia Ligresti[1], Klaus Toedter[2], Ludger Scheja[2] and Joerg Heeren[2]

[1,3]Endocannabinoid Research Group, [1]Istituto di Chimica Biomolecolare and [3]Istituto di Biochimica delle Proteine, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, Comprensorio Olivetti, 80078 Pozzuoli (NA), Italy; [2]Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany

The levels of endocannabinoids and/or CB1 receptors are elevated in the liver and epididymal white adipose tissue (WAT), and reduced in the subcutaneous WAT, of mice with high fat diet (HFD)-induced obesity. Furthermore, in obese rodents and humans, 2-arachidonoylglycerol levels are associated with fatty liver, high amounts of visceral adipose tissue, inflammatory markers and insulin resistance. On the other hand, apolipoprotein E (ApoE) deficiency is associated with non-alcoholic steatohepatitis and reduced fat accumulation in the WAT. Here we investigated, in apoE-/- and wild-type (WT) mice, the effect of a HFD on: 1) subcutaneous and epididymal WAT accumulation, liver triglycerides, inflammatory markers in the WAT and liver, and insulin resistance, and 2) WAT and hepatic fatty acid composition, endocannabinoid levels and CB1 receptor and endocannabinoid metabolic enzyme mRNA expression.

After a 16 week HFD, both WT and ApoE-/- mice exhibited higher amounts of WAT and liver triglycerides and impaired insulin resistance. However, ApoE-/- mice showed lower WAT accumulation and body weight, less fasting leptin, glucose and insulin levels, and more hepatic steatosis than WT mice. In glucose and insulin clearance experiments, the areas under the curve for both insulin and glucose after HFD were higher in WT than ApoE-/- mice, which also exhibited higher expression of inflammatory markers (TNFa, MCP-1, CD68, Emr1) in the liver, but lower in the epididymal WAT. The typical HFD-induced elevation of endocannabinoid in the liver or epididymal WAT was significantly higher or lower, respectively, in ApoE-/- than WT mice, whereas the HFD-induced decrease of endocannabinoid tissue and CB1 mRNA expression in the subcutaneous WAT was absent in the transgenic mice. These differential alterations in endocannabinoid levels reflected changes in the expression/activity of endocannabinoid catabolic enzymes in the WAT, or the availability of esterified arachidonic acid (the ultimate endocannabinoid biosynthetic precursor) in the liver.

In conclusion, we report that dysfunctional endocannabinoid tone in the liver and WAT of mice with diet-induced obesity is dependent on the presence of ApoE and is associated with hepatic steatosis, inflammation in the WAT and liver, and insulin resistance.

SELECTIVE ELEVATION OF AEA OR 2-AG, BUT NOT BOTH, ALTERS CALORIC INTAKE IN MICE

James J. Burston[1,2], Aron H. Lichtman[2], Jonathan Z. Long[3], Benjamin F. Cravatt[3] and Jenny L. Wiley[2,4]

[1] School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.
[2] Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298-0613, U.S.A.
[3] The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, USA.
[4] Research Triangle Institute, International, Research Triangle Park, NC 27709-2194, U.S.A

To date, it has been established that food restriction results in elevation of endocannabinoids and that local infusion of endocannabinoids into the nucleus accumbens shell can induce feeding behaviour. Despite this previous work, however, few studies have compared the effect of different endocannabinoids on food consumption. The aim of this study was to evaluate the effect of systemic administration of endocannabinoid agonists and analogs as well as inhibitors of endocannabinoid degradation on food intake in mice. For these experiments, adult male mice were administered an endogenous cannabinoid (or its analog) alone or in combination with an endocannabinoid metabolic inhibitor; e.g., fatty acid amide hydrolase (FAAH) for AEA and monoacylglycerol lipase (MAGL) inhibitor for 2-AG. Subsequently, consumption of a standard diet and a high sugar diet was measured.

The results of these studies showed that administration of AEA increased chow consumption at 10 mg/kg. When FAAH was pharmacologically inhibited with either URB-597 or PF-3845, AEA induced a significant increase in chow intake at a lower (3 mg/kg) dose, although neither inhibitor alone increased chow intake). A similar effect was observed in male FAAH knock-out mice, in that AEA produced an increase in chow consumption (at a dose of 1 mg/kg). The metabolically stable AEA analogue O-1860 also induced an increase in food consumption at 5.6 mg/kg. These data suggest that systemically administered AEA has limited ability to alter regular chow intake, possibly due to a short biological half life.

By comparison, administration of 16 mg/kg of the MAGL inhibitor JZL-184 induced a robust threefold increase in both chow intake and intake of a high sugar food (fruit loops cereal). Given this robust effect of JZL-184, the effects of the dual FAAH and MAGL inhibitor JZL-195 were examined. Surprisingly, JZL-195 did not induce an increase in the consumption of either chow or the high sugar food in a short time frame intake (1.5 hours access to food) or in a longer time frame intake (24 hours). Take together these findings suggest that administration of AEA combined with a FAAH inhibitor or to FAAH knockout mice increases food intake in mice, as does selective elevation of 2-AG, whereas selective dual elevation of AEA and 2-AG does not. Together, these results show that an increase in either AEA or 2-AG, but not both, may increase food intake under the experimental conditions used here, suggesting that endocannabinoid regulation of food intake involves a complex interplay between AEA and 2-AG rather than independent effects of individual endocannabinoids.

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BIOLOGICAL ROLE OF CB_1-RECEPTORS IN HUMAN ADIPOCYTES

Stefan Engeli[1], Nadine Friggemann[1], Jürgen Janke[2], Kerstin Gorzelniak[2], Mario Ost[2], Frauke Adams[2] and Jens Jordan[1]

[1]Institute of Clinical Pharmacology, Hannover Medical School, 30625 Hannover, Germany
[2] Experimental & Clinical Research Center, Charité, 13125 Berlin, Germany

Introduction: The identification of CB_1-Receptors in rodent adipocytes suggested that the endocannabinoid system (ECS) influences adipose tissue biology.

Methods: We treated primary cultured human adipocytes or SGBS adipocytes with CB_1-agonists and antagonists. Gene expression was determined by *Taq*Man-real-time RT-PCR, adipogenesis was monitored by Oil red O staining of lipid droplets, glycerol release was measured as a marker of lipolysis, and oxygen consumption was determined in the Seahorse XF24-3 instrument by fluorophore technology.

Results: All genes of the ECS were expressed in human SGBS adipocytes. The CB_1 gene was strongly induced during human adipogenesis. Neither CB_1-agonists (ACEA, HU210), nor CB_1-antagonists (AM251, rimonabant) influenced human adipogenesis. Expression of adipocyte genes was tested (e.g. leptin, adiponectin, LPL, FAS), but these genes were not regulated by CB_1-modulation. Lipolysis studies with and without isoproterenol revealed no significant influence of CB_1-receptors on basal or stimulated lipolysis. Treatment of differentiated SGBS adipocytes for 72h with HU210 significantly reduced maximal mitochondrial electron transport capacity, but did not influence basal oxygen consumption. The increase in electron transport capacity was completely prevented by the combination of HU210 with AM251, whereas AM251 alone did not change the bioenergetic profile of SGBS adipocytes.

Conclusion: In summary, human adipocyte biology is only marginally regulated by the ECS. We identified a specific effect of CB_1-activation on mitochondrial function, but the beneficial metabolic effects of CB_1-antagonists (e.g. rimonabant and taranabant) as described in clinical trials are most likely not mediated by specific effects in adipose tissue.

INVOLVEMENT OF THE CNR1 GENE IN RISK TAKING BEHAVIOR AND REWARD SEEKING: ENHANCED CB1 RECEPTOR ACTIVITY INDUCES A LASTING PUBERTAL PHENOTYPE IN ADULT RATS

M. Schneider[1], N. Bausbacher[3], M. Schreckenberger[3], F. Steindel[2], B. Lutz[2] and R. Spanagel[1]

[1]Central Institute of Mental Health, Mannheim, University of Heidelberg, Germany; [2]Institute of Physiological Chemistry and Pathobiochemistry, University of Mainz, Germany; [3]Clinic for Nuclear Medicine, University of Mainz, Germany

Introduction: Treatment with N-ethyl-N-nitrosourea (ENU), a powerful mutagen in rodent spermatogonial stem cells, is a highly efficient tool to generate new mutants in mice and rats. Here, we report the characterization of a rat line carrying one ENU-induced missense mutation in the CB1 receptor (Cnr1) gene.

Methods and Results: The Cnr1 mutant rat line was generated by target-selected ENU-based mutagenesis, resulting in a point mutation in exon 2 of the Cnr1 gene in a male founder rat (Fischer344 background). Cannabinoid agonist-stimulated [^{35}S]GTPgammaS binding was found to be increased in Cnr1 mutant rats compared to wild-type controls, indicating enhanced CB1 receptor signalling. In addition, differences between mutants and wildtype were observed in the expression of the anadamide degrading enzyme FAAH. Finally, a PET study for D2 receptor binding revealed a significant increased binding potential for D2 in mutant rats. Mutant rats show high novelty seeking and risk taking, are highly impulsive and show enhanced sensitivity for natural and cocaine reward compared to wildtype littermates, resembling a phenotype that is typically present during pubertal development. This is further supported by the finding of increased social play fighting behavior in adult mutant rats.

Conclusion: In conclusion, the present data indicate that the ENU-induced point mutation in the Cnr1 gene leads to enhanced CB1 receptor signalling. This Cnr1 rat line might serve as an useful animal model for a better understanding of the maturational changes in the brain that contribute to the age-specific behavioral characteristics of puberty, including an increase in risk taking and the predisposition to use and abuse drugs.

THE ZEBRAFISH *DANIO RERIO*: A MODEL SYSTEM FOR MECHANISTIC ANALYSIS OF THE BEHAVIOURAL CONSEQUENCES OF EMBRYONIC EXPOSURE TO CANNABINOIDS

Miralkumar Y. Parmar, Caroline H. Brennan and Maurice R. Elphick

School of Biological & Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom.

Cannabis is one of the most widely used non-medicinal drugs during pregnancy, but little is known about the long-term behavioural consequences of exposure to cannabinoids *in utero*. We are using the zebrafish *Danio rerio* as a model system to address this issue because exposure of the transparent embryos to drugs can be precisely controlled *in vitro*.

Sequencing of the *Danio rerio* genome has enabled identification of cannabinoid receptor genes in this species and previous studies have reported the discovery of a single CB_1 gene and two CB_2-like genes, CB_{2A} and CB_{2B}. However, our analysis indicates that CB_{2A} and CB_{2B} are in fact alleles of a single CB_2 gene located on chromosome 16, which we have named $CB_{2.1}$. Furthermore, we have identified a second CB_2 gene located on chromosome 11, which we have named $CB_{2.2}$. Analysis of cannabinoid receptor expression during zebrafish embryonic development using PCR revealed that CB_1 can be detected as early as the 4-128 cell stage, while $CB_{2.1}$ can be detected from the 50% epiboly stage and $CB_{2.2}$ can be detected from the 100% epiboly stage.

To establish a methodology for analysis of the long-term consequences of embryonic exposure to cannabinoids, we first tested the effect of exposure to Δ^9THC from 0-72 hr post-fertilisation (pf) on survival of zebrafish. With 0.1 μM Δ^9THC no significant effect on survival was observed, with 1 μM Δ^9THC a reduction in survival was observed and with 10 μM all of the Δ^9THC-exposed embryos had died by nine days pf. These results indicate that embryonic exposure to Δ^9THC in the range 0.1 - 1 μM is suitable for experiments analysing the long-term consequences of embryonic exposure to Δ^9THC. Importantly, this concentration range matches with the blood concentrations of Δ^9THC detected in cannabis users. We have also analysed the effect of Δ^9THC in adult zebrafish and have found that at 10 μM Δ^9THC causes a significant reduction in the locomotor activity, consistent with the inhibitory effect of Δ^9THC on locomotor activity in rodents. Ongoing experiments are investigating whether embryonic exposure to 0.1 or 1.0 μM Δ^9THC affects sensitivity to Δ^9THC in adult zebrafish. We are also using mRNA in situ hybridisation and immunocytochemistry to investigate whether embryonic exposure to Δ^9THC affects CB_1 receptor expression in the adult zebrafish brain.

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SEXUAL DIMORPHISM IN CB1R-MEDIATED BEHAVIORAL COPING STRATEGY IN THE FORCED SWIM TEST

Christopher J. Roberts, Amy L. Sasman and Cecilia J. Hillard

Department of Pharmacology and Toxicology, Medical College of Wisconsin
Milwaukee, WI 53226, United States of America

INTRODUCTION: Endocannabinod signaling (ECS) is known to control mood, which has human health implications for sexually dimorphic disorders, such as anxiety and depression. A growing body of evidence demonstrates that systemic activation of ECS promotes active coping in mice and rats in the forced swim test (FST). The ventromedial prefrontal cortex (vmPFC) is one brain region implicated in the affects of ECS since microinjection of a cannabinoid type 1 receptor (CB1R) agonist into this region decreases passive coping in male rats. Behavioral coping strategy in the FST has been shown to have sex differences. One goal of these studies was to determine whether sex differences in ECS could contribute to sex differences in FST behavior. A second goal of these studies was to examine the effects of chronic activation and inhibition of ECS on FST coping strategy in both male and female mice.

METHODS: Male and female ICR mice (6-9 weeks of age) were used in these studies. Animals were exposed to a twelve-day protocol consisting of FSTs on day one and twelve separated by 10 days of intraperitoneal (i.p.) injections on days two to eleven. Mice were injected with the CB1R antagonist, AM251 (1.0 mg/kg); the CB1R indirect agonist, URB597 (1.0 mg/kg); or vehicle. After the FST on day 12, mice were sacrificed and brains removed. CB1R binding parameters (Kd and Bmax) were determined in membranes prepared from PFC using radioligand binding assays with [^{3}H]CP55940.

RESULTS: Immobility did not differ between males and females at either the first or second FST ($p=0.15$; 0.32). However, vehicle-treated females exhibit a robust 23-second mean increase in immobility ($p=0.0012$), while vehicle-treated male mice do not display a change in immobility between the FST exposures ($p=0.42$). Vehicle-treated female mice exhibited a significant ($p=0.02$) positive correlation (Spearman r value=0.51) between the CB1R Kd in the PFC and the change in immobility between FST exposures. This correlation was lost after treatment with either AM251 ($p=0.64$) or URB597 ($p=0.43$). Male mice did not exhibit a correlation between CB1R Kd and the change in FST immobility in groups treated with vehicle, AM251 or URB597 ($p=0.72$; 0.20; 0.46).

CONCLUSIONS: These data indicate that in vehicle-treated, female ICR mice about 50% of the variability in the change in time immobile is due to differences in the affinity of CB1Rs in the PFC. Also, the correlation between CB1 receptor affinity in the PFC of females with FST immobility is lost if the receptor is blocked or endogenous ligand concentrations are increased. Furthermore, we have shown that although CB1R affinity in the PFC correlates with behavior in females; the same is not true in male mice. Nevertheless, acute CB1R activation within the vmPFC promotes active coping in male rats. Therefore, we hypothesize that within the PFC another component of ECS other than CB1R affinity, such as ligand concentrations or G protein coupling, is the rate-limiting factor that regulates FST behavior in male mice.

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ROLE OF CB1 RECEPTORS IN THE PSYCHONEUROENDOCRINE CONSEQUENCES OF REPEATED SOCIAL STRESS

Francis Chaouloff, Sarah Dubreucq, Isabelle Matias and Giovanni Marsicano

NeuroCentre Magendie, INSERM U862, Team Endocannabinoids & NeuroAdaptation, 33077 Bordeaux, France

Introduction: The use of the restraint stress model has led to propose that the endocannabinoid system plays a role in the adaptation to chronic stress. However, whether this adaptive function applies (i) to specific emotional (anxiety, fear, hedonia, despair) and neuroendocrine (hypothalamo-pituitary-adrenal axis) stress responses, and (ii) to more ethologically relevant stress models, such as social defeat stress is currently unknown. We thought to address this question by assessing the psychoneuroendocrine profile of constitutive/conditional CB1 receptor mutant mice exposed to social stress.

Methods: C57Bl6/N mice, constitutive CB1 knock-out mice, and conditional CB1 mutants lacking CB1 receptors either from cortical glutamatergic neurons or from GABAergic neurons were daily exposed to social defeat (according to a method slightly modified from the one we published previously: Berton et al., Neuroscience 82:147-159, 1998; Berton et al., Neuroscience 92:327-341, 1999). Thereafter, control and stressed mice were examined for several behavioural (anxiety, cued fear expression, behavioural despair, sucrose consumption) and neuroendocrine (adrenal weights) indices.

Results: A first series of experiments indicated that social stress increased anxiety (as assessed in the elevated plus-maze), fear expression on recall (cued fear conditioning), despair, and adrenal weights. Further, social stress increased the central levels of the endocannabinoid 2-arachidonoyl-glycerol after the last, but not the first, defeat. In keeping with this result, control and stressed C57Bl6/N mice were pretreated with the CB1 receptor antagonist rimonabant before each stress session. This treatment increased within- and between-session fear expression during recall in stressed mice but did not affect to a major extent the other behavioural/neuroendocrine responses to stress. Beside intrinsic influences of constitutive and conditional CB1 mutations on some of these responses, it was found that the lack of CB1 receptors decreased fear expression on recall in stressed, but not in control, mice. Further, the selective mutation of CB1 receptors in cortical glutamatergic neurons decreased fear expression in stressed animals, compared to control animals. An opposite result was found in mice lacking CB1 receptors from GABAergic neurons.

Conclusion: The full deletion of CB1 receptors increases fear expression, an effect partly accounted for by the deletion of CB1 receptors from cortical glutamatergic neurones. In addition, CB1 receptors located in cortical glutamatergic neurons or in GABAergic neurons modulate in an opposite manner the expression of fear after repeated stress.

POSTNATAL INHIBITION OF THE ENDOCANNABINOID SYSTEM IS ASSOCIATED WITH ADHD-LIKE SYMPTOMS IN ADULTHOOD

Shimon Rabichev[1], Sharon Anavi-Goffer[2], Ruth A. Ross[2] and Ester Fride[1]

Ariel University Center, Ariel[1], Israel, University of Aberdeen, Aberdeen, UK[2]

Attention Deficit Hyperactivity Disorder (ADHD) is characterized by inattention, impulsivity and hyperactivity. ADHD is a condition that becomes apparent in some children in preschool or early school years. It is estimated that 3-5% of children in USA and 5-10% in the Israel have ADHD. Although ADHD was identified over 80 years ago, the etiological and risk factors associated with ADHD are still unclear. Recently, low birth weight was found to be one of the most important predictive factors of ADHD. The CB1 receptor antagonist SR141716A (rimonabant) was developed for obesity to effectively reduce body weight. We hypothesised that treatment with SR141716A might lead to ADHD-like symptoms. Last year we demonstrated that a single injection of SR141716A within 24 hours after birth leads to hyperactivity ADHD-like behaviour in adult mice (model 1).

In this study, we continued to explore the effects of SR141716A given orally to mothers (0.06 mg/ml) while feeding their offspring between postnatal days 1 to 15 (model 2). In a third model we treated pregnant mothers orally (0.06 mg/ml) 12 hours before and 24 hours after giving birth (model 3). At 8 weeks of age, offspring mice were tested for pre-pulse inhibition (PPI) of the acoustic startle response. At the age of 9-10 weeks the mice were tested for motor activity in the open field and for anxiety in the 'plus-maze' tests.

Both male and female offspring mice of mothers that had been treated with SR141716A at the end of their pregnancy (model 3) showed a significant increase in acoustic response in adulthood. In contrast, the response to acoustic stimuli in male offspring of mothers that had been treated with SR141716A (model 2) was unchanged, while female offspring showed a significantly reduced response to acoustic stimuli compared with their vehicle control littler mates.

Interestingly, there was no difference in the response of offspring mice from pregnant mothers (model 3) to the startle stimuli but postnatal treatment with SR141716A (model 1 and 2) resulted in a significant difference in the response to the startle stimuli. In addition, both female and male offspring mice from each model showed a significantly reduced response in the PPI test, suggesting that their sensorimotor-gating system was affected. Both males and females displayed a significant hyperactivity in rearing and a significant increase in exploration behaviour in the open field test. In the plus-maze test, both males and females spent more time in the open arms than in the closed arms, suggesting a decreased vulnerability to anxiety-provoking situations. Taken together, these results suggest that a direct inhibition of the endocannabinoid system in offspring after birth or indirectly via the mother contributes to the development of ADHD–like behaviour in adult offspring.

ACUTE ALTERATION OF ANTI-CB_1 CANNABINOID RECEPTOR IMMUNOREACTIVITY IN ZEBRA FINCH SONG CONTROL REGIONS: DIFFERENTIAL RESPONSIVENESS FOLLOWING DEVELOPMENTAL CANNABINOID EXPOSURE

Ken Soderstrom

Department of Pharmacology and Toxicology, Brody School of Medicine,
East Carolina University, Greenville, NC 27834

We have found that developmental cannabinoid exposure alters normal zebra finch vocal development. Currently we are working to identify physiological changes responsible for this phenomenon. One possibility is that endocannabinoid signaling systems are altered by developmental cannabinoid exposure. To begin to test this hypothesis, we have measured densities of anti-CB_1 receptor immunoreactivity in several brain regions either; (1) known important to vocal development (lMAN, Area X, HVC, RA, Dlm and Ov) or (2) with distinctly-dense CB_1 receptor expression (cerebellum).

Groups of 12 animals were assigned to receive daily injections of WIN55212-2 (WIN, 1 mg/kg IM) or vehicle for 25 days. One group was treated during a period of sensorimotor vocal development (from 50 to 75 days) the other group was treated in adulthood (> 100 days of age). Following chronic treatments, animals were allowed to mature an additional 25 days before song recordings were made. Following recordings, animals were subdivided into acute treatment subgroups of six animals each: one to receive vehicle injections, and one to receive WIN (3 mg/kg). 90 min following acute treatments animals were transcardially perfused with paraform, and brains sectioned for immunohistochemistry with anti-CB_1 receptor antibody. Stained sections were photographed at 40X in grey scale under calibrated illumination and exposure conditions. OD was determined in each brain region from at least five sections. Mean OD +/- SEM were calculated and compared across treatment groups with ANOVA.

Anti-CB_1 densities were decreased in all brain regions following developmental WIN treatment, an effect not observed following treatment of adults. Greater differences were observed in vocal motor regions (HVC and RA) and cerebellum than in song regions of rostral telencephalon (lMAN and Area X) and thalamus (Dlm and Ov). Modest increases in staining densities were observed in HVC (vocal motor) of animals treated in adulthood, but not in other areas. Following developmental WIN treatment, acute WIN rapidly increased anti-CB_1 staining in all brain regions. This was not observed following chronic WIN treatment of adults. In control animals treated with vehicle during development, an opposite phenomenon was observed wherein acute cannabinoid treatments rapidly reduced anti-CB_1 immunoreactivity. Acute effects of WIN in adults following chronic treatments were to modestly increase staining density in HVC and cerebellum.

Results suggest that inappropriately-reduced CB_1 densities may be involved in WIN-altered vocal development. In addition, developmental WIN treatment alters CB_1 receptor regulation, an effect that may also contribute to persistent behavioral changes.

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EFFECTS OF PHYTOCANNABINOIDS ON THE ELEVATED PLUS MAZE IN MICE

Naoki Amada[a,b] , Akihito Watanabe[a,b] , Yuki Yamasaki[a,b],
Serena Deiana[a], Tetsuro Kikuchi[b] and Gernot Riedel[a]

[a] Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK
[b] Otsuka Pharmaceutical Co., Ltd., Tokushima, 771-0192, Japan

Introduction: The cannabis plant has acquired much interest in recent years, not only because of the problems associated with its abuse, but also because of the therapeutic potential of many of its constituents. Some of these ingredients have recently been proposed as effective anxiolytic compounds (Campos and Guimaraes, 2009. Prog. Neuropsychopharmacol. Biol. Psychiatry 33:1517). The elevated plus-maze is widely used test for measuring anxiolytic and anxiogenic-like activities of drugs in rodents. In an attempt to widen our understanding of the potential role of phytocannabinoids on anxiety, we tested mice in the presence of selected cannabinoid extracts (BDS) in the elevated plus maze.

Materials and Methods: Male NIH Swiss mice (Harlan, UK) were injected intraperitoneally 30 minutes before testing with the following drugs and doses: Diazepam (0.5, 1, 2, 4 mg/kg), rimonabant (0.3, 1, 3, 10 mg/kg), CBD pure (0.3, 1, 3, 10, 30 mg/kg), CBD BDS (0.3, 1, 3, 10, 30 mg/kg), CBG pure (0.3, 1, 3, 10, 30 mg/kg), CBG BDS (0.3, 1, 3, 10, 30 mg/kg), THCV pure (0.3, 1, 3, 10, 30 mg/kg), THCV BDS (1, 3, 10, 30 mg/kg). They were placed individually in the center of the maze and the number of entries into open / closed arms, and the time spent in open / closed arms were recorded on line (Ethovision System (Noldus, Holland).

Results: Diazepam significantly reduced the number of entries into closed arms at >2 mg/kg, and at the same time increased entries into open arms. This behaviour is consistent with anxiolytic properties of the drug and confirms the sensitivity of our system to anxiety modulating substances. Rimonabant (doses $\geq$ 1 mg/kg) and THCV pure (30 mg/kg) significantly increased time spent in closed arms. Similarly, THCV BDS (30 mg/kg) heightened the number of entries into closed arms. All other cannabinoids/doses had no effect.

Conclusion: Compared with Diazepam, a strong anxiolytic in this paradigm, cannabinoid effects were small. Furthermore, our data agree with similar work in hamsters (Moise et al., 2008. Psychopharmacology 200:333) and mice (Biala et al., 2009. J Physiol Pharmacol 60:113) and suggest that not low affinity but high affinity CB1 antagonists may have anxiogenic property. As for CBD and CBG, the mode of action still remains elusive but it appears that both compounds are devoid of effect on anxiety. This is consistent with our comparative approach in the Vogel conflict test, in which both candidates also failed to affect anxiety (Yamasaki et al., accompanying abstract).

SEX DIFFERENCES OF THC EFFECTS IN HUMANS

Linda E. Klumpers [1], Amita Sandhu[1], Roelof P. Soeter [2,3,4], Marieke L. de Kam[1], Serge R.A.B. Rombouts [2,3,4] and Joop M.A. van Gerven [1,5]

1 Centre for Human Drug Research; 2 Leiden University Medical Center (LUMC), Department of Radiology; 3 Leiden Institute for Brain and Cognition; 4 Institute of Psychology, Leiden University; 5 LUMC, Department of Neurology.
Leiden, The Netherlands

Sex differences regarding THC-response in humans, have not been reported accurately previously, whereas indications exist that THC could give response differences, for instance related to variations in adipose tissue composition and hormone levels. In this study we examined THC-effects on pharmacodynamics (PD) and pharmacokinetics (PK) in healthy male and female volunteers.

Healthy females (n=11) and males (n=11) inhaled 2, 6, and 6 mg THC or placebo with 90-minute intervals in a randomised, double blind, cross-over trial. Subjects rated mood and calmness (Bond and Lader), and 'feeling high', external and internal perception (Bowdle) on a visual analogue scale (VAS). In addition, heart rate, cortisol, prolactine, LH and FSH levels were measured. Blood samples were taken for PK analysis of THC and major metabolites THC-COOH and 11-OH-THC (psycho-active). Adverse events (AE's) were monitored continuously. Standard descriptive statistics were applied, as well as linear mixed effect modelling where necessary.

The analyses included all collected data. Females reported more treatment-related AE's compared to males (22 vs 6) such as anxiety (7 vs 0) and nausea (5 vs 0), particularly after the second THC inhalation. Consequently, many (n=7) female volunteers dropped out after the second THC inhalation. THC plasma concentration was larger in females (least square mean (LSM) concentration=11.0 ng/mL) than males (LSM concentration=13.2 ng/mL), but this reached significance only after the first (p=0.0087) and the third (p=0.0178) inhalation. Concentrations of the active metabolite 11-OH-THC showed a large increase in females compared to males (LSM concentration=3.3 vs 2.0 ng/mL; p=0.0116). No difference was found in THC-COOH concentration. Alertness decreased after THC administration, and for female subjects the decrease was significantly larger than for males (p=0.0331). Females showed a much larger effect for 'feeling high' after 2 mg inhalation (p=0.0001). External (p=0.0330) and internal perception (p=0.0037) also increased stronger in females. Heart rate increased after THC (p=0.0031), but no sex difference was found. No THC effects were found on cortisol, prolactin, LH or FSH, but all females used monophasic contraception, and the study was not adapted to the menstrual cycle.

Sex differences in humans were found on various PK and PD parameters after THC administration. The 11-OH-metabolite and clinical AE reports showed the largest gender differences. Also considering the time course of this metabolite, this suggests that the AE-differences (anxiety and/or nausea) after the second inhalation could be related to a sex-related difference in 11-OH-THC production. Further explorations of the relationships between concentrations of THC and its metabolites, the PD and clinical effects, and their sex-related differences will be performed by PK/PD-analyses

CHRONIC BUT NOT ACUTE ADMINISTRATION OF AM251 REDUCES PSYCHOTIC-LIKE SYMPTOMS IN ISOLATION-REARED RATS

Erica Zamberletti[1], Daniela Viganò[1,2], Cinzia Guidali[1],
Tiziana Rubino[1] and Daniela Parolaro[1,2]

[1]DBSF and Neuroscience Center, Univ. of Insubria, Via A. da Giussano 10, 21052 Busto Arsizio (VA), Italy
[2]Zardi-Gori Foundation, Milan, Italy

Increasing evidence has shown that schizophrenia is associated with certain disturbances that occur during individuals' early development and some early-life events can have substantial influences upon processes of brain maturation. In rats following isolation rearing both substantial changes in central neurotransmission and remarkable behavioural abnormalities can be observed, some of which have translational relevance to several core symptoms of schizophrenia. Moreover evidences suggest an involvement of the endogenous cannabinoid system in the pathophysiology of schizophrenia and we previously demonstrated that chronic administration of AM251, a CB1 receptor antagonist, antagonized psychotic-like symptoms induced by repeated PCP injections, a pharmacological model of schizophrenia.

In this study, we evaluated the effects of chronic and acute administration of a low dose of AM251 on isolation-induced cognitive impairment (object recognition test) and negative symptoms (social interaction test). Male Lister Hooded rats, PND 21, were randomly housed alone (isolated) or in groups of 4 (grouped) for 5 weeks. On PND 70 (6 weeks after isolation rearing commenced), rats were treated with AM251 (0.5mg/kg) acutely or chronically and the effect of AM251 on cognition and social behaviour was tested. In the object recognition test, an acute administration of AM251 did not affect the discrimination index impaired by social isolation, whereas chronic AM251 significantly improved the isolation-induced cognitive deficit. AM251 chronic exposure also reversed the increase in aggressive behaviour induced by social isolation, whereas an acute dose of AM251 had no such effect. The protocol of isolation rearing induced alteration in CB1 receptor's functional activity (measured by [^{35}S]GTPγS binding) and chronic AM251 restored CB1 receptor's functionality in all brain regions altered in reared rats.

These results suggest that chronic but not acute AM251 reduces cognitive and negative symptoms induced by isolation rearing, a neurodevelopmental animal model of schizophrenia. These findings, together with our previous results, strongly suggest a potential antipsychotic role of AM251.

Acknowledgments: EZ has a pre-doctoral fellowship from Compagnia di San Paolo, Turin, Italy

EVALUATION OF THE ENDOCANNABINOID SYSTEM IN POSTMORTEM HUMAN PREFRONTAL CORTEX OF ALCOHOLIC SUBJECTS

Amaia M Erdozain[1,2], Steve PH Alexander[3], David A Kendall[3], Elsa M Valdizan[2,4], J Javier Meana[1,2] and Luis F Callado[1,2]

[1]Department of Pharmacology, University of the Basque Country, 48940 Leioa, Bizkaia, Spain. [2]Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Spain. [3]School of Biomedical Sciences and Institute of Neuroscience, University of Nottingham Medical School, Nottingham NG7 7LP, UK.
[4]Department of Physiology and Pharmacology, Institute of Biomedicine and Biotechnology IBBTEC (Universidad de Cantabria-CSIC-IDICAN) 39011 Santander, Cantabria, Spain.

A wide variety of behavioural and biochemical studies in rodents have shown clear evidence of the involvement of the endocannabinoid system (ECS) in cerebral mechanisms underlying drug addiction, including alcoholism. In parallel, human genetic studies support the association of particular CB1 and FAAH coding polymorphisms with alcoholism. Additionally, an increase in CB1 receptor expression and density has been described in the prefrontal cortex of alcoholic suicide victims when compared with chronic alcoholics dying from causes other than suicide [1].

The purpose of this study was to evaluate different elements of the ECS in post-mortem human prefrontal cortex in four experimental groups: 1) non-suicidal alcoholic subjects (n=11), 2) suicidal alcoholic subjects (n=11), 3) non-alcoholic suicide victims (n=11), and 4) controls (n=11). All groups were matched for gender, age and post-mortem delay.

1. Immunoblot experiments showed a significant increase (126% of control) in CB1 immunoreactivity in the prefrontal cortex of the suicidal alcoholic subjects.
2. In qRT-PCR assays, no statistically significant differences were observed in the CB1 mRNA among the groups.
3. Functional coupling of CB1 receptor to G-proteins was evaluated by WIN 55,212-2 (10^{-12}-10^{-3} M, 10 concentrations) stimulated [^{35}S]GTPγS binding. The potency and the maximal effect were significantly unchanged among the different populations.
4. A significant decrease (42% of control) was observed in the basal adenylyl cyclase activity in the non-suicidal alcoholic subjects. However, no statistically significant differences were found either in the potency or the maximal effect of inhibition of forskolin-stimulated cAMP accumulation evoked by WIN 55,212-2 (10^{-8}-10^{-4} M).
5. FAAH activity, determined by the hydrolysis of [^{3}H]AEA (1, 5, 20 μM), was unaltered among the groups.
6. MAGL activity, determined by the hydrolysis of [^{3}H]-2-OG (100 μM), was significantly decreased in both non-suicidal and suicidal alcoholic groups (34% and 35% of control, respectively).

CONCLUSIONS. This is the first study reporting alterations in MAGL activity in the brain of human alcoholic subjects. A concurrent increase in CB1 receptor expression was observed in the cortex of alcoholic suicide subjects, which agrees with results previously reported [1]. Altogether these data support further the involvement of the ECS in alcoholism, as some of its elements are found to be altered in the brain of alcoholic subjects.

[1] Vinod KY et al. 2005. Biol Psychiatry 57, 480-486.

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BRAIN CANNABINOID CB_2 RECEPTORS INHIBIT COCAINE SELF-ADMINISTRATION AND COCAINE-ENHANCED EXTRACELLULAR DOPAMINE IN MICE

Zheng-Xiong Xi, Xiao-Qing Peng, Xia Li, Haiying Zhang, Jie Li and Eliot L. Gardner

National Institute on Drug Abuse, Intramural Research Program
Baltimore, MD, USA

The "lack" or low density of CB_2 receptors in the brain has led to a widely held belief that CB_2 receptors are not involved in the psychoactive effects of cannabinoids or other drugs of abuse. This view has been challenged recently by findings of functional CB_2Rs in the brain, and further, by findings of multiple CB_2R isoforms in both human and rodent brain. Such findings suggest that brain CB_2Rs may be involved in action of cannabinoids and other addictive drugs. We now report data appearing to support this hypothesis. Systemic administration of the selective CB_2 receptor agonist JWH133 dose-dependently inhibited intravenous cocaine self-administration under both fixed-ratio (FR) and progressive-ratio (PR) reinforcement conditions in both wild-type (WT) and CB_1 receptor-knockout ($CB_1^{-/-}$) mice, but not in CB_2 receptor-knockout ($CB_2^{-/-}$) mice. Intranasal JWH133 microinjection, by which the drug may directly enter the brain, also inhibited FR cocaine self-administration in WT mice. Systemic administration of JWH133 also inhibited cocaine-enhanced locomotion and extracellular dopamine (DA) in the nucleus accumbens (NAc) in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice. When administered systemically or locally into the NAc, JWH133 dose-dependently lowered basal levels of extracellular NAc DA in WT and $CB1^{-/-}$ mice, but increased extracellular NAc DA in $CB2^{-/-}$ mice. JWH133-induced inhibition of cocaine self-administration, locomotion and NAc DA was blocked by the selective CB_2 receptor antagonist AM630, but not by the selective CB_1 receptor antagonist AM251. JWH133 failed to alter oral sucrose self-administration or rotarod locomotor performance in any mouse strain tested. These findings suggest that brain CB_2 receptors functionally inhibit the acute rewarding and psychomotor-stimulating effects of cocaine in mice, likely by a dopamine-dependent mechanism.

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ADOLESCENT EXPOSURE TO THC INDUCED AN INCREASED SENSIBILITY TO PHENCYCLIDINE IN ADULT ANIMALS

Tiziana Rubino[1], Natalia Realini[1], Pamela Prini[1], Erica Zamberletti[1], Daniela Viganò[1,2] and Daniela Parolaro[1,2]

[1]DBSF and Neuroscience Center, Univ. of Insubria, Via A. da Giussano 10, 21052 Busto Arsizio (VA), Italy [2]Zardi-Gori Foundation, Milan, Italy

We have recently shown that adolescent THC exposure triggers the development of a complex depressive-like phenotype in adult female rats characterized by both emotional and cognitive symptoms. Since cognitive deficits and altered affective symptoms are also common traits to another psychiatric disorder, schizophrenia, whose relationship with cannabis abuse is still debated, we also checked for a major vulnerability to this disorder in animals pretreated with THC. To this aim we treated adolescent female rats with increasing doses of THC for 11 days (PND 35-45) and at adulthood (PND 75) we injected them with different doses of phencyclidine (PCP), a non competitive NMDA antagonist that is commonly used to reproduce psychotic-like symptoms in rodents. Indeed, hyperlocomotion induced by acute PCP-treatment is a well known animal model to study the positive symptoms of schizophrenia. When a low dose of PCP was used (2.5 mg/kg, ip) control animals did not show any locomotor activation whereas THC pre-treated animals exhibited significant hyperlocomotion. Similarly, PCP 5 mg/kg was able to induce significant hyperlocomotion and stereotyped behaviours in all animals, but they were more intense in THC pre-exposed group. These data suggest that THC exposed animals are more vulnerable to drugs able to precipitate psychosis-like behaviour. To explain the different sensitivity to PCP observed in THC pre-exposed animals, we hypothesized a difference in the molecular target of PCP, that is NMDA receptor. To check this hypothesis we monitored the density of NMDA receptors by autoradiographic binding studies. No significant alterations in the NMDA receptor densities were observed in THC exposed animals in comparison to controls. It is well known that PCP and related drugs (ketamine and dizocilpine) induce c-Fos protein in different brain areas and this may be involved in their psychotomimetic effects. We then decided to check c-Fos induction after PCP injection. As expected, PCP administration induced a significant c-Fos increase in the caudate-putamen and nucleus accumbens, however when this drug was injected in THC-exposed rats, it induced a greater activation.

As a whole, these data suggest the adolescent exposure to THC might act as a predisposing factor for developing psychotic-like symptoms.

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TARGETING ENDOCANNABINOID CATABOLIC ENZYMES FOR THE TREATMENT OF OPIOID WITHDRAWAL

Divya Ramesh[1], Joel E. Schlosburg[1], Gracious R. Ross[1], Rehab A. Abdullah[1], Steven G. Kinsey[1], Jonathan Z. Long[2], Benjamin F. Cravatt[2], Hamid I. Akbarali[1], Laura J. Sim-Selley[1] and Aron H. Lichtman[1]

[1] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA [2]The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA

It has long been known that cannabis sativa and its primary active constituent, Δ^9-tetrahydrocannbinol (THC), reduce opioid withdrawal symptoms. In the nervous system, THC produces its pharmacological actions predominantly via CB_1 cannabinoid receptors. The endogenous cannabinoids, anandamide (AEA) and 2-arachidonylglycerol (2-AG), also activate CB_1 receptors, but they are rapidly metabolized by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. The recent development of selective inhibitors of FAAH and MAGL provides pharmacological tools to investigate the role of endocannabinoids in modulating opioid dependence. The objective of this study was to determine whether elevating AEA or 2-AG, via inhibition of their respective metabolizing enzymes, reduces naloxone-precipitated morphine withdrawal symptoms. Male ICR mice were implanted subcutaneously with 75 mg morphine pellets and challenged 72 h later with naloxone (1 mg/kg) to precipitate withdrawal. The withdrawal signs included: number of jumping incidences, paw tremors, and head twitches, as well as body weight loss and the occurrence of diarrhea. Pretreatment with the MAGL inhibitor, JZL184 dose-dependently reduced the intensity of all naloxone-precipitated withdrawal measures with a similar efficacy as THC treatment. These effects of JZL184 were reversed by the CB_1 receptor antagonist, rimonabant, but not by the CB_2 receptor antagonist, SR144528, suggesting a CB_1 receptor specific mechanism of action. The FAAH inhibitor, PF-3845 reduced the intensity of naloxone-precipitated jumps and paw flutters in morphine-pelleted mice through a CB_1 receptor mechanism, but did not affect other withdrawal signs. Interestingly, MAGL inhibition significantly reduced the weight loss and production of diarrhea resulting from naloxone precipitated withdrawal, but FAAH inhibition did not produce these effects. Finally, to investigate further the differential effects of JZL-184 and PF-3845 in attenuating diarrhea and concomitant weight loss, we examined the ability of these inhibitors to modulate ileum contractions in vitro. Consistent with its in vivo actions, JZL184 attenuated the intensity of naloxone-induced longitudinal muscle contractions in morphine-dependent mouse ilea. The results from the present study are the first to show that inhibitors of endocannabinoid catabolic enzymes ameliorate the expression of opioid withdrawal. Thus, MAGL and FAAH offer promising targets to treat opioid dependence.

CANNABINOID CB_1 AND CB_2 RECEPTORS MODULATE BRAIN REWARD FUNCTION IN OPPOSITE DIRECTIONS IN RATS

Eliot L. Gardner, Krista Spiller and Zheng-Xiong Xi

National Institute on Drug Abuse, Intramural Research Program
Baltimore, MD, USA

Cannabinoids produce rewarding or aversive psychoactive effects in both humans and experimental animals. The underlying receptor mechanisms, however, remain unclear. At least three relevant receptors have been identified by molecular cloning: the G protein-coupled cannabinoid CB_1 and CB_2 receptors and the type 1 vanilloid receptors (TRPV1). Since CB_1Rs are highly expressed in the CNS and CB_2Rs are expressed predominantly in peripheral tissues, it is generally believed that the neurobehavioral and psychotropic effects of cannabinoids are mediated by activation of CB_1Rs. This view has been challenged recently by findings of CB_2Rs in the CNS, and further, by findings of multiple CB_2R isoforms in both human and rodent brain. Such findings suggest that brain CB_2Rs may be involved in cannabinoid behavioral actions. In the present study, we first investigated the effects of various cannabinoid agonists on electrical brain-stimulation reward (BSR), and then further determined receptor mechanisms underlying cannabinoid action on BSR. We found that: 1) systemic administration of the mixed CB_1/CB_2 receptor agonists Δ^9-THC or WIN55,212-2 produced biphasic effects, with a low dose significantly enhancing, and a high dose significantly inhibiting BSR; 2) the selective CB_1R agonist ACEA produced a BSR-enhancing effect, while the selective CB_2R agonist JWH133 produced a dose-dependent inhibition of BSR; 3) the BSR-enhancing effect produced by Δ^9-THC or WIN55,212-2 was blocked selectively by the CB_1R antagonist AM251, but not by the CB_2R antagonist AM630 or the TPRV1 receptor antagonist capsazepine. The inhibition of BSR produced by high-dose Δ^9-THC or WIN55,212-2 was blocked selectively by AM630, but not by either AM251 or capsazepine; and, 4) intranasal, but not intravenous, microinjections of JWH133 dose-dependently inhibited BSR, suggesting an effect mediated by activation of brain, but not peripheral, CB_2Rs. Together, the present data suggest that brain cannabinoid CB_1 and CB_2 receptors modulate brain reward function in opposite directions, i.e., CB_1R activation producing enhancement and CB_2R activation producing inhibition of BSR. This finding may in part explain previous conflicting results of cannabinoids in animal models of drug addiction, and suggests that brain CB_2Rs may be a new target for medication development for the treatment of drug addiction.

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PIMSR1 AS A NON-DYSPHORIC NEUTRAL CB1 ANTAGONIST

Herbert H. Seltzman[1], Patricia H. Reggio[2], Lauren Chun[3],
Zheng-Xiong Xi[3] and Eliot L. Gardner[3]

[1]Research Triangle Institute, Research Triangle Park, N. C. 27609 USA, [2]University of North Carolina, Greensboro, N. C. 27402 USA, [3]National Institute on Drug Abuse, Baltimore, MD. 21224 USA.

Introduction: The CB1 cannabinoid receptor is associated with appetitive behaviors including food intake, smoking and drug abuse. The application of CB1 antagonists to block such behaviors has shown considerable promise in treatment paradigms. Unfortunately, the leading candidate drug, the inverse agonist rimonabant, has been associated with suicidal ideation that has resulted in it being blocked and withdrawn from medical use. It is reasonable to consider that it is the inverse agonist properties of rimonabant that might be responsible for the dysphoric effect of suicidal ideation. Further, it is likely that other inverse agonists of the CB1 receptor would share this property as a class and thus be similarly unusable as a long-term treatment for appetitive disorders. Our findings of neutral antagonism in ligands for CB1 raised the question of whether neutral antagonists would be free of dysphoric side effects in its interaction with the constitutively active CB1 receptor.

Methods: Computational modeling to design neutral antagonists. Synthesis to provide test compounds. Calcium channel assay to establish neutral antagonism. Electrical brain stimulation reward threshold assay to discern dysphoria.

Results: Modeling suggested that hydrogen bonding to the carbonyl of the CB1 inverse agonist rimonabant is responsible for stabilization of the inactive state of the constitutively active CB1 receptor and that its removal would afford a neutral antagonist. Synthesis of ligands without the carbonyl but otherwise conformationally equivalent to rimonabant provided analogs that were neutral antagonists as shown by calcium channel assay. The highest affinity ligand, PIMSR1 (shown) (Ki = 17 nM, hCB1), was examined in rats and showed the absence of a shift in the electrical brain stimulation reward threshold assay indicative of the absence of dysphoric effects. This is in contrast to dysphoric shifts seen with rimonabant.

Conclusions: The structural modification of a ligand leading from an inverse agonist to a neutral antagonist of the CB1 receptor causes a loss of dysphoric response while retaining receptor blockade. This has potential implications in the development of treatments for appetitive disorders including food intake, smoking and drug abuse that are free of dysphoric side effects, which have kept the inverse agonists from medical use.

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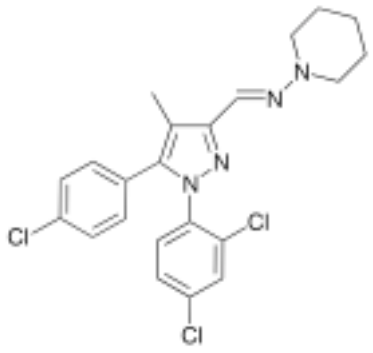


PRE-CLINICAL PHARMACOLOGICAL PROPERTIES OF NOVEL PERIPHERALLY-ACTING CB1-CB2 AGONISTS

Thierry Groblewski[1], Xiao Hong Yu[1], Etienne Lessard[1], Stéphane St-Onge[1], Hua Yang[1], Rosemarie Panetta[1], Chang Qing Cao[1], Michael Swedberg[2], Gvido Cebers[2], Svante Nyberg[2], Magnus Schou[2], Christer Halldin[3], Balazs Gulyas[3], Katarina Varnäs[3], Chistopher Walpole[1], Kemal Payza[1], Martin Perkins[1] and Julie Ducharme[1]

[1]AstraZeneca R&D Montréal, 7171 Fredrick Banting, H4S1Z9 Montréal, Qc, Canada
[2]AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden
[3]Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry Section, S-171 76 Stockholm, Sweden

There is a large body of pre-clinical evidence supporting a peripherally-mediated analgesic action of cannabinoids. We hypothesized that their therapeutic window could be improved, notably vs. their central side-effects, by limiting their access to the central compartment. To test the hypothesized peripheral analgesic mechanism and to assess the optimal level of peripheral restriction to combine efficacy with acceptable tolerability profile, we initiated pre-clinical studies with two novel orally active mixed CB1/CB2 agonists (AZD1940 & AZD1704) characterized by different extent of brain uptake (rat Cbr/Cpl ratio of ~0.1 & 0.01 for AZD1940 & AZD1704 respectively) and different CNS psychoactivity in a rat Δ9-THC drug discrimination test. Both compounds were orally active in various rat models of nociceptive and neuropathic pain and displayed improved safety margins vs. central side effects typically observed with various cannabinoids (e.g. Δ9-THC, WIN55212-2). Mechanistic studies involving local injection or recording of peripheral nerve activity in rat nociceptive and neuropathic pain models provided evidence for a peripheral site of action. Despite their mixed agonist activity at both CB1 and CB2 receptors, analgesic efficacy of this class of cannabinoids was found to be mainly driven by CB1 receptor. Indeed, the reported CB1 selective antagonist SR141716A completely reversed their analgesic efficacy in rat pain models. Additionally, activity of AZ11713908 (a mixed CB1/CB2 agonist tool compound) in the mouse FCA tail inflammation model was abolished in CB1 KO mice and remained unchanged in CB2 KO mice.

Imaging PET study conducted with [^{11}C]-AZD1940 in cynomolgous monkey confirmed low brain uptake previously evidenced in rodents. No pre-clinical safety issues preventing initiation of clinical studies with AZD1940 and AZD1704 were identified. Outcome of clinical studies are summarized in the next presentation.

This oral presentation will provide the chemical structure of these novel compounds and a summary of their pre-clinical in-vitro and in-vivo pharmacological properties.

PERIPHERALLY-ACTING CB1-CB2 AGONISTS FOR PAIN: DO THEY STILL HOLD PROMISE?

Thierry Groblewski[1], Rolf Karlsten[2], Märta Segerdhal[2], Jarkko Kalliomäki[2], Bror Jonzon[2], Margareta Bielenstein[2], Gvido Cebers[2], Michael Swedberg[2], Anita Annas[2], Greg Christoph[3], Pernilla Tellefors[2], Lars Ståhle[2], René Bouw[2], Urban Fagerholm[2], Agneta Berg[2], Stephen Butler[2], Michael O'Malley[2] and Gudrun Anstrén[2]

[1]AstraZeneca R&D Montréal, 7171 Fredrick Banting, H4S1Z9 Montréal, Qc, Canada
[2]AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden
[3]AstraZeneca R&D Wilmington, 1800 Concord Pike, PO Box 15437, Wilmington, DE, USA

To test the hypothesized peripheral analgesic mechanism and to assess the optimal level of peripheral restriction to combine efficacy with acceptable tolerability profile, we initiated clinical studies with the two novel orally active mixed CB1/CB2 agonists (AZD1940 & AZD1704) characterized by different extent of brain uptake (rat Cbr/Cpl ratio of ~0.1 & 0.01 for AZD1940 & AZD1704 respectively). In clinical phase I single ascending dose (SAD) study, AZD1940 maximum tolerated dose (MTD) was 0.8 mg with plasma exposure of 1.7 nM free. The clinical efficacy of AZD1940 as a pain relief agent was explored in two single dose phase II studies (human capsaicin and 3rd molar extraction models) and in the multiple ascending dose (MAD) study performed with volunteers affected by chronic low back pain. The 2 single dose phase II studies conducted with the MTD showed no effects on primary endpoints (pain intensity and heat pain threshold for capsaicin study). In the multiple ascending dose (MAD) study where AZD1940 was administered for 12 days, repeated dosing led to slow compound accumulation ($t_{1/2}$ ~ 80h). A daily 1mg dose led to a plasma exposure at steady state of 7 nM free. Significant weight gain and some hepatic enzymes increase were noticed. During the SAD study, AZD1704 exhibited hypotensive effects (up to 20 mm Hg supine systolic blood pressure drop with 2.4mg oral dose corresponding to a plasma exposure of ~2.0 nM free). No CNS adverse-events were noticed. The measured $t_{1/2}$ (6-8h) was too short to consider repeated dosing to investigate potential tolerance development to the blood pressure lowering effects. To conclude, CB1 agonists with limited CNS access have been observed to display a different tolerability profile in human compared to more brain-permeable CB1 agonists. After our limited studies, optimal clinical efficacy of peripherally restricted CB1 agonists remains to be proven. In addition, it is unclear how the hemodynamic and metabolic effects observed in these clinical studies could be completely managed for this class of cannabinoids.

TM38837 – A NOVEL SECOND GENERATION PERIPHERAL SELECTIVE CB1 RECEPTOR ANTAGONIST WITH EFFICACY AND POTENCY IN RODENT OBESITY MODELS EQUAL TO BRAIN-PENETRANT CB1 ANTAGONIST RIMONABANT

Pia K. Noerregaard, Marianne Fridberg and Christian E. Elling

7TM Pharma A/S, Hoersholm, Denmark

Introduction: The first generation brain-penetrant cannabinoid 1 (CB1) receptor antagonists (e.g. rimonabant) have proven efficacious in human obesity but therapeutic use is limited by its adverse CNS effects. TM38837 is a novel CB1 receptor antagonist which was designed to circumvent the CNS side effect profile through its restriction to peripherally located CB1 receptors in the body. An extensive panel of animal studies has been conducted to assess the therapeutic potential of TM38837 in obesity and to demonstrate the peripheral selectivity. TM38837 is currently in clinical development for treatment of obesity and related metabolic disorders.

Methods: Daily body weight and food intake were measured in Diet-Induced Obese (DIO) mice and rats treated orally once daily for 5 weeks with TM38837 or rimonabant. Drug measurement in brain homogenates, quantitative whole body autoradiography, brain CB1 receptor occupancy studies and behavioral CNS studies in rodents were employed to demonstrate peripheral selectivity of the compound.

Results: TM38837, 10 mg/kg po qd, induced weight loss in DIO mice and rats (26% and 14% vs. vehicle, respectively) as efficient as rimonabant, 10 mg/kg po qd (22% and 14% in DIO mice and rats, respectively). A spectrum of animal testing provided substantial evidence of peripheral selectivity of the compound and the differentiation to rimonabant.

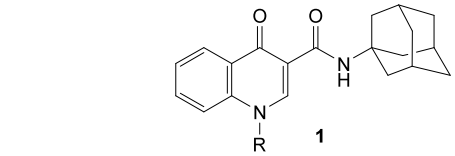
Conclusion: TM38837 is a novel peripheral selective CB1 antagonist that induces weight loss in rodents as efficiently as brain-penetrant rimonabant. This second generation CB1 antagonist therefore shows great promise as a safe therapy for obesity and related metabolic disorders.

DESIGN OF NOVEL SELECTIVE CB_2 RECEPTOR INVERSE AGONISTS BASED ON A 4-OXO-1,4-DIHYDROPYRIDINE SCAFFOLD

Didier M. Lambert[1] Jamal El Bakali,[2] Giulio G. Muccioli,[1] Nicolas Renault,[3] Delphine Pradal,[2] Mathilde Body-Malapel,[4] Madjid Djouina,[4] Virginie Andrzejak,[1] Pierre Desreumaux,[4] Philippe Chavatte,[3] and Régis Millet[2]

[1]Université catholique de Louvain, Louvain Drug Research Institute, B-1200 Bruxelles, Belgium, [2]Université Lille-Nord de France, Institut de Chimie Pharmaceutique Albert Lespagnol, [3] Laboratoire de Chimie thérapeutique & [4] Physiopathologie des maladies inflammatoires de l'intestin, Université, 59045 Lille, France

Growing evidence shows that CB_2 receptor is an attractive therapeutic target. Our groups previously described the synthesis, pharmacological characterization, and structure-activity relationships of selective CB_2 ligands based on a 4-oxo-1,4-dihydroquinoline-3-carboxamide scaffold (**1**)[1,2]. The structure-activity relationships studies highlighted a significant correlation between affinity and/or selectivity towards the CB_2 receptor and structural features such as an aliphatic moiety, especially an adamantyl substituent, on the C-3 carboxamide group as well as a *n*-pentyl chain in *N*-1 position. Concerning the functionality of these ligands, most of the compounds behaved as selective CB_2 agonists. Starting from this series, we describe here the medicinal chemistry approach leading to the development of simplified 4-oxo-1,4-dihydropyridine analogues as CB_2 receptor inverse agonists.



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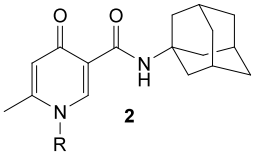

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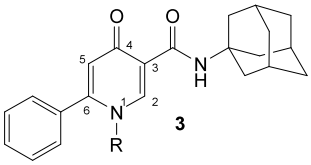

3

CB_2 selective agonist
R-CB_1: Ki > 3000 nM
R-CB_2: Ki = 20 ± 3 nM

CB_2 selective inverse agonist
R-CB_1: Ki = 592 ± 97 nM
R-CB_2: Ki = 4.0 ± 0.4 n M

The compounds reported here show high affinity and potency at the CB_2 receptor, with only a modest affinity for the centrally expressed CB_1 cannabinoid receptor. Further, we found that the functionality of this series is controlled by its C-6 substituent, since agonists bear a methyl (**2**) or a *tert*-butyl group and inverse agonists a phenyl (**3**) or 4-chlorophenyl group, respectively. Finally, *in silico* receptor-based studies also allow suggesting a putative binding mode for this series of high affinity CB_2 inverse agonists.

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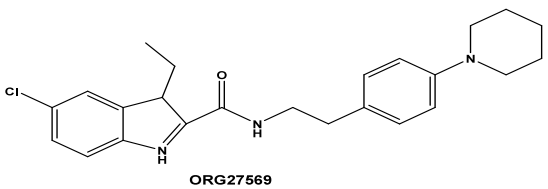
IDENTIFICATION OF ORG27569 BINDING SITE AT THE CB1 RECEPTOR

Patricia H. Reggio[1], Dow P. Hurst[1], Hadley A. Iliff[1], Teresa Barber[1], Gemma Baillie[2] and Ruth Ross[2]

[1]Center for Drug Discovery, University of North Carolina at Greensboro, Greensboro, NC 27402 USA [2] Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD Scotland, UK

Many G-protein-coupled receptors (GPCRs) contain *allosteric* binding sites for ligands, that are topographically distinct from the agonist-binding site, which is known as the *orthosteric site* (Christopoulos and Kenakin, *Pharmacol Rev* 2002). In contrast to the direct effects on receptor function that are mediated by orthosteric ligands, allosteric drugs act by modulating receptor activity through conformational changes in the receptor that are transmitted from the allosteric to the orthosteric site and/or to effector coupling sites (Christopoulos and Kenakin, *Pharmacol Rev* 2002). In 2005, ORG27569 was reported to act as a CB1 allosteric modulator by increasing the binding affinity of the agonist CP55940, but blocking its signaling (Price et al., *Mol Pharmacol* 2005). ORG27569 displays a ligand-dependent effect, whereby it enhances the specific binding of [^{3}H]-CP55940, while it inhibits the binding of the inverse agonist [^{3}H]-SR141716 (Horswill et al., *Br J Pharmacol* 2007). We have identified a binding mode for ORG27569 in our model of the CB1 activated state that satisfies the experimental data above, and localizes the allosteric binding site extracellular to the orthosteric binding site. A novel cavity-biased grand canonical Monte Carlo method (MMC), was initially used to identify likely binding regions for specific molecular fragments of ORG27569 (Guarnieri and Mezei, *J Am Chem Soc* 1996). Two extracellular binding sites were identified using piperidine and indole fragments, one near TMH1-2 and the other near TMH3-4. An ORG27569 *ab-initio* conformational analysis at the Hartree-Fock 6-31G* level indicated two major minimum energy conformations for ORG27569, a bent/L-shaped global minimum energy conformation and an extended conformation that was 0.75 kcal/mol above the global minimum. This data was combined with our current knowledge of the binding positions of CP55,940 (Kapur et al., *Mol Pharmacol* 2007) and SR141716 (Hurst et al., *Mol Pharmacol 2002*) and led to the conclusion that the L-shaped ORG27569 conformer binds in the TMH3-5-6-7 region of CB1 such that it would overlap the SR141716 binding site in the mono-chloro ring and piperidine ring regions of SR141716. At the same time, this allosteric binding site would not overlap the CP55940 orthosteric binding site. This ORG27569 binding site includes direct interactions with EC2 loop residues, as well as residues at the extracellular ends of TMH3-5-6 facing into the orthosteric binding crevice. [Support: NIH DA03934 (PHR) and DA021358 (PHR)].



ORG27569

DESIGN, SYNTHESIS AND NEUROPROTECTIVE POTENTIAL OF NOVEL CB_2 RECEPTOR SELECTIVE AGONISTS

Lital Magid[a], Catherine E. Goodfellow[b], Morris Srebnik[a], Michelle Glass[b], Alexander Alexandrovich[a], Lumir Hanuš[a], Esther Shohami[a] and Raphael Mechoulam[a]

[a] Institute of Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, 91120, Israel
[b] Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, 92019, New Zealand

There is increasing evidence that the cannabinoid receptor 2 (CB_2R) plays a functionally relevant role in the central nervous system (CNS) during neuroinflammation. We believe that selective activation of CB_2R has a therapeutic potential for the treatment of acute CNS trauma, such as traumatic brain injury (TBI).

The bicyclic pinene-derived structure of potent and highly selective agonist for CB_2R, HU-308, led us to replace the pinene moiety with a camphor one. We synthesized a number of enantiomeric pairs using synthetic approaches involving Suzuki cross-coupling and α-carbonyl arylation. The new compounds were evaluated for binding affinities for cannabinoid receptors, using a competitive radioligand binding assays. Inhibition of cAMP accumulation and guanosine 5'-3-*O*-(thio)triphosphate (GTPγS) binding were used to measure agonist-stimulated activation of CB_1 and CB_2 receptors. The most promising compounds were evaluated for inhibition of TNF-α production from murine primary macrophages by the Elisa method.

Several compounds exhibited a high to moderate potency, selectively bind and activate CB_2R. The most potent analogue in these series, HU-910, demonstrated a Ki = 6 nM for CB_2R and CB_1/CB_2 selectivity of 216. The carboxylic acid derivative, HU-914, inhibited 60-85% of TNF-α in a dose-dependent manner.

To examine the possibility that the novel CB_2R selective agonists will improve the recovery following TBI, Sabra mice were treated i.p. with HU-910 10 mg/kg and HU-914 5 mg/kg, 1 hr after TBI. The clinical status of the mice was followed for 28 days using a Neurological Severity Score (NSS) system testing motor and behavioral functions. Starting 48 hours, and until 28 days post injury the ΔNSS [the difference between the NSS (1 h) and NSS (t)] of the treated mice with HU-910 and HU-914 was significantly higher compared to vehicle-treated mice, indicating greater recovery of neurobehavioral function. In the presence of the selective antagonist for CB_2R, SR144528, the beneficial effect was abolished, indicating the involvement of CB_2R.

Following the ability to bind and to activate CB_2R together with an improvement in neurological outcome, the novel compounds offer a promising strategy in neuroprotective drug development.

THC SYNTHASE IN CANNABIS HAS UNDERGONE ACCELERATED EVOLUTION AND POSITIVE SELECTION PRESSURE

John M. McPartland and Geoffrey W. Guy

GW Pharmaceuticals, Salisbury, Wiltshire, SP4 0JQ, United Kingdom

INTRODUCTION: We hypothesized that the *Cannabis* genome has undergone two types of evolution: natural selection for 34 million years, and recent selection pressure by humans, for cannabinoids, fiber, and seed (McPartland and Guy, 2004, *The Medicinal Use of Cannabis,* pp. 71-102).

METHODS: The baseline evolutionary rate was calculated from two genes recognized as neutral to human selection, *rbcL* (chloroplast coding region) and *trnL-trnF* spacer (chloroplast noncoding region). Nucleotide sequences were obtained from the subspecies *Cannabis sativa* and *Cannabis indica,* plus eight related plants (*Humulus*, *Celtis, Pteroceltis, Morus, Ficus, Boehmeria, Cercropia, Ulmus*). Combined *rbcL* + *trnL-trnF* data were arrayed in a multiple sequence alignment (using ClustalX version 2.0), from which we constructed a phylogenetic tree (PAUP* version 4.0b10, ML algorithm). The number of nucleotide substitutions in each branch of the tree created a "molecular clock" (r8s version 1.70, nonparametric algorithm), calibrated with fossil data at three nodes in the tree: *Ulmus*, 65-56 million years ago (mya), *Celtis,* 58-49 mya; and *Humulus*, 28-23 mya. Next we examined a gene targeted by human selection: tetrahydrocannabinolic acid synthase (THCAS), in *C. sativa* (3 samples) and *C. indica* (3 samples).

RESULTS: Phylogenetic analysis with *rbcL* + *trnL-trnF* placed *Cannabis* and *Humulus* in a clade with *Celtis* and *Pteroceltis,* sister to the *Urticaceæ* and *Moraceæ* clades. Molecular clock analysis placed the divergence between *Cannabis* and *Humulus* at 27.8 mya (± 0.03 SEM), and the divergence between *C. sativa* and *C. indica* at 1.95 mya (± 0.04 SEM). The *C. sativa* and *C. indica* lineages diverged at a rate of 2.21×10^{-9} substitutions per site per year. In contrast, pairwise substitution rates for THCAS (at three polymorphisms) within the two *Cannabis* lineages were 1.94×10^{-8}, 2.00×10^{-8}, and 2.04×10^{-8} substitutions per site per year, a significant difference from the *rbcL* + *trnL-trnF* rate (unpaired Student *t* test, $t < 0.0001$).

CONCLUSIONS: The *Cannabaceæ* family should include *Celtis* and *Pteroceltis.* The calculated date of divergence between *Cannabis* and *Humulus* was younger than a previous estimate of 34 mya (McPartland and Guy, 2004). Like other domesticated crop plants, the *Cannabis* genome has experienced two types of selection pressure. The baseline evolutionary rate for genes neutral to human selection, 2.21×10^{-9}, is consistent with studies of many wild and domesticated plants. The ten-fold rate increase seen in THCAS is also consistent with other genes targeted for human selection in other crop plants. Mechanisms for this acceleration and evidence for positive selection pressure will be discussed.

WIN 55,212-2 REDUCES INTRAOCULAR PRESSURE IN MICE THROUGH A MECHANISM DEPENDANT ON β-ADRENERGIC RECEPTORS

Brian Hudson and Melanie Kelly

Department of Pharmacology, Dalhousie University,
Halifax, NS, Canada, B3H 1X5

Intraocular pressure (IOP) is maintained in the eye through the balance of aqueous humor secretion from the ciliary body epithelium and outflow through the trabecular meshwork and uveoscleral pathways. Elevated IOP is the primary risk factor for glaucoma, a blinding eye disease, and pharmacological reduction in IOP remains a major treatment for glaucoma. While it is well known that cannabinoid agonists reduce intraocular pressure, the specific mechanism behind this effect remains elusive. Therefore, the present study aimed to elucidate the mechanism of the cannabinoid-mediated reduction in IOP in mice.

In addition to cannabinoid agonists, β-adrenergic receptor (β-AR) antagonists also reduce IOP by decreasing aqueous humor secretion from the ciliary body epithelium. Given that activation of pre-synaptic CB_1 receptors inhibits neurotransmitter release both in the CNS and periphery, cannabinoids may be reducing IOP by inhibiting noradrenaline transmission in the ciliary body. In this respect, cannabinoids would be acting as indirect β-AR antagonists to reduce IOP. In order to test this hypothesis, the effect of cannabinoid agonists and β-AR antagonists on IOP were assessed in wild-type C57BL6, β_1AR/β_2AR dual knockout ($\beta\text{-AR}^{-/-}$) and CB_1 knockout ($CB_1^{-/-}$) mice.

In wild-type C57BL6 mice, topical application of either the cannabinoid agonist, WIN 55,212-2 (WIN), or the β-AR antagonist, timolol, significantly reduced IOP, each producing an 8% reduction ($p<0.05$). When WIN and timolol were co-applied they produced only a 4% drop in IOP ($p<0.05$), suggesting that not only is there no additive effect between these two compounds, but that they actually inhibit each other's actions when they are given together. As would be predicted if timolol reduces IOP through actions on the β-ARs, timolol had no significant effect on IOP in the $\beta\text{-AR}^{-/-}$ mice. More surprisingly, WIN also did not significantly affect IOP in $\beta\text{-AR}^{-/-}$ mice, suggesting that β-ARs are required for the cannabinoid-mediated reduction in IOP. Finally, it was found that not only did WIN not decrease IOP in $CB_1^{-/-}$ mice, but instead actually produced a 9% increase in IOP ($p<0.05$), suggesting that activation of non-CB_1 cannabinoid targets may increase the IOP of mice.

Taken together, the results of the present study demonstrate that WIN reduces IOP through a mechanism that is dependant on both CB_1 and the β-ARs. The most probable mechanism to account for this observation would be that CB_1 is inhibiting noradrenaline release in the ciliary body, however, future studies will be needed to directly test this hypothesis.

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GPR55 AND GPR35, PUTATIVE Δ^9-TETRAHYDROCANNABINOL RECEPTORS, AND THEIR ENDOGENOUS LIGANDS

Takayuki Sugiura, Ryo Ota, Mitsuru Shima, Atsushi Yamashita and Saori Oka

Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Sagamihara, Kanagawa 229-0195, Japan

Δ^9-THC interacted with the cannabinoid receptors (CB1 and CB2) thereby eliciting a variety of pharmacological responses. Recently, several groups reported that GPR55, a Class A G protein-coupled receptor, is a novel type of cannabinoid receptor. However, the details remain ambiguous. We started a search for the endogenous ligand for GPR55 using HEK293 cells expressing GPR55. We found that lysophosphatidylinositol (LPI) is an endogenous ligand for GPR55. LPI induced a Ca^{2+} transient, rapid phosphorylation of ERK, p38 MAP kinase and ATF-2 (*J. Biochem*., 2010, in press) and morphological changes in the GPR55-expressing cells. These results strongly suggest that GPR55 is a specific and functional receptor for LPI (*Biochem. Biophys. Res. Commun*., 362, 928-934, 2007). Notably, the biological activity of 2-arachidonoyl LPI was the highest among those of other molecular species of LPI, suggesting that 2-arachidonoyl LPI is the true natural ligand for GPR55 (*J. Biochem.*, 145, 13-20, 2009). Despite these previous data, however, it remains obscure whether GPR55 acts as a receptor or binding site for Δ^9-THC. In the present study, we examined in detail the biological activities of Δ^9-THC toward the GPR55-expressing cells and compared them with those of LPI. We found that Δ^9-THC induced Ca^{2+} transients and the phosphorylation of ERK at relatively high concentrations (5-10 μM), yet the level of the activity of Δ^9-THC was markedly lower than that of LPI. These observations suggest that GPR55 may act as a receptor or binding site for Δ^9-THC under special conditions. We then searched for another candidate for the receptor or binding site for Δ^9-THC. We found that a relatively high concentration of Δ^9-THC induced a Ca^{2+} response in HEK293 cells expressing GPR35 which has 30% homology with GPR55, suggesting that GPR35 may also act as a receptor or binding site for Δ^9-THC, like GPR55, in some case. It has been reported that kynurenic acid is a candidate for the endogenous ligand for GPR35. On the other hand, we found another candidate for the endogenous ligand for GPR35, its structure being different from that of kynurenic acid.

MODULATION OF GPR55 SIGNALLING BY PHYTOCANNABINOIDS USING THE ALPHASCREEN ASSAY

Sharon Anavi-Goffer[1], Andy J. Irving[2], Roger G. Pertwee[1] and Ruth A. Ross[1]

[1]School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, UK; [2]Division of Medical Sciences, Ninewells Hospital and Medical School, University of Dundee, DD1 9SY, UK

The orphan receptor, GPR55, belongs to the GPCR superfamily, but in contrast to cannabinoid CB_1 and CB_2 receptors, is coupled to $G\alpha_{12/13}$ and $G\alpha_q$ proteins. Recently, we have demonstrated that GPR55 is expressed in breast cancer cell lines and is involved in cell migration and polarization. Furthermore, GPR55 knock-out mice are resistant to inflammatory and neuropathic pain and have increased bone mass. These data suggest that GPR55 antagonists may have therapeutic potential. As part of our program to investigate the therapeutic potential of *Cannabis Sativa*, we examined the pharmacology of several of its constituents at GPR55. The phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2) is one of the main downstream signalling pathway that conveys agonist-induced activation of GPR55. A high-throughput system was established to test the ERK1/2 phosphorylation using the AlphaScreen Surefire assay.

We first confirmed that the endogenous lipid, L-α-lysophosphatidylinositol (LPI), stimulates ERK1/2 phosphorylation in HEK293 cells stably expressing the human GPR55 receptor. It induced a maximal stimulation of 91% ± 9.8 (E_{max}) after 20 min with an EC_{50} of 300 nM (95% CL 160-530). LPI-induced stimulation of GPR55 was blocked by 10 μM PD98059, a non-competitive ERK1/2 inhibitor. We then compared the ability of constituents of the plant, *Cannabis Sativa*, to stimulate ERK1/2 phosphorylation in hGPR55-HEK293 cells. Δ^9-tetrahydrocannabinol (Δ^9-THC) induced a 62% ± 18 stimulation at 10 μM while Δ^9-tetrahydrocannabivarin (Δ^9-THCV) produced a 110% ± 17 stimulation of ERK1/2 phosphorylation at the same concentration. Cannabidiol (CBD) and cannabigerol (CBG) and their structural analogues, cannabidiol acid (CBDA) and cannabigerol acid (CBGA), were also investigated in this bioassay. At a concentration of 10 μM, CBD induced a 35% ± 10 stimulation while CBDA produced a 7% ± 8 inhibition of basal, and CBG induced a 25% ± 25 stimulation while CBGA produced a 42% ± 4 inhibition of basal. Each of the phytocannabinoids was investigated at a sub-inhibitory concentration for the modulation of LPI-induced stimulation of ERK1/2 phosphorylation. CBDA and CBGA were more effective than their prototype compounds, significantly modulating LPI-stimulated ERK1/2 phosphorylation at 1 μM.

Our study reveals the ability of several compounds present in the *Cannabis Sativa* plant to modulate an endogenous GPR55 agonist, LPI. Furthermore, we provide the first evidence for a structure-activity relationship of cannabis constituents at GPR55, by demonstrating that the ability of CBDA and CBGA to modulate LPI-induced GPR55 stimulation of ERK1/2 phosphorylation can be enhanced by the presence of an acid group in the benzyl ring. These results have implications for developing GPR55 selective drugs for treating cancer, pain and bone disease.

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SYNTHESIS AND EVALUATION OF BIVALENT LIGANDS FOR CB1-OX1 RECEPTOR HETERODIMERS

Yanan Zhang, David Perrey, Brian P. Gilmour, Tiffany Langston, Keith Warner and Brian F. Thomas

Research Triangle Institute, Research Triangle Park, NC 27709, USA

CB1-OX1 receptor heterodimers have been shown to readily form *in vitro* and alter receptor trafficking and function compared to individual monomers. Furthermore, these receptors are colocalized in brain regions involved in feeding and appetitive behaviors and functional cross-talk between CB1 and OX1 receptors has been demonstrated *in vivo*. In order to recognize and take full advantage of the potential therapeutic utility of their modulation, a more basic understanding of the in vitro and in vivo pharmacology of the heterodimer targets is clearly needed. Bivalent ligands featuring a CB1 ligand and an OX1 ligand linked through spacers of proper lengths may be capable of binding with enhanced affinity to ligand recognition sites on CB1-OX1 heterodimers due to a thermodynamically more favorable binding interaction and may serve as probes for CB1-OX1 receptor heterodimer function. Thus, a series of bivalent ligands, composed of a SR141716 unit and an ACT-078573 moiety linked by spacers of various lengths, were designed and synthesized. For comparison purposes, the corresponding monovalent controls were also prepared. The functional activities of these bivalent ligands were determined using intracellular calcium mobilization assays at the CB1 and the OX1 receptors, respectively. Target compounds were then further evaluated in calcium assays using cells cotransfected with the CB1 and OX1 receptors. A number of compounds demonstrated Ke values that were pharmacologically relevant in the nanomolar range in these assays. Enhancement of potency was observed for several bivalent ligands in the cells cotransfected with CB1 and OX1 receptors compared to the individual cells. These novel compounds may assist the probe of the function and mechanism of receptor dimerization and further elucidate their physiological roles.

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TRANSCRIPTIONAL REGULATION OF CANNABINOID RECEPTOR-1 EXPRESSION IN THE LIVER BY RETINOIC ACID ACTING VIA RETINOIC ACID RECEPTOR-γ

Bani Mukhopadhyay, Jie Jie Liu, Douglas Osei-Hyiama, Grzegorz Godlewski, Partha Mukhopadhyay, Lei Wang, Won-Il Jeong, Bin Gao, Ken Mackie, Soichi Kojima and George Kunos

NIAAA.LPS.NIH, USA

Chronic alcoholism can result in fatty liver that can progress to steatohepatitis, cirrhosis and liver cancer. Mice fed alcohol develop fatty liver through endocannabinoid activation of hepatic CB_1 cannabinoid receptors (CB_1R), which increases lipogenesis and decreases fatty-acid oxidation. Chronic alcohol feeding also upregulates CB_1R in hepatocytes *in vivo*, which could be replicated *in vitro* by co-culturing control mouse hepatocytes with stellate cells (HSC) isolated from ethanol-fed mice, implicating HSC-derived mediator(s) in the regulation of hepatic CB_1R. HSC being a rich source of retinoic acid (RA), we tested whether RA and its receptors may regulate CB_1R expression in primary-cultured mouse hepatocytes. Incubation of hepatocytes with RA, ATR (all-trans retinoic acid) or RA receptor (RAR) agonists increased CB_1R mRNA and protein, the most efficacious being the RARγ-agonist CD437 and the pan-RAR agonist TTNPB. The endocannabinoid 2-arachidonoylglycerol (2-AG) also increased hepatic CB_1R expression, which was mediated indirectly via RA, as it was absent in hepatocytes from mice deficient in retinaldehyde dehydrogenase 1, the enzyme catalyzing the generation of RA from retinaldehyde. RAR regulates gene transcription by binding to RAR response elements in the promoters of target genes. The binding of RARγ to the CB_1R gene 5' upstream domain in hepatocytes treated with RAR agonists or 2-AG was confirmed by chromatin immunoprecipitation and electrophoretic mobility shift and antibody supershift assays. Finally, TTNPB-induced CB_1R expression was attenuated by siRNA knock-down of RARγ in hepatocytes. These findings indicate that RARγ regulates CB_1R expression and is thus involved in the control of hepatic fat metabolism by endocannabinoids.

LONG-LASTING EFFECTS OF AN ULTRA-LOW DOSE OF THC ON COGNITIVE FUNCTIONING IN MICE

Yosef Sarne, Fadi Asaf, Miriam Fishbein, Ora Keren and Mikhal Gafni

The Adelson Center for the Biology of Addictive Diseases, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978 Israel

Introduction: Our previous studies (1,2,3) indicated that a single administration of an ultra-low dose (0.002 mg/kg) of THC (delta-9 tetrahydrocannabinol) to mice induced long lasting (up to 5 months) cognitive deficits. These cognitive deficits were mild but reproducible and were detected by various behavioral assays that measured different aspects of memory and learning. These findings led us to test whether a low dose of THC can activate an endogenous compensatory mechanism that may protect the brain from more severe insults (pre- or post-conditioning).

Results: Injection of PTZ (pentylenetetrazole; metrazol) (60 mg/kg i.p) to male ICR mice induced a cognitive damage that was measured by three assays (the spatial "oasis" dry maze; the spatial "place recognition" test; and the non-spatial "object recognition" test) 3-6 weeks after the injection. Pretreatment with THC (0.002 mg/kg i.p) 24 hrs or 72 hrs before the injection of PTZ completely protected the mice from the deteriorating effect of PTZ. When THC preceded PTZ by 7 days, a partial protection was found. THC also protected the cognitive functioning of the mice when applied 24 hrs or 72 hrs after the injection of PTZ.
Biochemical experiments showed that 0.002 mg/kg of THC activated the ERK/MAPK pathway and elevated the levels of phosphorylated ERK (detected by Western blots) in various brain regions (cerebellum, hippocampus and frontal cortex) 12- 72 hrs after the administration of THC. This elevation was followed by a decrease in phosphorylated ERK that was observed even after 7 weeks. Interactions between the effects of THC and of PTZ (injected 24 hrs apart) on ERK were noticed 7 weeks after treatment.

Conclusions: An ultra-low dose of THC, which by itself causes a minor cognitive deficit, protects the brain from a more severe damage when applied either before or after the insult. These phenomena of "pre-conditioning" and "post-conditioning" evoked by THC can be used to prevent and treat cognitive damage due to various insults, or even to slow down ongoing cognitive decline.

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ENDOCANNABINOID MODULATION OF INTRACELLULAR CALCIUM IN RAT HIPPOCAMPAL NEURONS: IMPLICATIONS FOR ENDOCANNABINOID REGULATION OF NEURAL ACTIVITY AND BEHAVIOR

Sam A. Deadwyler and Robert E. Hampson

Wake Forest University School of Medicine, Winston-Salem, NC, USA

The cannabinoid receptor agonists Delta-9-THC and WIN 55,212-2 (WIN-2) have previously been shown to inhibit increases in intracellular calcium produced by NMDA-elicited depolarization of rat hippocampal principal cells (Zhuang et al. Neuropharmacology, 2005; Deadwyler et al. Behav. Pharmacol., 2007; Deadwyler & Hampson Psychopharmacology, 2008). The suppression of intracellular calcium results from cannabinoid (CB1) receptor activation and is mediated via Protein Kinase A and ryanodine-sensitive calcium receptors on endoplasmic reticulum. CB1 receptor antagonists Rimonabant and AM-251 block effects of endogenously-applied CB1 agonists, and enhance the increase in intracellular calcium by NMDA, implicating either inverse agonism or tonic endocannabinoid release that partially activates CB1 receptors. To test for the presence of the endogenous cannabinoids anandamide (AEA) or 2-arachidonyl glycerol (2-AG), fatty-acid amide hydrolase (FAAH) inhibitor URB597 (to enhance AEA) or monoacylglycerol lipase (MGL) inhibitor URB602 (to enhance 2-AG) were applied to hippocampal slices prior to application of NMDA to elicit intracellular calcium increase. URB597 had no effect, while URB602 exhibited dose-dependent decreases in intracellular calcium in a manner similar to WIN 55,212-2. The FAAH inhibitor was not competitive with rimonabant, while the MGL inhibitor was blocked by moderate doses of rimonabant, and increased the required dose of rimonabant required to produce facilitation. These results suggest that 2-AG may mediate glutamate-mediated excitation and depolarization in hippocampal principal cells.

Further studies in vivo applied either URB597 or URB602 intrahippocampally via intracerebral cannula and osmotic minipump. This laboratory has shown that CB1 agonists suppress neural firing and impair behavioral performance in a working memory task, while CB1 antagonism enhances neural firing, but only improves behavioral performance at very long delays (Deadwyler et al., 2007). As expected from the in vitro studies above, URB597 had no effect on neural firing or behavior, while URB602 suppressed hippocampal neural activity and impaired performance of a delayed-nonmatch-to-sample task. These results suggest a major role for 2-AG, but not anandamide, to modulate the hippocampal neural encoding of working memory and consequent mnemonic performance.

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DIFFERENTIAL SIGNALING IN HUMAN CANNABINOID CB_1 RECEPTORS AND THEIR SPLICE VARIANTS IN AUTAPTIC HIPPOCAMPAL NEURONS

Ken Mackie, Jim Wager-Miller and Alex Straiker

Department of Psychological and Brain Sciences, Gill Center for Biomolecular Science, Indiana University, Bloomington, IN 47405, USA.

Cannabinoids such as Δ^9-THC, the chief psychoactive component of marijuana and hashish, primarily act via cannabinoid receptors CB_1 and CB_2 and produce characteristic behavioral effects in humans. Due to the tractability of rodent models for physiological and behavioral studies, much of the work examining cannabinoid receptor action has made use of rodent cannabinoid receptors. While CB_1 is relatively well-conserved among mammals, human CB_1 (hCB_1) nonetheless differs from rCB_1 and mCB_1 at 13 residues, which may result in differential signaling. In addition, two hCB_1 splice variants (hCB_{1a} and hCB_{1b}) have been reported, diverging in their amino-termini relative to hCB_1. Autaptic cultured hippocampal neurons express a complete endogenous cannabinoid signaling system, with presynaptic CB1 receptors and the ability to synthesize and degrade endocannabinoids making them a useful model system to study cannabinoid receptor function.

We now report that when expressed in autaptic hippocampal neurons cultured from CB_1-/- mice, hCB_1, hCB_{1a}, and hCB_{1b} signal differentially from one another and from rodent CB_1. Specifically, hCB_1 inhibits synaptic transmission poorly relative to rCB_1, raising the provocative possibility that cannabinoid receptor signaling in humans is quantitatively very different from that in rodents. Since the problems of marijuana and hashish abuse occur in humans, our results highlight the importance to examine hCB_1 receptors. Our results also invite further study of the distribution and function of hCB_1 splice variants, given their differential signaling and potential impact on human health.

ABHD6: A NEW COMPONENT OF THE ENDOCANNABINOID SIGNALING SYSTEM

Nephi Stella, Ph.D.

University of Washington, Department of Pharmacology joint with Psychiatry & Behavioral Sciences, Seattle, WA 98195-7280, USA

In 2007, we found that the microglia cell line BV-2 hydrolyzes 2-AG without expressing MGL mRNA, suggesting the existence of a new enzyme capable of inactivating this endocannabinoid (eCB). In 2009, the Cravatt laboratory used functional proteomics (ABPP-MudPIT) to profile all the serine hydrolases expressed in mouse brain, and found two new enzymes, ABHD6 and ABHD12, that hydrolyze 2-AG when heterologously-expressed in COS-7 cells and their activity assessed in cell homogenate. In collaboration with the Cravatt laboratory, we performed ABPP-MudPIT analysis of BV-2 cell fractions and identified ABHD6, ABHD12, NTE and FAAH as viable candidates for the 2-AG hydrolyzing activity that we had found in this microglia cell line. Using shRNA knockdown, we demonstrate that ABHD6 is the enzyme that we were looking for, since only shRNA directed against ABHD6 reduce 2-AG hydrolysis in BV-2 cell homogenates and intact BV-2 cells in culture. Also using shRNA, we show that ABHD6 controls the efficacy of 2-AG at stimulating BV-2 cell migration through activation of CB2 receptors. Using qPCR and measuring ABHD6 hydrolyzing activity, we found that the expression of ABHD6 is extremely low in primary microglia, while being abundant in primary neurons. Thus, we focused the following experiments on neurons. In collaboration with the Mackie laboratory, we developed an ABHD6 antibody and found that this serine hydrolase is expressed in the soma and dendrites of neurons in culture, and is abundantly expressed by excitatory neurons in the prefrontal cortex of adult mice, while not being expressed by resting microglia. Using electron microscopy, we detected ABHD6 in post-synaptic densities of cortical neurons. Remarkably, when ABHD6 is pharmacologically inhibited in intact neurons in primary culture, 2-AG hydrolysis is inhibited, while activity-dependent production of 2-AG is enhanced. In collaboration with the Manzoni laboratory, we used mouse prefrontal cortical slices and showed that ABHD6 inhibition allows for the induction of CB1-dependent LTD by otherwise subthreshold stimulations. Together, our results show that ABHD6 constitutes a rate-limiting step in the hydrolysis of 2-AG in intact cells, controls the accumulation of 2-AG and its efficacy at cannabinoid receptors. Thus we propose that ABHD6 constitute a new component of the endocannabinoid signaling system.

SUSTAINED INACTIVATION OF MONOACYLGLYCEROL LIPASE PRODUCES FUNCTIONAL ANTAGONISM OF THE BRAIN ENDOCANNABINOID SYSTEM

Jacqueline L. Blankman[1], Joel E. Schlosburg[2], Jonathan Z. Long[1], Daniel K. Nomura[1], Aron H. Lichtman[2] and Benjamin F. Cravatt[1]

[1]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037 USA, [2]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA

Endocannabinoid signaling is terminated by enzymatic hydrolysis and endocannabinoid hydrolases are thought to be promising therapeutic targets for multiple pathological conditions including pain, inflammation and mood disorders. The endocannabinoid 2-arachidonoylglycerol (2-AG) is primarily hydrolyzed by monoacylglycerol lipase (MAGL) in the brain and our lab has recently shown that acute blockade of MAGL by a potent and selective inhibitor JZL184 raises 2-AG levels to elicit cannabinoid-mediated behaviors [Long et. al. 2009, Nat Chem Biol 5(1):37-44]. In order to study the effects of prolonged MAGL inactivation on 2-AG metabolism and cannabinoid behavior, we employed a mouse model in which MAGL activity has been genetically abolished. Mice containing a gene trap vector inserted into the third intron of the *Mgll* gene, upstream of the *Mgll* catalytic exon 4, were obtained from the Texas Institute of Genomic Medicine (TIGM). Mice homozygous for the gene trapped allele (MAGL -/- mice) are viable and largely indistinguishable from wild-type (MAGL +/+) littermates. Complete loss of active MAGL in MAGL -/- mice was confirmed by activity-based protein profiling (ABPP) analysis and 2-AG hydrolysis assays of MAGL +/+ versus MAGL -/- tissue homogenates. Importantly, both ABPP and 2-AG hydrolytic activity of MAGL -/- tissues is insensitive to further inhibition by JZL184. MAGL -/- mice display increased levels of 2-AG and other MAG species in the brain, spinal cord and many peripheral tissues. Anandamide and other NEA levels are unchanged in MAGL -/- tissues.

In contrast to acute blockade of MAGL, MAGL -/- mice do not exhibit CB1-dependent antinociception, hypomotility or hyperreflexia despite having >15-fold elevations in brain 2-AG levels. Furthermore, MAGL -/- mice display reduced sensitivity to the analgesic and hypothermic effects of the exogenous CB1 agonist WIN 55,5212-2. This striking phenotypic difference between acute blockade versus chronic ablation of MAGL is likely explained by desensitization of the CB1 receptor, evidenced by a ~50 % reduction in CB1 receptor expression and activity as measured by CB1 ligand and GTPγS binding, respectively. These data demonstrate that prolonged elevation in *endogenous* brain 2-AG levels is sufficient to cause functional antagonism of the central cannabinoid system, and argue against complete and prolonged blockade of MAGL as a cannabinoid-based therapeutic strategy.

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COX2 AND FAAH CAN REGULATE THE TIME COURSE OF DEPOLARIZATION INDUCED SUPPRESSION OF EXCITATION

Alex Straiker, Jim Wager-Miller and Ken Mackie

Department of Psychological and Brain Sciences, Gill Center for Biomolecular Science, Indiana University, Bloomington, IN 47405, USA.

Depolarization-induced suppression of inhibition (DSI) and excitation (DSE) are two forms of cannabinoid CB1 receptor-mediated inhibition of synaptic transmission. The duration of DSI and DSE are regulated by eCB degradation. We have recently shown that in cultured glutamatergic hippocampal neurons constitutively expressed monoacylglyceride lipase (MGL) controls the duration of DSE. In contrast, the duration of DSI in cultured inhibitory hippocampal neurons was determined by both MGL and cyclooxygenase 2 (COX2). This suggests that DSE might be attenuated during inflammation and in other settings where COX-2 expression is upregulated in excitatory neurons.

To investigate whether it is possible to sculpt the duration of endocannabinoid-mediated synaptic plasticity by varying expression levels of endocannabinoid degrading enzymes, we transfected excitatory autaptic hippocampal neurons with one of several 2-AG metabolizing enzymes: COX2, fatty acid amide hydrolase (FAAH), α/β hydrolyzing domain 6 (ABHD6), α/β hydrolyzing domain 12 (ABHD12), or MGL.

We found that expression of either COX2 or FAAH shortens the duration of DSE while expression of ABHD6 or ABHD12 does not. This indicates that induction of COX2 or FAAH will attenuate endocannabinoid signaling. In contrast, over-expression of MGL markedly interfered with endocannabinoid-mediated plasticity. However, exogenous cannabinoid agonists still induced a response, albeit diminished, at CB1 receptors. We conclude that both FAAH and COX2 can be trafficked to sites where they are able to degrade endocannabinoids to control DSE duration and, by extension, net cannabinoid signaling at a given synapse.

PURINERGIC RECEPTOR-MEDIATED ENDOCANNABINOID PRODUCTION AND INHIBITION OF GABAERGIC SYNAPTIC TRANSMISSION IN THE CEREBELLAR CORTEX

Bela Szabo and Flora E. Kovacs

Institut für Pharmakologie, Albert-Ludwigs-Universität, 79104 Freiburg, Germany

Introduction: Presynaptic CB_1 cannabinoid receptors can be activated by endogenous cannabinoids (endocannabinoids) synthesized by postsynaptic neurons. This phenomenon is termed endocannabinoid-mediated retrograde synaptic signaling (for review see Kano M et al., Physiol Rev 89: 309–380, 2009). The production of endocannabinoids in postsynaptic neurons can be triggered by calcium influx through voltage-gated calcium channels and by activation of $G\alpha_{q/11}$ protein-coupled receptors. The hypothesis of the present work was that activation of calcium-permeable transmitter-gated ion channels, specifically of P2X purinergic receptors (for review see Burnstock G, Cell Mol Life Sci 64:1471-1483, 2007), can also lead to endocannabinoid production and retrograde synaptic signaling.

Methods: GABAergic inhibitory postsynaptic currents (IPSCs) were recorded with patch-clamp techniques in Purkinje cells in mouse cerebellar slices. P2X receptors on Purkinje cells were activated by pressure ejection of ATP from a pipette.

Results: ATP evoked an inward current in Purkinje cells, most likely due to $P2X_4$ receptor activation. The ATP-evoked current (1.0-1.2 nA) was accompanied by currents mediated by voltage-gated calcium channels. Fluorometric calcium imaging indicated that the calcium concentration increase in the Purkinje cells was due to calcium influx through P2X receptor ion channels and through voltage-gated calcium channels. ATP suppressed electrical stimulation-evoked IPSCs (eIPSCs) and miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin, and these effects were sensitive to the CB_1 antagonist rimonabant and the calcium chelator BAPTA (applied into the Purkinje cell). ATP suppressed mIPSCs also when voltage-gated calcium channels were blocked by cadmium and intracellular calcium stores were depleted by thapsigargin.

Conclusions: Probably by activating P2X4 purinergic receptors, ATP elicits endocannabinoid production and endocannabinoid-mediated retrograde synaptic signaling. An increase in intracellular calcium concentration in the postsynaptic neuron is necessary for triggering the endocannabinoid production. ATP increases the calcium concentration by two mechanisms: calcium enters into the neuron via P2X receptor ion channels, and the ATP-evoked depolarization triggers voltage-gated calcium channels.

CARRIER-INDEPENDENT TRANSPORT OF ANANDAMIDE THROUGH SYNTHETIC VESICLES WITH INTERNALIZED FAAH

Dale G. Deutsch[1], Lindsay D Nelson[1], Michele K McKinney[2], Benjamin F. Cravatt[2], Erwin London[1] and Martin Kaczocha[1]

[1]Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794, USA and [2]Department of Cell Biology, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037, USA.

The endocannabinoid anandamide (AEA) mediates numerous physiological processes including pain, appetite and reproduction. AEA is inactivated through cellular uptake and intracellular hydrolysis by fatty acid amide hydrolase (FAAH). The mechanisms mediating transmembrane transport of AEA are controversial. Initial studies proposed that AEA uptake occurs through a process of facilitated diffusion. However, the putative AEA membrane transporter has never been molecularly identified. More recently, our laboratory and others have proposed that AEA transmembrane transport occurs through a process of simple diffusion. To provide further evidence supporting simple diffusion as a primary mode of AEA transmembrane transport, we engineered large unilamellar vesicles (LUVs) with FAAH enclosed within the vesicle interior. The LUVs afforded us the possibility to examine AEA transmembrane transport in a defined system without a contribution from putative transport proteins.

A 6:3:1 ratio of dioleoylphosphatidlycholine (DOPC): dioleoylphosphatidylglycerol (DOPG):Br-DOPC was mixed in a test tube. The lipids were dried down under nitrogen gas for 10 minutes and dried under vacuum for 1 hour. Dried lipids were hydrated with 500 µl of 0.1M Tris (pH 9) containing 10 mg/ml octyl glucoside, giving a final lipid concentration of 5 mM. 10 µg of purified FAAH was added and samples were dialyzed overnight in 3L of buffer at 4 ^{o}C, and again with 3L for 4 hours the following morning. LUVs containing internalized FAAH were treated with 500 µg/ml proteinase K for 20 min at 25 ^{o}C to inactivate non-internalized FAAH. The LUVs were subsequently pelleted by centrifugation at 4 ^{o}C. Following washing, the LUVs were resuspended in buffer and internalized FAAH was quantified by tryptophan fluorescence. Uptake and hydrolysis assays were carried out at 37 ^{o}C using ethanolamine labeled [^{14}C]AEA.

FAAH was stable within the vesicle interior and enzymatically active at 37 ^{o}C. [^{14}C]AEA was rapidly taken up and hydrolyzed by FAAH-containing LUVs, suggesting that AEA can rapidly partition into the lipid phase of vesicles and undergo transverse diffusion through the liposome membrane bilayer for hydrolysis. These data confirm previous cell-based results and point to simple diffusion as a process that is robust enough to account for AEA uptake. Current studies are underway to determine the contribution of cholesterol, a component of lipid rafts, upon the rate of AEA uptake and degradation.

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CLONING MAGL ISOFORMS FROM PUTATIVE TRANSLATION INITIATION SITES

Nadine M. Ulloa and Dale G. Deutsch

Biochemistry and Cell Biology, Stony Brook University,
Stony Brook, New York, 11794-5215, USA

Monoacylglycerol lipase (MAGL) is the principal enzyme responsible for 2-arachidonylglycerol (2-AG) hydrolysis (1). Originally cloned from rat adipocytes, MAGL is universally expressed as a ~33 kDa protein. However, western blots also show a ~35 kDa form of MAGL that is expressed only in testes and brain (1,2). Analysis of the MAGL mRNA sequence showed two putative translation initiation sites upstream of the start codon originally used for MAGL cloning (3). This may explain the ~35 kDa form of MAGL since translation from these start sites would add about 1-2 kDa to the final MAGL protein. MAGL is active in both soluble and membrane cell fractions because of its amphitrophic nature (4). However, the bulk of MAGL was shown to reside in the membrane fraction of brain homogenates in activity-based profiling studies (5). Additionally, immunohistochemistry studies show that MAGL localizes to the axons and not to the dendrites or cell bodies of neurons in the hippocampus, amygdala, and cerebellum (6). These data may be an indication of regulation of MAGL trafficking by post-translational modifications of the enzyme in neurons. Post-translational modifications offer a second explanation for the presence of the ~35 kDA form of MAGL because these modifications can cause an upward shift of a protein on SDS-PAGE. The larger form of MAGL may also be a result of post-translational modification of one of the putative MAGL transcripts.

MAGL was cloned from whole mouse brain cDNA using oligos designed against the putative upstream start codons as well as the originally characterized 5' end of MAGL. These MAGL transcripts were then sub-cloned into a pCDNA4/TO/myc-HisA vector and transiently transfected into COS-7 cells. A 6xHis/myc tag was added to the C-terminus of MAGL by removing its stop codon. MAGL was tracked in western blot and immunofluorescence experiments via this tag. Preliminary results show that all three constructs can be expressed in COS-7 cells with the longest MAGL transcript displaying an upward shift in molecular weight. All three constructs exhibit apparent cytosolic localization with strong perinuclear staining. Additionally, all constructs possess significant hydrolytic activity when compared to empty vector control cells. Current studies involve confirmation of construct localization in COS-7 cells by analyzing co-localization with sub-cellular markers and characterizing these constructs in neurons. Additionally, possible post-translational modifications are being examined by isolating the ~35 kDa band of MAGL via affinity chromatography with subsequent peptide sequence analysis.

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SYMPOSIUM – CANNABINOIDS, BONE REMODELING AND OSTEOPOROSIS

Vishnudutt Purohit and Rao Rapaka

National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland, USA

Increasing evidence suggests that cannabinoid system plays an important role in skeletal remodeling process comprised of bone formation and bone resorption, and thereby may impact the course of osteoporosis and other bone disorders. The cannabinoid receptor 1 (CB1) is mainly present in skeletal sympathetic nerve terminals, regulating the adrenergic tonic restrain of bone formation. Whereas cannabinoid receptor 2 (CB2) is mainly expressed in osteoblasts (bone forming cells) and osteoclasts (bone resorption cells). Both of these cells have been shown to produce endocannabinoids - anandamide and 2-arachidonoyl glycerol (2-AG). The connection between CB1 receptor and bone remodeling is based on the following information: 1) mice with inactivated CB1receptor have increased bone mass and they are protected from ovariectomy-induced bone loss; 2) CB1 receptor was shown to regulate bone formation in mice by modulating adrenergic signaling; and 3) CB1 receptor protected against age-related osteoporosis in mice by regulating osteoblast and adipocyte differentiation in marrow stromal cell. CB2 receptor has also been linked to bone remodeling on the basis of the following information: 1) CB2-deficient mice show a markedly accelerated age-related bone loss; 2) the CNR2 gene (encoding CB2) in women is associated with low bone mineral density and increased risk for osteoporosis; and 3) the activation of CB2 receptor attenuated ovariectomy-induced bone loss in mice by restraining bone resorption and enhancing bone formation. Recently, presence of endovanilloid/endocannabinoid system has been demonstrated in human osteoclasts, suggesting its possible involvement in bone remodeling. In addition, researchers have discovered a role of GPR55 in bone physiology by regulating osteoclast number and function. This symposium will provide an overview of the recent discoveries on the role of cannabinoid system in the modulation of bone remodeling process. Following topics will be covered in the symposium: 1) role of CB1 receptor in the regulation of bone formation; 2) CB2 regulation of bone metabolism in health and disease; 3) cannabinoid receptor GPR55 and osteoclast function; and 4) human cannabinoid receptor variants in osteoporosis.

ROLE OF CB1 RECEPTOR IN REGULATION OF BONE FORMATION

Yossef Tam

Laboratory of Physiologic Studies, National Institute on Alcohol Abuse & Alcoholism, Bethesda, MD 20892, USA

In mammals, including humans, bone metabolism is manifested by a continuous destruction/formation process, termed bone remodeling, whereby the bone mineralized matrix is being continuously renewed. This process has long been viewed as suggestive of a complex local, autocrine/paracrine, as well as endocrine regulation. Recently, several key components of the endocannabinoid (eCB) system have been identified in bone. The main eCBs, anandamide and 2-AG, are present in this tissue at high levels, in the same magnitude as their brain levels. Both ligands are produced by osteoblasts, the bone forming cells, and osteoclasts, the bone resorbing cells. In line with the occurrence of the eCB ligands in bone, the cannabinoid receptors CB_1, CB_2, TRPV1 and GPR55 are also present in the skeleton. CB_1 expression in osteoblasts and their precursors is very low, if any. However, it is expressed, in bone thyrosine hydroxylase positive sympathetic nerve terminals in close proximity to osteoblasts. Depending on the mouse line, CB_1 deficient animals have either a low or a high bone mass phenotype.

Traumatic brain injury (TBI)-induced heterotopic ossification and enhancement of fracture healing is the only clinically established, direct evidence for brain-to-bone communication. Since cumulative *in vitro* and *in vivo* evidence suggests that 2-AG, has a neuroprotective role following TBI, we have used a mouse TBI model, established in our lab, to specifically assess whether the eCB system has a role in the central regulation of bone remodeling. The TBI-induced stimulation of bone formation was absent in CB_1-null mice, regardless of the method of gene knockout or background strain. CB_2 mutant mice responded to TBI like WT animals, suggesting that CB_1, but not CB_2, is critically involved in the TBI-induced stimulation of bone formation. To further elucidate the role of CB_1 in bone, we tested the effect of TBI on bone 2-AG and norepinepherine (NE) levels, as the latter suppresses bone formation by binding to osteoblastic β2 adrenergic receptors. TBI induced an increase in femoral 2-AG levels peaking 8 h after trauma. This increase was accompanied by a decrease in NE. The peak decline in NE occurred 12 h post-TBI. The differential temporal changes in bone 2-AG and NE suggest that the increase in the former may lead to a decrease in the latter. Such a causal relationship is supported by the suppression of bone NE by exogenously administered 2-AG. Moreover, like TBI, the exogenously administered 2-AG stimulates bone formation in the distal femoral metaphysis in normal and CB_2, but not in CB_1 null mice. Both the TBI- and 2-AG-induced stimulation of bone formation can be blocked by the β-adrenergic receptor agonist, isoproterenol (ISO).

Jointly, the present findings confirm a role for CB_1 in bone remodeling. We propose that CB_1 controls osteoblast function by down regulating NE release from sympathetic nerve terminals in the immediate vicinity of these cells. NE suppresses bone formation by activating osteoblastic β2 adrenergic receptors, and this tonic suppression is apparently alleviated by activation of sympathetic CB_1. A direct action of 2-AG on osteoblasts is unlikely, since in these cells, which express CB_2, 2-AG acts as an inverse agonist.

These findings further suggest that 2-AG and NE levels in the osteoblast vicinity are subject to a negative feedback regulation. Indeed, exposure of osteoblasts to ISO stimulates DAGL expression in these cells, in particular DAGLα. In line with this elevation, 2-AG levels are markedly stimulated, with the latter increase being inhibited by the β-blocker, propranolol. Taken together, these data portray a negative feedback circuit whereby NE, released from sympathetic nerve terminals, stimulates 2-AG synthesis in osteoblasts. In turn, the increased 2-AG levels restrain the production and /or release of NE from the sympathetic nerves.

CB2 REGULATION OF BONE METABOLISM IN HEALTH AND DISEASE

Itai Bab

Bone Laboratory, The Hebrew University of Jerusalem, Jerusalem 91120, Israel

CB2 is expressed in osteoblasts, the bone forming cells, and osteoclasts, the bone resorbing cells. CB2 activation is mitogenic to osteoblasts through a Gi-protein – Erk1/2 – Mapkapk2 – CREB signaling pathway. Activation of CB2 also inhibits osteoclastogenesis by restraining the proliferation of osteoclast progenitors and RANK ligand expression in stromal cells/osteoblasts. CB2 deficient mice have a normal peak bone mass but exhibit a markedly accelerated age related bone loss. Reminiscent of postmenopausal osteoporosis in humans, this bone loss is associated with high turnover skeletal remodeling. Because this age-dependant low bone mass phenotype is the only spontaneous abnormality so far reported in CB2 mutant mice, it appears that the main physiologic involvement of CB2 is associated with maintaining bone remodeling at balance thus protecting the skeleton against age-related bone loss. Pre-clinical studies have shown that the synthetic CB2 specific agonists HU-308 and HU-433 (see Smoum et al, this symposium) rescue ovariectomy-induced bone loss. Taken together, these data pave the way for the development of cannabinoid-based anti-osteoporotic anabolic therapy.

GPR55: A NOVEL ROLE IN BONE PHYSIOLOGY

Ruth A. Ross[a], Lauren S. Whyte[a], Erik Ryberg[b], Natalie A. Sims[c], Susan Ridge[a], Ken Mackie[d], Peter J. Greasley[b] and Michael J. Rogers[a]

[a]*Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, UK,* [b]*AstraZeneca, MöIndal, Sweden.,* [c]*St. Vincent's Institute, Fitzroy VIC 3065, Australia,* [d]*Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN 47401, USA.*

GPR55 is a G-protein coupled receptor recently shown to be activated by certain cannabinoids and by lysophosphatidylinositol (LPI). However, the physiological role of GPR55 remains unknown. Given the recent finding that the cannabinoid receptors CB_1 and CB_2 affect bone metabolism, we examined the role of GPR55 in bone biology. GPR55 was expressed in human and mouse osteoclasts and osteoblasts; expression was higher in human osteoclasts than in macrophage progenitors. Although the GPR55 agonists O-1602 and LPI inhibited mouse osteoclast formation *in vitro,* these ligands stimulated mouse and human osteoclast polarisation and resorption *in vitro* and caused activation of Rho and ERK1/2. These stimulatory effects on osteoclast function were attenuated in osteoclasts generated from $GPR55^{-/-}$ macrophages and by the GPR55 antagonist cannabidiol (CBD). Furthermore, treatment of mice with this non-psychoactive constituent of cannabis significantly reduced bone resorption *in vivo*.

Consistent with the ability of GPR55 to suppress osteoclast formation but stimulate osteoclast function, histomorphometric and microcomputed tomographic analysis of the long bones from male $GPR55^{-/-}$ mice revealed increased numbers of morphologically-inactive osteoclasts, but a significant increase in the volume and thickness of trabecular bone and the presence of unresorbed cartilage.

These data reveal a hitherto unrecognised role of GPR55 in bone physiology by regulating osteoclast number and function. In addition, this study also brings to light a newly identified effect of both the endogenous ligand, LPI, on osteoclasts and of the cannabis constituent, CBD, on osteoclasts and bone turnover *in vivo*.

FATTY ACID AMIDE HYDROLASE IMMUNOREACTIVITY IN PROSTATE CANCER – ASSOCIATION WITH DISEASE SEVERITY AND OUTCOME

Christopher J. Fowler[1], Lina Thors[1] and Anders Bergh[2]

Departments of [1]Pharmacology and Clinical Neuroscience, [2]Medical Biosciences, Umeå University, SE90187 Umeå, Sweden

Introduction*:* Recently, Nomura *et al.* (*Cell* 140 [2010] 49-61) reported that monoacylglycerol lipase (MGL) was involved in the pathogenesis of cancer due to its function as a provider of free fatty acids. Fatty acid amide hydrolase (FAAH) hydrolyses *N*-acylethanolamines to their corresponding fatty acids, and it has been reported that FAAH expression is higher in prostate cancer tumours than in normal tissue, and that its overexpression in androgen-insensitive PC3 prostate cancer cells increases their invasivity *in vitro* (Endsley *et al., Int J Cancer* 123 [2008] 1318-26). Here we investigated FAAH expression in a tissue microarray from 412 patients with prostate cancer to determine whether it was correlated with disease outcome.

Method: FAAH immunoreactivity (FAAH-IR) was assessed using an antibody kindly provided by Dr. K. Mackie (see Harkany *et al., Eur J Neurosci* 18 [2003] 1979-92 for antibody details). Formalin-fixed, paraffin-embedded specimens of non-malignant tissue (median 4 cores/patient) and tumour tissue (median 5 cores/patient) obtained at diagnosis from patients who underwent transurethral resection surgery for prostatic enlargement were scored for FAAH-IR (for details of the patient material and the scoring method used, see Chung *et al., Eur J Cancer* 45 [2009] 174-82).

Results*:* In the non-malignant prostate tissue, FAAH-IR was found in the glandular epithelial cells (basal > luminal) and in blood vessel walls. In the tumour epithelial tissue, there was a significant correlation between the disease severity (Gleason score) and the epithelial FAAH-IR. Tumour, but not non-malignant, FAAH-IR correlated with CB_1IR measured previously (Chung *et al., ibid*.), consistent with the hypothesis that a component of the tumour microenvironment can regulate both their levels. One candidate component is IL-4, and IL-4 receptor immunoreactivity was also detected in tumour epithelial cells. Preincubation of PC3 prostate cancer cells with IL-4 increased their FAAH activity.

Of the cases investigated, 281 had been followed up for up to 21 years by watchful waiting until the appearance of metastases, this being the standard treatment at the time, thereby allowing the correlation of biomarkers obtained at diagnosis with disease outcome in an essentially untreated population. ROC (receiver operating characteristic) curves using a 15 year cutoff gave an area of the curve of 0.60 ($p<0.02$) for the tumour epithelial FAAH-IR. No significant value for the tumour blood vessel FAAH-IR was found. Using the optimum tumour epithelial FAAH-IR score obtained from the ROC analysis as a cutoff, a significant contribution of FAAH-IR upon the median survival was seen (10 *vs.* 18 years for the patients with an FAAH-IR below *vs.* above the optimum score, Kaplan-Meyer analyses; $p<0.005$ log-rank test).

Conclusion*:* A high expression of FAAH was associated with a more severe form of prostate cancer and a poorer prognosis. The cell culture data is consistent with the hypothesis that the tumour microenvironment can play a role in determining the tumour epithelial FAAH activity.

THE CANNABINOID RECEPTOR CB_2 PROTECTS AGAINST BALLOON-INDUCED NEOINTIMAL PROLIFERATION AND INFLAMMATION IN ATHEROSCLEROSIS-PRONE MICE

Filippo Molica[1], Fabienne Burger[1], Christian M. Matter[2], Andreas Zimmer[3], Pal Pacher[4] and Sabine Steffens[1]

[1]Division of Cardiology, Department of Internal Medicine, University Hospital, 1211 Geneva, Switzerland; [2]Cardiovascular Research, Institute of Physiology, University of Zurich and University Hospital, 8057 Zurich, Switzerland; [3]Institute of Molecular Psychiatry, University of Bonn, 53127 Bonn, Germany; [4]Section on Oxidative Stress Tissue Injury, Laboratory of Physiological Studies, National Institutes of Health, NIAAA, Bethesda, MD 20892-9413, USA

Vascular inflammation and smooth muscle cell proliferation contribute to vascular remodelling and obstructive vasculopathies such as atherosclerosis and restenosis following acute vascular injury. Cannabinoids are known for their immunomodulatory properties, due mainly to cannabinoid type 2 (CB_2) receptors. It is known that CB_2 activation with selective CB_2 receptor agonists inhibited the TNF-α-induced proliferation and migration of human coronary artery smooth muscle cells. The potential in vivo relevance of these findings and putative effects on endothelial cells after acute vascular injury, however, remain unclear. The aim of our study was to test the role of CB_2 receptors in an experimental mouse model mimicking human angioplasty.

We performed carotid balloon distension injury in ApoE-/- mice fed on high cholesterol diet for two weeks. Mice were randomly assigned to receive daily i.p. injection of either the CB_2-selective synthetic cannabinoid JWH-133 (5 mg/kg) or vehicle control, with the first injection given 30 min before balloon injury. After 7 days, DAPI staining of frozen cross-sections revealed significantly reduced numbers of total intimal nuclei in injured vessels of JWH-133-treated mice (vehicle: 57.09 ± 1.985, n=9; JWH: 43.26 ± 5.44, n=7; p=0.0199). Immunohistochemical analysis revealed reduced staining for PCNA-positive proliferating cells and macrophages within the artery wall of JWH-133-treated mice. However, we did not observe differences in arterial re-endothelialisation, as determined by CD31 staining after 7 and 14 days post-ballooning. To confirm the role of CB_2 in neointima formation, we performed balloon distension injury in wildtype and CB_2-/- mice under normal diet. Two weeks after injury, intimal nuclei were significantly increased in CB_2-/- mice as compared to wildtypes. In addition, we found significantly higher numbers of circulating lymphocytes in operated CB_2-/- mice, indicating an enhanced systemic inflammatory state.

Our data indicate that CB_2 receptors play a role in neointima formation in response to acute arterial injury. Thus, selective targeting of CB_2 receptors may offer a new therapeutic strategy for reducing restenosis in response to balloon angioplasty in humans.

ANTIANGIOGENIC PROPERTIES OF THE NON PSYCHOACTIVE CANNABINOID COMPOUND CANNABIDIOL: IN VITRO AND IN VIVO STUDIES

Marta Solinas[a], Paola Massi[b], Marta Valenti[a], Daniele Bolognini[a] and Daniela Parolaro[a]

[a] DBSF and Neuroscience Center, Univ. of Insubria, Via A. da Giussano 10, 21052 Busto Arsizio (VA), Italy - [b] Dept. of Pharmacology, Chemotherapy and Toxicology, Univ. of Milan, Via Vanvitelli 32, 20129 Milano, Italy

Malignant gliomas are the most common and aggressive primary tumors of the CNS, characterized by resistance to the conventional chemotherapy, invasion and extensive angiogenesis. Recently, we have reported that the non-psychoactive cannabinoid compound cannabidiol (CBD) is highly effective at inhibiting *in vitro* and *in vivo* gliomas growth and at down-regulating some pro-angiogenic signal produced by human glioma cells. Thus, in the present work we aim at studing CBD ability to modulate tumor angiogenic process, to support its possible use to reduce new blood vessel formation and tumor growth itself. At first, we evaluated CBD anti-proliferative effect on human endothelial HUVEC cells treated with increasing doses of the drug for 16 and 24 h. MTT assay showed that CBD was able to inhibit in a dose-related manner cell proliferation in a concentration range of 1-19 μM. Besides, we found that *in vitro* CBD induced a significant inhibition of HUVEC cells motility in scratch wound healing assay and a significant decrease in the level of metalloproteinase MMP-2 in the supernatants of cell cultures. Through the use of the Biocoat Angiogenesis System, we evaluated the ability of endothelial cells to invade extracellular matrix and create new vessels. As a consequence of CBD treatment, the capillary-like tube structures formation was far less organized than control. We next employed an angiogenic Array Kit to evaluate the effects of CBD on different proteins implied in angiogenic process and released by endothelial cells. CBD significantly down-regulated the expression profile of a set of proteins specifically involved in basal membrane degradation and neovascularization. Based on these *in vitro* results, we further characterized CBD effects in *in vivo* angiogenesis assay, using Matrigel sponge model of angiogenesis. Matrigel sponges containing increasing doses of the drug were inoculated subcutaneously into the flank of C57Bl6 mice. Four days after injection, striking inhibition of angiogenesis by CBD was observed both microscopically and by spectrophotometric analysis of haemoglobin content, with respect to the control. In the whole, the present study reveals that CBD inhibits angiogenesis by multiple mechanisms and its dual effect both on glioma and endothelial cells reinforces the hypothesis that CBD could represent an effective tool in glioma therapy.

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PHYTOCANNABINOIDS IN THEIR ACID FORMS ARE POTENT INHIBITORS OF HUMAN BREAST CANCER CELL VIABILITY

Lesley A. Ford-Taylor, Selina M.Y. Chiu, Jasmeer Renoo,
Roger G. Pertwee and Ruth A. Ross

University of Aberdeen, Institute of Medical Sciences, Scotland. UK. AB25 2ZD

Cannabis has been used medicinally for thousands of years, and some of its constituent phytocannabinoids have recently been shown to induce apoptosis in tumour cells, making them promising new therapeutic agents for the treatment of cancer cell proliferation. While their ability to cause tumour cell apoptosis is clear, the receptor(s) and/or effector proteins which mediate these effects remain to be fully identified. In the unheated cannabis plant, most phytocannabinoids are present in their respective acid forms, which differ from their non-acidic counterparts by containing a salicylic acid moiety. Until recently, the pharmacology of these acidic compounds has remained little investigated.

In the present study, we used an AlamarBlue® cell viability assay to investigate the anti-proliferative actions of a number of phytocannabinoids, including acidic forms, in the human breast cancer cell line, MDA-MB-231. We also investigated the role of ERK1/2 in the responses seen. All of the phytocannabinoids under investigation dose-dependently inhibited MDA-MB-231 cell viability. Interestingly, acidic phytocannabinoids were more effective at reducing cell viability than their respective non-acidic counterparts. CBD and CBDV reduced viability with an IC_{50} of around 5 μM, while CBDA was more potent with an IC_{50} of 3.9 μM. CBGA was the most potent inhibitor of cell viability, with an IC_{50} of 2.2 μM. Δ^9-THC and Δ^9-THCV were the least potent with IC_{50} values of >10 μM and 6.5 μM, respectively. Investigations into the potential signalling pathways associated with the viability-reducing properties of phytocannabinoids showed that the ERK1/2 inhibitor, PD98059, completely reversed the reduction in cell viability induced by CBD and CBDA, suggesting that ERK1/2 plays a crucial role in these effects. Further investigations into the downstream signalling pathways associated with plant cannabinoid-induced reductions in cancer cell viability are ongoing.

Phytocannabinoids were supplied by GW Pharmaceuticals.

PROTECTIVE EFFECT OF CANNABIDIOL AND CANNABIS EXTRACT IN COLON CARCINOGENESIS

Gabriella Aviello, Barbara Romano, Francesca Borrelli,
Fabiana Piscitelli[§], Vincenzo Di Marzo[§] and Angelo A. Izzo

Endocannabinoid Research Group, Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy; [§]Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy.

Background/Aim Cannabidiol (CBD) has been shown to exert pro-apoptotic properties in several cell lines and to inhibit glioma growth and experimental breast carcinoma *in vivo*[1]. Here, we have evaluated the effect of CBD (1 and 5 mg/kg) and CBD-enriched *Cannabis* extract (5 mg/kg, standardized to contain 65.6% CBD, here named CBD-BDS) on the formation of pre-neoplastic lesions [i.e. aberrant crypt foci (ACF)], polyps and tumours in the mouse colon *in vivo*.

Methods ACF, polyps and tumours were induced by the carcinogenic substance azoxymethane (AOM). Cannabinoid drugs (IP) were given three times a week for a period of three months. Endocannabinoid colonic levels were determined by isotope dilution liquid chromatography–mass spectrometry.

Results AOM induced the appearance of ACF, polyps and tumours in the mouse colon and these effects were associated with a significant increase of both anandamide and 2-arachidonoylglycerol levels. CBD (1 and 5 mg/kg) and CBD-BDS (5 mg/kg) significantly reduced the number of ACF with four or more crypts as well as polyps formation. In addition, CBD 1 mg/kg significantly reduced the number of tumours, but had little effect on endocannabinoid levels. CBD 5 mg/kg and CBD-BDS 5 mg/kg showed a trend towards inhibition of tumour formation.

Conclusions Various steps of colon carcinogenesis, which are accompanied by elevation of colon endocannabinoid levels, are counteracted by CBD and CBD-BDS. Unlike what previously observed for the effect of a FAAH inhibitor on colon carcinogenesis[2], CBD does not seem to act *via* inhibition of endocannabinoid inactivation.

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REGULATION AND POSSIBLE FUNCTION OF THE ENDOCANNABINOID SYSTEM IN MYOGENESIS

Cristoforo Silvestri, Fabiana Piscitelli, Andrea Martella and Vincenzo Di Marzo

Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy

Skeletal muscle arises from stem cells originating in the embryonic mesoderm. These same stem cells are the source of satellite cells, which are associated with skeletal muscle and are crucial to muscle growth and regeneration. The pluripotent murine myoblast cell line C2C12 is often utilized as a model of skeletal muscle differentiation and regeneration due to its ability to undergo myogenesis. Upon withdrawal from the cell cycle, C2C12 cells initiate a muscle specific gene programme resulting in cellular fusion and formation of multinucleated myotubes. Recent studies have identified the expression of cannabinoid receptors (*Cnr*) in skeletal muscle and the role of the endocannabinoid system (ECS) as a regulator of insulin sensitivity, oxidation and glucose metabolism. The role of the ECS in muscle cells differentiation remains to be determined. Utilizing C2C12 cells, quantitative PCR and isotope-dilution LC-MS, we analysed the expression of various components of the ECS during myogenesis, and obtained preliminary results on its possible role in skeletal muscle differentiation.

We report that C2C12cells express *Cnr1*, and much less, if any, *Cnr2*, and show that *Cnr1* expression increases drastically during the early stages of myogenesis and then decreases. Interestingly, expression of 2-arachidonoylglycerol (2-AG) anabolic and catabolic enzymes, *Dagla* and *Mgll*, decreases and increases, respectively, during myogenesis. Analysis of 2-AG levels in differentiating C2C12 cells showed that, consistent with the changes observed in *Dagla* and *Mgll* expression, its levels decrease significantly with myogenesis. Incubation of C2C12 cells during the first 4 days of differentiation with 2-AG or its catabolite arachidonic acid (AA) resulted in significantly lower levels of the differentiated muscle marker *Myh1*. Observation of differentiated C2C12 cells did not reveal any obvious morphological differences between 2-AG- or AA-treated cells. In contrast to this, treatment with anandamide did not result in consistent changes in *Myh1* expression in differentiating C2C12 cells; however it did result in the formation of thickened and branched myotubes, indicating an increase in cell fusion and/or protein synthesis, which affect myotube size.
These data show that the ECS is tightly regulated during the process of myogenesis and represent the first evidence of the emerging role that the ECS may play in muscle differentiation and/or regeneration, with likely different functions exerted by 2-AG and AEA.

ALLERGEN CHALLENGE INDUCES ANANDAMIDE IN BRONCHOALVEOLAR FLUID OF ALLERGIC ASTHMA PATIENTS

Alexander A. Zoerner[1], Dirk O. Stichtenoth[1], Sandor Batkai[1,2], Stefan Engeli[1], Frank Schaumann[3], Norbert Krug[3], Dimitrios Tsikas[1], Jens Jordan[1] and Jens Hohlfeld[3]

[1]Institute of Clinical Pharmacology, Hannover Medical School, 30625 Hannover, Germany
[2]NIAAA, Bethesda 20892, MD, USA
[3]Fraunhofer Institute of Toxicology and Experimental Medicine (Fraunhofer ITEM), 30625 Hannover, Germany

Introduction: Some animal and *in vitro* studies suggest that endocannabinoids exert anti-inflammatory effects. Specifically in the lung, inhaled AEA reduced the obstructive effect of leukotrien D4 in airways, and a specific CB1- and CB2-agonist significantly reduced pulmonary inflammation in guinea pigs. We therefore hypothesized that AEA might be involved in acute inflammatory reactions in asthmatic patients challenged by allergens.

Methods: Fifteen symptom-free patients with a known history of allergic asthma were challenged 3 times by selective bronchoscopy and sampling of bronchoalveolar fluid (BAL). In the first session, unstimulated BAL was obtained without bronchoscopy, in the second session, saline without allergens was administered by bronchoscopy and BAL obtained after 4h. In the third session, saline with allergens was administered. AEA was measured by stable isotope dilution GC-MS/MS. Eosinophil number was determined by FACS analysis.

Results: AEA concentrations in BAL were not significantly different in unstimulated and saline provocation samples (3.1 ± 0.7 pM *vs.* 3.8 ± 0.8 pM; mean ± SEM). In some patients, AEA concentration was near or below the detection limit under these conditions. However, allergen provocation lead to a significant increase in AEA to 17.1 ± 5.1 pM. Furthermore, a highly significant correlation was found between the number of eosinophils and AEA concentration in BAL ($r^2 = 0.63$, $p = 0.0007$).

Conclusion: This is the first study to demonstrate allergen stimulated anandamide in bronchoalveolar fluid of patients with asthma. Whether increased AEA exerts protective or deleterious effects during bronchoalveolar inflammation induced by allergen provocation remains to be demonstrated. Future studies should address which cells are the source of AEA in BAL and which cells in the bronchoalveolar tree are the target of AEA.

CANNABIDIOL ATTENUATES CARDIAC DYSFUNCTION, OXIDATIVE STRESS, FIBROSIS, INFLAMMATORY AND CELL DEATH SIGNALING PATHWAYS IN DIABETIC CARDIOMYOPATHY

Pál Pacher, Mohanraj Rajesh, Partha Mukhopadhyay, Sándor Bátkai, Vivek Patel, Keita Saito, Shingo Matsumoto, Bani Mukhopadhyay, Lauren Becker, György Haskó, Lucas Liaudet, Wilmarie Flores-Santana, David A Wink, Murali C. Krishna, Aristidis Veves and Raphael Mechoulam

National Institutes of Health (NIAAA and NCI), Bethesda, Maryland, USA; UMDNJ-New Jersey Medical School, Newark, NJ 07103, USA; University Hospital, 1011 Lausanne, Switzerland; Microcirculation Laboratory, the Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA; Department for Medicinal Chemistry and Natural Products, Faculty of Medicine, Hebrew University of Jerusalem, Ein Kerem, Jerusalem 91010

INTRODUCTION: Cannabidiol (CBD), the most abundant nonpsychoactive constituent of Cannabis sativa (marijuana) plant, exerts antiinflammatory effects in various disease models and alleviates pain and spasticity associated with multiple sclerosis in humans.

OBJECTIVES: In this study, we have investigated the effects of cannabidiol (CBD) on myocardial dysfunction, inflammation, oxidative/nitrosative stress, cell death and interrelated signaling pathways, using a mouse model of type I diabetic cardiomyopathy or primary human cardiomyocytes exposed to high glucose.

METHODS: Left ventricular function was measured by pressure-volume system. Oxidative stress, cell death and fibrosis markers were evaluated by molecular biology/biochemical techniques, *electron paramagnetic resonance* and flow cytometry.

RESULTS: Diabetic cardiomyopathy was characterized by declined diastolic and systolic myocardial performance associated with increased myocardial reactive oxygen and nitrogen species generation, NFκB and MAPK (JNK and p-38) activation, enhanced expression of adhesion molecules (ICAM-1, VCAM-1), TNFα, iNOS, markers of fibrosis (TGFbeta, CTGF, fibronectin, collagen-1, MMP-2 and MMP-9), and augmented cell death (caspase 3/7 and PARP activity and chromatin fragmentation). Remarkably, both pre- or post treatment with CBD attenuated myocardial dysfunction, cardiac fibrosis, oxidative/nitrosative stress, inflammation, cell death, and interrelated signaling pathways. Furthermore, CBD also attenuated the high glucose-induced increased reactive oxygen and nitrogen species generation, NFκB activation and cell death in primary human cardiomyocytes.

CONCLUSIONS: Collectively, these results coupled with the excellent safety and tolerability profile of cannabidiol in humans, strongly suggest that it may have great therapeutic potential in the treatment of diabetic complications, and perhaps other cardiovascular disorders, by attenuating oxidative/nitrosative stress, inflammation, cell death and fibrosis.

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INHIBITION OF MONOACYLGLYCEROL LIPASE AND FATTY ACID AMIDE HYDROLYSE *IN VIVO* MODULATES LPS-INDUCED INCREASES IN CYTOKINE EXPRESSION IN THE RAT PREFRONTAL CORTEX

Michelle Roche[1], Daniel M. Kerr[1,2], Nikita Burke[1], Weredeselam M. Olango[2], Brendan Harhen, Gemma K. Ford[2] and David P. Finn[2]

[1]Physiology, [2]Pharmacology and Therapeutics, NCBES Neuroscience Cluster and Centre for Pain Research, National University of Ireland, Galway, Ireland.

The endogenous cannabinoid system plays an important role in regulating both the nervous and immune system thereby representing an attractive alternative for the treatment of neuroinflammatory disorders. This study investigated the effects of URB597, a selective inhibitor of fatty acid amide hydrolase (FAAH), and JZL184, a selective inhibitor of monoacylglycerol lipase (MGL), the enzymes that preferentially metabolise the endocannabinoids anandamide and 2-AG, respectively, on lipopolysaccharide (LPS)-induced increases in cytokine expression in the prefrontal cortex. URB597 (1mg/kg i.p.) or JZL184 (10mg/kg i.p.) were administered to male Sprague Dawley rats 30 min prior to LPS (100μg/kg i.p.) administration. Two hours post LPS, the brain was removed, the prefrontal cortex dissected out and stored at -80^{o}C. Cytokine (TNFα, IL-1β or IL-6) gene expression was determined using quantitative RT-PCR. Concentrations of the endocannabinoids, anandamide and 2-AG, and the entourage compounds, N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA), were assessed using LC-MS/MS.

LPS significantly increased IL-6, IL-1β and TNFα mRNA expression in the prefrontal cortex, an effect attenuated by JZL184. In contrast, URB597 significantly enhanced LPS-induced IL-6 mRNA expression in the prefrontal cortex. URB597 did not alter anandamide or 2-AG levels but significantly increased OEA and PEA concentration in the prefrontal cortex of both saline and LPS-treated rats. JZL184 significantly increased 2-AG concentration in both saline- and LPS-treated rats, without affecting anandamide, OEA or PEA levels. LPS did not alter concentrations of the endocannabinoids or entourage compounds when compared to vehicle-saline-treated controls.

In conclusion, enhanced 2-AG in the prefrontal cortex following the administration of a MGL inhibitor attenuates the pro-inflammatory response to LPS. In comparison, administration of a FAAH inhibitor *in vivo* is associated with enhanced levels of endocannabinoid entourage compounds, OEA and PEA, and augmentation of LPS-induced IL-6 mRNA in the prefrontal cortex. The present study provides evidence for a differential role for FAAH and MGL in the regulation of LPS-induced IL-6 expression in the prefrontal cortex. Improved understanding of endocannabinoid-mediated regulation of neuroimmune function has fundamental physiological and potential therapeutic significance.

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THE ENDOCANNABINOID 2-ARACHIDONOYL-GLYCEROL ACTIVATES HUMAN NEUTROPHILS FUNCTIONS: CRITICAL ROLE OF LEUKOTRIENE B_4

François Chouinard, Samuel Poirier, Claudine Ferland,
Julie S. Lefebvre and Nicolas Flamand

Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec,
Faculté de médecine, Université Laval, Québec, Canada, G1V 4G5.

BACKGROUND – Human neutrophils are the most abundant circulating leukocytes to migrate toward infectious sites. They indeed play an important role in host defense and participate to pathogen clearance by phagocytosis (and killing), and by releasing microbicidal peptides such as α-defensins and the cathelicidin LL-37. Neutrophils also contribute to host defense by releasing bioactive lipid mediators derived from arachidonic acid. These mediators, particularly leukotriene (LT) B_4, recruit and activate circulating and tissue leukocytes to fight pathogens. Immunomodulatory effects of endocannabinoids have been documented but their involvement in the regulation of neutrophil functions has not been thoroughly investigated yet. Importantly, the endocannabinoids 2-arachidonoyl-glycerol (2-AG) and N-arachidonyl-ethanolamine include a molecule of arachidonic acid in their structure and are rapidly hydrolyzed (within minutes). In the present study we investigated the effect of endocannabinoids and their metabolites on human neutrophil functions along with the cellular and molecular mechanisms involved.

RESULTS – N-arachidonyl-ethanolamine did not activate human neutrophils. In sharp contrast, 2-AG potently stimulated an important release of the microbicidal peptides LL-37 and α-defensins, concomitantly with a robust biosynthesis of LTB_4. 2-AG did not elicit the migration of neutrophils but induced the release of a potent chemotactic activity from and for neutrophils. All the effects of 2-AG on neutrophil functions occurred within seconds and were maximal within 2 minutes. In agreement with the results mentioned above, 2-AG rapidly induced the mobilization of calcium ions from intracellular stores as well as activation of the p38 and the ERK pathways. Experiments with labeled 2-AG confirmed the newly biosynthesized LTB_4 derived from the arachidonoyl moiety of 2-AG. CB receptor agonists/antagonist did not mimic/prevent the stimulatory effects of 2-AG on neutrophil functions. Importantly, the 2-AG hydrolysis inhibitors MAFP and JZL-184 prevented all the effects of 2-AG on neutrophils' functional responses, indicating that arachidonic acid was implicated in the effects of 2-AG. In line with an critical role of arachidonic acid in the activation of neutrophil functions by 2-AG, the inhibition of enzymes involved in the biosynthesis of LTB_4 or the blockade of the LTB_4 receptor BLT_1 prevented all the effects of 2-AG on neutrophil functions and cell signaling.

CONCLUSIONS – 2-AG activates human neutrophils. This activation is the consequence of 2-AG hydrolysis, the subsequent biosynthesis of LTB_4 and an autocrine amplification loop involving the LTB_4 receptor BLT_1. This study suggests that endocannabinoids and their metabolites might promote host defense *in vivo.*

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THE CANNABINOID RECEPTOR AGONIST CP 55,940 INDUCES A RECIPROCAL PATTERN OF GENE EXPRESSION IN HEPATIC STELLATE CELLS ENGINEERED TO EXPRESS CB1 OR CB2 RECEPTORS

Rangan Maitra[1] and Huaqin Pan[2]. RTI International, [1]Department of Pharmacology and Toxicology, [2]Department of Social, Statistical and Environmental Sciences, Research Triangle Park, NC 27709, U.S.A.

Introduction: Recent studies indicate that 7.6% of the US population aged 12 or older (18.2 million) abuse alcohol. Alcohol abuse has detrimental effects on many organ systems, most notably the liver, causing both acute and chronic liver disease. Hepatic cirrhosis resulting from progressive fibrosis is a leading cause of alcohol-related morbidity and mortality worldwide. Further, hepatitis increases the probability of developing hepatocellular carcinoma, which is usually fatal. Development of alcoholic liver disease (ALD) is dependent on the interplay between several different hepatic cell types but alcohol-induced injury to hepatocytes, accumulation of fat by these cells and activation of hepatic stellate cells (HSC) to a fibrogenic phenotype are important steps. Activation of HSC leads to a transition of these cells from a quiescent vitamin A-rich cell type to a vitamin A-deficient, proliferative, fibrogenic and contractile myofibroblast. Activated HSC demonstrate characteristic phenotypic changes including excess production of collagen and decreased degradation of matrix proteins. Failure of matrix degradation leads to accumulation of fibrotic proteins and progressive hepatic fibrosis. Therefore, inhibition of HSC activation is a promising therapeutic strategy to treat liver fibrosis induced by alcohol and other toxicants. Previous reports indicate that expression cannabinoid receptors and hepatic endocannabinoid levels are increased in several types of liver disease. Past studies also indicate that inhibition of CB1R activity using drugs or genetic manipulation has a positive outcome on ALD and other related fibrogenic disorders of the liver. By contrast, agonism of CB2 receptor (CB2R) activity has a beneficial effect. However the roles played by CB1R and CB2R in the development and resolution of liver disease, particularly within the context of HSC activation and signaling remain to be fully elucidated. The goals of these studies were to establish disease relevant model systems to evaluate the effects of signaling through CB1R and CB2R in HSC on gene expression using microarray analyses.

Methods: An immortalized human hepatic stellate cell line (LX2) was engineered to over-express either CB1R or CB2R using lentiviral transduction. Clonal cell lines stably expressing either receptor were isolated and characterized using various approaches including immunoblotting, flow-cytometry and immunofluorescence microscopy. Consequently, microarray-based gene expression profiling (GEP) studies were conducted in LX2-CB1R or LX2-CB2R cells stimulated with the synthetic cannabinoid receptor agonist CP 55,940 alone or following pre-treatment with specific antagonists of each receptor. Vehicle-treated cells were used as controls. Supervised analyses of GEP data were performed to identify cellular pathways and processes affected by CP 55,940 in LX2 cells over expressing either CB1R or CB2R.

Results: Immortalized human stellate cells were engineered to stably express CB1R or CB2R and fully characterized. Microarray analyses of gene expression in these cells indicated a reciprocal pattern of regulation of various pathways critical to initiation and progression of liver disease. Clear differences were noted in the regulation of genes involved in cytoskeletal remodeling. For example, Collagen IV expression was up-regulated in cells expressing CB1R (pro-fibrotic) but decreased in CB2R expressing cells (anti-fibrotic) upon stimulation with CP 55,940. Similarly, CB1R agonism up-regulated the expression of genes associated with cellular proliferation and inhibition of apoptosis including Cyclin B1, HSP70, XIAP and c-IAP1. In contrast, these genes were down-regulated in LX2-CB2R cells. Significant differences were also noted in pathways related to NFkappaB and TGF-beta receptor signaling, which are important regulators of liver disease development.

Conclusions: These studies support past reports indicating that CB1R and CB2R reciprocally regulate development and resolution of liver fibrosis. Creation and characterization of HSC stably expressing CB1R or CB2R will enable studies aimed at elucidating the role of endocannabinoid signaling in regulation of liver fibrosis. Further elaboration of gene expression changes noted in these cells upon activation of CB1R and CB2R may lead to innovative therapeutic strategies to treat liver diseases in the future.

ANANDAMIDE STABILISES LYSOSOMES AGAINST AMYLOID-β TOXICITY BY ACTING ON INTRACELLULAR CB_1 RECEPTORS

Janis Noonan, Allan Klompas and Veronica Campbell

Department of Physiology and Institute of Neuroscience,
University of Dublin, Trinity College

Inappropriate apoptosis, triggered by amyloid-beta (Aβ) toxicity, is thought to underlie the neuronal cell death characteristic of Alzheimer's disease (AD). Whilst the precise intracellular mechanisms responsible for apoptosis are not fully understood, lysosomal membrane permeabilisation (LMP) is emerging as a possible initiating factor in this event. It was recently demonstrated that CB_1 receptors can be targeted to lysosomal membranes where they retain the ability to mediate signal transduction (Rozenfeld and Devi, 2008). Based on this finding, coupled with numerous reports highlighting the neuroprotective effects of endocannabinoids, the aim of this study was to (i) determine whether anandamide (AEA) has the proclivity to prevent LMP and thus the onset of the apoptotic pathway, and (ii) to investigate the role of the CB_1 receptor in AEA-mediated neuroprotection against Aβ toxicity. Cultured cortical neurones were exposed to $A\beta_{1\text{-}40}$ (2μM) ± AEA (10nM) and assessed for apoptosis using TUNEL technique and caspase-3 activation (48 hr). LMP was determined using acridine orange (AO) following 6 hr of treatment. To determine the role of plasma membrane and intracellular CB_1 receptors in AEA-mediated neuroprotection, neurones were exposed to the cell impermeable CB_1 receptor antagonist, hemopressin (10μM), or the cell permeable antagonist, SR 141716A, for 15 min prior to exposure to $A\beta_{1\text{-}40}$ ± AEA, and assessed for apoptosis and LMP.

In control neurones DNA fragmentation was 9.04±1.41% (mean±SEM) and this was significantly increased to 25.83±2.19% by Aβ, and prevented by co-treatment with AEA. Caspase-3 activation was 0.03±0.005 pmol pNA produced/mg protein/minute (mean±SEM) in control neurones and increased to 0.08±0.02 by Aβ, and this was also prevented by co-treatment with AEA. Lysosomal stability was assessed by monitoring fluorescence intensity at 633nm, whereby a decrease in emission was indicative of LMP. In control cells, fluorescence intensity was 130±18.21 relative fluorescence units (RFU) and this was decreased to 51.68±10.02 RFU by Aβ. In the presence of AEA, the Aβ-mediated decrease in lysosomal stability was prevented. Finally in the presence of SR 141716A, AEA could not prevent the Aβ-induced DNA fragmentation and LMP. However, in the presence of hemopressin, AEA maintained the ability to confer neuroprotection.

The results of this study indicate that AEA, by acting on intracellular CB_1 receptors, can stabilise lysosomes against Aβ-induced LMP to sustain cell survival.

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OPPOSING EFFECTS OF CANNABINOID-1 RECEPTOR INHIBITION AND CANNABINOID-2 RECEPTOR ACTIVATION ON INFLAMMATION, OXIDATIVE/NITROSATIVE STRESS, AND CELL DEATH IN NEPROPATHY

Partha Mukhopadhyay, Mohanraj Rajesh, Hao Pan, Sándor Bátkai, Vivek Patel, György Haskó, Judith Harvey-White, Bani Mukhopadhyay, Ken Mackie, Bin Gao and Pál Pacher

National Institutes of Health/NIAAA, Bethesda, Maryland, USA; UMDNJ-New Jersey Medical School, Newark, NJ 07103, USA; Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

INTRODUCTION: Cisplatin is an important chemotherapeutic agent; however, its nephrotoxicity limits its clinical use. Enhanced inflammatory response and oxidative/nitrosative stress play a key role in the development of cisplatin-induced nephropathy. Accumulating recent evidence suggests that cannabinoid 1 (CB_1) receptor activation may promote inflammation and cell death and its pharmacological inhibition is associated with anti-inflammatory and tissue protective effects in various preclinical disease models, as well as in humans. In contrast, accumulating evidence supports the anti-inflammatory role of Cannabinoid 2 (CB_2) receptor activation in various clinically relevant inflammatory disease models.

OBJECTIVES: In this study, using molecular biology and biochemistry methods, we have investigated the effects of genetic deletion or pharmacological inhibition of CB_1 receptors on inflammation, oxidative/nitrosative stress and cell death pathways associated with a clinically relevant model of nephropathy induced by cisplatin. We also studied the role of CB_2 receptors in tissue injury by using knockout mice and a CB_2 receptor agonist.

RESULTS: Cisplatin significantly increased endocannabinoid anandamide content, activation of p38 and JNK MAPKs, apoptotic and poly(ADP-ribose)polymerase-dependent cell death, enhanced inflammation (leukocyte infiltration, TNF-α and IL-1β), and promoted oxidative/nitrosative stress (increased expressions of superoxide generating enzymes (NOX2(gp91phox), NOX4), iNOS, and tissue 4-HNE and nitrotyrosine levels) in the kidneys of mice, accompanied by marked histopathological damage and impaired renal function (elevated creatinine and serum BUN) 3 days following the administration of the drug. Both genetic deletion and pharmacological inhibition of CB_1 receptors with AM281 or SR141716 markedly attenuated the cisplatin-induced renal dysfunction and interrelated oxidative/nitrosative stress, p38 and JNK MAPK activation, cell death and inflammatory response in the kidney. The above mentioned cisplatin-induced pathological processes were also attenuated by CB_2 receptor agonist HU-308, and tended to be enhanced in CB_2 receptor knockout mice.

CONCLUSIONS: The endocannabinoid system through CB_1 receptors promotes the cisplatin-induced tissue injury by amplifying MAPK activation, cell death, and interrelated inflammation and oxidative/nitrosative stress. In contrast, CB_2 receptor activation appears to limit the inflammation-induced injury. Thus CB_1 inhibition or CB_2 activation may exert beneficial effects in renal (and most likely other) diseases associated with enhanced inflammation, oxidative/nitrosative stress and cell death.

THE CANNABINOID WIN 55,212-2-MEDIATED NEUROPROTECTION IS DRIVEN BY CB1 RECEPTORS AND MODULATED BY TRPA1 CHANNELS

Marco Koch, Susanne Kreutz, Charlotte Böttger[*], Urszula Grabiec, Chalid Ghadban, Horst-Werner Korf[*] and Faramarz Dehghani

Institute of Anatomy, Leipzig University, Leipzig 04103, Germany
[*]Institute of Anatomy, Goethe University, Frankfurt 60590, Germany

Within secondary neuronal damage, excitotoxicity - meaning the immoderate release of glutamate - reflects the essential damaging mechanism that occurs in most brain pathologies. The neuroprotective potential of cannbinoids is determined by neuronal CB1 and microglial CB2 receptors. However, CB1 and CB2 receptor deficient mice and synthetic cannabinoids have revealed the involvement of further cannabinoid receptors. The well established synthetic CB1 and CB2 receptor agonist WIN 55,212-2 (WIN) might also act upon putative novel cannabinoid receptors since WIN displayed CB1-receptor independent effects on glutamate release in the hippocampus and acts on transient receptor potential (TRP)A1 channels in sensory neurons.

Here, we tested the neuroprotective efficacy of WIN (0.001µM-10µM) in excitotoxically (NMDA)-lesioned organotypic hippocampal slice cultures that were derived from Wistar rats.

Interestingly, lower WIN concentrations (10nM) had a more profound effect on neuroprotection when compared to higher WIN concentrations (10µM). WIN-mediated neuroprotection was blocked by the CB1 receptors antagonist AM251 but not by the CB2 receptor antagonist AM630. Selective inhibition of TRPA1 channels by HC-030031 enhanced the neuroprotective efficacy of 10µM WIN and the number of degenerating neurons became equal to that observed after application of the most effective WIN concentration of 10nM. Selective activation of TRPA1 channels by AITC led to an even stronger neurodegeneration after NMDA-lesion. HC-030031 abolished this enhanced neurodegeneration.

In summary, our data provides evidence that physiological and pharmacological effects of cannabinoids strongly depend on their concentration and the neuroprotective efficacy of WIN 55,212-2 is determined by interaction of activated CB1 receptors and TRPA1 channels.

CHARACTERIZATION OF THE ENDOCANNABINOID SYSTEM IN MOUSE EMBRYONIC STEM CELLS

Mauro Maccarrone[1,2] , Monica Bari[3], Marianna Tedesco[4], Nicoletta Pasquariello[1], Mariangela Pucci[1], Valeria Gasperi[3], Maria Lucia Scaldaferri[4], Donatella Farini[4] and Massimo De Felici[4]

[1]Department of Biomedical Sciences & StemTeCh Group, University of Teramo, Italy
[2]European Center for Brain Research (CERC)/ Santa Lucia Foundation, Rome, Italy
[3]Department of Experimental Medicine and Biochemical Sciences, and [4]Department of Public Health and Cell Biology, University of Rome Tor Vergata, Rome, Italy

In this study we have ascertained the presence and functionality in mouse embryonic stem cells (ESCs) of members of the endocannabinoid system, that have been proposed as possible modulators of the survival and differentiation of various stem cells. We show that mouse ESCs, in addition to classical CB1 and CB2 cannabinoid receptors, express also the transient receptor potential vanilloid receptor (TRPV1), both at mRNA and protein levels. Remarkably, we demonstrate that ESCs have the mRNA, protein and activity of the enzymes to synthesize and degrade the prominent endocannabinoids anandamide (through *N*-acyl-phosphatidylethanolamine-specific phospholipase D, NAPE-PLD, and fatty acid amide hydrolase, FAAH), and 2-arachidonoylglycerol (through diacylglycerol lipase, DAGL, and monoacylglycerol lipase, MAGL). In addition, ESCs are shown to constitutively release a FAAH activating compound. Finally, we document that stimulation of ESCs by methanandamide, a non-hydrolysable analogue of anandamide, does not lead to overt alteration of the expression of *Oct3/4*, *Nanog* and *Cdx2*, genes that are involved in early cell fate in the preimplantation embryo and stemness, nor of the expression patterns of *Brachyury* and *Hnf4*, genes that are used as later markers of lineage differentiation capability of ESC-derived embryoid bodies. Taken together, these results confirm and extend the notion that ESCs express several functional members of the endocannabinoid system, but leave open the question about their role in stem cells as modulators of stemness and differentiation potential.

AN ENDOTHELIAL MECHANISM OF ANANDAMIDE-INDUCED VASORELAXATION IN THE HUMAN AND RAT PULMONARY ARTERY

Marta Baranowska–Kuczko[1], Hanna Kozlowska[1], Miroslaw Kozlowski[2], Eberhard Schlicker[3], Margaret R. MacLean[4] and Barbara Malinowska[1]

[1]Department of Experimental Physiology and [2]Department of Thoracic Surgery, Medical University of Bialystok, Bialystok, Poland,
[3]Department of Pharmacology and Toxicology, University of Bonn, Bonn, Germany,
[4]Integrative and Systems Biology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, Scotland.

Introduction: The endocannabinoid anandamide (AEA) is involved in the pathogenesis of hypertension and hypotension associated with various forms of shock, including hemorrhagic, endotoxic and cardiogenic shock as well as in advanced liver cirrhosis. So far, the influence of endocannabinoids on the pulmonary circulation remains unknown. Thus, **the aim** of present study was to elucidate the physiological contribution of AEA on the regulation of human and rat pulmonary artery tone, as well as the underlying mechanism(s).

Methods: Human lung tissue was obtained from patients undergoing lobectomy or pneumonectomy during resection of lung carcinoma. Rat pulmonary arterial segments were isolated from male Wistar rats. The vasodilator effects of AEA were examined on the isolated human and rat pulmonary arteries pre-constricted with U-46619 (0.01 – 0.03 μM; 0.1 - 0.3 μM, respectively) .

Results: AEA (0.1 - 100 μM) caused almost full concentration-dependent relaxation of both human and rat pulmonary arterial segments pre-constricted with U-46619 (pEC_{50}=5.00±0.13, n=10; 5.00±0.09, n=15; respectively). The vasodilator effect of AEA was reduced by denudation of endothelium in the human and rat pulmonary vessels, by around 70% and 20%, respectively. In endothelium-intact rat vessels, inhibition of cyclooxygenase (indomethacin, 10 μM), NO synthase (L-NAME, 300 μM) and fatty acid amide hydrolase (URB 597, 1 μM) administered alone or together reduced the AEA-induced relaxation. The antagonist of the novel endothelial receptor O-1918 (10 μM) given in the presence of URB 597 (1 μM) diminished the relaxation evoked by AEA in both human and rat pulmonary arteries.

Conclusion: The present study shows that AEA relaxes human and rat pulmonary arteries. These effects are partially endothelium-dependent and result from two mechanisms: (1) an activation of a putative endothelial cannabinoid receptor and (2) a cyclooxygenase-derived prostacyclin-like vasoactive compound formed from the AEA metabolite arachidonic acid. Moreover, results obtained in rat and human pulmonary vessels were comparable.

MYOCARDIAL INFARCTION AFFECTS RESPONSES MEDIATED VIA CARDIAC TRPV1 AND CB_1 RECEPTORS IN RATS

Barbara Malinowska[1], Radosław Rudź[1], Sebastian Łukasz Łupiński[1], Eberhard Schlicker[2]

[1]Department of Experimental Physiology, Medical University of Białystok, Poland
[2]Department of Pharmacology and Toxicology, University of Bonn, Germany

Introduction. Anandamide induces complex cardiovascular responses including the reflex bradycardia (the Bezold-Jarisch reflex) mediated via TRPV1 receptors located on the sensory vagal nerves in the heart; Malinowska et al., *Naunyn-Schmiedeberg's Arch. Pharmacol. 2001;364:562*) and an inhibition of the neurogenic tachycardia mediated via presynaptic CB_1 receptors located on the sympathetic nerve endings innervating heart (Malinowska et al., *Naunyn-Schmiedeberg's Arch. Pharmacol. 2001;364:233*). Acute myocardial infarction (MI) has been demonstrated to increase anandamide content in monocytes and platelets isolated after MI and to induce hypotension in rats (Wagner et al., *J. Am. Coll. Cardiol., 2001;38:2048*). The aim of the present study was to examine the influence of the MI on responses mediated via cardiac TRPV1 and CB_1 receptors.

Methods. MI was induced via ligation of the left coronary artery in rats anaesthetized with urethane or in pithed and vagotomized rats for the examination of TRPV1 and CB_1 receptor function, respectively. The Bezold-Jarisch reflex was stimulated via rapid intravenous (*i.v.*) injection of anandamide 0.2 mg/kg, which decreased heart rate (HR) by about 25 beats/min. Increase in HR by about 50 beats/min in pithed rats was induced via electrical stimulation (0.75 Hz, 1 ms, 50 V, 5 pulses) of the preganglionic sympathetic nerve fibers or *i.v.* injection of isoprenaline (0.1 nmol/kg).

Results. The anandamide-stimulated reflex bradycardia was elevated by about 110 and 70% - 10 and 20 min after MI, respectively. The amplificatory effect of MI was not modified by the CB_1 receptor antagonist rimonabant (0.1 μmol/kg) but it was diminished by the TRPV1 receptor antagonist capsazepine (1 μmol/kg). The anandamide-elicited Bezold-Jarisch reflex was not changed 30 min after MI.

The electrically- but not the chemically-induced tachycardia in pithed rats was reduced by about 30-40% - 10, 20 and 30 min after MI. The inhibitory effect of MI was prevented by rimonabant (0.1 μmol/kg), but not by the CB_2 receptor antagonist SR 144528 (3 μmol/kg), capsazepine (1 μmol/kg) and the endothelial cannabinoid receptor antagonist O-1918 (3 μmol/kg).

Conclusion. Our results demonstrate that acute myocardial infarction amplifies the TRPV1 receptor mediated reflex bradycardia and inhibits the neurogenic tachycardia via the activation of presynaptic CB_1 receptors.

INFLUENCE OF FEMALE AND MALE SEX HORMONES ON VASORELAXATION TO ENDOCANNABINOIDS

W. -S. Vanessa Ho

Division of Basic Medical Sciences, St George's University of London, London, SW17 0RE, United Kingdom

Emerging evidence suggests that cannabinoid functions might demonstrate sex differences. It has been reported that vasorelaxation to the endocannabinoid, anandamide is potentiated by 17β-estradiol treatment, possibly via Transient Receptor Potential Vanilloid receptors, in male rats (Peroni et al. 2004). However, interaction between sex hormones and endocannabinoids in the vasculature remains to be clarified. Here, we have examined the *in vitro* effects of 17β-estradiol, progesterone or testosterone on endocannabinoid-induced vasorelaxation in male and female rats.

Male Wistar rats (200-350g) were killed by cervical dislocation. Small mesenteric arterial segments were mounted in a wire myograph and maintained at 37^{o}C in gassed (95% O_2/5% CO_2) Krebs-Henseleit solution. Relaxations to cumulative addition of an endocannabinoid to vessels precontracted with methoxamine (10μM) were expressed as mean±s.e.m ($n \geq 4$ rats) and analysed by two-way analysis of variance of the whole data set. Hormones were incubated with vessels for 2h before determination of a concentration-response curve. $P<0.05$ was considered statistically significant.

In males, 17β-estradiol, but not its stereoisomer 17α-estradiol, significantly potentiated relaxation to anandamide, although the effect was more prominent in vessels without endothelium (control: pEC_{50}=6.2±0.1, E_{max}=96±1%; +1μM 17β-estradiol: pEC_{50}=6.5±0.1, E_{max}=95±4%, $P<0.01$; +1μM 17α-estradiol: pEC_{50}=6.3±0.1, E_{max}=97±1%). In females (in estrous or proestrous stages of estrous cycle as identified by smear test), 17β-estradiol or testosterone had no effect on anandamide relaxations (control: pEC_{50}=6.4±0.2, E_{max}=101±1%; +1μM 17β-estradiol: pEC_{50}=6.4±0.3, E_{max}=102±2%; +1μM testosterone: pEC_{50}=6.3±0.1, E_{max}=98±1%). Interestingly, 17β-estradiol slightly attenuated relaxation to another endocannabinoid, 2-AG in females, but not males (males, control: pEC_{50}=5.5±0.1, E_{max}=86±7%; +1μM 17β-estradiol: pEC_{50}=5.5±0.1, E_{max}=87±2%; females, control: pEC_{50}=5.5±0.1, E_{max}=89±7%; +1μM 17β-estradiol: pEC_{50}=5.4±0.1, E_{max}=95±2%, $P<0.05$). As in the case for anandamide, testosterone had no effect on 2-AG relaxations in females (data not shown). However, in males, progesterone potentiated relaxation to 2-AG (control: pEC_{50}=5.4±0.2, E_{max}=88±6%; +1μM progesterone: pEC_{50}=5.8±0.2, E_{max}=93±5%), but not anandamide (data not shown).

This study shows that prolonged, *in vitro* treatment with 17β-estradiol and progesterone, but not testosterone, differentially modulate the mesenteric relaxation to anandamide and 2-AG. It remains to be determined if this applies to other vascular regions. The long-term effects of sex hormones *in vivo* on vascular actions of the endocannabinoids also warrant further investigation.

Peroni RN et al. (2004). Eur J Pharmacol 493: 151-160

EVIDENCE THAT HUMAN PLATELETS EXPRESS AUTHENTIC CB_1 AND CB_2 RECEPTORS

Mauro Maccarrone[1,2] , Maria V. Catani[3], Valeria Gasperi[3], Giuseppina Catanzaro[1,2], Samantha Baldassarri[4], Alessandra Bertoni[4,5], Fabiola Sinigaglia[4,5] and Luciana Avigliano[3]

[1]Department of Biomedical Sciences, University of Teramo, Teramo, Italy

[2]European Center for Brain Research (CERC)/Santa Lucia Foundation, Rome, Italy

[3]Department of Experimental Medicine & Biochemical Sciences, University of Rome Tor Vergata, Rome, Italy

[4]Department of Clinical and Experimental Medicine, University of Eastern Piedmont, Novara, Italy

[5]Center of Biotechnology for Applied Medical Research (BRMA), University of Eastern Piedmont, Novara, Italy

In the last decade, the cardiovascular effects exerted by endocannabinoids (eCBs) have attracted growing interest, because they hold the promise to open new avenues of therapeutic intervention against one of the major cause of death in Western society. Several actions of eCBs are mediated by type-1 (CB_1) or type-2 (CB_2) cannabinoid receptors, yet there is no consensus on the presence of these proteins on the platelet surface. To obtain conclusive proofs of the presence of CB_1 and CB_2, we analysed their protein level by Western blotting, visualized their intracellular localization by confocal microscopy, as well as by FACS analysis, and performed functional assays of their binding activity.

Collected evidence demonstrates that CB_1, and to a lesser extent CB_2, are expressed in highly purified human platelets. Both receptor subtypes are predominantly confined within the cell compartments, thus explaining why they might remain undetected in preparations of platelet plasma membranes.

The identification of authentic CB_1 and CB_2 in human platelets supports the potential exploitation of selective agonists or antagonists of these receptors as novel therapeutics to combat cardiovascular diseases. It seems all the more important that some of these substances, like the CB_1 selective antagonist rimonabant, have been already used in humans as oral anti-obesity drugs.

2-ARACHIDONOYLGLYCEROL CONCENTRATION IN HUMAN SERUM IS INCREASED BY BLOOD WITHDRAWAL

Kara L. Stuhr[1], Andrea Dlugos[2], Cecilia J. Hillard[1], and Harriet de Wit[3]

1 Medical College of Wisconsin, Milwaukee, WI, 53226, USA
2. University of Muenster, Muenster, 48149, Germany
3. University of Chicago, Chicago, IL, 60637, USA

Numerous studies have demonstrated that the endocannabinoids, N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), are present in human serum and that their content changes with stress, hormonal status, and in various diseases and disorders. However, no study, to our knowledge, has investigated the impact of blood withdrawal per se on circulating endocannabinoids. In the present study, ten Caucasian healthy, consenting, male subjects were recruited and had an intravenous catheter placed during a morning session. Thirty minutes after catheter placement, blood was collected at varying time points over the subsequent 2 hour period. The intervals between blood draws were varied between 5 and 30 min. The volume of blood withdrawn at each time point was 10 ml; blood was held at 4°C until harvest of serum. Serum was frozen shortly after harvest. Serum lipids were extracted and the concentrations of 2-AG, AEA as well as other structurally related compounds 2-oleoylglycerol (2-OG), palmitoylethanolamide (PEA), oleolyethanolamide (OEA) and docosatetraenoylethanolamide (DEA) were determined using isotope dilution, LC/MS. We found that the contents of 2-AG and 2-OG were 3.2-fold and 2.7-fold higher, respectively, in samples that were drawn within 10 min of a previous draw. The concentrations of monoacylglycerols were unaffected by a blood withdrawal that occurred 20 or 30 minutes previously. The concentrations of ethanolamide compounds were not affected by previous blood withdrawal. These findings, particularly the large change in 2-AG concentrations, are quite remarkable. We do not think that the changes are due to events occurring after the blood collection, to time of day or to the level of stress of the subject. We hypothesize that the process of blood withdrawal results in a transient mobilization of 2-AG. Consideration should be given to these data for the construction of future studies in which multiple blood withdrawals over a short period of time will be made. These data may also provide insight into the role of endocannabinoids in hemodynamic and vascular function as well as blood loss.

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CANNABINOIDS AND PROSTAMIDES ATTENUATE INFLAMMATORY DAMAGE IN A HUMAN EXPLANT COLITIS MODEL

Scott D. Smid and Lauren Nicotra

Discipline of Pharmacology, School of Medical Sciences, University of Adelaide, South Australia, 5005 Australia

The gastrointestinal endocannabinoid system partly protects against inflammatory damage in experimental colitis models. Endocannabinoids such as anandamide also act as substrates for cyclooxygenase-2 (COX-2), generating prostaglandin ethanolamides (prostamides). However, prostamide effects on colitis and colonic function are unknown. In this study, a tissue culture explant model of acute colitis was utilised, whereby healthy human colonic mucosal tissue obtained from colorectal resections was incubated with the pro-inflammatory cytokines TNF-α and IL-1β (10 ng/ml, 20hrs, 37 deg. C). Explants then underwent histological determination of crypt destruction, luminal epithelial damage and lamina propria lymphocyte numbers. Cytokine-evoked inflammatory damage was then compared against select pharmacological interventions in cannabinoid and COX-2 pathways. Cytokine incubation resulted in a significant increase in all colitis parameters compared to incubation controls. Anandamide (10^{-6}M) reduced all indices of tissue damage. This was unaltered when anandamide was co-incubated with the FAAH inhibitor URB 597 (10^{-6}M) or CB_1 receptor antagonist AM251 (10^{-6}M), indicating (i) stability of anandamide in culture and (ii) lack of CB_1 receptor involvement. Incubation of explants with the CB_2 receptor agonist JWH-015 (10^{-6}M) was also effective in reducing colitis indices; this effect was reversed in the presence of the CB_2 inverse agonist JTE-907 (10^{-6}M). While co-incubation with nimesulide (COX-2 selective inhibitor; 10^{-6}M) did not alter the protective effects of anandamide, incubation of explants with the exogenous prostamide $F_{2\alpha}$ analog bimatoprost (10^{-6}M) alone markedly reduced colitis scores, which was reversed when co-incubated with a prostamide-selective antagonist (10^{-6}M). Human mucosal FAAH activity was unaltered following cytokine and drug incubations, and was only diminished when URB597 was present. These results demonstrate a direct role for CB_2 receptors and prostamides in the protection against colitis development. Ligands targeting these pathways may have therapeutic potential in the treatment of clinical colitis.

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EFFECTS OF CB2 RECEPTOR MODULATION ON THE INTESTINAL MICROCIRCULATION IN EXPERIMENTAL SEPSIS

Mandana Kianian, Juan Zhou, Mel Kelly, Sara Whynot, Orlando Hung, Michael Murphy and Christian Lehmann

Departments of Anesthesia and Pharmacology, Dalhousie University, Halifax, NS, Canada

Introduction: Sepsis is a disease of the microcirculation and impairment of the intestinal microcirculation during sepsis may cause a breakdown of gut barrier function thus releasing bacteria and their toxins into the systemic circulation [1]. Consequently, the protection of the intestinal microcirculation represents a pivotal therapeutic target in severe systemic inflammation. Cannabinoids that interact with Cannabinoid receptors (CB1R and CB2R) have been shown to have immunomodulatory properties in *in vivo* and *in vitro* studies and the endocannabinoid system has been shown to be involved during systemic inflammation [2]. The aim of the present study was to examine the effects of CB2 receptor modulation on the intestinal microcirculation in experimental sepsis (endotoxemia) using intravital microscopy (IVM).

Methods: We studied four groups of animals (Lewis rats, n=5 per group): healthy controls (CON), endotoxemic animals (20 mg/kg lipopolysaccharide; LPS), endotoxemic animals treated with CB2 agonist, HU308 (10 mg/kg IV), and endotoxemic animals treated with CB2 antagonist, AM630 (2.5 mg/kg IV). Intravital microscopy of the intestinal microcirculation was performed following 2 hours LPS/placebo administration. Leukocyte adhesion and functional capillary density (FCD) were measured offline in a blinded fashion.

Results: Following two hours of endotoxemia, a significant increase of leukocyte adhesion in the intestinal submucosal venules (e.g., V1 venules: CON 86.8±16.8 n/mm^2, LPS 189.8±27.6 n/mm^2, $p<0.05$) was observed. Capillary perfusion of the muscular and mucosal layers of the intestinal wall was significantly reduced (e.g., circular muscular layer: CON 109.9±7.8 cm/cm^2, LPS 82.4±3.5 cm/cm^2). Treatment of endotoxemic animals with the CB2 receptor agonist, HU308, further increased leukocyte adhesion (V1 venules: 259.9±20.0 n/mm^2), whereas CB2 receptor inhibition by AM630 significantly reduced leukocyte activation (V1 venules: 142.4±16.1 n/mm^2) and restored capillary perfusion (circular muscular layer: 107.7±8.9 cm/cm^2).

Conclusion: The data support the hypothesis, that CB2 receptor signalling is involved in the impairment of the intestinal microcirculation during sepsis. Blocking CB2 receptor signalling reduces leukocyte activation and improves capillary perfusion in acute endotoxemia in rats. The long-term effect of modulating CB2 receptors in more clinical sepsis models needs further investigation.

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SYNTHESIS OF FATTY ACID AMIDES OF CATECHOL METABOLITES WITH ANTIOXIDANT AND ANTIOBESITY PROPERTIES

Bruno A. Cotrim,[a,b] Jesús Joglar, [c] Maria Jesus L. Rojas, [d] Juan Manuel Decara, [d]Francisco Javier Bermudez-Silva, [d] Manuel Macias-Gonzalez, [d,f] Montserrat Fito, [e,f] Miguel Romero, [d] Magí Farré, [a,g] Maribel Covas, [e,f] Fernando Rodriguez de Fonseca, [d] Rafael de la Torre[a,b,f]

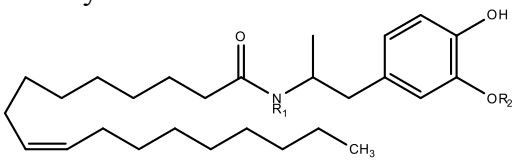
[a]IMIM-Hospital del Mar, Neuropsychopharmacology Research Program, Dr Aiguader 88, Barcelona 08003, Spain; [b]Universitat Pompeu Fabra, Dr Aiguader 88, Barcelona 08003, Spain; [c]Departamento de Química Biológica y Modelización Molecular, (IQAC-CSIC), Jordi Girona 18-26 08034 Barcelona, Spain; [d]Fundación IMABIS, Hospital Carlos Haya de Málaga, Spain; [e]IMIM-Hospital del Mar, Inflammation and Cardiovascular Research Program Dr Aiguader 88, Barcelona 08003, Spain; [f]CIBEROBN, Instituto de Salud Carlos III, Madrid, Spain: [g]UDIMAS-UAB, Dr Aiguader 88, Barcelona 08003, Spain.

All endogenous cannabinoids known up to date are composed of a large fatty acid chain and a polar head. The linkage between the fatty acid and the polar head could be an amide (anandamide, *N*-oleyl-dopamine and *N*-arachidonyl-dopamine), an ester (2-arachydonyl glycerol) or an ether (noladin ether) linkage.

It is already accepted that the analgesic effect of acetaminophen is derived from the compound known as AM404 resulting from the conjugation of *p*-hydroxyaniline (an acetaminophen metabolite) with arachidonic acid and its subsequent interaction with the endocannabinoid system.

By analogy, for what is known for fatty acid derivatives of dopamine and the potential of xenobiotics to form analogous derivatives with relevant biological activities, it is postulated that fatty acid derivatives of several catechol type metabolites of MDMA (HHA: 3,4-dihydroxyamphetamine and HHMA: 3,4-dihydroxymethamphetamine) and their *O*-methyl derivatives (HMA: 3-hydroxy-4-methoxyamphetamine and HMMA: 3-hydroxy-4-methoxymethamphetamine) could display biological activities of interest for a better understanding of MDMA pharmacology if never proven their formation *in vivo* or for potential therapeutic applications or as pharmacological tools.

In accordance with this, a series of fatty acid amides of MDMA metabolites were synthesized in order to evaluate their biological activities.



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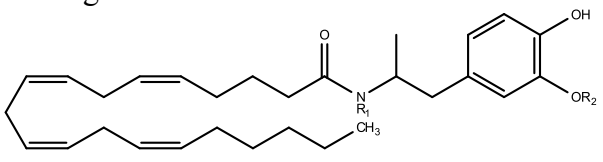

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R_1 = R_2 = H Oleyl-HHA (**1**); Arachidonyl-HHA (**4**); R_1=H, R_2=Me Oleyl-HMA (**2**), Arachidonyl-HMA (**5**); R_1=Me, R_2=H; Oleyl-HHMA (**3**), Arachidonyl-HHMA (**6**)

All synthesized compounds displayed antioxidant activity in an *ex vivo* experiment with human LDL and negative modulation of food intake in rats. The most active compounds had affinity for the CB1 and/or PPAR-α receptors and part of their biological activity may have been due to those double interactions.

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BPR0912, A NOVEL PERIPHERAL CB1 INVERSE AGONIST AND ITS METABOLIC EFFECTS

Ming-Shiu Hung, Kak-Shan Shia, Yi-Ting Wang, Yinchiu Lin, Chien-Huang Wu
Yen-Nan Yeh, Jen-Shin Song, Wenchi Hsiao and Yu-Sheng Chao

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli 350, Taiwan

Cannabinoid receptor 1 (CB1), a G protein-coupled receptor, regulates appetite and metabolic pathways via both central and peripheral mechanisms, and has become a potential therapeutic target for treating metabolic diseases. To limit the psychological side effects resulted from the central action of CB1 receptors, identification of peripheral CB1 antagonists are being actively pursued. A novel alkynylthiophene series of CB1 antagonists identified demonstrated low brain/plasma (B/P) ratio, and BPR0912 is a representative of this series. BPR0912 (IC50 = 8.5 nM, EC50 = 18.5 nM) is an orally active inverse agonist with a B/P ratio of 0.03. Administration of BPR0912 at 20 mg/kg to normal C57BL6/J mice did not reverse CP55,940-induced hypothermia and analgesia; while the dose was increased to 50 mg/kg, these responses were only mildly reversed. BPR0912 was further examined in the diet-induced obese mouse model. In a three-week study, BPR0912 significantly reduced body weight at a dose as low as 3 mg/kg, and the serum levels of insulin and glucose and the liver triglyceride level also decreased dose-dependently. These results suggest that BPR0912 is a peripheral acting CB1 inverse agonist and can be served as a promising lead compound for later drug development. Additional metabolic changes will be reported.

FATTY ACID AMIDE HYDROLASE ACTIVITY IN HUMAN ADIPOCYTES DOES NOT CORRELATE WITH METABOLIC MARKERS OR ANTHROPOMETRIC MEASUREMENTS

Jemma C. Cable[1], Garry D. Tan[1], Stephen P. H. Alexander[2], Saoirse E. O'Sullivan[1]

1. School of Graduate Entry Medicine and Health, University of Nottingham, Derby, DE22 3DT, UK
2. School of Biomedical Sciences, University of Nottingham, Nottingham, NG7 2UH, UK

Blood serum concentrations of endocannabinoids are elevated in obesity. Studies investigating the effects of obesity on fatty acid amide hydrolase (FAAH) mRNA levels have shown that FAAH mRNA is downregulated, upregulated or not different in subcutaneous adipose tissue between obese and lean controls. No studies to date have examined the effects of body weight on FAAH enzyme activity. The aim of the present study is to investigate any correlation between FAAH activity and markers of body fat and metabolism in subcutaneous mature adipocytes taken from healthy people over a range of body mass indices (BMI).

Ethical approval was granted by the University of Nottingham Medical School Ethics Committee, and volunteers were recruited from staff and students of the University of Nottingham (BMI range 19-34). Exclusion criteria included type 2 diabetes and hypertension. Anthropometric measurements, such as height, weight and various skinfold thicknesses, were taken on all volunteers. Following an overnight fast, a venous blood sample was taken and a subcutaneous abdominal adipose sample obtained via a needle biopsy under local anaesthetic. Blood serum was separated and stored at -80^{o}C prior to glucose, insulin and adiponectin assays. Adipose tissue was digested with collagenase to release mature adipocytes, which were then homogenised and centrifuged (20,000 *g*, 20 minutes). The particulate and cytosolic fractions were stored at -80^{o}C until assay of FAAH activity using 2 μM *N*-arachidonoyl-^{3}H-ethanolamine as substrate.

FAAH activity was detected in the particulate fractions and was completely eradicated in the presence of the FAAH inhibitor URB597 (1 μM). There were no significant correlations between FAAH activity and fasting serum glucose, insulin or adiponectin. Similarly, there were no significant correlations between FAAH activity and estimates of total body fat and fat distribution, such as BMI, waist to hip circumference ratio or skinfold thickness at various sites.

In this sample of healthy people, FAAH activity in mature adipocytes does not correlate with any of our measured metabolic markers or estimations of adiposity. Further work in this research will focus on obese and morbidly obese patients to assess whether any trends in FAAH activity and metabolic or body fat markers are observed within these populations.

ENDOCANNABINOIDS AND BODY MASS INDEX DURING PREGNANCY - IS THERE A RELATIONSHIP? A LONGITUDINAL STUDY

Holiday A. Durham[1], Alexandros Makriyannis[2], Jodi T. Wood[2], John S. Williams[2] and Carol J. Lammi-Keefe[1]

[1]AgCenter, Louisiana State University; Baton Rouge, LA, 70803, USA,
[2]Center for Drug Discovery, Northeastern University, Boston, MA, 02115, USA.

Introduction: Pregnancy is a unique period for weight gain; however, evidence indicates women of childbearing age have experienced a substantial increase in the rate of overweight and obesity, increasing inflammatory response and exacerbating metabolic changes. Endocannabinoids are emerging as important regulators of physiological processes, including functions in reproduction, inflammation and weight regulation. However, little is known about regulation of these metabolites during the course of pregnancy, especially among women with different body mass indexes (kg/m2; BMI).

Objective: In a longitudinal design, compare plasma endocannabinoids of pregnant women who were underweight, normal, overweight or obese prior to pregnancy.

Methods: We quantified plasma arachidonoylethanolamine (AEA), palmitoylethanolamine, (PEA), oleoylethanolamine (OEA) and 2-arachidonolglyerol (2-AG) of 58 women at 20-22, 23-26, 32 and 38-40 weeks of pregnancy using liquid chromotography-mass spectrometry. Women were categorized into BMI categories based on self-reported pre-pregnancy body weights [underweight (n=3), normal (n=24), overweight (n=15) or obese (n=16)]. Differences in endocannabinoid concentrations during pregnancy, between BMI categories and time by group interactions, were tested using repeated measures analysis.

Results: During pregnancy the measured ethanolamides increased by delivery among all groups of women (LSM ± SE): AEA (0.70 ± 0.04 pg/µl to 0.89 ± 0.04 pg/µl, $p<0.01$), PEA (4.54 ± 0.22 pg/µl to 5.52 ± 0.25 pg/µl, $p<0.01$) and OEA(4.29 ± 0.27 pg/µl to 5.71 ± 0.32 pg/µl, $p<0.01$). The glycerol, 2-AG, was not significantly different at delivery (175.49 ± 12.91 pg/µl to 142.38 ± 15.26 pg/µl, $p =0.23$). AEA, OEA and 2-AG concentrations did not differ among underweight, normal, overweight or obese women, nor was there a time by group interaction. PEA significantly increased among overweight women by delivery; however, concentrations did not differ from other BMI groups over time.

Conclusions: This exploratory longitudinal study points to an increase in ethanolamides from the second to third trimesters of pregnancy. Based on these data, BMI was not a significant factor in regulating response of the endocannabinoids measured. There is a need in future work to: i.) investigate the inflammatory indicators that may be more sensitive markers of weight/adiposity than BMIs calculated from self-reported pre-pregnancy weights and ii.) examine the endocannabinoids longitudinally from pre-pregnancy and throughout pregnancy. To our knowledge, this is the first report of endocannabinoid concentrations during pregnancy using a longitudinal rather than a cross-sectional design.

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INHIBITORY EFFECT OF CANNABICHROMENE ON INTESTINAL MOTILITY IN MICE

Barbara Romano, Raffaele Capasso, Gabriella Aviello, Luciano De Petrocellis°, Antonio Pescatore, Angelo A. Izzo and Vincenzo Di Marzo§

Endocannabinoid Research Group, Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy; °Institute of Cybernetics and §Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy.

Background/Aim Cannabichromene (CBC), together with Δ^9-tetrahydrocannabinol, is the major cannabinoid in freshly harvested dry-type plant material[1]. Although CBC has been shown to exert anti-inflammatory, antimicrobial and a modest analgesic activity[1], the pharmacological actions of this non-psychotropic cannabinoid are largely unexplored. Here, we have investigated the possible effects of this non-psychotropic cannabinoid on intestinal motility in mice.

Methods Motility *in vivo* was measured by evaluating the distribution of an orally-administered fluorescent marker along the small intestine either in control mice or in mice with croton-oil induced intestinal inflammation; *in vitro*, CBC was evaluated on the contractions induced by electrical field stimulation (EFS) or acetylcholine.

Results Compared to control mice, croton oil administration *in vivo* produced an increase in intestinal transit. CBC (10 mg/kg, IP) did not affect motility in control mice, but normalized croton oil-induced hypermotility. *In vitro*, CBC (10^{-8}-10^{-4} M) reduced acetylcholine- and EFS-induced contractions.

Conclusions: CBC reduces intestinal contractility *in vitro*. *In vivo*, CBC inhibits intestinal transit in the inflamed gut, without causing constipation in control mice. Studies on the molecular mechanism(s) of action of CBC, and, in particular, on the role in the described effects of transient receptor potential ankyrin type-1 (TRPA1) channels and of the putative endocannabinoid membrane transporter, both of which CBC targets[2], are ongoing.

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CENTRAL CB1 RECEPTORS DIFFERENTIALLY MODULATE FASTING-INDUCED HYPERPHAGIA IN RATS

Edgar Soria-Gómez[1], Federico Massa[1], Pavel E. Rueda-Orozco[2], Giovanni Marsicano[1] and Oscar Prospéro-García[3]

1AVENIR Group "Endocannabinoids and Neuroadaptation" U862 INSERM, NeuroCentre Magendie, Bordeaux 33077, France.
2Departamento de Biofísica, Instituto de Fisiología Celular, UNAM, México DF 04510, México.
3Grupo de Cannabinoides, Facultad de Medicina, UNAM. México DF 04510, México.

Recent evidence showed that CB1-dependent control of brain glutamatergic transmission mediates at least in part the hyperphagic functions of the endocannabinoid system, whereas the control of GABAergic signaling exerts a paradoxical opposite effect (Bellocchio et al., Nature Neuroscience, 2010). However, the sites where this complex control is exerted are not fully understood yet.

In this work we hypothesized that the activation of the ECS in different central nervous structures could mediate these diverse functions. To this aim, we decided to perform intra-cerebral injections of the CB1 antagonist, AM251, in 24h food deprived rats just before re-feeding, in order to affect the increase in food consumption induced by the food deprivation. The targeted nuclei were the nucleus accumbens (NAc), the dorsal striatum (DS), the lateral hypothalamus (LH) and the paraventricular nucleus of the hypothalamus (PVN).

We found that AM251 intra-NAc partially blocks the hyperphagia induced by food deprivation. There were no effects in the DS and the LH. However, CB1 blockade in the PVN induced a potentiation of the food deprivation-induced hyperphagia.

Such results indicate that in conditions of energy deficit the ECS could have differential roles in feeding behavior depending of the brain region (NAc vs. PVN). We are performing in vitro electrophysiological studies in order to better understand the cellular basis of these results.

CHRONIC ADMINISTRATION OF SR141716 EXACERBATES SEIZURES IN R6/2 MICE, A MODEL OF HUNTINGTON'S DISEASE

Eric A. Horne, William Marrs, Jonathon Coy, Xiaocui Sun and Nephi Stella

Department of Pharmacology, University of Washington
Seattle, WA, USA

Huntington's disease (HD) is a neurodegenerative disease caused by a CAG trinucleotide repeat expansion in exon 1 of the huntingtin gene. One of the earliest changes observed in human patients is a decrease in the mRNA of the CB1 receptor in the caudate and putamen, however it is unclear how this change effects the progression of HD. Similar changes in CB1 expression have been observed in presymptomatic R6/2 mice, a rapidly progressing mouse model of HD whose phenotype has been compared to symptoms observed in children with early-onset HD. We hypothesized that if CB1 loss contributes to the progression of HD, inhibition of CB1 should exacerbate the HD phenotype observed in these mice.

To test this hypothesis, we treated presymptomatic (4 week old) R6/2 mice with SR141716 (SR1: 10mg/kg; n=12) or vehicle (n=12) injected daily s.c., a route and regimen that provides optimal brain penetration of SR1 according to our pharmacokinetic study. The chronic treatment of R6/2 mice with SR1 did not affect their motor deficits, since we found no change in their decrease in rotarod performances and the occurrence of tremors at 8 weeks of age. Remarkably, SR1-treated R6/2 mice had much shorter life spans, with SR1 treated R6/2 mice starting to die at 7 weeks of age. By 10 weeks of age, when the first vehicle treated R6/2 mouse died, 70% of SR1 treated mice had died. In addition, seizures were observed in SR1 treated R6/2 mice starting at 5 weeks of age, with about 40% of these mice having seizures by week 7. Seizures have been reported at later ages in R6/2 mice and in agreement with those reports we observed that 16% of the vehicle-treated mice exhibited seizures by 9 weeks of age, with the first seizure observed in an 8 weeks old mouse. Immunohistochemical analysis of animals treated with SR1 indicated increased staining for both active astrocytes (GFAP) and microglia (Iba-1) in the hippocampus.

In conclusion, chronic administration of SR1 decreases the life span and increases the onset of seizures in R6/2 mice without effecting motor deficits normally observed in these mice. The ability for SR1 to increase seizure onset in HD mice is especially relevant for juvenile HD, since epileptic seizures are more common for patients in this age group then for adults with HD. In addition, children with epilepsy from early-onset HD do not respond to classic anticonvulsants, such as Carbamazepine and Phenobarbital. Thus, administration of a CB1 agonist could be an effective therapy for treating these seizures and will be investigated in future experiments.

POTENTIAL OXIDATION OF 2-AG BY COX-2 ENHANCES MALONATE TOXICITY IN THE STRIATUM: RELEVANCE FOR CANNABINOID TREATMENTS IN HUNTINGTON'S DISEASE

Onintza Sagredo[1], María Ruth Pazos[1], Tiziana Bisogno[2], Fabiana Piscitelli[2], Stefania Petrosino[2], Mariluz Hernández[1], Vincenzo Di Marzo[2] and Javier Fernández-Ruiz[1]

[1]Departamento de Bioquímica y Biología Molecular and CIBERNED, Facultad de Medicina, Universidad Complutense, 28040-Madrid, Spain; [2]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, Comprensorio Olivetti, 80078 Pozzuoli, Naples, Italy

The cannabinoid signaling system encompasses the CB_2 receptor, which protects striatal neurons from the apoptotic death caused by the local administration of malonate, a rat model of Huntington's disease (HD). In the present study, we investigated if endocannabinoids provide tonic neuroprotection in this HD model, by examining first the effect of O-3841, an inhibitor of diacylglycerol lipases (DAGLs), the enzymes that catalyse the biosynthesis of 2-arachidonoyl-glycerol (2-AG). The local administration of O-3841 produced a significant reduction in the striatal levels of 2-AG in animals lesioned with malonate, without affecting the levels of anandamide. Neither anandamide nor 2-AG were altered by the lesion of the striatum with malonate alone. Given the neuroprotective properties previously described for 2-AG, a reduction of its levels by O-3841 was expected to enhance the magnitude of malonate-induced lesion. However, we observed that, rather than being harmful, the inhibitor enhanced the survival of striatal neurons since it partially attenuated the malonate-induced GABA and BDNF deficits, whilst reducing the number of dead neurons and glial activation. By contrast, OMDM169, an inhibitor of monoacylglycerol lipase (MAGL), which maintained elevated 2-AG levels, was unable to protect striatal neurons, and it even aggravated striatal damage. These data agree with previous information indicating that 2-AG may be neurotoxic under certain circumstances through its transformation into prostaglandin glyceryl esters by the action of cyclooxygenase-2 (COX-2). Indeed, we found here that COX-2 is induced in vivo in the striatum 24 hours after the lesion, a fact also reproduced in vitro in cultured M-213 cells exposed to malonate. In vivo, using a sensitive ESI-IT-ToF LC-MS technique, we could not detect the major 2-AG oxygenated metabolite, prostaglandin E_2 glyceryl ester (PGE2-Gs), presumably because the generation of this compound is strictly localised to the lesioned areas, thus reaching levels (<0.1 pmol/g tissue) that cannot be detected in the whole striatum with our method. Interestingly, however, the treatment of M-213 cells with malonate combined with the inhibitor of DAGL O-3841 attenuated cell death, whereas the blockade of MAGL with OMDM169 caused the opposite effect as in the in vivo experiments. We are presently analyzing whether both effects were accompanied by the expected reduction or increase in the generation of PGE2-Gs, respectively. The lesion with malonate also caused up-regulation of inducible nitric oxide synthase in the striatum, as well as a marked decrease in nuclear receptors with anti-inflammatory action, like PPAR-α and $-\gamma$, this latter effect being partially corrected by the treatment with O-3841. In summary, the inhibition of 2-AG biosynthesis is neuroprotective in rats lesioned with malonate, possibly because this effect might have counteracted some pro-neuroinflammatory actions of 2-AG.

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THERAPEUTIC EFFECTS OF ABHD6 INHIBITION IN R6/2 MICE, A MODEL OF HUNTINGTON'S DISEASE

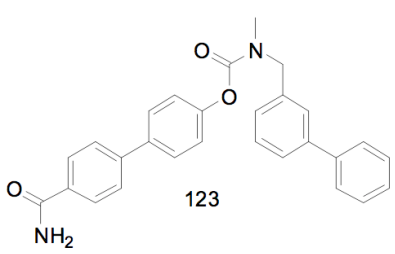
William Marrs[1], Eric Horne[1], Karen Sun[1], Weiwei Li[2], Ben Cravatt[2] and Nephi Stella[1]

[1]Department of Pharmacology, University of Washington, Seattle, WA, USA
[2]Department of Chemical Physiology, Scripps Research Institute, La Jolla, CA, USA

Huntington's Disease (HD) is a progressive neurological disorder characterized by cognitive impairment and involuntary movements, all of which results from the inheritance of mutated huntingtin. Neuropathologically, HD is primarily associated with selective neuronal loss in striatum and cortex. Although it has been believed that the progression of symptoms in HD is due to this neurodegeneration, evidence suggests that severe neuronal dysfunction precedes degeneration and is likely involved in many symptoms. With this early neuronal dysfunction in mind, it is interesting to note that there is a dramatic loss of CB_1 receptors throughout the basal ganglia in HD patients, and that this occurs before the loss of other receptor types and before the appearance of major HD symptoms.

One of the best-characterized animal models of HD is the R6/2 mouse line, which expresses a pathological form of the first exon of human huntingtin under the control of its endogenous promoter. These mice develop a progressive neurological phenotype with motor disturbances and cognitive impairments reminiscent to what is found in HD patients, and this mouse model is commonly used for pre-clinical therapeutic testing. Importantly, one of the earliest documented molecular events in R6/2 mice is the loss of CB_1 receptors in the striatum. In order to assess the therapeutic potential of counteracting this early decrease in CB_1 signaling, we sought to pharmacologically augment the level of the endogenous cannabinoid agonist 2-AG in the brains of R6/2 mice by targeting the newly discovered 2-AG-hydrolyzing enzyme ABHD6.

R6/2 mice and their wild-type (WT) littermates were given daily injections (i.p.) of the CNS-permeable ABHD6 inhibitor WWL123 (10 mg/kg; chemical structure below) or its vehicle starting at 4 weeks of age. Disease progression was assayed every two weeks using a standardized behavioral assessment protocol approved for HD mouse models. Compared to vehicle-treated R6/2 mice, WWL123-treated R6/2 mice did not exhibit clasping and their spontaneous activity levels were completely normalized to WT levels. However, treatment with WWL123 did not affect the locomotor impairment assessed by rotarod, nor did it significantly affect the life span of R6/2 mice. Taken together, our results suggest that there may be therapeutic potential in targeting ABHD6 in HD.



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METABOLISM OF ANANDAMIDE AND 2-OLEOYLGLYCEROL BY GPNT RAT BRAIN ENDOTHELIAL CELLS

Antonio Rodríguez-Gaztelumendi, Stig O.P. Jacobsson and Christopher J. Fowler

Departments of Pharmacology and Clinical Neuroscience, Umeå University, SE90187 Umeå, Sweden

Introduction: The blood-brain barrier plays an important role in controlling the access to the brain of potentially harmful agents, and a dysfunctional blood-brain barrier has been implicated in several neurodegenerative disorders (Zlokovic *et al., Neuron* 57 [2008] 178-201). A promising cell line that models some of the characteristics of the blood-brain barrier is the immortalized rat brain endothelial line GPNT. These cells express P-gp and GPNT monolayers have a low permeability to agents such as vincristine (Régina *et al*., *J Neurochem* 73 [1999] 1954-63). However, it is not known whether these cells express components of the endocannabinoid system. This has been investigated here.

Method: GPNT cells were a kind gift from Dr. John Greenwood (Institute of Ophthalmology, University College London, U.K.). They were cultured in α-minimal essential medium / Ham's F-10 Ham medium (1:1 v/v) containing 10% FBS and 1% PEST. Hydolytic activities towards 0.5 μM [^{3}H] anandamide ([^{3}H]AEA) and 0.5 μM [^{3}H]2-oleoylglycerol ([^{3}H]2-OG) were determined in the cell lysates by the method of Boldrup *et al.* (*J Biochem Biophys Meth* 60 [2004] 171–7). Inhibitors were tested without a preincubation phase.

Results: GPNT cell lysates hydrolysed 0.5 μM [^{3}H]AEA in a time- and protein concentration-dependent manner. The hydrolysis was inhibited by the selective FAAH inhibitor URB597. Thus, the AEA hydrolysis was 23 and 2% of control in the presence of 0.1 and 1 μM URB597 respectively. The lysates also hydrolysed 0.5 μM [^{3}H]AEA in a time- and protein concentration-dependent manner. However, the hydrolysis was only partially inhibited by the selective MGL inhibitor JZL184: concentrations of 0.1 and 1 μM gave mean % of control values of 86 and 77%, respectively. However, 0.1 and 1 μM URB597 gave mean % of control values of 61 and 39%, respectively. Preliminary data using a commercial kit indicated that CP55,940 (0.1-10 μM) did not affect the cAMP response to forskolin, suggesting that the cells do not have robust expression of CB_1 receptors, but further experiments are needed to confirm this finding.

Conclusion: GPNT cells can hydrolyse both AEA and 2-OG. The hydrolysis of AEA is entirely sensitive to URB597, suggesting that it is primarily metabolised by FAAH in these cells. 2-OG appears to be metabolised by MGL, but at the concentration tested, FAAH plays a more important role.

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NEUROPROTECTIVE CONCENTRATION OF ANANDAMIDE INDUCES NICASTRIN EXPRESSION IN PRIMARY NEURONAL CULTURES

Riffat Tanveer and Veronica A. Campbell

Department of Physiology & Trinity College Institute of Neuroscience,
Trinity College, Dublin 2, Ireland.

Introduction: γ-secretase is a high molecular weight multi protein complex composed of at least four components, namely presenilin (PS1/2), nicastrin (Nct), anterior pharynx-defective phenotype 1 (APH1) and PS enhancer 2 (PEN2). Functionally active γ-secretase is involved in processing numerous type-1 membrane associated proteins, including amyloid precursor protein (APP) and Notch. Nct acts as a receptor site for substrate recognition, maturation and stabilization of the γ-secretase complex. Loss of Nct elicits an apoptotic phenotype in mouse embryos and conditional forebrain inactivation of Nct causes progressive memory impairment and age related neurodegeneration (Nguyen et al., 2006; Tabuchi et al., 2009). Whilst over expression of Nct is not associated with excess processing of neuronal APP, in telencephalon specific murine neurons (TSMI) and human embryonic kidney (HEK) 293 cell lines over expression of Nct increases their viability (Pardossi-Piquard et al., 2004; Brijbassi et al., 2007). We have previously demonstrated a neuroprotective effect of the endocannabinoids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), against ß-amyloid-induced neurodegeneration (Noonan et al., 2010, in review) and in the current study we examine the influence of the cannabinoid system on nicastrin expression in cultured cortical neurones since this may represent a novel neuroprotective target.

Methods: Primary neuronal cultures were prepared from neonatal rat cerebral cortices and were treated with AEA (10nM) or 2-AG (10nM) for 1, 6, 24 or 48 hours. Following treatment the cells were fixed in methanol for fluorescent immunostaining or harvested in lysis buffer for western immunoblot in order to assess nicastrin protein expression. mRNA was also extracted for analysis of nicastrin gene expression using real timer PCR.

Results: AEA treatment significantly induced Nct protein expression at 6 hours (**$p < 0.01$, n=6, ANOVA) and this induction was persistent 24 and 48 hours later. No change in Nct was observed at the earlier time point of 1 hour treatment with AEA. In contrast, 2-AG treatment was not associated with any change in Nct protein expression at any of the time points examined.

Conclusion: Nct has been proposed to be anti-apoptotic and neuroprotective promoting different pro-survival pathways. The significant induction of Nct by AEA may represent a target for the neuroprotective effect of AEA. Our current work is investigating whether pharmacological enhancement of endogenous AEA tone using inhibitors of fatty acid amide hydrolase can produce similar results. The consequences of Nct induction remain to be resolved but Notch signalling, which is altered in Alzheimer's disease, is proposed to be vital for neuronal outgrowth and viability. So our project also aims to investigate whether the cannabinoid-mediated induction of Nct impacts on the Notch pathway *in-vitro* and *in-vivo*.

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INHIBITION OF ANANDAMIDE UPTAKE BY DORSAL ROOT GANGLION NEURONS REDUCES MECHANICAL HYPERALGESIA IN TUMOR-BEARING MICE

I.A. Khasabova, M.A, Holman, D.A. Simone and V.S. Seybold

University of Minnesota, 321 Church St. S.E., Minneapolis, MN 55455

The analgesic effects of the endogenous cannabinoid ligand anandamide (AEA) are limited by its cellular uptake and degradation. Previous studies established that an increase in AEA degradation contributes to the development of mechanical hyperalgesia in a murine model of bone cancer (Khasabova et al., 2008). Subsequently we determined that injection of AM404, a putative inhibitor of AEA transport, into plantar skin ipsilateral to tumors reduced mechanical hyperalgesia in tumor-bearing mice. Primary cultures of dorsal root ganglion (DRG) neurons were used to test the hypothesis that an increase in AEA uptake in to cells contributes to the increase in AEA degradation and is independent of enzymatic degradation of AEA. [3H]AEA and a fluorescent AEA analog were used to measure AEA uptake in vitro. Fluorescence microscopy resolved that AEA was taken up predominately by neurons in the DRG cultures. AEA uptake was greater into DRG neurons from tumor-bearing mice in comparison to neurons from naïve mice. When DRG neurons were co-cultured with fibrosarcoma cells, AEA uptake also increased compared to control cultures, demonstrating that factors released from fibrosarcoma cells are sufficient to alter AEA uptake in neurons. AEA uptake was attenuated by pre-incubation of DRG neurons with AM404 (10 μM) or OMDM-1 (5 μM), an inhibitor of the putative AEA transporter with low affinity for fatty acid amide hydrolase (FAAH). Inhibition occurred in a cannabinoid receptor-independent manner. URB597 (100 nM), an inhibitor of FAAH, also decreased AEA uptake. Importantly, incubation of DRG neurons with OMDM-1 plus URB597 decreased AEA uptake into neurons to a greater extent than each drug alone. Together, the data provide evidence that the cellular process of AEA uptake is independent from AEA degradation and can be an important target in the management of bone cancer pain.

EXAMINATION OF THE NEUROPROTECTIVE EFFECTS OF URB597 IN YOUNG AND AGED RAT RETINA

Joanna E Slusar[1], Anna-Maria Szczesniak[1], Melanie EM Kelly[1,2]

Departments of Pharmacology[1] and Ophthalmology[2], Dalhousie University, Halifax, NS, B3H 1X5 Canada.

A number of age-related eye diseases, including glaucoma, diabetic retinopathy and vascular occlusive diseases, share the risk factors of age and vascular dysregulation, resulting in retinal neuron death and vision loss. Cannabinoids and endocannabinoids have vasodilatory and neuroprotective actions in the eye and manipulation of the endocannabinoid system may have therapeutic potential in the treatment of retinal disease (Nucci et al., 2008 *Prog Brain Res*). However, the therapeutic use of endocannabinoids, such as anandamide (AEA) is limited due to rapid degradation by the enzyme fatty acid amide hydrolase (FAAH). Therefore, this research examined the effects of a FAAH enzyme inhibitor, URB597, in aged (18-24 months) and young animals (12 weeks) in two experimental models of retinal neurodegeneration: 1) Optic nerve transection (axotomy) and 2) Transient retinal ischemia-reperfusion.

Retinal ganglion cells (RGCs) were retrogradely labeled using fluorogold (FG) 7 days prior to axotomy or ischemia-reperfusion. For axotomy, the optic nerve was transected 1 mm behind the eye globe. To produce acute retinal ischemia/reperfusion, the pterygopalantine artery, which gives rise to the ophthalmic artery, was ligated for 60 minutes and the external carotid artery was ligated and transected to prevent anastomoses. URB597 (0.03 mg/kg i.p.) was administered daily until sacrifice. Enucleated eyes were then processed for histology and quantitative analysis. FG+ RGCs, phagocytic microglia (MG) and lectin-stained capillaries were then quantified in retinal whole-mounts across four retinal quadrants.

Aged animals showed a reduction in capillary density and an increased number of microaneurysms, compared to young animals ($p<0.01$), suggesting that aged retinas may be more vulnerable to insult. URB597 increased RGC survival after 2 weeks of axotomy ($p<0.05$) in young animals. Ongoing experiments are now investigating whether URB597 can increase RGC survival in aged animals after axotomy. In contrast, URB597 did not produce a significant change in RGC, MG or lectin-stained capillary density in either young or old animals at 14 days after acute ischemia/reperfusion.

These results demonstrate that treatment with URB597 is able to provide RGC neuroprotection after axotomy, but is unable to protect RGCs after acute ischemia-reperfusion injury, irrespective of age. The differential actions of endocannabinoids in these two models suggests that endocannabinoid neuroprotection may depend on the mechanisms leading to RGC loss. Further studies are underway to identify the cellular targets underlying endocannabinoid-mediated neuroprotection after optic nerve injury.

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ROLE OF THE ENDOCANNABINOID-CB1 RECEPTOR PATHWAY IN SYNAPTIC DYSFUNCTION OBSERVED BY BETA-AMYLOID 1-42 AND IN AD MICE

Basavaraj S. Balapal and Ralph A. Nixon

NYS Psychiatric Institute, NKI and Department of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, NY 10032. Center for Dementia Research, NKI, Psychiatry and Cell Biology, Langone School of Medicine, New York University, New York, NY 10016

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of synaptic function and progressive loss of memory, but specific AD-related changes in synaptic transmission are not well understood. To better understand the role of synaptic deficits during the early stages of AD, we must study the effects of AD-related proteins on synaptic transmission in a mammalian central nervous system preparation. Substantial evidence has established the central role of beta-amyloid 1-42 (Abeta42) protein in the AD pathology, which has been found to inhibit long-term potentiation (LTP), a physiologic correlate of memory. Studies have established a critical role for the cAMP signaling pathway in different forms of synaptic plasticity related to learning. Available literature suggests that levels of endocannabinoids (ECs) such as 2-arachidonyl glycerol (2-AG), which acts through the cannabinoid type-1 receptor (CB1R)/cAMP pathway, were significantly elevated in the hippocampus as a result of Abeta42 exposure. The impairment in memory evoked by Abeta42 in rodents is also found to be reversed by CB1R blockade. Despite the above information, no studies have been carried out to ascertain the mechanism by which the CB1R system contributes to the negative effects of Abeta42 on synaptic function in AD. Research in our laboratory suggests that Abeta42 enhances 2-AG levels in hippocampal neurons in a dose and time dependent manner. Ca2+ chelator prevents the Abeta42-induced formation of 2-AG. These results suggest that Abeta42-induces formation of 2-AG through Ca2+ pathways. In addition, the CB1R antagonist SR141617A rescues synaptic dysfunction observed in a ß-amyloid depositing animal model, the double transgenic mouse (APP/PS1). These results suggest that endocannabinoid-CB1R pathways may be involved in Abeta42-induced synaptic dysfunction. These findings identify a new potential therapy targeting the synaptic dysfunction in AD.

FUNCTIONAL INTERACTION BETWEEN PERIAQUEDUCTAL GREY CANNABINOID SUBTYPE 1 AND METABOTROPIC GLUTAMATE SUBTYPE 1 AND 5 RECEPTORS IN NEUROPATHIC RATS

Enza Palazzo[1], Livio Luongo[1], Luigia Cristino[2], Vito de Novellis[1], Francesco Rossi[1], Vincenzo Di Marzo[2] and Sabatino Maione[1]

[1, 2] Endocannabinoid Research Group.
[1] Department of Experimental Medicine, Faculty of Medicine and Surgery, Second University of Naples, Via Costantinopoli 16, 80138, Napoli, Italy.
[2] Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli (Napoli), Italy.

Metabotropic glutamate (mGlu) and cannabinoid receptors interaction has important implications both for control of synaptic transmission and for novel therapeutic strategies, such as those ones for treating pain. Periaqueductal grey (PAG) and rostral ventromedial medulla (RVM) represent important supraspinal sites within the antinociceptive descending pathway. The study has analyzed the effect of intra-periaqueductal grey (PAG) microinjections of cannabinoid or group I mGlu receptor ligands on thermoceptive responses and on rostral ventromedial medulla (RVM) ON and OFF cell activities in neuropathic rats.

Neuropathic pain was induced by the chronic constriction injury (CCI) of the sciatic nerve in rats. Levels of the anadamide (AEA) and 2-arachidonoylglycerol (2-AG) and expression of the protein network involved in the endocannabinoid biosynthesis in glutamate and GABA terminals have been investigated within PAG in healthy and CCI rats. The neural activity of "pronociceptive" ON and antinociceptive "OFF" cells of the RVM has been monitored by in vivo single unit extracellular recording. Thermoceptive responses have been also measured through a tail flick unit.

Intra-PAG microinjection of WIN 55,212-2 (WIN**),** a cannabinoid receptor agonist, increased, in a dose-dependent manner, tail flick latencies, delayed the tail flick related onset of the ON-cell burst and decreased the OFF-cell pause duration. Furthermore, WIN decreased RVM ON-cell and increased OFF-cell ongoing activities. These effects were prevented by SR141716A, a CB1 cannabinoid receptor antagonist, or by MPEP, a selective mGlu5 receptor antagonist. CPCCOEt and LY367385, selective mGlu1 receptor anatgonists, were ineffective in preventing WIN-induced effects. Increased levels of AEA and 2-AG within the PAG, changes in the protein network involved in the endocannabinoid biosynthesis on glutamate and GABA terminals, and 3) changes on the ongoing and tail-flick-related ON- and OFF-cell activities have been found in nerupathic rats by the chronic constriction of the sciatic nerve. We have also found that the lowest dose of WIN capable to modify tail flick latencies and change RVM ON and OFF activities in neuropathic rats was twofold that one used in healthy animals. MPEP was however effective in preventing WIN-induced effects also in CCI rats.

This study shows that CCI generates tolerance to cannabinoids at PAG level either on behavioural pain response and RVM neural activities. Moreover, this study shows that stimulation of PAG mGlu5, but not mGlu1 receptor, is required for the cannabinoid-induced antinociceptive effect within the PAG-RVM circuitry.

NEURONAL FAAH INHIBITION VIA PF-3845 REVERSES LPS INDUCED TACTILE ALLODYNIA

Lamont Booker[1], Steven G. Kinsey[1], Rehab Abdullah[1], Jonathan Z. Long[2], Dale Boger[2], Benjamin F. Cravatt[2] and Aron H. Lichtman[1]

[1]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA, [2]The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA

Inflammatory pain results from the immune response to insults, such as physical injury or bacterial infection. The result is a potentially debilitating condition that adversely affects the quality of life. A common hallmark of inflammatory pain is the occurrence of allodynia (i.e., a painful response to a normally non-noxious stimulus). Currently, there are few therapeutic options used to treat allodynia, however due to their limited efficacy and negative side effects, alternative treatments are needed. The endocannabinoid system (ECS) has been implicated in a variety of acute pain models. The ECS is comprised of signaling lipids including anandamide (AEA) and 2-arachidonoylglycerol (2-AG), two receptors (CB_1 and CB_2) that bind these endocannabinoids, and the enzymes responsible for the biosynthesis and degradation of the endocannabinoids. AEA signaling is short-lived, due to its rapid degradation by its chief catabolic enzyme fatty acid amide hydrolase (FAAH). However, genetic deletion or pharmacological inhibition of FAAH increases AEA levels globally and reduces nociception in a wide range of animal models. In the present study, we used complementary genetic and pharmacological approaches to determine whether FAAH is a viable target for reversing mitogen-induced allodynia. Intraplantar (i.pl) administration of lipopolysaccharide (LPS) caused tactile allodynia, as tested with von Frey filaments. Pharmacological blockade of FAAH via inhibitors PF-3845, URB597, or OL-135 reversed LPS-induced allodynia. FAAH (-/-) mice treated with LPS showed an anti-allodynic phenotype, as compared with wild type or FAAH-NS mice, which express FAAH only in nervous tissue [$F(2,21)=8.99$, $p<0.01$], suggesting that the FAAH (-/-) anti-allodynic phenotype is mediated by FAAs in the nervous system. Indeed, PF-3845 treatment in FAAH-NS mice produced anti-allodynic effects similar to the phenotypic allodynia seen in FAAH (-/-) mice, consistent with the idea that FAAH inhibition reduces allodynia by elevating fatty acid amides in the central or peripheral nervous tissue. Furthermore, we found that the anti-allodynic effects of FAAH inhibition or genetic deletion were mediated through a mechanism of action that requires both CB_1 and CB_2 receptors. Lastly, LC/MS/MS analysis revealed that PF-3845 administration increased AEA but not 2-AG in the brain, and spinal cord. Taken together, these data suggest that stimulating the endocannabinoid system, by inhibiting neuronal FAAH, represents a potential strategy for therapeutic treatments of inflammatory pain.

IDENTIFICATION OF *N*-ACYLETHANOLAMINES AND CANNABINOID 1 RECEPTORS IN HUMAN TRAPEZIUS MYALGIA

Nazdar Ghafouri[1, 2] Bijar Ghafouri[1], Maria Turkina[1], Britt Larsson[1], Bo AG Jönsson[3], Christopher J Fowler[2] and Björn Gerdle[1]

[1]Department of Clinical and Experimental Medicine, Linköping University, SE-581 85 Linköping, Sweden; [2]Department of Pharmacology and Clinical Neuroscience, Umeå University, SE-901 87 Umeå, Sweden; [3]Department of Laboratory Medicine, Lund University, SE-221 85 Lund, Sweden

Introduction: N-acylethanolamines (NAEs) and endocannabinoids are a group of endogenous lipid mediators that play important roles in the reegulation of inflammation and pain. However, most studies into their function have been undertaken using animal models, and little is known as to the roles played by these lipids in the human skeletal muscle. The aims of this study were: 1) to develop sensitive, high-throughput strategies for analysis of NAEs and endocannabinoids in dialysate samples from healthy and myalgic subjects; 2) to investigate the expressed level of CB_1 receptors in human muscle biopsies from patients with trapezius myalgia.

Methods: A triple quadrupole tandem mass spectrometer was used to detect N-acylethanolamines and 2-arachidonoyl glycerol (2-AG) in pooled dialysate samples from myalgic and healthy trapezius muscle respectively. CB_1 receptor expression was investigated by immunoblotting.

Results: Seven NAE's ; Anandamide (AEA), oleoyl-ethanolamine (OEA), palmitoyl ethanolamide (PEA), docasatertraenoylethanolamide (DEA), stearoyl ethanolamide (SEA), linoleoyl ethanolamide (LEA), linolenoyl ethanolamide (LNEA) and 2-AG were detected in a single chromatographic run. The levels of PEA and SEA were higher in myalgic subjects compared to healthy controls: SEA 5.28 nM (myalgic) vs. 0.21 nM (healthy); PEA 3.6 nM (myalgic) vs. 0.53 nM (healthy). Further development of the method with an ion-trap mass spectrometer allowed the detection of 2-AG, AEA, SEA and PEA in dialysates from single individuals. In the immunoblotting experiments, a clear immunoreactive band corresponding to CB_1 receptor was detected in muscle biopsies. The identification was verified by MALDI-TOF mass spectrometry and sequenced using MS/MS.

Conclusion: We have developed, adjusted and validated the method for the targeted LC-MS/MS detection of NAEs and 2-AG from human muscle microdialysates. This provides the methodology to allow us to determine whether myalgia is associated with altered levels of NAEs.

ACTIVATION OF SPINAL TRPV1 OR CB_1 RECEPTORS IN NEUROPATHIC RATS DEPENDS ON ANANDAMIDE CONCENTRATION

Katarzyna Starowicz[1], Wioletta Makuch[1], Fabiana Piscitelli[2], Stefania Petrosino[2], Vincenzo Di Marzo[2] and Barbara Przewłocka[1]

[1] Dept. of Pain Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna str, 31-343 Krakow, Poland; [2] Endocannabinoid Research Group, Istituto di Chimica Biomolecolare CNR, via Campi Flegrei 34, 80078 Pozzuoli (Naples), Italy

Evidence on the relations between cannnabinoid CB_1 receptors and transient receptor potential vanilloid type 1 (TRPV1) channels is accumulating. They are frequently co-expressed in neuronal cells and share at least one endogenous agonist: anandamide (AEA). However, no data are published on the potential role of spinal TRPV1 activation by AEA in neuropathic pain, and our present study was carried out to fill this gap.

Rats chronically implanted with intrathecal (i.t.) catheters underwent sciatic nerve ligation (CCI model), and seven days after CCI in a time-course study mechanical allodynia (von Frey test) and thermal hyperalgesia (Hargreaves test) were measured. We tested the effect of AEA (50 μg i.t.) and elevated level of endogenous AEA (following inhibition of FAAH) in CCI rats, and the involvement of TRPV1 or cannabinoid CB_1 receptors in the observed effects by blocking these receptors with I-RTX (2 μg i.t.) or AM251 (10 μg i.t.), respectively. Additionally, since up-regulated spinal AEA levels may inhibit pain (as postulated by Petrosino et al., Neuropharmacology 2007) we tested if increased endogenous AEA level may change the response curve of low doses of exogenous AEA. Finally, we determined the levels of AEA in the spinal cord of CCI rats following all treatments.

AEA (50 μg i.t.) displayed an antiallodynic and antihyperlagesic effect. The analgesic action of AEA was abolished by pretreatment with I-RTX but not AM251, suggesting the involvement of spinal TRPV1 receptors in this effect. Depending on the administered dose the selective inhibitor of AEA enzymatic hydrolysis, URB597 (10-100 μg i.t.), reduced thermal and tactile nociception via CB_1 or CB_1/TRPV1 receptors, as demonstrated by the attenuation of its effects by pretreatment with the respective antagonists, AM251 or I-RTX. When *per se* ineffective doses of URB597 (5 μg i.t.) and AEA (5 μg i.t.) were coadministered, a clear antinociceptive effect was observed that was abolished by pharmacological ablation of CB_1 but not TRPV1 receptors. After nerve injury AEA levels were increased both in the ipsi- and contralateral side. The levels of endogenous AEA were only slightly increased by URB597 at the dose of 10 μg i.t., and strongly elevated by URB597 at the dose of 100 μg i.t. Injection of AEA (50 μg i.t.) into the lumbar spinal cord led to its dramatic elevation in this tissue, whereas when a lower dose was used (5 μg i.t.) its endogenous levels were elevated only in the presence of URB597 (5 μg i.t.).

Consistent with the earlier observation that the concentrations required for AEA to stimulate TRPV1 are higher than those necessary for CB_1 stimulation, we suggest that i.t. AEA reduces neuropathic pain by acting as an endocannabinoid or endovanilloid, depending on its concentration in the lumbar spinal cord. Up-regulation of TRPV1, such as that occurring in neuropathic pain, might regulate the functional balance between CB_1 and TRPV1, although in either case AEA produces anti-hyperalgesic effects.

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MODULATION OF NOCICEPTION AND FEAR-CONDITIONED ANALGESIA BY THE ENDOCANNABINOID SYSTEM IN THE RAT DORSOLATERAL PERIAQUEDUCTAL GREY

Weredeselam M.Olango[1], Michelle Roche[2] and David P. Finn[1]

[1]Pharmacology and Therapeutics, [2]Physiology, NCBES Neuroscience Cluster and Centre for Pain Research, National University of Ireland, Galway, Ireland.

Fear-conditioned analgesia (FCA), the profound suppression of pain during exposure to conditioned aversive stimuli, is an important survival response mediated by the endocannabinoid system (Butler et al., 2008 *Pain* 140:491-500; Finn et al., 2004 *Eur J Neurosci* 20:848-852). Endocannabinoids in the midbrain periaqueductal grey (PAG) are involved in modulating nociception and unconditioned stress-induced analgesia (Hohmann et al., 2005 *Nature* 435:1108-1112), however, their role in conditioned fear-induced analgesia has not been examined. The present study examined the effect of intra-dorsolateral (dl)PAG administration of the fatty acid amide hydrolase (FAAH) inhibitor, URB597, or the CB_1 receptor antagonist/inverse agonist, rimonabant, on formalin-evoked nociceptive behaviour, conditioned fear and FCA in rats, and associated alterations in the expression of the signal transduction molecule phosphorylated ERK1/2 (pERK1/2) in the dlPAG .

Male Lister-hooded rats (230-300g, n=6-9) were implanted with guide cannulae above the right dlPAG under isoflurane anaesthesia. The fear-conditioning paradigm used was footshock paired with context (10x1s footshocks (FC), 0.4mA, 1min intervals; non-footshocked (NFC) controls also included). The formalin test was used to assess nociceptive behaviour during re-exposure to the conditioned context 24hrs post-footshock. Rats received intra-dlPAG administration of URB597 (0.1 mM), rimonabant (Rim) (2mM) or vehicle (Veh) (100% DMSO) 15 minutes prior to re-exposure to the fear-conditioning arena. Pain- and fear- (freezing and 22kHz ultrasonic vocalisation) related behaviours were assessed over a 15 min period, 30 minutes following intra-plantar formalin. Levels of pERK1/2 were measured using Western immunoblotting. All data were analysed by ANOVA followed by Fisher's LSD post hoc test where appropriate and $p<0.05$ was deemed statistically significant.

Re-exposure of rats to the arena previously associated with footshock resulted in a significant reduction of formalin-evoked nociceptive behaviour (Composite pain score (CPS): NFC-Veh 0.68 ± 0.15 *vs* FC-Veh 0.19 ± 0.11, $p<0.05$). Intra-dlPAG administration of URB597 significantly reduced formalin-evoked nociceptive behaviour in NFC rats (CPS: NFC-Veh 0.68 ± 0.15 *vs* NFC-URB 0.20 ± 0.06, $p<0.01$) and tended to enhance FCA. Intra-dlPAG administration of Rim did not alter formalin-evoked nociceptive behaviour in NFC rats but significantly attenuated FCA (FC-Veh 0.19 ± 0.11 *vs* FC-Rim 0.70 ± 0.19, $p<0.05$). Intra-dlPAG administration of URB597 did not alter fear-related behaviour, however, rimonabant significantly attenuated contextually-induced freezing (FC-Veh 499.11 ± 62.94s *vs* FC-Rim 184.88 ± 56.58s, $p<0.01$) and ultrasonic vocalisation (FC-Veh 449.78 ± 78.38s *vs* FC-Rim 143.25 ± 57.31s, $p<0.01$). Neither fear-conditioning nor drug treatment significantly altered levels of pERK1/2 in the dlPAG of these formalin-treated rats at the time-point investigated.

In conclusion, these data suggest that second phase formalin-evoked nociceptive behaviour may be modulated by FAAH substrates in the dlPAG. The data also suggest that dlPAG mechanisms mediating FCA are sensitive to the CB_1 receptor antagonist/inverse agonist rimonabant. Further work is needed to identify the intracellular signalling molecules mediating the effects observed.

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THE ABILITY OF NABILONE TO INTERACT WITH CANNABINOID CB_1 AND CB_2 RECEPTORS

Gemma L. Baillie, Ruth A. Ross and Roger G. Pertwee
Institute of Medical Sciences, University of Aberdeen, Scotland. AB25 2ZD. UK

Nabilone (Figure 1), a licensed cannabinoid medicine, was initially discovered by Eli Lilly and is approved for use against nausea induced by anti-cancer medications. It was developed before the discovery of cannabinoid CB_1 and CB_2 receptors and its ability to interact with these receptors has been little investigated.

This study was directed at investigating the ability of nabilone to interact with CB_1 and CB_2 receptors *in vitro*. Activation of CB_1 receptors by nabilone and CP55940 was monitored by measuring their ability to stimulate [^{35}S]GTPγS binding to mouse whole brain membranes (n = 4). Displacement binding assays were performed with [^{3}H]CP55940 using membranes obtained from mouse whole brain (CB_1) or from Chinese hamster ovary (CHO) cells in which human CB_2 receptors were highly expressed (n = 4).

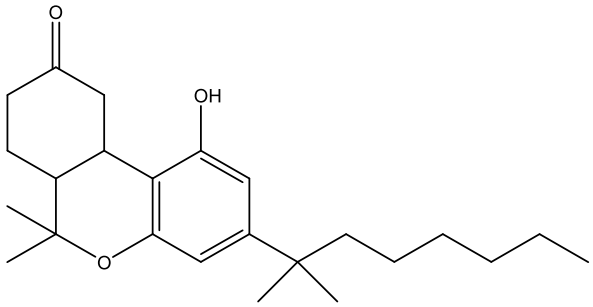

Figure 1: Structure of nabilone

K_i values of nabilone for [^{3}H]CP55940 displacement from brain membranes and from human CB_2 receptors were 0.64nM (95% confidence limits, 0.45 & 0.94) and 52nM (95% confidence limits, 37 and 72), respectively. Corresponding values for CP55940 were 0.57nM (95% confidence limits, 0.25 & 1.3) and 26nM (95% confidence limits, 18 and 39), respectively. In the [^{35}S]GTPγS binding assay, nabilone displayed a pEC50 value of 7.6 ± 0.2 (mean ± s.e.) and an E_{max} of 61.7% (95% confidence limits, 54.6 & 68.7). Corresponding values for CP55940 were 7.9 ± 0.1 and 87.8% (95% confidence limits, 80.9 & 94.7), respectively.

In conclusion, this investigation has provided evidence that nabilone is a CB_1 receptor agonist that possesses similar potency but significantly lower efficacy than CP55940. Our data also suggest that the affinity of nabilone for mouse CB_1 receptors is significantly greater than its affinity for human CB_2 receptors. However, it will be important to determine the affinity of nabilone for human CB_2 receptors in membranes in which these receptors are expressed at "physiological" levels, especially since it has been reported previously that nabilone displays more or less the same affinity for human CB_1 and CB_2 receptors (Gareau *et al*., 1996). Further experiments are also required to establish whether or not nabilone can activate human CB_2 receptors.

Gareau, Y. *et al* (1996) Bioorg Med Chem Lett 6(2), 189-194

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CANNABIDIOL INJECTED INTO THE BED NUCLEUS OF THE STRIA TERMINALIS INDUCES ANXIOLYTIC-LIKE EFFECTS IN THE ELEVATED PLUS MAZE VIA 5-HT1A RECEPTOR-DEPENDENT MECHANISMS

Felipe V. Gomes[1]; Leonardo Resstel[1] and Francisco S. Guimarães[1]

[1]Department of Pharmacology, FMRP-USP, Ribeirão Preto, SP, 14049-900, Brazil

Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* which induces anxiolytic- and antipsychotic-like effects in rodents and humans after systemic administration. Despite the brain structures of CBD acts remain poorly understood, previous results from our group suggest that the bed nucleus of the stria terminalis (BNST) may be involved in the anxiolytic effects of the CBD. The mechanisms of CBD effects are still poorly understood but may involve activation of 5-HT1A receptors. In the present study, we have investigated the anxiolytic-like effects of CBD into BNST of rats submitted to an elevated plus maze (EPM) and if these effects are mediated by 5-HT1A receptors.

Methods: Male Wistar rats (240-270g, n= 5-8) with cannulae implanted bilaterally into the BNST received injections of CBD (15, 30 or 60 nmol) or vehicle (V, 0.2μL) and, 10 minutes later, were submitted to the EPM. Animals receiving the active doses of CBD outside the BNST were joined in an OUT group. The second experiment was similar to the first one except that animals received microinjections of the 5-HT1A receptor antagonist WAY100635 (WAY; 0.37 nmol) 5 min before CBD (60 nmol) treatment. The number of entries and time spent in open and enclosed arms was recorded for 5 min. The results were analyzed by one-way ANOVA followed by the Bonferroni post-hoc test and the criterion for statistical significance was considered to be $P<0.05$.

Results: CBD (60 nmol) significantly increased the % of entries [$F(4,30)= 4,634$; $p<0,01$] and time [$F(4,30)= 3,405$; $p<0,05$] spent in the open arms. WAY by itself did not change the % of entries and time spent in the open arms, but blocked the effects of CBD. Moreover, no behavioral changes were observed when CBD was microinjected into structures surrounding. **Conclusion:** These results suggest that BNST could be involved in the anxiolytic-like effects of CBD observed after systemic administration by facilitating 5-HT1A receptor-mediated neurotransmission.

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NON-Δ^9THC PHYTOCANNABINOID-INDUCED MODULATION OF RAT FEEDING PATTERNS

Jonathan A. Farrimond, Benjamin J. Whalley and Claire M. Williams

Schools of Pharmacy and Psychology, University of Reading, Whiteknights, Reading, Berkshire, RG6 6UR, United Kingdom.

Introduction: The appetite-stimulating effects of cannabis have been well-documented and attributed to partial agonism of CB1 receptors by Δ^9-tetrahydrocannabinol (Δ^9THC). Recently, we reported that the pattern of cannabis-induced hyperphagia is dependent upon cannabinoid composition (Farrimond *et al*, Psychopharmacology, in press). We now show that non-Δ^9THC phytocannabinoids produce hyperphagia.

Methods: 10 adult male Lister Hooded rats were dosed with a Δ^9THC-rich standardised botanical drug substance (Δ^9THC-BDS; GW Pharmaceuticals; 4.0mg/kg) and two combinations of pure cannabinoids exactly matching the proportions present in Δ^9THC-BDS (0.5 – 4.0mg/kg); mixture 1 (Δ^9THC: 67%; CBD: 0.3%; CBG: 1.7%; CBC: 1.6%; THCV: 0.9%; THCA: 0.3%; CBN: 1.5%; and sesame oil vehicle: 26.7%) and mixture 2 (0.5 – 4.0mg/kg; as mixture 1 except Δ^9THC is replaced with sesame oil vehicle). Rats were pre-satiated using a highly palatable wet mash diet before randomised drug administration commenced. Food intake data were then recorded for 2 hours. Data were analysed using 2-way ANOVA, followed by Tukey's HSD post-hoc tests. Δ^9THC-BDS results were compared to control using paired t-tests.

Results: At doses ≥2.0mg/kg, mixture 1 significantly increased consumption during the first hour; which can be attributed to a significant increase in the duration of the first eating bout. No other changes in meal pattern were seen. Similarly, mixture 2 (Δ^9THC-free) increased intake during the first hour at doses ≥2.0mg/kg. However, few changes to the meal pattern were seen at these doses, instead, a 0.5mg/kg dose produced an increase in duration of the first eating bout but this was not concomitant with an increase in overall chow consumption. A 4.0mg/kg dose of Δ^9THC-BDS induced a significant increase in intake during the first hour when compared to vehicle-treated animals in a fashion similar to our previous study (Farrimond *et al*, Psychopharmacology, in press).

Conclusion: Mixture 1 produced results similar to those previously described in literature. However, mixture 2 which did not contain any Δ^9THC, also produced significant hyperphagic actions in pre-fed rats suggesting that non-Δ^9THC phytocannabinoids can also stimulate feeding. While further feeding studies using the purified non-Δ^9THC phytocannabinoids in mixtures 1 and 2 are needed to fully elucidate these effects; we suggest non-Δ^9THC phytocannabinoids may be of future clinical use for the stimulation of feeding and appetite.

CANNABIDIOL EXERTS ANTI-CONVULSANT EFFECTS IN ANIMAL MODELS OF TEMPORAL LOBE AND PARTIAL SEIZURES

Nicholas A. Jones, Samantha E. Weston, Andrew J. Hill, Gary J. Stephens, Benjamin J. Whalley and Claire M. Williams

Reading Schools of Pharmacy and Psychology, University of Reading, Whiteknights, Reading, RG6 6UB, UK

Introduction: Recently, we have shown that the phytocannabinoid cannabidiol (CBD) reduced seizure severity and lethality in the well-established *in vivo* model of pentylenetetrazole-induced generalised seizures, suggesting that earlier, small-scale clinical trials for CBD in treated epilepsy warrant renewed investigation (Jones *et al.,* (2010) JPET, doi: 10.1124/jpet.109.159145). Here, effects of pure CBD in two other established seizure models were assessed.

Methods: Seizure behaviour following 1, 10 and 100 mg/kg CBD in the pilocarpine model of temporal lobe seizure and the penicillin model of partial seizure was video recorded and scored offline using seizure severity scales appropriate for each model. All data were obtained from adult (>P21) male Wistar Kyoto rats with statistical significance assessed by one-way ANOVA or binomial tests where $P \leq 0.05$ was considered significant.

Results: In the pilocarpine model, CBD (all doses) significantly reduced the proportion of animals experiencing the most severe seizures. In the penicillin model, CBD significantly increased the proportion of seizure-free animals (all doses), decreased the proportion of animals experiencing the most severe seizures (100 mg/kg), decreased median seizure severity (100 mg/kg) and showed a strong trend to reduce mortality at 100 mg/kg ($P<0.1$).

Conclusion: Whilst the CBD mechanism(s) of action remain(s) unknown, these results extend the range of anti-convulsant properties exerted by CBD. When combined with a reported absence of psychoactive effects, they suggest that CBD is a strong candidate therapeutic treatment for different forms of epilepsy.

EFFECT OF SEVEN PHYTOCANNABINOIDS ON CATECHOLAMINE TRANSPORTERS AND RECEPTORS

Carolyn Tanner, Maria-Grazia Cascio, Lesley Stevenson,
Ruth A. Ross and Roger G. Pertwee
Institute of Medical Sciences, University of Aberdeen, Scotland

To date, two phytocannabinoids, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (Δ9-THC), have been found to modulate the neuronal uptake of the catecholamines, dopamine and noradrenaline (reviewed in Pertwee, 2008). This study has confirmed and expanded these findings by exploring the ability of five other phytocannabinoids to target noradrenaline and dopamine transporters and also dopamine receptors. Such targeting might have therapeutic relevance as, for example, these transporters and receptors are known to be involved in depression and schizophrenia.

Experiments were performed with whole brain membranes and synaptosomes derived from adult male MF1 mice. The synaptosomes were employed for uptake assays with [3H]dopamine and [3H]noradrenaline, and the membranes for [3H]dopamine displacement assays. Seven phytocannabinoids were investigated: Δ9-tetrahydrocannabinol (Δ9-THC), Δ9-THC acid, Δ9-tetrahydrocannabivarin (Δ9-THCV), cannabidiol (CBD), cannabidiolic acid (CBD acid), cannabidivarin (CBDV) and cannabigerol (CBG).

CBD and Δ9-THC each inhibited both [3H]dopamine and [3H]noradrenaline synaptosomal uptake at concentrations of 1μM and 10μM. CBG inhibited the synaptosomal uptake of dopamine at 100nM, 1μM and 10μM, and also slightly at 0.1nM, but the synaptosomal uptake of noradrenaline only at a concentration of 10μM. At this concentration (10μM), each of the other four phytocannabinoids tested inhibited both noradrenaline and dopamine uptake, suggesting that at this concentration, a non-specific phytocannabinoid-induced inhibition of catecholamine uptake occurs. In the [3H]dopamine displacement binding assay, none of the seven phytocannabinoids tested produced any displacement of dopamine at concentrations of up to 10μM.

In conclusion, the results obtained with CBD and Δ9-THC in this investigation are in concurrence with previous reports that both these compounds have the ability to modulate the synaptosomal uptake of noradrenaline and dopamine. CBG inhibited the synaptosomal uptake of dopamine at concentrations that did not inhibit noradrenaline uptake, indicating that it may target dopamine transport in a selective manner. None of the seven phytocannabinoids investigated were able to displace dopamine in the [3H]dopamine binding assay, implying that these phytocannabinoids do not activate or block dopamine receptors directly. However, further experiments are required to establish whether any of these phytocannabinoids are dopamine receptor allosteric modulators and, indeed, whether they target the orthosteric or allosteric sites of any other receptors with significant potency.

Pertwee R.G. (2008). Br J Pharmacol, 153, 199-215

Funded by GW Pharmaceuticals.

REPEATED ADMINISTRATION OF CANNABIDIOL PRODUCES PANICOLYTIC RESPONSE BY ACTIVATING 5HT1A RECEPTORS IN THE DORSAL PERIAQUEDUCTAL GRAY

A.C. Campos[1]; V. P. Soares[2]; H. Zangrossi Jr.[1], A. W. Zuardi[2] and F. S. Guimarães[2]

1 Department of Pharmacology and 2 Neuroscience and Behavioral Sciences, School of Medicine of Ribeirão Preto, Campus USP, 14049-900, Ribeirão Preto, SP, Brazil
email: allinecampos@usp.br

Cannabidiol (CBD) is a nonpsychotomimetic constituent of Cannabis sativa plant that produces anxiolytic-like effects after acute systemic administration in the elevated plus maze and Vogel punished tests, probably by interacting with 5-HT1A receptors. The elevated T-maze (ETM) is another model of anxiety that evokes two types of defensive behaviors: inhibitory avoidance, which has been related to generalized anxiety disorder (GAD), and escape response, related to panic disorder. In the present study we investigate the effects of repeated administration of CBD in rats submitted to the ETM and if these effects could be mediated by activating 5HT1A receptors located into dorsal periaqueductal gray (dPAG). Experiment 1: Male Wistar rats received repeated (21 days) i.p. daily injections of CBD (5mg/Kg), vehicle (V) or fluoxetine (FLX, 10mg/Kg) and were tested in the ETM and open field tests 3h after the last injection. Experiment 2: Male Wistar rats received a similar repeated treatment with CBD or V. However, seven days before the ETM test they are submitted to a stereotaxic surgery to implant a unilateral cannula into the dPAG. Three hours after the last injection of CBD or V the animals received a single intra-dPAG injection of the 5HT1A receptor antagonist WAY100635 (WAY, 0.37nmol/0.2 uL) or saline (SAL). Ten minutes later they were submitted to the tests. Similar to fluoxetine, CBD increased escape latency (V: 6.9+/-1.4; CBD 13.3+/-1.6; 15.2+/-2.8; n=9-10/group). The effect of CBD on escape latency was prevented by intra-dPAG injection of WAY100635 (V-SAL: 3.6+/-0.6; CBD-SAL: 13.8+/-1.9; V-WAY: 7.6+/-1.6; CBD-WAY: 7.2+/- 0.9). Results are expressed in mean +/- SEM. No significant effects were found in the open field test. The results suggest that CBD could exert a panicolytic effect probably by interacting with 5HT1A receptors in the dPAG.
Financial support: FAPESP, CNPq, CAPES.

EFFECT OF A CANNABIS EXTRACT ON RAT AND HUMAN BLADDER CONTRACTILITY IN VITRO

Gabriella Aviello, Raffaele Capasso, Beniamino Vaccaro, Vincenzo Altieri[§], Vittorino Montanaro[§] and Angelo A. Izzo

Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy; [§]Department of Urology, University of Naples Federico II, Naples Italy

Background/Aim Sativex®, which contains approximately 1:1 ratio Δ^9-tetrahydrocannabidiol- and cannabidiol (CBD)-enriched extracts is marketed for the symptomatic relief of neuropathic pain in adults with multiple sclerosis (MS). Sativex® has been shown to reduce urinary urgency, incontinence episodes, frequency and nocturia in MS patients[1]. The aim of the present study is to evaluate the effect of one of the two Sativex® components, namely CBD-enriched Cannabis extract (standardized to contain 65.6% CBD, here named CBD BDS) on rat and human bladder contractility in vitro.

Methods Strips were cut from the human and rat bladder body and placed in organ baths containing Krebs solution. Contractions were induced by electrical field stimulation (EFS) and by acetylcholine.

Results CBD BDS (0.1-100 μg/ml) significantly reduced acetylcholine-, but not EFS-induced contractions in the isolated rat bladder. The inhibitory effect of CBD BDS was not significantly modified by cannabinoid or opioid receptor antagonists [(rimonabant (1 μM), SR144528 (0.1 μM) and naloxone (1 μM)], by modulators of Ca^{2+} channels [cyclopiazonic acid (10 μM) and nifedipine (0.1 μM)], but it was increased by ruthenium red (10 μM) and by capsazepine (10 μM), two TRPV1 blockers. In humans, CBD BDS significantly reduced acetylcholine-induced contractions.

Conclusions Our data suggest that CBD BDS reduces acetylcholine-induced contractions and this effect is modulated by TRPV1. Such results 1) might provide a pharmacological basis able to explain the efficacy of Sativex® in reducing urinary symptoms associated to MS and 2) open the possibility to evaluate CBD BDS for its possible use in treating urinary incontinence.

[1] Brady CM, DasGupta R, Dalton C, Wiseman OJ, Berkley KJ, Fowler CJ. An open-label pilot study of cannabis-based extracts for bladder dysfunction in advanced multiple sclerosis. *Mult Scler.* 2004;10:425-33.

THC-EFFECTS MEASURED IN HUMAN BY RESTING STATE-FMRI

Linda E. Klumpers [1], Roelof P. Soeter [2,3,4], Naj Khalili-Mahani [2,3,4], Serge A.R.B. Rombouts [2,3,4], Mark A. van Buchem [2,3] and Joop M.A. van Gerven [1,5]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Leiden University Medical Center, Department of Radiology, Leiden, The Netherlands
3 Leiden Institute for Brain and Cognition (LIBC), Leiden, The Netherlands
4 Institute of Psychology, Leiden University, Leiden, The Netherlands
5 Leiden University Medical Center, Department of Neurology, Leiden, The Netherlands

Resting state-functional magnetic resonance imaging (RS-fMRI) is a neuroimaging technique that allows repeated assessments of functional connectivity in *resting state*. While tasks show a limited range of indirectly measured effects, resting state more objectively shows direct CNS effects. Therefore, RS-fMRI could be an *objective* measure for compounds affecting the CNS. Several studies on the effects of cannabinoid receptor type 1 (CB_1)-receptor agonist Δ^9-tetrahydrocannabinol (THC) on task dependent fMRI have been performed. However, no studies on the effects of cannabinoids on resting state networks using RS-fMRI have been published. Therefore, we investigated the effects of THC on functional brain connectivity using RS-fMRI.

Nine male volunteers inhaled 2, 6 and 6 mg THC or placebo with 90-minute intervals in a randomised, double blind, cross-over trial. Eight RS-fMRI scans of 8 minutes were obtained per occasion. Subjects rated subjective 'feeling high' on a visual analogue scale after each scan, as a pharmacodynamic effect measure. Drug-induced effects on functional connectivity were examined using double regression (Beckmann OHBM 2009) with FSL software (FMRIB Analysis Group, Oxford)). Eight maps of voxelwise connectivity throughout the entire brain were provided per RS-fMRI series with eight predefined resting-state networks of interest. These maps were used in a mixed effects model group analysis to determine brain regions with a statistically significant drug-by-time interaction, and regions associated with the subjective effect scores ($p<0.05$, cluster corrected).

THC administration decreased functional connectivity in different brain regions, including cerebellum and some cortical regions. The subjective effect scores correlated with effects on functional connectivity measurements in the brainstem, cerebellum, medial frontal gyrus and parietal lobe. Not all THC-related decreases in connectivity correlated with subjective effect scores.

This study shows that THC induces decreases in functional brain connectivity, mainly in brain regions with high densities of CB_1-receptors. The regions, in which some of the changes in connectivity occur, could be functionally related to THC-induced changes in CNS-test-measurements, found in previous studies (Zuurman et al, 2008). However, not all changes in connectivity correlated with subjective THC effects.

Effect of intrapulmonary tetrahydrocannabinol administration in humans.
Zuurman L, et al., J Psychopharmacol. 2008 Sep;22(7):707-16

THE EFFECTS OF CHRONIC CANNABINOID TREATMENT ON BEER CONSUMPTION IN PUBERTAL AND ADULT RATS

Chris M. Friemel, Rainer Spanagel and Miriam Schneider

Central Institute of Mental Health, Mannheim, J5, 68159 Germany

Introduction: Consumption of psychoactive substances is commonly initiated at puberty with nicotine, cannabis and alcohol being the most commonly used drugs among adolescents. An early age of drug onset that is highly correlated with a more severe progression of addiction disorders indicates a higher vulnerability of teenagers towards long-lasting neurotoxic effects of psychoactive compounds. Given that drugs are often consumed in mixture with other drugs the present study aimed to investigate the acute and lasting effects of the cannabinoid receptor agonist WIN 55,212-2 on beer intake and the motivation for beer in pubertal (PND 40 – 60) and adult (> PND 100) male Wistar rats. A total of 48 animals received for 18 days a daily injection of either vehicle or an escalating dose of WIN (1.0-1.6 mg/kg bodyweight) and beer intake was measured every second day in a limited access paradigm for 30 minutes. Furthermore, long-term changes of this treatment on spontaneous consumption and motivational incentive for beer were monitored in these animals at a later timepoint.

Results: Chronic administration of WIN enhanced beer intake significantly in young and adult rats but the pubertal group displayed a much higher increase in consumption in response to the treatment. The lowest dosage of WIN caused only in pubertal animals a rise in beer drinking. Statistical analysis revealed a main effect for age with elevated beer ingestion during puberty. Further testing in adulthood revealed that only rats that received access to beer during pubertal development showed higher consummatory behavior and increased motivation for beer.

Conclusion: The effects of chronic cannabinoid treatment on beer drinking were more pronounced in pubertal than in adult rats. Additionally, only pubertal beer exposure persistently increased later consumption and motivational incentive of beer in adult rats.

SLEEP DISRUPTION FOLLOWING DAILY CANNABIS USE

Ryan Vandrey*, Una McCann, Michael Smith and Alan Budney

Sleep difficulty is a common complaint of heavy cannabis users when they abruptly stop using cannabis. Controlled laboratory and clinical survey studies suggest that this occurs as part of a pharmacologically specific cannabis withdrawal syndrome, and that sleep difficulty significantly predicts relapse following a period of abstinence. A limitation of this research is that it has been largely limited to self-report. While some objective studies have been conducted, interpretation of the findings are limited in that results were somewhat inconsistent across studies, sample sizes were very small, and most measured sleep following administration of oral THC rather than smoked cannabis.

A within-subject crossover study (N=20) was conducted to characterize the effects of cannabis withdrawal on sleep architecture in daily cannabis users. Participants completed two 5-day residential study visits. Each visit consisted of a 2-day baseline assessment period, during which participants were allowed to smoke cannabis ad-libitum, followed by a 3-day cannabis abstinence period. Sleep EEG recordings were collected each night. Participants were also administered an oral capsule each night at bedtime. Placebo was administered during both baseline periods, and during one of the abstinence periods. Extended-release zolpidem was administered during the other abstinence period. Administration of placebo and zolpidem occurred in a counterbalanced order across participants.

Repeated measures analysis indicated that, compared with when participants were allowed to use cannabis, abrupt abstinence in the absence of active medication resulted in a reduction in total sleep time, sleep efficiency, REM latency, and Stage 1sleep, and an increase in sleep latency and REM sleep. Administration of zolpidem during abstinence attenuated the reduction in sleep efficiency and alterations in REM sleep. A second study (N=2) has been initiated using a similar protocol, but in which the duration of abstinence was increased. Data from the first 2 participants have replicated the effects from the initial study, and suggests that sleep disturbance persists for at least 16 days and may be more severe after the first 3 days of abstinence when self-reported withdrawal severity typically peaks.

These data provide objective evidence, in controlled laboratory studies, that sleep function is disrupted in heavy cannabis users when they abruptly quit. The magnitude of sleep disruption observed was clinically significant. These studies also indicate that some aspects of the sleep disruption associated with cannabis withdrawal can be attenuated by approved hypnotic medications, suggesting that such medications might be useful in the treatment of cannabis use disorders. The exact time course and severity of these effects remains to be determined.

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PRECIPITATED VERSUS NATURAL WITHDRAWAL IN OUTPATIENTS WITH CANNABIS DEPENDENCE: IMPLICATIONS FOR TREATMENT

Barbara J. Mason, Rebecca Crean and Vivian Goodell

The Scripps Research Institute
La Jolla, CA, USA 92037

Introduction: Marijuana (mj) withdrawal is not included in the DSM-IV because its "clinical significance is uncertain." However rats chronically treated with cannabinoids, but not drug naïve rats, showed marked withdrawal when administered the CB1 antagonist rimonabant. We aim to characterize precipitated (rimonabant) versus naturally-occurring (placebo) mj withdrawal across emotional, behavioral, and biochemical measures in paid volunteers with mj dependence and in relation to untreated non mj-using controls, to provide clear evidence of a mj withdrawal syndrome. ***Methods:*** This is a midpoint analysis of a 28-day longitudinal study with two components: a single dose, double-blind, placebo-controlled study of precipitated mj withdrawal in the lab using rimonabant 90mg po, followed by outpatient assessments of key symptoms of mj withdrawal with financial compensation for monitored abstinence over the subsequent 28 day period. ***Results:*** Subjects are 21 mj dependent volunteers (age 23.4 years, 81% male) and 10 non mj-using controls (age 24.3 years, 70% male). Mj subjects averaged 5.4 years of daily mj use at 1.3 grams of mj per day and had a baseline THCCOOH/creatinine concentration of 430 ng/mL. Rimonabant-treated subjects showed significant elevations on the Marijuana Withdrawal Checklist and Spielberger State-Trait Anxiety Inventory relative to placebo on the challenge day, but then averaged lower scores on these measures, as well as on the Beck Depression Inventory, over the subsequent period of monitored abstinence. However untreated, non mj-using controls scored lower than mj groups on all scales at all time points. The THCCOOH/creatinine concentrations confirmed mj abstinence over the 28-day study. ***Conclusion:*** Rimonabant 90mg precipitated a significant acute increase in mj withdrawal symptoms which then significantly decreased over the 28-day study, relative to placebo. Both mj groups showed elevated mood, anxiety, and mj withdrawal symptoms relative to non mj-using controls. These data suggest mj withdrawal has motivational components symptoms akin to other drugs of abuse which may be significantly altered by a single dose of rimonabant.

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THE DISCRIMINATIVE STIMULUS EFFECTS OF THE CANNABINOID AGONIST NABILONE ALONE AND IN COMBINATION WITH Δ^9-THC IN HUMANS

Joshua A. Lile, Thomas H. Kelly and Lon R. Hays

University of Kentucky College of Medicine
Lexington, KY 40536-0086, USA

The central effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary active constituent of cannabis, are attributed to cannabinoid CB_1 receptor activity, although clinical evidence is limited. Studies that have tested novel, centrally acting cannabinoid agonists in humans have typically not included Δ^9-THC as a comparator. Drug discrimination has proven useful for examining the clinical neuropharmacology of drugs, as data from this procedure are concordant with the actions of a drug at the receptor level; therefore, it was chosen as the primary outcome for these studies. The aim of these studies was to determine the effects of the cannabinoid agonist nabilone, alone and in combination with Δ^9-THC in humans trained to discriminate Δ^9-THC. In the first study, six subjects (N=5 male, 1 female) who reported moderate cannabis use learned to identify when they received 25 mg oral Δ^9-THC or placebo and then received a range of doses of the cannabinoid agonists nabilone (1, 2, 3 and 5 mg) and Δ^9-THC (5, 10, 15 and 25 mg). The dopamine reuptake inhibitor methylphenidate (5, 10, 20 and 30 mg) was included as a negative control. Subjects completed the Multiple-Choice Procedure to assess drug reinforcement, and self-report, task performance and physiological measures were collected. Δ^9-THC functioned as a discriminative-stimulus and increased subject ratings associated with cannabis such as Good Drug Effects, High and Stoned. Nabilone shared discriminative-stimulus effects with the training dose of Δ^9-THC, produced subject-rated drug effects that were comparable to those of Δ^9-THC, and increased heart rate. Methylphenidate did not engender Δ^9-THC-like discriminative-stimulus effects. In the second study, six subjects (N=4 male, 2 female) who reported moderate cannabis use learned to discriminate 30 mg oral Δ^9-THC from placebo and then received nabilone (0, 1 and 3 mg) and Δ^9-THC (0, 5, 15 and 30 mg) separately and in combination. Nabilone and Δ^9-THC produced overlapping effects that were comparable to what was observed in the first study, and these effects were significantly enhanced when the two cannabinoid agonists were administered together. These data demonstrate that the interoceptive effects of nabilone are similar to Δ^9-THC in cannabis users. The overlap in their behavioral effects, and enhancement of their effects when combined, is likely due to their shared mechanism as CB_1 receptor agonists. Given the relative success of agonist replacement therapy to manage opioid, tobacco and stimulant dependence, these results also support the evaluation of nabilone as a potential medication for cannabis-use disorders. Supported by USA National Institutes of Health grants K01 DA18772 and P20 RR015592.

REACTIVITY TO *IN VIVO* MARIJUANA CUES AMONG TREATMENT-SEEKING CANNABIS DEPENDENT ADOLESCENTS

Kevin M. Gray, Steven D. LaRowe, Noreen L. Watson and Matthew J. Carpenter

Medical University of South Carolina (MUSC), Charleston, South Carolina, USA

Introduction: Cannabis dependence is a common but poorly understood condition in adolescents. Craving has been posited as a potential contributing factor to continued use and relapse, but minimal prior investigation has focused on the measurement of craving and reactivity to marijuana cues. **Methods:** Thirty treatment-seeking cannabis dependent adolescents (ages 13-20) completed a cue reactivity session, consisting of exposure to and manipulation of *in vivo* marijuana cues ("joint" and lighter) and matching neutral cues (pencil and eraser), in counterbalanced order. Subjective craving was measured using a single-item ("I have a desire to smoke marijuana") visual analog scale rating and the Marijuana Craving Questionnaire (MCQ). Physiological reactivity was assessed using skin conductance and heart rate measurement. **Results:** Participants reported increased visual analog scale craving ratings in response to *in vivo* marijuana cues, as compared to neutral cues ($p < 0.05$). Skin conductance reactivity was significantly greater in response to marijuana cues, as compared to neutral cues ($p < 0.01$). MCQ ratings and heart rate reactivity did not significantly differ between marijuana and neutral cues. **Conclusion:** *In vivo* marijuana cues appear to elicit significant subjective and physiological reactivity, at least by some measures, among cannabis dependent adolescents. Further work is needed with a larger sample and with a wider variety of cues.

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CHRONIC DELTA9-TETRAHYDROCANNABINOL EXPOSURE RESULTS IN A SUBTLE MOTOR COORDINATION DEFICIT THROUGH CEREBELLAR MICROGLIA ACTIVATION

Laura Cutando[1], Emma Puighermanal[1], Arnau Busquets-Garcia[1], Rafael Maldonado[1] and Andrés Ozaita[1].

[1]Laboratori de Neurofarmacologia. Departament de Ciències Experimentals i de la Salut (CEXS). Universitat Pompeu Fabra (UPF). 08003 Barcelona. Spain

Introduction:

Heavy cannabinoid exposure has been associated to cerebellar dysfunction in humans. On the other hand, it has been previously demonstrated a relation between proinflammatory cytokines and the induction of the cerebellar ataxia.

Results:

We observe that chronic administration of delta9-tetrahydrocannabinol (THC) produces morphological changes specifically in the microglia of the cerebellar cortex that are correlated with the increased expression of the microglial activation marker CD11b, the phosphorylation of Akt/PKB protein, a kinase involved in the activation of resting microglia, and the activation of the NF-κB pathway in these activated cells. Cerebellar cortex microglia remained in the activated state five days after the spontaneous withdrawal of THC or after the withdrawal precipitated by the cannabinoid antagonist rimonabant. The fine motor coordination assessment measured as the coordination index by the coat-hanger test showed a subtle deficit in THC withdrawn mice. Interestingly, inhibition of microglial activation by minocycline during the THC withdrawal period reduced the characteristic markers of microglial activation and reversed the locomotor coordination deficits associated to the cannabinoid withdrawal. Finally, we observe that chronic THC administration altered the expression of cerebellar glial glutamate transporter EAAT1.

Conclusion:

We hypothesize that the change in glutamate function in the cerebellum resulting of chronic THC exposure would produce the activation of microglial cells in the cerebellar cortex, underlying a subtle long term deficit in fine motor coordination.

AN IMPORTANT ROLE FOR CANNABINOID CB1 RECEPTORS IN AMPHETAMINE-INDUCED IMPULSIVITY

Joost Wiskerke, Dustin Schetters, Nicky Stoop,
Anton N.M. Schoffelmeer and Tommy Pattij

Neuroscience Campus Amsterdam, Dept. of Anatomy and Neurosciences, VU university medical center., v/d Boechorststraat 7, 1081 BT Amsterdam, the Netherlands

Introduction: Acute challenges with the psychostimulant amphetamine affect impulsive behavior in both rodents and humans. Little is known about the neuronal mechanisms underlying this phenomenon, other than that it critically depends on enhanced mesolimbic dopamine transmission. Here, we investigated a putative role of the endogenous cannabinoid system, particularly CB1 receptor activity, in these effects of amphetamine using two rat models of impulsivity, a 5-choice serial reaction time task (5-CSRTT) and a delayed reward task (DRT).

Methods: Groups of male Wistar rats were trained in the 5-CSRTT or DRT. In the 5-CSRTT, rats have to respond to visual stimuli that appear pseudo-randomly in one of five apertures in a curved wall, but refrain from responding before stimulus onset, i.e. during the intertrial interval, to prevent a time penalty. Hence, this task provides measures of visuospatial attention and inhibitory response control. In the DRT, rats are given the choice between a small, immediate or a larger, delayed food reward. This task primarily measures impulsive decision-making (impulsive choice) as reflected by tolerance/intolerance to delay of reward. Following stable baseline performance in these two tasks, effects of systemic injections of amphetamine, alone or in combination with different doses of the CB1 receptor inverse agonist SR141716A or the neutral CB1 antagonist O-2050, were studied on impulsivity using a within-subjects design.

Results: Results showed that blockade of CB1 receptors using either SR141716A or O-2050 dose-dependently attenuated amphetamine-induced decrements in inhibitory control and completely prevented an amphetamine-induced reduction in impulsive choice. Data further reconfirmed that under baseline conditions CB1 receptors play a modulatory role in inhibitory control, albeit the effects of CB1 blockade under these conditions can sometimes be masked by a 'floor-effect'. In contrast, CB1 receptors do not seem to play a role in impulsive choice under baseline conditions.

Conclusion: Together, these results indicate an important role for cannabinoid CB1 receptor activation in amphetamine-induced impulsivity. Thus, we show that the cannabinoid system is not only involved in inhibitory control as was recently published (Pattij *et al.* 2007, Psychopharmacology 193), but can under certain conditions also be recruited for mediating impulsive decision-making.

THE RELATIONSHIP BETWEEN CHRONIC ILLNESS AND CANNABIS USE

Amanda Reiman, MSW PhD

Berkeley Patients Group, Berkeley California, 94702

Introduction: Concern exists over the long term effects of prescription drug use for those with chronic illness. Regular prescription drug use over long periods of time can increase the likelihood of drug dependence, contribute to complications in organ functioning, and potentially interact with other medications being taken. Medical cannabis patients include those with both chronic and acute illnesses. This study poses the question; Do medical cannabis patients with a chronic illness report different cannabis use patterns than patients without chronic illness?

Methods: Anonymous surveys were administered to 350 medical cannabis patients at Berkeley Patients Group (BPG), a social-model dispensary in Berkeley, California. Participants were asked to indicate if they had a chronic illness. Additionally, data were collected on frequency of visits to BPG, frequency of cannabis use; amount of cannabis used per week, preferred method of cannabis ingestion, and the use of other treatments in addition to cannabis. Chi-Square tests were run to determine differences in cannabis use patterns between those with and without chronic illness.

Results: Sixty eight percent of the sample reported having a chronic medical condition and those with a chronic condition were significantly more likely to report a physical condition/not chronic pain, compared to chronic pain or a mental health condition ($p < .01$) Having a chronic medical condition was not significantly associated with how often patients reported visiting BPG. However, those with a chronic medical condition (N= 239) reported visiting BPG a couple of days per week most often (34%) and those without a chronic medical condition (N=101) were most likely to visit once a week (35%). Similar results were found concerning chronic condition and frequency of cannabis use. Although the groups were not significantly different, those with a chronic illness most often reported using cannabis 3 times per day (24%), and those without a chronic condition most often reported using cannabis twice a day (33%). There was also no significant difference in preferred method of ingestion between the groups. Joints were the method indicated most often by both groups; however, the chronic condition group reported more use of vaporizers (16% vs. 8%). Finally, those without a chronic condition were significantly more likely to report a decrease in cannabis use in the past six months ($p < .05$), and those with a chronic illness were significantly more likely to report needing treatments in addition to cannabis to control their symptoms ($p < .01$). In all cases, the data are normally distributed.

Conclusion: This study supports that, although medical cannabis patients with and without chronic illness have similar patterns of cannabis use, there are some marked differences. Patients with chronic illness are more likely to have a physical health issue, outside of chronic pain. The absence of chronic illness is associated with a pattern of cannabis use that is more situational in nature, and is likely to decrease over time. Those with chronic illness are more likely to be undergoing multiple treatments, and are more likely to use methods such as vaporizations to ingest cannabis.

AUTOMATED ION TRAP SCREENING METHOD FOR THE DETECTION OF SYNTHETIC CANNABINOIDS IN COMMERCIAL INCENSE PRODUCTS

Paul D. Kennedy, William R. Collin and Thomas Buddenborg

Cayman Chemical Co., Ann Arbor, MI 48108 USA

Introduction

Since 2008 there have been several confirmed reports of synthetic cannabinoids identified in over-the-counter incense products. This had led to legislation in Europe banning several incense products and regulation of the synthetic cannabinoids that have been identified in these products. Although no legislation currently exists in the United States to regulate these cannabinoids or the incense products, the story is beginning to attract media and regulatory attention. In an effort to determine the scope of this new designer drug phenomenon our lab has prepared several synthetic cannabinoids standards and developed an automated method to screen for and positively identify these compounds in commercial herbal incense products.

Method

In the present study, 13 commercially available synthetic cannabinoids were included in the screening method. A standard mixture of these 13 compounds was used to optimize the HPLC separation on an Agilent Technologies 1100 HPLC equipped with an Agilent 1100 MSD/Trap SL. An automated, data-dependent MSn method was optimized to generate a searchable library of retention time, MS spectra, and MS/MS spectra for the 13 analytes. This method was then used to screen 5 commercially available incense blends for the 13 synthetic cannabinoids.

Results

Of the five incense samples analyzed, four tested positive for multiple synthetic cannabinoids. The K2 incense product contained JWH-200, JWH-073, and JWH-018 at very high levels. Tribal Warrior contained a very high level of JWH-018 and much lower levels of CP 47497-C8, JWH-250, and JWH-073. Spike99 contained the largest number of synthetic cannabinoids and included high levels of JWH-250, JWH-073, and JWH-018 along with lower levels of CP 47,497-C8 and JWH-019. The Neder Gold sample did not yield any positive hits upon analysis.

Conclusion

These data confirm the presence of a variety of synthetic cannabinoids in commercially available incense products and demonstrates the effectiveness of this rapid screening approach for this application. Further studies are ongoing to identify and synthesize additional cannabinoids and to extend this methodology to additional incense blends.

FRESH CANNABIS: A NON-PSYCHOACTIVE THERAPEUTIC MODALITY

Kristen N. Peskuski

Cannabis International, Mendocino, CA USA

Over 525 different chemical compounds have been identified in the cannabis plant. These substances act as primarily feedback modulators facilitating the Endogenous Cannabinoid System's regulation of cellular physiology. The plant's primary constituent is THCa, which along with other phytocannabinoids, interact to modulate the immune system.

Patients become dysphoric or euphoric on 10-20 mg of THC, well before they can take in a full dose (200 to 1,000 mg) of the other non-psychoactive cannabinoids. Many middle-aged patients cannot tolerate THC, even if it alleviates their symptoms, due to the dysphoria that interferes with their day-to-day functions. Age or heat decarboxylate THCa to THC, reducing the tolerable dose from 2000 mg to 10 mg, and resulting in the loss of the inflammatory, anti-spasmodic and anti-proliferative activities of the cannabinoids.

US Patent 6,630,507 states that certain cannabinoids can have useful therapeutic effects, which are not mediated by cannabinoid receptors, and are therefore not accompanied by psychoactive side effects. Furthermore, the absence of psychoactivity in some cannabinoids allows for very high doses to be used without encountering unpleasant side effects or potentially dangerous complications.

In October 2009, we confirmed that the 14,500 μgm / ml of non-psychoactive THCa was potently active in modulating the immune system and was tolerated because the 90 μgm / ml of free THC does not cross the CB1 stimulation threshold. This has supported widespread interest in juicing the whole plant, diluting that juice 10:1 for palatability and then consuming the juice in divided doses up to 5 times / day. We have discovered that dietary leaf therapy is a gradual process that increases over the first two months or regular use. Once the plant is absorbed, cannabinoids clear in 50 minutes supporting a q3-4 hour dosing.

This research examines whether THCa and other phytocannabinoids, such as CBD, exhibit any psychotropic effect in human patients, using self-reporting on eating fresh cannabis leaf and flowers. February 1, 2009 until February 1, 2010, roughly 2731 of patients were consulted on their use of fresh cannabis leaf, which Dr. William Courtney had prescribed the previous year. One patient reported a mildly psychoactive effect from eating raw leaf, though it may be attributed to aging the material.

Five subjects ate the fresh flowers of the plant to test whether they felt a psychoactive effect. Two volunteers were heavy users, two were accustomed to only leaf, and one was naive to cannabis. Raw flowers were eaten for a two-week period; only psychoactive effects were examined. None of the patients felt euphoria or dysphoria from fresh cannabis flower consumption.

CANNABIDIOL REDUCED MARIJUANA WITHDRAW SYMPTOMS

Mateus M. Bergamaschi[1], Marcos H. N. Chagas[2], Regina H. C. Queiroz[1], José A. B. Martinez[3], Glício R. Oliveira[2], Jaime E. C. Hallak[2], José A. S. Crippa[2] and Antonio W. Zuardi[2]

1) Department of Clinical, Bromatological and Toxicological Analysis, School of Pharmaceutical Science of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, 14040-903, Brazil
2) Department of Neuroscience and Behaviour, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, 14048-900, Brazil.
3) Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, 14048-900, Brazil.

Introduction: Recent studies showed that withdrawal of cannabis in humans induces abstinence symptoms and that these symptoms considerably contribute to the repeated use of cannabis. Cannabidiol (CBD) inhibits the reuptake and hydrolysis of anandamide and has a broad spectrum of pharmacological effects, what could be useful in the treatment of cannabis abstinence.

Aim: Evaluate the effect of CBD in a patient dependent of cannabis during the drug withdraws.

Methods: L.O., 18 years old, female, student, started to smoke marijuana 4 years ago. Three month after started to smoke marijuana, she started to use cocaine and quit it in November 2007. She had a previous episode of depression that improved with psychotherapy. Nowadays she had no obsessive-compulsive disorder, no social anxiety disorder and no generalized anxiety disorder. The first attempt to quit smoking was in November 2007 for 9 days, she had a lot of fissure, anger to feel and smell, increased anxiety, nervousness, decreased appetite with weight loss. The second attempt was in May 2008 for 2 days, she could not deal with the withdraw phase and felt anxiety, anger and nervousness explosions. Due to the previous unsuccessful attempt to quit smoking, it was proposed her to be interned at Hospital for up 10 days, receiving 600mg CBD per day. She was submitted to clinical evaluation; Hamilton anxiety scale, Hamilton depression scale, Marijuana withdrawal symptom checklist, Beck anxiety inventory, Beck depression inventory and Clinical administered dissociative states scales were applied.

Results: It has been observed a significant improvement from the second day of the treatment. In the 6^{th} day, it has been observed no symptoms related to marijuana withdraw and symptoms on all scales applied.

Conclusion: CBD could mitigate the marijuana withdraw symptoms. This result further suggests that CBD can be used to treatment for marijuana addiction, although future studies are still necessary.

GENETIC DEACTIVATION OF FATTY ACID AMIDE HYDROLASE PRODUCES ANXIOLYTIC-LIKE AND ANTIDEPRESSANT-LIKE BEHAVIOURS AND MODIFIES SEROTONERGIC TRANSMISSION IN THE DORSAL RAPHE, PREFRONTAL CORTEX AND HIPPOCAMPUS

Francis Rodriguez Bambico[1], Tommaso Cassano[2], Noam Katz[1], Sergio Dominguez Lopez[1], Jean-Philippe Garant[1], Daniele Piomelli[3] and Gabriella Gobbi[1]

[1]Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada;
[2]Department of Pharmacology, University of Foggia, Foggia, Italy;
[3]Department of Pharmacology and Center for Drug Discovery, University of California, Irvine, CA, USA

Pharmacological enhancement of the endocannabinoid system has been argued to be beneficial in the treatment of primary and secondary depression, as well as, of anxiety disorders (Bambico and Gobbi, *Expert Opinion in Therapeutic Targets* 2008). The inhibition of the enzyme fatty acid amide hydrolase (FAAH), which degrades the endocannabinoid anandamide, has been shown to produce CB_1 receptor-mediated analgesic, anxiolytic-like and antidepressant-like effects in rodents (Kathuria et al., *Nat Med* 2002; Gobbi et al., *PNAS* 2005). Genetic deletion of FAAH also increases anandamide-CB_1 receptor signalling. Using a battery of behavioural tests for emotional reactivity, we have characterized the emotional phenotype of mice lacking the FAAH gene in comparison to wildtype, as well as their response to a challenge of the CB_1 receptor antagonist rimonabant (1.0 mg/kg, intraperitoneal). Since the monoamine transmitter serotonin (5-HT) is mainly involved in the mode of action of antidepressants and anxioltyics, we also performed *in vivo* extracellular single-neuron recordings in order to characterize 5-HT activity in these mice. FAAH null-mutant ($FAAH^{-/-}$) mice exhibited reduced immobility in the forced swim and tail suspension tests, predictive of antidepressant activity, which was attenuated by rimonabant. $FAAH^{-/-}$ mice showed an increase in the duration of open arm visits in the elevated plus maze (EPM), and a decrease in thigmotaxis and an increase in exploratory rearing displayed in the open field (OF), indicating anxiolytic-like effects that were reversed by rimonabant. Rimonabant also prolonged initiation of feeding in the novelty-suppressed feeding test (NSFT). Single-neuron recordings in the dorsal raphe (DR) nucleus, the major source of 5-HT innervation of the brain, revealed a significant increase in the spontaneous firing activity of 5-HT neurons; this was reversed by rimonabant in a subgroup of neurons exhibiting high firing rates. Microiontophoresis and electrophysiological recordings of pyramidal neurons in the ventromedial prefrontal cortex showed desensitized $5\text{-}HT_{2A/2C}$ receptors in $FAAH^{-/-}$ mice, indicated by a significant decrement in the response of these neurons to the $5\text{-}HT_{2A/2C}$ agonist (±)-DOI. This may likely be associated with the enhanced anxiolytic-like response in the EPM, OF and the NSFT. In the hippocampus of $FAAH^{-/-}$ mice, the increased disinhibition of pyramidal neurons in response to the $5\text{-}HT_{1A}$ receptor antagonist WAY100635 indicated enhanced tonic activity of $5\text{-}HT_{1A}$ receptors, an effect associated with antidepressant-like activity. Similarly, chronic treatment with the FAAH inhibitor URB597 (0.3 mg/kg, intraperitoneal) also increased tonic $5\text{-}HT_{1A}$ receptor activity. This effect in $FAAH^{-/-}$ mutant mice and URB597-treated wildtypes were both blocked by chronic co-treatment with rimonabant (1.0 mg/kg, intraperitoneal). Together, these findings suggest that genetic deletion of FAAH produces anxiolytic-like and antidepressant-like activity, paralleled by modifications in 5-HT neurotransmission and in postsynaptic $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A/2C}$ receptor function.

THE DUAL ROLE OF THE ENDOCANNABINOID SYSTEM AS A REGULATOR OF ANXIETY RESPONSES

Alejandro Aparisi[2], Maria Paz Viveros[1] and Beat Lutz[2]

[1] Departamento de Fisiología (Fisiología Animal II), Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain
[2] Department of Physiological Chemistry, University Medical Center Johannes Gutenberg-University Mainz, Duesbergweg 6, D-55099 Mainz, Germany

Introduction: During the last years, the biphasic effect of cannabinoids has been shown in a great variety of processes such as memory processing, feeding behavior and also anxiety responses. The effects of synthetic or plant-derived cannabinoid receptor type 1 (CB1) agonist on these behaviors have been related to the inhibitory properties of cannabinoids on different neurotransmitter systems. Here, we demonstrate the relevance of CB1 localization and its role in the regulation of GABAergic and glutamatergic transmission.

Methods and Results: Using common tasks for anxiety-related behavior (Elevated Plus Maze) and exploratory activity (Holeboard test), we identified a range of doses of the CB1 agonist CP-55,940 (1, 10, 50 and 75 μg/kg) with anxiolytic (lower doses) and anxiogenic properties (higher doses) in C57BL/6N mice. Testing the more significant doses (1 and 50 μg/kg) in two different knock-out (KO) mouse lines; GABA-CB1-KO (lack of CB1 in GABAergic interneurons) and Glu-CB1-KO (lack of CB1 in glutamatergic forebrain neurons) a significant difference occurred in the anxiolytic-like effect, between the Glu-CB1-KO mice and their wild-type littermates; and in the anxiogenic-like effect, between the GABA-CB1-KO mice and their wild-type littermates.

Conclusion: These results suggest the presence of CB1 receptor on the glutamatergic terminals as a requirement in order to produce an anxiolytic-like effect 30 minutes after a treatment (i.p. injection) with a low dose of the CB1 synthetic agonist. On the other hand, the CB1 receptor on the GABAergic terminals apparently plays a completely different role and it is indeed required to produce an anxiogenic-like effect under a high dose treatment. These experiments showed a consistent connection between the anxiolytic behavior of the subjects and their ability to explore in the Holeborad test and viceversa, demonstrating as a matter of fact a logic influence of anxiety state on innate behaviors such as exploratory activity. Furthermore, the study was carried out in both genders and no differences occurred with the doses tested in the KO mice. However, the highest dose (75 μg/kg) tested in the dose-response curve (C57BL/6N mice) showed a different response to an "overdose" of this drug, increasing the locomotion activity in females and decreasing it in males. The molecular mechanism underlying these processes are still unknown, thus further experiments should be done to clarify the precise role of the endocannabinoid system in anxiety related behaviours.

ENDOCANNABINOIDS MODULATE OBJECT RECOGNITION MEMORY IN THE RAT

Áine M. Kelly, Andreea Petrasca, Ita Shanahan and Veronica Campbell
Department of Physiology & Trinity College Institute of Neuroscience,
Trinity College Dublin, Ireland

The endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2AG) play diverse roles in biological function, and increasing evidence suggests that they may act as modulators of cognitive function. The CB1 subtype of cannabinoid receptor is highly expressed in the hippocampus, a region of the medial temporal lobe that plays a pivotal role in several forms of learning and memory. Here, we have assessed the roles of endocannabinoids on acquisition and consolidation of recognition memory in the rat by use of object substitution (OS) and object displacement (OD) tasks, both of which are hippocampus-dependent. In the OS task, the ability of rats to discriminate between a novel and a familiar object 10min or 24h following exploration of two novel objects (training phase) is assessed. Rats are tested 10min and 24h after training in order to discriminate between acquisition and consolidation respectively. The OD task is a form of spatial learning that tests the ability of rats to identify the novel position of a familiar object.

In vivo, inhibitors of the enzymes FAAH and MAGL, which hydrolyse AEA and 2AG respectively, have been demonstrated to enhance endocannabinoid tone. Rats were injected intraperitoneally (i.p.) with the FAAH inhibitor URB597 (300µl, 0.3mg/kg), the MAGL inhibitor URB602 (300µl,3mg/kg) or vehicle either 30 min prior to, or immediately following, training in the OS task. Vehicle-treated rats successfully learned the OS task, exploring a novel object significantly more than a familiar object both 10min and 24h post-training ($p<0.01$, 2-way ANOVA). Analysis revealed that pre-training injection of URB597 enhanced acquisition of the OS task when compared with vehicle-treated controls, as evidenced by increased exploration of a novel object 10min post-training ($p<0.001$; 2-way ANOVA), while URB602 had no effect on acquisition. Injection of either enzyme inhibitor pre- or post-training blocked consolidation of OS learning, as evidenced by the inability of treated rats to discriminate between a novel and a familiar object at 24h.

These data demonstrate that consolidation of OS learning was blocked by elevation of endocannabinoid tone. We then assessed the effect of blockade of the CB1 receptor on this learning task by injection of AM251(300µl, 3mg/kg i.p.) or vehicle 30min prior to training. Both groups preferentially explored a novel compared with a familiar object at 24h ($p<0.001$, 2-way ANOVA). When exploration of the novel object was normalised to total exploration time, AM251-treatment was demonstrated to significantly enhance OS learning ($p<0.001$, Students *t*-test). Having established an effect of AM251 on OS learning, we tested its effects on performance of the OD task. Vehicle-injected rats were tested in a 3-object OD task (third object is displaced), in which they were given 1x5min trial of exploration on the training day, and failed to preferentially explore the displaced object compared with the stationary objects at 24h, indicating they did not learn the task. In contrast, rats treated with AM251 prior to training successfully learned the task, exploring the displaced object significantly more than the stationary objects during testing ($p<0.001$, ANOVA).

These data are consistent with the hypothesis that endocannabinoids can negatively regulate some forms of hippocampus-dependent memory in the male Wistar rat.

CHANGES IN THE ENDOCANNABINOID SYSTEM FOLLOWING BILATERAL VESTIBULAR DEAFFERENTATION

Jean-Ha Baek, Yiwen Zheng, Cynthia L. Darlington and Paul F. Smith

Department of Pharmacology & Toxicology, School of Medical Sciences, University of Otago, Dunedin, New Zealand

Electrophysiological, neurochemical, and imaging studies provide evidence for a vestibular contribution to higher cognitive function, especially that related to the hippocampus. It has been shown that the hippocampus undergoes significant changes following damage to the peripheral vestibular system. Unilateral or bilateral peripheral vestibular lesions in animals result in deficits in a number of spatial and non-spatial learning and memory tasks. These results suggest that the loss of vestibular function may change the way the hippocampus integrates spatial and non-spatial information. It is well known that CB1 receptors in the hippocampus are critically involved in synaptic plasticity. Therefore, the aims of this study were to investigate whether CB1 receptors in different regions of the hippocampus change following bilateral vestibular deafferentation (BVD) and whether treatment with a CB1 receptor agonist, WIN 55212-2 (WIN), can modulate the vestibular lesion-induced cognitive deficits.

Rats were sacrificed at 24 h, 72 h, or 1 week after the BVD or sham surgery. Using western blotting, the CB1 receptor protein levels in the CA1, CA3, and dentate gyrus (DG) areas of the hippocampus were determined. Two-way ANOVAs were performed followed by Bonferroni's post hoc comparisons. The levels of the CB1 receptor protein were significantly reduced in all regions of the hippocampus in a time-dependent manner (P = 0.000). Post hoc comparisons indicated significant differences between all time points, where 24 h post-op had the highest and 1 week post-op had the lowest level of the CB1 receptor (P = 0.000). However, there was no difference in the levels of the CB1 receptor protein between BVD and sham animals, except in the CA3 area, where 24 h and 1 week post-op sham animals had lower CB1 receptor protein level than BVD animals, but higher level in 72 h post-op (P = 0.05). Furthermore, there was no surgery x time interaction. The foraging task was employed to assess the effect of WIN (1-2 mg/kg, *s.c.*) on spatial learning and memory in 14-month post-op. animals. Twenty-eight rats were randomly divided into 4 treatment groups: 1) BVD-vehicle; 2) BVD-WIN; 3) sham-vehicle; 4) sham-WIN. Searching and homing time, distance, velocity, and number of errors made before reaching home were measured. The data were analysed by a mixed model analysis. BVD animals took a significantly longer time and travelled a longer distance to return home but at a higher velocity (P = 0.000) compared to sham. However, WIN treatment had no significant effect on any of above parameters. These studies suggest that few changes occur in the endocannabinoid system in the hippocampus following BVD, despite the electrophysiological and neurochemical changes that have been documented in previous studies.

EFFECTS OF ENDOCANNABINOID MODULATORS ON SPONTANEOUS FIRING RATE, BURSTING AND CELL SYNCHRONY OF HIPPOCAMPAL PRINCIPAL CELLS IN RATS

John Sesay, Anushka V.Goonawardena and Robert E. Hampson

Wake Forest University School of Medicine, Winston-Salem, NC, USA

Cannabinoid receptor agonists Delta-9-THC; WIN 55,212-2 (WIN-2) and HU-210 have been shown to alter hippocampal neuronal activity and disrupt spatial learning and memory task.(Hampson and Deadwyler, J. Neuroscience, 2003; Robinson, et al., Bri. J. Pharm, 2007). Moreover, *in vivo* recordings of local field potentials and single neurons have demonstrated that Delta-9-THC and CP 55,940 disrupt the synchrony of action potentials between hippocampal cells without altering average firing rates. Although endocannabinoids such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) have also been shown to impair memory, to date no studies have looked at how hippocampal principal cell firing is modulated when these endogenous cannabinoids are pharmacologically elevated *in vivo*. Adult, male Long-Evans rats were implanted with multi-electrode arrays to CA3 and CA1 regions of the hippocampus. Following recovery from surgery, all subjects were anesthetized by inhalation (isoflurane) and single-units (firing rate: 0.25-6Hz) were isolated. Thereafter, each isolated principal cell activity was recorded following intra-peritoneal injections of vehicle (1:19; Cremaphor:Saline) plus subsequently either *R*-methanandamide (10.0 mg/kg; stable analog of AEA), URB597 (3.0 mg/kg; FAAH inhibitor), VDM-11 (10.0 mg/kg; putative AEA transporter blocker) or URB602 (3.0 mg/kg; MAG Lipase inhibitor). Results will be presented in terms of how these modulators of the endocannabinoid system affect the overall hippocampal principal cell 1) 'firing' rate; 2) 'bursting' characteristics and 3) synchronous firing within and between pairs of cells located in CA3 and CA1 regions. Furthermore, to determine whether any of these effects were CB1 receptor mediated, all of these drugs were tested following pre-treatment of the CB1 receptor antagonist, SR141617 (2.0 mg/kg). Results from these experiments should will increase understanding about how the endogenous cannabinoid system is involved in modulating hippocampal neuronal activity and provide a neurophysiological basis as to how exogenous cannabinoids such as Delta-9-THC could be producing learning and memory deficits in rodents and humans.

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ACUTE TREATMENT WITH DELTA-9-TETRAHYDROCANNABINOL IMPAIRS COGNITIVE FUNCTION IN RHESUS MACAQUES

M. Jerry Wright Jr., Renee Croce and Michael A. Taffe

The Scripps Research Institute
Committee on the Neurobiology of Addictive Disorders
La Jolla, CA 92037 U.S.A.

Some studies suggest that smoking marijuana produces discrete cognitive deficits in humans and that these cognitive deficits may persist for up to 28 days (Bolla *et al*., 2002). Other studies suggest that marijuana-related cognitive deficits are generally mild (Brown, McKone, & Ward, 2010). Cannabis abuse produces a variety of public health concerns, including the poorly understood cognitive impact of cannabis exposure. To that end, a series of experiments were undertaken to measure the degree to which Δ^9-THC (0 to 0.3 mg/kg) affects cognitive function in primates. In these experiments, adult male Rhesus macaques (*N*=7) were trained to perform a paired-associates learning task (PAL). The PAL task measures associative learning and memory. All of these experiments were conducted using touch-sensitive computer monitors running CANTAB® software (Lafayette Instruments, Lafayette, Indiana, USA). In the PAL task, subjects are presented with between 1 and 4 visual stimuli, each occupying a unique position on a touch-screen. To be reinforced, subjects are required to recall the screen position associated with each of the previously-viewed stimuli. Changes in response accuracy that are produced by manipulation of delay between the initial and subsequent presentation of the stimuli may be selective measures working memory (Brown, 1958). As the trials progressed, the delay between the initial and subsequent presentation of the stimuli (i.e., the retention interval) was systematically lengthened (1-10 seconds). Under these conditions, there was a significant main effect of Δ^9-THC on response accuracy. After treatment with Δ^9-THC, response accuracy declined significantly. A significant main effect of retention interval on response accuracy was also noted. Response accuracy declined significantly as retention interval increased. While a significant interaction of the main effects of Δ^9-THC and retention interval on response accuracy was absent, the data suggest that an interaction may become apparent if the retention interval is lengthened beyond 10 seconds. However, the current data indicate that while Δ^9-THC inhibits associative learning and memory, the degree to which these functions are impaired is not dependent on the retention interval.

ACUTE EFFECTS OF CANNABINOID AGONISTS, ANTAGONISTS AND NEUTRAL ANTAGONISTS ON SLEEP-WAKE CYCLE IN MICE

Anushka V Goonawardena[1], Andrea Plano[2], Matt Elverman[1], Bettina Platt[2], Robert E Hampson[1,2] and Gernot Riedel[1,2]

1. Dept. Physiology and Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC, USA
2. School of Medicine and Life Science, University of Aberdeen, Aberdeen, Scotland, UK

Background: Endocannabinoids (e.g. Anandamide, 2-Arachidonylglycerol) are fatty acid derivates that have a variety of biological actions, most notably via the activation of cannabinoid receptors. They actively modulate diverse neurobiological functions such as learning and memory, feeding, pain perception and sleep generation. Experimental evidence shows that administration of cannabinoid receptor agonists such as anandamide and Δ^9-THC promote sleep via the activation of CB1 receptors, since this effect is blocked by the CB1 receptor antagonist, rimonabant. Moreover, rimonabant on its own dose-dependently increases the time spent in wakefulness at the expense of slow-wave sleep (SWS) and rapid eye movement (REM) sleep in rats. However, the modulation of cannabinoids on the sleep-wake cycle in mice has not been thoroughly investigated. Here we assessed the effects of the potent cannabinoid agonist, WIN55,212 (WIN-2); the CB1 receptor antagonist, AM251 and the novel CB1 receptor neutral antagonist, ABD459 (ABD) on circadian activity in freely-moving mice using time scoring and power spectral analysis of the quantitative electroencephalogram (EEG). **Methods:** Adult, C57/BL6 mice were surgically implanted with epidural gold screws anchored to the skull directly over the medial pre-frontal cortex, left and right dorsal hippocampus. EEG was recorded using wireless Neurologger microchips attached directly to their heads in home cages. The effects of three drugs (3mg/kg WIN-2; 3mg/kg AM251 and 3mg/kg ABD459; i.p.) were compared for alterations in their sleep patterns over a 6h recording period (1.00-7.00pm). **Results:** Vigilance analysis [Wakefulness, non-rapid-eye-movement (NREM) and REM)] yielded a WIN-2 mediated reliable increase in NREM (not REM) sleep at the expense of wakefulness. Furthermore, WIN-2 reduced the latency to the 1st NREM episode, but increased the latency to the 1st REM episode. In contrast, AM-251 increased wakefulness and reduced both NREM and REM sleep. ABD459 had no effect on either wakefulness or NREM but reduced REM sleep. FFT transformation and normalization of EEG spectral power revealed that WIN-2 increased the power of delta and theta frequencies in the hippocampus during NREM and REM sleep respectively. Moreover, AM-251 increased the power of theta and alpha in the hippocampus during the wake period. **Conclusions:** Taken together, these results demonstrate that WIN-2 induces sleep whilst AM251 but not ABD459, enhance wakefulness. This study suggests that an endogenous cannabinoid-like system is involved in the regulation of the sleep-waking cycle in mice.

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MATERNAL INTAKE OF HEMPSEED AS A *"JUNK NUT"* DID NOT ALTER STRESS, DEPRESSION, AND PSYCHOMOTOR PROFILES IN RAT OFFSPRING AT POST-WEANING

Isaac Karimi[1] and Lora A. Becker[2]

1- Department of Biochemistry, Physiology and Pharmacology, College of Veterinary Medicine, Razi University, Kermanshah, PO box: 67156-85414, Iran; 2- Department of Psychology, University of Evansville, IN 47722, USA.

Introduction: There are many evidences to suggest that maternal nutrition or drug-abusing can influence the offspring's predilection for developing psycho-metabolic disorders. The present study investigated whether maternal intake of a "hempseed" diet can alter postnatal psychomotor fitness in the rat offspring.

Methods: On 30th postnatal day, normal (NC; n=6) and hempseed-treated (HS; n=5) offspring- that their moms had free access to normal diet, and normal diet plus hempseed respectively- were tested with forced swimming test: FST, novelty stress-induced fecal pellet output test: NT and balance beam test: BTT that used for scoring of depression, stress and psychomotor fitness, respectively. Data were reported as mean±SEM. All parameters were analyzed using one-way *ANOVA* with SPSS *ver.*16 at a significant level of $p<0.05$.

Results: In BTT; foot fault number in HS group (13.0±2.03) increased in comparison to NC (8.5±2.20; p=0.164), rear number in HS group (0.3±0.33) decreased in comparison to NC (0.5± 0.34; p=0.734), grooming number in HS group (1.8±0.91) increased in comparison to NC (1.6±0.42; p=0.871), direction exchanges through beam in HS group (9.6±1.87) decreased in comparison to NC (10.0±1.89; p=0.903), and total motility time (sec) in HS group (54.0±8.51) increased in comparison to NC (40.3±9.12; p=0.299). In FST; floating time (sec) in HS group (99.3±7.29) increased in comparison to NC (88.6±6.56; p=0.311), fecal boli number during 6min in HS group (2.6±0.55) was lesser than in NC group (4.0±0.89; p=0.222), and sniffing number during 6min in HS group (1.1±0.54) was lesser than in NC group (2.0±1.09; p=0.489). In NT, fecal boli number in dark box as novel media during 1 hour in HS group (3.6±1.25) was lesser than in NC group (5.8±2.80; p=0.478).

Conclusion: The results of this study showed that hempseed as a junk nut is safe during gestation and breast feeding in rats. Due to apparent reduced explorative motor activities, increased anxious behaviors in BTT paradigm and reduced swimming time and sniffing number in FST paradigm may result in deleterious outcomes. However, the decrement of fecal boli numbers in both NT and FST paradigms led us to consider hempseed as a weak anti-stress or adoptogen.

INTERACTION BETWEEN CB1 AND TRPV1 RECEPTORS IN THE MODULATION OF PANIC-LIKE REACTIONS IN RATS

Fabricio A. Moreira (1); Plinio C. Casarotto, Daniele C. Aguiar, Ana Terzian, Helio Zangrossi, Francisco S. Guimaraes (2); and Carsten T. Wotjak (3)

(1) Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil
(2) University of Sao Paulo, Ribeirao Preto, SP, 34049-900, Brazil
(3) Max Planck Institute of Psychiatry, Munich, BY, 80804, Germany

Introduction: The cannabinoid type-1 receptor (CB1) and the transient receptor potential vanilloid type-1 channel (TRPV1) are expressed in the dorsal periaqueductal gray (dPAG), a structure proposed to elaborate anxiety and panic reactions. CB1 inhibits, whereas TRPV1 facilitates, anxiety-like responses. Thus, we tested the hypothesis that these receptors would also have opposing functions in panic-like responses induced by local electrical stimulation.

Methods: Male Wistar rats (n=5-9/group) received intra-dPAG administration of a CB1 agonist, ACEA, a TRPV1 antagonist, capsazepine, or a CB1 antagonist, AM251. Ten minutes afterwards, the current threshold to induce escape reaction was recorded. In addition, double-staining immunofluoresce was performed to verify whether these receptors would be complementarily expressed. The data were analyzed by one-way ANOVA followed by the Duncan test.

Results: Local injection of either ACEA (0.05, but not 0.01 or 0.5 pmol) or (capsazepine, 1-10 nmol), increased the threshold to induce panic-like responses. Moreover, we identified that CB1 and TRPV1 may be complementarily expressed in the dPAG, providing morphological basis for a possible interaction. Accordingly, pre-treatment with AM251 (75 pmol), prevented the anti-panic effect of both ACEA (0.05 pmol) and capsazepine (1 nmol).

Conclusion: CB1 inhibits, whereas TRPV1 facilitates, panic-like reactions. Importantly, the present work provides morphological and pharmacological evidences for an interaction between these receptors. They could be simultaneously activated by an endogenous agonist, possibly anandamide. Depending on its local levels, anandamide might restrain or promote panic-like reactions through CB1 or TRPV1 activation, respectively.

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BLOCKING DAM'S CANNABINOID CB1 RECEPTOR AFFECTS PUP'S SOCIAL BEHAVIOR

Michal Schechter[1, 3], Albert Pinhasov[1], Aron Weller[4] and Ester Fride[1, 2]
[1]Department of Molecular Biology, [2]Department of Behavioral Sciences, Ariel University Center, Ariel. Faculty of Life Sciences[3], Department of Psychology[4], Bar Ilan University, Ramat-Gan, Israel

Introduction: Early postnatal development has long-lasting influences on the behavior of individuals in adulthood. Alteration in maternal behaviors such as grooming and licking, during the first 10 days of the mouse's life, a sensitive period for establishment of attachment processes, may impact offspring social behavior. We have previously shown that blocking of maternal endocanabinoid CB1 receptors significantly affected attachment processes. The purpose of the current study was to evaluate effects of blocking these receptors in lactating dams on the offspring's social behavior during later developmental stages. **Methods**: Offspring of dams treated on postpartum days 1-8 with SR141716 (Rimmonaband) 10 mg/kg (SRO) or vehicle (VO) underwent preference and social behavior tests post-weaning and in adulthood, respectively. *Preference test*: At the age of 24-28 days, 17 male and 17 female offspring were tested in a Y-maze that included an entrance box, right & left arms. Different targets were placed at the edge of each arm. Three different preference tests were performed: Dam vs. Milk (DM), Dam vs. Pup (sibling) (DP) and Pup (sibling) vs. Milk (PM). In each 4 min test, the time offspring spent in each maze was measured. The three tests were performed in succession, with a 1 min interval between the tests, keeping the same order in every repeat. *Social behavior test*: At the age of 13-14 weeks, 48 female and 38 male SRO and VO mice underwent a social behavior test. In each test, randomly chosen SRO and VO of unfamiliar individuals were placed in separate cages for 30 min, then animals were placed together in a new cage for 5 min and their behavior was video-recorded for further analysis. The same test was repeated in 30 min intervals. Active social interactions were measured by sniffing the partner while passive social interactions were measured from the observed time that animals were ignoring each other. Rearing was also assessed. **Results**: Preference test: 1) both SRO and VO females preferred the dam over milk, while SRO males preferred staying more with the dam compared to VO males. 2) Both SRO males and females preferred dam over pup and pup over milk in comparison to VO mice. Social behavior test: SRO males and females were more active, showing higher levels of social sniffing and rearing than VO mice. **Conclusions**: It is commonly accepted that changes in maternal behavior are related to modification in behavioral response later in life. This study shows that blocking CB1 receptors in lactating mouse dams causes behavioral changes in the offspring's social behavior. We speculate that the high degree of social interactions during weaning and adulthood of SRO males and females result from reduced maternal care, contact and milk during the neonatal period (Schechter et al., ICRS abstract, 2009), The present study further indicates an important role of the endocanabinoid system in attachment processes and their impact on the individual's future social behavior.

INHIBITION OF MONOACYLGLYCEROL LIPASE: ASSAY COMPARISON

Emmelie Björklund and Christopher J. Fowler

Departments of Pharmacology and Clinical Neuroscience, Umeå University, SE90187 Umeå, Sweden

Introduction*:* A number of assays for monoacylglycerol lipase have been published in recent years, using different substrates, different enzyme preparations and different extraction techniques. However, little comparative data is available for data derived from the same laboratory. In the present study, we compare the sensitivity to inhibition of MGL in two different assays.

Method: The assays used were radiochemical (rat cerebellum cytosolic preparations and commercially available recombinant human MGL (lysates and purified enzyme), substrate 0.5 μM [^{3}H]2-oleoylglycerol (2-OG), see Cisneros *et al., J Med Chem* 50 [2007] 5012-23) and spectrophotometric (recombinant human MGL, substrate 0.25 mM 4-nitrophenyl acetate (NPA), Muccioli *et al., ChemBioChem* 9 [2008] 2704-10).

Results*:* JZL184 produced a potent and time-dependent inhibition in both assay systems. For the radiochemical assay, IC_{50} values of 350 and 5.8 nM were found using preincubation times of 0, 60 min, respectively with the cytosols as enzyme source and 2-OG as substrate. For the purified enzyme, the IC_{50} values following a 60 min preincubation period were 17 nM and 9.6 nM for NPA and 2-OG as substrate, respectively. With the hMGL lysates, the IC_{50} value for the inhibition of NPA hydrolysis (4.7 nM, 60 min preincubation) was about fourfold lower than that for the inhibition of 2-OG hydrolysis (IC_{50} value 18 nM). *N*-arachidonoyldopamine was found to inhibit the hydrolysis of NPA and 2-OG by the MGL lysates with IC_{50} values of 0.78 and 2.2 μM, respectively. The compound was less potent towards the hydrolysis of 2-OG by the cytosolic preparations cytosol (IC_{50} value 20 μM). The potency of troglitazone as an inhibitor of human lysate MGL activity was highly dependent upon the assay conditions, with a much lower potency being seen in the radiochemical assay than in the spectrophotometric assay, where an IC_{50} value of 1.2 μM was found. One difference between the assays is the presence of fatty acid-free BSA in the radiochemical assay to stabilise the substrate. Troglitazone exihibits a high degree of albumin binding, and addition of fatty acid-free BSA to the spectrophotometric assay not only produced a time-dependent hydrolysis of NPA *per se* (due to the esterase activity of BSA), but also reduced the observed potency of both troglitazone and *N*-arachidonoyldopamine as inhibitors of NPA hydrolysis by the MGL lysates.

Conclusion*:* From the data provided here, it can be concluded that it is important to test compounds with respect to their MGL inhibitory activity in more than one assay system, since assay and/or species differences may be important.

PREDICTIVE MODELS OF ACTIVITY AND SELECTIVITY FOR CB1 ANTAGONISTS AND CB2 AGONISTS

Robert J. Doerksen*[1,2], Ronak Y. Patel[1] and Pankaj R. Daga[1]

[1] Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, MS, 38677-1848, USA; [2] Research Institute of Pharmaceutical Sciences, University of Mississippi

Introduction. The human cannabinoid receptor subtypes CB1 and CB2 have been targeted for a variety of disease conditions such as obesity, drug abuse disorders, modulation of the immune system, inflammatory diseases, anorexia and vomiting. There has been much interest in the development of CB1 antagonists and CB2 agonists, but accurately-predictive models which are not chemical class-specific are lacking.

Methods. In the present study, classification models were generated for prediction of activity and subtype selectivity of CB1 antagonists and CB2 agonists using support vector machines (SVM). The models were built to predict activity in two classes and selectivity in three classes. From literature reporting activity at both the receptors, 223 CB1 antagonists and 295 CB2 agonists were selected and divided into training and test sets. Special emphasis was placed on having an approximately equal number of compounds in each class for a particular model, either by removal of compounds from the category having excess available structures or by addition of putative inactive decoys.

Results. The models provided accuracy of 94.4%, 75.0%, 78.0% and 73.3% for CB1 antagonist activity, CB1 antagonist selectivity, CB2 agonist activity and CB2 agonist selectivity prediction models, respectively, for all compounds in the external test set which fit within the local applicability domain. The models were used for screening of three databases, including the ZINC natural product subset, and provided hit rates comparable to those reported studies for other targets, < 10% in all cases, typically ~ 1% and in some cases << 1%, with lower hit rates for CB1.

Conclusion. Overall this study showed the capability of machine learning methods to predict the CB1 antagonist and CB2 agonist activity and selectivity of chemically diverse sets of compounds. Discovery and development of new active and selective CB ligands is a difficult task; in this context, the models we report will be useful in virtual screening to predict a small number of hits to proceed into *in vitro* screening and to increase the chances of finding target-selective compounds compared to screening hits resulting from activity-only prediction models.

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NOVEL CANNABIDIOL DERIVATIVES AND THEIR USE AS ANTI-INFLAMMATORY AGENTS

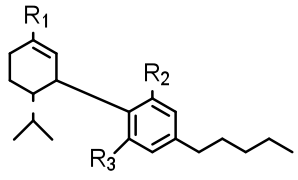
Christeene Haj[1], Ruth Gallily[2], Aviva Breuer[1] and Raphael Mechoulam[1]

[1]Institute of Drug Research and [2]Institute of Immunology, Medical faculty, Hebrew University of Jerusalem 91120, Israel

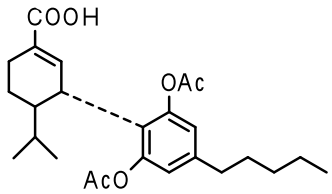
Cannabidiol (CBD) is a nonpsychoactive component of cannabis with a high potential for use in several therapeutic areas. It binds very weakly to the CB1 and CB2 cannabinoid receptors. It has been evaluated both *in vitro* and *in vivo* in anti-inflammatory assays. Thus, it lowers the formation of TNF-α, a proinflammatory cytokine and was found to be an oral anti-arthritic therapeutic in murine collagen-induced arthritis (CIA) *in vivo*. Its numerous potentially therapeutic pharmacological effects may be due in part to its metabolites. Indeed, a derivative of such a metabolite, CBD-dimethylheptyl (DMH)-7-oic-acid (HU-320) is more potent than CBD as an anti-arthritic agent in the above model of CIA. However the reported synthesis of HU-320 is complicated and is not practical. The mechanism of the cannabinoid anti-inflammatory effect is not fully understood, but most probably it has to do with its anti-oxidative action, its ability to enhance adenosine signaling through inhibition of adenosine uptake and by lowering of the levels of proinflammatory cytokines and the enhancement of Lipoxin A4 (LXA4) levels.

Contrary to the natural (-)CBD itself, several synthetic enantiomeric (+) CBD derivatives, have been found to bind to the CB1 receptor, but have only peripheral action.

The synthesis of HU-320 was repeated and improved. However it is still presumably too difficult for therapeutic application. The synthesis of HU-320 is complicated by the existence of a reactive Δ8 double bond. We found that this double bond is not required for the anti-inflammatory activity, hence we proceeded with syntheses in the Δ8 dihydro series.Some presumed metabolites of Δ8-dihydro-CBD were synthesized and submitted for testing: Δ8-dihydro-7-hydroxy-CBD (HU-446) ,Δ8-dihydro-CBD-7-oic acid-di acetate (HU-444), Δ8-dihydro-7-oic acid CBD (HU-445), Δ8-dihydro-7-hydroxy-dimethoxy-CBD and (+)-Δ8-dihydro-7-oic acid CBD. *In vivo* and *in vitro* data showed that both HU-444 and HU-446a have more potent anti-arthritic effects *in vitro* than CBD and HU-444 is more active than CBD in vivo in the CIA assay.



R_1=CH_2OH, R_2=OH, R_3=OH HU-446
R_1=COOH, , R_2=OAc, R_3=OAc HU-444
R_1=COOH, R_2=OH, R_3=OH HU-445
R_1=CH_2OH, R_2=OMe, R_3=OMe
Δ8-dihydro-7-hydroxy-dimethoxy-CBD



(+) Δ8-dihydro-7-oic acid-CBD

HU-433, ENANTIOMER OF THE CB2 AGONIST HU-308, IS A HIGHLY POTENT REGULATOR OF BONE MASS

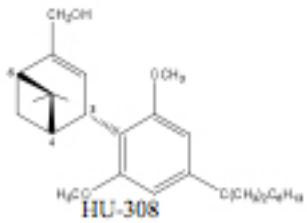
Reem Smoum[1, 2], Aviva Breuer[1], Lumir Hanuš[1], Naama Mussai[1,2], Orr Ofek[2], Malka Attar-Namdar[2], Raphael Mechoulam[1] and Itai Bab[2]
[1]Institute of Drug Research and [2]Bone Laboratory, the Hebrew University of Jerusalem, Jerusalem 91120, Israel.

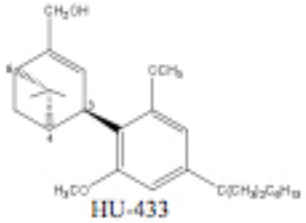
Bone mass is determined by a continuous remodeling process whereby the mineralized matrix is being removed by osteoclasts and subsequently replaced with newly formed bone tissue produced by osteoblasts. Osteoporosis, the most prevalent degenerative disease in developed countries, results from the impairment of this balance, leading to bone loss and increased fracture risk. We recently reported that these bone cells express CB2 receptors and that the CB2 agonist HU-308 stimulates osteoblast number and rescues ovariectomy (OVX) induced bone loss at 10^{-8} M and 20 mg/Kg/day, respectively. Here we report that the HU-308 enantiomer, code numbered HU-433, retains the HU-308 CB2 binding specificity and potency. In spite of this similarity, by comparison to HU-308, HU-433 demonstrates a markedly increased skeletal efficacy *in vitro* in mouse osteoblasts and *in vivo* in OVX animals. Fig. 1 and Table 1 show a comparison of the structural and pharmacological properties between HU-308 and HU-433.

Table 1. Comparison between HU-308 and HU-433

Enantiomer	Configuration	Binding (Ki)		Peak stimulation of osteoblast* no.	Bone loss rescue**
		CB1	CB2		
HU-308	3*S*, 4*S*, 6*S*	> 5 µM	3.2±.3 nM	10^{-8} M	20 mg/Kg/d
HU-433	3*R*, 4*R*, 6*R*	> 5 µM	7.9±.5 nM	10^{-11} M	0.2 mg/Kg/d

*MC3T3 E1 cell line
** Six-week agonist treatment commenced 6 weeks after OVX


HU-308


HU-433

The discrepancy between the similarity in binding efficacy and markedly enhanced skeletal activity could be explained by the occurrence of two domains: a CB2 binding domain that consists of the aromatic and alkyl part (similar in both enantiomers) of the molecule and an activation domain that consists of the bicyclic pinene part.

A SAFETY AND TOLERABILITY STUDY OF SINGLE ASCENDING DOSES OF TM38837 - A NOVEL SECOND GENERATION PERIPHERAL SELECTIVE CB1 RECEPTOR ANTAGONIST IN HEALTHY MALE SUBJECTS

Fridberg M, Noerregaard PK, Little PB, Jensen NO, and Elling CE

7TM Pharma A/S, Hoersholm, Denmark

Introduction: TM38837 is a novel peripheral selective cannabinoid 1 (CB1) receptor antagonist in clinical development for treatment of obesity and related metabolic disorders. This double-blind, randomized, placebo-controlled, single ascending oral dose study investigated safety, tolerability and pharmacokinetics (PK) in healthy male subjects.

Methods: Forty-eight healthy normal weight adults (aged 19-55 years) were randomized to a single oral dose of either 5, 15, 30, 100, 300 or 900 mg TM38837 or placebo. In each dose group six subjects received TM38837 and two subjects received placebo. Blood was drawn before dosing and up to 48 hours post-dosing for determination of plasma TM38837 concentration by LC-MS/MS. PK parameters were calculated according to standard methods. Safety data included physical examination, adverse event reporting, vital signs, 12-lead ECGs, POMS_65, ARCI_49, Bond&Lader VAS, and safety laboratory measurements.

Results: TM38837 was absorbed relatively slowly with median t_{max} values ranging from 4.0-7.0 hours. C_{max} and $AUC_{0-\infty}$ increased in a less than dose proportional manner, especially at the two highest doses. $T_{½}$ was close to 17 hours at all studied doses. TM38837 was particularly well tolerated with no significant changes in any of the safety assessments employed. Nine adverse events were reported in seven subjects. Most of these were of gastrointestinal origin (abdominal pain, nausea or diarrhea) and all were mild and transient.

Conclusion: TM38837 has an attractive pharmacokinetic profile suitable for a once-daily dosing regimen. No safety concerns were raised.

SEMINAL PLASMA LEVELS OF *N*-ACYLETHANOLAMIDES ARE DECREASED IN MEN WITH ABNORMAL SPERM MOTILITY

Akwasi A. Amoako[1], Timothy H. Marczylo[1], Patricia M.W. Lam[1], Amanda Derry[2], Jonathon Willets[1], Janine Elson[2] and Justin C. Konje[1]

[1]Endocannabinoid Research Group, Reproductive Science Section, Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, LE2 7LX, United Kingdom. [2]Assisted Conception Unit, University Hospitals of Leicester NHS Trust, Leicester Royal Infirmary, Leicester, LE1 5WW, United Kingdom.

Introduction: Endocannabinoids are lipid signalling molecules which are associated with an array of physiological functions and pathological conditions. Anandamide (AEA) is the most widely studied of these lipid messengers and activates the G-protein coupled cannabinoid receptors (CB1 and CB2) and the transient receptor potential vanilloid (TRPV1). AEA is co-synthesised with other *N*-acylethanolamides (NAE) such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) that potentiate its effect either by inhibiting its enzymatic hydrolysis or by potentiating its effects at the receptors sites. NAEs have previously been detected in human seminal plasma. *In-vitro* studies suggest that AEA reduces sperm fertilizing potential by inhibiting motility and capacitation. PEA and OEA possess antioxidant and anti-inflammatory potentials and recent studies suggest they may exert their effects via the peroxisome proliferator-activated receptors (PPARα and PPARγ). Exposure of human spermatozoa *in-vitro* to OEA and PEA increase sperm motility and capacitation. The aim of this study was to determine the concentrations of these compounds in human seminal plasma and relate them to sperm quality.

Methods: Seminal Fluid was obtained from 80 human donors. They were divided into 2 groups based on percentage sperm motility irrespective of other abnormalities. 45 men had normal sperm motility whilst 35 men had abnormal sperm motility. AEA, OEA and PEA were extracted by a solid-phase method and quantified by UPLC-MS/MS utilizing an isotope dilution method and selective ion monitoring.

Results: The (mean ± SEM) concentrations were 0.196 ± 0.033 versus 0.083 ± 0.007 for AEA, 1.793 ± 0.270 versus 0.604 ± 0.053 for OEA and 15.56 ± 2.538 versus 6.329 ± 0.760 for PEA for seminal plasma obtained from samples with normal and abnormal motilities, respectively. A significant decrease in the seminal plasma concentrations was noted in men with abnormal sperm motility compared to those with normal sperm motility ($p= 0.0003$, $p= 0.0002$ and $p< 0.0001$ for AEA, OEA and PEA, respectively; unpaired Student's t-test with Welch correction for unequal variances.

Conclusion: We propose on the basis of these findings, that maintenance levels of AEA, OEA and PEA may be necessary for the preservation of normal sperm motility.

ENDOCANNABINOID SIGNALING IN GASTRULATION AND EARLY NEURODEVELOPMENT OF CHICK AND MOUSE EMBRYOS

Delphine Psychoyos-Diakakis[a], K. Yaragudri Vinod [b,e] Jin Cao[a], Richard L. Hyson[c], Weimin He[a], Thomas B. Cooper[b,d,e], Basalingappa L. Hungund[b,d,e] and Richard H. Finnell[a]

[a]Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, Texas 77030; [b]Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962; [c]Department of Psychology, Florida State University, Tallahassee, FL 32306; [d]Department of Psychiatry, College of Physicians & Surgeons, Columbia University, New York, NY10032; [e]New York State Psychiatric Institute, New York, NY 10032 USA

Introduction: Marijuana is the illicit drug most commonly abused by pregnant women. *In utero* exposure to THC (Δ^9-tetrahydrocannabinol), the psychoactive component of marijuana, has been reported to be associated with an increased risk for miscarriage, anencephaly, and subtle neurodevelopmental defects in exposed offspring, including ADHD, psychiatric disorders, learning disabilities and memory impairment. Recent evidence suggests that THC mediates its effects by interfering with an endogenous cannabinoid system. At present, the components of this system have been characterized during implantation and in late fetal development, but it is not known whether this system is functional in early embryonic development, and if so, what its potential function(s) might be. **Methods:** We used chick and mouse animal models to determine whether components of the endocannabinoid system are present during early neural development. To this end, we examined embryonic tissue from which the brain originates using **LC-MS (for detection of endocannabinoid 2-AG), whole mount immunohistochemistry, Western blot analysis (for detection of CB1 receptor), and real-time RT-PCR (for detection of CB1 receptor as well as metabolic enzymes of the endocannabinoids; MAGL, DAGLα and FAAH). Results: W**e report for the first time that components of the endocannabinoid system are present at all stages studied (XIII to HH35 in chick and GD7.0 to GD18 in mouse). Our LC-MS data shows that 2-AG is present as early as at HH10 (earliest stage studied by this methodology), with levels of around 1 nmol/gm tissue. Whole mount immunohistochemistry in early chick embryos reveals that CB1 receptor is present at all stages studied, with high density in the neural plate and developing neural folds (i.e. the embryonic region which will give rise to brain primordia). Western blot and real-time RT-PCR methods corroborate these results, with levels of CB1 receptor mRNA (in copies/10 ng) of 70521 (gastrulation HH4$^-$) and 148216 (HH10), compared to 182496 at HH23. Finally, analysis of enzymes of endocannabinoid metabolism reveals that **MAGL, DAGLα and FAAH are all present during early embryonic development (i.e. prior to the formation of brain primordia) at levels comparable to those in late brain development. Conclusion:** Our results demonstrate that the endocannabinoid system is indeed present at embryonic stages which precede brain development. The function of this system at these stages is currently unknown. Finally, our results point to the possibility that THC might interfere with the endocannabinoid system present in the embryo during early stages of pregnancy. **Acknowledgements:** This research is supported by NRSA Long Term Fellowship Award 1F32DA021977-01 (D.P-D), NIH R21DA020531 (K.Y.V) and NIH R56DC000858 (R.L.H.)

N-ARACHIDONOYL GLYCINE POTENTLY INDUCES HUMAN ENDOMETRIAL CELL MIGRATION

Douglas McHugh, Emily J. Dunn and Heather B. Bradshaw

Department of Psychological & Brain Sciences,
Indiana University, Bloomington, IN 47405, USA.

Endometriosis is a condition where endometrium, which normally provides an inner lining to the uterus, grows in other areas of the body, typically on the ovaries, bowel, rectum, bladder and pelvic lining. It is a leading **cause** of chronic recurring pelvic pain**,** dysmenorrhea (painful menstruation) and infertility in women of reproductive age, and is estimated to affect over 5.5 million women in North America. The precise pathogenesis of endometriosis is unknown, but the disorder does exhibit a positive correlation with estrogens and symptom severity is often synchronous with the menstrual cycle. Proposed explanations for the development of endometriosis favour mechanisms that allow endometrial cells to exit the uterine cavity, implant in other tissues and proliferate.

Endometrium is a dynamic tissue under the influence of lipid hormones that vary through the female menstrual cycle. Differential gene expression has been shown to occur in endometrial cells in accordance with the proliferation, secretion and regression events of the cycle. Among those upregulated were genes encoding for cell surface receptors and key elements of signal transduction pathways responsible for cell motility, including mitogen-activated protein kinases (MAPKs). Estradiol, the major human estrogen, varies across the menstrual cycle, signals via a GPCR and induces directed migration in human endometrial cells. Recent data from our lab has shown that the endogenous anandamide (AEA) metabolite, *N*-arachidonyl glycine (NAGly), potently induces directed cell migration (chemotaxis) in microglia *in vitro* via a G-protein coupled receptor (GPCR) and mitogen-activated protein kinase (MAPK) signaling. Additional data showed that NAGly is present in rat uterine tissue and that its levels oscillate predictably across the hormonal cycle. These observations support the following hypothesis: NAGly drives endometrial cell chemotaxis through a GPCR, which activates a MAPK pathway, and dysregulation of this signaling cascade could contribute to endometriosis.

Using a 96-well Boyden chamber migration assay, we have shown that HEC-1B cells (an endometrial cell line) are migratory *in vitro*. Low nanomolar (0.1nM – 10nM) concentrations of NAGly and AEA more potently induce HEC-1B cell migration than equivalent amounts of estradiol. Future work will be directed toward establishing whether NAGly, AEA, and estradiol activate the same GPCR as in microglial cells, which then drives changes in MAPK enzymes to initiate endometrial cell chemotaxis, and if this contributes to endometrial cell migration within and outside the uterus *in vivo*.

DISTRIBUTION OF THE ENDOCANNABINOID SYSTEM IN THE RAT AND HUMAN BLADDER

Evangelia Bakali, Ruth A. Elliott, Jonathon M. Willetts, Anthony H. Taylor, Justin C. Konje and Douglas G. Tincello

Prolapse & Incontinence Group & Endocannabinoid Group, Reproductive Sciences Section, CSMM, University of Leicester, LE2 7LX, UK

Background:
Cannabis has an effect on urge incontinence probably mediated through endocannabinoid system-dependent mechanisms. We examined the distribution of the cannabinoid receptors (CB1 & CB2), transient receptor potential vanilloid type 1 (TRPV1) receptor, and cannabinoid-modulating enzymes, fatty acid amide hydrolase (FAAH) and N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD) in the rat and human bladder.

Methods:
Immunohistochemistry was performed on formalin fixed paraffin-embedded sections using polyclonal antibodies to to CB1, CB2, NAPE-PLD, FAAH & TRPV1, with brain as positive control. Western Blots were performed after SDS-PAGE on homogenised tissues probed with the same antibodies.

Results:
Immunoreactivity for CB1, FAAH, NAPE-PLD & TPVR1 was observed in rat and human urothelium and detrusor. CB2 immunostaining was observed in detrusor from both species but only in human urothelium.

Immunoblots indicated an identical molecular mass of 45kDa for CB2 in rat bladder and brain. CB1 produced specific bands of 30kDa and 40kDa in the bladder and 76kDa in the brain. Immunoblots for FAAH showed specific bands at 33kDa and 45kDa in the bladder but only 33kDa in brain. Specific bands were seen at 35kDa in the rat bladder with NAPE-PLD antibodies.

Conclusion:
For the first time, the main components of the endocannabinoid system (receptors and enzymes) have been localised in the rat and human bladder Cannabinoid and TRPV1 receptors and the modulating enzymes were expressed. CB2 expression in the rat bladder was different to that previously described.

1 Freeman RM, *et al.* (2006) Int Urogyecology Journal 17: 636-41.
2 Hayn MH, *et al.* (2008) Urology 72: 1174-78.

THE CANNABINOID, WIN55,212, REDUCES PACEMAKER FREQUENCY IN THE GASTRIC ANTRUM OF CONSCIOUS FERRET; A POTENTIAL MECHANISM FOR CANNABINOID-INDUCED GASTROPARESIS

Nathalie Percie du Sert [1,2], W. -S. Vanessa Ho [1],
John A. Rudd [2] and Paul L. R. Andrews [1,2]

[1] Division of Basic Medical Sciences, St George's University of London, London, SW17 0RE, United Kingdom; [2] Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

Gastric motility arises from a pacesetter potential termed gastric myoelectric activity (GMA), which is generated by the interstitial cells of Cajal (ICCs). In rodents, cannabinoids have been shown to inhibit gastrointestinal motility but cannabinoid (CB) receptors have not been identified on the ICCs and the effect of cannabinoids on GMA is unknown. This study used telemetry to identify the effect of the CB_1/CB_2 receptor agonist, WIN55,212-2, in freely moving, conscious ferrets. Ferrets were surgically implanted with telemetry transmitters; 2 biopotential electrodes were sutured in the antral serosa. Spectral analysis was carried out on the GMA recordings to identify the dominant frequency. Following WIN55,212-2 (1 mg/kg, i.p.) the frequency of the GMA was reduced (8.2 ± 0.4 cpm) compared to vehicle (9.6 ± 0.1 cpm). WIN55,212-2 also decreased body temperature by 2.2 ± 0.6˚C and the heart rate by 19.4 ± 7.7% ($p<0.05$, two-way analysis of variance, n=5-6). The results suggest that, in the ferret, modulation of the ICC network could contribute to the inhibition of gastric motility commonly associated with cannabinoids. The mechanism could be concomitant to previously identified mechanisms such as CB_1 receptor-mediated inhibition of acetylcholine release from nerve terminals of the autonomic nervous system. The observed hypothermia and bradycardia is also consistent with CB_1 receptor activation, as reported in rodents. Further investigation is warranted to identify how the effect of cannabinoids on the ICCs is mediated; possible mechanisms include the release of an inhibitory neurotransmitter or a direct effect on the ICC via CB receptors.

CELLULAR TARGETS FOR ANANDAMIDE REUPTAKE INHIBITORS

Lina Thors, Johanna Ranberg and Christopher J. Fowler

Department of Pharmacology and Clinical Neuroscience, Umeå University, SE90187 Umeå, Sweden

Introduction*:* Recent studies using both artificial liposomes and cultured cells are consistent with a model of anandamide (AEA) reuptake whereby the endocannabinoid binds to membrane cholesterol and thereafter translocates the membrane before being transported by intracellular proteins (serum albumin, hsp70, fatty acid binding proteins) to fatty acid amide hydrolase (FAAH) in the endoplasmic reticulum or FAAH-2 in lipid rafts (Di Pasquale *et al., Plos One* 4 [2009] e4989; Oddi *et al., Chem Biol* 16 [2009] 624-32; Kaczocha *et al., PNAS* 106 [2009] 6375-80; Kaczocha *et al., J Biol Chem* 285 [2010] 2796-806). What is not clear is how AEA reuptake inhibitors block this process. In the present study, we have investigated the effects of four commonly used AEA reuptake inhibitors (AM404, VDM-11, UCM707 and OMDM-2) upon a variety of cellular and intracellular targets.

Methods: Cyclooxygenase (COX) assays were performed using purified commercially available enzymes and an oxygen electrode. Peroxisome proliferator-activated receptor α (PPARα) assays were conducted using a Lanthascreen assay kit. The interaction of AEA with fatty acid-free bovine serum albumin was assessed by DEAE binding assay of Oddi *et al.* (*ibid.*). Artificial liposomes comprising cholesterol, POPC and POPG were made using a Lipofast extruder, and AEA retention determined by the method of Thors *et al.* (*Br J Pharmacol* 155 [2008] 244-52).

Results*:* The uptake inhibitors tested are generally used in the 1-10 μM concentration range *in vitro*. Over the range of 1-30 μM, none of the compounds affected the retention of AEA by synthetic cholesterol-containing liposomes. Neither AM404 (0.1-30 μM) nor OMDM-2 (0.1-10 μM) affected the binding of AEA to serum albumin preadsorbed to DEAE. However, AM404 and VDM-11 interacted with PPARα, producing 49 and 24% inhibition of the Lanthascreen signal at concentrations of 30 μM, whereas OMDM11 (10 μM) and UCM707 (10 & 30 μM) were without effect. AM404 concentration-dependently inhibited the activity of ovine COX-1 and human COX-2 towards 10 μM arachidonic acid, producing 37/30, 46/43 and 48/53% inhibition (COX-1 / COX-2) at concentrations of 3, 10 and 30 μM, respectively, a finding consistent with the study of Högestätt *et al.* (*J Biol Chem* 280 [2005] 31405-12). AM404 also inhibited COX-2 when 2-arachidonoylglycerol (10 μM) was used as substrate. UCM707 (30 μM) produced 24/40% inhibition of the COX-1 / COX-2 catalysed metabolism of arachidonic acid. VDM11 inhibited the activity of COX-1 by 16, 23 & 19% at 3, 10 and 30 μM, respectively.

Conclusion*:* The present data indicate that whilst the uptake inhibitors do not influence the binding of AEA to cholesterol-containing membranes or affect its ability to bind to serum albumin, the compounds do interact with intracellular proteins over an above their well established ability to inhibit FAAH. Whether these are "off targets" of the compounds or contribute to their ability to slow down the rate of cellular uptake of AEA remains to be elucidated.

HUMAN TUMOR SUPPRESSORS HAVE A NAPE-FORMING N-ACYLTRANSFERASE ACTIVITY

Toru Uyama[1], Xing-Hua Jin[1], Naoki Shinohara[1], Kazuhito Tsuboi[1], Takeharu Tonai[2] and Natsuo Ueda[1]

[1]Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan;
[2]Department of Orthopedic Surgery and Clinical Research Institute, National Zentsuji Hospital, Zentsuji, Kagawa, 765-0001, Japan.

Ca2+-independent N-acyltransferase (iNAT) is an enzyme capable of transferring an acyl chain of phosphatidylcholine (PC) to the amino group of phosphatidylethanolamine (PE) and generates N-acyl-PEs (NAPEs) known as precursors of anandamide and other N-acylethanolamines. Recently, we found that the tumor suppressor H-rev107 acts as a Ca2+-independent cytosolic phospholipase A1/2 with a low N-acyltransferase activity. iNAT and H-rev107 show structural similarity to each other and belong to the LRAT (lecithin retinol acyltransferase) family. Our database search revealed that HRASLS2 and TIG3 are also tumor suppressors belonging to this protein family. However, they have not been characterized as enzymes. In this study, we purified recombinant proteins of human HRASLS2 and TIG3 from COS-7 cells and examined their possible enzyme activities. The purified proteins showed Ca2+-independent phospholipase A1/2 activity toward various PCs and PEs. They also catalyzed the O-acylation of lyso PC, using PC as a donor substrate. In addition, HRASLS2 showed an N-acyltransferase activity to generate NAPE, while TIG3 had a very low N-acyltransferase activity. RT-PCR analyses revealed ubiquitous expression of HRASLS2 and TIG3 in human tissues, but their expression profiles were different. Together with our previous results, these results indicated that the LRAT family members including iNAT, H-rev107, HRASLS2 and TIG3 are a novel class of phospholipid-metabolizing enzymes. Among them, iNAT and HRASLS2 showed a relatively high NAPE-forming N-acyltransferase activity.

DESIGN OF ANTAGONISTS FOR PGF2α-ETHANOLAMIDE AND PGE2-GLYCERYL ESTER BY USING A SINGLE OXABICYCLOHEPTANE SCAFFOLD

J.L. Martos[3], D.F. Woodward[1], J.W. Wang[1], R.A. Ross[2], C. Cornell[3], S.N. Pettit[3], R.W. Carling[3] and H. Fliri[3]

[1]Department of Biological Sciences, Allergan, Inc., Irvine, CA, U.S.A.;
[2]Department of Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland;
[3]Discovery Department, Selcia Ltd., Fyfield, Essex, England

The mammalian endocannabinoids, 2-Arachidonyl glycerol and Arachidonyl ethanolamide, are substrate for cyclo-oxygenase-2 (COX-2), with the resultant formation of prostaglandin (PG) glyceryl esters and ethanolamides (prostamides). PGE2-glyceryl ester and PGF2α-ethanolamide have received particular attention, since agonist comparison studies with PGs have suggested pharmacologically distinct receptors that preferentially recognize PGE2-glyceryl ester and prostamide F2α.

In an attempt to further elucidate the pharmacology of the anti-glaucoma drug Bimatoprost, antagonists for prostamide F2α were targeted. The TP receptor antagonist BMS180,291 provided a lead structural template. The core oxobicycloheptane was retained, the first key structural modification being replacement of the anionic carboxylate with a neutral monoalkylamide. This produced the prototypical prostamide antagonists AGN 204396-7. Antagonist potency was subsequently improved 100 fold by replacing the benzylic methylene at position 3 with an oxygen atom.

To further elucidate PGE2-glyceryl ester pharmacology, a cell model (monocyte derived human osteoclasts) that elicited a Ca2+ signal response to PGE2-glyceryl, but not PGE2, was employed. A PGE2-glyceryl antagonist AGN 217673 was prepared by substituting with serinolamide at the C1 position of the oxabicycloheptane scaffold. The PGE2-glyceryl ester antagonist AGN 217673 did not block DP1-2, EP1-4, FP or IP receptors, although activity at TP receptors was retained. Importantly, AGN 217673 did not antagonize the effects of prostamide F2α or Bimatoprost in vitro or in vivo. AGN 217673 is, to the best of the authors knowledge, the first compound shown to selectively block the effects of PGE2-glyceryl ester and may provide a useful pharmacological “tool”.

POSSIBLE "ENTOURAGE" EFFECT OF PALMITOYLETHANOLAMIDE IN DOG AND HUMAN PLASMA

Stefania Petrosino[1,4], Pilar Brazis[2], Anna Puigdemont[3], Mariella Fusco[4], Francesca Comelli[5], Barbara Costa[5] and Vincenzo Di Marzo[1]

[1]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, CNR, Pozzuoli, NA, Italy; [2]UNIVET. Spain; [3]Dept Farmacología, Universitat Autònoma de Barcelona, Spain ; [4]Epitech Group S.r.l., Padova, Italy; [5]Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano, Italy

Introduction: Palmitoylethanolamide (PEA) has emerged recently as an important local pro-homeostatic mediator that can be also administered exogenously as the active principle of anti-inflammatory and analgesic preparations. Several mechanisms have been proposed to explain these effects of PEA. One of these is the "entourage effect" on the tissue levels or CB1-, CB2- or TRPV1-mediated effects of endocannabinoids. Here we have studied the plasma bioavailability of an acute oral administration of PEA to either dogs or humans and its effects on plasma endocannabinoid levels.

Methods: Four Beagle dogs spontaneously hypersensitive to Ascaris suum, with mean body weights of 14.0±0.6 kg were used in this study. The animals were fasted overnight before per os administration of ultra-micronized PEA (30 mg/kg). Ten fasted healthy human volunteers (4M/6F, age 43±7, mean body weight 73±12) were also recruited and administered with either 300 or 1200 mg (2x600 mg/tablet) of ultra-micronized PEA, after giving informed consent. In dogs, blood sample collection was carried out before administration of PEA (T0), and 1 (T1), 2 (T2), 4 (T4) and 8 (T8) hours after administration. In human volunteers, instead, blood sample collection was carried out before (T0), and after 2 (T2), 4 (T4) and 6 (T6) hours after administration of PEA, in the experiment with 300 mg of the compound; and before (T0), and 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after administration of PEA, in the experiment with the 1200 mg dose. After blood sample collection, plasma lipids were extracted, pre-purified on silica gel and submitted to isotope dilution LC-MS analysis for anandamide (AEA), 2-arachidonoylglycerol (2-AG), PEA and oleoylethanolamide (OEA).

Results: In dogs, a significant 6-fold elevation of PEA plasma levels vs. T0 was observed at T1 and T2, accompanied by a strong (15-20-fold) elevation of 2-AG, but not AEA or OEA, levels. Both PEA and 2-AG levels returned to normal between T4 and T8. In healthy volunteers, PEA plasma levels were increased 2-fold at T2 with 300 mg PEA, whereas 2-AG levels were slightly reduced at T2, and enhanced between T4 and T6 vs. T0. PEA plasma levels were strongly increased (9-fold) at both T1 and T2 with the higher dose of 1200 mg, and in this case 2-AG levels were slightly increased only at T6 vs. T0. No changes were observed at any time or dose for AEA and OEA levels.

Conclusions: This is the first demonstration that orally administered PEA reaches the bloodstream in dogs and human subjects, and that PEA elevates circulating 2-AG levels. The stronger and more rapid "entourage" effect of PEA observed in dogs, compared to healthy patients, might be due to the former being sensitised to allergic reactions to A. suum, or to the more favourable bioavailability of PEA in this species.

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ANALYSIS OF THE STIMULATORY EFFECT OF RIMONABANT ON THE GABAERGIC SYNAPTIC TRANSMISSION IN THE CEREBELLAR CORTEX

Bela Szabo and Sophia Linder

Institut für Pharmakologie, Albert-Ludwigs-Universität, 79104 Freiburg, Germany

Introduction. CB_1 cannabinoid receptors are typically localized on axon terminals, and their activation leads to inhibition of neurotransmitter release (Szabo B & Schlicker E, Handb Exp Pharmacol 168:327–365, 2005). The presynaptic CB_1 receptors are frequently targeted by endocannabinoids synthesized in postsynaptic neurons (endocannabinoid-mediated retrograde synaptic signaling; for review see Kano M et al., Physiol Rev 89: 309–380, 2009). In a recent publication, the CB_1 receptor antagonist AM251 elicited a surprizing effect in the cerebellar cortex: it markedly increased the spontaneous GABAergic synaptic input to Purkinje cells (Ma YL, Brit J Pharmacol 154: 204–215, 2008). The aim of the present study was to clarify the mechanism of this effect.

Methods. GABAergic inhibitory or glutamatergic excitatory postsynaptic currents (IPSCs or EPSCs) were recorded with patch-clamp techniques in Purkinje cells in mouse cerebellar brain slices. If not otherwise stated, patch-clamp pipettes were filled with a CsCl-based intracellular solution.

Results. The cumulative amplitude of miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin was markedly enhanced (more than twofold) by rimonabant (10^{-6} M). Importantly, spontaneous calcium spikes were not observed in these recordings. Rimonabant failed to enhance mIPSCs in slices prepared from brains of CB_1/CB_2 double-knockout mice (generated by Andreas Zimmer, Bonn, Germany). Spontaneous IPSCs (sIPSCs) recorded with pipettes containing K-gluconate were not affected by rimonabant. EPSCs evoked by electrical stimulation of parallel fibers (eEPSCs) were also not affected by rimonabant.

Conclusions. The results of the experiments on CB_1/CB_2 double-knockout mice point to involvement of CB_1 receptors in the effect of rimonabant. The lack of effect of rimonabant on eEPSCs indicates that endocannabinoids are not diffusely present in the extracellular space around the Purkinje cells, equally inhibiting GABAergic and glutamatergic transmission. Rather, the CB_1 receptor-mediated inhibition is restricted to GABAergic axon terminals. The exact mechanism of this inhibition remains enigmatic. Further experiments will be carried out to test the possibility that CB_1 receptors on GABAergic axon terminals are constitutively active. We will also search for mechanisms triggering endocannabinoid production selectively in the vicinity of GABAergic axon terminals.

INSULIN INCREASES ANANDAMIDE UPTAKE IN CULTURED HUMAN ADIPOCYTES

Jemma C. Cable[1], Saoirse E. O'Sullivan[1] and Christopher J. Fowler[2]

1. University of Nottingham Medical School at Derby, Uttoxeter Road, Derby, UK
2. Department of Pharmacology and Clinical Neuroscience, Umea University, Sweden

In cultured human adipocytes, cannabinoid receptor (CB_1) activation stimulates insulin-induced glucose uptake (1). In 3T3-L1 cells, insulin increases fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) mRNA and reduces intracellular levels of anandamide and 2-AG (2). The aim of this study was to investigate whether cellular uptake, the first step in the termination of endocannabinoid signalling, is stimulated by insulin or glucose.

Differentiated human adipocytes grown in 24-well plates were incubated with glucose (15 mM) and/or insulin (1 μM) for 2 or 24 hours. After washing and preincubation with a FAAH inhibitor (URB597, 1 μM), a MGL inhibitor (JZL184, 1 μM) or vehicle, [^{3}H]-AEA or [^{3}H]-2-AG was added and intracellular tritium counted (3,4).

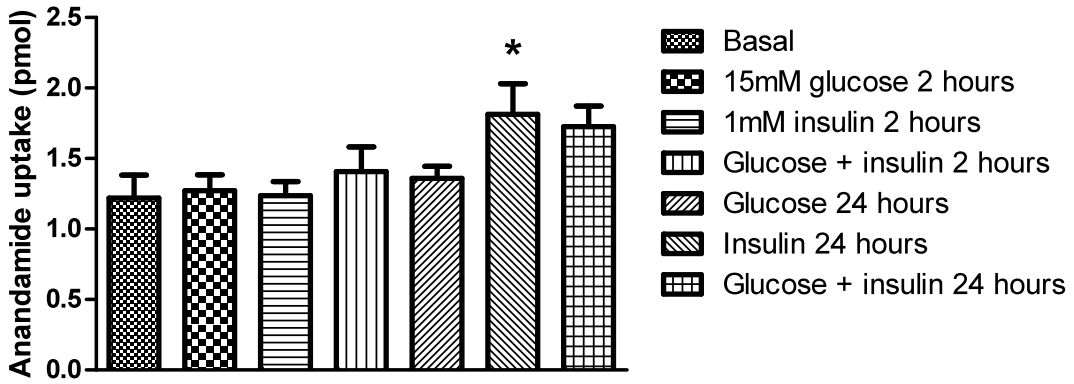

Fig. 1. Anandamide uptake in differentiated human adipocytes

Anandamide uptake was not affected by either 2 or 24 hours' exposure to glucose. 24 h, but not 2 h, treatment with insulin significantly increased anandamide uptake by adipocytes. URB597 did not affect basal anandamide uptake, indicating that anandamide uptake in human adipocytes is FAAH independent. Additionally, URB597 did not affect insulin-stimulated anandamide uptake. 2-AG uptake was not affected by glucose or insulin over 2 or 24 hours. JZL184 did not affect 2-AG uptake, indicating that 2-AG uptake in human adipocytes is not concentration dependent.

Our data indicate that endocannabinoid uptake in human adipocytes is not concentration dependent. The stimulatory effect of insulin on anandamide uptake is further evidence for a relationship between endocannabinoid and metabolic signalling, and more extensive studies are needed to explore the mechanisms behind this.

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2. D'eon et al. (2008) Diabetes **57**: 1262-1268.
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4. Sandberg and Fowler (2005) Chemistry and Physics of Lipids **134**: 131-139.

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF ARYLDITHIOCARBAMATES AS SELECTIVE MONOACYLGLYCEROL LIPASE INHIBITORS.

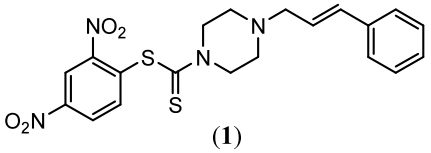
Coco N. Kapanda, Geoffray Labar, Jacques H. Poupaert and Didier M.Lambert

Université catholique de Louvain, Louvain Drug Research Institute, Cannabinoid and endocannabinoid research group, Pharmaceutical Chemistry Dpt, B-1200 Bruxelles, Belgium.

Introduction: Modulation of endocannabinoid system is of utmost therapeutic relevance owing to its implication in several major physiopathological processes. In this connection, the inhibition of enzymes responsible for endocannabinoid degradation (MAGL, FAAH and others) represents an attractive approach to modulate this system. A previous report from our group showed that arylthioamide scaffold represents a useful template for designing new MAGL inhibitors[1-2]. We now wish to report on synthesis, pharmacological evaluation and SAR of a series of aryldithiocarbamate derivatives, originated from pharmacomodulations of arylthioamides.

Methods: Target compounds were synthesized by reacting aromatic halides with dithiocarbamates. The inhibition of MAGL and FAAH was measured using pure human recombinant MAGL and human recombinant FAAH, respectively.

Results: 2, 4-dinitrophenyl 4-cinnamylpiperazine-1-carbodithioate (**1**) was found to inhibit human MAGL with a pI_{50} of 6.03 ± 0.09, along with a good selectivity compared to FAAH inhibition (40% maximal inhibition at 10^{-3}M). MAGL inhibition within this series was dependent on the presence of the lipophilic group, as similar compounds lacking, for instance, the cinnamylpiperazine moiety were found less potent inhibitors. Beside this, evaluation of the inhibitory potential of **1** on MAGL cysteine mutants revealed that inhibition of the enzyme occurs, at least in part, through an interaction with Cys208 and/or Cys242. The pharmacological profile of **1** was further characterized using 4-nitrophenyl propionate as substrate. Using this assay, a rapid dilution experiment allowed revealing the irreversible nature of the inhibition.



(**1**)

Conclusion: We have shown that aryldithiocarbamate derivatives are promising human MAGL inhibitors that display a comfortable selectivity profile when compared to the FAAH. Additionally, these compounds may constitute pharmacological tools useful to explore the endocannabinoid system. Further work is now devoted to delineating the SAR of this original series of aryldithiocarbamates.

References : [1]Kapanda *et al.* J. Med. Chem. 2009; 52:7310-4. [2]Kapanda *et al.* Med. Chem. Res. 2009; 18:243-54.

FREE FATTY ACID LEVELS IN HUMAN PLASMA CORRELATE WITH *N*-ACYLETHANOLAMINES

Michiel GJ Balvers[1,2], Michel M Joosten[1,2], Kitty CM Verhoeckx[2], Heleen M Wortelboer[2], Jocelijn Meijerink[1], and Renger F Witkamp[1,2]

[1] Wageningen University, Division of Human Nutrition. PO Box 8129, 6700 EV Wageningen, The Netherlands, and [2] TNO Quality of Life, Zeist, The Netherlands

N-acylethanolamines (NAEs), such as the endocannabinoid anandamide (AEA), are a group of endogenous lipids with important biological functions including regulation of food intake and inflammatory processes. It has been documented that the relative abundance of dietary fatty acids correlates with changes in levels of specific NAEs. In addition, plasma AEA levels are increased in obesity, which is also characterized by chronically elevated plasma free fatty acid (FFA) levels. Finally, both plasma FFA and AEA levels decrease postprandially. This made us hypothesize that NAE levels in human plasma are a general reflection of their specific precursor FFAs.

Data from three human studies was investigated. In the first study, blood was drawn from female subjects after an overnight fast. In the second study, blood was drawn before and frequently after a standardized lunch to investigate postprandial changes in FFA and NAE levels over time. In the third study, two subjects started consuming fish oil preparations for 4 weeks and one regular fish-oil user refrained from consuming fish oil capsules. Levels of several NAEs were quantified using an LC-MS/MS method for human plasma described earlier [1]. Total FFA levels were determined using Olympus analytical equipment and reagents. Individual FFA levels were quantified using a high-resolution UHPLC-MS technique reported elsewhere [2]. Statistical analysis was performed using SAS version 9.0.

Fasting AEA levels correlated with total FFAs ($r=0.84$; $p<0.001$) and arachidonic acid levels ($r=0.42$; $p<0.05$). Similar results were observed for other NAEs with both total FFAs and their corresponding fatty acid precursors. Changes over time in postprandial AEA were also correlated with changes in total FFA ($r=0.73$; 0.41-0.89). Again, comparable correlations were found between total FFA and other NAEs and also for specific FFAs with their respective NAEs. Finally, 4 weeks consumption of fish oil capsules only slightly altered NAE levels, whereas termination of consumption resulted in a marked decrease in plasma DHEA levels and an increase in OEA, PEA and SEA levels.

In conclusion, in fasting and non-fasting states circulating *N*-acylethanolamine patterns were found to be a reflection of their corresponding free fatty acids. Considering their diverse biological effects it seems useful to study the biological significance of NAE patterns in addition to that of individual components.

[1] *Balvers et al, J Chrom B 2009*
[2] *Kleemann et al, PLOS One 2010*

PITFALLS AND SOLUTIONS IN ASSAYING ANANDAMIDE TRANSPORT IN CELLS

Giuseppina Catanzaro, Sergio Oddi, Filomena Fezza, Chiara De Simone, Mariangela Pucci, Daniele Piomelli, Alessandro Finazzi-Agrò and Mauro Maccarrone

Department of Biomedical Sciences/IRCCS S. Lucia Foundation

The nonspecific binding of anandamide to plastic exhibits many features that could be mistaken as biological processes, thereby representing an important source of conflicting literature data on the uptake and release of this lipophilic substance. In the present study, we sought to minimize the errors associated with the nonspecific binding of AEA to plastic ware, by trying a methodological alternative to traditional protocols. To this aim, we carried out the experiments on glass support (coverslips) instead of plastic, exploiting the limited tendency of hydrophobic molecules to bind the hydrophilic surface of borosilicate glass. In this investigation we used radioactive and immmunocytochemical assays to measure the uptake and the export of AEA. Concerning radioactive assays, in order to compare the results obtained using different supports, we used [^{3}H]AEA in the presence or in the absence of coverslips,. The background, that is the uptake and the export carried out at 4°C, was subtracted from the results obtained at 37°C. As for immunocytochemical assays, cells were treated with bAEA in serum-free medium at 37°C and 4°C, the latter for background subtraction, and the fluorescence was detected by incubating the coverslips with the streptavidin Alexa Fluor 488-conjugated antibody.

Although the results obtained using plastic do not differ significantly from those obtained using glass, the new procedure has the advantage to be faster, simpler and more accurate. In fact, the lack of aspecific adsorption of anandamide to the glass surface yields a lower background, a higher precision and accuracy in determination of transport kinetics, especially for the export process. Remarkably, when measured with this procedure the K_m constant for anandamide uptake is ~40% smaller than that found with the traditional method, while the V_{max} value does not change significantly. This study describes a fast, simple, and reliable glass-based assay for measuring *in vitro* AEA uptake and release. This approach is aimed at avoiding some of the artefacts generated by the nonspecific binding of AEA to plastic and the complicated kinetics of AEA/BSA interaction. Considering the fact that AEA does not bind appreciably borosilicate glass, the use of coverslips seems particularly indicated to perform export experiments. More importantly, the new procedure can be also extended to subcellular level, employing b-AEA as a non-radioactive probe to look at anandamide trafficking within intact cells by means of light, electron and fluorescence microscopy techniques.

TEMPORAL SPECIFIC CHANGES THAT OCCUR IN ADOLESCENT LIMBIC ENDOCANNABINOID SIGNALING: RELEVANCE TO EMOTIONAL BEHAVIOR IN ADULTHOOD

Tiffany T.-Y. Lee[1], Matthew N. Hill[2], Silvain Dang[1],
Cecilia J. Hillard[3] and Boris B. Gorzalka[1]

[1]University of British Columbia, Vancouver, V6T 1Z4, Canada;
[2]Rockefeller University, New York, 10065, USA;
[3]Medical College of Wisconsin, Milwaukee, 53226, USA

Adolescence is a critical stage of life in which the brain undergoes developmental changes leading to sexual, cognitive and emotional maturation. There is a growing amount of evidence to suggest that the endocannabinoid (eCB) system is key in early pre- and post-natal development. With respect to adolescence, it has been shown that escalating doses of delta-9-tetrahydrocannabinol (THC) during this period can result in long lasting neuroanatomical, emotional and cognitive impairments in adulthood. However, little is known about the naturally occurring biochemical changes and activity of the eCB system throughout adolescence, within corticolimbic structures subserving cognition and emotionality.

We examined tissue content of the two eCB ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in the hippocampus, prefrontal cortex, hypothalamus and amygdala on post-natal days (PND) 25, 35, 45 and 70. While we did not detect any changes in 2-AG content, AEA levels dramatically increased from pre-adolescence (PND 25) to early adolescence (PND 35) in every structure except the PFC. AEA levels declined by late adolescence (PND 45) but then increased again by adulthood (PND 70).

To determine if this spike in limbic AEA in early-adolescence modulated the development of emotional behaviour into adulthood, we examined the effects of CB1 receptor blockade throughout adolescence on tests of emotionality in adulthood. Rats were administered CB1 receptor antagonist, AM-251 (1 mg/kg), or vehicle from PND 35-47 and then left undisturbed until adulthood (PND 75) at which point they were tested on behavioural coping responses in the forced swim test and locomotion in the open field test. Surprisingly, a significant reduction in immobility with a corresponding increase in struggling behaviour was seen in the forced swim test; a shift towards active coping responses suggestive of an “antidepressant-like” response. Open field test data showed no significant differences in average speed or total line crosses indicating that the changes seen in the forced swim test were not due to changes in motor function. Ongoing studies will examine the effects of this treatment on other aspects of emotional behaviour, stress responsivity and neural markers of plasticity and resilience.

CONCENTRATIONS OF Δ^9-TETRAHYDROCANNABOLIC ACID DURING GROWTH AND DEVELOPMENT OF MEDICINAL GRADE CANNABIS PLANTS

M. de Wit, L Saddik and S.F.A.J. Horsten

Maripharm BV, Nieuw-Mathenesserstraat 33, 3029 AV Rotterdam, The Netherlands

Introduction

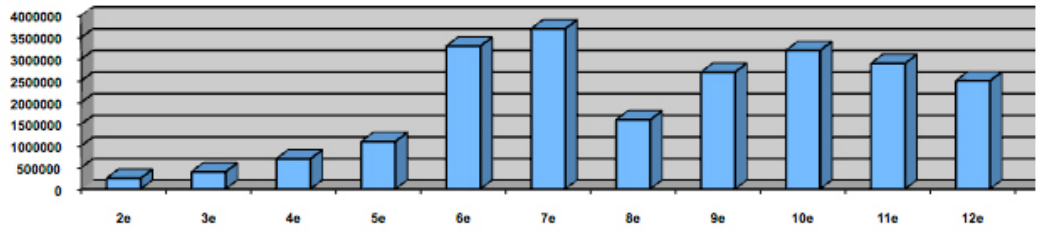
Under the general conditions applied for growing Medicinal Grade Cannabis (MGC 1001), the point when about 50% of the stigmas on the inflorescences have turned brown marks the start to harvest. Normally this happens when plants are 9-10 weeks old, after the cuttings have been fully rooted and transferred to the cultivation cel. To correlate this rule of thump applied by the growers to the actual content of Δ9-tetrahydrocannabolic acid (THC-A) in the plants, the following investigation was undertaken.

Material and Methods

From the content of 1 production batch (Total of 200 plants) 6 individual plants were randomly selected. At weekly intervals, from week 2 till week 12, one flowering top from each plant was obtained. The total of 6 tops were finely cut, extracted, and analysed for their THC-A content by HPLC. Since at the time of this investigation, no certified THC-A standard, was available, the results are expressed as areas. However, by means of a in house standard the HPLC method used was shown linear over the range analyzed, with good precision (Rsd < 1.5%).

Results and Conclusion

The results of the experiment are reproduced in figure 1.



Although 7 weeks old plants contain the highest absolute amounts of THC-A, they only have a few branches with inflorescences, and small primary flowers. Therefore, as a whole, the relative THC-A concentration is still rather low. To stimulate branching and development of many more inflorescences, plants undergo a calculated water and fertilizer management scheme. The results of this procedure can be seen as a drop of THC-A concentration in week 8, followed by a gradual increase again in week 7; however, due to excessive branching of 10 weeks old plants they contain relative the most of Δ9-tetrahydrocannabolic acid. This furthermore correlates exactly with the point when about 50% of the stigmas of the inflorescences have turned into brown. Δ9-tetrahydrocannabolic analyzed concomitantly by HPLC is only present in very small quantities when compared to its acid form. Its absolute concentrations generally follow the pattern of THC-A.

EFFECT OF TOPICAL AND SYSTEMIC ADMINISTRATION OF ABNORMAL CANNABIDIOL ON INTRAOCULAR PRESSURE IN BROWN NORWAY RATS

Szczesniak AM, Slusar JE and Kelly MEM[†]

Laboratory for Optic Nerve and Retina Research, Dalhousie University
5850 College St, Halifax, Nova Scotia, B3H 1X5
Canada

In humans, intraocular pressure (IOP) is determined by the secretion of aqueous humor, from the ciliary body, and outflow resistance, via the trabecular and uveoscleral routes. Cannabinoid receptors are present on tissues of both these inflow and outflow pathways and cannabinoid ligands decrease IOP in humans. Abnormal cannabidiol **(**Abn-CBD) is a synthetic, non-psychoactive cannabinoid, which does not activate cannabinoid 1 (CB1) or cannabinoid 2 (CB2) receptors. This study investigated the effect of topical and systemic administration of Abn-CBD on IOP.

IOP was measured with a rebound tonometer (TonoLab; Icare, Finland), in Brown Norway rats, at 15 minute intervals for 2 hours. Animals were placed on the countertop and the TonoLab was gently placed against the anesthetized surface of the cornea. All drugs were administered either by intraperitoneal injections (i.p.) or topically. O1918 (2.5 mg/kg), the endothelial cannabinoid receptor antagonist, and the CB_1 specific receptor antagonist, AM251 (2.5 mg/kg), were administered i.p. 20 minutes prior to the injection with Abn-CBD.

Topical administration of 1.5% and 2% Abn-CBD significantly decreased IOP in the rat eye, from 19.7 ± 0.5 mmHg to 17.4 ± 1.1 mmHg, and from 20.7 ± 0.25 mmHg to 19.2 ± 0.13 mmHg, respectively, (mean ± SEM). The i.p. administration of Abn-CBD, also reduced IOP in a dose-dependent manner (0.025 mg/kg-10mg/kg). A significant drop in IOP, from 21.3 ± 0.29 mmHg to 20.1 ± 0.29 mmHg, and from 21.3 ± 0.12 mmHg to 19.8 ± 0.12 mmHg, respectively, was seen with 2.5 and 10 mg/kg doses of Abn-CBD. The IOP-lowering effect of i.p. Abn-CBD was significantly reduced by pre-administration of O1918 (2.5 mg/kg). Pre-administration of AM251 (2.0 mg/kg) did not block the IOP-lowering effect of Abn-CBD.

The IOP lowering effects of Abn-CBD were insensitive to AM251, but sensitive to O-1918. This implies that Abn-CBD does not exert its effects though the CB1, but may act at non-CB1/CB2 target(s), such as the endothelial cannabinoid receptor. Both topical and i.p. routes of administration of Abn-CBD effectively decreased IOP in the rat eye, suggesting that this atypical cannabinoid has the potential to function as a novel ocular hypotensive cannabinoid devoid of psychotropic activity. Further studies are required to determine the ocular tissue targets for IOP-lowering actions of Abn-CBD.

PERIPHERAL ANTINOCICEPTIVE EFFECTS OF INHIBITORS OF MONOACYLGLYCEROL LIPASE IN A RAT MODEL OF INFLAMMATORY PAIN: A COMPARATIVE ANALYSIS

Josée Guindon[1] and Andrea G. Hohmann[1]

Neuroscience and Behavior Program[1], Department of Psychology, University of Georgia, Athens, GA 30602-3013

Introduction: The endocannabinoid 2-arachidonoylglycerol (2-AG) is degraded primarily by monoacylglycerol lipase (MGL). MGL thus represents a potential analgesic target. We compared peripheral antinociceptive effects of JZL184, a novel selective MGL inhibitor, with the MGL-preferring inhibitor URB602 and exogenous 2-AG. Antinociceptive effects of JZL184 have not been reported in rats.

Methods: Nociception was assessed in the formalin test in 26 experimental groups receiving dorsal paw injections of: (1) vehicle; (2-10) JZL184 (0.001-300μg); (11-19) URB602 (0.001-600 μg); (20) 2-AG (ED_{50}); (21) 2-AG+JZL184 (at their ED_{50}); (22) 2- AG+URB602(at their ED_{50}); (23) AM251 (80 μg); (24) AM251 + JZL184 (10 μg); (25) AM630 (25 μg); (26) AM630 + JZL184(10 μg).

Results: Intra-paw administration of JZL184, URB602 and 2-AG suppressed both early and late phases of formalin pain. JZL184 (ED_{50} (Phase 1; Phase 2): 0.06 ± 0.028; 0.03 ± 0.011 μg) produced greater antinociception than either URB602 (ED_{50} (Phase 1; Phase 2): 120 ± 51.3; 66 ± 23.9 μg) or 2-AG. Both MGL inhibitors produced additive antinociceptive effects when combined with 2-AG. Similar slopes of the dose response curves suggest that JZL184 and URB602 acted through a common mechanism, albeit with different potencies. JZL184 combined with 2-AG produced a greater antinociceptive effect than that of URB602 with 2-AG. Like URB602, antinociceptive effects of JZL184 were blocked by both CB_1 and CB_2 antagonists. These results are consistent with our immunohistochemical studies showing the distribution of enzymes that regulate 2-AG levels in dorsal root ganglion and peripheral nerve.

Conclusions: MGL inhibitors (JZL184, URB602) suppress formalin-induced pain behaviour through peripheral CB_1 and CB_2 receptor mechanisms. MGL represents a therapeutic target for the treatment of inflammatory pain.

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CYCLOOXYGENASE-2-CATALYZED OXYGENATION OF 2-ARACHIDONOYLGLYCEROL IS MORE SENSITIVE TO PEROXIDE TONE THAN OXYGENATION OF ARACHIDONIC ACID

Joel Musee and Lawrence J. Marnett
Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 U.S.A.

Introduction: The endocannabinoid 2-arachidonoyl glycerol (2-AG) is a selective substrate for the inducible isoform of cyclooxygenase (COX), COX-2, *in vitro*. Its turnover leads to formation of glyceryl analogs of traditional prostaglandins (PG-Gs), a subset of which elicit agonism at unique receptors, at picomolar to nanomolar concentrations. While 2-AG is approximately one-tenth as abundant as arachidonic acid (AA) in zymosan-stimulated macrophages, the ratio of PGs to PG-Gs recovered from these macrophages is about 1000:1 (Rouzer, C.A. and Marnett, L.J. (2008) *J. Biol. Chem.* **47**:3917.). The activation of oxygenase activity in COX is dependent on the turnover of the peroxide product of AA or 2-AG oxygenation (PGG_2 and PGG_2-G respectively). We hypothesized that PGG_2-G is a less efficient POX substrate than PGG_2 and therefore a poor activator of 2-AG turnover. We have previously demonstrated that PGG_2 and PGG_2-G surrogates (15-HpETE and 15-HpETE-G) are reduced comparably as peroxidase substrates. Here we extend this finding to *in situ* generated PGG_2 and PGG_2-G, demonstrating that both peroxidase substrates are reduced equally effectively by the peroxidase active site of hCOX-2. Further than this, preliminary findings in 3T3 fibroblast demonstrate that stable knock-down of the phospholipid hydroperoxide glutathione peroxidase (PHGPx or GPx4) leads to 2-4 fold increase in PG-G production in these cells, with little to no change in PG production.

Methods: The reduction of *in situ* generated PGG_2 or PGG_2-G by hCOX-2 was measured by monitoring the consumption of the peroxidase reductant 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) at 414 nm and via LC/MS. Knock-down of GPx1 and GPx4 in 3T3 cells was achieved by stable transfection of shRNA plasmids corresponding to either gene, with selection for stable transfectants via puromycin. PG and PG-G production from 3T3 fibroblasts was determined via LC/MS/MS.

Results: While the extent of reduction of *in situ* generated PGG_2 and PGG_2-G by hCOX-2 was the same, steady-state determination of the reduction of either species by hCOX-2 via the ABTS assay demonstrated differing substrate availabilities with PGG_2-G seemingly lagging in its availability for reduction, relative to PGG_2. Murine 3T3 fibroblasts demonstrate that shRNA modulation of the endogenous peroxide detoxification system, Glutathione and glutathione peroxidase, lead to a 2 and 4 fold increase in PGE_2-G and $PGF_{2\alpha}$-G production in TPA/Ionomycin stimulated cells respectively, with little effect on concomitant PG production.

Conclusions: Here we further observations presented last year, where the oxygenation of 2-AG was demonstrated to be exquisitely sensitive to peroxide tone relative to AA; and demonstrate that this relative lag in PGG_2-G versus PGG_2 availability for reduction at the peroxidase active site, is responsible for 2-AGs sensitivity to peroxide tone for continued oxygenation by COX-2. Furthermore, *ex vivo* experiments demonstrate that this dependence on peroxide tone is partly responsible for the diminished production of PG-Gs in cell culture, in response to chemical agonism.

TISSUE-SPECIFIC DIFFERENCES IN THE REGULATION OF N-ACYL ETHANOLAMINES AND N-ACYL TAURINES BY FATTY ACID AMIDE HYDROLASE

Jonathan Z. Long, Xin Jin and Benjamin F. Cravatt

The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92073

In the central nervous system (CNS), fatty acid amide hydrolase (FAAH) regulates the endogenous levels of the fatty acid amide family of lipid transmitters, which include the endocannabinoid anandamide (*N*-arachidonoyl ethanolamine), the anti-inflammatory factor *N*-palmitoyl ethanolamine, and the satiating signal *N*-oleoyl ethanolamine, the sleep-inducing lipid oleamide, and the transient receptor potential (TRP) calcium channel agonists the *N*-acyl taurines. The genetic or pharmacological inactivation of FAAH causes dramatic accumulations of fatty acid amides in the nervous system and a range of therapeutically useful phenotypes, which consequently has resulted in considerable pharmaceutical interest in FAAH for the treatment of disorders such as chronic pain, anxiety, and depression. However, the regulation of fatty acid amides in peripheral tissues by FAAH remains poorly characterized. Here, we investigated the changes of two classes of fatty acid amides, the *N*-acyl ethanolamines (NAEs) and *N*-acyl taurines (NATs), in both the CNS and a panel of peripheral tissues upon administration to mice of PF-3845, a highly selective, urea-containing covalent inhibitor of FAAH. Anandamide hydrolysis (FAAH) activity was detected in all tissues tested except heart and brown fat, and this activity was entirely ablated upon PF-3845 treatment. Anandamide elevations were highly tissue-dependent, with brain and testis showing the most dramatic changes (> 10-fold), and kidney showing no changes, despite the presence of FAAH activity in this organ. In contrast, changes in shorter chain NAEs such as palmitoyl- or oleoyl-NAEs were most dramatically elevated in brain and liver (~10-fold) but far more modest in all other tissues. Acute FAAH blockade produced dramatic NAT accumulations (~20-60-fold) that were almost entirely restricted to the liver, kidney, and plasma, with very modest changes in all other organs. These data suggest that at least two different biosynthetic pathways exist for the formation of NAEs, one that produces anandamide (present in brain and testis) and another that produces shorter chain NAEs (present in brain and liver), and that the major NAT biosynthetic enzymes are likely restricted to liver and/or kidney, with NAT transport through circulatory system potentially elevating NATs in other organs.

DIFFERENTIAL ROLE OF ENDOCANNABINOIDS IN MEMORY CONSOLIDATION, ANALGESIA AND ANXIETY

Arnau Busquets-Garcia, Emma Puighermanal,
Rafael Maldonado and Andrés Ozaita

Laboratori de Neurofarmacologia (Neurophar). Departament de Ciències experimentals de la salut (CEXS). Universitat Pompeu Fabra (UPF). 08003 Barcelona, Spain.

Introduction:
Drugs based on the agonistic modulation of the endocannabinoid system (ECS) are potential therapeutic agents, although an important caveat to their use is the possible adverse effects related to memory impairment. An alternative approach to this caveat was based on the inhibition of the enzymatic degradation of the endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG).

Results:
Using the specific inhibitors of the endocannabinoid metabolizing enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), JZL184 and URB597 respectively, we report that the endocannabinoid AEA is a central component of the ECS in the modulation of memory consolidation, while the endocannabinoid 2-AG is not involved in such a process. This dichotomy is correlated by the modulation of mTOR activity by AEA and not by 2-AG in the hippocampus, an effect recently related to the amnesic responses induced by cannabinoids. Moreover, we show that the acute analgesic effects produced by JZL184 administration are maintained after chronic administration in three different pain paradigms (tail immersion, hot plate and acid acetic tests). In addition, the increase in 2-AG levels has anxiolytic-like effects in the elevated-pluz maze and the elevated-zero maze.

Conclusions:
These results segregate the role of endocannabinoids in memory function and support the therapeutic intervention over 2-AG degradation, in contrast to AEA metabolism, to preserve the therapeutic effects of 2-AG modulation avoiding the cognitive impairment promoted by those acting on AEA degradation.

INTERACTIONS OF NON-THC PHYTOCANNABINOIDS AND *CANNABIS* EXTRACTS WITH TRP CHANNELS AND ENDOCANNABINOID METABOLIC ENZYMES

Luciano De Petrocellis[1], Alessia Ligresti[2], Aniello Schiano Moriello[1], Marco Allarà[2], Tiziana Bisogno[2], Stefania Petrosino[2], Colin Stott[3] and Vincenzo Di Marzo[2]

Endocannabinoid Research Group, [1]Institute of Cybernetics and [2]Institute of Biomolecular Chemistry, CNR, Pozzuoli (NA), Italy; [3]GW Pharmaceuticals, UK

The endocannabinoid system is defined as the signalling system composed of: *i*) cannabinoid CB_1 and CB_2 receptors, *ii*) the endocannabinoids, i.e. anandamide and 2AG, and *iii*) proteins for endocannabinoid biosynthesis and degradation. More recently, it was suggested that this definition should encompass other non-CB_1, non-CB_2 targets for endocannabinoids. In particular, the transient receptor potential vanilloid type-1 (TRPV1) channel was demonstrated to be a target for anandamide, and recent evidence suggests that anandamide also antagonizes another TRP channel, the menthol/"cold" receptor, TRPM8. Furthermore, several pathways, and hence several alternative enzymes, were shown to participate in anandamide biosynthesis or 2-AG degradation. It is also emerging that phytocannabinoids interact with several different types of TRP channels as well as with endocannabinoid inactivating mechanisms (Ligresti et al., *J. Pharmacol. Exp. Ther*., 2006; De Petrocellis et al., *J. Pharmacol. Exp. Ther*., 2008). In the present study, we have investigated, by means of fluorescence-based TRP channel activity assays in transfected cells, and of specific radiolabelled substrate-based enzymatic assays, the effects of eleven non-THC phytocannabinoids, and, in most cases, of the corresponding botanical extracts (BDS), on TRPV1, TRPM8 and TRPA1 channels, as well as on human recombinant diacylglycerol lipase α (DAGLα), rat brain fatty acid amide hydrolase (FAAH), COS cell monoacylglycerol lipase (MAGL), human recombinant *N*-acylethanolamine acid amide hydrolase (NAAA), and anandamide cellular uptake (ACU) by RBL-2H3 cells. For cannabidiol (CBD) and cannabigerol (CBG) we tested the corresponding acids (CBDA, CBGA) and C_3 analogues (CBDV, CBGV), and for THC-acid (THCA) the corresponding C_3 analog (THCVA). Cannabinol (CBN), cannabichromene (CBC) and C_3-Δ^9-THC (i.e. tetrahydrocannabiverin, THCV), were also studied.

The main results obtained can be summarized as follows: 1) Only CBD, CBG and THCV, and much less so their analogues, potently stimulated TRPV1 (EC_{50}=0.7-1.5 μM), with the BDS being less active except for THCV; 2) none of the pure compounds was more potent than CBC, CBD and CBN as TRPA1 agonist, but THCV-BDS was the most potent on this target (EC_{50}=0.06 μM); 3) likewise, some BDS (CBG, THCV) could be very potent (IC_{50}<0.1 μM) at antagonizing TRPM8; 4) CBDV, and all the acids tested, exhibited inhibitory effects on DAGLα (IC_{50}=16.6-64.3 μM), this activity being retained only in the BDS of CBDA and THCA; 5) some BDS, but not the pure compounds, exhibited significant MAGL inhibitory efficacy (IC_{50}=6.2-29.6 μM), THCA-BDS being the most potent; 6) CBD was the only compound that inhibited FAAH, whereas the BDS of CBC>CBG>CBGV inhibited NAAA (IC_{50}=14.2-24.4 μM); 7) CBC=CBG>CBD inhibited ACU (IC_{50}=11.3-25.0 μM), but some BDS's (i.e. those of THCVA, CBGV, CBDA, THCA) were more potent (IC_{50}=5.8-12.5 μM) than the corresponding pure compounds in this assay; 8) a synergistic effect between CBG and "CBG-free" CBG-BDS appears to underlie the MAGL-inhibitory and TRPM8-antagonistic activity of CBG-BDS.

The reported effects might be relevant to the suggested analgesic, anti-inflammatory, anxiolytic and anti-cancer effects of phytocannabinoids and *Cannabis* extracts.

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GLUCOCORTICOID EFFECTS ON NEURONAL MONOACYLGLYCEROL LIPASE ACTIVITY

Lalita D. Shrestha and Cecilia J. Hillard

Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, USA

Monoacylglycerol lipase (MAGL) is a serine hydrolase that hydrolyzes endocannabinoid, 2-arachidonoylglycerol (2-AG), into arachidonic acid, and glycerol. Activity based protein profiling (ABPP) has shown that MAGL contributes to ~85% of 2AG hydrolysis in mouse brain [Blankman et. al. 2007, Chemistry and Biology 14(12): 1347]. MAGL-mediated hydrolysis of 2-AG is one of the major mechanisms to regulate (turn off) cannabinoid receptor type 1 (CB1R) signaling; however, very little is known about the mechanisms by which MAGL activity is regulated.
Glucocorticoids, steroid hormones released from the adrenal cortex in response to stress, exert both rapid and delayed effects on behavior by altering neuronal physiology. Delayed effects of glucocorticoids result from shuttling of cytosolic glucocorticoid receptor:ligand complex to the nucleus where it acts as a transcription factor. On the other hand, short-term glucocorticoid mediated effects are thought to result from activation of a G-protein coupled receptor. Multiple studies have shown that stress causes significant increases in 2-AG concentration in mouse brain. The underlying mechanism of increased 2-AG concentration is not known. We hypothesized that glucocorticoids decrease MAGL activity, resulting in decreased clearance of 2-AG and prolonged CB1 receptor activation.

Rat cerebellar granule neurons in culture (day 7-8), were treated with or without 100 nM dexamethasone (Dex), a glucocorticoid receptor agonist, at 37˚C for 15 minutes. Whole cell lysate was prepared by harvesting CGNs in PBS buffer, followed by sonication and passing the cells via a 27 gauge needle. MAGL activity was determined using radiolabeled 2-mono-oleyolglycerol as the substrate.

Our results indicate that MAGL-activity was decreased significantly by Dex treatment. In particular, Dex treatment resulted in a significant decrease in the Vmax of neuronal MAGL. These findings support the hypothesis that MAGL activity is regulated by glucocorticoids via a fast, nongenomic mechanism. Furthermore, these results provide a mechanism by which stress, via glucocorticoids, can enhance endocannabinoid signaling.

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THE ROLE OF THE ENDOCANNABINOID SYSTEM IN MESENCHYMAL STEM CELL DIFFERENTIATION, SURVIVAL AND MIGRATION

Aoife Gowran, Siobhan Hussey, Katey K McKayed and Veronica A Campbell

Department of Physiology and Institute of Neuroscience, University of Dublin, Trinity College, Ireland

Mesenchymal stem cells (MSCs) are adult stem cells isolated from bone marrow which can differentiate into different cell types *e.g.,* osteoblasts, chondrocytes and adipocytes. The exact physiological function of MSCs remains unclear at present, however, MSCs do play a role in tissue healing. MSCs represent an ideal cell population for use in regenerative medicine due to their easy isolation, ability to differentiate and immunosupressive effects. The aim of this study was to examine the role of the endocannabinoid system in the differentiation, survival and migration of MSCs. MSCs were isolated from the bone marrow of adult Wistar rats and expanded in culture. For differentiation studies MSCs were treated with osteogenic factors (OFs; 10 mM β-glycerolphosphate, 10 nM dexamethasone, 50 μM ascorbic acid) in the presence or absence of SR141716A (SR1; 1 μM), Δ^9-THC (1 μM) or an inhibitor of ERK (U0126; 2 μM). Osteogenesis was assessed by measuring osteocalcin mRNA and by quantifying matrix mineralisation. For migration experiments MSCs were grown in a Boyden chamber and were allowed to migrate for 24hr.

Osteocalcin mRNA was significantly increased from 0.3 ± 0.1 (fold change of average control) to 2.5 ± 0.4 by OFs and further significantly increased to 15.7 ± 5.1 when MSCs were exposed to SR1 in the presence of OFs (mean ± SEM; $p<0.05$, unpaired *t* test, n=5). Hydroxyapaptite deposits were significantly increased from 2.2 ± 0.2 (Relative Fluorescent Units (RFU) at 518 nm) to 7.2 ± 0.5 by OFs and further significantly increased to 11.2 ± 1.2 when MSCs were exposed to SR1 in the presence of OFs (mean ± SEM; $p<0.05$, unpaired *t* test, n=6). CB_1 mRNA expression was significantly increased from 0.4 ± 0.2 (fold change of average control) to 6.2 ± 1.3 by OFs (mean ± SEM; $p<0.01$, unpaired *t* test n=5). The increased level of CB_1 receptor expression was essential for the survival of differentiated MSCs, since SR1 prevented the ability of differentiated MSCs to survive growth factor withdrawal. Treatment with Δ^9-THC and U0126 had a negative effect on the differentiation and survival of MSCs. MSC migration was significantly induced by AEA (1 μM), the response being 240.6 ± 25.3% of the response to TGF-β (5 ng/ml; mean ± SEM; $p<0.05$, unpaired *t* test, n=5). This response was CB_1 dependent and of chemotactic nature as determined by using SR1 and checkerboard experiments respectively.

These novel findings show that the endocannabinoid system is upregulated during MSC osteogenesis and has an essential role in the survival and migration of MSCs.

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VALIDATION OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF AEA, OEA AND PEA IN HUMAN SEMINAL PLASMA BY UPLC-MS/MS

Akwasi A. Amoako[1], Timothy H. Marczylo[1], Patricia M.W. Lam[1], Amanda Derry[2], Jonathon Willets[1], Janine Elson[2] and Justin C. Konje[1] *

[1]Endocannabinoid Research Group, Reproductive Science Section, Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, LE2 7LX, United Kingdom. [2]Assisted Conception Unit, University Hospitals of Leicester NHS Trust, Leicester Royal Infirmary, Leicester, LE1 5WW, United Kingdom

Introduction: The endocannabinoids are a group of bioactive lipid signalling molecules which have been implicated in the signalling transduction pathways involved in mammalian sperm motility, capacitation and acrosome reaction. Anandamide (*N*-arachidonoylethanolamide, AEA), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) are N-acylethanolamide (NAE) endocannabinoids present in human seminal plasma. There is a growing interest in the seminal plasma levels of these endocannabinoids and their biological activity in relation to human sperm function. Although several analytical methods have been developed to measure these compounds in biological fluids and tissues, none of these methods has been validated for analysis in human seminal plasma. We describe a highly sensitive, robust, reproducible method for the simultaneous quantification of AEA, OEA and PEA in human seminal plasma.

Materials and methods: Seminal fluid was obtained from 30 human donors with normal semen parameters. Seminal plasma was separated from spermatozoa by centrifugation and lipids extracted using both liquid and solid phase extraction techniques. AEA, OEA and PEA were quantified by UPLC-MS/MS using isotope dilution.

Results: Extraction efficiencies using SPE were 54%, 49% and 30% for AEA, OEA and PEA respectively whilst LPE yielded lower extraction efficiencies of 21%, 25% and 14% for AEA, OEA and PEA respectively. The inter-day (n=20) and intraday (n=5) variability for AEA, PEA and OEA were 6.3% and 6.6% for AEA, 10.2% and 11.2% for OEA and 12.5% and 17.7% for PEA. For all analytes, the inter-day precision was within a CV% of 6.6% - 17.7% and intraday CV% was 6.3% -12.5%, all were within acceptable limits. ***Regression analysis indicated that the analyses of AEA, PEA and OEA were linear over the range 0.237-19nM for AEA and OEA and 0.9-76 nM for PEA.*** LOD were 50 fmol/ml, 100 fmol/ml and 100 fmol/ml and LOQ were 100 fmol/ml, 200 fmol/ml and 200 fmol/ml for AEA, PEA and OEA, respectively. Whilst AEA and OEA remained relatively stable at -80^{o}C for up to 4 weeks, the levels of PEA showed a significant decline at 7 days. Following validation we assessed the NAE concentrations in seminal plasma from 30 human donors with normozoospermia and found mean concentrations of 0.164nM, 1.723nM and 11.65nM for AEA, OEA and PEA, respectively.

Conclusions: This method was shown to be linear, precise and accurate for the quantification of NAEs in human seminal plasma. The mean concentrations of the endocannabinoids were lower than those levels previously reported.

DIFFERENTIAL MODULATION OF PTSD SYMPTOMATOLOGY VIA GENETIC MODULATION OF ENDOCANNABINOID RECEPTORS

Caitlin J. Riebe and Carsten T. Wotjak

Max-Planck-Institute of Psychiatry, Neuronal Plasticity Group
Kraepelinstrasse 2, D-80804, Munich

Endocannabinoid system functioning in various aspects of fear and anxiety has been quite widely investigated; however, the role of the endogenous cannabinoid system in more specific anxiety-related disorders, such as post-traumatic stress disorder (PTSD), has yet to be explored. Clinical PTSD is characterized by clusters of symptoms including hyperarousal, contextual fear and avoidance. We have developed a mouse model of PTSD that generates all three symptom clusters and a subsequent battery of behavioural tests for the discrimination among specific aspects of fear and anxiety inherent in the disorder.

Induction of PTSD in mice was achieved through a brief, unsignaled foot shock in a distinctive chamber after which animals were left undisturbed for 28 days to allow incubation of PTSD-like symptoms. Behavioural testing took place after this incubation period and included measurements of startle and avoidance behaviour as well as fear responses to the shock chamber and other environments.

By employing this model on several lines of CB_1 and $TRPV_1$ transgenic mice we have gained insight into the differential effects of distinct receptor ablation on particular aspects of the PTSD model symptomatology. For example, total CB_1 knockouts show distinct changes in PTSD-like symptoms with tendencies towards reduced fear responses and hyperarousal. $TRPV_1$ knockout mice also show specific reductions in generalized fear response as well as differences in avoidance behaviour. In contrast, conditional CB_1 mutant lines, such as the D_1-CB_1 knockout mice, show increased fear in some contexts despite an overall reduction in hyperarousal and a lack of avoidance behaviour.

Such results help to shed light on the differential involvement of the endogenous cannabinoid system in specific features of PTSD-like symptoms. Development of novel treatments targeting the endocannabinoid system may make it possible to relieve individual symptoms or clusters of symptoms resistant to conventional treatment methods.

SEXUAL DIMORPHISM IN CB1R-MEDIATED BEHAVIORAL COPING STRATEGY IN THE FORCED SWIM TEST

Christopher J. Roberts, Amy L. Sasman and Cecilia J. Hillard

Department of Pharmacology and Toxicology, Medical College of Wisconsin
Milwaukee, WI 53226, United States of America

INTRODUCTION: Endocannabinod signaling (ECS) is known to control mood, which has human health implications for sexually dimorphic disorders, such as anxiety and depression. A growing body of evidence demonstrates that systemic activation of ECS promotes active coping in mice and rats in the forced swim test (FST). The ventromedial prefrontal cortex (vmPFC) is one brain region implicated in the affects of ECS since microinjection of a cannabinoid type 1 receptor (CB1R) agonist into this region decreases passive coping in male rats. Behavioral coping strategy in the FST has been shown to have sex differences. One goal of these studies was to determine whether sex differences in ECS could contribute to sex differences in FST behavior. A second goal of these studies was to examine the effects of chronic activation and inhibition of ECS on FST coping strategy in both male and female mice.

METHODS: Male and female ICR mice (6-9 weeks of age) were used in these studies. Animals were exposed to a twelve-day protocol consisting of FSTs on day one and twelve separated by 10 days of intraperitoneal (i.p.) injections on days two to eleven. Mice were injected with the CB1R antagonist, AM251 (1.0 mg/kg); the CB1R indirect agonist, URB597 (1.0 mg/kg); or vehicle. After the FST on day 12, mice were sacrificed and brains removed. CB1R binding parameters (Kd and Bmax) were determined in membranes prepared from PFC using radioligand binding assays with [3H]CP55940.

RESULTS: Immobility did not differ between males and females at either the first or second FST ($p=0.15$; 0.32). However, vehicle-treated females exhibit a robust 23-second mean increase in immobility ($p=0.0012$), while vehicle-treated male mice do not display a change in immobility between the FST exposures ($p=0.42$). Vehicle-treated female mice exhibited a significant ($p=0.02$) positive correlation (Spearman r value=0.51) between the CB1R Kd in the PFC and the change in immobility between FST exposures. This correlation was lost after treatment with either AM251 ($p=0.64$) or URB597 ($p=0.43$). Male mice did not exhibit a correlation between CB1R Kd and the change in FST immobility in groups treated with vehicle, AM251 or URB597 ($p=0.72$; 0.20; 0.46).

CONCLUSIONS: These data indicate that in vehicle-treated, female ICR mice about 50% of the variability in the change in time immobile is due to differences in the affinity of CB1Rs in the PFC. Also, the correlation between CB1 receptor affinity in the PFC of females with FST immobility is lost if the receptor is blocked or endogenous ligand concentrations are increased. Furthermore, we have shown that although CB1R affinity in the PFC correlates with behavior in females; the same is not true in male mice. Nevertheless, acute CB1R activation within the vmPFC promotes active coping in male rats. Therefore, we hypothesize that within the PFC another component of ECS other than CB1R affinity, such as ligand concentrations or G protein coupling, is the rate-limiting factor that regulates FST behavior in male mice. **Supported by R21 DA02243**

REGION-SPECIFIC AND SELECTIVE ABNORMALITES IN THE ENDOCANNABINOID SYSTEM IN WISTAR KYOTO RAT: A GENETIC MODEL OF DEPRESSION

K. Yaragudri Vinod,[1,2,3] Shanaz M. Tejani-Butt,[4] Basalingappa L. Hungund,[1,3,5] and Thomas B. Cooper,[1,3,5]

[1]Division of Analytical Psychopharmacology, Nathan Kline Institute for Psychiatric Research Orangeburg, New York; [2]Department of Child and Adolescent Psychiatry, New York University Langone Medical Center , New York; [3]Department of Analytical Psychopharmacology, New York State Psychiatric Institute, New York; [4]Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, USA.[5]Department of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, USA.

Introduction: Recent studies have suggested a role of the central endocannabinoid system in the pathophysiology of depressive behavior. However, the genetic basis of this disorder is currently unknown. The present study investigated the status of the endocannabinoid system in a genetic model of depression, Wistar Kyoto (WKY) rat.

Methods: The CB1 receptor-stimulated G-protein signaling, protein levels and activity of metabolic enzymes of the endocannabinoids were measured in the frontal cortex and hippocampus of WKY and Wistar rats (control group). The pharmacological inhibition of the fatty acid amide hydrolase (FAAH) on sucrose preference was also investigated.

Results: The findings of this study revealed for the first time that there are region-specific and selective abnormalities in the component of the endocannabinoid system in the brain of WKY. The CB1 receptor-mediated G-protein signaling was found to be significantly higher in the frontal cortex and hippocampus of WKY compared to WIS rats. There were no differences in the levels of N-arachidonyl phosphatidyl ethanolamine specific phospholipase-D (NAPE-PLD), whereas, diacyl glycerol lipase (DAGL) was found to be significantly lower in the frontal cortex and hippocampus of WKY compared to WIS rats. The monoglycerol lipase (MGL) was higher in the frontal cortex whereas the FAAH was elevated in both frontal cortex and hippocampus of WKY rats. The lower level of DAGL could reduce the 2-AG content in WKY rats. In addition, the elevated levels of FAAH and MGL enzymes might lead to a greater degradation of the endocannabinoids (AEA and 2-AG) in these rats. The pharmacological inhibition of FAAH enzyme resulted to a greater preference for sucrose in WKY rats, which appears to be related to a decrease in the hedonic response leading to an increased sensitivity to reward.

Conclusion: Selective alterations in metabolic enzymes of the endocannabinoids might reduce the endocannabinoid-mediated CB1 receptor signaling, which could be one of the genetic factors associated with depressive-like behavior in WKY rats. These findings might have etiological and therapeutic implications for the treatment of genetic basis of depressive behavior.

QUANTIFICATION OF LYSOPHOSPHATIDYLINOSITOL-STIMULATED [^{35}S]GTPγS AUTORADIOGRAPHY IN THE RAT BRAIN. COMPARISON WITH RESPONSE TO CP55,940

Maria Luisa Rojo and Christopher J. Fowler

Departments of Pharmacology and Clinical Neuroscience, Umeå University, SE90187 Umeå, Sweden

Introduction: There is some debate as to whether or not the orphan receptor GPR55 is a cannabinoid receptor. Recent work, however, has supported the finding of Oka *et al.* (*Biochem Biophys Res Commun* 362 [2007] 928-34) that GPR55 is primarily responsive to lysophosphatidylinositol (LPI) rather than cannabinoids. However, most studies have been undertaken using transfected cells, and there is little known as to the sensitivity of the naturally-occuring receptor to stimulation by LPI. In the rat brain, expression of GPR55 found (Sawzdargo *et al*., *Mol Brain Res* 64 [1999] 193-8), but no functional data was reported by these authors. One way of assessing the function of a G-protein coupled receptor is to measure the stimulation of [^{35}S]GTPγS binding produced by an agonist. This has been undertaken in the present study.

Method: Agonist-stimulated [^{35}S]GTPγS binding to coronal brain sections (20 μm) was measured autoradiographically as desribed by Rodríguez-Gaztelumendi *et al.* (*J Neurochem* 108 [2009] 1423-33).

Results: The effects of LPI and as a positive control, the CB receptor agonist CP55,940 upon [^{35}S]GTPγS binding are shown in the Table. Under conditions where robust responses to CP55,940 are seen, there is no stimulation of [^{35}S]GTPγS binding by any of the concentrations of LPI tested.

Brain region	[^{35}S]GTPγS binding (% of unstimulated) in presence of:			
	0.5 μM LPI	1 μM LPI	10 μM LPI	10 μM CP55,940
Prefrontal cortex	77±7	99±5	92±4	335±10
Caudate-putamen	86±7	101±7	101±6	377±28
Hypothalamus	80±5	87±10	90±5	175±11
Hippocampus	87±6	96±8	88±6	301±54
Cerebellum	109±9	95±9	95±2	1250±108

Data are means ± s.e.m., n=3-4.

Conclusion: Under the conditions and in the brain regions used here, the expression of GPR55 and/or its responsiveness to LPI is not sufficient for a measurable stimulation of [^{35}S]GTPγS binding to be found.

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NOVEL ENDOGENOUS TRPV1 AND TRPV4 AGONISTS ARE STRUCTURAL ANALOGS TO ANANDAMIDE

Heather B. Bradshaw and Siham Raboune

Psychological and Brain Sciences, Indiana University, Bloomington IN, 47405 USA

Introduction *N*-acyl amides (also known as lipoamino acids) are a large family of endogenous lipids consisting of over ~60 identified compounds and the combinatorial potential for ~60 additional members. The most studied *N*-acyl amide is the endogenous cannabinoid, *N*-arachidonoyl ethanolamine (Anandamide), which is active at the CB1, CB2, and TRPV1 receptors. Functional relevance of the majority of the additional Anandamide structural analogs is unknown; however, there are data to suggest some may play a role in pain and inflammation. Here, we test the hypothesis that members of the *N*-acyl amide family activate TRPV1, TRPV3, TRPV4, and TRPM8 receptors. **Methods** The following *N*-acyl amides that typically consisted of 6 members (*N*-docosahexaenoyl; *N*-arachidonoyl, *N*-linoleoyl, *N*-oleoyl, *N*-palmitoyl, and *N*-stearoyl amides) where used in each screen: *N*-acyl tyrosine, *N*-acyl aspartic acid, *N*-acyl proline, *N*-acyl Isoleucine, *N*-acyl GABA, *N*-acyl phenylalanine, *N*-acyl tryptophan, *N*-acyl serine, *N*-acyl glycine, *N*-acyl methionine, *N*-acyl valine, *N*-acyl threonine, *N*-acyl beta-alanine, *N*-acyl alanine, *N*-acyl leucine, *N*-palmitoyl glutamic acid, and *N*-palmitoyl lysine. Calcium mobilization using Fura 2AM with standard protocols was measured in non-transfected HEK cells and HEK cells transfected with the following: TRPV1, TRPV3, TRPV4, and TRPM8 calcium channels. Cells were challenged with vehicle (1% DMSO in buffer), 1 and 10μM of lipids listed above or appropriate positive controls. Where activity was identified in the initial screens, dose-response curves were performed. **Results** None of the compounds tested caused calcium mobilization in non-transfected HEK cells, TRPV3, or TRPM8-HEK cells. *N*-acyl GABA compounds caused calcium mobilization in TRPV1-HEK cells with a strict SAR as well as dose dependency. Rank order of potency is as follows: *N*-docosahexaenoyl GABA > *N*-arachidonoyl GABA > *N*-linoleoyl GABA, whereas, *N*-oleoyl, palmitoyl and stearoyl GABA had no effect. Likewise, *N*-docosahexaenoyl serine and *N*-docosahexaenoyl glycine showed calcium mobilization at TRPV1, whereas, the other members of those *N*-acyl amide families had no effect, suggesting an SAR specific for *N*-docosahexaenoyl amides. *N*-acyl tryptophan compounds caused calcium mobilization in TRPV4-HEK cells with an SAR as well as dose dependency. Rank order of potency is as follows: *N*-arachidonoyl tryptophan ≥ *N*-docosahexaenoyl tryptophan > *N*-linoleoyl tryptophan, whereas, *N*-oleoyl, palmitoyl and stearoyl tryptophan had no effect. *N*-acyl tyrosine compounds, likewise, drive calcium mobilization in TRPV4-HEK cells, however, with a different SAR with *N*-arachidonoyl tyrosine ≥N-linoleoyl tyrosine = *N*-palmitoyl tyrosine > *N*-stearoyl tyrosine and only *N*-oleoyl tyrosine having no effect. *N*-docosahexaenoyl tyrosine was not available for these screens. **Conclusions** The specificity of these *N*-acyl amides at TRPV1 and TRPV4 demonstrate novel routes for activation of these calcium channels. Future work will determine if these endogenous lipids are regulated in cases of acute and chronic inflammatory pain.

BIOLOGICAL ACTIVITIES OF LYSOPHOSPHATIDYLINOSITOL AND SEVERAL CANNABINOID RECEPTOR LIGANDS AND RELATED MOLECULES AS GPR55 LIGANDS

Saori Oka, Shinji Kimura, Mitsuru Shima, Ryo Ota,
Atsushi Yamashita and Takayuki Sugiura

Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko,
Sagamihara, Kanagawa 229-0195, Japan

GPR55 is a seven-transmembrane, G protein-coupled receptor identified in 1999. Evidence is gradually accumulating that GPR55 plays a number of essential roles in various mammalian tissues and cells, yet details remain to be determined. Recently, we conducted a search for the endogenous ligand for GPR55 using HEK293 cells which express GPR55. Finally, we found that lysophosphatidylinositol (LPI) acted as an agonist toward GPR55. LPI induced rapid phosphorylation of ERK and a Ca^{2+} transient in the GPR55-expressing cells. Such cellular responses were not observed in the vector-transfected cells. Treatment of the GPR55-expressing cells with siRNA against GPR55 markedly reduced the cellular responses induced by LPI. These results strongly suggest that GPR55 is a specific and functional receptor for LPI (*Biochem. Biophys. Res. Commun*., 362, 928-934, 2007). We also found that LPI induced the phosphorylation of p38 MAP kinase and a transcription factor ATF-2 in HEK293 cells expressing GPR55 (*J. Biochem*., 2010, in press). LPI also provoked rapid morphological changes and actin stress fiber formation in the GPR55-expressing HEK293 cells. In the present study, we compared in detail the biological activities of LPI with those of several cannabinoid receptor ligands and related molecules. LPI exhibited the highest agonistic activity among various cannabinoid receptor ligands and related molecules. CP55940 and WIN55212-2 did not elicit apparent cellular responses in both the vector-transfected cells and GPR55-expessing cells. On the other hand, AM251 induced a Ca^{2+} transient in the GPR55-expressing cells. AM251 also evoked rapid phosphorylation of ERK and p38 MAP kinase in the GPR55-expressing cells. AM251-induced cellular responses were detected from 100 nM and reached a plateau at 1 μM. The magnitudes of the cellular responses induced by AM251 were almost comparable to those induced by soybean LPI. These effects of AM251 were not observed in the vector-transfected cells. AM251 is known as a CB1 antagonist/inverse agonist. The pharmacological effects of AM251 observed *in vitro* and *in vivo* have been assumed to be due to its antagonistic/inverse agonistic activity toward the CB1 receptor. However, at least a part of the pharmacological effects of AM251 may be attributed to the agonistic activity of AM251 toward GPR55.

CHARACTERIZATION OF WELL-KNOWN LIGANDS ON TISSUES NATURALLY EXPRESSING CB_2 RECEPTORS

P. Marini and Ruth A. Ross

School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 3ZD, Scotland, UK

The human cannabinoid CB_2 receptor was discovered by sequence homology. It was predominantly detected in the immune system (spleen, tonsils, and immune cells) and further cloned from mouse and recently from rat. Although the spleen plays a crucial role in the immune response and the CB_2 receptors are widely expressed in it, a functional characterization of these receptors in this tissue has not been done yet.

The aim of this study was to evaluate how some of the well known cannabinoid CB_2 receptor ligands (i.e. CP-55940, JWH-133, JWH-015, AM-1241, GW-405833, SR-144528, AM-630 and JTE-907) behave when tested in tissues naturally expressing cannabinoid CB_2 receptors. Specifically, human and rat spleen membranes and, as functional assay, [^{35}S]GTPγS binding assay, have been used.

Some of our results have shown that: in both human and rat spleen membranes 1) CP-55940 (EC_{50}: 4.92nM and 8.73nM; E_{max}: 19.41% and 36.57%, respectively) and JWH-133 (EC_{50}: 5.43nM and 2.05nM; E_{max}: 28.46% and 20.19%, respectively) behave as CB_2 receptor full agonists 2) SR-144528 (IC_{50}: 1.34nM and 14.1nM; E_{max}: -22.13% and -28.17%, respectively) and JTE-907 (IC_{50}: 8.04nM and 1.96nM; E_{max}: -18.52% and -18.93%, respectively) behave as CB_2 inverse agonists; 3) AM-1241, JWH-015 and AM-630 have shown interesting behaviours, different from their expected pharmacological activity. In particular, in both human and rat spleen membranes, AM-1241, that has been reported to be a protean agonist behaves, in our experimental conditions, as an agonist (EC_{50}:12.96nM and 26.23nM; E_{max}: 16.49% and 26.96%, respectively); JWH-015, a well known CB_2 receptor agonist, has shown high potency, but only low efficacy (EC_{50}: 14.09nM and 30.18nM; E_{max}: 9.60% and 11.02%, respectively), and AM-630, despite of its well known strong inverse agonism, has shown a very low inverse agonism (IC_{50}: 2.8nM and 3.93nM; E_{max}: -8.39 and -11.61, respectively). Finally, 4) GW-405833, a protean agonist, like JWH-015, albeit with lower potency, has shown low efficacy both in human and rat spleen membranes (EC_{50}: 747.0nM and 495.30nM; E_{max}: 11.53% and 7.05%, respectively).

Here we demonstrate that CB_2 receptor ligands show different pharmacological behaviours in spleen, a tissue which natively expresses the CB_2 receptor. For certain ligands, the effect differ from those observed in assays performed using recombinant CB_2 receptor. The results may have implications for the discovery of new therapeutic CB_2 receptor agonists.

THE ENDOCANNABINOID VIRODHAMINE ACTIVATES G-PROTEIN COUPLED RECEPTOR 55

June Penman, Christopher M. Henstridge and Andrew J. Irving

Division of Medical Sciences, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, UK.

The endocannabinoid O-arachidonoyl ethanolamine (virodhamine) is thought to be partial agonist at the type 1 cannabinoid receptor (CB_1) and a full agonist of the type 2 cannabinoid receptor (CB_2). Recent studies using a GTPγS binding assay suggest that virodhamine may also be a potent agonist at G-protein coupled receptor 55 (GPR55), a putative cannabinoid receptor. Interestingly, virodhamine is found at comparable levels to anadamide in brain tissue and high concentrations are present in the spleen, which is also has prominent expression of GPR55 mRNA.

In the present study we have used a previously characterised HEK293 cell line stably over-expressing recombinant human GPR55 (hGPR55-HEK) to investigate the effects of virodhamine on GPR55-mediated Ca^{2+} signaling events. We find that in these cells virodhamine is able to induce oscillatory Ca^{2+} transients that are characteristic of GPR55 receptor activation. These effects were concentration-dependent over the range 3μM - 30μM. In control HEK293 cells, treatment with virodhamine induced a transient Ca^{2+} rise, but no sustained, oscillatory activity. Treatment of hGPR55-HEK cells with GPR55 siRNA (24-48 hrs) significantly reduced the oscillatory activity, but did not affect the initial response to virodhamine. However, treatment of cells with control siRNA was without effect.

We conclude that the sustained, oscillatory Ca^{2+} responses induced by virodhamine in hGPR55-HEK293 cells are likely to be mediated by GPR55, suggesting that virodhamine acts as an agonist at this receptor, albeit at high concentrations. These data are consistent with the notion that GPR55 exhibits sensitivity to a subset of cannabinoid ligands, although a potent endocannabinod-like agonist remains to be identified.

N-ACYL AMIDES PROMOTE GPR119-MEDIATED CALCIUM SIGNALING

Amy Alexander, Natalia Reglinska, June Penman and Andrew J. Irving

Division of Medical Sciences, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, UK.

G-protein coupled receptor 119 (GPR119) is sensitive to certain N-acyl amides and is thought to play a role in the regulation of glucose homeostasis and insulin signalling. GPR119 is expressed at high levels in the pancreas (β-cells) and gastrointestinal tract (enteroendocrine cells), although there is also some evidence for neuronal expression. Phylogenetically, GPR119 has been assigned to the MECA (*m*elanocortin; *e*ndothelial differentiation gene; *c*annabinoid; *a*denosine) receptor cluster, which designates cannabinoid receptors among its closest relatives. GPR119 is known to signal via G-protein Gs, which typically stimulates adenylate cyclise leading to cAMP accumulation. In the present study we have used fura 2 microfluorimetry and digital epifluorescence imaging to investigate whether GPR119 activation can also promote Ca^{2+} signaling in HEK293 cells transfected with recombinant, human GPR119. Treatment of cells with the previously characterised GPR119 ligands, oleoylethanolamide (OEA; 10-30 μM) and palmitoylethanolamide (PEA; 30 μM) induced Ca^{2+} transients in cells expressing GPR119, with OEA being the most potent ligand. However, no response were observed in control HEK293 cells. These data suggest that recombinant GPR119 can regulate Ca^{2+} signalling as well as cAMP levels in HEK293 cells. Although the signaling pathway underlying this effect remains to be established, this could provide a useful, alternative assay for evaluating putative GPR119 ligands.

CANNABINOID RECEPTOR INTERACTING PROTEIN (CRIP) 1A: SIGNAL TRANSDUCTION AND EPIGENETIC PHENOMENA

Lawrence C. Blume[1], Caroline E. Bass[1], George D. Dalton[1], Dana E. Selley[2], and Allyn C. Howlett[1]

[1]Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Winston-Salem, NC 27157 USA; [2] Department of Pharmacology and toxicology, Virginia Commonwealth University, Richmond, VA 23298 USA

The novel protein CRIP1a is highly expressed in the brain of vertebrates and has been shown to suppress the tonic inhibition of voltage-gated Ca^{2+} channels induced by CB_1 in neurons [Niehaus et al., 2007 Mol. Pharmacol. 72:1557–1566]. The focus of this study was to evaluate the role of CRIP1a on signaling via ERK phosphorylation, and to elucidate the epigenetic phenomena manifest by CRIP1a overexpression.

N18TG2 cells were stably transfected with the CRIP1a-pcDNA3.1 plasmid, yielding two separate stable clones that possessed a 12:1 and 1:1 CRIP1a:CB1 cDNA ratios (by qPCR using ribosomal 18S and neuron-specific enolase as standards), compared with wild-type cells expressing at a 1:7 ratio. Expression of the CB_1 receptor was the same in CRIP1a-overexpressing clones as it was in wild-type cells. N18TG2 cells and those over-expressing CRIP1a, were treated with tetrahydrolipstatin (to block 2-arachidonoyl glycerol production) and challenged with 10 nM WIN55212-2, 10 nM CP55940, or 10 nM rimonabant for 5 minutes, to determine levels of total ERK protein and phosphoERK using a 96-well format in-cell-Western technique. CB_1-mediated ERK phosphorylation in WT N18TG2 cells was reduced by rimonabant, consistent with "constitutive activation" of signal transduction. In the CRIP1a-overexpressing clones, basal pERK levels were low compared with WT, and rimonabant had no ability to further reduce this level, suggesting the absence of the constitutive activation resulting from CRIP1a-overexpression. Stimulation of ERK phosphorylation by CB_1 agonist was unaltered in CRIP1a overexpressing clones.

ERK phosphorylation is one of several signal transduction pathways that can lead to regulation of gene expression. CRIP1a-overexpressing clones exhibited significant decreases in the mRNA levels of the opioid peptide precursor PENK; concomitantly, mRNA levels of the delta opioid receptor DOR1 were significantly upregulated. CRIP1a-overexpressing clones exhibited significant increases in NOS1 and GPR55 RNAs, and differences in RNA levels for proteins associated with the NF-kB pathway.

In order to investigate the impact of overexpression of CRIP1a in vivo, a CRIP1a-AAV10 viral vector was unilaterally injected into the striatum of Sprague Dawley rats. Animals were sacrificed after 3, 5, 10, or 17 days, and 2 mm brain slices were taken at the site of injection and the contralateral side, RNAs isolated from striata, and qPCR was performed. Comparing injected to contralateral side, CRIP1a overexpression had no effect on CB_1 receptor RNA levels. As was seen in the neuronal cells, PENK and PDYN RNAs were decreased and DOR1 was increased. These studies indicate that in vivo and in vitro overexpression of CRIP1a has no effect on CB_1 expression; however significant alterations can be seen in the opioid GPCR systems as well as other signaling proteins. Because of the potential for altered non-G-protein signaling via the NF-Kb pathway, changes in genomic regulation may be due to multiple transcription regulation pathways.

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DOWN-REGULATION OF ROSTRAL VENTROLATERAL MEDULLA PI3K/AKT SIGNALING UNDERLIES THE CENTRAL CB_1R-EVOKED SYMPATHOEXCITATION IN CONSCIOUS RATS

Badr M. Ibrahim and Abdel A. Abdel-Rahman

Brody School of Medicine, East Carolina University, Greenville, NC, USA

We have shown that intracisternal (i.c.) injection of the cannabinoid receptor (CB_1R) agonist WIN55,212-2 (3-30 μg) elicited dose-related increases in MAP. However, the exact cellular mechanisms that underlie CB_1R-evoked pressor/sympathoexcitatory effects are unclear. Studies have implicated PI3K/Akt signaling in some pharmacological responses mediated by CB_1R activation both in *vivo* and in *vitro*. Therefore, we hypothesized that CB_1R modulation of PI3K/Akt signaling in the brainstem underlies its centrally mediated sympathoexcitation in freely moving rats. WIN55,212-2 reduced Akt phosphorylation (PI3K downstream molecular target) in the rostral ventrolateral medulla (RVLM) and nucleus tractus solitarius (NTS) 5 min post injection, which coincides with or precedes WIN55,212-2-evoked peak pressor response. The basal level of activity was restored 30 min post WIN55,212-2 injection. The hemodynamic and neurochemical responses were mediated via CB_1R because they were abrogated by the selective CB_1R antagonist AM251 (30 μg, i.c.). More intriguingly, Akt phosphorylation was significantly elevated in the NTS of animals that received AM251 prior to WIN55,212-2. Furthermore, i.c. Wortmannin (PI3K inhibitor, 0.42 μg) pretreatment dose dependently augmented WIN55,212-2 (7.5 and 15 μg, i.c) evoked pressor and neurochemical effects. Taken together, our findings suggest that CB1R-evoked sympathoexcitation is mediated, at least partly, via inhibition of brainstem PI3K/Akt signaling pathway.

FIRST INVESTIGATIONS OF ENDOCANNABINOID FUNCTION IN HUMAN OCULAR CELLS USING CELLULAR DIELECTRIC SPECTROSCOPY

D. Piwnica, W.D. Stamer, A.B. Kharlamb, J.W. Wang and D.F. Woodward

Cannabis and the endocannabinoids have long been known to reduce intraocular pressure. Thus, in the glaucomatous monkey model, anandamide, abu-cannabidiol both lower intraocular pressure. Potent and selective Fatty acid amide hydrolase inhibitors do not affect intraocular pressure, thereby suggesting no control of aqueous humor dynamics by endogenous endocannabinoid. This scenario was investigated at the primary human ocular cell level using a new high-throughput technology, cellular dielectric spectroscopy. This is a label-free HTS format, which measures changes in impedance across a cell layer according to changes in cell shape/volume. Two human cell types, key to control of aqueous humor outflow, were examined: TM (trabecular meshwork) and ECSC (endothelial cells of Schlemm's canal). Anandamide was inactive in TM cells but was active in ECSC cells, indicating the potential for endocannabinoid/endovanilloid control only at the level of Schlemms' canal.

EFFECT OF CANNABINOIDS AND GPR55 AGONISTS ON MICROGLIAL CELLS MIGRATION IN CULTURE

Khalil Eldeeb[1,2], Stephen Alexander[1], David Pritchard[1]* and David Kendall[1]

[1]School of Biomedical Sciences, [1*]School of Pharmacy, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, UK. [2] Pharmacology Department, Faculty of Medicine, Al-Azhar University, Damietta, Egypt

Migration of microglial cells is one of the key elements of microglial activation in a variety of neuroinflammatory CNS disorders such as stroke and multiple sclerosis. Cannabinoid compounds have well known immunomodulatory properties, via their actions predominantly on CB2 cannabinoid and, as yet unidentified atypical cannabinoid receptor, which are regarded as promising pharmaceutical targets. Recent reports suggest that the orphan G protein-coupled receptor 55 (GPR55) is an atypical cannabinoid receptor. The purpose of the present study was to test the effect of phytocannabinoid ligands, in comparison with lysophosphatidylinositol (LPI), (the putative endogenous ligand for GPR55), and GSK-GPR55 agonist on BV-2 cell migration, a microglial cell line commonly used to study microglial function. BV-2 cells were resuspended and stained with 700 nM DRAQ5 for 20 min at 4°C. Migration was measured in a 96-well chemotaxis chamber using filters with a pore diameter of 10 μm. Upper wells of the chamber were loaded with 7×10^4 cells in 390 μL, while lower wells contained chemoattractant solutions. Once loaded, the chamber was left untouched on the bench top for 10 min before being moved to an incubator for 3 h at 37°C and 5% CO_2. Migration was measured by scanning the filter with an Odyssey Infrared Imaging System to detect DRAQ5-labeled cells that had migrated toward the bottom of the filter. Using this method, we found that ATP (100 μM) strongly stimulate microglial migration (351±75 % of basal), whereas LPI (25μM), GSK-GPR55 agonist (20μM) had produced modest increases in microglial migration (137±22, 134±30 % of basal respectively) the phytocannabinoids, cannabidiol and THC (10μM) were unable to induce a significant change in migration (105±21,110±16% of basal respectively). On other side when it's combined, CBD was able to attenuate ATP- (193±67% of basal), LPI- (71±10 % of basal) and GSKGPR55 agonist (82±14 % of basal) - induced migration. THC also founded to inhibit ATP- (228±65% of basal), LPI- (98±16 % of basal) and GSKGPR55 agonist (65±10 % of basal) - induced migration. Whilst these results are consistent with a non-selective inhibition of evoked Ca^{2+} release by cannabinoid ligands in BV-2 microglial cells, further pharmacological and biochemical investigation is required.

This work was made possible by scholarship to Khalil Eldeeb from the Egyptian government.

ANTI-INFLAMMATORY ACTIVITY OF ANANDAMIDE IN MURINE MACROPHAGE RAW264.7 CELLS: INVOLVEMENT OF HEME OXYGENASE-1 EXPRESSION

Man Hee Rhee, Mehari Endale and Parth Vyas

College of Veterinary Medicine, Kyungpook National University,
Daegu 702-701, Republic of Korea

Anandamide also known as N-arachidonoylethanol-amine or AEA, is an endogenous cannabinoid neurotransmitter in animal and human organs which exhibit anti-inflammatory properties. However information on anti inflammatory activity of anandamide and its mechanism is limited. The objective of this study was to investigate the anti inflammatory properties of anandamide in RAW264.7 cells and the possible mechanism involved in it. Treatment of RAW264.7 cells with anandamide markedly inhibited nitric oxide (NO) production, inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2) and interleukin-6 (IL-6) cytokine expression in cells stimulated with lipopolysaccharide (LPS, 0.2μg/ml). Anandamide's anti-inflammatory activities are related to the induction of HO-1 expression. Inhibition of HO-1 activity by treatment with Tin protoporphyrin (SnPP), a specific HO-1 inhibitor, abrogated the inhibitory effects of anandamide on the production of NO in LPS-stimulated RAW264.7 cells. SnPP, also reversed anandamide mediated suppression of iNOS, COX-2, and IL-6 expression, suggesting that HO-1 induction could be implicated in the suppression of pro inflammatory mediators and cytokines. In addition, anandamide treatment along with either CB1 or CB2 receptor antagonist, AM281 and AM630, respectively, resulted in blockage of HO-1 expression by AM281, suggesting that anandamide induces HO-1 via CB1 receptor and not through CB2 receptor. Taken together, the anti inflammatory properties of anandamide may suppresses NO production and iNOS, IL-6, COX-2 expression in LPS stimulated macrophages by induction of HO-1 expression via CB1 receptor activation.

ANTI-INFLAMMATORY CANNABINOIDS WITHOUT PSYCHO-ACTIVE EFFECTS

Marcel de Wit

Maripharm, Rotterdam, Netherlands 3029AV

Fytogoras BV, a company owned by TNO (Leiden and Zeist, the Netherlands) and Maripharm BV (Rotterdam, the Netherlands) have discovered a group of Cannabinoids with potent anti-inflammatory and immuno-modulating properties. These compounds are acidic (carboxylated-) forms of the cannabinoids as present in *Cannabis spp*, with delta-9-tetrahydrocanabinoid-acid (THCa) as the major representative discovered until now. Thus far, only heated cannabis (200 °C or more), decarboxylated cannabinoids purified from heated cannabis extracts or synthetic derivatives derived from decarboxylated cannabinoids are used for medicinal applications. The disadvantage of using heated (e.g. smoking) or decarboxylated cannabinoids (e.g. Δ^9 tetrahydrocannabinol (THC)) is that they give rise to psychoactive side effects.

We demonstrated that the biological activity of unheated extracts on inflammatory and immune system responses is higher as compared to heated extracts and probably not mediated by CB1 or CB2 receptors. This means that we can generate a cannabis extract with high medicinal value that is devoid of psycho-active side effects.

We also demonstrated that the carboxylic derivative of THC, THC-acid is of major importance for the effects seen with unheated extracts.

The cannabinoids, for example as purified THC-acid or cold extract, have great potential for further development to a formulation to be used in the treatment of inflammatory diseases (including skin diseases, inflammatory bowel disease, arthritis) with or without a pain component.

R(+)WIN55,212-2 REGULATES TLR3 AND TLR4 SIGNALLING: THERAPEUTIC IMPLICATIONS FOR MULTIPLE SCLEROSIS

Eric J. Downer,[1] Eileen Clifford,[1] Bruno Gran,[2]
Hendrik J. Nel,[3] Padraic G. Fallon,[3] and Paul N. Moynagh[1]

[1]Institute of Immunology, Department of Biology, National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland.
[2]Division of Clinical Neurology, University of Nottingham, Nottingham, UK.
[3]Institute of Molecular Medicine, Trinity Centre for Health Sciences, Trinity College Dublin, St. James's Hospital, Dublin 8, Ireland.

Cannabinoids are effective in palliating experimental autoimmune encephalomyelitis (EAE) symptoms and have significant therapeutic potential in multiple sclerosis (MS) patients. Despite this, the precise molecular mechanism for these effects is not understood. Toll-like receptor (TLR) signalling is central in controlling innate immune responses and TLRs have been implicated as key players in the pathogenesis of neuroinflammatory disorders, including MS. Here we examined the impact of the synthetic cannabinoid *R*(+)WIN55,212-2 on signalling events induced by TLR3 in response to the double-stranded RNA mimic poly(I:C) and by TLR4 in response to lipopolysaccharide (LPS). Using HEK 293 cells, human U373-CD14 astrocytoma cells and primary mouse astrocytes as cell models, we provide evidence that *R*(+)WIN55,212-2, in the presence of TLR3 and TLR4 ligands, targets downstream transcription factors in the TLR signalling cascade. We investigate these signalling events in the context of neuroinflammatory events linked with MS by pinpointing the anti-inflammatory patterns exerted by *R*(+)WIN55,212-2 in EAE mice and in PBMCs isolated from both healthy donors and MS patients. These findings delineate a novel mechanism by which *R*(+)WIN55,212-2 may act therapeutically, particularly in the context of MS.

NOVEL ANTI-INFLAMMATORY ANALOGS OF ANANDAMIDE

Sumner Burstein, Catherine McQuain and Rebecca Salmonsen
Department of Biochemistry and Molecular Pharmacology,
University of Massachusetts Medical School, Worcester, MA 01605

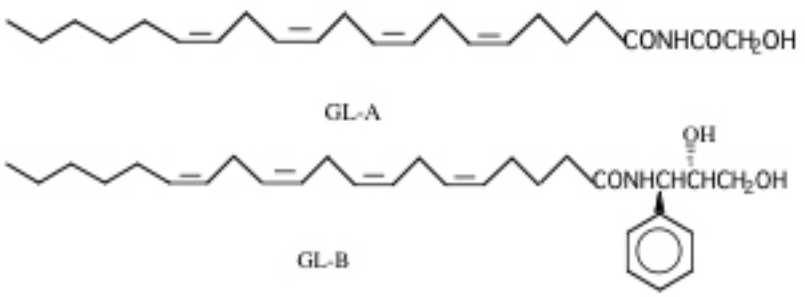
Teng Ai, Venkatesh Kattamuri and Guigen Li
Department of Chemistry & Biochemistry
Texas Tech University, Lubbock, TX 79409

Recently, data were presented supporting the possibility that analogs of anandamide called elmiric acids are potential anti-inflammatory agents [Burstein SH, et al. *Bioorganic & medicinal chemistry* **2007**;*15*:3345-55]. We have developed a mechanism-based *in vitro* assay for screening elmiric acids for potential anti-inflammatory activity based on their stimulatory action on prostaglandin J_2 levels and have applied this to the novel compounds described in this report.

Using this assay, we have determined a rank order of anti-inflammatory potencies for a series of analogs of the endocannabinoid anandamide. Here we would like to report some preliminary results of two new candidates (5Z,8Z,11Z,14Z)-*N*-(2-hydroxyacetyl)icosa-5,8,11,14-tetraenamide (GL-A) and (5Z,8Z,11Z,14Z)-*N*-((1S,2R)-2,3-dihydroxy-1-phenylpropyl)icosa-5,8,11,14-tetraenamide (GL-B) and several of their analogs.

The following conclusions can be reached. GL-B and its enantiomer showed a potency difference of approximately 20 fold. This degree of stereospecificity suggests that the stimulation of PGJ levels in RAW264.7 cells is probably receptor mediated. Because of their similarity to anandamide, the cannabinoid receptors are a possibility. Regarding the effect of the fatty acid group, as with the elmiric acids, palmitoyl derivatives have little or no effect on prostaglandin production whereas arachidonoyl analogs are all relatively active. The significance of this finding is not known at this time; however, the palmitoyl compounds may have other activities such as inhibition of cell proliferation. Halogen substituents on the phenyl ring show a range of potencies all of which are lower than the parent GL-B. Pretreatment of the cells with SR141716a or GW9662 reduced the PGJ response to 3 uM GL-B by 90.6% and 86.5% respectively suggesting involvement of both CB1 and PPAR-g in the mechanism of action.

Support for this project was provided by NIDA (1R03DA026960).



EFFECT OF CANNABIDIOL ON LPS ACTIVATE GLIAL CELL

Daniele De Filippis [1°], Giuseppe Esposito [2], Carla Cirillo[3]
Mariateresa Cipriano[1°], Benedicte De Winter[4], Caterina Scuderi [2],
Rosario Cuomo[3], Luca Steardo[2], Joris De Man[4] and Teresa Iuvone[1°]

°Endocannabinoid Research Group [1]Dep.tment of Experimental Pharmacology, University of Naples Federico II, Naples, Italy [2] Dep.tment of Physiology and Pharmacology V. Erspamer, University of Rome "La Sapienza" Rome, Italy, [3] Department of Clinical and Experimental Medicine, Division of Hepatogastroenterology, University Federico II, Via Sergio Pansini 5, 80131 Naples, Italy. [4]Laboratory of Experimental Medicine and Pediatrics, Division of Gastroenterology, Faculty of Medicine, University of Antwerp, Antwerp, Belgium

Introduction. Glial cells, commonly named neuroglia or simply glia (Greek for "glue"), are non-neuronal cells that provide support and nutrition, maintain homeostasis, myelin production and participate in signal transmission in the nervous system. Recent studies evidenced the *dark side* of glia, since chronic glial activation has been demonstrated an important component of neurodegenerative diseases. During neurodegeneration, chronically activated glial cells likely contribute to neuronal dysfunction, injury, loss and, hence, to the disease starting and progression both in Central (CNS) and Peripheral Nervous System (PNS).

In the gut, enteric glia cells (EGCs) from the enteric nervous system (ENS) actively contribute to an inflammatory condition, via antigen presentation and cytokine synthesis. During gut inflammation, EGC proliferate and release neurotrophins, growth factors, and proinflammatory cytokines which, in turn, may amplify the immune response. Therefore, of great interest seems to find molecules able to control glial cell activation, thereby reducing inflammatory condition, in CNS, PNS and ENS. In this respect, cannabinoids represent an interesting family of compounds because their ability to control neuroinflammation both in CNS and ENS. Cannabidiol (CBD) is a cannabis derivative, lacking in unwanted psychotropic effects, thus representing a promising agent with high prospective for therapeutic use.

The aim of our study was to investigate the effect of CBD on LPS-induced glial cells activation.

Methods. C6, rat astocytoma cells and intestinal biopsies from patients with ulcerative rectocolitis (URC), separately, were stimulated with LPS plus INF-γ for 24h and the effect of CBD was studied. Mice with LPS-induced acute intestinal inflammation were treated 15 minutes before and 2 h after LPS with CBD. C6 cells and intestinal tissues, both from human and from mice, were processed for biochemical markers..

Results. Stimulation of C6 cells with LPS induced a significant nitrite production, as inflammatory marker, after 24 h, an effect counteracted by CBD treatment. Moreover, zimography assay revealed that CBD was able to prevent LPS-induced activation of matrix metal proteases, a key enzymes in the degrading of extracellular matrix, which may contribute to tissue injury. Similar results were obtained in URC biopsies, where the stimulation with LPS+INF increased nitrite production and the effect was reversed by CBD treatment. CBD also reduced the expression of S100B, a marker of glial cell activation and iNOS protein, suggesting an anti-inflammatory effect of CBD in URC biopsies. These data were confirmed in *in vivo* experiments in mice; a significant increase of S100B protein levels was found in the intestine of LPS-treated mice, while CBD pre-treatment prevented glial cell activation, as revealed by the reduced expression of S100B protein.

Conclusion Our results suggest that CBD, by modulating glial cells, is able to control the inflammatory environment and therefore open the way to new therapeutic strategies for all those inflammatory disorders, recognizing in the activation of glial cells their aethiopatogenesis.

CANNABINOIDS ARE NEUROPROTECTIVE IN A HUMAN CELL CULTURE MODEL OF PARKINSON'S DISEASE

Camille B Carroll, Marie-Louise Zeissler,
Virginia Stone, C Oliver Hanemann and John P Zajicek

Peninsula College of Medicine and Dentistry, University of Plymouth, Tamar Science Park, Plymouth, UK. PL6 8BU

Introduction: Cannabinoids have neuroprotective effects which could be exploited for the treatment of Parkinson's disease (PD). Upregulation of the CB1 receptor observed in the striatum of PD patients animal models may reflect neurochemical compensatory mechanisms or a response to neuronal damage. We investigated whether upregulation of the CB1 receptor occurred in human cell culture models of PD and whether CB1 receptor agonists have neuroprotective effects.

Methods: SH-SY5Y neuroblastoma cells were differentiated with retinoic acid and exposed to PD-relevant toxins: MPP^+, lactacystin and paraquat. Cannabinoids were co-administered with toxins. Cell death was assessed by the LDH assay. Quantitative real-time PCR was performed to assess fold change in expression levels of mRNA for the CB1 receptor. Enzymes involved in anandamide metabolism were detected by RT-PCR, immunohistochemistry and Western blotting.

Results: We found that MPP^+, paraquat and lactacystin resulted in an increase in CB1 receptor mRNA level. 10μM Δ^9THC protected against the toxic effects of all three toxins. This protective effect was not prevented by co-administration of the selective CB1 receptor antagonist AM251 nor could it be reproduced by the CB1 agonist, WIN 55,212-2. 0.01μM Cannabidiol, which is antioxidant but has little affinity at the CB1 receptor, exerted a significant protective effect against MPP^+ but did not protect against the toxic effects of paraquat or lactacystin. Cannabidiol may be acting via modulation of anandamide hydrolysis. We demonstrated the presence of enzymes involved in anandamide synthesis and hydrolysis, N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) and fatty-acid amide hydrolase (FAAH) respectively, in differentiated SH-SY5Y cells. We found that co-administration of URB597, a FAAH inhibitor, was protective against MPP^+.

Conclusion: This is the first demonstration of upregulation of the cannabis CB1 receptor in response to PD-relevant toxins. However, the protective effects of the phytocannabinoids, Δ^9THC and cannabidiol, are not mediated by the CB1 receptor, and may be related to modulation of endocannabinoid function.

THE NOVEL ENDOCANNABINOID N-ARACHIDONOYL-DOPAMINE EXERTS NEUROPROTECTIVE EFFECTS AFTER EXCITOTOXIC NEURONAL DAMAGE

Urszula Grabiec, Marco Koch, Sonja Kallendrusch,
Robert Kraft* and Faramarz Dehghani

Institute of Anatomy, Leipzig University, Leipzig 04103, Germany
*Carl-Ludwig Institute of Physiology, Leipzig University, Leipzig 04103, Germany

Pathological events within the central nervous system like trauma or stroke involve inflammatory processes that strongly account for further neurodegeneration. Classical endocannabinoids like 2-AG are known to exert neuroprotective effects after brain injuries. N-arachidonyl-dopamine (NADA), a recently identified endocannabinoid, was shown to activate CB1 receptors and/or transient receptor potential (TRP)V1 channels. Since NADA displayed anti-oxidative and anti-inflammatory effects in primary cultures of microglia cells, we asked whether NADA affects microglial cells and protects dentate gyrus granule cells after excitotoxic lesion.

Organotypic hippocampal slice cultures (OHSC) derived from Wistar rats were excitotoxically lesioned by application of NMDA (50µM) and subsequently treated with different NADA concentrations (0.1nM-10µM) alone or in combination with CB1/ TRPV1 antagonists.

NADA reduced the amount of microglial cells and degenerating neurons within the dentate gyrus. To identify the responsible receptor type mediating the neuroprotective effect, we applied the CB1 receptor antagonist AM251 and the TRPV1 channel antagonist 6-iodonordihydrocapsaicin. Neuroprotective properties of NADA were effectively blocked by AM251 whereas 6-iodonordihydrocapsaicin did not influence neuroprotection of NADA. Patch Clamp experiments in HEK cells stably transfected with human TRPV1 channel revealed that NADA evoked inward and outward currents. The NADA-induced response was relatively small compared to responses induced by the well-accepted TRPV1 agonist capsaicin.

In conclusion, our findings show that NADA protects dentate gyrus granule cells after excitotoxic lesion. NADA-mediated neuroprotection is driven by CB1 receptors and seemingly independent of TRPV1 channels.

CELL LINE SPECIFIC ENHANCEMENT OF SENSITIVITY TO ADRIAMYCIN BY PHYTOCANNABINOIDS IN BREAST CANCER

Sean Emery, Xu Di, David A. Gewirtz and Aron H. Lichtman

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA

While oral administration of Δ9-tetrahydrocannabinol (THC), also referred to as Marinol, is currently used in combination with cancer chemotherapy in the United States, there is surprisingly little understanding about how these drugs interact with current cancer chemotherapeutics. To address this concern, we evaluated the interaction between the cannabinoids, THC and Cannabidiol (CBD), and chemotherapeutic agents, starting with Adriamycin (ADR) in various breast cancer cell lines, including MCF-7 (human p53 wild type), MDA-MB-231 (human p53 wild type), and 4T1 (mouse p53 null) cells. THC was chosen because of its use in the clinic, and CBD was chosen because of its non-traditional profile, as well as the fact that it is more potent than THC at inhibiting cancer cell growth in our hands. Based on the well-documented antiproliferative effects cannabinoids are known to have on cancer, we hypothesized that there would be positive interactions between these agents. However, since these compounds are used in the clinic, it was also of interest to eliminate the possibility that the cannabinoids antagonized the antiproliferative effects of ADR. The Crystal Violet Sensitivity assay was used to quantify the extent of drug-induced growth inhibition. Neither THC nor CBD antagonized the antiproliferative effects of ADR. To the contrary, these drugs enhanced the effects of ADR in 4T1 cells and MDA-MB-231 cells. 4T1 cells are p53 null and therefore do not represent the vast majority of clinical breast cancers, many of which are more likely to be p53 mutant. However, 4T1 cells transfected with mutant p53 did not appear to alter the interaction between ADR and either cannabinoid compared to the parental 4T1 cells. We also examined the anti breast tumor activity of the full cannabinoid receptor agonist, W55212-2 and its enantiomer WIN55212-3, which does not bind to cannabinoid receptors. WIN55212-2 was highly efficacious in inhibiting cell growth in all three cell lines at concentrations ranging between 5 and 20uM, which were significantly lower than those for THC (15, 30 and 60 μM) and CBD (10, 20 and 40 μM), reducing concerns about possible off-target effects. In contrast, WIN55212-3 failed to elicit antiproliferative effects in any of the cell lines. This demonstration of stereoselectivity suggests that WIN55212-2 is operating through a specific receptor mediated (?) mechanism of action. Of importance, our studies indicate that neither THC nor CBD antagonize the antiproliferative effects of ADR. Moreover, these compounds may increase the efficacy of ADR, an effect that appears to be cell line specific. We speculate that specific genetic factors in the breast tumor cells are responsible for the drug interaction that could ultimately be relevant for predicting the conditions under which enhancement from the drug combination would occur in the clinic.

ANANDAMIDE SYNTHESIS IS ALTERED BY DEXAMETHASONE

Cecilia J. Hillard and Kara L. Stuhr

Medical College of Wisconsin; Milwaukee, Wisconsin, 53226; USA

The study and understanding of the endocannabinoid system has been made possible by the development of biochemical assays such as those used to quantify receptor and enzyme activity, mRNA expression, ligand presence, and others. Among the least well-understood biochemical mechanisms of endocannabinoid signaling are the processes and mechanisms of regulation of the synthesis of *N*-arachidonylethanolamine (anandamide). Study of these mechanisms is complicated by the fact that anandamide can be synthesized through multiple enzymatic pathways. Most of these pathways utilize the same precursor, N-arachidonylphosphatidylethanolamine (N-arachPE). We have developed an assay that can be used to determine the conversion of N-arachPE to anandamide by brain tissues using thin layer chromatography to separate chemical species. This method has the advantage that it is unbiased with respect to the pathway of conversion, thus modeling synthesis as it occurs in the brain. Using this method, we have found that anandamide synthesis from N-arachPE is affected biphasically by incubation with dexamethasone. Anandamide synthesis is significantly increased by 1 μM dexamethasone and significantly decreased by a lower 0.01 μM concentration ($p < 0.005$ for each, $n = 3$). These changes are rapid, being seen within 90 minutes, and occur in lysates suggesting that the mechanism is not transcription-dependent but likely the result of changes in signaling.

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CO_2 ASSIMILATION AND STOMATAL RESPONSE TO ELEVATED CO_2 IN *CANNABIS SATIVA* L.

Zlatko Mehmadic[1], Suman Chandra[1], Hemant Lata[1],
Ikhlas Khan[1, 2]and Mahmoud A. ElSohly[1, 3]

[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi,University, MS 38677, USA,
[2]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA,
[3]Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

The increase in environmental CO_2 and global warming are major environmental concern around the world. Atmospheric CO_2, from 270 μmol mol^{-1} in 1970 has climbed to a level above 360 μmol mol^{-1} at present and, is likely to be doubled by the end of the century (Houghton et al 1996). This increase may have considerable direct and indirect effect on various life forms, in particular on vegetation, since CO_2 is a substrate for photosynthesis (P_N) in plants and also, a major green house gas. Doubling in CO_2 concentration is reported to increase yield by 30% or more in crops (Poorter, 1993). A close correlation between P_N and crop yield is reported as more than 90% of dry matter of live plants is derived from photosynthetic CO_2 assimilation (Zelitch, 1975). Therefore, studying photosynthetic characteristic can reflect the biomass production potential of these species under changed climatic conditions due to elevated CO_2. Such studies are lacking in *Cannabis sativa* L., an important medicinal species worldwide. In this regard, present study was conducted to evaluate CO_2 assimilation and stomatal response of four high Δ^9-THC yielding varieties *Cannabis sativa* L. namely HPM, K2, MX and W1, under ambient CO_2 concentration (Ca, 360 μmol mol^{-1}) and elevated CO_2 level (720 μmol mol^{-1}). Elevated CO_2 stimulated P_N, water use efficiency (*WUE)* and internal CO_2 concentration (C_i) by an average of 44%, 123% and 87%, respectively and on the average suppressed transpiration (E) and stomata conductance (g_s) ~ 34% and 39%, respectively as compared to ambient CO_2 concentration. No significant difference in the ratio of internal to ambient C_i/C_a was observed in these varieties under elevated CO_2 as compared to ambient CO_2 concentration. Higher P_N, WUE and nearly constant C_i/C_a ratio under elevated CO_2 concentrations in *C. sativa* L. reflect a potential for better growth and productivity under CO_2 rich environment.

Studying plant growth and yield under elevated CO_2 fertilization using open top chambers (OTCs) or free air CO_2 enrichment (FACE) models for a complete life cycle in *Cannabis sativa* L. however, is needed in order to evaluate optimum conditions for highest biomass yield of each variety and the effects of those conditions on the chemical profile of each variety. This work will be the subject of our next report.

Acknowledgements

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NOVEL DRUG DERIVATIVES OF ISOPROPYLAMINO PROPANOL (AIPs) SUPPRESS HEPATOCELLULAR CARCINOMA VIA CANNABINOID RECEPTORS AND MULTIKINASE MODULATION

Stanislav I. Svetlov[1,2], Anatolyi Vakulenko[3], Mengde Cao[3] and David R. Nelson[3]

Center of Innovative Research, Banyan Biomarkers, Inc., Alachua, FL and Departments of Physiological Sciences[2] and Hepatology[3], University of Florida, Gainesville, FL, USA

Existing chemotherapy for liver cancer, particularly hepatocellular carcinoma (HCC), has limited utility, mainly due to significant toxicity of the drugs and the heterogeneity and resistance of malignant cells. The main problem is that most such patients have underlying cirrhosis, so chemotherapy can easily kill remaining normal liver cells. Thus, developing a therapy that would impair proliferation/survival of malignant cells, while being minimally toxic for primary hepatocytes, is of vital importance for treatment of HCC. Ideally, the therapeutics to be developed should selectively and safely inhibit growth of the bulk of HCC cells, as well as obliterate a small pool of putative cancer stem cells.

We developed novel modality- fatty acid derivatives of isopropylamino propanol, named jointly **AIP**s, for liver cancer treatment and prevention. In our experiments, AIPs inhibited proliferation/survival of human HCC and mouse hepatoma cells *in vitro,* and suppressed HCC-driven tumor induction in *in vivo* models. AIP-1 (long fatty acid) and AIP-3 (short fatty acid) analogs induced cell death in human HCC and mouse hepatoma cells in culture, while being minimally toxic for normal hepatocytes. AIPs completely blocked activation and growth of putative cancer stem cells, while AIP-1 halted tumor xenograft induction in Scid mice injected with HUH-7 cells.

AIPs utilize multifaceted mechanisms promoting cancer cell death, including inhibition of sphingosine phosphate kinase 2 (SPHK2), calcium-calmodulin kinaseI (CAMK-I) as well as modulation of endocannabinoid receptors CB1 and CB2. CB1 signaling through adenylate cyclase or PLC was assessed by modulation of specific transcriptional response elements placed upstream of the beta-lactamase. CHO cells overexpressing CB1 and co-transfected with different variants of G-proteins were used as the cell-based system and CP-55940 as standard agonist. AIP-1 has shown a significant CB1 antagonistic activity with IC50 of 3.5 μM, which is nearly 2-fold lower than IC50 for AIP-1 against HCC cells, and a modest CB2 receptor antagonism. In contrast, AIP-3 potentiated the effect of CP-55940, predominantly on the CB1 receptor signaling. In ATP-dependent kinase activity assay, AIP-1 strongly suppressed CAMK-I, while modestly inhibited SPHK2 activity. AIP-3 substantially inhibited SPHK2, but not SPHK-1, suppressed PDK1, and slightly affected CAMK-I.

Multifaceted drugs are required to overcome the molecular diversity of HCC and resistance of putative stem/progenitor cells to chemotherapy, and addition of AIPs may provide a new angle in prevention and treatment strategies. The active drugs are lipid-fatty acid derivatives, thus capable of forming liposomes, which enhances self bioavailability and delivery via either oral or locoregional transarterial hepatic tumor chemoembolization. Mechanisms of AIP action, effectiveness, and safety of nano-liposomal formulation of the drugs and potential clinical implication will be discussed.

CANNABIDIOL, A NON PSYCHOTROPIC PLANT CANNABINOID, REDUCES HUMAN ADENOCARCINOMA CELL PROLIFERATION

Barbara Romano, Gabriella Aviello, Francesca Borrelli and Angelo A. Izzo

Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy

Background/Aim Cannabidiol (CBD) is a safe and non-psychotropic ingredient of *Cannabis sativa* which exerts potent inhibitory effects on growth of several cancer cell lines[1]. In the present study, we have evaluated its effect on cell proliferation in human colon adenocarcinoma (Caco-2) cells. **Methods** Caco-2 cell survival was measuring using the MTT assay; cell proliferation was evaluated through ^{3}H-thymidine incorporation; p42/44 ERK expression was measured by western blot analysis; intracellular calcium levels were evaluated by using the Fura-2-acetoxymethyl ester (FURA-2AM) loading. **Results** CBD (up to 10^{-5} M) had no significant cytotoxic effects on Caco-2 cells after 24-hours exposure. Pre-incubation of Caco-2 cells for 24 hours with CBD (10^{-8}-10^{-5} M) significantly reduced ^{3}H-thymidine incorporation, $pERK_{1/2}$ expression and intracellular calcium levels. **Conclusions** CBD reduces human colon adenocarcinoma cell proliferation via a mechanism which could involve MAP kinase pathway.

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STRESS-CANNABINOID INTERACTIONS IN THE CENTRAL EXTENDED AMYGDALA

Sachin Patel[1], Cecilia J. Hillard[2] and Danny G. Winder[3]

Department of [1]Psychiatry, [3] Molecular Physiology and Biophysics, Vanderbilt University, Nashville, and [2]Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI

Clinical and preclinical data indicate an interaction between environmental stress and cannabinoid administration at the behavioral level. For example, anxiogenic and panic-like reactions to cannabis are more likely to occur under stressful conditions in humans, and pre-exposure of laboratory animals to stress shifts the effects of cannabinoids towards producing anxiogenic effects. We have previously shown that the central amygdala may be a key neural substrate subserving the interaction between stress and cannabinoid exposure. The central amygdala is one subregion of a larger functional nucleus termed the "extended amygdala", which has been implicated in stress responses, neuroendocrine modulation, and negative reinforcement.

Male ICR mice, 21-24 g were used as subjects. We used Fos immunohistochemistry as a marker of neuronal activation to identify brain regions within the extended amygdala-identified based on corticotropin releasing factor expression- showing interactions between 30 minute restraint stress exposure and tetrahydrocannabinol (THC) exposure (2.5 mg/kg i.p.). We used whole-cell ruptured patch clamp electrophysiology to determine the effects of CB_1 receptor activation of excitatory transmission in the central lateral amygdala.

Two-way ANOVA revealed significant interactions between stress and THC exposure on neuronal activation within the dorsal lateral (dl) and ventral (v) bed nucleus of the stria terminalis (BNST). A trend toward a significant interaction was observed within the central lateral nucleus. Irrespective of treatments, significant positive correlations in neuronal activation were observed between the dlBNST and the central lateral amygdala, the dlBNST and the vBNST, and the capsular and central lateral amygdala. Initial electrophysiological studies indicate that CB_1 receptor activation strongly inhibits brainstem excitatory inputs to the central lateral amygdala. Ongoing studies are aimed at determining the effects of CB_1 receptor activation on excitatory transmission in other regions of the extended amygdala, as well as examining endocannabinoid-mediated synaptic signaling in this region.

These data implicate the extended amygdala as a neural substrate that could subserve the interaction between stress and cannabinoids in the generation of anxiety responses. Understanding the role of eCB signaling in the extended amygdala could reveal mechanisms underlying cannabinoid-induced anxiety responses, and the role of this signaling system in affective disorders.

CANNABINOID-1 RECEPTOR ACTIVATION INDUCES REACTIVE OXYGEN SPECIES-DEPENDENT AND–INDEPENDENT MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION AND CELL DEATH IN HUMAN CORONARY ARTERY ENDOTHELIAL CELLS AND CARDIOMYOCYTES: IMPLICATIONS FOR CARDIOVASCULAR DYSFUNCTION

Partha Mukhopadhyay, Mohanraj Rajesh, Sándor Bátkai, Vivek Patel, Yoshihiro Kashiwaya, György Haskó, Lucas Liaudet, Oleg V. Evgenov, Ken Mackie and Pál Pacher

National Institutes of Health/NIAAA, Bethesda, Maryland, USA; UMDNJ-New Jersey Medical School, Newark, NJ 07103, USA; University Hospital, 1011 Lausanne, Switzerland; Harvard Medical School, Boston, Massachusetts, USA; Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN

INTRODUCTION: Impaired function of endothelial cells and/or cardiomyocytes plays a critical role in
the development of cardiovascular dysfunction associated with heart failure, diabetic complications,
hypertension, coronary artery disease and atherosclerosis. Increasing evidence has suggested that cannabinoid 1(CB_1) receptor inhibition is beneficial in atherosclerosis and cardiovascular inflammation both in experimental models, as well as in humans.

OBJECTIVES: Here, we investigated the effects of CB_1 receptor activation with the endocannabinoid anandamide(AEA) or synthetic agonist HU210 on cell death and interrelated signal transduction pathways in human primary coronary artery endothelial cells(HCAECs) and cardiomyocytes (HCMs).

METHODS: Cell death, CB_1 receptor expression, reactive oxygen species (ROS) generation, and activation of signal transduction pathways in cells were determined by flow cytometry and molecular biology tools.

RESULTS: In HCAECs or HCM expressing CB_1 receptors (demonstrated by Western immunoblot and flow cytometry) AEA or HU210 triggered concentration- and time-dependent activation of p38 and JNK MAPKs, cell death, and ROS generation. The AEA- or HU210-induced cell death and MAPK activation were attenuated by CB_1 antagonists (SR141716(rimonabant) and AM281), inhibitors of p38 and JNK MAPKs or the antioxidant Nacetylcysteine. N-acetylcysteine alone prevented AEA- or HU210-induced ROS generation, but only partially attenuated MAPK activation and cell death. In contrast, in combination with CB_1 antagonists Nacetylcysteine completely prevented these effects.

CONCLUSIONS: CB_1 receptor activation in endothelial cells and cardiomyocytes may amplify the reactive oxygen species-MAPK activation-cell death pathway in pathological conditions when the endocannabinoid synthetic or metabolic pathways are dysregulated by excessive inflammation and/or oxidative/nitrosative stress, thereby contributing to the development of endothelial dysfunction and pathophysiology of multiple cardiovascular diseases.

GPR55 HOMOLOGY MODEL BASED ON THE CRYSTAL STRUCTURES OF HUMAN A2A-ADENOSINE AND β2-ADRENERGIC RECEPTORS

Sari Yrjölä, Tuomo Laitinen and Antti Poso

School of Pharmacy, Faculty of Health Sciences,
University of Eastern Finland, Kuopio, Finland

The intronless gene coding for an orphan receptor GPR55 was first identified in 1999.[1] It has been suggested that GPR55 is a third cannabinoid receptor, since it has been reported to be activated by certain cannabinoid ligands, such as, AM251 and SR141716A.[2,3] Later studies have demonstrated, however, that GPR55 is not activated by Δ^9-THC or endocannabinoids indicating that GPR55 should be classified as an atypical cannabinoid receptor at best.[4] In addition, the strongly proposed endogenous ligand for GPR55 is phospholipid, lysophosphatidylinositol (LPI).[5]

In order to find novel GPR55 ligands using structure-based virtual screening, we have developed a three dimensional model of the receptor. We used the crystal structures of human A_{2A}-adenosine[6] and β_2-adrenergic receptors[7] as the templates for homology modeling. The sequence similarity between GPR55 and A_{2A}-adenosine and β_2-adrenergic receptors is rather low, but receptors share a common fold of seven transmembrane helices. The sequences of GPR55 and templates were aligned by comparing the most conserved residues of helices. The raw version of GPR55 model was refined by minimizations and molecular dynamic (MD) simulations. During the simulations the model was surrounded by a lipid bilayer and water environment mimicking the true environment of the receptor. The model held up its structure in MD simulations for tens of nanoseconds which indicates the successful alignments of the model sequence and template sequences. The known GPR55 ligands were docked into the receptor models in order to locate the binding site. Several putative binding modes were identified.

Sequence alignments and construction of the model was done with Discovery Studio by Accelrys; minimizations, MD simulations and rearrangement of the side chains with Desmond and Prime by Schrödinger Maestro; and docking was done with GOLD.

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PROBING THE GPR55 LIGAND BINDING SITE WITH LPI AND 2-AGPI

Evangelia Kotsikorou[1], Karla E. Madrigal[1], Dow P. Hurst[1],
Mary E. Abood[2] and Patricia H. Reggio[1]

[1]Center for Drug Discovery, University of North Carolina at Greensboro, Greensboro, NC 27402 USA
[2]Department of Anatomy and Cell Biology and Center for Substance Abuse Research, Temple University, Philadelphia, PA 19140 USA

Introduction: GPR55 is a Class A G protein-coupled receptor that has been shown to be activated by cannabinoids (Henstridge et al. FASEB J. 2008). This receptor is also activated by lysophosphatidylinositol (LPI) found in rat brain (Oka et al. BBRC, 2007), with 2-AGPI having the highest activity (Oka et al. J Biochem. 2009). Kapur et al. (J Biol Chem. 2009) have confirmed that LPI is a GPR55 agonist using a β-arrestin green fluorescent protein biosensor as a direct readout of agonist-mediated receptor activation. Here we report the docking and molecular dynamics (MD) simulations of LPI and 2-AGPI interaction with a computer model of the GPR55 activated state.

Methods: Outputs from NAMD MD simulations of LPI and 2-AGPI in a POPC lipid bilayer were used as input for receptor docking studies. Each compound was docked in an activated GPR55 receptor model that had been pre-equilibrated for 50 ns in a POPC bilayer. This model includes intra- and extracellular loops, as well as N- and C-termini. MD simulations of the docked ligands in the receptor were run for 300ns each to explore ligand-receptor interactions.

Results: The binding site for LPI and 2-AGPI is located between transmembrane helices (TMHs) 2, 3, 6 and 7 and the primary interaction site for LPI or 2-AGPI involves a phosphate oxygen interaction with K2.60. Additionally, S2.64 can hydrogen bond to another LPI/2-AGPI phosphate oxygen and Q2.65 can hydrogen bond to one of the LPI/2-AGPI inositol hydroxyl groups. LPI's glycerol hydroxyl can also hydrogen bond to the EC2 residue H170, while the corresponding 2-AGPI hydroxyl can hydrogen bond to E3.29.

Conclusions: We have identified the TMH 2, 3, 6 and 7 region of GPR55 as the putative binding site for LPI and 2-AGPI. This binding site includes interactions with the amino acids K2.60, S2.64, Q2.65, E3.29 and H170.

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CREDIBLE EVIDENCE FOR THE CNS EFFECTS OF CB2 CANNABINOID RECEPTORS

Emmanuel Shan Onaivi

Biology Department, William Paterson University, Wayne, USA.

Endocannabinoids, cannabinoids and marijuana use activate two well characterized cannabinoid receptors (CBRs), CB1-Rs and CB2-Rs. Previously, it was thought that CBRs in CNS were predominantly the CB1-Rs and that CB2-Rs were expressed in peripheral and glia tissues. Therefore, the expression of CB1-Rs in the brain and peripheral tissues has been well studied and characterized, but functional neuronal CB2-Rs have received much less attention than CB1-Rs. There is however mounting credible evidence for the functional neuronal CB2-Rs in the CNS. Here, we used immunoblotting, genotyping, immunoelectron microscopy, mouse behavioral assessment and quantified human and rodent CB2-R specific isoforms in different tissues and brain regions as well as in mice treated with CBR ligands. Association studies were performed between CB2-R SNPs in schizophrenia and depression in human population. We discovered the peripheral and CNS CB2-R subtype specific expression patterns in human and rodents that resolved the ambiguity and controversy over the neuronal expression of CB2-Rs that are localized mainly in post-synaptic elements of rat hippocampus and substantia nigra. Species comparison found that the CB2-R gene of human, rat and mouse genomes deviated in their gene structures and isoform expression patterns. Naive BTBR mice that have been reported to have autism-like behavioral phenotypes have an upregulated high level of CB2A gene expression in the cerebellum. There is an increased risk of schizophrenia for people with low CB2 receptor function. Indeed, our studies provide the first evidence for the neuronal CNS effects of CB2-Rs and its possible involvement in drug addiction and neuropsychiatric disorders. Thus the results provides much improved information about *CB2* gene structure and its human and rodent variants that should be considered in developing CB2-R-based therapeutic agents. Support NIDA-NIH, WPUNJ.

WIN55212-2 MODULATES CELL SURVIVAL IN MOUSE EMBRYOID BODIES VIA CB1 AND CB2 RECEPTORS

Marilia Z. P. Guimarães, Jader Nones, Tania C. L. S. Spohr, Daniel R. Furtado, Rafaela C. Sartore, Bruna S. Paulsen and Stevens K. Rehen

Instituto de Ciências Biomédicas,
Universidade Federal do Rio de Janeiro,
Rio de Janeiro, 21941-902, Brazil.

Embryonic stem cells (ESCs) are potentially able to replace damaged cells in animal models of neural pathologies such as Parkinson's disease, stroke, and spinal cord lesions. Nevertheless, many issues remain unsolved regarding optimal culturing procedures of these cells. For instance, on their path to differentiation *in vitro*, which usually involves the formation of embryoid bodies (EBs), they may present chromosomal instability, loss of pluripotency, or simply die. Therefore, finding strategies to increase the survival of cells within EBs is of great interest. Cannabinoid receptors have many roles in the physiology of the adult body, but little is known about their role in the biology of ESCs, even though they are known to be present very early in development.

In the present study we investigated how two cannabinoid receptors, CB1 and CB2, may affect the outcome of ESCs aggregated as EBs, using USP1 and R1 cell lines. RT-PCR was performed to check for the expression of cannabinoid receptors as well as stemness markers. EBs were treated for 2 days with WIN 55212-2 (WIN) in various concentrations, with or without antagonists. Next cell death and proliferation were evaluated by TUNEL and Phospho H3 histone labeling, respectively.

RT-PCR revealed that EBs expressed both CB1 and CB2 receptors. Incubation with WIN reduced cell death by approximately 45%, which was reversed by a CB1 antagonist. A specific CB2 agonist also reduced cell death by approximately 20%. . No increase in proliferation, neural differentiation or changes in chromosomal stability were observed.

These data indicate that both cannabinoid receptors, CB1 and CB2, are involved in reducing cell death in EBs mediated by exogenous cannabinoids. This could mean that cannabinoid signaling is functionally implicated in the biology of differentiating ESCs.

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EXPRESSION OF CB_1 RECEPTOR IMMUNOREACTIVITY IN COLORECTAL CANCER

Sofia B. Gustafsson[1], Richard Palmqvist[2] , Stig O.P. Jacobsson[1] and Christopher J. Fowler[1]

Departments of [1]Pharmacology and Clinical Neuroscience, [2]Medical Biosciences, Pathology, Umeå University, SE90187 Umeå, Sweden

Introduction*:* In both prostate and pancreatic cancer, a high expression of CB_1 receptor immunoreactivity (CB_1IR) is associated with a poor prognosis (Michalski et al., Int J Cancer 122 [2008] 742-50; Chung et al., *et al., Eur J Cancer* 45 [2009] 174-82). In contrast, a markedly decreased CB_1 expression compared to matched normal tissue has been seen in a small series of grade 2-3 colon carcinomas due to hypermethylation of the promotor region of the receptor (Wang *et al., Cancer Res* 68 [2008] 6468-76). In the present study, we have examined CB_1IR in a large series of well characterised tissue samples from patients with colorectal cancer.

Method: Formalin-fixed, paraffin-embedded specimens from >450 patients diagnosed with colorectal adenocarcinoma were used in the study (details, see Forssell *et al*., *Clin Cancer Res* 13 [2007] 1472-9). CB_1IR was assessed using a commercially available antibody (AbCam ab23703) which produces the appropriate pattern of staining in human cerebellum, but does not produce immunoreactivity in forebrains from $CB_1^{-/-}$ mice (see Chung *et al., ibid*.). CB_1IR was assessed in both non-malignant and tumour regions of the sections, the latter both in central parts and at the tumour front. Each area was scored for CB_1IR on a unitary scale from 0 (absent) – 3 (intense).

Results*:* All the specimens have been stained and of these 154 have been scored for tumour CB_1IR intensity. In the non-malignant tissue, expression of CB_1IR was found in the epithelial cells of the crypts, with scattered positivity in subepithelial inflammatory cells. This is consistent with the pattern of staining seen with other antibodies in the normal colon (Wright *et al., Gastroenterology* 129 [2005] 437-53; Marquéz *et al., PLoS One* 4 [2009] e6893). In the tumours, the CB_1IR was found in the epithelial cells but not the stroma. In both the central parts and fronts of the tumour, there was a large variation in the CB_1IR. Thus, for the central parts, the number of patients were 11 (7%), 72 (47%), 53 (34%) and 18 (12%) with scores of 0, 1, 2 and 3, respectively. For the 112 cases where tumour front CB1IR could be scored, the distribution was 5 (4%), 54 (48%), 36 (32%) and 17 (15%), respectively. This distribution was not significantly different from the distribution in the central parts of the tumour ($\chi^2 = 1.5$, df = 3, $p>0.6$), but the correlation between the scores for the 112 pairs was highly significant (Spearman's $r = 0.74$, $p<0.0001$).

Conclusion*:* The present data indicates that the dramatic reduction in tumour CB_1 receptor expression reported by Wang *et al.* (*ibid.*) is not a universal event, that approximately half of the cases have a medium-high level of CB_1IR in the tumours, and that the CB_1IR score in the central part of the tumour is highly correlated with the score in the tumour front. Upon completion of the analyses, the codes will be broken to allow determination of whether tumour CB_1IR is associated with disease outcome.

ISOLATION AND PHARMACOLOGICAL CHARACTERIZATION OF MINOR CANNABIS CONSTITUENTS

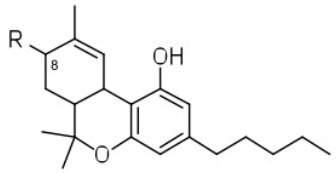
Samir A. Ross[a,b], Mohamed M. Radwan[a], Abir T. El-Alfy[c], Susan, P. Manly[a], Safwat A. Ahmed[a], Desmond Slade[a], Anwen Eslinger[c], Lisa Wilson[c], Suzanna Seale[c], Olivia Dale[d], Stephen Cutler[d], Ikhlas A. Khan[a,b] and Mahmoud A. ElSohly[a,e]

[a] National Center for Natural Products Research, Departments of [b]Pharmacognosy, [c]Pharmacology, [d]Medicinal chemistry and [e]Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

Many Natural product classes have been identified from Cannabis plant including: monoterpenes, sesquiterpenes, flavonoids, steroids and nitrogenous compounds but the C_{21} terpenophenolics, namely cannabinoids are uniquely found structures in *Cannabis sativa* L. To date more than 534 constituents have been isolated and/or detected in cannabis, out of which 100 are Cannabinoids.

The onset of HIV as a worldwide problem refocused marijuana as a possible symptom management drug and led to the discovery of the endocannabinoid system. Features of this system include that cannabinoids act through CB1 and CB2receptors.The CB1 receptor, uniquely recognized by cannabinoids, is found in brain and peripheral tissue of the central nervous system (CNS), while the CB2 receptor is primarily found outside the CNS in tissues associated with immune function.

As part of our program to study the constituents of high potency cannabis and their pharmacology we herein report the isolation and structure elucidation of seven minor cannabinoid constituents including six new (**1-6**) and one known (**7**) compounds. The structures of the isolates were determined by 1D and 2D NMR spectroscopic analyses, GCMS and ESIMS to be 8α-hydroxy-Δ^9-THC (**1**), 8β-hydroxy- Δ^9-THC (**2**), 10α-hydroxy- Δ^8-THC (**3**), 10β-hydroxy- Δ^8-THC (**4**), 9β,10β-epoxy-hexahydrocannabinol (**5**), 11-acetoxy- Δ^8-tetrahydrocannabinoic acid A (**6**), and cannabiripsol (**7**). The binding affinity of the isolated compounds (**1-7**) and Δ^9-THC, Δ^8-THC, CBC and CBD toward CB1 and CB2 receptors, functional activity as well as their behavioral effects in the mouse tetrade assay were studied.



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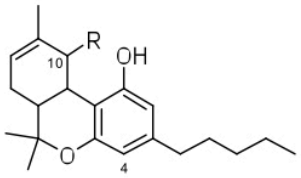
2 R = β-OH



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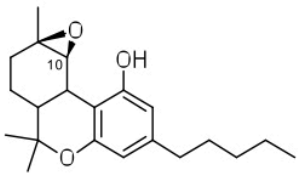

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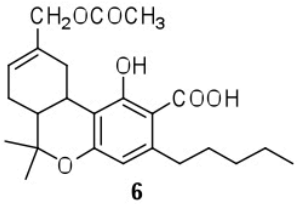

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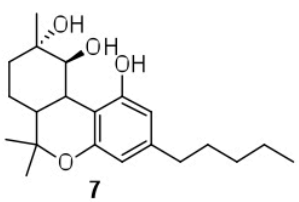

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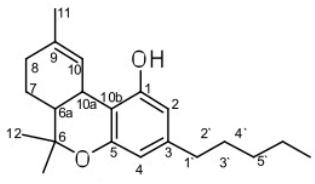

Δ^9-THC

EFFECTS OF CANNABIGEROL ON ALPHA $_{2A}$- AND ALPHA $_{2C}$-ADRENOCEPTORS

Maria G. Cascio, Carolyn Tanner, Lesley Stevenson, Daniele Bolognini, Ruth A. Ross and Roger G. Pertwee

School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK

Cannabigerol (CBG) is a little-investigated phytocannabinoid, which was first detected in *Cannabis sativa* and synthesized by Gaoni and Mechoulam (1964), and subsequently found not to induce Δ^9-THC-like psychopharmacological effects *in vivo* (Grunfeld and Edery, 1969; Mechoulam et al., 1970).

We have previously (Cascio et al., 2010) reported evidence from *in vitro* experiments that CBG is a potent α_2-adrenoceptor agonist. We have also obtained evidence that CBG can block 5-HT_{1A} and cannabinoid CB_1 receptors albeit with potency lower than that with which it appears to activate α_2-adrenoceptors. In the present study, with the aim of exploring further the *in vitro* pharmacology of CBG, we have performed β-arrestin assays using cells containing α_{2A} or α_{2C}-adrenoceptors. Some of our results have shown that: 1) CBG, tested alone at increasing concentrations (1 nM to 10 μM), is unable to activate by itself either α_{2A}- or α_{2C}-adrenoceptors; 2) at both 1 μM or 10 μM, CBG tested in combination with the well-known α_2-adrenoceptor agonist, clonidine (1 nM to 10 μM), is able to antagonize clonidine-induced activation of α_{2C}-adrenoceptors; finally, 3) CBG (0.01 nM to 10 μM) is able to counteract apparent desensitization of α_{2A}-adrenoceptors induced by 10 μM clonidine.

Further experiments are required to establish whether this phytocannabinoid is an α_2-adrenoceptor allosteric modulator or an α_2-adrenoceptor "indirect agonist".

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Funded by GW Pharmaceuticals

EFFECTS OF CANNABIDIOL (CBD) AND CANNABIGEROL (CBG) ON VOGEL'S CONFLICT TEST IN RATS

Yuki Yamasaki[a,b], Naoki Amada[a,b], Akihito Watanabe[a,b] ,
Serena Deiana[a], Tetsuro Kikuchi[b] and Gernot Riedel[a]

[a]Institute of Medical Sciences, University of Aberdeen, Foresterhill, AB25 2ZD, Aberdeen, UK
[b] Otsuka Pharmaceutical Co. Ltd., Tokushima, 771-0192, Japan

Introduction: Phytocannabinoids are thought to convey various benefits for human health. Recent reports indicated that cannabinoids have anxiolytic effects as shown in rodents tested in the elevated plus maze paradigm (Campos and Guimaraes, 2009. Prog. Neuropsychopharmacol. Biol. Psychiatry). Vogel's Conflict Test (VCT) is another anxiety paradigm well-established and pharmacologically validated for the evaluation of anxiolytic properties of drugs. Previous studies (Moreira et al., 2006. Prog. Neuropsychopharmacol Biol Psychiatry; Lisboa et al., 2008. Eur J Pharmacol.) also confirmed the anxiolytic potential of cannabidiol in this model, possibly through modulation of the periaqueductal grey. Here, we initially aimed at confirmation of the results using pure CBD in the VCT and then extend the work to plant extracts rich in CBD (CBD extracts) or those rich in cannabigerol (CBG extracts).

Method*:* Eight week old male Wistar rats were used, handled regularly and habituated to the test chamber for 20 min with free access to the drinking bottle. Then, animals were water deprived overnight and placed in the test chamber and drinking attempts were punished by electric shocks (0.50 mA, 500 msec) delivered every 20 licks between the grid floor and the spout of the drinking bottle. The number of licks throughout a 5-min experimental session was recorded. All cannabinoids in this study were extracted and provided by GW Pharmaceuticals.

Results: Pure CBD administered acutely in doses of 4-40mg/kg failed to reduce anxiety in rats. Similarly, both CBD extract (4-40mg/kg) and CBG extract (0.3-10 mg/kg) also failed to increase of licking rate. By contrast, diazepam (3 mg/kg) as a positive control reliably enhanced licking confirming its anxiolytic properties.

Conclusions*:* This is the first report to analyse the efficacy of CBD extracts and CBG extracts as anxiolytic drugs in VCT in rats. Interestingly, these data confirm results obtained in the elevated plus maze (see Abstract by Amada et al., this conference) and suggest that these extracts are devoid of anxiety relieving properties.

CHRONIC PUBERTAL CANNABINOID TREATMENT AS A VALID ANIMAL MODEL FOR ASPECTS OF SCHIZOPHRENIA: BENEFICIAL EFFECTS OF THE ATYPICAL ANTIPSYCHOTIC QUETIAPINE

M. Schneider[1] and F.M. Leweke[2]

[1]Department of Psychopharmacology, Central Institute of Mental Health, Mannheim, University of Heidelberg, Germany; [2] Department of Psychiatry, Central Institute of Mental Health, Mannheim, University of Heidelberg, Germany;

Chronic pubertal cannabinoid treatment in rats has been suggested as a possible animal model for schizophrenia since it results in long-lasting behavioral alterations reflecting certain aspects of schizophrenic symptomatology. Lasting deficits in sensorimotor gating, impaired short-term mnemonic processing, reduced motivation as well as inapproriate and deficient social behavior have been reported after chronic cannabinod treatment during pubertal development. In addition, sensorimotor gating deficits could be restored by acute injections of the typical antipsychotic haloperidol. The aim of the present study was to examine possible acute as well as lasting beneficial effects of the atypical antipsychotic drug quetiapine in adult animals that underwent chronic treatment of the synthetic cannabinoid receptor agonist WIN 55,212-2 (WIN) (1.2 mg/kg) during puberty. Therefore, animals were tested repeatedly for their performance in social interaction and social recognition after acute and chronic quetiapine treatment. Chronic pubertal WIN treatment induced persistent deficits in social recognition and impaired social interaction. Acute quetiapine treatment was able to completely restore those deficits in social behavior and social memory. Social recognition memory was affected again one week after cessation of chronic quetapine treatment, in social interaction however, persistent improvements could be detected. In conclusion, the results are indicating that the atypical antipsychotic drug quetiapine is able to acutely restore deficits in social behavior induced by developmental cannabinoid exposure and even excerts some persistent beneficial effects. Furthermore, the present data are further supporting and validating the suitability of chronic pubertal cannabinoid administration as an animal model for aspects of the etiology of schizophrenia.

THE PROTECTIVE EFFECT OF PROPOFOL AGAINST EXPOSURE TO ANANDAMIDE IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

Yasunori Mishima, Takahiko Ito, Asuka Ito and Kazuo Ushijima

Department of Anesthesia, Oita Sanai Medical Center
Oita, 870-11511, JAPAN

Propofol works as not only an anesthetic agent, but also an inhibitor of fatty amide acid hydrolase (FAAH). Furthermore, it has been reported that propofol can reduce cytokine-induced cell death. Anandamide (AEA) is generated during septic shock and is believed to be a causative mediator of shock. It has also been shown that AEA induces death of several cells types. Therefore, it is hypothesized that pretreatment with propofol may affect in AEA-induced cell death.

Human umbilical vein endothelial cells (HUVECs) were used in this study, and the cells were stimulated with 10μM of AEA to induce cell death. Survival and viability were tested by a cell proliferation assay using propofol, CB1 and CB2 antagonists (AM251 and AM630, respectively), and FAAH inhibitor URB597. An FAAH inhibitor screening assay evaluated the magnitude of FAAH inhibition by propofol and URB597.

Although 10μM of AEA induced significant cell death, 100μM of propofol maintained cell viability. AM251 and AM630 did not block AEA-induced cell death. However, 10nM of URB597 enhanced AEA-induced cell death. Although FAAH inhibitor screening assay showed that both propofol and URB597 inhibit FAAH activity in a dose dependent manner, propofol did not inhibit FAAH activity at the clinical concentrations. Propofol blocks AEA-induced cell death, but does not act as an FAAH inhibitor at clinical concentrations, and therefore, does not affect the metabolism of AEA clinically. In instances of clinically high level of AEA secretion, such as in septic shock and reperfusion injury, propofol may protect cells from damage caused by AEA.

ROLE OF PHOSPHORYLATION IN THE CONFORMATION OF THE FOURTH CYTOPLASMIC LOOP OF CB1 CANNABINOID RECEPTOR

Anjali D. Ganjiwale[1], Indu R. Chandrashekaran[1], Allyn C. Howlett[2] and Sudha M Cowsik[1]

[1]Jawaharlal Nehru University, New Delhi110067, India, [2] Wake Forest University Health Sciences, Winston-Salem, North Carolina 27157

Phosphorylation is an important regulatory mechanism in receptor signaling. It has been shown that the phosphorylation of the CB1 receptor disrupts modulation of ion channels by the receptor.CB1 cannabinoid receptor intracellular C-terminal tail domain (amino acids 401-417) is critical for G(i/o) protein coupling. Peptide fragments that represent the key intracellular domains of the receptor demonstrated that the proximal portion of the C-terminus of the cannabinoid CB1 receptor is a primary determinant for G-protein activation. The synthetic peptide fragment of the C-terminal juxtamembrane region (CB1 401-417) referred here as IL4 peptide mimicked the receptor's response of inhibiting adenylate cyclase. In the present study, we have used phosphorylated analogues of IL4 peptide to analyze the effect of phosphorylation on the conformation of the peptide and G-protein activation using CD spectropolarimetry and NMR Spectroscopy. Unambiguous proton NMR assignments have been carried out with the aid of correlation spectroscopy (DQF-COSY and TOCSY) experiments and nuclear Overhauser effect spectroscopy (NOESY and ROESY) experiments. The distance constraints obtained from the NMR data have been used in torsion angle dynamics algorithm for NMR applications (DYANA) to generate a family of structures which have been refined using restrained energy minimization and dynamics. These data show that in water the IL4 peptide prefers to be in an extended conformation. In the presence of sodium dodecylsulfate (SDS) micelles, a membrane model system, helical conformation is induced. The conformational range of the phosphorylated IL4 peptide revealed by NMR studies has been analyzed in terms of characteristic secondary structural features. The results obtained provide insight into the mechanism by which the peptide activates G-proteins, as a first step in signal transduction.

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THE KEY ROLE OF THE ENDOCANNABINOID SYSTEM IN CONTROLLING MALE REPRODUCTIVE POTENTIAL

Giuseppina Catanzaro, Natalia Battista, Gianna Rossi, Mariangela Pucci, Cinzia Rapino, Filomena Fezza, Sandra Cecconi and Mauro Maccarrone

Department of Biomedical Sciences/European Center for Brain Research/S. Lucia Foundation, Via del Fosso di Fiorano 64, Rome, Italy 00143

During the past few years accumulated evidence has shown that endocannabinoids have a prominent role in regulating reproductive events, and consistently the essential components of the endocannabinoid system have been demonstrated in male and female reproductive cells and tissues. Here, we sought to extend to mouse previous observations on other mammalian sperm cells, because genetically modified mice could help to clarify the impact of distinct elements of the endocannabinoid system on male reproduction. In addition, we ascertained the effect of sperm capacitation on the endocannabinoid system, by using immunochemical and functional assays, as well as confocal microscopy.

We show that mouse sperm have the biochemical machinery to bind (CB1R, CB2R and TRPV1), synthesize (NAPE-PLD and DAGL) and degrade (FAAH and MAGL) the two most prominent endocannabinoids (anandamide and 2-arachidonoylglycerol). Moreover, we demonstrate that capacitation markedly increases 2-arachidonoylglycerol content (~4-fold over controls), by increasing DAGL activity (~10-fold over controls) without affecting the activity of MAGL. Also anandamide levels were increased in capacitated sperm cells, but this was not due to any overt alteration in NAPE-PLD or FAAH activity.

Overall, our data suggest that 2-arachidonoylglycerol and its synthetic enzyme DAGL might play a major role in controlling mammalian sperm cells, an unprecedented observation that might open new therapeutic opportunities to correct anomalies in male reproductive functions.

CONVERSION OF AM630 INTO AN APPARENT NEUTRAL CB_2 RECEPTOR ANTAGONIST

Daniele Bolognini[1,2], Maria Grazia Cascio[1], Ruth A. Ross[1] and Roger G. Pertwee[1]

[1]School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK, AB25 2ZD. [2]DBSF and Neuroscience Center, University of Insubria, Busto A. (VA) Italy.

We have found previously that Δ^9-tetrahydrocannabivarin (THCV) binds to hCB_2 receptors with significant affinity (K_i = 225nM), and that by itself, it behaves as a CB_2 receptor partial agonist in both GTPγS binding and cyclic AMP assays (Bolognini et al., 2010). Here we describe experiments in which we were able to induce signs of neutral antagonism of CP55940- and THCV-induced activation of hCB_2 receptors with the inverse agonist/antagonist, AM630.

Activation of CB_2 receptors by CP55940 and THCV was monitored by measuring their ability to inhibit cyclic AMP production stimulated by 10μM forskolin in Chinese hamster ovary (CHO) cells transfected with hCB_2 receptors (n = 4). Displacement binding assays were performed with membranes from these cells using [^{3}H]CP55940 (n = 4). Further details of the methods we used can be found in Bolognini et al. (2010). In some experiments hCB_2 CHO cells were incubated with 10μM of AM630 for up to 24h and then subjected to intense washing (Mancini et al., 2009). THCV was obtained from GW Pharmaceuticals and all compounds were dissolved in DMSO.

First we showed that AM630 (100nM) induced a marked downward shift in both CP55940 and THCV log concentration-response curves. Next, we showed that AM630 by itself stimulated the production of cyclic AMP by hCB_2 CHO cells (EC_{50} = 151nM). No such an effect was detected in cells that had been preincubated with AM630, suggesting that it might behave as a neutral antagonist in cells that are pretreated in this way. We also found that inhibition of cyclic AMP production by CP55940 and THCV was similar in AM630-preincubated and unpreincubated cells. We then went on to investigate the ability of AM630 to antagonize CP55940 and THCV in AM630-preincubated cells. We found that in these cells, AM630 (25μM) produced significant rightward shifts in the log concentration-response curves of both CP55940 and THCV, but no downward shift of either curve. The two dextral shifts were similar in magnitude. No significant antagonism of these agonists was induced by AM630 at 0.1, 1 or 10μM. We also found that the K_i value of CP55940 for [^{3}H]CP55940 displacement from hCB_2 receptors was similar in membranes from AM630-preincubated cells (K_i = 16.8nM) and membranes from unpreincubated cells (K_i = 13.4nM). Corresponding K_i values for AM630 were 273nM and 143nM, respectively.

In conclusion, this investigation has provided further evidence that THCV can activate CB_2 receptors. It has also shown that by pre-exposing CB_2 receptors to AM630, it is possible to convert this CB_2-selective inverse agonist/antagonist into an apparent neutral antagonist of CB_2 receptor agonists. Further experiments are required to establish why AM630 displays rather low potency as a CB_2 receptor antagonist in AM630-preincubated cells.

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DIAGNOSTIC CRITERIA FOR CANNABIS WITHDRAWAL

David A. Gorelick,[1] Kenneth H. Levin,[1] Marc L. Copersino[2], Stephen J. Heishman[1], Fang Liu[3], Douglas L. Boggs[3] and Deanna L. Kelly[3]

[1]Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, MD 21224 USA; [2]McLean Hospital/Harvard Medical School, Belmont, MA 02478 USA; [3]Maryland Psychiatric Research Center, University of Maryland School of Medicine, Catonsville, MD 21228 USA

Introduction: Cannabis withdrawal is not recognized in the US DSM-IV and is recognized in the WHO ICD-10, but without diagnostic criteria. Diagnostic criteria have been proposed for DSM-V.

Methods: We collected from a convenience sample of 469 cannabis smokers retrospective self-report data on their most "difficult" (self-defined) quit attempt without formal treatment. The questionnaire assessed the experience of the index quit attempt, including the occurrence and response (any action taken to relieve) to 40 potential cannabis withdrawal symptoms.

Results: Subjects were 79.5% African-American, 58% male, and not seeking treatment. 71.2% had used cannabis more than 1,000 times. The index quit attempt started 2 days to 35 years (median 12 months) before the interview, and lasted 1 day to 35 years (median 2 months). At the start of the quit attempt, subjects were (mean [SD]) 27.7 [9.1] years old (range 10-64 years) and had been using cannabis at least weekly for 11.3 [8.8] years (range 0-42 years). Almost all (95.5%) subjects endorsed at least one withdrawal symptom; the mean was 9.5 [6.1] (median 9.0, range 1-38). The 7 proposed DSM-V withdrawal symptoms were reported by the following proportion of subjects (experienced symptom/took action to relieve it): irritability/anger/aggression—58.4%/45.2%, anxiety—50.1%/39.9%, insomnia—64.8%/48.0%, decreased appetite/weight loss—42.9%/20.3%, restlessness—33.7%/24.1%, depression—45.1%/34.0%, physical symptoms—34.5%/24.7%. Limiting symptoms to those causing enough distress to prompt action (a surrogate for proposed DSM-V Criterion B "clinically significant distress or impairment"), 41.1% of subjects met the proposed DSM-V criterion ($\geq$3 symptoms) for cannabis withdrawal (38.4% if physical symptoms omitted). Subjects meeting the criterion, compared to those who did not, had longer duration of lifetime use (14.0 vs. 12.3 years), greater frequency of use in the 6 months (75.6% vs. 58% $\geq$ daily use) and month (24.2 vs. 21.5 days) prior to start of the quit attempt, and were more likely to relapse (92.8% vs. 83.3%), but did not differ significantly in duration of quit attempt or other cannabis use or demographic characteristics.

Conclusions: These findings suggest that the proposed DSM-V diagnostic criteria identify a clinically significant withdrawal syndrome in a large minority (41.1%) of non-treatment-seeking, chronic cannabis users who try to quit, and that physical symptoms contribute little to the diagnosis.

Acknowledgements: Supported by the Intramural Research Program, NIH, National Institute on Drug Abuse and NIDA Residential Research Support Services Contract HHSN271200599091CADB.

PRE-TREATMENT WITH CANNABIDIOL ENHANCES Δ^9-THC BEHAVIOURAL EFFECTS AND BLOOD LEVELS IN ADOLESCENT RATS

Charlotte Klein, Emily Karanges, Thanh Huynh, Jarrah Spencer, Nathan Gunasekaran, Jonathon Arnold, Leonora Long, Tim Karl and Iain McGregor

Schools of Psychology and Pharmacology, University of Sydney, Australia
Prince of Wales Medical Research Institute, University of New South Wales, Australia

The psychotropic effects of *Cannabis sativa* are mainly produced by Δ^9-tetrahydrocannabinol (THC), while another cannabis constituent, cannabidiol (CBD), lacks significant psychoactive effects when given alone. However, the interactions between THC and CBD may have important implications for understanding the long-term health consequences of chronic marijuana use and are relevant for the development of THC- and CBD-based therapeutics such as *Sativex*. Studies examining the interactions between THC and CBD in laboratory animals and humans have revealed contradictory results, with some studies showing attenuation and others potentiation of THC-effects by CBD. To further investigate this issue, the current study treated adolescent male Wistar rats daily with THC or CBD plus THC (CBD/THC) for 21 consecutive days. The starting dose of both drugs was 1mg/kg (IP), which was increased to 3 mg/kg after 7 days, and to 10 mg/kg after a further 7 days. A 20 min interval separated the injection of CBD and THC. During the drug administration period the rats were tested in the emergence test, elevated plus maze (EPM), social interaction (SI) test and conditioned place preference (CPP) paradigm. In the emergence test the 3 mg/kg CBD/THC dose, but not THC alone, had an anxiogenic effect, while the EPM revealed a THC-induced anxiogenic effect that was more pronounced in CBD/THC rats. Both THC and CBD/THC decreased SI in rats at the 3 mg/kg dose. The CPP test showed a trend towards a preference for CBD/THC but not THC. During CPP conditioning, decreased locomotor activity was seen in CBD/THC but not THC-treated rats. Lower body weight was observed in THC-treated rats and this effect was significantly potentiated by CBD. Overall, these results suggested that CBD may potentiate some THC effects, perhaps through a pharmacokinetic interaction. To investigate this hypothesis, we acutely injected an additional 16 rats with either THC or CBD/THC and analysed blood THC levels using GC-MS. Results showed higher THC concentrations in acute CBD/THC rats than in THC rats. A trend towards the same effect was also seen with chronic administration. These findings suggest that it is not necessarily the case that CBD acts as a functional antagonist of THC, but that it can potentiate the effects of THC most likely through inhibition of THC-metabolizing hepatic cytochrome P450 enzymes.

Supported by the Australian Research Council

INVESTIGATION OF RESIDUES CRUCIAL FOR SELECTIVE LIGAND RECOGNITION AND SIGNAL TRANSDUCTION IN THE HUMAN CB1 CANNABINOID RECEPTOR

Jahan P. Marcu[1], Ankur Kapur[1], Patricia H. Reggio and Mary E. Abood[1]

[1]Temple University Philadelphia, PA 19140,
[2]University of North Carolina, Greensboro, NC 27412

The cannabinoid receptors belong to the Class A rhodopsin-like superfamily of G-protein coupled receptors (GPCRs). In the absence of a crystal structure of the CB_1 or CB_2 proteins, much of the structural information on these proteins has been gained from biochemical, mutational, and modeling studies. Highly conserved, charged residues are of interest as they may be important for structural or functional aspects. For instance, in the CB1 receptor, the third transmembrane domain contains a highly conserved lysine residue at position K3.28 (K192). Previous research on an alanine substitution of K3.28 (K3.28A) showed that only WIN55,212 (WIN) could maintain CB1 receptor binding (Song and Bonner, 1996; Chin et al, 1998). CP55,940 (CP) binding was eliminated, as was that for HU210 and anandamide. These and subsequent mutation and computational studies suggest that specific residues are important for recognizing different types or classes of cannabinoids. Aminoalklyindole derivatives appear to require different residues for receptor activation than the bicyclic, fatty acid amide, and classical cannabinoids. D2.63 is a highly conserved aspartate in a nonbinding region of the second transmembrane domain of CB1. We have previously demonstrated that mutation of D2.63 to N does not affect ligand binding but does play a crucial role in modulating signal transduction (Kapur et al, 2008). K373 is another lysine residue of interest, located on the 3rd extracellular loop of the CB1 receptor. Together, D2.63 and K373 are thought to form a salt bridge which constrains the receptor under normal receptor activation, stabilizing the binding pocket. Disrupting this salt bridge may alter receptor dynamics, effectively preventing bicyclic cannabinoids from achieving high binding affinities. Hence, disruption of this salt bridge should alter CP binding but not WIN.

We used site-directed mutagenesis to investigate the role of highly conserved amino acids resides at position K3.28A, D2.63, and K373 of the CB1 receptor. Stable CB1 mutant cells lines were established with HEK293 cells and expression was confirmed by immunostaining. We substituted alanine for each of our mutations: D2.63A, K3.28A, and D2.63A-K373A. Preliminary GTPγS binding data demonstrates markedly lower activity for CP in all three mutant receptors: K3.28A, D2.63A and the double mutant D2.63A-K373A. WIN binding was only slightly lowered versus the wild type receptor. Furthermore, the K3.28A mutation maintained high levels of receptor stimulation by WIN, while CP could not significantly affect receptor activity. Our results suggest these three key residues are highly involved in CP binding mechanisms and receptor stimulation.

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ANTAGONISM OF Δ^9-THC BY RIMONABANT: TIME-COURSE STUDIES IN RATS

Sherrica Tai, Roger Gifford, Torbjörn U.C. Järbe and Alexandros Makriyannis

Center for Drug Discovery, Northeastern University,
Boston, MA 02115, USA

Rimonabant is a cannabinoid receptor 1 (CB1R) antagonist/inverse agonist originally launched as an anti-obesity medication. Although now withdrawn as a medication, the compound still has utility as a pharmacological tool in uncovering roles of the endocannabinoid system in normal and pathophysiological body functions. The half-life of orally administered rimonabant has been estimated to be 1 to 2 weeks, depending on body weight mass – the larger the body weight, the slower the excretion. The slow elimination is thought to reflect preference for uptake by adipose tissue and subsequent sequestration. In acute studies using food restricted rats that were lever pressing for food, the *in vivo* half-life after systemic administration has been estimated to be around 15 hrs (McLaughlin et al., 2003). The time-course of rimonabant as an antagonist of cannabinergic induced CB1R agonism has been less investigated. We used two behavioral operant procedures that are sensitive to the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC): rat drug discrimination and suppression of fixed ratio responding (FR). Two training doses of Δ^9-THC (1.8 and 3 mg/kg) served as discriminative cues in two groups discriminating between Δ^9-THC and vehicle; injections were ip 20 min before session onset. Once trained to criterion, test sessions were interspersed between the regular training sessions to assess the dose-response functions of Δ^9-THC as well as the time-course for rimonabant in its ability to block the discriminative stimulus effects of Δ^9-THC. For these antagonism tests the training doses of Δ^9-THC were used and the rimonabant dose was 1 mg/kg. Testing occurred 20, 60, 120, and 240 post rimonabant administration; Δ^9-THC was administered 20 min prior to testing. Non-linear regression analyses (Prism software v.5) were used to estimate the median effective dose (ED_{50}) and 95% confidence limits (± 95% C.L.) as well as the *in vivo* half-life in antagonizing the discriminative stimulus effects of Δ^9-THC (regression model: log dose – variable slope constraining top and bottom of the curves to 100 and 0). The ED_{50} estimates for the dose-response functions were 0.38 (± 0.28-0.51) and 0.50 (± 0.40-0.63) mg/kg for the training doses of 1.8 and 3 mg/kg Δ^9-THC. The time-course studies suggested half-life estimates of 128.4 (± 95.7-172.2) and 98.4 (± 64.2-150.7) min for the two training conditions, respectively. For the ongoing FR studies only one lever was activated and every 10^{th} (FR-10) press on the lever resulted in food delivery. When the rate of responding had stabilized, test with Δ^9-THC, rimonabant and combinations of the two drugs occurred twice weekly, customarily on Tuesdays and Thursdays. On interim days, rate of responding was assessed when the rats were non-drugged. These additional findings will also be presented at the symposium.

P.J. McLaughlin, K. Winston, L. Swezey, A. Wisniecki, J. Aberman and D.J. Tardif *et al.*, The cannabinoid CB1 antagonists SR141716A and AM251 suppress food intake and food-reinforced behavior in a variety of tasks in rats, *Behav Pharmacol* **14** (2003), pp. 583–588.

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LATE BREAKING ABSTRACT

PERFORANT PATHWAY TRANSSECTION INDUCES DIFFERNTIAL REGULATION OF ENDOCANNABINOID LEVELS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

Sonja Kallendrusch, Marco Koch, Urszula Grabiec and Faramarz Dehghani

Institute of Anatomy, Leipzig University, Leipzig 04103, Germany

Endocannabinoids (eCB) are involved in the preservation of the homeostatic and regulatory balance within the central nervous system. The potency of eCB to control neuronal destruction and immune reactions that are associated with secondary neuronal damage has been recently proven. However, the knowledge of the temporal regulation of eCB levels within different brain areas is an indispensible requirement to fully utilize the neuroprotective potential of distinct eCB during brain pathologies. The perforant pathway provides a well-defined fibre tract connecting the entorhinal cortex with the dentate gyrus (DG). Since the perforant pathway transection (PPT) in organotypic hippocampal slice cultures (OHSC) reflects a well established model to investigate denervation-induced gliosis, trans-neuronal degeneration and plasticity-related changes in the hippocampal formation, the region and time-dependent regulation of eCB levels were analyzed in the present study.

Dentate gyrus (DG) and entorhinal cortex (EC) were dissected from OHSC 5 minutes, 1 hour, 4 hours, 12 hours, 24 hours, 48 hours or 72 hours after PPT and the eCB levels were measured by combined liquid chromatography/tandem mass spectrometry (LC/MS-MS).

Generally, all investigated eCBs displayed a temporal variability in the DG of lesioned OHSC in relation to untreated control OHSC whereas all eCB levels remained comparable in EC. The levels of palmitoylethanolamide (PEA), arachidonoylethanolamide (AEA), oleoylethanolamide (OEA) and 2-arachidonoylglycerol (2-AG) were maximally increased 24 hours after PPT and decreased to control levels 48 hours after PPT.

In conclusion, all investigated eCB showed a region-specific elevation after PPT since all eCB concentrations were elevated in the DG, the target area of the perforant pathway. This observation is in line with other studies showing that eCB levels are up-regulated after brain damage. The here shown data further demonstrate that the endocannabinoid system is activated within 24 hours after the induced damage, indicating a defined temporal activation of the endocannabinoid system. Thus, our data may help to characterize a clear therapeutic window for eCB being necessary for the possible development of clinical trials.

ABSTRACT REVISION

FATTY ACID AMIDE HYDROLASE ACTIVITY CORRELATES WITH BMI IN ADIPOCYTES FROM METABOLICALLY HEALTHY HUMANS

Jemma C. Cable[1], Garry D. Tan[1],
Stephen P. H. Alexander[2] and Saoirse E. O'Sullivan[1]

1. School of Graduate Entry Medicine and Health, University of Nottingham, Derby, DE22 3DT, UK
2. School of Biomedical Sciences, University of Nottingham, Nottingham, NG7 2UH, UK

Blood serum concentrations of endocannabinoids are elevated in obesity. Studies investigating the effects of obesity on fatty acid amide hydrolase (FAAH) mRNA levels have shown that FAAH mRNA is downregulated, upregulated or not different in subcutaneous adipose tissue between obese and lean controls. No studies to date have examined the effects of body weight on FAAH enzyme activity. The aim of the present study is to investigate any correlation between FAAH activity and markers of body fat and metabolism in subcutaneous mature adipocytes taken from healthy people over a range of body mass indices (BMI).

Ethical approval was granted by the University of Nottingham Medical School Ethics Committee, and volunteers were recruited from staff and students of the University of Nottingham (BMI range 19-34). Exclusion criteria included type 2 diabetes and hypertension. Anthropometric measurements, such as height, weight and various skinfold thicknesses, were taken on all volunteers. Following an overnight fast, a venous blood sample was taken and a subcutaneous abdominal adipose sample obtained via a needle biopsy under local anaesthetic. Blood serum was separated and stored at -80^{o}C prior to glucose, insulin and adiponectin assays. Adipose tissue was digested with collagenase to release mature adipocytes, which were then homogenised and centrifuged (20,000 *g*, 20 minutes). The particulate and cytosolic fractions were stored at -80^{o}C until assay of FAAH activity using 2 μM *N*-arachidonoyl-^{3}H-ethanolamine as substrate.

FAAH activity was detected in the particulate fractions and was completely eradicated in the presence of the FAAH inhibitor URB597 (1 μM). There were no significant correlations between FAAH activity and fasting serum glucose, insulin or adiponectin. Similarly, there were no significant correlations between FAAH activity and estimates of total body fat and fat distribution, such as waist to hip circumference ratio or skinfold thickness at various sites. However, FAAH activity did show a positive relationship with BMI ($r^2 = 0.14$, $P < 0.05$).

In this sample of healthy people, FAAH activity in mature adipocytes does not correlate with many of our measured metabolic markers or estimations of adiposity, but does correlate with BMI. Further work in this research will focus on obese and morbidly obese patients to assess whether any trends in FAAH activity and metabolic or body fat markers are observed within these populations.

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