26TH ANNUAL SYMPOSIUM OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

BUKOVINA

POLAND

JUNE 26 – JULY 1, 2016

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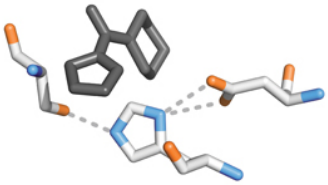

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26TH Annual Symposium of the International Cannabinoid Research Society
26 June - 1 July, 2016 Bukovina, Poland

SCHEDULE OF EVENTS

Sunday, 26 June

16:00 – 18:00 **REGISTRATION** (Hotel Bukovina, Bukowina Tatrzanska)
18:30 – 20:00 **WELCOME RECEPTION**

DAY 1: Monday, 27 June

08:15 - 08:30 **WELCOME AND OPENING REMARKS** (Hotel Bukovina)
08:30 – 10:00 **ORAL SESSION 1:** Medicinal *Cannabis* I
10:00 – 10:30 **COFFEE BREAK**
10:30 – 11:00 **ICRS CAREER ACHIEVEMENT AWARD**
11:00 – 12:15 **ORAL SESSION 2:** Endocannabinoid Transport / Dynamics
12:15 – 13:30 **LUNCH/NIDA Trainee lunch**
13:30 – 14:45 **ORAL SESSION 3:** Cardiovascular System
14:45 – 15:30 **ORAL SESSION 4:** Behavioural Neuroscience 1 – Pain and Sleep
15:30 – 16:00 **COFFEE BREAK**
16:00 – 16:45 **ORAL SESSION 4** (continued)
16:45 – 17:00 **BREAK**
17:00 – 19:30 **ORAL SESSION 5:** Focus on CBD
19:30 – **DINNER**

DAY 2: Tuesday, 28 June

08:30 – 10:30 **ORAL SESSION 6:** Focus on CB2
10:30 – 11:00 **COFFEE BREAK**
11:00 – 12:00 **ICRS PRESIDENTIAL ADDRESS:** Moving from Mechanisms of GPCR Functionality to New Drugs
12:00 – 13:00 **LUNCH**
13:00 – 15:00 **ORAL SESSION 7:** Receptors, Probes and Signalling
15:00 – 17:15 **COFFEE & POSTER SESSION 1**
17:15 – 19:30 **ORAL SESSION 8:** Neuroprotection / Neuroinflammation / Neurogenesis
19:30 – **DINNER**

DAY 3: Wednesday, 29 June

08:30 – 10:15 **ORAL SESSION 9:** Metabolism, Endocrine, Diabetes
10:15 – 10:45 **COFFEE BREAK**
10:45 – 12:15 **ORAL SESSION 10:** *Cannabis*, Medicinal
12:15 – 13:00 **KANG TSOU MEMORIAL LECTURE:** Human Exosome Complex in Health and Disease
13:00 – 14:00 **LUNCH**
14:00 – **OUTING**

DAY 4: Thursday, 30 June

08:30 – 09:30 **ORAL SESSION 11:** Addiction
09:30 – 10:00 **YOUNG INVESTIGATOR AWARD PRESENTATION:** Endocannabinoids as Homeostatic Signal Counteracting Adverse Effects of Stress
10:00 – 10:30 **COFFEE BREAK**
10:30 – 11:45 **ORAL SESSION 12:** Behavioural Neuroscience II – Anxiety, Stress
11:45 – 13:00 **LUNCH**
13:00 – 15:00 **POSTER SESSION 2**
15:00 – 16:15 **ORAL SESSION 13:** Endocannabinoid Metabolism
16:15 – 16:45 **COFFEE BREAK**
16:45 – 17:45 **ORAL SESSION 13** (continued)
17:45 – 20:00 **ICRS BUSINESS MEETING**
20:00 – **AWARDS CEREMONY & ICRS BANQUET**

Friday, 1 July **DEPARTURE**

Registration: June 26th, 2016, 16.00 – 18.00

Hotel Bukovina, Bukowina Tatrzanska

Welcome Reception: 18.30 – 20.00

Day 1
Monday, June 27th

Breakfast at your accommodations			
8.15	Welcome and Opening Remarks **Hotel Bukovina - *Sala Kongresowa***		
Oral Session 1. Medicinal *Cannabis* I *Chairs*: Dror Robinson and Mark Ware			
8.30	Mark A. Ware, Julie Desroches, William Barakett, Pierre Beaulieu, Andrée Néron, Yola Moride and Antonio Vigano	PHARMACOVIGILANCE OF MEDICAL CANNABIS: PRELIMINARY RESULTS FROM THE QUEBEC CANNABIS REGISTRY	1
8.45	Dror Robinson	EFFECT OF CANNABIS USE ON SEVERITY OF CHRONIC LOW BACK PAIN AND SCIATICA	2
9.00	Philippe Lucas and Zach Walsh	SELF-REPORTED MEDICAL CANNABIS ACCESS, USE, AND SUBSTITUTION FOR OTHER SUBSTANCES IN 301 CANADIAN MEDICAL CANNABIS PATIENTS	3
9.15	Lihi Bar-Lev Schleider, Raphael Mechoulam, Inbal Sikorin, Timna Naftali, Zvi Bentwich and Victor Novack	EPIDEMIOLOGICAL CHARACTERISTICS OF PATIENTS TREATED WITH MEDICAL CANNABIS	4

9.30	Lee Saynor, Kunal Sudan and Henry J. Moller	TOWARDS A NATURALISTIC CHARACTERIZATION OF A MEDICAL CANNABIS POPULATION: EMERGING CANADIAN DATA	5
9.45	Mikael A. Kowal and Arno Hazekamp	THE DEVELOPMENT OF A CANNABIS PLACEBO	6
10.00	**COFFEE BREAK**		
10.30	**ICRS CAREER ACHIEVEMENT AWARD** **ANANDAMIDE UPTAKE, TRANSPORT AND INACTIVATION: STUDIES BRIDGING TWO CENTURIES** **DALE DEUTSCH, PH.D.** *Department of Biochemistry and Cell Biology* *Stony Brook University, Stony Brook, NY 11794, USA* *CHAIR*: ALLYN HOWLETT		
ORAL SESSION 2. ENDOCANNABINOID TRANSPORT / DYNAMICS *CHAIRS*: MARTIN KACZOCHA AND MATT HILL			
11.00	Jennifer Bialecki, Nicholas L. Weilinger, Haley Vecchiarelli, Alexander W. Lohman, Matthew N. Hill and Roger J. Thompson	PANNEXIN-1 MODULATES GLUTAMATERGIC TRANSMISSION BY REGULATING SYNAPTIC ANANDAMIDE CONCENTRATION	7
11.15	Matthew W. Elmes, Dale G. Deutsch and Martin Kaczocha	REGULATION OF CANNABINOID ACTIVITY BY HEPATIC FATTY ACID BINDING PROTEINS	8

11.30	Martin Kaczocha, Matthew W. Elmes, Keith Studholme, Jeremy T. Miyauchi, Stella E. Tsirka, Dale G. Deutsch, Panayotis K. Thanos, and Samir Haj-Dahmane	FATTY ACID BINDING PROTEINS MEDIATE RETROGRADE ENDOCANNABINOID SIGNALING	9
11.45	Yao Chen, Xiaojie Liu, Casey R. Vickstrom, Michelle J. Liu, Andreu Viader, Benjamin F. Cravatt and Qing-song Liu	NEURONAL AND ASTROCYTIC MONOACYLGLYCEROL LIPASE LIMIT THE SPREAD OF ENDOCANNABINOID SIGNALING IN THE CEREBELLUM	10
12.00	Cecilia J. Hillard and Elizabeth Sabens Liedhegner	STEROL CARRIER PROTEIN 2 DELETION ENHANCES FEAR EXTINCTION THROUGH MODULATION OF THE ENDOCANNABINOID SYSTEM	11
12.15	**LUNCH** **NIDA LUNCH & MENTORING**		
ORAL SESSION 3. CARDIOVASCULAR SYSTEM *CHAIRS*: SAOIRSE O'SULLIVAN AND VANESSA HO			
13.30	Eugen Brailoiu, G. Cristina Brailoiu and Mary E. Abood	NOVEL EFFECTS OF CANNABIDIOL ON NUCLEUS AMBIGUUS NEURONS	12
13.45	Barbara Malinowska, Anna Pędzińska-Betiuk, Jolanta Weresa, Michał Biernacki, Marek Toczek, Marta Baranowska-Kuczko and Elżbieta Skrzydlewska	DIFFERENT EFFECTS OF CHRONIC ADMINISTRATION OF THE FATTY ACID AMIDE HYDROLAZE INHIBITOR URB597 ON CARDIAC PERFORMANCE AND OXIDATIVE STRESS IN NORMOTENSIVE AND HYPERTENSIVE RATS	13

14.00	Catherine T. Choy, Stephen P Alexander and W.S. Vanessa Ho	PROTECTIVE EFFECTS OF FAAH SUBSTRATES ON AGE-RELATED ENDOTHELIAL DYSFUNCTION	14
14.15	Rajesh Mohanraj, Partha Mukhopadhyay, Sandor Batkai, Zoltan Varga and Pal Pacher	CANNABINOID RECEPTOR 2 ACTIVATION ALLEVIATES DIABETES-INDUCED CARDIAC DYSFUNCTION, INFLAMMATION, OXIDATIVE STRESS AND FIBROSIS	15
14.30	Eilidh E McNaughton, Catherine T. Choy, Charles E Sudlow and W.S. Vanessa Ho	ROLE OF GPR55 AND GPR18 RECEPTORS IN THE VASCULAR ACTIONS OF ENDOCANNABINOIDS AND RELATED LIPIDS	16
ORAL SESSION 4. BEHAVIOURAL NEUROSCIENCE I - PAIN AND SLEEP *CHAIRS*: ARON LICHTMAN AND SARA JANE WARD			
14.45	Xiaolong Sun, Ning Gu and Xia Zhang	ROLE OF HYPOTHALAMIC WAKE CIRCUITRY IN CANNABINOID MODULATION OF SLEEP	17
15.00	Daniel J. Morgan, Matthew B. Yuill, Michael L. Zee, Rebecca LaFleur and Josée Guindon	MECHANISMS OF TOLERANCE TO Δ^9-THC IN RODENT MODELS OF PATHOLOGICAL PAIN	18
15.15	Jenny Wilkerson, Travis Grim, Rehab Abdullah, Daisuke Ogasawara, Benjamin F. Cravatt and Aron H. Lichtman	DIACYLGLYCEROL LIPASE ALPHA: INFLAMMATORY AND NEUROPATHIC PAIN RELIEF IN MICE	19
15.30	**COFFEE BREAK**		
16.00	Alyssa M Myers, Kirsten King, Ariele Soroka-Monzo, Ronald Tuma, Ellen A Walker and Sara Jane Ward	SINGLE AND COMBINATION EFFECTS OF CANNABINOIDS ON NEUROPATHIC PAIN AND COGNITION	20

16.15	Christopher R. McCurdy, Lisa L. Wilson, Jacqueline Morris, Jennifer M. Miller and Stephen J. Cutler	SIGMA RECEPTOR BLOCKADE POTENTIATES THE ANALGESIC EFFECTS OF CANNABINOIDS	21
16.30	Lawrence M. Carey, Richard A. Slivicki, Emma Leishman, Ben Cornett, Ken Mackie, Heather Bradshaw and Andrea G. Hohmann	A PRO-NOCICEPTIVE PHENOTYPE REVEALED IN MICE LACKING THE ANANDAMIDE HYDROLYZING ENZYME FATTY-ACID AMIDE HYDROLASE	22
16.45	**BREAK**		
ORAL SESSION 5. FOCUS ON CBD *CHAIRS:* JOHN ASHTON AND ALESSIA LIGRESTI			
17.00	Ronald Tuma, Hongbo Li, Weimin Kong, Christina Chambers, Doina Ganea and Sara Jane Ward	TARGETING SPINAL CORD INJURY-ASSOCIATED NEUROPATHIC PAIN WITH CANNABIDIOL	23
17.15	James T. Toguri, Dinesh Thapa, Robert B. Laprairie, Eileen M. Denovan-Wright, Christian Lehmann, Mary Lynch and Melanie E.M. Kelly	EFFICACY OF CANNABIDIOL AND CANNABIDIOL DERIVATIVES IN THE TREATMENT OF OCULAR PAIN	24
17.30	Ewa Kozela, Nathali Kaushansky, Giovanni Coppola, Ana Juknat and Zvi Vogel	CANNABIDIOL EFFECTS ON MRNA LEVELS AND SIGNALING PATHWAYS IN MOG-35-55 ACTIVATED ENCEPHALITOGENIC T CELLS	25
17.45	Douglas R. Smith, Netsanet Gebremedhin, Christine Stanley, Vasisht Tadigotla, Eric Marsh, Orrin Devinsky and Kevin McKernan	INVESTIGATION OF GENETIC FACTORS IN CBD RESPONSE	26

18.00	María Ceprián, Carlos Vargas, Andrea Cora, Lorena Barata, William Hind, José Martínez-Orgado and Laura Jiménez-Sánchez	NEUROPROTECTIVE EFFECT OF CANNABIDIOL IN A NEWBORN RAT MODEL OF ACUTE ISCHEMIC STROKE	27
18.15	Timothy Bakas, Steven Devenish, Petra Van Nieuwenhuizen, Jonathon Arnold, Iain McGregor and Mary Collins	THE ACTIONS OF CANNABIDIOL AND 2-ARACHIDONYL GLYCEROL ON GABA-A RECEPTORS	28
18.30	Daniel Couch, Elena Theophilidou, Jon Lund and Saoirse O'Sullivan	CANNABIDIOL (CBD) AND PALMITOYLETHANOLAMIDE (PEA) DO NOT MODULATE CYTOKINE PRODUCTION IN CACO-2 INTESTINAL CELLS BUT DO IN HUMAN COLON EPITHELIUM	29
18.45	Alessia Ligresti, Roberto Ronca, Viviana Marolda, Daniela Coltrini and Vincenzo Di Marzo	ROLE OF CANNABINOIDS IN A MULTISTAGE MURINE MODEL OF PROSTATE CARCINOMA TO INVESTIGATE POTENTIAL CLINICAL APPLICATIONS	30
19.30	**DINNER**		

NOTES:

Day 2
Tuesday, June 28th

Breakfast at your accommodations			
Oral Session 6. Focus on CB2 *Chairs:* Scott Graham and Katarzyna Starowicz			
8.30	Francisco Espejo-Porras, Carmen Rodríguez-Cueto, Laura García-Toscano, Irene Santos-García, Eva de Lago and J. Fernández-Ruiz	TARGETING THE CB2 RECEPTOR TO DELAY THE PROGRESSION OF THE PATHOLOGICAL PHENOTYPE IN TDP-43 TRANSGENIC MICE	31
8.45	Alicia López, Carmen Vázquez, RM Tolón, Noelia Aparicio, Erlantz Pinto, Cecilia J. Hillard and Julián Romero	CANNABINOID CB2 RECEPTORS ARE PRESENT IN PERIPHERAL TISSUES OF THE MOUSE, BUT NOT IN THE CNS, UNDER NORMAL CONDITIONS	32
9.00	Zheng-Xiong Xi, Hai-Ying Zhang, A. Vanessa Stempel, Andreas Zimmer and Dietmar Schmitz	IDENTIFICATION OF FUNCTIONAL CANNABINOID CB2 RECEPTORS IN HIPPOCAMPAL PRINCIPAL NEURONS IN MICE	33
9.15	William John Redmond, Umit Keysan, Destiny Lu-Cleary, Bruno Cécyre, Sébastien Thomas, Christian Casanova and Jean-François Bouchard	CANNABINOID TYPE 2 RECEPTORS MODULATE VISUAL RESPONSES OF NEURONS IN THE PRIMARY VISUAL CORTEX	34
9.30	Anne-Caroline Schmöle, Ramona Lundt, Gregor Toporowski, Eva Beins, Jan Niklas Hansen, Annett Halle and Andreas Zimmer	CANNABINOID RECEPTOR 2 DEFICIENCY ALTERS NEURONAL LOSS AND IMPROVES COGNITIVE FUNCTION IN AN ALZHEIMER'S DISEASE MOUSE MODEL	35

9.45	Natalia Malek, Magdalena Kostrzewa, Agnieszka Pająk, Julia Borowczyk, Justyna Drukala and Katarzyna Starowicz	LOCAL ADMINISTRATION OF CB2 AGONISTS FOR PREVENTION AND TREATMENT OF OSTEOARTHRITIS (OA) – STUDIES IN MIA MODEL OF OA	36
10.00	Dan Kho, Elyce du Mez, Julie MacIntosh, Rebecca Johnson, Natasha Grimsey, Michelle Glass, Catherine E Angel and E Scott Graham	ARE HUMAN CIRCULATING MONOCYTES REGULATED BY CB2 RECEPTORS?	37
10.15	Zoltán V. Varga, Sandor Batkai, Partha Mukhopadhyay, Katalin Erdélyi, Resat Cinar, Andrea Cicca, George Kunos, Jürg Gertsch and Pal Pacher	HEPATOPROTECTIVE EFFECTS OF BETA-CARYOPHYLLENE: ROLE OF CANNABINOID 2 RECEPTORS	38
10.30	**COFFEE BREAK**		
11.00	**ICRS PRESIDENTIAL ADDRESS** **MOVING FROM MECHANISMS OF GPCR FUNCTIONALITY TO NEW DRUGS** **ANDREW TOBIN, PH.D.** *MRC Toxicology Unit* *University of Leicester* *Hodgkin Building, Leicester* *United Kingdom LE1 9HN* *CHAIR*: MICHELLE GLASS		
12.00	**LUNCH**		

Oral Session 7. Receptors, Probes and Signalling

Chairs: Natasha Grimsey and Sharon Anavi-Goffer

13.00	Marjolein Soethoudt, Uwe Grether, Jürgen Fingerle, Travis W. Grim,e Filomena Fezza, Luciano de Petrocellis, Christoph Ullmer, Benno Rothenhäusler, Camille Perret, Noortje van Gils, David Finlay, Christa MacDonald, Andrea Chicca, Marianela Dalghi Gens, Jordyn Stuart, Henk de Vries, Nicolina Mastrangelo, Lizi Xia, Georgios Alachouzos, Marc P. Baggelaar, Andrea Martella, Elliot D. Mock, Hui Deng, Laura H. Heitman, Mark Connor, Vincenzo Di Marzo, Jürg Gertsch, Aron H. Lichtman, Mauro Maccarrone, Pal Pacher, Michelle Glass and Mario van der Stelt	CANNABINOID CB2 RECEPTOR LIGAND PROFILING REVEALS BIASED SIGNALING AND OFF-TARGET ACTIVITY: IMPLICATIONS FOR DRUG DISCOVERY	39
13.15	Uwe Grether, Simon M. Ametamey, Erick M. Carreira, Caitlin Davies, Jürgen Fingerle, Luca Gobbi, Wolfgang Guba, Thomas Hartung, Atsushi Kimbara, Julian Kretz, Andrea Martella, Rainer E. Martin, Alistair Mason, Matthias Nettekoven, Anne de Paepe, Antonio Pedrina-McCarthy, Camille Perret, Mark Rogers-Evans, Stephan Röver, Arne Rufer, Manuel Tzouros, Christoph Ullmer, Dmitry B. Veprintsev and Matthias Westphal	DESIGN, SYNTHESIS AND CHARACTERIZATON OF CANNABINOID RECEPTOR 2 SELECTIVE CHEMICAL PROBES	40

13.30	Khalil Eldeeb, Victor M. Pulgar and Allyn C. Howlett	THE CANNABINOID AGONIST WIN55212-2 MODULATES INTRACELLULAR CALCIUM VIA CB1 RECEPTOR DEPENDENT AND INDEPENDENT MECHANISMS IN NEUROBLASTOMA CELLS	41
13.45	Bela Szabo, Marco Spehl, Sophia Linder and Eszter Boros	PRESYNAPTIC CB1 CANNABINOID RECEPTORS ARE CONSTITUTIVELY ACTIVE AT SYNAPSES BETWEEN INTERNEURONS AND PURKINJE CELLS IN THE CEREBELLAR CORTEX	42
14.00	Travis W. Grim, Maciej Gonek, Jenny L. Wiley, Brian F. Thomas, Laura J. Sim-Selley, Dana E. Selley, S. Stevens Negus and Aron H. Lichtman	STRATIFICATION OF CANNABINOID 1 RECEPTOR (CB1R) AGONIST EFFICACY: MANIPULATION OF CB1R DENSITY THROUGH USE OF TRANSGENIC MICE REVEALS CONGRUENCE BETWEEN *IN VIVO* AND *IN VITRO* ASSAYS	43
14.15	Sharon Anavi-Goffer, Andrew J. Irving, Raphael Mechoulam and Ruth A. Ross	PHARMACOLOGY OF CB2 RECEPTOR-SELECTIVE AGONISTS AT GPR55	44
14.30	Mary A. Lingerfelt, Ping Wei Zhao, Dow P. Hurst, Mary E Abood and Patricia H. Reggio	THE GPR55 BINDING POCKET: LIGAND COMMONALITIES AND REQUIREMENTS	45
14.45	Brian F. Thomas, Timothy W. Lefever and Jenny L. Wiley	KEEPING UP WITH THE CHEMISTS: CONTINUING *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF THE PHARMACOLOGY OF SYNTHETIC CANNABINOIDS	46
15.00 - 17.15	**COFFEE** POSTER SESSION 1		P1

Oral Session 8.

Neuroprotection / Neuroinflammation / Neurogenesis

Chairs: Andras Bilkei-Gorzo and Jose Martínez-Orgado

17.15	Anastasia Piyanova, Carlo Alberto Rossi, Laura Bindila, Ermelinda Lomazzo, Monika Feliszek, Pierluigi Nicotera, Beat Lutz, Eberhard Schlicker, Andreas Zimmer and Andras Bilkei-Gorzo	AGE-RELATED DECREASE IN ENDOCANNABINOID SIGNALLING CONTRIBUTES TO IMPAIRED PROTEOSTASIS	47
17.30	Tomoyuki Kino, Rania Abutarboush, Ye Chen, Esther Shohami, Toshiki Tomori, Paola Castri, Alois Strasser, Raphael Mechoulam, Richard M. McCarron and Maria Spatz	THE ROLE OF ENDOCANNABINOID SUBSTANCES ON HUMAN CEREBROMICROVASCULAR ENDOTHELIAL CELLS FUNCTIONS	48
17.45	Kelly Shevonne De Coteau, Alfonso Calle-Perez, Volker Behrends, Laurent Lacroix and Francisco Molina-Holgado	CROSS-TALK BETWEEN INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1RA) AND THE BRAIN CANNABINOID SYSTEM IN NEUROGENESIS	49
18.00	Bogna Ignatowska-Jankowska, Douglas J. Hermes, Changqing Xu, Ken Mackie, Micah J. Niphakis, Ben F. Cravatt, Aron H. Lichtman and Sylvia Fitting	NEUROPROTECTIVE POTENTIAL OF ENDOCANNABINOIDS IN MODELS OF NEURONAL DAMAGE INDUCED BY HIV-1 TAT PROTEIN	50
18.15	Lorena Barata, Luis Arruza, Maria José Rodríguez, Eva Vierge, Esther Aleo, Enrique Criado, Elena Sobrino, Alberto Cabañas, Laura Jimenez-Sanchez, Maria Ceprián, Hector Lafuente, Will Hind, Francisco José Alvarez and Jose Martínez-Orgado	CEREBRAL AND EXTRACEREBRAL EFFECTS OF COMBINING CANNABIDIOL AND HYPOTHERMIA AFTER HYPOXIA-ISCHEMIA IN NEWBORN PIGLETS	51

18.30	Paula Morales, Maria Gómez-Cañas, Gemma Navarro, Laura Lagartera, Dow P. Hurst, Francisco J. Carrillo-Salinas, Ruth Pazos, Pilar Goya, Carmen Guaza, Rafael Franco, Javier Fernández-Ruiz, Patricia H. Reggio and Nadine Jagerovic	NOVEL CB2 RECEPTOR AGONISTS: SYNTHESIS, MOLECULAR MODELING, *IN VITRO* AND *IN VIVO* NEUROPROTECTIVE PROPERTIES	52
18.45	Yosef Sarne, Roni Toledano and Ravid Doron	ULTRA-LOW DOSES OF TETRAHYDROCANNABINOL (THC) REVERSE AGE-DEPENDENT COGNITIVE DECLINE IN MICE	53
19.30	**DINNER**		

NOTES:

Day 3
Wednesday, June 29th

Breakfast at your accommodations			
Oral Session 9. Metabolism, Endocrine, Diabetes *Chairs*: Claire Williams and Pal Pacher			
8.30	Tony Jourdan and George Kunos	CANNABINOID-1 RECEPTORS (CB1R) ON MYELOID CELLS DRIVE BETA-CELL LOSS IN TYPE 2 DIABETES	54
8.45	Liad Hinden, Shiran Udi, Adi Drori, Rivka Hadar, Saja Baraghity, Anna Permyakova, Matan Geron, Merav Cohen, Sabina Tsytkin, Yaakov Nahmias, Avi Priel and Joseph Tam	PERIPHERALLY-RESTRICTED CANNABINOID-1 RECEPTOR BLOCKADE ATTENUATES DIABETIC NEPHROPATHY VIA THE REGULATION OF PROXIMAL TUBULE GLUT2	55
9.00	Daniel I Brierley, Joe R Harman, Natasa Giallourou, Emma Leishman, Heather B Bradshaw, Jonathan R Swann, Ketan Patel, Benjamin J Whalley and Claire M Williams	PROHOMEOSTATIC EFFECTS OF CANNABIGEROL ON CHEMOTHERAPY-INDUCED METABOLIC DYSREGULATION	56
9.15	Claudio D'Addario, Mariangela Pucci, Maria Vittoria Micioni Di Bonaventura, Valeria Vezzoli, Massimo Scacchi, Luca Persani, Stefania Mai, Carlo Cifani and Mauro Maccarrone	REGULATION OF TYPE-1 CANNABINOID RECEPTOR (*CNR1*) GENE IN OBESITY	57

9.30	Ibrahim Knani, Brian J. Earley, Shahar Azar, Saja Baraghithy, Shiran Udi, Harry J Hirsch, Talia Eldar-Geva, Varda Gross-Tsur, Daniela P. Reyes-Capo, Joan C. Han, Andrea M. Haqq, Rachel Wevrick and Joseph Tam	ROLE OF THE ENDOCANNABINOID SYSTEM IN PRADER-WILLI SYNDROME	58
9.45	Ya Wang, Michiel Balvers, Sophie Schutte, Diederik Esser, Lydia A. Afman, Marco Mensink, Jean-Paul Vincken, Renger Witkamp and Jocelijn Meijerink	ENDOCANNABINOIDS AND RELATED COMPOUNDS IN PERIPHERAL PLASMA OF HUMANS WITH ABDOMINAL OBESITY; A RANDOMIZED CONTROLLED TRIAL COMPARING DIFFERENT LOW-CALORIE DIETS	59
10.00	Suzanne E.M. de Bruijn, Anouk J. Andeweg, Cees de Graaf, Renger F. Witkamp and Gerry Jager	PROFILING THE ENDOCANNABINOID RESPONSE TO HEDONIC EATING	60
10.15	**COFFEE BREAK**		
ORAL SESSION 10. *CANNABIS,* MEDICINAL *CHAIRS*: ETHAN RUSSO AND JOHN MCPARTLAND			
10.45	John M. McPartland and Geoffrey W. Guy	*CANNABIS* MAY HAVE EVOLVED IN THE NORTHEASTERN TIBETAN PLATEAU, BASED ON AN INTERDISCIPLINARY STUDY OF GENETICS, FOSSIL POLLEN, AND ECOLOGY	61
11.00	Arno Hazekamp, Katerina Tejkalova and Stelios Papadimitriou	A METABOLOMICS APPROACH TO THE SATIVA-INDICA DILEMMA	62

11.15	Margaret Haney, Evan Herrmann, Ilaria Buonomo, Isabel Matias, Monique Vallee, Stéphanie Monlezun and Piervi Piazza	ENDOGENOUS CANNABINOIDS LEVELS PREDICT THE POSITIVE SUBJECTIVE EFFECTS OF SMOKED CANNABIS	63
11.30	Gerry Jager, Suzanne de Bruijn, Carl Roberts and Tim Kirkham	INVESTIGATING CANNABIS RELATED APPETITE CHANGES TO CHARACTERISE THE ROLE OF ENDOCANNABINOIDS IN HEDONIC HUNGER	64
11.45	Darryl Hudson	OPTIMIZING CANNABIS VARIETIES FOR THE MANAGEMENT OF PTSD IN MILITARY VETERANS	65
12.00	Ethan Russo	PESTICIDE CONTAMINATION OF CANNABIS IN THE LEGAL MARKET	66
12.15	**KANG TSOU MEMORIAL LECTURE** **HUMAN EXOSOME COMPLEX IN HEALTH AND DISEASE** **ANDRZEJ DZIEMBOWSKI, PH.D.** *Institute of Biochemistry and Biophysics, PAS &* *Department of Genetics & Biotechnology* *Warsaw University* *Warsaw, Poland 02-106* *CHAIR*: **KATARZYNA STAROWICZ**		
13.00	**LUNCH**		
14.00 -	**OUTING**		

Day 4
Thursday, June 30th

Breakfast at your accommodations			
Oral Session 11. Addiction *Chairs:* Joel Schlosburg and Eliot Gardner			
8.30	Javier Orihuel, Roberto Capellan, Marcos Ucha, Raquel Santos-Toscano, David Roura-Martínez, Mónica Fernández Cabrera, Emilio Ambrosio and Alejandro Higuera-Matas	CHRONIC Δ^9-TETRAHYDROCANNABINOL TREATMENT DURING ADOLESCENCE IN RATS ENHANCES ACQUISITION OF COCAINE SELF-ADMINISTRATION AND ESCALATION OF DRUG INTAKE	67
8.45	Eliot L. Gardner, Xiang-Hu He, Guo-Hua Bi, Ganesh Thakur, Alexandros Makriyannis, Herbert H. Seltzman and Zheng-Xiong Xi	TARGETING THE CB1 RECEPTOR FOR ANTI-DRUG ADDICTION THERAPEUTIC POTENTIAL: CB1R ANTAGONISTS/INVERSE AGONISTS, CB1R NEUTRAL ANTAGONISTS, OR CB1R NEGATIVE ALLOSTERIC MODULATORS (NAMS)?	68
9.00	Jonathan Lovelace, Alex Corches, Philip Vieira, Alex Hiroto, Ken Mackie and Edward Korzus	ADOLESCENT CANNABINOID EXPOSURE TRIGGERS A PERMANENT DEFICIT IN PRESYNAPTIC LONG-TERM PLASTICITY	69
9.15	Joel E. Schlosburg, Leandro F. Vendruscolo and George F. Koob	INTRACRANIAL SELF-STIMULATION RESPONSE TO INTRAVENOUS CANNABINOIDS AS A METHOD TO MODEL EXCESSIVE INTAKE	70

9.30	**YOUNG INVESTIGATOR AWARD PRESENTATION** **ENDOCANNABINOIDS AS A HOMEOSTATIC SIGNAL COUNTERACTING ADVERSE EFFECTS OF STRESS** **SACHIN PATEL, M.D., PH.D.** *Department of Psychiatry and Behavioral Sciences* *Department of Molecular Physiology & Biophysics* *Vanderbilt University Medical Center, Nashville, TN, USA* *CHAIR*: **CECILIA HILLARD**
10.00	**COFFEE BREAK**

ORAL SESSION 12. BEHAVIOURAL NEUROSCIENCE II - ANXIETY, STRESS
CHAIRS: STEVE KINSEY AND SACHIN PATEL

10.30	Kristen R. Trexler, Sara R. Nass, Austin W. McKitrick and Steven G. Kinsey	MAGL INHIBITION ATTENUATES Δ^9-THC SOMATIC WITHDRAWAL, BUT NOT ALTERED EMOTIONALITY-RELATED BEHAVIORS	71
10.45	Vincenzo Micale, Tibor Štark1, Vladimír Pekařík, Fabio Arturo Iannotti, Martina Di Bartolomeo, Jana Ruda-Kucerova, Fabiana Piscitelli, Claudio D`Addario, Filippo Drago, Vincenzo Di Marzo, Raphael Mechoulam and Alexandra Sulcova	EARLY PHARMACOLOGICAL MODULATION OF THE ENDOCANNABINOID TONE COUNTERACTS THE LATE BEHAVIORAL AND MOLECULAR ALTERATIONS IN A RODENT DEVELOPMENTAL MODEL OF SCHIZOPHRENIA	72
11.00	Haley A. Vecchiarelli, Maria Morena, Martin Sticht, Catherine M. Keenan, Winnie Ho, Keith A. Sharkey and Matthew N. Hill	INHIBITION OF ANANDAMIDE HYDROLYSIS REVERSES ANXIETY INDUCED BY SUSTAINED PERIPHERAL INFLAMMATION	73

11.15	Ning Gu, Haixing Zhong and Xia Zhang	ENDOCANNABINOID MODULATION OF UNCONSCIOUSNESS AND CONSCIOUSNESS	74
11.30	Rebecca J. Bluett, Rita Báldi, Andre Haymer, Nolan D. Hartley, David J. Marcus and Sachin Patel	AN ENDOCANNABINOID MECHANISM SUPPORTING RESILIENCE TO TRAUMATIC STRESS	75
11.45	**LUNCH**		
13.00 - 15.00	POSTER SESSION 2		P2
ORAL SESSION 13. ENDOCANNABINOID METABOLISM *CHAIRS*: HEATHER BRADSHAW AND MARIO VAN DER STELT			
15.00	Marc P. Baggelaar, Annelot C. M. van Esbroeck, Bogdan I. Florea, Herman S. Overkleeft, Giovanni Marsicano, Francis Chaouloff and Mario van der Stelt	CHEMICAL PROTEOMICS MAPS BRAIN REGION DEPENDENT ACTIVITY OF ENDOCANNABINOID HYDROLASES	76
15.15	Michael Garle, Pragya Mehrotra, Oliver Sandy-Hindmarch, Nahed Alharthi and Stephen Alexander	N-ACYLGLYCINE HYDROLYSIS BY RAT LIVER MICROSOMES	77
15.30	Molly S. Crowe, Ramesh Gujjar, Anu Mahadevan, Matthew Banks and Steven G. Kinsey	MAGL INHIBITION SYNERGISTICALLY POTENTIATES THE ANTIALLODYNIC EFFECTS OF GABAPENTIN AND DICLOFENAC	78

15.45	Emma Leishman, Michelle Murphy, Ken Mackie and Heather B Bradshaw	BRAIN-REGION DEPENDENT EFFECTS OF THC ON THE ENDOCANNABINOID AND WIDE-RANGING-RELATED LIPIDOME IN THE MOUSE BRAIN	79
16.00	Attila Oláh, Nóra Zákány, Arnold Markovics, Erika Takács, Simon Nicolussi, Jürg Gertsch, Fabiana Piscitelli, Vincenzo Di Marzo, Zouboulis C. Christos and Tamás Bíró	EFFECTS OF MODULATION OF THE ENDOCANNABINOID TONE ON HUMAN SEBOCYTES	80
16.15	**COFFEE BREAK**		
16.45	Hui Deng, Daisuke Ogasawara, Andreu Viader, Marc P. Baggelaar, Arjen C. Breman, Hans den Dulk, Adrianus M.C.H. van den Nieuwendijk, Marjolein Soethoudt, Tom van der Wel, Juan Zhou, Hermen S. Overkleeft, Benjamin F. Cravatt and Mario van der Stelt	RAPID AND PROFOUND REWIRING OF BRAIN LIPID SIGNALING NETWORKS BY ACUTE DIACYLGLYCEROL LIPASE INHIBITION	81
17.00	Jason J. McDougall, Milind Muley, Allison Reid and Eugene Krustev	EARLY INTERVENTION USING URB597 TO REDUCE JOINT INFLAMMATION CAN ALLEVIATE END STAGE OSTEOARTHRITIS PAIN IN MICE	82
17.15	Kiri L. Wills, Gavin N. Petrie, Geneva Millet, Cheryl L. Limebeer, Erin M. Rock, Micah J. Niphakis, Benjamin F. Cravatt and Linda A. Parker	DOUBLE DISSOCIATION OF CB1 ANTAGONISM AND MONOACYLGLYCEROL LIPASE INHIBITION IN THE CENTRAL AMYGDALA, BASOLATERAL AMYGDALA AND THE INTEROCEPTIVE INSULAR CORTEX ON THE AFFECTIVE PROPERTIES OF ACUTE NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL IN RATS	83

17.30	Allyn Howlett, Deborah Luessen, Haiguo Sun, Meimei Wan, Rong Chen and Colin Bishop	ENDOCANNABINOID SYSTEM RECEPTORS AND ENZYMES ARE EXPRESSED IN EARLY DEVELOPING HUMAN BRAIN ORGANOIDS	84
17.45	**ICRS BUSINESS MEETING**		
20.00	**AWARDS CEREMONY** **AND** **ICRS BANQUET**		

DEPARTURE: **FRIDAY, JULY 1ST**

Poster Session 1: Topics A – H

DAY 2, Tuesday June 28th, 15.00 – 17.15

TOPIC A. Peripheral Inflammation and Immune System

Adam A. Marentes and Nancy E. Buckley	THC AND FUNGAL IMMUNITY: HOW AN IMMUNOCOMPROMISED MOUSE'S ABILITY TO CLEAR A FUNGAL INFECTION IS AFFECTED BY Δ^9-TETRAHYDROCANNABINOL	P1-1
Sara R. Nass, Mary K. Cardullo, and Steven G. Kinsey	THE CB2-SELECTIVE AGONIST HU-308 ATTENUATES INFLAMMATION AND HYPERALGESIA IN CHRONIC AND ACUTE MODELS OF INFLAMMATORY ARTHRITIS	P1-2
Agnieszka Pajak, Katarzyna Dudek and Katarzyna Starowicz	THE ROLE OF DORSAL ROOT GANGLIA NEURONS INNERVATING THE RAT KNEE JOINTS DURING OSTEOARTHRITIS DEVELOPMENT: IMPLICATIONS FOR THE ENDOCANNABINOID SYSTEM	P1-3
Carmen del Río, Irene Cantarero, M.Luz Bellido, Giovanni Appendino, María Gómez-Cañas, Ruth Pazos, Javier Fernández-Ruiz, Marco A Calzado and Eduardo Muñoz	CANNABIDIOL QUINOL DERIVATIVES FOR THE TREATMENT OF SCLERODERMA	P1-4
Hinanit Koltai, Yoram Kapulnik, Marcelo Fridlender, Ahmad Nasser, Oded Sagee, Nave Firestein and Timna Naftali	SPECIFIC VARIATIONS BETWEEN *CANNABIS SATIVA* LINES CONFER DIFFERENT ANTI-INFLAMMATORY ACTIVITY ON INFLAMED COLON CELLS	P1-5
Torsten Lowin and Georg Pongratz	TNF INCREASES TRPA1 EXPRESSION AND FUNCTION IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS: TRPA1 AS THERAPEUTIC TARGET TO REDUCE JOINT INFLAMMATION IN RA?	P1-6
Viola Brugnatelli and Andrew J. Irving	CANNABIS TERPENES WITH A ROLE IN INFLAMMATION: A MOLECULAR PERSPECTIVE	P1-7

Stefania Petrosino, Aniello Schiano Moriello, Roberta Verde, Roberta Imperatore, Marco Allarà, Alessia Ligresti, Pierangelo Orlando, Matilde Valeria Ursini and Vincenzo Di Marzo	ANTI-INFLAMMATORY ACTIONS OF PALMITOYLETHANOLAMIDE IN AN *IN VITRO* MODEL OF NEUROGENIC INFLAMMATION	P1-8
Tiziana Bisogno, Luca Navarini, Pamela Mozetic, Domenico Paolo Emanuele Margiotta, Fabiana Piscitelli, Fabio Basta, Stefano Saracini, Antonella Afeltra and Mauro Maccarrone	ENDOCANNABINOID SYSTEM AND SYSTEMIC LUPUS ERYTHEMATOSUS	P1-9
Sarah J. Kent and Nancy E. Buckley	MODULATION OF CANNABINOID RECEPTORS AND CYTOKINES EXPRESSION BY SEX HORMONE TREATMENT OF MACROPHAGES	P1-10
Magdalena Kostrzewa, Natalia Małek, Agnieszka Pajak, Julia Borowczyk, Zbigniew Madeja, Justyna Drukala, Marcin Binkowski, Martyna Czaja, Jakub Staron and Katarzyna Starowicz	NEW APPROACHES TO TREATING OSTEOARTHRITIS IMPLICATIONS FOR THE ENDOCANNABINOID SYSTEM IN OSTEOBLAST METABOLISM	P1-11
Jakub Mlost, Magdalena Kostrzewa, Natalia Malek and Katarzyna Starowicz	MOLECULAR UNDERSTANDINGS ON THE ACTIVATION OF CB1 AND BLOCKADE OF TRPV1 RECEPTORS: IMPLICATIONS FOR NOVEL TREATMENT STRATEGY IN OSTEOARTHRITIS	P1-12

TOPIC B. Behavioural Neuroscience I

Jimit G. Raghav, Torbjörn U. C. Järbe, Subramanian K. Vadivel and Alexandros Makriyannis	BEHAVIORAL PROFILING OF AM1346, A HIGH AFFINITY CB1R ANANDAMIDE ANALOG, USING DRUG DISCRIMINATION	P1-13
Erica Zamberletti, Massimo Grilli, Alberto Catanese, Mario Marchi, Tiziana Rubino and Daniela Parolaro	SEX- AND REGION-DEPENDENT CONSEQUENCES OF ADOLESCENT THC EXPOSURE ON BEHAVIOR AND SYNAPTIC PLASTICITY	P1-14

Zoltán Kristóf Varga, Manó Aliczki, Zoltán Balogh and József Haller	CONTEXT DEPENDENT INVOLVEMENT OF 2-ARACHIDONOYLGLYCEROL SIGNALING IN THE REGULATION OF SOCIAL CHALLENGE RESPONDING	P1-15
Eva Beins and Andreas Zimmer	THE ROLE OF THE CANNABINOID RECEPTOR 1 IN STRESS-INDUCED INFLAMMATION AND BEHAVIOUR	P1-16
Cheryl L Limebeer, Erin M Rock, Nirushan Puvanenthirarajah, Micah J Niphakis, Benjamin F Cravatt and Linda A Parker	MONOACYLGLYCEROL LIPASE INHIBITION IN THE VISCERAL INSULAR CORTEX SELECTIVELY ELEVATES 2-AG WHICH INTERFERES WITH ANTICIPATORY NAUSEA IN A RAT MODEL	P1-17

TOPIC C. CARDIOVASCULAR

Nuha Anajirih, WS Vanessa Ho, Saoirse O'Sullivan and Steve PH Alexander	2-OLEOYLGLYCEROL HYDROLYSIS IN THE RAT VASCULATURE BY MONOACYLGLYCEROL LIPASE ACTIVITY	P1-18
Marta Baranowska-Kuczko, Hanna Kozłowska, Monika Kloza, Olga Karpińska, Marek Toczek, Mirosław Kozłowski and Barbara Malinowska	CANNABIDIOL-INDUCED VASORELAXATION IN ISOLATED HUMAN PULMONARY AND SMALL MESENTERIC ARTERIES OF HYPERTENSIVE RAT – PRELIMINARY STUDY	P1-19
Jordan S. Farrell, Roberto Colangeli, Matthew N. Hill and G. Campbell Teskey	POTENTIAL INVOLVEMENT OF COX-2 MEDIATED BREAKDOWN OF ENDOCANNABINOIDS ON POST-SEIZURE ISCHEMIA/HYPOXIA	P1-20
Edward S Bentley, Saoirse E O'Sullivan and Tim J England	CANNABINOIDS AND EXPERIMENTAL MYOCARDIAL INFARCTION: A SYSTEMATIC REVIEW AND META ANALYSIS	P1-21

TOPIC D. CB1 Receptors - Signalling, Novel Probes, Etc.

David B. Finlay, Morag R. Hunter, Natasha L. Grimsey, V. Kiran Vemuri, Alexandros Makriyannis and Michelle Glass	GS COUPLING OF CB1: LIGAND BIAS AND THE INFLUENCE OF RECEPTOR NUMBER	P1-22
Chris Breivogel, Bonnie Brenseke, Amreen Jonas and Artik Mistry	COMPARISON OF REPRESENTATIVE SYNTHETIC CANNABINOIDS TO THC	P1-23
Luigi Bellocchio, Etienne Hebert-Chatelain, Tifany Desprez, Román Serrat, Edgar Soria-Gomez, Anna Delamarre, Peggy Vincent, Arnau Busquets-Garcia, Laurie M. Robin, Geoffrey Terral, Mª Dolores García-Fernández, Michelangelo Colavita, Wilfrid Mazier, Filippo Drago, Nagore Puente, Leire Reguero, Izaskun Elezgarai, Jean-William Dupuy, Daniela Cota, Maria-Luz Lopez-Rodriguez, Gabriel Barreda-Gómez, Federico Massa, Pedro Grandes, Giovanni Bénard and Giovanni Marsicano	A CANNABINOID LINK BETWEEN MITOCHONDRIA 1 AND MEMORY	P1-24
Robert Laprairie, Abhijit Kulkarni, Pushkar Kulkarni, Dow Hurst, Diane Lynch, Patricia Reggio, David Janero, Roger Pertwee, Lesley Stevenson, Melanie Kelly, Eileen Denovan-Wright and Ganesh Thakur	MAPPING CANNABINOID RECEPTOR 1 ALLOSTERIC SITE(S): CRITICAL MOLECULAR DETERMINANT AND SIGNALING PROFILE OF GAT100 - A NOVEL, POTENT AND IRREVERSIBLY BINDING PROBE	P1-25
Allison Griffith, Herbert Seltzman, Zhao-Hui Song, Jagjeet Mnpotra, Dow Hurst and Patricia Reggio	COVALENT ANALOGS OF THE ALLOSTERIC LIGAND ORG 27569 – MOLECULAR DYNAMICS, SYNTHESIS, AND PHARMACOLOGY STUDIES DIRECTED AT IDENTIFYING THE CB1R ALLOSTERIC BINDING SITE	P1-26
Pushkar Kulkarni, Sumanta Garai, Robert Laprairie, Melanie Kelly, Eileen Denovan-Wright, Lesley Stevenson, Roger Pertwee and Ganesh Thakur	FLUORINE-WALK ON GAT211, A POSITIVE ALLOSTERIC MODULATOR OF THE CANNABINOID 1 RECEPTOR: IDENTIFICATION OF CRITICAL SITES FOR ADVANCING STRUCTURE-ACTIVITY RELATIONSHIP STUDIES	P1-27

TOPIC E. *CANNABIS*

Kevin McKernan, Jessica Spangler, Lei Zhang, Vasisht Tadigotla, Yvonne Helbert, Ryan Lynch, Theodore Foss, Cindy Orser and Doug Smith	CANNABIS MICROBIOME SEQUENCING REVEALS *PENICILLIUM PAXILLI* AND THE POTENTIAL FOR PAXILLINE DRUG INTERACTIONS WITH CANNABIDIOL	P1-28
Kevin J McKernan, Yvonne Helbert, Adrian Devitt-Lee, Vasisht Tadigotla, Stephen McLaughlin, Jessica Spangler, Lei Zhang, Ryan Lynch and Douglas Smith	SINGLE MOLECULE SEQUENCING OF THCA AND CBDA SYNTHASE REVEALS COPY NUMBER VARIATION IN MODERN DRUG-TYPE *CANNABIS SATIVA* L. AND SHEDS NEW LIGHT ON THE BT:BD ALLELE	P1-29
Ryan Lynch, Jessica Spangler, Jeremy Rualo, Vasisht Tadigotla, Ted Foss, Stephen McLaughlin, Roberto Lopesino, Yvonne Helbert and Kevin McKernan	GENOMIC AND CHEMICAL DIVERSITY IN *CANNABIS*	P1-30
Šárka Vlachová, Jana Pexová Kalinová, Vítězslav Březina, Šárka Beranová and Pavlína Tláskalová	USE OF LIVE CELL IMAGING SYSTEM TO EVALUATE BIOLOGICAL ACTIVITY OF HEMP EXTRACT	P1-31

TOPIC F. CANCER

Katrin Winkler, Robert Ramer, Sophie Dithmer, Igor Ivanov, Jutta Merkord and Burkhard Hinz	FATTY ACID AMIDE HYDROLYSE INHIBITORS CONFER ANTI-INVASIVE AND ANTIMETASTATIC EFFECTS ON LUNG CANCER CELLS	P1-32
Jessica Karlsson, Sandra Gouveia-Figueira, Mireille Alhouayek and Christopher J. Fowler	2-ARACHIDONOYLGLYCEROL LEVELS IN DU145 PROSTATE CANCER CELLS FOLLOWING TUMOUR NECROSIS FACTOR-α TREATMENT	P1-33

Massimo Nabissi, Maria Beatrice Morelli, Consuelo Amantini, Claudio Cardinali, Massimo Offidani, Pietro Leoni and Giorgio Santoni	CANNABINOIDS AND MULTIPLE MYELOMA – TARGETING IMMUNOPROTEASOME AS NEW POTENTIAL THERAPY	P1-34
Paweł Śledziński, Joanna Zeyland, Agnieszka Nowak and Ryszard Słomski	CURRENT STATE OF KNOWLEDGE OF CANNABIDIOL'S INFLUENCE ON CANCER CELLS	P1-35
Stefano Gigli, Luisa Seguella, Nicola Nobile, Marcella Pesce, Alessandra D'Alessandro, Luca Steardo, Giovanni Sarnelli and Giuseppe Esposito	CANNABIDIOL ATTENUATES *CLOSTRIDIUM DIFFICILE* TOXIN A (TCDA) DAMAGE IN HUMAN COLON CARCINOMA CACO-2 CELL LINE	P1-36

TOPIC G. Receptors - Signalling, Novel Probes, Etc.

Andrea Martella, Arne Rufer, Uwe Grether, Mario van der Stelt, Juergen Fingerle, Christoph Ullmer, Thomas Hartung, Adriaan P. IJzerman and Laura H. Heitman	A NOVEL PYRIDINE INVERSE AGONIST AS A RADIOLABELED TOOL COMPOUND FOR CB2 RECEPTOR BINDING KINETIC STUDIES.	P1-37
Julian Kretz, Uwe Grether, Luca Gobbi, Irene Knuesel, Michael Honer and Simon M. Ametamey	DETAILED STRUCTURE ACTIVITY RELATIONSHIP STUDIES FOR THE DESIGN OF NOVEL AND HIGHLY SELECTIVE CB2 PET TRACERS	P1-38
Natasha L. Grimsey, Mahalakshmi Razdan, Samuel King and Michelle Glass	FUNCTIONAL CHARACTERISATION OF CANNABINOID RECEPTOR 2 SINGLE NUCLEOTIDE POLYMORPHISMS	P1-39
Jagjeet Mnpotra, Zhuanhong Qiao, Dow Hurst, Patricia Reggio and Zhao-Hui Song	IDENTIFICATION OF THE RESIDUES AT THE CANNABINOID CB2 RECEPTOR HOMODIMER INTERFACE	P1-40

Matthew Yuill, Daniel Morgan and Josée Guindon	MECHANISMS OF CROSS-TOLERANCE BETWEEN MORPHINE AND CANNABINOID RECEPTOR 2 AGONISTS	P1-41
Kimberley M. Zorn, Diane L. Lynch, Dow P. Hurst, and Patricia H. Reggio	CONSTRUCTION OF A REALISTIC PHOSPHOLIPID BILAYER FOR CB2 SIMULATIONS	P1-42
Irene Reyes-Resina, Gemma Navarro-Brugal, Katarzyna Kiec-Kononowicz, Christa Müller and Rafael Franco	HETEROMERIZATION OF GPR18 AND CANNABINOID G-PROTEIN-COUPLED RECEPTORS	P1-43
Marcus R. Goetz, Bernd L. Fiebich, Ulrike Holzgrabe, Laura García-Toscano, Oskar Koch, Eduardo Munoz Blanco and Maria Ruth Pazos	EFFECTS OF NOVEL SEMI-SYNTHETIC CANNABIDIVARIN-DERIVATIVES ON CB1 AND CB2 RECEPTORS THROUGH BINDING AND SIGNALLING	P1-44
TOPIC H. ENDOCANNABINOIDS - SYNTHESIS AND METABOLISM		
Kazuhito Tsuboi, Tatsuya Tai, Yoko Okamoto, Ryouhei Yamashita, Iffat Ara Sonia Rahman, Toru Uyama, Hitoshi Houchi, Tamotsu Tanaka, Akira Tokumura and Natsuo Ueda	A POSSIBLE INVOLVEMENT OF ACID CERAMIDASE IN THE DEGRADATION OF N-ACYLETHANOLAMINES	P1-45
Boris Ferger, Christoph Porazik, Serena Deiana and Anke Witting	NEUROCHEMICAL AND BEHAVIOURAL TARGET ENGAGEMENT FOR MONOACYLGLYCEROL LIPASE (MAGL) INHIBITION USING TANDEM MASS SPECTROMETRY (LC-MS/MS) AND AUTOMATED BEHAVIORAL ANALYSIS IN MICE	P1-46
Jonathon C. Arnold, David Clarke, Jordyn Stuart and Iain S. McGregor	PARTIAL GENETIC DELETION OF NRG1 INFLUENCES ENDOCANNABINOID CONCENTRATIONS IN THE MOUSE BRAIN	P1-47

Nahed Alharthi and Stephen PH Alexander	POLYUNSATURATED FATTY ACIDS ETHANOLAMIDES AS ENDOGENOUS CANNABINOIDS	P1-48
Nada Mahmood, Andy J Bennett, Vicky Chapman and Steve PH Alexander	2-OLEOYLGLYCEROL HYDROLYSIS IN THE RAT BRAIN	P1-49
Wei Tuo, Natascha Leleu-Chavain, Amélie Barczyk, Frédérique Klupsch, Nicolas Renault, Lucas Lemaire, Aurélien Tourteau, Philippe Chavatte and Régis Millet	DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL 3-CARBOXAMIDO-5-ARYL-ISOXAZOLES AS POTENTIAL FAAH INHIBITORS	P1-50
Rangan Maitra, Danni Harris, Scott Runyon, Keith Warner and Elaine Gay	DISCOVERY OF NOVEL FAAH INHIBITORS BY VIRTUAL SCREENING	P1-51
Natalia Battista, Arcangelo Barbonetti, Tiziana Bisogno, Fabiana Piscitelli, Alessandro Micillo, Sandro Francavilla, Felice Francavilla and Mauro Maccarrone	EFFECT OF LEUKOCYTOSPERMIA ON SEMINAL LEVELS OF 2-ARACHIDONOYLGLYCEROL	P1-52
Christina Miyabe, Nikolai Zvonok, Alexander Zvonok and Alexandros Makriyannis	PROTEOMIC CHARACTERIZATION OF RECOMBINANT HUMAN α/β HYDROLASE DOMAIN 6	P1-53

NOTES:

Poster Session 2:
Topics I – N

DAY 4, Thursday June 30TH, 13:00 – 15:00

TOPIC I. Behavioural Neuroscience II - Pain, Anxiety, and Fear

Maria Morena, Kira Leitl, Haley Vecchiarelli, Megan Gray, Patrizia Campolongo and Matthew Hill	MODULATION OF ANXIETY BY ENDOCANNABINOID SIGNALING IN THE AMYGDALA IS DEPENDENT ON AROUSAL STATE	P2-1
Erin M. Rock, Gavin N. Petrie, Cassidy Connolly, Lauren Williams, Cheryl L. Limebeer and Linda A. Parker	EFFECT OF Δ^9-TETRAHYDROCANNABINOL (THC) AND CANNABIDIOLIC ACID (CBDA) ON RAT MODELS OF NAUSEA AND ANXIETY	P2-2
Jie Zhu, Ning Gu and Xia Zhang	FAAH INHIBITORS INDUCES ANTI-ANXIETY RESPONSES THROUGH ASTROGLIAL-MEDIATED LTD	P2-3
Gaurav Bedse, Philip Kingsley, Nolan Hartley, Emily Neale, Andre Haymer, Jashim Uddin, Niels Plath, Lawrence Marnett and Sachin Patel	THERAPEUTIC ENDOCANNABINOID AUGMENTATION FOR MOOD AND ANXIETY DISORDERS: COMPARATIVE PROFILING OF FAAH, MAGL AND DUAL INHIBITORS	P2-4
Regimantas Jurkus, Harriet Day, Jonathan Lee, Francisco Guimarães and Carl Stevenson	CANNABIDIOL DAMPENS THE EXPRESSION OF AUDITORY FEAR MEMORY WITHOUT AFFECTING ITS EXTINCTION IN RATS	P2-5
Zoltán Balogh, László Szente, Zoltán Kristóf Varga, László Biró, József Haller and Manó Aliczk	DIFFERENTIAL INVOLVEMENT OF ENDOCANNABINOIDS ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL IN THE ACQUISITION AND EXTINCTION OF LEARNED FEAR	P2-6

Jeremy M. Thompson, Henry Blanton, Christian Bezboruah, Volker Neugebauer and Josée Guindon	DIFFERENTIAL EFFECTS OF IBUPROFEN AND URB937 ON FRONT AND HIND PAW MECHANICAL AND COLD SENSITIVITY IN CISPLATIN-INDUCED NEUROPATHY	P2-7
Ephraim Brener, Adi Zuloff-Shani and Elran Haber	PALMITOYLETHANOLAMIDE (PEA) IMPROVES PHARMACOLOGICAL PROFILE OF TETRAHYDROCANNABINOL (THC) IN MURINE PAIN PERCEPTION MODEL	P2-8
Louise Corcoran, Kieran Rea, Michelle Roche and David. P Finn	IMPAIRED EXPRESSION OF FEAR-CONDITIONED ANALGESIA IN THE STRESS- AND PAIN-HYPERRESPONSIVE WISTAR-KYOTO RAT STRAIN	P2-9
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ICRS Career Achievement Award

Monday, June 27, 2016, 10:30 – 11:00

Anandamide Uptake, Transport and Inactivation: Studies Bridging Two Centuries

Dale Deutsch, Ph.D.

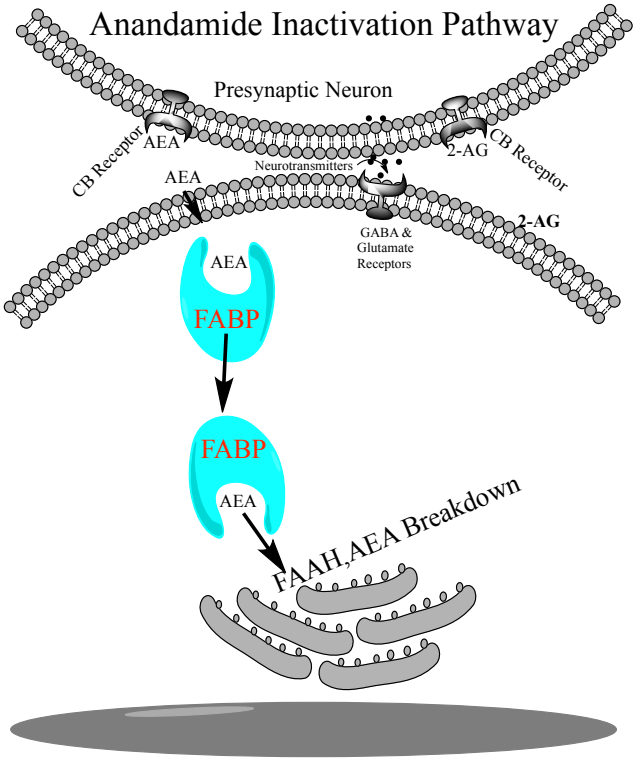
Department of Biochemistry and Cell Biology
Stony Brook University, Stony Brook, NY 11794, USA

As a biochemist working in the neurosciences, I was always fascinated with neurotransmitter inactivation. In 1993 I was thrilled to identify an enzyme activity that breaks down anandamide (AEA). We called the enzyme anandamide amidase, now called FAAH. We and other laboratories developed FAAH inhibitors that were useful reagents that also proved to have beneficial physiological effects and, until recently, new generations of inhibitors were in clinical trials.

Nearly all neurotransmitters are water soluble and, as such, require a transmembrane protein transporter to pass through the lipid membrane for inactivation inside the cell. However, using model systems, we and others have shown that this is unnecessary for anandamide, an uncharged hydrophobic molecule that readily diffuses across the cellular membrane. Most interestingly, its uptake is driven by the concentration gradient resulting from its breakdown by FAAH (or other enzymes such as COX-2) localized on the endoplasmic reticulum.

We identified FABPs as intracellular carriers that bind and "solubilize" anandamide, transporting anandamide to FAAH. Compounds that bind to FABPs tend to block anandamide breakdown, raising anandamide levels. Thus FABPs have become a target for drug discovery. The cannabinoids (THC and CBD) also were discovered to bind FABPs and this may be one mechanism by which CBD works in childhood epilepsy, raising anandamide levels. Likewise, other cannabinoid type drugs, such as ajulemic acid, may be acting by binding to the FABPs and blocking anandamide breakdown.

Targeting FABPs may be advantageous since they have some tissue specificity and do not require reactive serine hydrolase inhibitors, as does FAAH, with potential for off-target reactions.



AEA (Anandamide) FAAH (fatty acid amide hydrolase)
FABP (human fatty acid binding protein), CB (cannabinoid receptor)

Acknowledgements: This work would not have been possible without the support of the undergraduate and graduate students, the postdocs and technical staff as well as the many collaborators and the National Institute on Drug Abuse for funding (current grant DA035923).

Presidential Plenary Speaker

Tuesday, June 28, 2016, 11:00 – 12:00

Moving from Mechanisms of GPCR Functionality to New Drugs

Andrew Tobin, Ph.D.

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University of Leicester
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Over the last three decades our understanding of the signalling properties and mechanisms of drug action at GPCRs has risen at a rapid rate. Despite this, the attrition rate of GPCR drug discovery programmes has remained alarmingly high. Is it the case that our knowledge base, largely centred on in vitro studies, has painted an inaccurate picture of the functionality of GPCRs or is it that we have yet to learn how GPCRs operate, and how GPCR ligands act, in a physiological and disease context. In this presentation I will be describing our work on a range of GPCRs, from free fatty acid receptors through to muscarinic receptors, aimed at understanding novel paradigms of GPCR drug action in the context of physiology and disease. In so doing I will ask the question of how close are we to a rational design strategy for GPCR ligands that will lead to an impact on the attrition rate of GPCR-drug discovery programmes.

Kang Tsou Memorial Speaker

Wednesday, June 29, 2016, 12:15 – 13:00

HUMAN EXOSOME COMPLEX IN HEALTH AND DISEASE

Andrzej Dziembowski, Ph.D.

Institute of Biochemistry and Biophysics PAS &
Faculty of Biology, University of Warsaw

The multisubunit RNA exosome complex is a major ribonuclease of eukaryotic cells that participates in the processing, quality control and degradation of virtually all classes of RNA in Eukaryota. All this is achieved by about a dozen proteins with only three ribonuclease activities between them. In this talk I will provide an overview of our recent results describing the role of the nuclear exosome complex in the regulation of transcriptome homeostasis. Moreover, I will describe an interesting connection between the exosome and the pathogenesis of multiple myeloma, a cancer of plasma cells.

Young Investigator Award Presentation

Thursday, June 30, 2016, 9:30 – 10:00

Endocannabinoids as a Homeostatic Signal Counteracting Adverse Effects of Stress

Sachin Patel, M.D., Ph.D.

Department of Psychiatry and Behavioral Sciences
Department of Molecular Physiology & Biophysics
Vanderbilt University Medical Center, Nashville, TN, USA

Environmental, psychological, and physical stressors are key risk factors for the development of a variety of psychiatric disorders and medical illnesses. Over the past 15 years, the role of endogenous cannabinoids (eCBs) in the regulation of stress responsivity and adaptation has been heavily investigated at the behavioral, hormonal, neuronal, and synaptic level. This work has culminated in two seminal conceptual advances in the field. First, in most cases eCB signaling via CB1 receptors appears to counteract acute stress responses and the adverse effects of chronic stress exposure, while functional antagonism of this system impairs the ability of organisms to appropriately cope with stress. Second, preclinical efficacy studies, aided by development of novel and selective chemical probes, has provided compelling support for the notion that eCB degradation inhibitors (of FAAH, MAGL, and possibly COX-2) could represent novel approaches to the treatment of mood and anxiety disorders and potentially other disorders affected by stress. Here we will summarize data form our laboratory implicating 2-arachidonoylglycerol (2-AG) as a key eCB regulating stress response physiology, stress adaptation, and emotional behavior. Findings supporting pharmacological 2-AG augmentation as a potential treatment approach for stress-related neuropsychiatric disorders will be presented. Novel insights in to the synaptic and circuit-level mechanisms by which 2-AG regulates these processes will also be discussed, while future directions and critical gaps in current knowledge will be highlighted.

Acknowledgements:

Work presented was supported by National Institutes of Health and Vanderbilt University Medical Center.

PHARMACOVIGILANCE OF MEDICAL CANNABIS: PRELIMINARY RESULTS FROM THE QUEBEC CANNABIS REGISTRY

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Since April 1st 2014, legal access to dried cannabis in Canada has been regulated by the *Marihuana for Medical Purposes Regulations* (MMPR). The MMPR obliges the medical profession to authorise cannabis outside the usual framework of prescription drugs, as there is a lack of scientific evidence required for good medical practice. The Quebec Cannabis Registry (QCR) was launched in May 2015 as a pharmacovigilance initiative to allow adult patients in Quebec to access cannabis for medical purposes, with the support of their physician, as part of a research framework. The main objectives of the QCR are to: (1) systematically collect data on indications, dosages, benefits or side effects of the products used; (2) stimulate future research; and (3) support the ongoing development of a drug monitoring program.

We performed a preliminary extraction of key variables to present the structure and content of the QCR. As of March 9th 2016, 213 patients have been enrolled. The average age was 46.8 years (range 19 to 83 years), with a higher proportion of men (58.7%). More than half of the participants reported symptoms of neuropathic, somatic, visceral or mixed pain (54.5%), whereas almost one quarter suffer from HIV, cancer or chronic pain (23.5%). The main reported diagnoses were chronic neuropathic pain (17.4%), chronic lumbar neuropathic pain (14.6%), chronic pain (8.0%) and fibromyalgia (4.7%).

At initial visit, the mean (SD) Brief Pain Inventory (BPI) pain severity score (4 items) was 5.5 (± 2.1) and the mean (SD) BPI pain interference score (7 items) was 5.9 (± 2.4). Among the pain-related pharmacotherapy used by patients, opioids (36.8%), analgesic antidepressants (21.8%) and analgesic antiepileptics (15.5%) were the most common treatments. There were 100 patients using opioids, with an average morphine equivalent (MEQ) daily dose of 290.4 ± 536.3 mg (range 3.75 – 4208). The average daily cannabis dose prescribed to patients was 2.1 ± 1.4 g (range 0.3 – 10).

After the three month visits, BPI scores, opioid dose (MEQ) and cannabis dose were compared to baseline data in subjects with available data at both time points. The mean BPI pain severity score and the mean BPI pain interference score were significantly lower at the three months visits compared to the initial visits ($p = 0.009$ and $p = 0.02$, respectively). However, of the patients who had data at both time points, there was no significant difference in the mean MEQ daily dose between the initial (128.7 ± 121.8 mg) and 3 month visits (108.5 ± 109.2 mg) ($p = 0.10$) and no significant difference in the daily cannabis dose between the initial (2.1 ± 1.2 g) and 3 month visits (2.2 ± 1.0 g) (P = 0.58).

The QCR gathers valuable data on the use of medical marijuana in Quebec. We are beginning to see reductions in pain severity and interference, and opioid doses appear to be reducing. The analysis of the data collected over time will identify new research questions and will increase knowledge about the clinical effects, including safety and effectiveness, of cannabis used for medical purposes.

Acknowledgements: Funded by the Canadian Consortium for the Investigation of Cannabinoids with support from the College des Medecins du Quebec, Bedrocan (Canada), Mettrum, and Tweed.

EFFECT OF CANNABIS USE ON SEVERITY OF CHRONIC LOW BACK PAIN AND SCIATICA

Dror Robinson

Department of Orthopedics, Hasharon Hospital,
Rabin Medical Center, Petah Tikwa, Israel.
Affiliated with the Sackler School of Medicine,
Tel Aviv University

Background: Anecdotal evidence indicates the possible efficacy of cannabis use as an adjunctive treatment in chronic low back pain. The purpose of the current study was to assess the results of treatment of patients suffering from chronic low back pain of at least 12 months duration by medicinal cannabis.

Methods: A cohort of 39 patients was followed for a minimum of six months. They were evaluated at baseline, 3 months and 6 months by patient reported outcome questionnaire (SF-12), visual analogue scale and the Brief Pain Inventory. Inclusion criteria included: age over 25 years, sciatica with documented treatment for at least 12 months, evidence on CT or MRI scan of disc herniation or spinal stenosis, failure of at least two narcotic drugs, and consent to use medicinal cannabis. Exclusion criteria included evidence of bone cancer, evidence of diabetic neuropathy, evidence of prior psychotic reactions.

Treatment protocol: Cannabis usage was at a fixed dosage of 20 grams per month, dose increase was considered at least after 4 months of treatment. The cannabis was smoked at a recommended rate of 4 dosages per day.

Results: VAS decreased from 85±12 to 32±17 at six months. SF12-PCS imrproved from 21±14 to 52±11. SF12-MCS improved from 23±12 to 53±10.

In conclusion, short term usage of smoked medicinal cannabis appear to improve both physical and mental function while decreasing pain levels of chronic low back pain sufferers.

SELF-REPORTED MEDICAL CANNABIS ACCESS, USE, AND SUBSTITUTION FOR OTHER SUBSTANCES IN 301 CANADIAN MEDICAL CANNABIS PATIENTS

Philippe Lucas and Zach Walsh

Tilray, Nanaimo, BC/Social Dimensions of Health, University of Victoria, BC, Canada

Background: Canada has had a federally-regulated medical cannabis program in place since 2001. In 2014 Health Canada replaced the Marihuana for Medical Access Regulations (MMAR) with the Marihuana for Medical Purposes Regulations (MMPR). One of the primary changes in the new program has been to move from a single Licensed Producer (LP) of cannabis to multiple large-scale Licensed Producers. This is the first comprehensive survey of patient experiences, patterns of use and cannabis substitution effect as self-reported by patients enrolled in Canada's federal medical cannabis program.

Methods: Tilray is a federally authorized Licenced Producer within the MMPR. In an effort to learn more about patient experiences and patterns of use within the federal program, a 107 question survey was made available to Tilray patients online in French and English for 2 weeks in July 2015. The survey included questions on patient demographics, patterns of use, and cannabis substitution effect, and gathered 301 responses.

Results: Results suggest a very high self-reported use of cannabis as a substitute for prescription drugs (63%), particularly pharmaceutical opioids (31%) and benzodiazapines (11%). Patients also reported substituting cannabis for alcohol (25%), cigarettes/tobacco (12%), and illicit drugs (5%). Additionally, a large percentage of patients (42%) reported currently accessing cannabis from illegal/unregulated sources, and over half (55%) we're charged to receive a medical recommendation by a physician to use cannabis, with nearly 25% paying $300 or more.

Interpretation: These findings suggest that medical cannabis is primarily used to treat chronic pain and mental health issues, and may prove to be an effective adjunct or substitute treatment to prescription drugs used to treat these conditions. Longitudinal research would help identify the specifics and context of cannabis substitution effect, and further elucidate the potential impact of cannabis substitution on the quality of life of patients. The finding that patients continue to purchase cannabis from unregulated sources and that a significant percentage of patients have had to pay high rates for medical cannabis recommendations also highlights areas of concern for both patients and policy-makers.

EPIDEMIOLOGICAL CHARACTERISTICS OF PATIENTS TREATED WITH MEDICAL CANNABIS

Lihi Bar-Lev Schleider MA[1,2], Raphael Mechoulam PhD[3], Inbal Sikorin RN[2], Timna Naftali MD[4], Zvi Bentwich MD[2,5] and Victor Novack MD PhD[1]

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[2] Research Department, Tikun Olam LTD.
[3] School of Pharmacy, the Hebrew University of Jerusalem, Israel.
[4] Department of Gastroenterology and Hepatology, Meir Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Kfar Saba.
[5] Center for Emerging Tropical Diseases and AIDS, Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel.

Background: In 2007, Israeli Ministry of Health began providing registrations for medical cannabis. Today there are ~23,000 medical cannabis patients in Israel; 6,500 (28%) receive treatment at Tikun-Olam (TO). The aim of this study is to characterize the patient population receiving medical cannabis treatment.

Methods: We analyzed the data routinely collected as part of the treatment program on 2,119 medical cannabis patients out of 2,671 registered in TO during 2015 (80%).

Results: The most frequent indications were: cancer (56.6%), non-cancer pain (26.9%), post-traumatic stress disorder (5.5%), Parkinson's disease (2.6%), epilepsy (2.2%), inflammatory bowel disease (2.0%) and multiple sclerosis (0.8%). The median disease duration was 4 years (1-21). The average age was 56±19 years with 34.3% patients being older than 65 and 2.2% younger than 18, 51.5% were males, 22.7% were employed and 29.4% retired. Thirty per cent of the patients reported previous experience with cannabis. The main symptoms requiring therapy were: pain (80.2%, median intensity 8/10), insomnia (74.1%), weakness (71%), fatigue (49.2%), movement limitation (45.1%), vomiting (44.1%), anxiety (42%), digestive problems (41.8%), constipation (40.8%) and depression (39.9%). At one month follow up of 1979 patients, 1,184 (60%) patients reported significant improvement in their condition; 159 (8%) died, 57 (2.8%) stopped the treatment and 94 (4.7%) had side effects.

Conclusions: This is the first study describing the characteristics of the medical cannabis users in Israel. The treatment appears to be safe and efficacious. Establishing a national clinical investigation program to elucidate the safety and effectiveness of the therapy is imperative.

TOWARDS A NATURALISTIC CHARACTERIZATION OF A MEDICAL CANNABIS POPULATION: EMERGING CANADIAN DATA

Lee Saynor [1,2], Kunal Sudan [1] and Henry J. Moller [1,2,3]

PRAXIS Holistic Health [1], Digital Futures Initiative, OCAD University [2], and Dept of Psychiatry, University of Toronto [3], Toronto, ON, Canada

We present a demographic synopsis of a population attending health and wellness care under Canada's federal Marihuana for Medical Purposes Regulations (MMPR, est. by Gov of Canada, 2013). We have previously presented the utility of the electronic health record Wellpad, which has been previously discussed as being of high utility for demographic and clinical outcome data gathering in a medical cannabis population (Moller et al., 2014).

Data for 427 consecutive MMPR patients (males=79%, females= 21%) attending our clinic between November 2013 and February 2016 were retrospectively examined in Wellpad. Geolocating data established four clinical groups: A) Toronto-urban B) Toronto- suburban C) within Ontario province D) out-of-province

Out of 419 patients who entered their Primary Clinical Indication, 243 (56.9%) added a second, 103 (24.1%) added a third, and 26 (6.1%) added 4 or more comorbidities. Clinical Indications reported were: Pain, which accounted for 41.0%, anxiety at 27.6%, sleep disturbances 26.9%, depression 12.9%, arthritis 10.5%, ADD/ADHD 7.3%, migraines/headaches 5.6%, PTSD 4.7%, spinal injury 3.5% and other (eg. Epilepsy, diabetes, IBS/Crohn's, etc.)

Complete demographics are described in this presentation.

References:

1. Government of Canada, Marihuana for Medical Purposes Regulations (2013).

http://www.laws-lois.justice.gc.ca/eng/regulations/SOR-2013-119/

2. Moller HJ, Saynor L, Sudan K, Tabak D Wellpad: A New Standardized Assessment and Data Visualization Tool for Clinicians and Researchers. IACM 2015 8th Conference on Cannabinoids in Medicine 17-19 September 2015 in Sestri Levante, Italy

Acknowledgement: we are grateful for the kind assistance of Robelyn Soriano and Daniel Tabak in assisting in data collection and data system design assistance.

THE DEVELOPMENT OF A CANNABIS PLACEBO

Mikael A. Kowal and Arno Hazekamp

Bedrocan, Veendam, The Netherlands

A placebo may be defined as a substance or procedure which possesses no inherent power to produce an effect that is sought or expected. In contemporary clinical trials placebos are crucial in securing a reliable, controlled research design. However, clinical research involving cannabis frequently faces the challenge of obtaining a proper placebo. Due to the herbal form of cannabis and the specific administration forms (predominantly smoking or vaporizing), it is difficult to find a placebo that may 'trick' a research subject into believing that he or she is using a real medicine, while in fact it does not contain the active substances responsible for the intended effect.

Moreover, cannabis contains terpenes that are responsible for the characteristic smell and taste of cannabis. This specific smell is easily recognizable by most individuals who have ever had contact with the plant. On the other hand, looking into the scientific literature, one may notice that clinical research involving cannabis frequently uses placebos which have virtually no resemblance to cannabis both in terms of appearance, as well as smell and taste. This includes various herbal mixtures, sometimes containing industrial hemp flowers, or even tobacco. For that reason, researchers frequently fail at properly blinding their study conditions to the subjects.

A solution to this problem would be to have a cannabis placebo that does not contain the biologically active cannabinoids (like THC and CBD), while retaining exactly the same terpene profile as the original, active plant. This solution has been adopted by the company Bedrocan, resulting in a method that allows to produce placebo cannabis out of any strain of active cannabis.

The presentation will provide an introduction into the concept of a placebo and describe the entire process of developing the currently GMP-compliant cannabis placebo method. Moreover, it will review the use of different placebos in clinical trials involving cannabis and show data on the applicability of the newly-developed placebo.

PANNEXIN-1 MODULATES GLUTAMATERGIC TRANSMISSION BY REGULATING SYNAPTIC ANANDAMIDE CONCENTRATION

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Anandamide (AEA) is an endogenous fatty acid which modulates synaptic activity. AEA is able to decrease or increase synaptic activity based on its concentration in the synaptic cleft which dictates its actions as endocannabinoid, through cannabinoid 1 receptor (CB1R), or as an endovanilloid, leading to activation of the transient receptor potential 1 (TRPV1). Currently, the mechanism by which AEA concentration is regulated in the synaptic cleft is unknown. Fatty acid amide hydrolase, FAAH, is the enzyme responsible for AEA degradation to arachadonic acid and other metabolites. How AEA is transported into the cell for breakdown by FAAH is under investigation. Suggested mechanisms include transporters, lipid rafts or direct movement via passive diffusion through membranes. We hypothesize that AEA is transported through the large pore ion and metabolite channel, Pannexin-1 (Panx1). Pannexin -1 is able to flux molecules up to 1 kilodalton in size, possibly including lipid derivatives. Using whole-cell patch clamp recordings from CA1 pyramidal neurons, we show that blocking Panx1 prevents the access of AEA to FAAH for degradation. This leads to the accumulation of AEA in the synaptic cleft and subsequent TRPV1 receptor activation that increases presynaptic glutamate release.

To determine if AEA is active at excitatory synapses, 50μM AEA was loaded into a single post-synaptic CA1 pyramidal neuron. This augmented pre-synaptic glutamate release, evident by increased excitatory postsynaptic potential (EPSP) frequency. This increase was abolished by inclusion of a Panx1 blocker in the patch pipette, suggesting that AEA leaves the post-synaptic neuron via Panx1 channels. Increase glutamate release was also inhibited by bath application of capsazepine (10μM), a TRPV1 receptor antagonist, suggesting that AEA increases glutamate release by activation of the cation specific TRPV1 channel. An increase in EPSP frequency was observed with post-synaptic Panx1 blocked, in the absence of 50μM AEA; but this required afferent stimulation. Together, we have unveiled a novel form of short-term synaptic plasticity that requires AEA activation of presynaptic TRPV1, which is regulated by AEA transport through postsynaptic Panx1.

REGULATION OF CANNABINOID ACTIVITY BY HEPATIC FATTY ACID BINDING PROTEINS

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The increasing use of medical marijuana highlights the importance of developing a more complete understanding of cannabinoid metabolism. Following administration, cannabinoids, including Δ^9-tetrahydrocannabinol (THC), are metabolized and inactivated by cytochrome P450 (CYP450) enzymes primarily within the liver. The lipophilic nature of cannabinoids necessitates protein-mediated cytoplasmic transport to intracellular CYP450 enzymes. In a recent publication, our group identified a role for brain-expressed fatty acid-binding proteins (FABPs) in facilitating cytoplasmic transport of the phytocannabinoids THC and cannabidiol (Elmes et. al. *Journal of Biological Chemistry* 290.14 (2015): 8711-8721). Currently, no hepatic proteins that facilitate intracellular shuttling of exogenous cannabinoids have been described, highlighting a major gap in our knowledge of phytocannabinoid inactivation. The high expression level of fatty acid binding protein 1 (FABP1, L-FABP) in the liver coupled to its promiscuous binding to structurally diverse lipophilic xenobiotics renders this protein as an ideal candidate to mediate hepatic phytocannabinoid transport.

The work presented here tests the central hypothesis that FABP1, the major FABP subtype expressed in liver, is an important regulator of phytocannabinoids transport and subsequent inactivation at the CYP450s. We show that purified recombinant human FABP1 binds both THC and CBD with low micromolar affinities *in vitro*. The *in vivo* contributions of FABP1 to phytocannabinoid activity was assessed by comparing cannabimimetic effects (e.g., hypothermia and hypomotility) in FABP1-knockout (FABP1-KO) and wild type (WT) mice following treatment with THC. FABP1-KO mice exhibit significantly potentiated hypothermic responses following intraperitoneal THC administration compared to their WT counterparts. Furthermore, WT animals pretreated with the pharmacological FABP inhibitor BMS-309403 were found to exhibit a significantly heightened sensitivity to THC effects. Our ongoing work seeks to directly address the role of FABP1 in THC metabolism *in vitro* and *in vivo*. Collectively, these results suggest that FABP1 influences phytocannabinoid inactivation, likely by facilitating cytoplasmic transport to the intracellular CYP450 enzymes.

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FATTY ACID BINDING PROTEINS MEDIATE RETROGRADE ENDOCANNABINOID SIGNALING

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The endocannabinoid (eCB) system regulates diverse biological functions including stress, pain, and reward. The eCB 2-arachidonoylglycerol (2-AG) is the principal endogenous ligand at brain cannabinoid receptor 1 (CB1). Upon depolarization, postsynaptic neurons release 2-AG, which subsequently activates CB1 on presynaptic axon terminals. The rapid release of 2-AG suggests that a transport mechanism(s) may mediate its translocation across the synaptic cleft. However, the nature of this mechanism(s) is not known. Intracellular transport of lipophilic eCBs is governed by carriers including fatty acid binding proteins (FABPs). Herein, we examined whether FABPs likewise mediate retrograde transport of eCBs.

To that end, we examined the impact of FABP inhibition on constitutive and phasic eCB signaling at glutamate synapses onto dorsal raphe nucleus (DRn) serotonin (5-HT) neurons. We found that treatment of DRn slices with the FABP inhibitor SBFI26 (10 μM) prevented the potentiation of glutamate-mediated excitatory postsynaptic currents (EPSCs) induced by the CB1 receptor antagonist/reverse agonist AM251 (3μM). Inhibition of FABPs also impaired eCB long-term depression (eCB-LTD) induced by stimulation of α1-adrenergic receptors (α1-ARs) without altering the ability of the CB1 receptor agonist WIN 515,212-2 to reduce the amplitude of EPSCs. Knockout of FABP5 profoundly reduced the magnitude of AM251-induced potentiation of EPSC amplitude and α1-AR-induced eCB-LTD. SBFI26 did not reduce 2-AG levels in the slices, arguing against an off-target effect upon 2-AG biosynthesis. These findings suggest that FABP5 is required for the retrograde transport of eCBs at glutamate synapses in the DRn.

To further substantiate that FABPs could serve as synaptic eCB carriers, FABP5 secretion from cells and its localization to synapses was examined. Our data indicate that primary cultures of mouse astrocytes secrete FABP5. Furthermore, immunogold electron microscopy indicated clustering of FABP5 at sites proximal to and within synapses of the dorsal raphe, which was abolished in slices from FABP5 KO mice. We confirmed that synaptic localization of FABP5 was not a unique feature of the dorsal raphe by demonstrating a similar distribution pattern in the hippocampus. Collectively, our results ascribe novel functions to FABPs in synaptic eCB signaling and suggest that FABPs serve as synaptic carriers for eCBs.

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NEURONAL AND ASTROCYTIC MONOACYLGLYCEROL LIPASE LIMIT THE SPREAD OF ENDOCANNABINOID SIGNALING IN THE CEREBELLUM

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Endocannabinoids are diffusible lipophilic molecules that may spread to neighboring synapses. Monoacylglycerol lipase (MAGL) is the principal enzyme that degrades the endocannabinoid 2-arachidonoylglycerol (2-AG). Using knockout mice in which MAGL is deleted globally or selectively in neurons and astrocytes, we investigated the extent to which neuronal and astrocytic MAGL limit the spread of 2-AG-mediated retrograde synaptic depression in cerebellar slices. A brief tetanic stimulation of parallel fibers in molecular layer induced synaptically evoked suppression of excitation (SSE) in Purkinje cells, and both neuronal and astrocytic MAGL contribute to the termination of this form of endocannabinoid-mediated synaptic depression. The spread of SSE among Purkinje cells occurred only after global knockout of MAGL or pharmacological blockade of either MAGL or glutamate uptake, but no spread was detected following neuron- or astrocyte-specific deletion of MAGL. The spread of endocannabinoid signaling was also influenced by the spatial pattern of synaptic stimulation as it did not occur at spatially dispersed parallel fiber synapses induced by stimulating the granular layer. The tetanic stimulation of parallel fibers did not induce endocannabinoid-mediated synaptic suppression in Golgi cells even after disruption of MAGL and glutamate uptake, suggesting that heightened release of 2-AG by Purkinje cells does not spread the retrograde signal to parallel fibers that innervate Golgi cells. These results suggest that both neuronal and astrocytic MAGL limit the spatial diffusion of 2-AG and confer synapse-specificity of endocannabinoid signaling.

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STEROL CARRIER PROTEIN 2 DELETION ENHANCES FEAR EXTINCTION THROUGH MODULATION OF THE ENDOCANNABINOID SYSTEM

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BACKGROUND: CB1 cannabinoid receptors (CB1R) are widely expressed in the brain, particularly regions involved in emotional regulation. Previous studies suggest that the lipid transport protein, sterol carrier protein 2 (SCP-2), contributes to the transmembrane movement of the endocannabinoids, *N*-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG). These data led us to hypothesize that SCP-2 regulates endocannabinoid concentrations, thereby affecting CB1R signaling. The purpose of these studies was to test this hypothesis.

METHODS: All of these studies were carried out in male wild type and SCP-2 null mice. Biochemical assays were used to endocannabinoid contents and CB1R binding site density and mRNA expression in amygdala, hippocampus, PFC and cerebellum. Fear conditioning was used to probe the behavioral consequences of SCP-2 deletion in the fear circuit.

RESULTS: Contrary to our hypothesis, AEA contents were not different between SCP-2 knock out and wild type mice in any of the brain regions examined; 2-AG content was significantly reduced in amygdala. On the other hand, CB1R binding site density was increased in amygdala, and decreased in PFC and cerebellum; while CB1R mRNA was significantly increased in the amygdala alone. SCP-2 knock out mice exhibit reduced anxiety-like behaviors in elevated plus maze and following an acute stress. SCP-2 knock out mice also exhibit an increased rate of fear extinction that reverts to wild type by treatment with the CB1R antagonist, rimonabant.

CONCLUSIONS: Loss of SCP-2 results in an up-regulation of CB1R density in the amygdala and CB1R-mediated enhancement of fear extinction. These results suggest that SCP-2 inhibition could provide a therapeutic target for the manipulation of amygdalar endocannabinoid signaling, perhaps for the treatment of anxiety disorders.

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NOVEL EFFECTS OF CANNABIDIOL ON NUCLEUS AMBIGUUS NEURONS

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Cannabidiol (CBD) is a component of marijuana (*Cannabis*), which lacks the psychotomimetic effects of Δ^9-tetrahydrocannabinol (THC). Unlike THC and other cannabinoid compounds which act through CB1 and CB2 cannabinoid receptors, CBD activates TRPV2 channels; Ca^{2+}-permeable, non-selective cation channels, members of the transient receptor potential (TRP) channel family. We have recently reported that functional TRPV2 channels are present in cardiac vagal neurons (CVNs) of nucleus ambiguus (Amb), a critical site for the cardiac vagal tone (1). Axons of cardiac vagal neurons of nucleus ambiguus project to the cardiac ganglia, providing the parasympathetic innervation at this level. Our central hypothesis is that CBD, via TRPV2 activation in cardiac-projecting neurons of nucleus ambiguus, increases cardiac vagal tone and elicits bradycardia. Using a multidisciplinary approach including calcium and voltage imaging, and microinjection in the Amb with concomitant telemetric measurement of heart rate, together with pharmacological and molecular approaches, we are studying the effects and underlying mechanisms of activation of Amb neurons by CBD. Our results indicate that 1 and 10 μM CBD increase $[Ca^{2+}]_i$ in CVNs of Amb; the response was abolished in Ca^{2+} free saline or by the TRPV2 blocker, tranilast. Similarly, 1 and 10 μM CBD elicits depolarization of Amb neurons. In vivo, CBD microinjection in nucleus ambiguus produces a dose-dependent bradycardia. Depolarization of Amb neurons leads to acetylcholine release to cardiac ganglia and consequent bradycardia. To exclude any possible effects of anesthesia on cardiovascular response, this effect was determined using bilateral microinjection of CBD in nucleus ambiguus on heart rate in conscious rats. Understanding the effects of CBD on cardiac vagal tone is highly relevant, as marijuana is the most widely used illicit recreational drug; in addition, its medical use has been legalized in much of the US and several countries, which make the full understanding of its effects imperative.

Reference

1. Brailoiu, E., Deliu, E., Sporici, R. A., Benamar, K., and Brailoiu, G. C. (2014a) HIV-1-Tat excites cardiac
parasympathetic neurons of nucleus ambiguus and triggers prolonged bradycardia in conscious rats.
Am J Physiol Regul Integr Comp Physiol 306, R814-822.

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DIFFERENT EFFECTS OF CHRONIC ADMINISTRATION OF THE FATTY ACID AMIDE HYDROLAZE INHIBITOR URB597 ON CARDIAC PERFORMANCE AND OXIDATIVE STRESS IN NORMOTENSIVE AND HYPERTENSIVE RATS

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The aim of the study was to determine the influence of chronic administration of the inhibitor of fatty acid amide hydrolase [FAAH; an enzyme responsible for the degradation of anandamide (AEA)] URB597 (1 mg/kg; for 14 days twice daily) on cardiac performance and oxidative stress in primary (SHR) and secondary (DOCA-salt) hypertension.

Twelve hours after the final dose of URB597 or its solvent we determined (1) the isoprenaline (ISO; non-selective β-adrenoceptor agonist)-induced increases in heart rate, left ventricular pressure (LVP), the maximum rate of positive (inotropism) or negative (lusitropism) changes in LVP and decreases in coronary perfusion pressure (CPP) in isolated hearts (Langendorff model); (2) increases and decreases in isolated paced left atria contractile force induced by ISO and the cannabinoid receptor agonist CP55940, respectively (isometric force transducer) and (3) parameters of cardiac oxidative stress (biochemical methods).

All cardiostimulatory effects of ISO in heart and in atria and the negative inotropic effect of CP55940 were diminished in hypertension compared with normotensive control rats. URB597 did not affect basal parameters of isolated hearts and atria and the ISO-induced changes in CPP. It slightly improved the reduced contractility to ISO in DOCA-salt but not in SHR and inotropic effects of ISO in atria of SHR (but not in DOCA-salt). It almost restored the negative inotropic effect of CP55940. In normotensive rats it reduced the positive inotropic and lusitropic effects of ISO.

Hypertension was accompanied by an increase in reactive oxygen species (ROS) level, decrease in the activity/level of antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione, vitamin A, E) and enhanced level of products of lipid peroxidation (4-hydroxynonenal, isoprostanes) and protein oxidation (carbonyl groups, dityrosine). Unexpectedly, URB597 induced similar changes in normotension, and partially in a model-specific manner, increased oxidative stress in hypertension. The comparable trend of changes in components of the endocannabinoid system (e.g. enhanced level of AEA and 2-arachidonyl glycerol or expression of CB_1 and CB_2 receptors) were noticed in normotensive rats treated with URB597 and in hypertension.

Conclusions: Chronic URB597 administration only partially restored the changed values of parameters of cardiac function and oxidative stress induced by hypertension. It diminished the β-adrenoceptor-mediated cardiostimulation and enhanced cardiac oxidative stress in normotensive rats. Thus, caution should be taken in potential therapeutic application of FAAH inhibitors in normotensive patients, because of side effects connected with their off-target response.

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PROTECTIVE EFFECTS OF FAAH SUBSTRATES ON AGE-RELATED ENDOTHELIAL DYSFUNCTION

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The endocannabinoid, N-arachidonoyl ethanolamine (AEA) is a potent vasodilator that improves blood flow. Recent evidence suggests that targeting the degradative enzyme of AEA, fatty acid amide hydrolase (FAAH) might be beneficial in age-related pathological conditions. However, the vascular function of AEA and FAAH during ageing remains undetermined. In this study, we investigated the effects FAAH inhibitors and FAAH substrates on endothelial dysfunction, a key feature of ageing and cardiovascular disease.

In isolated small mesenteric arteries from male Wistar rats, ageing (3-12 months) was associated with progressively smaller endothelium-dependent relaxation, and appearance of endothelium-dependent contraction in response to the muscarinic agonist, carbachol (3 month, pEC_{50}=7.0±0.1 R_{max}=102±8%; 12 month, pEC_{50}=6.6±0.1 R_{max}=47±4%). Treating vessels from older rats (for 45 min in a myograph, or for 3 days in organ culture) with two structurally distinct FAAH inhibitors (1μM URB597 or 20μM AA-5-HT) restored the endothelium-dependent relaxation, augmenting the responses by about 30%. This was mimicked by combined treatment with AEA and its congeners (30nM AEA, 10μM PEA and 1μM OEA), but not AEA alone. WIN55212-2 (1μM; $CB_{1/2}$ agonist) or capsaicin (1nM; TRPV1 agonist) also had no effect. The protective effect of URB597 was inhibited by the GPR55/GPR18 antagonist O-1918 (3μM), but not $CB_{1/2}$ receptor antagonists (AM281 and JTE907, 1μM) or the PPAR-gamma antagonist GW9662 (1μM). In vessels from younger rats (3 month), URB597 had no effect on carbachol-induced relaxation despite the presence of URB597-sensitive FAAH activity. Quantitative RT-PCR analysis also demonstrated the expression of GPR55, GPR18, CB_1, CB_2, TRPV1 and FAAH in rat mesenteric arteries.

In conclusion, AEA and its congeners improve dilator function and protect against endothelial dysfunction in ageing. Although the underlying mechanisms are yet to be clarified, these data suggest that targeting FAAH may be a novel protective strategy against vascular ageing.

CANNABINOID RECEPTOR 2 ACTIVATION ALLEVIATES DIABETES-INDUCED CARDIAC DYSFUNCTION, INFLAMMATION, OXIDATIVE STRESS AND FIBROSIS

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Cardiomyopathy and heart failure are among the most serious complications of diabetes. In this study, we explored the role of anti-inflammatory cannabinoid 2 receptor (CB2R) signaling in myocardial dysfunction, inflammation, oxidative/nitrative stress, and cell death associated with type 1 diabetic cardiomyopathy in mice. The CB2R is under intensive investigation of both academic and industrial laboratories for its potential to treat various devastating disease conditions, including ischemia/reperfusion injury, and diabetic nephropathy, among many others.

Diabetic cardiomyopathy was characterized by impaired myocardial contractility, increased inflammation (increased expression of tumor necrosis factor-a, interleukin-1ß, and intracellular adhesion molecule 1), enhanced myocardial oxidative/nitrative stress (3-nitrotyrosine, and 4-hydroxynonenal formation, and enhanced expression of gp22phox, gp67phox, and gp91phox), fibrosis (picrosirius red staining, and collagen-1, CTGF, fibronectin, and TGF- ß expression), and cell death (caspase-3 and -7 and PARP activation). Pharmacological activation of CB2R with JWH133 attenuated diabetes-induced inflammation, oxidative/nitrative stress, fibrosis and cell demise, and consequent systolic and diastolic cardiac dysfunction without affecting hyperglycemia. In contrast, genetic deletion resulted in aggravation of myocardial pathology.

Thus, selective activation of CB2R ameliorates diabetes-induced myocardial tissue injury and preserves the functional contractile capacity of the myocardium in the diabetic milieu. This is particularly encouraging, since unlike CB1R agonists, CB2R agonists do not elicit psychoactive activity and cardiovascular side effects, and therefore are potential clinical candidates for the treatment of diabetic cardiovascular and other complications.

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ROLE OF GPR55 AND GPR18 RECEPTORS IN THE VASCULAR ACTIONS OF ENDOCANNABINOIDS AND RELATED LIPIDS

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Previous studies have suggested that the endocannabinoid N-arachidonoyl ethanolamine (AEA) induces arterial relaxation through a novel target distinct from the classical cannabinoid CB_1 and CB_2 receptors. In some assays, GPR55 and GPR18 receptors, both G protein-coupled, are activated by AEA and other endogenous lipids but their role in vascular tone regulation remains speculative. In this study, we compared the vasorelaxant effects of AEA and other putative GPR55/GPR18 ligands in mesenteric arteries from wild-type (WT; C57BL/6J) and GPR55 knockout (KO) mice.

Mesenteric relaxation to AEA was reduced in KO compared to WT mice, with a 10-fold rightward shift in the concentration response curves and 20% less relaxation to 30μM AEA. This inhibition was mimicked by the GPR55 antagonists O-1918 (3μM) and ML191 (10μM), but not TRPV1 receptor desensitization by capsaicin (10μM). The other major endocannabinoid, 2-arachididonoyl glycerol (2-AG) similarly induced mesenteric relaxation, but genetic deletion of GPR55 or capsaicin had little or no effect. The GPR55/GPR18 agonist, abnormal-cannabidiol (abn-CBD) was much less potent, while the putative GPR18 agonist, N-arachidonoyl glycine (NAGly) evoked no relaxation until 30μM (rank order of potency, AEA ~ 2-AG > abn-CBD >> NAGly). Responses to abn-CBD and NAGly were unaffected in KO mice. Cannabidiol (CBD, 10μM), a GPR55 antagonist and GPR18 partial agonist, also induced comparable relaxation in WT and KO mice. On the other hand, the GPR55 agonist, L-lysophosphotidylinositol (LPI, 30μM) induced mesenteric relaxation that was inhibited by ML191 or GPR55 KO, but such a response was sensitive to the contractile agents used. We also found that prior exposure of vessels to LPI (up to 30μM) attenuated AEA-induced relaxation, possibly due to GPR55 desensitisation. Expression of GPR55 and GPR18 in mouse mesenteric arteries was demonstrated by quantitative RT-PCR analysis.

In conclusion, AEA and LPI evoke GPR55-mediated relaxation in mouse mesenteric arteries. Some GPR18 agonists are vasorelaxants but the role of GPR18 in endocannabinoid responses require further investigation.

ROLE OF HYPOTHALAMIC WAKE CIRCUITRY IN CANNABINOID MODULATION OF SLEEP

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Sleep occupies approximately 1/3 of humans' life span, and sleep problems impair brain function. Cannabinoids are able to improve sleep, but its underlying mechanism is unknown. Here, we observe that cannabinoids prolong non-rapid eye movement (NREM) sleep and shorten wakefulness, which are inhibited by CB_1 cannabinoid receptor (CB_1R) antagonism in the perifornical area (Pef) and dorsomedial nucleus of the hypothalamus (DMH). Surprisingly, cannabinoids selectively activate CB_1R at excitatory glutamatergic but not inhibitory GABAergic synapses onto DMH glutamatergic but not GABAergic output neurons. We are conducting further studies to explore whether inactivation or activation of the glutamatergic DMH-Pef pathway respectively mimics or counteracts cannabinoid effects on NREM sleep and wakefulness. These results suggest that CB_1R-mediated deactivation of the wake-promoting glutamatergic DMH-Pef circuitry contributes to cannabinoid promotion of NREM sleep, the stage of deep sleep without dream.

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MECHANISMS OF TOLERANCE TO Δ^9-THC IN RODENT MODELS OF PATHOLOGICAL PAIN

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The use of cannabinoids in pain management is of significant interest due to the antinociceptive efficacy of Δ^9-THC. However, tolerance to the antinociceptive effects of Δ^9-THC develops with repeated treatment. The objective of this study was to examine tolerance to daily administration of Δ^9-THC in clinically relevant rodent models of pathological pain. The effect of Δ^9-THC in pathological pain was assessed in male wild-type and cannabinoid receptor 1 (CB_1) desensitization-resistant S426A/S430A mutant mice. First, we assessed tolerance to Δ^9-THC in mice subjected to the formalin test of inflammatory pain. Intraplantar formalin injection (10 µl at 2.5 %) produces a biphasic nociceptive response consisting of acute and inflammatory pain phases. Experimental groups were subjected to the formalin test after receiving daily injections of Δ^9-THC (6 mg/kg) ranging from zero to twelve days. Wild-type mice exhibited complete tolerance to the antinociceptive effects of Δ^9-THC in the formalin test after eight days of daily Δ^9-THC. Interestingly, we find that tolerance to the antinociceptive effects of Δ^9-THC is attenuated in S426A/S430A mutants for the inflammatory pain phase but not for the acute pain phase. The effect of the c-jun N-terminal kinase (JNK) inhibitor, SP600125, on Δ^9-THC tolerance was also examined. Pre-treatment with SP600125 (3 mg/kg) attenuates tolerance for the antinociceptive effects of Δ^9-THC in both the acute and inflammatory pain phases. Tolerance to the anti-allodynic effects of Δ^9-THC was examined in a mouse model of post-surgical pain. Injection of Δ^9-THC completely alleviates thermal and mechanical allodynia following a 5 mm hindpaw surgical incision. Consistent with our previous work, tolerance to the anti-allodynic effects of Δ^9-THC is attenuated in S426A/S430A mutant mice. These results demonstrate that Δ^9-THC exhibits robust anti-nociceptive effects in two separate rodent models of clinically relevant, pathological pain and are consistent with previous studies showing the rapid onset of tolerance to the antinociceptive effects of Δ^9-THC in the tail-flick and hotplate tests.

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DIACYLGLYCEROL LIPASE ALPHA:
INFLAMMATORY AND NEUROPATHIC PAIN RELIEF IN MICE

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Diacylglycerol lipase (DAGL), the enzyme responsible for generation of 2-arachidonylglycerol (2-AG), represents a particularly interesting component of the endogenous cannabinoid (endocannabinoid) system as a potential therapeutic target to treat pain. This enzyme also regulates formation of arachidonic acid in mouse peritoneal macrophages in response to inflammatory insults. As DAGL-α inhibition leads to decreases in lipopolysaccharide (LPS)-induced prostaglandins (PGs) and proinflammatory cytokines, we hypothesized that the DAGL-α inhibitor DO-34 will reverse nociceptive behavior in laboratory animal models of inflammatory and neuropathic pain. Both the inflammatory model of intraplantar LPS (2.5 μg) and chronic constriction injury of the sciatic nerve (CCI) model of neuropathic pain produce robust increases in sensitivity to light mechanical touch, or allodynia, as assessed with von Frey filaments, and increased thermal sensitivity, or thermal hyperalgesia, as assessed in the hotplate test and acetone induced cold allodynia. To examine general pharmacological effects of DO-34, we also tested this compound in the tetrad assay, which consists of assessments of locomotor behavior, catalepsy, thermal antinociception, and hypothermia.

Intraperitoneal injection of DO-34 reversed LPS-induced allodynia in a time- and dose-dependent manner. The ED50 (95% confidence interval) dose of DO-34 in the LPS model was determined to be 4.15 mg/kg (2.43-7.09 mg/kg). DAGL-α inhibition also reversed acetone induced cold-allodynia and thermal hyperalgesia. Repeated administration of DO-34 retained its anti-allodynic effects in the LPS model. Additionally, systemic DO-34 (30 mg/kg) reversed CCI-induced allodynia and thermal hyperalgesia. Whole brains taken from naïve mice treated with DO-34 revealed decreases in 2-AG (~83%), anandamide (~42%), as well as arachidonic acid (~58%). However, DO-34 did not produce general pharmacological effects in the tetrad assay. Taken together, these findings suggest that DAGL-α represents a provocative target to treat both inflammatory and neuropathic pain.

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SINGLE AND COMBINATION EFFECTS OF CANNABINOIDS ON NEUROPATHIC PAIN AND COGNITION

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Rationale: It has been suggested that the phytocannabinoid Cannabidiol (CBD), a non-psychoactive component in Cannabis sativa, can impact some of the pharmacological effects of delta-9-trtrahydrocannabinol (THC). Currently, a combination of Cannabidiol and THC (Sativex) is an approved medication in Europe for the treatment of conditions such as chronic pain, and has been fast tracked by the US FDA for late stage clinical trials. **Objectives**: The objectives of the present study were to determine the effects of CBD and THC alone or in combination on chemotherapy-induced neuropathic pain and spatial and discrimination learning. We hypothesized that CBD would be as effective as THC in mitigating neuropathic pain but would not affect cannabinoid-agonist induced cognitive impairment. **Methods**: Mechanical allodynia was used to assess chemotherapy induced peripheral neuropathy (CIPN) using the Von Frey test. This assay tests for heightened tactile sensation caused by innocuous stimuli. Mice were treated with Paclitaxel (8.0 mg/kg) on days 1, 3, 5 and 7. To determine the efficacy of CBD, THC or CBD+THC, mice were pretreated with cannabinoids alone or in combination prior to behavioral testing. Learning and memory was assessed in C57BL/6 mice in two mouse models of cognition: 1) conditional discrimination, and 2) Barnes Maze. In the conditional discrimination assay, mice were trained to differentiate between two tones (2s vs. 8s). After mice were trained to criteria, animals were treated with the partial CB agonist THC, the full CB agonist WIN 55212, the non-psychoactive cannabinoid CBD, or combinations of CB agonists + CBD. The Barnes Maze is a spatial memory task that requires animals to learn the position of an escape tunnel that can be used to flee from aversive stimuli on the surface of the maze. On Day 5 of the Barnes Maze task (retention), mice were pretreated with CBD, THC or a combination of CBD + THC and placed in the maze for five minutes. **Results**: Both CBD and THC alone attenuated mechanical allodynia in mice treated with paclitaxel. When evaluated in combination, very low ineffective doses of CBD and THC were found to have supra-additive/synergistic effects when given in combination whereas higher, individually effective doses exhibited sub-additive effects in combination. Results of the conditional discrimination task showed subtle cognitive impairment in mice treated with WIN-55212. Consequently, CBD did not show a reversal of impairment induced by WIN-55212. Additionally, CBD alone did not impact cognitive performance in this task. THC produced subtle impairments in spatial learning in the Barnes Maze task, and these impairments were not attenuated by co-administration with CBD. **Conclusions**: Our present findings suggest that CBD+THC combinations may produce synergistic and sub-additive effects depending on the dose. Additionally, subtle cognitive deficits seen with CB agonists were not reversed by CBD.

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SIGMA RECEPTOR BLOCKADE POTENTIATES THE ANALGESIC EFFECTS OF CANNABINOIDS

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CP 55,940 is a synthetic full agonist of the cannabinoid (CB) receptors that is similar to Δ^9-tetrahydrocannbinol (Δ^9-THC). CP 55,940 has been shown to be 10 times more potent in behavioral assays and has a 10-fold higher affinity in receptor binding assays than Δ^9-THC. Sigma receptor ligands have shown to potentiate the analgesic effects of opiates. Sigma receptors and cannabinoid receptors share similar effects on the body such as appetite regulation, depression, and analgesia; however, there is little data that indicates whether or not the endocannabinoid system and the sigma receptors work in correlation or independent pathways. Compound AZ66 is a sigma receptor antagonist. Sigma receptor ligands have shown to potentiate the analgesic effects of opiates; however, there is little known about the interaction between these ligands and the CB_1 receptors. The purpose of this study is to evaluate AZ66 for potentiation of CB_1 related analgesic effects. A tetrad assay was performed for AZ66 using doses 5-20mg/kg (i.p.) to evaluate any possible analgesic effects. CP 55,940 was evaluated in the tetrad battery to find a dose that caused little to no analgesic effect. The potentiation study was completed by evaluating a 20mg/kg dose of AZ66 against a 0.1mg/kg dose of CP 55940 in the tail-flick and hotplate assays. Studies found that AZ66 increased the antinociceptive effects of low dose CP 55,940 by 10-fold in the hotplate assay and 20-fold in the tail-flick assay. These studies provide, for the first time, direct evidence that sigma antagonists can significantly enhance the analgesic action of cannabinoids thus, opening up new avenues for cannabinoid research in pain. This can serve as a potentially very important part of future pain management research. Future studies involve determination of the sigma receptor subtype involved in this activity as well as evaluation of effects on conditioned place preference.

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A PRO-NOCICEPTIVE PHENOTYPE REVEALED IN MICE LACKING THE ANANDAMIDE HYDROLYZING ENZYME FATTY-ACID AMIDE HYDROLASE

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Fatty-acid amide hydrolase (FAAH) is the main catabolic enzyme responsible for degradation of anandamide, an endogenous ligand for cannabinoid receptors. Pharmacological inhibition or genetic deletion of FAAH produces antinociceptive effects in preclinical pain models. Therapeutic efficacy of FAAH inhibition is largely attributed to disruption of anandamide deactivation and its subsequent activation of cannabinoid receptors. However, FAAH is not selective in its degradation of anandamide, but also metabolizes a wide range of structurally related, biologically active lipid signaling molecules whose functions remain largely unknown. Some of these endogenous lipids, including anandamide itself, may exert pro-nociceptive effects under certain conditions. In mice with a genetic deletion of FAAH, intradermal injection of capsaicin increased nocifensive behavior as well as hypersensitivity to heat and mechanical stimulation compared to their wild-type counterparts. The pro-nociceptive phenotype exhibited by the knockout mice was accompanied by capsaicin-induced increases in Fos-like immunoreactive cells with expression preferentially localized to spinal dorsal horn regions implicated in nociceptive processing. Increased pain behaviors in mice lacking FAAH were attenuated by the CB_1 antagonist AM251 and the TRPV1 antagonist AMG9810. At one hour post-capsaicin, when central sensitization was established, FAAH KO mice displayed increased levels of anandamide, certain endogenous TRPV1 agonists and prostaglandins in both the paw skin and lumbar spinal cord relative to wild type mice. Genetic deletion of FAAH increases pain hypersensitivity and spinal cord neuronal activation in response to intradermal capsaicin. The heightened nociceptive response was mediated by CB_1 and TRPV1 receptors. Moreover, genetic deletion of FAAH has a profound impact on both the peripheral and central lipidome. Our observations suggest that deletion of FAAH may predispose animals to increased sensitivity to certain types of pain. More work is necessary to determine whether such changes could explain the lack of efficacy of fatty-acid amide hydrolase inhibitors in clinical trials.

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TARGETING SPINAL CORD INJURY-ASSOCIATED NEUROPATHIC PAIN WITH CANNABIDIOL

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Rationale: The incidence of chronic neuropathic pain after spinal cord injury (SCI-NP) is estimated at 50 – 80% significantly impacts the patient's life quality. Recent clinical trials have shown that combined THC+CBD can alleviate chronic cancer pain, central neuropathic pain, and peripheral neuropathic pain. The aim of this study was to investigate the therapeutic effect of CBD alone on SCI-NP. **Methods:** Female C57Bl/5 mice were exposed to moderate spinal cord contusion injury (level T9-10) and received repeated vehicle or CBD (1.5 mg/kg IP) injections. Locomotor and bladder function and changes in thermal and mechanical hindpaw sensitivity were evaluated for 10 weeks. Additional groups of mice were used to assess the effect of SCI and CBD treatment on inflammatory mediators via microarray and qRT-PCR at 48h post injury and microglial activation and peripheral immune cell invasion using flow cytometry at 2 weeks post injury. **Results:** Fewer SCI mice in the CBD-treated group went on to develop moderate to severe thermal sensitivity as compared with vehicle-treated mice. However, CBD treatment failed to improve locomotor and bladder function following SCI. Microarray and qRT-PCR results showed a decrease in pro-inflammatory cytokines and chemokines associated with T cell differentiation and invasion. Flow cytometry data showed a decrease in T cell invasion into the injured cord. **Conclusion:** CBD treatment attenuated the development of thermal sensitivity following spinal cord injury and this effect may be related to protection against pathological T cell invasion.

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EFFICACY OF CANNABIDIOL AND CANNABIDIOL DERIVATIVES IN THE TREATMENT OF OCULAR PAIN

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Background: Ocular pain plays an integral role in protecting our eyes from damage, however in some pathologies nociceptive signaling can become dysfunctional resulting in neuropathic pain. The cornea possesses the one of the highest concentrations of sensory nerve endings in the body and injury to the cornea is frequently associated with corneal hyperalgesia and neuropathic pain (CNP). Currently, there are few adequate treatments available for CNP and many of these treatments are ineffective and/or can result in significant adverse effects which limit their prolonged use. Cannabinoid receptor ligands have been demonstrated to decrease neuropathic pain and hyperalgesia. The actions of these cannabinoids occurs through modulation of both cannabinoid 1 receptor (CB_1R) and the cannabinoid 2 receptor (CB_2R). Given that corneal cells express both CB_1R and CB_2R, activation of these receptors by cannabinoid ligands, may show utility in the treatment of CNP.

Purpose: To investigate the *in vivo* pharmacology and antinociceptive properties of cannabidiol (CBD) and cannabidiol derivatives, CBD-DMH and HU308, in experimental corneal hyperalgesia.

Methods: Experimental hyperalgesia was generated using silver nitrate cauterization of the cornea in wild type (WT) and CB_2R knockout ($CB_2R^{-/-}$) mice. Cauterized eyes were treated with topical cannabinoids (1-5% w/v), in the presence or absence the CB_1R antagonist, AM281 (2.5mg/kg ip). The ocular blink response, indicative of corneal pain, was recorded at 6 hrs-post injury following chemical stimulation by capsaicin.

Results: Cauterized eyes had an increased blink response to capsaicin (1μM) at 6 hrs post-injury. Topical application of cannabinoids decreased the number of blinks following corneal sensitization in WT mice. Application of CBD, CBD-DMH and HU-308 significantly decreased the number of blinks following corneal sensitization in WT mice ($p < 0.0001$, $p< 0.001$, $p< 0.0001$, respectively). In $CB_2R^{-/-}$, CBD was still able to decrease the blink response in sensitized mice, albeit to a lesser extent than that observed in WT mice ($p < 0.05$). HU-308 failed to reduce the ocular blink response in CB2R-/- mice ($p >0.05$) while the antinocipetive effects of CBD-DMH remained similar in $CB_2R^{-/-}$ mice compared to WT mice compared to their respective untreated controls ($P<0.001$). The CB_1R antagonist, AM281, exacerbated the ocular blink response ($p <0.001$). In the presence of AM281, CBD reduced the number of blinks to levels comparable to cauterized untreated animals ($p >0.05$). CBD-DMH in the presence of AM281 failed to reduce the sensitization and the exacerbated blink response, with the number of blinks in response to capsaisin remaining elevated compared to cauterization untreated alone ($p <0.001$).

Conclusion: CBD and CBD derivatives reduced corneal pain in a model of corneal hyperalgesia. CBD antinociceptive effects were mediated in part by CB_2R and likely involved other non-cannabinoid receptors since the antinociceptive actions of CBD were still apparent in the presence of the CB1 antagonist, AM281. The CBD derivatives, CBD-DMH and HU-308 produced their anti-nociceptive effects via activation of CB_1R and CB_2R, respectively.

CANNABIDIOL EFFECTS ON mRNA LEVELS AND SIGNALING PATHWAYS IN MOG-35-55 ACTIVATED ENCEPHALITOGENIC T CELLS

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Cannabinoids exert potent immunoregulatory activities. Our previous studies showed that the non-psychoactive cannabinoid, cannabidiol (CBD, a component of *Sativex*), ameliorated clinical symptoms in murine Myelin Oligodendrocyte Glycoprotein (MOG)35-55-induced Experimental Autoimmune Encephalomyelitis model of Multiple Sclerosis. Moreover, CBD decreased MOG-specific T cell proliferation and cytokine secretion including of IL-17. The mechanisms of these activities are poorly understood.

Herein, we describe gene networks and intracellular pathways that are mediating the suppressing effects of CBD in an activated MOG35-55-specific T cell lineage. Encephalitogenic MOG35-55-specific T cells were stimulated with MOG35-55 in the presence of spleen-derived APCs with or without CBD, then separated using magnetic-bead CD4+ selection and subjected to microarray analysis of mRNA levels. Ingenuity Pathway Analysis and Gene Ontology identified IL-17 polarization, IL-6 and IL-10-signalling as top canonical pathways affected by the CBD treatment. Main upstream regulators affected by CBD were recognized as EGR2, STAT5A and NRF1. The effects of CBD on activated encephalitogenic T cell were linked to the regulation of selective T cell activation molecules (BTLA, CD40, CD69, IFNGR1), intracellular modulators of MAPK (DUSP6, DUSP2), PKA (CREM) and Jak/STAT (SOCS3, STAT5) pathways. Gene targets within cell cycle regulation pathways (PTPN6, SLC3A2, VAV3, DDR1) and modulators of oxidative stress (MT1A, HMOX1, SLC30A1) were also significantly affected by the CBD treatment. The microarray results were confirmed using qPCR on selected gene targets. Immunoblotting demonstrated that CBD reduces IL-17 by decreasing STAT3 phosphorylation and increasing that of STAT5. Our observations increase our understanding of the mechanisms of the anti-inflammatory activities of CBD.

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INVESTIGATION OF GENETIC FACTORS IN CBD RESPONSE

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Cannabidiol (CBD) is currently being evaluated in clinical trials to reduce seizure frequency in patients with epilepsy. Preliminary data from the GW Pharmaceuticals open label extension study shows a broad gradation in the magnitude of response to CBD in different patients, from negative to complete cessation of seizures. In collaboration with several physicians who are treating patients in these trials, we are evaluating genetic variants for possible association with inter-individual differences in CBD response. To accomplish this, de-identified patient samples are being analyzed using a next generation sequencing diagnostic panel (epiSEEK® comprehensive - 471 genes) that includes all major epilepsy genes and P450 AED metabolism variants, plus 36 genes associated with the endocannabinoid system.

Initial results from 55 patients with 16-week response data revealed rare mutations in known epilepsy-associated genes in approximately 35% of the patients, and multiple variants of uncertain significance in another 24% of patients. None of the genes harboring these rare variants is preferentially associated with responders or non-responders. The analysis was, therefore, extended to look at common variants. Approximately 2/3 of the sequencing data generated from the epiSEEK panel is derived from noncoding DNA flanking exons, regions that are enriched for common variants. We utilized linear regression to test for association of approximately 6,000 such variants with CBD response using an additive model.

A number of variants showed an apparent association with CBD response. Although none of the hits reached statistical significance, we observed that 3 out of the 5 strongest hits had known associations with the endocannabinoid system (ECS). This is surprising, given that only ~8% of the genes in the panel have ECS associations. Restricting the analysis to just the subset of genes having a published association with the ECS revealed 4 variants approaching the required significance level (~1 x 10^{-4}) with a false discovery rate of <15%. Details of these findings, including the genes, variants and regulatory sequences affected will be presented. Additional work is underway to add ~150 additional patients to the study, which should enable us to confirm or rule out these potential associations.

NEUROPROTECTIVE EFFECT OF CANNABIDIOL IN A NEWBORN RAT MODEL OF ACUTE ISCHEMIC STROKE

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Background and Aims: Acute arterial ischemic stroke affects 4/1000 live newborns, leading to long-lasting sequelae. There is currently no treatment available for patients. Cannabidiol (CBD) has demonstrated neuroprotective effects in models of acute hypoxic-ischemic brain damage in newborn animals and in adult rodent stroke models. The aim of the present work was to determine the possible beneficial effect of CBD in a model of stroke in newborn rats.

Methods: A nylon filament was introduced through the left carotid artery which occluded the left middle cerebral artery (MCA) for 3 h in 7 day-old Wistar rats. The occluder was then removed and 15 min after reperfusion CBD (GW Pharmaceuticals, Cambridge UK) single dose 5mg/kg (SC, n=10) orvehicle (SV, n=11) were administrated i.p. Similarly manipulated but non-occludedrats servedas controls (SHM, n=12). One week (P7) and one month(P37) after the stroke, behavioral tests were conducted: P14: negative geotaxis (coordination), rope and grip test (strength); P37: beam crossing (coordination), cylinder rear test (CRT, hemiparesis) andadhesive removal (sensorimotor). MRI analysiswas performed to quantify the volume of damage (% brain volume) and that of a hyperintense signal corresponding to gliosis.

Results: Volume of infarct was not modified by CBD, but CBD reduced the volume of perilesionalgliosis. CBD administration improved neurobehavioral function regarding strength, hemiparesis, coordination and sensorimotor performance. Beneficial effects of CBD were apparent already one week after MCA occlusion and were sustained in the long term.

	Item	***SHM***	***SV***	***SC***
	Infarct area (MRI) (%)	-	13.1 (0.9)	12.2(1.6)
	Gliosis (MRI) (%)	-	11.7(0.8)	8.4 (1.3)#
P14	Negative geotaxis (sec)	6.5 (0.7)	11.1 (1.2)*	6.5 (1.5)#
	Rope test (sec)	32.5(4.8)	21.2 (3.1)*	22.2 (3.2)*
	Grip test (pts)	5.8 (0.4)	3.8 (0.4)*	5.5 (0.4)#
	Infarct Area (MRI) (%)	-	19.4 (2.1)	20.5 (2.9)
	Gliosis (MRI) (%)	-	13.5 (1.2)	7.4 (1.4)#
P37	Beam crossing (sec)	8.2 (1.5)	13.7 (2.7)*	7.8 (1.2)#
	CRT (%)	37.3 (2.4)	50.6 (3.4)*	38.3 (5.4)#
	Adhesive (sec)	32.4 (5.3)	64.1 (7.6)*	43.0 (4.5)#

Mean (SEM). Kruskall-Wallis (Dunne's test). (*): p<0.,05 vs. SHM; (#) p<0.05 vs. HV

Conclusions: In a model of severe stroke in newborn rats, post-insult CBD administration reduces peri-infarct brain damage, restoring neurobehavioral function in the short and long term.

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THE ACTIONS OF CANNABIDIOL AND 2-ARACHIDONYL GLYCEROL ON GABA-A RECEPTORS

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Introduction: Cannabidiol (CBD) is considered the major non-psychoactive component of cannabis. It has been found however, to possess anti-epileptic, anxiolytic and anti-psychotic properties in humans which may be suggestive of GABAergic involvement. While the actions of endogenous and synthetic cannabinoids on GABA-A receptors have previously been investigated, the actions of the phytocannabinoid CBD have not. Additionally, the current clinical trials of CBD for the treatment of pediatric epilepsy makes such elucidation compelling. As such, we present a study on the direct actions of CBD at specific GABA-A receptor combinations.

Objectives: We aimed to assess the direct actions of CBD and 2-arachidonyl glycerol (2AG) on GABA-A receptor subtypes, specifically 1) between α and β subunits of synaptic GABA-A receptors, i.e. α1-6β1-3γ2L; 2) on extra-synaptic δ subunit containing GABA-A receptors, i.e. α4β2δ; 3) on binary receptors devoid of a γ or δ subunit, i.e. α1β2; and 4) on a mutant previously shown to affect endocannabinoid activity, i.e. α2β2(V436T)γ2L.

Methods: Recombinant DNA techniques and *two-electrode voltage clamp electrophysiology* of receptors expressed in *Xenopus laevis* oocytes.

Results: CBD and 2AG enhance the actions of GABA significantly, with CBD generally acting more efficaciously. An α2 subunit selectivity was observed, with greater than fourfold modulation of the GABA EC_5 with 100μM CBD. At the physiologically relevant dose of 10μM CBD, the modulation of the GABA EC_5 upon α2β2γ2L receptors was approximately twice that of other α subunit receptor combinations (i.e. 241% vs 160-180%, $p < 0.05$, n=6). In regards to β subunit selectivity, modulatory activity was abolished when β1 was introduced. The β1 subunit homologous mutant β2(V436T) reduced the enhancement seen by CBD (334.2% vs 125.2%, $p < 0.05$) and 2AG (285.9% vs 85.0%, $p < 0.05$) as compared to wildtype β2 subunit combinations. Exploration of the extra-synaptic α4β2δ receptor revealed far greater enhancement of GABA responses by both CBD and 2AG as compared to synaptic γ2L containing subtypes (i.e. for CBD; 895% vs 190%, $p < 0.05$). Additionally, it was found that the γ2L subunit was not necessary for the activity of CBD and 2AG.

Discussion: Both CBD and 2AG directly potentiate GABA-A receptors, with CBD generally producing more pronounced effects than 2AG, the major central endocannabinoid. An α2 and β2/3 subunit selectivity profile was observed for synaptic receptor combinations without a requirement for γ2L, while greater modulatory effects were observed for extrasynaptic δ containing receptors. Additionally, the β1 subunit homologous mutant β2(V436T) substantially diminished the actions of both drugs. Together these results suggest a mutual mode of action and may account for some of the intriguing central effects seen with CBD.

CANNABIDIOL (CBD) AND PALMITOYLETHANOLAMIDE (PEA) DO NOT MODULATE CYTOKINE PRODUCTION IN CACO-2 INTESTINAL CELLS BUT DO IN HUMAN COLON EPITHELIUM

Daniel Couch, Elena Theophilidou, Jon Lund and Saoirse O'Sullivan

BACKGROUND: We have previously shown in a Caco-2 monolayer model that increased permeability in response to pro-inflammatory cytokines is prevented by application of N-palmitoylethanolamide (PEA) and cannabidiol (CBD). We aimed to determine if the mechanism underlying this observation is related to the inhibition of a local inflammatory response in both Caco-2 cell lines and ex vivo human appendix and if so, via which intracellular pathways.

METHODS: Caco-2 cells were cultured for 15 days until fully confluent in 12-well plates. Inflammatory conditions were simulated by adding to the media IFNγ (10ng/ml) for 18 hours, followed by TNFα (10 ng/ml) for 6 hours. PEA (10μM), CBD (10μM) or vehicle were added simultaneously with IFNγ. The effect of PEA or CBD on IL-8 and monocyte chemoattractant protein 1 (MCP)-1 secretion (with or without inflammatory cytokines) was measured via ELISA (R&D systems). Multiple cell-signalling phosphoprotein levels, such as NF-KB were measured in cell lysates using a Multiplex protocol (Merck Millipore Catalogue No. 48-680). Cytokine and cell signalling phosphoprotein concentrations were corrected for total protein content with bicinchoninic acid assay. In order to compare these results to human tissue we performed *ex vivo* experiments on human colonic tissue. Ethical approval was gained via the local Health Research Authority. Samples of normal human appendix (n=6) were obtained from elective bowel cancer resections after giving informed consent. Sections of mucosa (2x2mm) were incubated in culture media. After 24 hours media was changed and samples were treated with inflammatory cytokines in the presence or absence of cannabinoids as above for 24 hours.

RESULTS: In Caco-2 cell media, IL-8 levels rose 3-fold ($p<0.001$, one-way ANOVA) compared to control and MCP-1 levels rose three fold ($p<0.0001$) after the application of IFNγ and TNFα. The increase in cytokine secretion in Caco-2 cells was not affected by administration of PEA or CBD. Addition of PEA or CBD alone did not affect cytokine secretion compared to control. However, inflammation induced rises in the following cell-signalling phosphoproteins were prevented by addition of PEA; CREB ($p<0.05$), JNK ($p<0.001$), NF-KB ($p<0.05$), p38 ($p<0.05$), p70s6K (p0.05), STAT3 ($p<0.05$) and STAT5 ($p<0.05$). Within explant human tissue media IL-8 levels rose two fold ($p<0.01$) and MCP-1 levels three fold ($p<0.001$) under inflammatory conditions. In this case, the increase in the above cytokine levels caused by IFNγ & TNFα *was* prevented by PEA and CBD (MCP-1; $p<0.05$, IL-8; $p<0.01$).

CONCLUSIONS: PEA and CBD are inhibit the inflammatory response in ex vivo human colonic tissue, but not in cultured Caco-2 monolayers. However, despite not inhibiting a cytokine response in cultured cells, proinflammatory intracellular signalling molecules are down regulated by these drugs. We may therefore suggest that the effect of PEA and CBD on permeability is conducted through NF-KB and STAT down regulation.

ROLE OF CANNABINOIDS IN A MULTISTAGE MURINE MODEL OF PROSTATE CARCINOMA TO INVESTIGATE POTENTIAL CLINICAL APPLICATIONS

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Strong evidence indicates the existence of a neuroendocrine pathway in which endocannabinoids, cannabinoid receptors and TRP channels control prostate growth and maintain normal prostatic architecture and function. The key role exerted by the endocannabinoid system (ES) is evident especially during the progression of prostate cancer, when changes in the expression of ES components mostly occur. We have previously explored the capability of non-THC cannabinoids of inhibiting prostate carcinoma growth in vitro and in vivo (De Petrocellis et al., Br J Pharmacol. 2013). We found that cannabidiol (CBD), in particular, causes apoptosis via a combination of cannabinoid receptor-independent cellular and molecular mechanisms. Furthermore, a cannabis extract enriched in CBD potentiated the effects of bicalutamide and docetaxel against xenograft tumors. We thus decided to validate the efficacy of CBD (alone or in combination with cannabigerol [CBG]) in a mouse model that more accurately mimics the human disease. Since naturally occurring prostate carcinoma (PCa) is uncommon in the mouse and genetically engineered mouse models can accurately mimic all of these stages of human disease, we decided on the Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) as an ideal candidate for this research. The expression of both large and small SV40 tumor antigens (T/tag) in this mouse is regulated by the probasin (PB) promoter, specific for prostatic epithelial cells. For this reason, male TRAMP mice uniformly and spontaneously develop autochthonous (orthotopic) prostate tumors following the onset of puberty with a short latency period and with 100% frequency. The tumor tissue histologically and biochemically resembles the human disease with prostatic epithelial neoplasia (PIN) and cribriform structures appearing as early as 10-12 weeks (the model can be used to follow the progression/multi-stage development of disease within a 10 – 30 week period).

We investigated the antitumor properties of cannabinoids in 12-weeks old TRAMP mice treated (i.p) with CBD (alone and in combination with CBG) up to 18 weeks. The percentage of pathological adenomers was significantly reduced and the combination CBD+CBG appeared slightly more efficacious than the two compounds alone. The comparison between the histopathological analyses of 12-weeks old TRAMP mice (day 0) with 18-weeks old TRAMP mice (end-point) revealed that treatment with these compounds, and especially with the combination CBD+CBG, caused a reduction of tumor burden in terms of transformed areas and also slowed tumor progression from PIN towards well-differentiated cancer. The chronic treatment confirmed the safety of these compounds, except for a slight increase in white blood cell (WBC) number and ALT transaminase (mainly attributable to CBG). The general hematology and clinical biochemistry of C57BL/6 male mice (same strain as the TRAMP mice) was not compromised. We further investigated the possible synergisms between [1:1/CBD:CBG] mix and Enzalutamide (MDV3100), a standard chemo used for metastatic castration-resistant prostate cancer. Individually, the single treatments were efficacious in restoring the tumor progression to the initial phase. When given together, the [1:1/CBD:CBG] mix enhanced the efficacy of the standard chemotherapeutic (MDV3100) by reducing the percentage of pathological adenomers. In particular, we observed a synergistic effect in terms of transformed areas and slower tumor progression from PIN towards well-differentiated cancer. Finally, we set up an in vivo model of hormone refractory prostate with TRAMP mice by taking the animals under uninterrupted treatment with Enzalutamide from 12 weeks to 30 weeks, with a relapse in tumor progression around 22-25 weeks of age. The effect of cannabinoids on hormone refractory status is currently under investigation together with their cellular and molecular mechanism(s) of action in an *in vitro* model (TRAMP-C2 cells) derived from TRAMP tumors.

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TARGETING THE CB_2 RECEPTOR TO DELAY THE PROGRESSION OF THE PATHOLOGICAL PHENOTYPE IN TDP-43 TRANSGENIC MICE

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We recently described that CB_2 receptors became up-regulated, predominantly in reactive microglial cells, in the spinal cord of TDP-43 transgenic mice (Espejo-Porras et al., J Neuroimmune Pharmacol 10, 233-244, 2015), an experimental model of amyotrophic lateral sclerosis. In order to determine whether such up-regulatory response may be pharmacologically exploited, we have conducted a pharmacological study using the selective CB_2 receptor agonist HU-308, which was chronically administered to male TDP-43 transgenic mice from the age of 65 days after birth up to 90 days. We found that HU-308 improved the rotarod deficits observed in TDP-43 transgenic mice and this benefit is likely associated with a preservation of spinal motor neurons detected with Nissl staining in the ventral horn of the spinal cord at the lumbar level. HU-308 was also able to attenuate the reactive astrogliosis (labelled with GFAP immunostaining) observed in the affected areas within the spinal cord of TDP-43 transgenic mice. Paradoxically, this was not observed for the reactive microgliosis (labelled with Iba-1) detected in these mice, which was not altered (dorsal white matter) or even was elevated (ventral white matter) after the treatment of TDP-43 transgenic mice with HU-308. Such paradoxical response was also seen for a series of biochemical markers (e.g. IL-1β, TNF-α, and even CB_2 receptors) measured in spinal cord samples by qPCR, possibly suggesting a possible HU-308-induced shift of M1-type cells towards M2, which would be compatible with the benefits found in neurological parameters and spinal motor neuron survival. We also conducted a parallel pharmacological study using the non-selective cannabinoid agonist WIN55,212-2, alone or combined with antagonists for the two cannabinoid receptor types, with results that confirmed the relevance of CB_2 receptors but also a certain contribution of CB_1 receptors. In summary, our data support the relevance of CB_2 receptors as a potential neuroprotective target in TDP-43 transgenic mice, an experimental model of amyotrophic lateral sclerosis.

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CANNABINOID CB_2 RECEPTORS ARE PRESENT IN PERIPHERAL TISSUES OF THE MOUSE, BUT NOT IN THE CNS, UNDER NORMAL CONDITIONS

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Cannabinoid CB_2 receptors are candidate targets for the development of novel therapies, mainly in the context of inflammation and, specifically, in Alzheimer's Disease. We recently reported that the expression of cannabinoid CB_2 receptors is induced in microglial cells in areas of amyloid-triggered neuroinflammation. Further, this expression takes place in activated microglial cells only, located in close proximity to neuritic plaques. These data were obtained with our recently-developed transgenic mouse model, $CB_2^{eGFP/f/f}$. This mouse model was generated by inserting an eGFP reporter gene preceded by an IRES sequence in the 3' UTR of the *Cb2* mouse gene. This approach results in the expression of the reporter gene under the control of the endogenous mouse *Cb2* promoter and transcript from the same bicistronic mRNA as the CB_2 protein. In addition, the whole exon 3, including the 3' UTR and the knocked-in reporter was flanked by *loxP* sites, allowing the conditional inactivation of the *Cb2* gene. The mouse model ($CB_2^{eGFP/f/f}$) was generated by homologous recombination in embryonic stem cells, in the C57BL/6J genetic background. In this context, we analyzed the pattern of expression of CB_2-controlled eGFP expression in different organs and tissues, including brain, spinal cord, spleen, liver, lung, kidney, bladder, testicle, heart, thymus, etc. We found that GFP staining was most frequent in B- and T-lymphocytes, as well as in endothelial cells and resident macrophages. Importantly, no eGFP signal was observed in any region of the brain or the spinal cord. These data confirm previous data in the literature indicating that cannabinoid CB_2 receptors are restricted to peripheral cells, under physiological conditions.

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IDENTIFICATION OF FUNCTIONAL CANNABINOID CB2 RECEPTORS IN HIPPOCAMPAL PRINCIPAL NEURONS IN MICE

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We have recently reported that cannabinoid CB2 receptors are expressed in midbrain (VTA) dopamine neurons and functionally modulate dopamine neuronal activity and dopamine-related behavior (Xi et al., Nat Neurosci, 2011; Zhang et al., PNAS, 2014; Zhang et al., Neuropsychopharmacology, 2015; Zhang et al., Addiction Biology, 2016). This finding raises a question on whether functional CB2 receptors are also expressed in neurons in other brain regions. In the present study, we used multiple neural imaging, pharmacology, electrophysiolgy, and transgenic approaches to explore CB2 receptor expression and function in hippocampus. We found that CB2 gene (mRNA) is detected in the hippocampus, particularly in CA2 and CA3 regions) by classical *in situ* hybridization (ISH) assays. This CB2 mRNA-staining is co-localized with NeuN- (a neuronal marker) or vGluT2- (a glutamatergic neuronal marker) immunostaining in hippocampal neurons, suggesting (glutamatergic) neuronal CB2 expression. Similar CB2 mRNA-staining was also seen in constitutive CB1-KO mice, but not in constitutive CB2-KO mice. To confirm this finding, we used a novel highly-sensitive RNAscope ISH technique to examine CB2 gene expression. We found that CB2 mRNA is also detectable in the hippocampus of WT mice, while the density is substantially reduced in neuron-specific CB2-KO mice (using synapsin-cre/floxed CB2R approach). We note that weak CB2 mRNA signaling was still detectable in hippocampal slices of neuronal CB2-KO mice, suggesting glial CB2 expression in hippocampus. To test this hypothesis, we used fluorescence-activated cell sorting (FACS) technique to sort hippocampal neurons and glial cells, and then quantitatively measured CB2 mRNA expression in both cell populations by qRT-PCR. We detected significant CB2 mRNA expression in both hippocampal neurons (NeuN-positive) and glial cells (NeuN-negative) in WT mice. In consistent with the above finding by RNAscope, neuronal CB2-KO mice displayed a 70% reduction in CB2 mRNA expression in NeuN-positive neurons compared to WT mice. Finally, we used patch-clamp electrophysiological methods to record neuronal firing in hippocampal slices. We found that bath-applied 2-AG (10 μM), WIN55212-2 (1 μM), or HU308 (1 μM) produced significant membrane hyperpolarization in WT mice, but not in constitutive CB2-KO mice or neuronal CB2-KO mice (syn-CB2-KO). Taken together, all these findings provide convincing evidence demonstrating that cannabinoid CB2 receptors are expressed in hippocampal principal neurons and functionally modulate neuronal excitability.

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CANNABINOID TYPE 2 RECEPTORS MODULATE VISUAL RESPONSES OF NEURONS IN THE PRIMARY VISUAL CORTEX

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Cannabinoid receptors (CBRs) are present at every level of the visual system, from the retina to the visual cortex. However, their functional role in vision, especially at the cortical level, remains poorly understood. Recently, using immunohistochemistry, we found that CB2Rs are present in the primary visual cortex (V1). The aim of this study was to determine the functional impact of these receptors on neural properties of mouse V1. In a first step, intrinsic and voltage-sensitive dye optical imaging were performed on CB2R KO and WT mice. Retinotopic organization, contrast response function and spatial frequency selectivity were analyzed and compared. Subsequently, the impact of the administration of JWH 133 (CB2R agonist, 1-10 μM) and AM 630 (CB2R antagonist, 30 μM) on optical signals and single cell responses was investigated.

When compared to WT, optical responses to retinotopic stimulations in CB2R KO mice were reduced in amplitude. Similar results were obtained following bath application of AM 630. The latter also reduced the sensitivity of the contrast-response function. On the opposite, activating CB2Rs with JWH 133 caused a two-fold increase in responses to all visual stimuli, independent of their parameters. Furthermore, CB2 KO animals had a lowered contrast sensitivity function, an effect that could be mimicked by blocking CB2R with AM 630. Conversely, JWH 133 significantly decreased the attenuation of the contrast sensitivity curve seen for higher spatial frequencies. At the single cell level, JWH 133 increased the spontaneous activity and the visual responses in V1. Local field potential analysis indicate that JWH 133 increased the power spectrum of theta, alpha and beta bands during spontaneous activity, and delta and gamma bands under visual stimulation. Our results suggest that CB2Rs play an important modulatory role in neural processes taking place in V1, potentially through layer V neurons.

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CANNABINOID RECEPTOR 2 DEFICIENCY ALTERS NEURONAL LOSS AND IMPROVES COGNITIVE FUNCTION IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Alzheimer´s disease (AD) is one of the most common forms of dementia. In addition to Aβ depositions and intracellular tangles, AD is accompanied by an inflammatory response, which includes activation and recruitment of microglia to sites of Aβ-deposition and secretion of pro-inflammatory cytokines. The endocannabinoid system (ECS) is implicated in (patho-) physiological events in the CNS and changes in this system are related to many neurological diseases, including AD. Independent studies have shown that activation of the CB2 receptor reversed Aβ-induced memory impairments and neuroinflammation. However, the exact molecular mechanism of CB2 signalling in AD remains elusive.

In previous studies we demonstrated microglia from $CB2^{-/-}$ mice are less responsive to pro-inflammatory stimuli than WT microglia. Furthermore, APP/PS1x$CB2^{-/-}$ mice showed reduced numbers of microglia cells as well as infiltrating macrophages and lower expression levels of pro-inflammatory chemokines and cytokines. However, diminished neuroinflammation did not affect basic learning and memory abilities in APP/PS1x$CB2^{-/-}$ mice.

Here, we revealed in a more stringent paradigm of Morris Water Maze (MWM) an improved cognitive and learning phenotype in APP/PS1x$CB2^{-/-}$ mice in 3 different age groups. This was accompanied by a rescue of the neuronal loss compared to APP/PS1 mice at the age of 14 months. In addition we observed reduced plaque levels in the cortex of APP/PS1x$CB2^{-/-}$ mice that correlates with increased expression of enhanced Aβ degrading enzymes.

Taken together, we show that diminished neuroinflammation affected spatial learning and memory in aged APP/PS1*$CB2^{-/-}$ mice. This is probably based on a reduction of the neuronal loss in APP/PS1*$CB2^{-/-}$ mice compared to APP/PS1 mice. Our data suggest an important role for CB2 in AD-associated neuroinflammation and cognitive/neuronal impairment.

LOCAL ADMINISTRATION OF CB2 AGONISTS FOR PREVENTION AND TREATMENT OF OSTEOARTHRITIS (OA) – STUDIES IN MIA MODEL OF OA

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Osteoarthritis (OA) is the most common degenerative joint disease, characterized i.e. by gradual destruction of articular cartilage, exposure of innervated subchondral bone, which leads to pain during joint loading and chronic physical disability. Currently there are no drugs available that would modify the disease development, pharmacological therapy only alleviates the OA pain symptoms. Therefore there is an increasing need for basic research to focus on OA therapeutic discovery. Endocannabinoid system is widely described as involved in modulation of pain perception and changes in cannabinoid receptors' expression were observed in the OA-affected joints. CB2 receptor is expressed both in neuronal tissue as well as in cartilage that makes it interesting target in OA studies. Our aim was to determine the possible action of JWH-133, selective CB2 agonist, on cartilage regeneration during the development of OA using both *in vitro* (cultured chondrocytes) and *in vivo* (MIA model of OA) studies.

Primary human chondrocytes treated with MIA compound were used to investigate action of JWH-133, potent selective CB2 agonist, on chondrocytes viability (LDH assay), proliferation (BrdU assay) and migration (wound-healing assay). Intraarticular (i.a.) injection of monosodium iodoacetate (MIA, 1 mg) has been used to induce OA in rats. Pain symptoms were assessed by behavioral testing: pressure application measurements (PAM) and von Frey's test. JWH-133 was administered i.a. for 2 weeks starting on day 14 after model induction. Cannabinoid system components and inflammatory cytokines expression were measured on mRNA (qPCR) and protein (Western blot) levels both in chondrocytes *in vitro* and animal cartilage *in vivo*.

The protective effect of JWH-133 on chondrocyte culture treated with MIA has been shown. JWH-133 administration reduced LDH release (reduced toxicity), increased BrdU incorporation (proliferation increased) and increased chondrocytes' migration in the wound-healing assay. We proved, that JWH-133 action is dependent on the activation of CB2 receptor, by using AM-630 – CB2 antagonist to block JWH-133 effects. We observed elevated levels of CB2 expression after MIA treatment in cultured chondrocytes that was restored after JWH-133 administration. Elevation of CB2 receptor was observed also in cartilage during development of OA in rats. Repeated, i.a. administration of JWH-133 showed antinociceptive potential in PAM measurements, which suggest it as a novel drug to modify progression of OA.

Our studies demonstrate antinociceptive action of JWH-133 after local administration. Based on results from *in vitro* studies and poor availability of compound after i.a. treatment, we postulate that analgesic effect of JWH-133 is due to the regenerative effect on damaged joint's cartilage. Activation of CB2 receptors may be of benefit for chondrocytes' proliferation and may delay disease development. Our results propose innovative therapy, which can benefit in management of human OA, acting on disease development rather than symptoms.

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ARE HUMAN CIRCULATING MONOCYTES REGULATED BY CB2 RECEPTORS?

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It has now been demonstrated in various studies that primary human monocytes and monocyte-derived cells express CB2 receptors. Our own studies suggest that CB2 is expressed at low levels by classical $CD14^{+ve}$ monocytes and at slightly higher levels by the non-classical $CD16^{+ve}$ monocytes ($CD14^{low}$). In humans, the classical $CD14^{+ve}$ subset represents 80-85% of all monocytes. The aim of this study was to assess the effects of CB2 activation on the circulatory monocytes and assess whether CB2 influences key innate functions of monocytes. This is an important question because any future anti-inflammatory CB2 mimetic delivered systemically would not just target the monocytes involved in the inflammatory response, but would also have access to all circulatory monocytes.

Monocytes have a highly dynamic phenotype and are innately programmed to responding quickly to a range of danger signals that occur during infection, tissue damage and other inflammatory responses. Therefore, in order to investigate the circulatory monocyte phenotype, monocytes were isolated as quickly (and carefully) as possible to minimise any potential differentiation. Thus the time from phlebotomy to functional assays was typically less than 2.5 hours. We have previously presented at ICRS that various CB2 ligands (HU308, JWH015 and JWH133) had no effect on basal or cytokine induced monocyte secretions or phagocytic activity. Here we build on these observations looking at monocyte engagement with vascular endothelial cells measured using Electric Cell substrate Impedance Sensing (ECIS). Using ECIS Zθ we monitored the real-time interaction of freshly isolated monocytes with brain microvascular endothelial cells under basal conditions and also during pro-inflammatory conditions induced with IL-1β and TNFα. We have shown previously that IL-1β and TNFα induce pronounced pro-inflammatory activation of the brain endothelial cells, conducive to leukocyte migration (O'Carroll et al, 2015). The highly sensitive nature of ECIS reveals monocyte-endothelial engagement leading to increased barrier permeability, which is expected to occur during para-cellular migration. This is very apparent under the pro-inflammatory conditions, and to a lesser (but detectable) extent under basal conditions. Treatment of the monocytes with CB2 agonists (up to 10μM) at the time of addition to the endothelial cells had no influence on monocyte engagement, or endothelial barrier disruption. We did however observe a pronounced inhibitory effect of WIN55,212 on monocyte functions (chemokine secretion and monocyte adhesion), which later resulted in the death of the monocytes. These effects were only observed at supra-high concentrations (typically above 5μM) and occurred in a CB2-independent manner. We conclude that although circulatory monocytes express low levels of CB2 receptors, that CB2 does not appear to be majorly involved in regulating key innate functions of circulatory monocytes.

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References: S O'Carroll, DT Kho, V Nelson, L Rotimi, **E Scott Graham.** Differential regulation of the secretory profile and inflammatory activation of brain microvascular endothelial cells following pro-inflammatory activation by IL1β and TNFα **(2015)**. **Journal of Neuroinflammation. 12:131 DOI: 10.1186/s12974-015-0346-0**

HEPATOPROTECTIVE EFFECTS OF BETA-CARYOPHYLLENE: ROLE OF CANNABINOID 2 RECEPTORS

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Beta-caryophyllene (BCP) is a bicyclic sesquiterpene found in various essential oils from cloves, rosemary, black pepper and marijuana, and also an FDA approved food additive. Previous studies have implicated that BCP may exerts its anti-inflammatory effects in part by activating cannabinoid 2 receptors (CB2). In this study we aimed to test the potential of BCP treatment in clinically relevant murine models of liver injury (hepatic ischemia/reperfusion and alcoholic steatohepatitis).

Acute BCP treatment (10 mg/kg) ameliorated markers of hepatic ischemia/reperfusion injury, oxidative/nitrative stress, inflammatory response, and liver histopathological damage. Chronic treatment with BCP also decreased the hepatic inflammatory response, tissue injury, and beneficially influenced the alcohol-induced metabolic dysregulation in a model of alcoholic steatohepatitis. The protective effects of chronic BCP treatment against alcoholic steatohepatitis and hepatic I/R injury were largely attenuated in CB2 knockout mice, suggesting involvement of CB2.

Collectively, BCP as a dietary food additive has promising therapeutic potential in a multitude of diseases associated with hepatic inflammation, oxidative stress and metabolic dysregulation for preventing or attenuating liver injury.

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CANNABINOID CB_2 RECEPTOR LIGAND PROFILING REVEALS BIASED SIGNALING AND OFF-TARGET ACTIVITY: IMPLICATIONS FOR DRUG DISCOVERY

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Highly selective, well-characterized, *in vivo* active chemical probes are essential for target validation in drug discovery. The cannabinoid CB_2 receptor (CB_2R) is a promising therapeutic target for tissue injury and inflammatory diseases. Selectivity against the psychoactive cannabinoid CB_1 receptor (CB_1R) in the CNS is hereby essential. Although a multitude of chemical tools have been developed and widely used to target CB_2R, their selectivity, molecular mode of action and pharmacokinetic properties have been poorly characterized.

Therefore, we aimed to comprehensively profile the most widely used ligands for CB_2R in several independent academic and industry laboratories on receptor binding of human and mouse CB_2R, and on multiple signal transduction pathways (GTPγS, cAMP, β-AR, pERK and GIRK). We also determined their physico-chemical, *in vitro* ADME, pharmacokinetic parameters, and their cross-reactivity in the CEREP panel of 64 common off-targets. Common ligands of CB_1R, including Δ^9-THC and the endocannabinoids 2-AG and anandamide, were also tested. The top 3 candidate CB_2R agonists were further investigated *in vivo* to exclude potential interactions with CB_1R at higher doses.

We found marked differences in the ability of certain agonists to activate distinct signaling pathways and uncovered a rich poly-pharmacology of widely used ligands. Our results may also have important consequences on the interpretation of prior *in vivo* studies with some of these ligands. Our consensus is that HU910, HU308 and JWH133 are the recommended selective CB_2R agonists to investigate the role of CB_2R in biological and disease processes.

DESIGN, SYNTHESIS AND CHARACTERIZATON OF CANNABINOID RECEPTOR 2 SELECTIVE CHEMICAL PROBES

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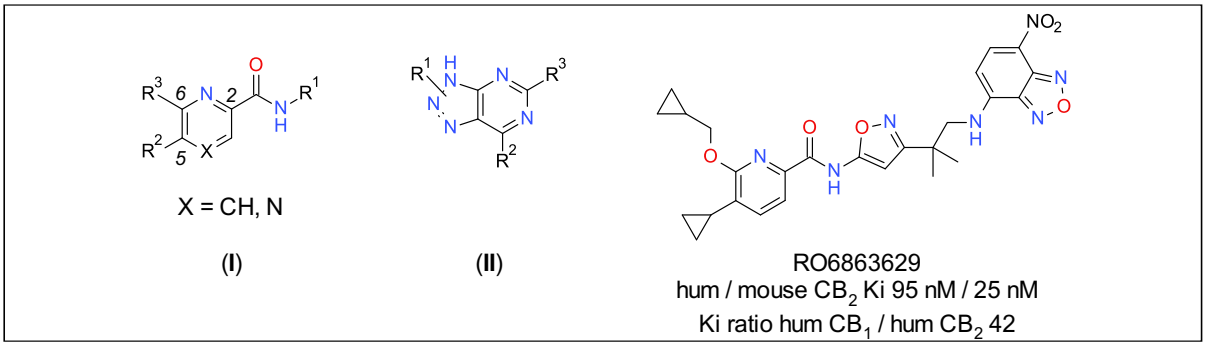
Despite many years of intense cannabinoid receptor 2 (CB_2) research, many fundamental questions regarding target location, exact mechanism of action and receptor trafficking are still unanswered. The inducible nature of the receptor and the lack of highly specific antibodies may partially explain these uncertainties. Novel improved small molecule derived CB_2 probes such as e.g. fluorescence- and radio-labels might help to answer some of the unknown questions.

2,5,6-Trisubstituted pyridines/pyrazines (**I**)[1] as well as triazolopyrimidines (**II**)[2] were found to be novel, highly potent and selective CB_2 ligands. Both series offer three independent exit vectors which have been used for exploring the introduction of labels as exempflied for the nitrobenzofurazan moiety in fluorescence label RO6863629.



In this communication we report details en route to novel CB_2 specific radio- and fluorescence-labeled probes. Additionally, we describe efforts towards covalently binding as well as Raman-active molecules. Further, we will show data on: i) the structure activity relationship (SAR) with regard to human CB_2 and CB_1 binding and functional assays; ii) mouse CB_2 binding and functional data; and iii) early ADME properties of advanced probes including e.g. solubility, membrane permeability and lipophilicity data.

[1] R. Slavik, U. Grether, A. Müller Herde, L. Gobbi, J. Fingerle, C. Ullmer, S. D. Krämer, R. Schibli, L. Mu, S. M. Ametamey, *J. Med. Chem.* **2015**, *58*, 4266-4277.

[2] M. Nettekoven, J.-M. Adam, S. Bendels, C. Bissantz, J. Fingerle, U. Grether, S. Grüner, W. Guba, A. Kimbara, G. Ottaviani, B. Püllmann, M. Rogers-Evans, S. Röver, B. Rothenhäusler, S. Schmitt, F. Schuler, T. Schulz-Gasch, C. Ullmer, *ChemMedChem* **2016**, *11*, 179-189.

THE CANNABINOID AGONIST WIN55212-2 MODULATES INTRACELLULAR CALCIUM VIA CB_1 RECEPTOR DEPENDENT AND INDEPENDENT MECHANISMS IN NEUROBLASTOMA CELLS

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CB_1 cannabinoid receptors (CB_1R) are found in abundance in the neurons where they can affect neuronal plasticity by modulating intracellular signal transduction mechanisms including cAMP (1) and Ca^{2+} (2,3) dynamics. The presence of the G-protein coupled calcium-sensing receptor (CaS) has been previously demonstrated in N18TG2 neuroblastoma cells (4) which endogenously express CB_1R. The aim of the present studies was to explore the mechanisms involved in CB_1R-mediated increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in N18TG2 neuroblastoma cells using the Fura-2 fluorescence method. Cells grown on coverslips were loaded with Fura-2 (5μM) in Krebs-Henseleit Buffer (KHB) containing 0.25mM Ca^{2+} for 15 min then perfused with drugs prepared in KHB containing either 0.25mM Ca^{2+} or 2.5mM Ca^{2+} using a perfusion valve control system. Cells were imaged using an upright Olympus BX51WI microscope equipped with a xenon arc lamp, a manual stage, and a cooled charge-couple device (CCD) camera. Average changes in fluorescence after excitation at 340nm (F_{340}) and 380 nm (F_{380}) were recorded continuously and $[Ca^{2+}]_i$ responses were determined using the ratiometric method (ratio between F_{340} and F_{380}). Results were expressed as the difference between baseline and peak response ($\Delta F_{340/380}$). N18TG2 cells exposed to 2.5mM extracellular (Ca_e^{2+}) responded with a 304% increase in ($[Ca^{2+}]_i$). Addition of WIN55212-2 (5μM) increased $[Ca^{2+}]_i$ in 0.25 and 2.5mM Ca_e^{2+} by 700 and 350% respectively ($p<0.05$). This increase was not replicated by treatment with CB_1R synthetic agonist CP55940 or CB_1R partial agonist meth-anandamide ($p>0.05$). The WIN55212-2 response in 0.25mM Ca_e^{2+} was blocked by co-incubation with the CB_1R antagonist SR141716, the CaS antagonist NPS2143, and the store operated calcium channels (SOCs) blocker MRS1845. The response to WIN55212-2 in 2.5mM Ca_e^{2+} was unaffected by either SR141716A or NPS2143, but was significantly reduced by co-incubation with MRS1845. We conclude that the cannabinoid agonist WIN55212-2 can modulate $[Ca^{2+}]_i$ in neuroblastoma cells via at least two different mechanisms. At low Ca_e^{2+} the effects of WIN55212-2 are CB_1R-dependent and involve CaS and SOCs. The second mechanism at high Ca_e^{2+} is CB_1R-independent and includes SOCs. Potential targeting of additional CB_1R-independent pathways involved in the WIN55212-2 effects and also the interactions between CaS- and cannabinoid-dependent pathways modulating $[Ca^{2+}]_i$ warrant further investigation.

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1. Howlett, A. C. (2005) Handb. Exp. Pharmacol. 53-79

2. Sugiura, T., Kodaka, T., Nakane, S., Miyashita, T., Kondo, S., Suhara, Y., Takayama, H., Waku, K., Seki, C., Baba, N., and Ishima, Y. (1999) J. Biol. Chem. 274, 2794-2801

3. Sugiura, T., Kodaka, T., Kondo, S., Nakane, S., Kondo, H., Waku, K., Ishima, Y., Watanabe, K., and Yamamoto, I. (1997) J. Biochem. (Tokyo) 122, 890-895

4. Sesay, J. S., Gyapong, R. N., Najafi, L. T., Kabler, S. L., Diz, D. I., Howlett, A. C., and Awumey, E. M. (2015) Neurochem. Int. 90, 142-151

PRESYNAPTIC CB_1 CANNABINOID RECEPTORS ARE CONSTITUTIVELY ACTIVE AT SYNAPSES BETWEEN INTERNEURONS AND PURKINJE CELLS IN THE CEREBELLAR CORTEX

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Introduction. CB_1 cannabinoid receptors are typically localized on axon terminals, and their activation leads to inhibition of neurotransmitter release. These presynaptic receptors are frequently activated by endocannabinoids synthesized in postsynaptic neurons. Analyzing the effects of CB_1 receptor antagonists in the cerebellar cortex, Ma et al. observed that three antagonists (rimonabant, AM251 and Δ^9-tetrahydrocannabivarin) stimulated the GABAergic synaptic transmission between interneurons and Purkinje cells in the cerebellar cortex (Brit J Pharmacol 154: 204–215, 2008). The aim of the present study was to clarify the mechanism of this stimulatory effect. We tested the hypotheses that (a) the CB_1 antagonists acted as inverse agonists and reversed the activity of constitutively active CB_1 receptors in the axon terminals, and (b) that CB_1 receptors were continuously activated by endocannabinoids.

Methods. 250 μm-thick slices containing the cerebellum were prepared from the brains of mice. Miniature GABAergic inhibitory postsynaptic currents (mIPSCs) were analyzed in superfused brain slices with patch-clamp electrophysiological techniques.

Results. The inverse CB_1 agonists rimonabant (10^{-6} M), ibipinabant (10^{-6} M) and taranabant (10^{-6} M) increased the frequency and the cumulative amplitude of mIPSCs. In parallel experiments, the three inverse agonists abolished the 2-arachidonoylglycerol-mediated suppression of mIPSCs elicited by depolarization of the postsynaptic Purkinje cells. The neutral CB_1 antagonists O-2050 (10^{-6} M) and ABD-459 (2×10^{-5} M) did not affect the frequency and the cumulative amplitude of mIPSCs. Importantly, O-2050 and ABD-459 abolished the 2-arachidonoylglycerol-mediated suppression of mIPSCs elicited by depolarization of the postsynaptic Purkinje cells. The stimulation of mIPSCs by rimonabant (10^{-6} M) persisted in brain slices pretreated with the diacylglycerol lipase inhibitor orlistat (10^{-5} M). It was verified in additional experiments that orlistat prevented 2-arachidonoylglycerol production elicited by depolarization or activation of mGluR1 receptors.

Conclusions. Two observations argue against the hypothesis that endocannabinoids continuously inhibited GABAergic synaptic transmission in the cerebellar cortex. a) The neutral antagonists did not affect the synaptic transmission. b) The stimulation by the inverse agonists persisted when 2-arachidonoylglycerol production was inhibited by orlistat. Therefore, the most likely explanation of the stimulation of the synaptic transmission between interneurons and Purkinje cells by the inverse agonists is that they reversed the constitutive activity of CB_1 receptors in the axon terminals of interneurons.

Acknowledgement: CB_1/CB_2 double-knockout mice were generated and kindly supplied to us by Andreas Zimmer (Bonn, Germany).

STRATIFICATION OF CANNABINOID 1 RECEPTOR (CB_1R) AGONIST EFFICACY: MANIPULATION OF CB_1R DENSITY THROUGH USE OF TRANSGENIC MICE REVEALS CONGRUENCE BETWEEN *IN VIVO* AND *IN VITRO* ASSAYS

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Synthetic cannabinoids (SCs) represent an emerging new class of abused drugs that widely differ from each other and from the phytocannabinoid Δ^9-tetrahydrocannabinol (THC) in cannabinoid-1 receptor (CB_1R) selectivity, potency, and efficacy. As these pharmacological parameters offer critical information to understand drug action, the present study investigated *in vivo* CB_1R efficacy and selectivity of THC, two well-characterized SCs (WIN55,212-2 and CP55,940), and three abused SCs possessing largely unknown pharmacology (JWH-073, CP47,497, and A-834,735-D) in CB_1 (+/+), (+/-), and (-/-) mice. Whereas (+/+) mice displayed dose-dependent effects in three CB_1R-sensitive assays (i.e., catalepsy, hypothermia and antinociception), these effects were essentially eliminated in CB_1R (-/-) mice. CB_1 (+/-) mice, which express 50% CB_1Rs as (+/+) mice, showed reduced hypothermic and antinociceptive potencies of all drugs and reduced E_{max} values for THC, CP47,497, and JWH-073. An *in vivo* metric of relative efficacy (i.e., potency ratios between (+/+) and (+/-) mice) strongly correlated with *in vitro* efficacy assessed by agonist-stimulated [^{35}S]GTPγS binding in spinal cord tissue (r=0.95 and 0.84 for antinociception and hypothermia, respectively). Conversely, CB_1 (+/+) and (+/-) mice displayed similar catalepsy dose-response relationships for each respective cannabinoid. This pattern of findings suggests that low efficacy CB_1R agonists show substantial E_{max} and potency reductions in pharmacological measures mediated by low receptor reserve compared with an effect mediated by high receptor reserve. Thus, evaluating antinociception and hypothermia in CB_1R transgenic mice offers a useful *in vivo* approach to determine CB_1R selectivity, potency, and efficacy of emerging SCs, which shows strong congruence with *in vitro* efficacy.

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PHARMACOLOGY OF CB_2 RECEPTOR-SELECTIVE AGONISTS AT GPR55

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GPR55 is coupled to $G\alpha_{12/13}$ and propagate the signal via multiple intracellular pathways. The AlphaScreen® SureFire® assay for MAP kinase has been used to investigate the pharmacology of cannabinoids at GPR55, a receptor for the endogenous lipid L-α-lysophosphatidylinositol (LPI). We have previously investigated the actions of arylpyrazole CB_1 receptor antagonists and a selection of phytocannabinoids at GPR55-mediated ERK1/2 phosphorylation (Anavi-Goffer et al., 2012). Here we have focused our study around the activity at GPR55 of cannabinoid ligands with known CB_2 receptor-selectivity. GPR55-mediated MAP kinase signalling was evaluated in HEK293 cells expressing GPR55. Specifically, we have compared the actions of HU-308 with those of its (-)-enantiomer (HU-433), and that of HU-910 with its carboxylic analogue (HU-914). We have found that LPI-induced ERK1/2 phosphorylation is inhibited by HU-433 and HU-914 but not with HU-308. Our study further directs the development of selective ligands to GPR55 and has identified novel ligands which modulate both the CB_2 receptor and GPR55 activity.

THE GPR55 BINDING POCKET:
LIGAND COMMONALITIES AND REQUIREMENTS

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The research presented here details validation of the current GPR55R* (activated state) model using a combination of *in silico* experimentation, *in vitro* single point mutations and superposition of structures generated (for GPR55 SAR) from the original Burnham screen used to identify GPR55 selective ligands. The initial model was constructed using the 1.8 Å crystal structure of the hDOR as the template to which refining modifications were made to reflect any hDOR/GPR55 sequence differences. The present iteration of this receptor has several residues which, pointing into the interior of the binding pocket, were hypothesized to interact with GPR55 agonist ML184 (3-[4-(2,3-dimethylphenyl)piperazine-1-carbonyl]-N,N-dimethyl-4-pyrrolidin-1-ylbenzene sulfonamid, CID2440433, EC_{50} 263nM). GPR55 mutants, (Y3.32F/L, F6.55A/L, Q6.58M, C10A, H170F, C260A, Q7.36A/N, M3.36A, F6.48A, K2.60A, and E3.29A/L) designed to test select residue involvement in either binding or function, were created and then evaluated in HEK293 cells by measuring the serum response element (SRE) signal produced by the receptor upon exposure to ML184. The results of these assays indicate that K2.60 and E3.29 are crucial for ML184 signaling and that Y3.32 and H170 engage in hydrogen bonding and aromatic stacking with the ligand. The loss of function seen with the complimentary cysteine mutations indicates the presence of a disulfide bond that tethers the N-terminus of GPR55 to the top of the EC3 loop (near the top of TMH7) and we hypothesize that the functional perturbations seen with the F6.55A/L mutants are linked to a specific aromatic cascade that occurs upon receptor activation. The results from the final three mutations, Q7.36A/N, M3.36A, F6.48A, did not indicate a direct role for these residues in response to ML184.

As a demonstration of the viability of our model and to generate hypotheses for the next round of receptor mutations and selective GPR55 agonists, ten compounds were chosen from 4 different sources and superimposed, along with ML184, in the GPR55 active binding site. The sets of compounds used were chosen based on 1) their ability to selectively activate GPR55; 2) the availability of a partner analog that differed only in the placement of a single moiety (or atom) that rendered the second compound inert at the GPR55 receptor. The ligand based superposition was done manually and then confirmed by the automated docking program Glide (Schrodinger). The combined overall area occupied by the ligands evaluated within the receptor hints at a very specific shape and orientation of agonists in the binding pocket even though various core modifications (multi-cyclic/heterocyclic) are seen with each ligand pair.

Predicting the overall three dimensional structure of GPR55 and any specific residues it uses for ligand recognition will create a powerful and specific tool with which to understand and exploit this receptor as a therapeutic target.

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KEEPING UP WITH THE CHEMISTS: CONTINUING *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF THE PHARMACOLOGY OF SYNTHETIC CANNABINOIDS

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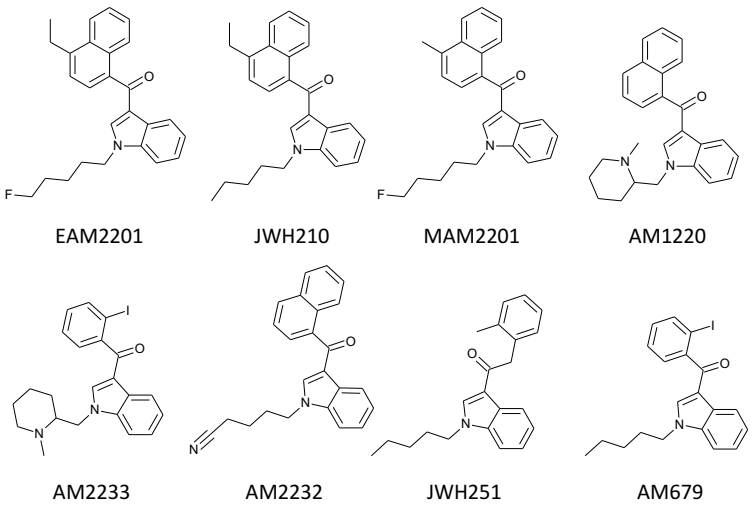
The manufacture and use of novel synthetic cannabinoids as intoxicants persists despite the obvious health risks associated with exposure to chemicals of unknown toxicity and legislation making their sale and possession illegal. The progression in the chemical diversity of synthetics may involve commonly employed strategies used in pharmaceutical drug discovery and development but fails to include the nonclinical and clinical toxicity screening required to ensure their safety for human use. In fact, compounds containing structural elements that are known to be hazardous to health are being synthesized, and adverse effects and death continue to be associated with their use. To classify and ban these novel compounds and protect public health, the US Drug Enforcement Administration (DEA) often requires information about the ability of a new chemical entity to bind to and stimulate cannabinoid receptors. These in vitro properties of chemicals are highly correlated with potencies in producing characteristic cannabimimetic in vivo effects (Compton et al., 1993; Wiley et al., 1998), including suppression of locomotor activity, antinociception, hypothermia, and catalepsy in mice (Martin et al., 1991), and Δ^9-tetrahydrocannabinol-like (Δ^9-THC) discriminative stimulus effects in rats and mice (Barrett et al., 1995; Vann et al., 2009). In this study, eight compounds provided by the DEA (shown below) were evaluated in in vitro and in vivo assays of cannabimimetic activity to facilitate their scheduling as controlled substances. All of them bound to the hCB1 receptor with nanomolar affinity, activated G-proteins in a manner similar to that exhibited by CP55940, and produced Δ^9-tetrahydrocannabinol-like (Δ^9-THC) discriminative stimulus effects in mice. This research was funded by RTI International Internal Research and Development Funds, the Drug Enforcement Administration, and the National Institute on Drug Abuse R01DA00372.



AGE-RELATED DECREASE IN ENDOCANNABINOID SIGNALLING CONTRIBUTES TO IMPAIRED PROTEOSTASIS

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Impaired proteostasis is one of the critical hallmarks of aging, and so is the decreased activity of autophagic-lysosomal system. Since cannabinoids have a major impact on autophagy, we asked whether endocannabinoid signalling might contribute to changes in autophagy and lysosomal degradation during aging.

We first showed that lack of CB1 receptors lead to increased lipofuscin accumulation and reduced levels of cathepsin D in the hippocampus of old mice. We also found altered levels of autophagic markers, as well as increased autophagic flux in CB1 knockout cells, indicating compensatory upregulation of autophagy due to CB1 receptor deletion. Next, we assessed the levels of 2 endogenous cannabinoids, 2-arachidonoyl glycerol (2-AG) and anandamide (AEA), in the hippocampus of young and old mice, and found an age-related decrease in 2-AG but not AEA levels. In order to identify the cause for 2-AG decrease, we investigated relevant biosynthetic and degradation enzymes in the hippocampus and found a selective decrease in diacylglycerol lipase (DAGL) α levels, as well as elevated monoacylglycerol lipase (MAGL) activity in aged animals. Finally, we compared the basal and CP 55,940-induced ^{35}S-GTPγS binding capacity in hippocampal membranes from young and old mice. CP 55,940 concentration-dependently increased ^{35}S-GTPγS binding in both groups, but the maximum effect was higher in young mice, suggesting that the Gi-protein coupling of CB1 receptors declined during aging. Thus, the decreased CB1-receptor coupling and the diminished levels of 2-AG synergistically lead to a decrease endocannabinoid signalling, which may in turn contribute to the impairment of proteostasis during aging.

Our results underline the importance of endocannabinoid signalling in the aging brain and suggest that CB1 agonists can be used as therapeutic agents to normalize disturbed proteostasis.

THE ROLE OF ENDOCANNABINOID SUBSTANCES ON HUMAN CEREBROMICROVASCULAR ENDOTHELIAL CELLS FUNCTIONS

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Endocannabinoids have vasoactive and cytoprotective effects among other various biological activities. Human cerebromicrovascular endothelial cells (HBEC), the main constituent of the blood-brain barrier (BBB), have been shown to react to many endocannabinoids [i.e., anandamide, 2-arachidonoyl glycerol (2-AG)], mediated by CB1, CB2, and vanilloid 1 (TRPA1) receptors present in these cells. Previous reports indicate that these substances activate calcium uptake and most importantly they interact with one of most potent vasoconstrictive compounds, endothelin-1 (ET-1). In addition, they affect actin assembly by a direct effect as well as via interacting with ET-1. This report will describe the published and unpublished data concerning the effects of cannabinoids and/or cannabinoid-like substances on calcium and actin as functional markers associated with the vasoactive properties of HBEC. In addition, results will demonstrate that linoleoyl ethanolamide (LEA) has vasodilatory effects on brain pial microvessels, as investigated by intravital microscopy which has not been heretofore described.

The endocannabinoid 2-AG, and other related compounds [anandamide, methanandamide, N-(4-hydroxyphenyl-arachidonyl-ethanolamide)] dose-dependently stimulated Ca^{2+} influx in HBEC. The selective CB1 receptor antagonist (SR141716A), CB2 receptor antagonist (SR144528) and TRPV1 receptor antagonist (capsazepine) inhibited these responses. 2-Lino-G and 2-Palm-G ('ENTOURAGE' monoacylglycerols) augmented 2-AG-induced Ca^{2+} influxes which were inhibited by CB1 and CB2 antagonists and by the TRPV1 antagonist, capsazepine. These effects are mediated by PI_3, PKC and PKA signal transduction pathways as indicated by inhibitory effects upon treatment with their respective inhibitors. 2-Lino-G or 2-Palm-G inhibit intracellular Ca^{2+} induced by ET-1, Mas7, or IP_3; the reduction of ET-1 induced Ca^{2+} by 2-Lino-G or 2-Palm-G is partially prevented by CB1, CB2, and TRPV1 antagonists and completely inhibited by Ly294002 (PI3/Akt inhibitor). Endogenous lipid endocannabinoid like compounds ARA-S and LEA, activate MAPK, Akt, JNK and c-JUN phosphorylation; these responses were inhibited by CB1, CB2, and TRPV1 receptor antagonists. In addition, all of above mentioned pathways except Akt were inhibited by H1152, which is an inhibitor of Rho/ROCK kinase. In contrast, the phosphorylation of Akt by ARA-S and LEA was increased two-fold by H1152. It was demonstrated that LEA, by itself and in conjunction with ET-1, increased the microvascular pial vessel diameter.

These findings strongly suggest that the above mentioned endocannabinoids and endocannabinoid-like substances act as modulators involved in vasorelaxation and may play a role in BBB functions and in the endothelium-dependent regulation of microcirculation in the brain.

DISCLAIMER: The views here are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. Some of the authors are military service members or employees of the U.S. Government. This work was funded by USAMRMC work unit number 603115HP.3520.001.A1411. The study protocol was reviewed and approved by the WRAIR/NMRC Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research. This work was prepared as part of official duties. Title 17 U.S.C. §105 provides that 'Copyright protection under this title is not available for any work of the U.S. Government.' Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member of employee of the U.S. Government as part of that person's official duties.

CROSS-TALK BETWEEN INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1RA) AND THE BRAIN CANNABINOID SYSTEM IN NEUROGENESIS

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Neuroimmune networks and the brain endocannabinoid system contribute to the maintenance of neurogenesis. Moreover, the endocannabinoid system directs cell fate specification of neural stem cells (NSC) in the central nervous system (CNS). We have previously shown that the activation of cannabinoid receptors suppressed chronic inflammatory responses through the attenuation of pro-inflammatory mediators such as interleukin-1β (IL-1β) by increasing the expression of IL-1 receptor antagonist (IL-1RA), an endogenous antagonist for the actions of IL-1 in the CNS. Endogenous IL-1RA mediates the neuroprotective and anti-inflammatory actions of CBs in primary neurons and glia[1]. These effects appear to be mediated by both CB1 and CB2 receptors. CB-induced IL-RA release may negatively regulate IL-1 actions in the brain, via IL-1RA blocking the IL-1 receptor (IL-1RI), after inflammatory or excitotoxic insults. Interestingly, receptors for cannabinoids (CB1 and CB2 receptors) and interleukin-1 co-expressed in NSC[2]. It is tempting to speculate therefore that the neurogeneic, neuroprotective and anti-inflammatory actions of CBs depend in part on modification of the balance between proinflammatory and anti-inflammatory cytokines.

In order to further explore the effects of IL-1RA on endocannabinoid signalling in NSC the levels of the endocannabinoids 2-arachidonylglycerol (2-AG), 1-AG and anadamide (AEA) were detected using liquid chromatography-mass spectrometry (LC-MS) on a Waters Acquity H-Class UPLC coupled to TQSmicro triple quadrupole mass spectrometer following IL-1RA treatment. Treatment with IL-1RA caused marked increases in the levels of AEA (approximately three-fold) in NSC supernatants and (approximately two-fold) in NSC cellular extracts respectively, compared to the control group. Whereas the levels of 2-AG and 1-AG were similar to that obtained in the control group.

In this study we show for the first time that acute administration with IL-1RA significantly increases levels of AEA in NSC. Thus it may be hypothesised that IL-1RA increases proliferation by increasing the levels of AEA, which acts via CB1 or CB2 receptors. These results provide crucial new insights into the effects of IL-1RA in regulating NSC proliferation and the pathways involved, and highlight the therapeutic potential of their interplay with endocannabinoid signalling in brain repair.

[1]Molina-Holgado, F., Pinteaux, E., Moore, J.D., Molina-Holgado, E.,Guaza, C., Gibson, R.M. and Rothwell, N.J. (2003) Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. J. Neurosci. 23, 6470–6474

[2]García-Ovejero D, Arévalo-Martín Á, Navarro-Galve B, Pinteaux E, Molina-Holgado E, Molina-Holgado F. Neuroimmmune interactions of cannabinoids in neurogenesis: focus on interleukin-1β (IL-1β) signalling. Biochem Soc Trans. 2013, 41(6):1577-82.

NEUROPROTECTIVE POTENTIAL OF ENDOCANNABINOIDS IN MODELS OF NEURONAL DAMAGE INDUCED BY HIV-1 TAT PROTEIN

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In the era of combined antiretroviral therapy (cART), human immunodeficiency virus type 1 (HIV-1) is now considered a chronic disease that specifically targets the brain and causes HIV-associated neurocognitive disorders (HAND). Endocannabinoids elicit neuroprotective and anti-inflammatory actions in several central nervous system (CNS) disease models, but their effects in HAND are poorly understood. HIV-1 does not infect neurons, but produces viral toxins, such as transactivator of transcription (Tat), that have been shown to disrupt cellular calcium equilibrium resulting in excitotoxic conditions. The increase in intracellular calcium can give rise to synaptodendritic injuries and cell death, the former of these is highly correlated with HAND. To address this issue, we investigated the neuroprotective actions of the endocannabinoids N-arachidonoyl ethanolamine (anandamide/AEA) and 2-arachidonoyl-glycerol (2-AG) using a neuronal culture model. Specifically, we examined the neuroprotective actions of endocannabinoids on Tat excitotoxicity in primary prefrontal cortex neurons, and the mechanisms mediating this neuroprotection. Neurons were cultured and treated with or without HIV-1 Tat in the absence or presence of endocannabinoid ligands or inhibitors of endocannabinoid catabolic enzymes. Tat-induced excitotoxicity was measured by assessing intracellular calcium, synaptodendritic damage, cell excitability, and neuronal survival. The direct application of AEA and 2-AG reduced excitotoxic levels of intracellular calcium and promoted neuronal survival following Tat exposure. Upregulating endogenous AEA levels using PF3845, a highly selective and potent inhibitor of its major catabolic enzyme, fatty acid amide hydrolase (FAAH), also blunted Tat-induced intracellular calcium increase and neuronal death. However, MJN110, a selective inhibitor of monoacylglycerol lipase, the major enzyme responsible for degradation of 2-AG, did not elicit this protective effect. This may be due to insufficient 2-AG elevation induced by applied concentrations of MJN110 at a given pretreatment time, and requires further testing. The CB_1 receptor selective antagonist rimonabant abolished the neuroprotective effects of AEA, 2-AG and PF3845. This pattern of finding indicates that CB_1 receptors are required for the neuroptotective actions of endocannabinoids against Tat-induced neuronal damage. The results indicate that endocannabinoids AEA and 2-AG elicit neuroprotective actions that protect against Tat-induced excitotoxicity and promote neuronal survival. Furthermore, our studies suggest that endocannabinoid catabolic enzymes should be further examined as a promising target for treatment of neurodegenerative disorders associated with HIV/AIDS.

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CEREBRAL AND EXTRACEREBRAL EFFECTS OF COMBINING CANNABIDIOL AND HYPOTHERMIA AFTER HYPOXIA-ISCHEMIA IN NEWBORN PIGLETS

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Background and aim: Hypothermia is the gold standard for treating newborn infants after a hypoxic-ischemic (HI) insult. However, hypothermia does not provide benefit in approximately 40% of treated newborns. Cannabidiol (CBD) has demonstrated neuroprotective effects in animal models of neonatal HI encephalopathy (NHIE). We aimed to study whether the addition of CBD augments hypothermic neuroprotection.

Methods: 1 day-old piglets were studied for 48 h after a HI insult (carotid clamp and FiO_2 10% for 20 min). After HI, piglets were randomized to receive hypothermia (HH, n=4), CBD 1 mg/kg iv (GW Pharmaceuticals, Cambridge UK) at 0.5, 24 and 48 h post-HI (HC, n=5) or both (HHC, n=5). Haemodynamic (cardiac output –CO-, mean blood pressure –MBP- urine output, and systemic or cerebral regional tissue oxygen saturation –$srSO_2$ and $crSO_2$, respectively), respiratory (oxygenation index- OI- and alveolo-arterial gradient –$AaDO_2$) and cerebral (aEEG: basal and mean amplitude, background-scored from 5: normal, to 0: flat- and seizures) parameters were monitored throughout.

Results (mean(SEM)): At the end of the experiment basal aEEG amplitude was 21.9 (6.1), 52.5 (15.0) and 65.8 (10.1) % basal in HH, HC and HHC, respectively ($p<0.05$ for HH vs. HC and HHC); mean aEEG amplitude was 21.1(1.1), 47.3 (14.4) and 61.7 (10.4) % basal ($p<0.05$ for HH vs. HC and HHC). aEEG background was 1.7 (0.7), 2.8 (0.4) and 3.4 (0.4) ($p<0.05$ for HH vs. HC and HHC). Seizures were observed in the first day after HI at aEEG in 2/4 HH, 1/5 HC and 1/6 HHC. CBD, in particular when combined with hypothermia, modulated HI-induced increase of regional cerebral blood flow in the first hours after HI. CBD treatment was well tolerated, without side effects on haemodynamic or respiratory parameters.

Conclusions: Our preliminary results suggest that CBD administration after HI in newborn piglets is more effective than hypothermia in protecting brain function, with some additive effect when combining both treatments. CBD by itself tended to reduce the incidence of HI-induced seizures.

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NOVEL CB2 RECEPTOR AGONISTS: SYNTHESIS, MOLECULAR MODELING, *IN VITRO AND IN VIVO* NEUROPROTECTIVE PROPERTIES

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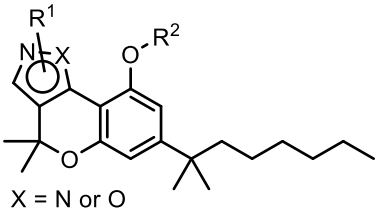
The cannabinoid receptor type 2 (CB_2R) offers an attractive opportunity for treating neuroinflammatory events in neurological disorders, while avoiding the adverse psychotropic effects related to the modulation of CB_1R in the brain. In this context, we proposed the development of novel CB_2R selective ligands based on the chromenopyrazole scaffold previously reported by us.[1]

Structural modifications on the pyrazole and the phenol substituents, as well as a replacement of the heterocycle moiety of the chromenopyrazole core led to the synthesis of novel derivatives (Figure 1). In the course of these studies, molecular modeling helped us to identify the key structural features necessary to fine-tune CB_2R affinity and selectivity. Docking studies using the active CB_1R* and CB_2R* models provided structural information related to ligand-receptor interactions, allowing us to optimize our scaffold and validate the experimental SAR. These studies confirmed that the phenolic hydroxyl lone pairs of electrons are crucial for binding affinity to CB_1R* through a K3.28(192) main interaction, whereas, *O*-alkylation creates steric hindrance of these lone pairs in this receptor type and prevents the ligand from binding at CB_1R. Modeling studies of the CB_2R*/ligand complexes, showed that S6.58(268) and/or K3.28(109) were crucial for ligand recognition depending on the chromenopyrazole derivative.



X = N or O
R^1= Alk or Ar
R^2= Alk or Ar

Figure 1

Radioligand binding experiments were performed to determine the affinity of the novel compounds for CB_1R and CB_2R. Functional activity of the best CB_2R ligands was tested by cAMP accumulation and GTPγS binding assays.

Among this series of compounds, we have identified a very potent, efficacious and selective CB_2R agonist [K_i (CB_1R): >40 μM; K_i (CB_2R): 12.8 nM; EC_{50}: 4.2 nM]. Interestingly, this lead compound has shown neuroprotective properties in M213 neurons. The neuroprotective capacity of this novel derivative was further confirmed in two *in vivo* models of Huntington´s disease and multiple sclerosis. Therefore, herein we have discovered a promising neuroprotective agent useful for those neurodegenerative pathologies in which the activation of CB_2R has a therapeutic value. [Support: SAF2012-40075-C02-02, SAF2015-68580-C2 and CANNAB-CM S2011/BMD-2308, NIH DA003934 (PHR)]

1. Cumella, J. *et al.* Chromenopyrazoles: non-psychoactive and selective CB1 cannabinoid agonists with peripheral antinociceptive properties. *ChemMedChem* **7,** 452–463 (2012).

ULTRA-LOW DOSES OF TETRHYDROCANNABINOL (THC) REVERSE AGE-DEPENDENT COGNITIVE DECLINE IN MICE

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We have previously shown that an ultra-low dose of delta-9 tetrahydrocannabinol (THC) protects the mice brain from a variety of brain insults (Ref. 1, 2, 3). A single injection of 0.002 mg/kg of THC (3-4 orders of magnitudes lower than doses that induce the conventional cannabinoid effects in mice) prevented the cognitive damage that was induced by either hypoxia, deep anesthesia, MDMA-toxicity, epileptic seizures or neuroinflamation. THC was applied either 1-3 days before or 1-7 days after the insult, thus providing a wide therapeutic time window. The protective effect of the single injection of ultra-low THC lasted for at least 7 weeks. The protective effect of THC was accompanied by long-lasting elevation in the levels of pERK, pCREB and BDNF in the hippocampus and frontal cortex of the THC-treated mice.

In the present study we tested whether the same ultra-low dose of THC reverses age-dependent cognitive decline in mice. Old (18-24 months) mice performed significantly worse than young (3-4 months) mice in a battery of cognitive assays, including Morris Water Maze, Y maze, Object Recognition and Place Recognition Tests. Old mice that had been injected once with 0.002 mg/kg THC performed significantly better than vehicle-treated old mice, and performed similar to naive young mice in all applied assays. The improvement in cognitive functioning lasted for at least 7 weeks following a single injection of ultra-low THC.

These findings suggest that extremely low doses of THC, that devoid any psychotropic effect and do not induce desensitization, may provide a safe and effective treatment for mild cognitive impairment in ageing humans.

References: (1) Behav. Brain Res. 220, 194 (2011); (2) Exp. Brain Res. 221, 437 (2012); (3) J. Neurosci. Res. 92, 1669 (2014)

CANNABINOID-1 RECEPTORS (CB1R) ON MYELOID CELLS DRIVE β-CELL LOSS IN TYPE-2 DIABETES

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Endocannabinoids acting via CB1R in peripheral tissues play a pathogenic role in metabolic diseases including type-2 diabetes (T2D). In previous studies using Zucker diabetic fatty (ZDF) rats, a model of T2D, we reported that activation of CB1R on proinflammatory macrophages contribute to the loss of β-cells in pancreatic islets and the resulting hyperglycemia (Nat Med 19:1132, 2013), whereas the associated diabetic nephropathy could be attributed to overactive CB1R in glomerular podocytes with no indication of macrophage infiltration in the kidney (PNAS 111:E5420, 2014). To find out whether macrophage CB1R are both necessary and sufficient for the development of T2D in ZDF rats, we generated CB1R knockout rats on a ZDF background (ZDF-Cnr1$^{-/-}$) using the Zn finger technology, and monitored their glycemic and renal functions up to the age of 30 weeks. At the age of 6 wks, both ZDF and ZDF-Cnr1$^{-/-}$ rats were normoglycemic and hyperinsulinemic, indicating insulin resistance, as supported by an elevated HOMA-IR index. During the next 6 weeks, ZDF rats developed extreme hyperglycemia due to progressive β-cell loss, and their islets became heavily infiltrated by CB1R-expressing M1 macrophages. In parallel, they also developed nephropathy, indicated by polyuria, albuminuria and decreased glomerular filtration rate. In contrast, ZDF-Cnr1$^{-/-}$ rats remained normoglycemic throughout the 30-week observation period, with maintained β-cell function and no significant macrophage infiltration, and also had normal renal function. This indicates that CB1R are both necessary and sufficient for the development of T2D and diabetic nephropathy, but doesn't indicate their cellular localization Adoptive transfer of bone marrow (BM) from ZDF donors to ZDF recipients did not alter their diabetic phenotype or nephropathy. In contrast, ZDF rats receiving BM from ZDF-Cnr1$^{-/-}$ donors remained normoglycemic and displayed minimal macrophage infiltration of islets, but did develop impaired renal functions. These findings demonstrate that CB1R on cells of myeloid origin drive β-cell loss and the development of T2D, but not the associated nephropathy.

PERIPHERALLY-RESTRICTED CANNABINOID-1 RECEPTOR BLOCKADE ATTENUATES DIABETIC NEPHROPATHY VIA THE REGULATION OF PROXIMAL TUBULE GLUT2

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Diabetic nephropathy (DN) is a worldwide, progressive kidney disease that affects both type 1 and 2 diabetic patients. Overactivity of the endocannabinoid/cannabinoid-1 receptor (CB_1R) system contributes to the development of DN, and chronic treatment with globally-acting CB_1R antagonists improves renal function in murine models of DN. However, their clinical use is halted because of centrally-mediated adverse neuropsychiatric effects. Recently, the development of peripherally-restricted CB_1R antagonists, such as JD5037, have revised the potential clinical use of CB_1R antagonism for the treatment of the metabolic syndrome. However, its therapeutic efficacy and molecular mechanism in type 1 DN has not elucidated yet.

Here, we report that in a streptozotocin-induced DN mouse model the increased structural and functional injuries in the kidney, along with enhanced fibrosis and inflammation were completely attenuated by a selective blockade of CB_1Rs in periphery. Daily chronic treatment (15 weeks) with JD5037 (3 mg/kg, po) was equieffective as its brain penetrant parent compound, SLV319 (3 mg/kg, po). The improved kidney function by JD5037 was associated with a significant reduction in the expression of the glucose transporter GLUT2 in the brash border membrane (BBM) of the renal proximal tubule cells (RPTCs). In primary human RPTCs, JD5037 blocked the high glucose- or CB_1R-induced translocation of GLUT2 to the BBM via the inhibition of Ca^{2+}/PKC-β1 signaling pathway.

These results demonstrate for the first time that RPTC-CB_1R regulates the hyperglycemia-induced renal dysfunction and the development of DN via affecting GLUT2 dynamics in these cells. Moreover, this study highlights the therapeutic relevance of blocking CB_1Rs in periphery for the management of diabetic kidney disease, and may further support the clinical development and testing of peripherally-restricted CB_1R antagonists in this pathology.

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PROHOMEOSTATIC EFFECTS OF CANNABIGEROL ON CHEMOTHERAPY-INDUCED METABOLIC DYSREGULATION

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The majority of cancer patients exhibit some degree of cachexia, a debilitating wasting condition characterised by anorexia and involuntary weight loss, particularly affecting skeletal muscle. A key hallmark of cachexia pathophysiology is chronic systemic inflammation, resulting in dysregulation of energy homeostasis and pathways involved in maintenance of muscle mass. While this cancer-induced cachexia is driven by both tumour-derived and host-response factors, more recent evidence has revealed that cytotoxic chemotherapy agents can themselves elicit a cachectic response, even in the absence of tumour, similarly characterised by systemic inflammation and tissue catabolism. Such chemotherapy-induced cachexia can compromise cancer treatment efficacy and have detrimental effects on patients' quality of life, morbidity and mortality. There is an urgent unmet clinical need for effective adjunct therapies to attenuate the dose- and compliance-limiting cachectic effects of chemotherapy.

A possible candidate for this indication is the non-psychoactive phytocannabinoid cannabigerol (CBG), which has shown anti-inflammatory and anti-tumour properties *in vitro* and *in vivo*. We have previously demonstrated that acute administration of CBG to healthy, pre-satiated rats stimulates multiple aspects of feeding behaviour, without detrimental motoric side effects. Previously we presented preliminary data showing CBG efficacy in a rodent model of chemotherapy-induced cachexia, whereby CBG significantly attenuated the anorexia, weight loss and muscle atrophy induced by the chemotherapy agent cisplatin. Using post-mortem plasma, hypothalamus and muscle samples from these animals, we have recently sought to characterise the effects of cisplatin and CBG on central and systemic levels of endocannabinoids and related lipid species, systemic metabolic phenotype and anabolic and catabolic pathways in muscle. Using a HPLC/MS/MS targeted lipidomics platform, we observed extensive differential cisplatin- and CBG-induced changes in plasma levels of *N*-acyl ethanolamines (including anandamide), *N*-acyl glycines and free fatty acid precursors, but not in 2-AG or other 2-acyl-*sn*-glycerols. In hypothalamic samples, both cisplatin and CBG predominantly modulated levels of *N*-acyl ethanolamines and *N*-acyl GABAs. ^{1}H-NMR spectroscopy based untargeted metabonomic analysis revealed that cisplatin induced a wide-ranging aberrant metabolic phenotype, characterised by impaired glucose homeostasis, oxidative stress, nephrotoxicity and muscle catabolism. CBG effectively reversed this metabolic phenotype to one resembling that of control animals. Gene and protein expression analyses in muscle tissue confirmed previously reported atrophic effects of cisplatin on anabolic and catabolic signalling, and demonstrated protective effects of CBG on autophagy and protein synthesis pathways.

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REGULATION OF TYPE-1 CANNABINOID RECEPTOR (*CNR1*) GENE IN OBESITY

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Obesity is an urgent public health problem, potentially affecting emotional and physical health, with a relevant mortality rate and a high burden of disease for our societies. Environment and heritability factors certainly contribute to individual vulnerability, although the mechanisms at the basis of the interaction between these two factors in the development of obesity are still largely unexplored. Endocannabinoid signaling through the type-1 cannabinoid receptor (CB_1R) plays a key role in energy balance regulation, and its physiological functions are influenced by the diet [1].

Here, we used the Diet-induced obesity (DIO) rat model to analyze the differences in the epigenetic regulation of CB_1R gene (*CNR1*) expression between rats placed on a high-fat diet (HFD) becoming obese, compared to their diet-resistant (DR) counterparts [2]. Moreover, in a subset of obese human subjects and controls, we assessed DNA methylation level at *CNR1* promoter, and also genotyped two single nucleotide polymorphisms (SNPs) within this gene (rs806368 and rs6454674) that were previously associated with obesity [3].

Male Sprague Dawley rats were fed with HFD for up to 21 weeks. After 5 weeks of diet, when the average body weight of DIO rats began to be significantly higher compared to DR rats, *CNR1* mRNA was significantly reduced in the hypothalamus of DR respect to DIO animals, whereas no changes were observed in rats sacrificed after 21 weeks. Epigenetic studies showed a selective and consistent significant increase in DNA methylation at *CNR1* gene promoter in DR respect to DIO rats. Alterations in DNA methylation levels were also observed in early stage of obesity onset in human subjects, where we also observed the significant association of rs6454674 SNP with obesity.

Overall, we identified time-dependent epigenetic regulation of *CNR1* in DIO rats as well as in obese human subjects. Changes have been observed at the earliest time-point in the animal model and at the shortest obesity onset in human subjects. We can thus hypothesize that it is crucial to identify epigenetic alterations in gene transcription at the beginning of obesity development, tohelp to predict disease trajectories and choose the most effective therapy.

References

[1] Matias, I., Di Marzo, V., 2007. Endocannabinoids and the control of energy balance. Trends Endocrinol Metab. 18(1), 27-37.

[2] Sclafani, A., Springer, D., 1976. Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol Behav 17(3), 461-471.

[3] Benzinou, M., Chèvre, J.C., Ward, K.J., Lecoeur, C., Dina, C., Lobbens, S., Durand, E., Delplanque, J., Horber, F.F., Heude, B., Balkau, B., Borch-Johnsen, K., Jørgensen, T., Hansen, T., Pedersen, O., Meyre, D., Froguel, P., 2008. Endocannabinoid receptor 1 gene variations increase risk for obesity and modulate body mass index in European populations. Hum Mol Genet 17(13), 1916-21.

ROLE OF THE ENDOCANNABINOID SYSTEM IN PRADER-WILLI SYNDROME

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Extreme obesity is a core phenotypic feature of Prader-Willi syndrome (PWS). Among the numerous metabolic regulators, the endocannabinoid (eCB) system is critically involved in the control of feeding, body weight, and metabolism, and globally-acting cannabinoid-1 receptor (CB_1R) blockade reverses obesity in both animals and humans. However, due to common neuropsychiatric side effects, global CB_1R blockers are not considered to be a feasible treatment for obesity in humans.

Using an established mouse model for adiposity in PWS, *Magel2*-null mice, we measured the expression of CB_1R as well as the endogenous levels of the main eCBs (anandamide (AEA) and 2-arachidonoylglycerol (2-AG)). We then determined the efficacy of the peripherally-restricted CB_1R antagonist, JD5037, in treating obesity in female and male *Magel2*-null mice fed high-fat diet. To assess the relevance of our findings to humans, we measured eCB levels in the serum of two different cohorts of individuals with PWS and healthy controls matched by age, sex and BMI.

Increased eCB 'tone', as reflected by changes in the expression of CB_1R and/or elevated levels of AEA in the circulation and adipose tissue were found in *Magel2*-null mice. Daily oral treatment of obese *Magel2*-null mice and their controls with JD5037 (3 mg/kg/d for 28 days) resulted in significant and comparable reductions in body weight, food intake and metabolic parameters in both mouse strains. Human patients with PWS showed increased levels of 2-AG, but not AEA.

In conclusion, using a pre-clinical animal model for PWS and human data, our results document that dysregulation of the eCB/CB_1R system may contribute to obesity of *Magel2*-null mice and PWS patients. Our findings with JD5037 in *Magel2*-null mice may provide rationale for future clinical testing of peripherally-restricted CB_1R antagonists (which avoid the unwanted risks of psychiatric side-effects) in the treatment of obesity in PWS.

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ENDOCANNABINOIDS AND RELATED COMPOUNDS IN PERIPHERAL PLASMA OF HUMANS WITH ABDOMINAL OBESITY; A RANDOMIZED CONTROLLED TRIAL COMPARING DIFFERENT LOW-CALORIE DIETS

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Context and Objective: Obesity and obesity-associated metabolic changes are linked to a dysregulation of the Endocannabinoid system (ECS). Levels of endocannabinoids (ECs) and endocannabinoid (EC)-like compounds in obese people might be influenced by diet and/or weight loss. The objective of this study was to compare the effects of two calorie-restricted diets differing in their nutrient composition on EC(s) levels in peripheral plasma from subjects with abdominal obesity.

Study Design and Method: This study was a randomised parallel trial in which 100 men and women (aged 40-70 yrs) with abdominal obesity (BMI: 31.3 ± 3.5 kg m^{-2}) were randomly assigned to either a standard western calorie restricted (CR) diet group (n=39), a targeted CR diet group (n=34) or a control group (n=27). The standard CR diet was based on a traditional, more western-style diet which included both saturated as well as unsaturated fats, while the targeted CR diet was enriched in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) based on their presumed positive health effects. The control group did not receive any dietary advice and participants were instructed to maintain their habitual diet. Plasma samples were collected before and after 12 weeks of intervention under fasting conditions. ECs and EC-like compounds, anandamide (AEA), 2-arachidonoylglycerol (2-AG), palmitoylethanolamide (PEA), oleoylethanolamide (OEA), stearoylethanolamide (SEA), docosahexaenoylglycine (DHAGly) and docosahexaenoylethanolamide (DHEA) were measured by liquid chromatography coupled to tandem mass spectrometry.

Results: Body weight was significantly decreased by 6.3 ± 3.9 kg and 8.4 ± 3.2 kg in western CR diet and targeted CR diet, respectively. Plasma endocannabinoid levels were not changed within the three groups. However, there was a trend of decreasing SEA levels (P=0.074) and increasing 2-AG levels (P=0.087) in the combined CR groups after weight loss. Changes in individual levels of AEA, OEA, PEA, SEA and DHEA were significantly correlated before and after calorie restriction, while 2-AG was inversely correlated with these ECs.

Discussion: Our data showed that plasma levels of ECs and EC-like compounds were not changed in a randomized parallel trial including 100 men and women. However, we observed a trend for decreasing levels of SEA and increasing levels of 2-AG in the combined CR groups. Moreover, different nutrient composition of CR diets did not affect peripheral ECs levels in obese subjects. Measurements of ECs in adipose tissue are currently ongoing and will yield additional data on the effects of calorie restriction and nutrient quality on EC tone in obesity.

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PROFILING THE ENDOCANNABINOID RESPONSE TO HEDONIC EATING

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Just thinking about palatable food can trigger a feeling of appetite even when a person is satiated. This leads to eating for pleasure, also called hedonic eating. There is evidence that the eCB system plays an important role in both the anticipation phase and the consumption phase of hedonic eating. The main objective of the current study was to further elucidate the role of the eCB system during anticipation of food intake and the consumption phase of hedonic eating by measuring eCBs prior to, during and after consumption of a palatable (tasty) and a neutral brownie. We expected to see an increase in 2-arachidonoyl glycerol (2-AG) levels after hedonic eating compared to non-hedonic eating. In addition, we expected levels of anandamide (AEA) and oleoyl ethanolamide (OEA) to be decreased after eating, irrespective of palatability of the brownie.

The study had a randomized, cross-over design. Participants first came to the test location for a screening, during which they rated liking of six different brownies that differed in salt content, but not in macronutrient composition, on a 100 point visual analogue scale (VAS). Only those participants who rated at least one brownie as palatable, VAS score $\geq$ 70, and at least one brownie as neutral, 40 $\leq$ VAS score $\leq$ 60, were included in the study and invited for the two test sessions that were two weeks apart.

The evening before each test session, participants consumed a standardized dinner. Both test sessions started with a standardized breakfast, after which an indwelling cannula was placed in a vein in participants' forearms to facilitate blood drawings. Two hours after participants finished breakfast, baseline blood samples were collected. Participants were then presented with 100 grams of brownie that they had either rated as palatable or as neutral during screening. To avoid anticipation effects, participants did not know how much they would like the brownie. Participants were instructed to look at and smell the brownie, but to not yet eat it. Blood samples were collected at two and four minutes after brownie presentation. Participants were then instructed to eat the brownie and additional blood samples were collected at one and three minutes after participants started eating. After five minutes, participants finished eating the brownie and another blood sample was collected. Additional blood samples were collected at 10, 15, 30, 45, 60, 90, and 120 minutes after participants started eating. The endocannabinoids AEA, 2-AG and dihomo-γ-linolenoyl ethanolamide (DLE), and the related *N*-acylethanolamines OEA, docosahexaenoyl ethanolamide (DHEA), palmitoyl ethanolamide (PEA) and stearoyl ethanolamide (SEA) were measured using LC-MS/MS.

So far, endocannabinoid levels have been analysed for eight male participants. These participants had an average age of 21.8 years (SD=3.3) and an average BMI of 22.6 kg/m^2 (SD=1.3). The preliminary analyses suggest a decrease in AEA at sixty and ninety minutes after eating of a brownie, irrespective of palatability; with average AEA plasma levels of 0.22 ng/ml (SD=0.08), 0.16 ng/ml (SD=0.06) and 0.15 ng/ml (SD=0.06) at T=0, T=60 and T=90 respectively. No differences in eCB levels between the palatable and neutral brownie were found for this subset of participants. This could indicate that differences in eCB levels after consumption of palatable versus neutral food are dependent on the anticipation of hedonic versus non-hedonic eating. Endocannabinoid levels will be measured for seventeen additional participants. These results will be presented at ICRS2016.

CANNABIS MAY HAVE EVOLVED IN THE NORTHEASTERN TIBETAN PLATEAU, BASED ON AN INTERDISCIPLINARY STUDY OF GENETICS, FOSSIL POLLEN, AND ECOLOGY

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INTRODUCTION: *Cannabis* and *Humulus* diverged 27.8 mya (million years ago), estimated from chloroplast DNA sequences (McPartland and Guy 2010). The fossil record cannot confirm this date—no convincing *Cannabis* print (rock) fossils exist. Microfossils (pollen) have been found, but *Cannabis* and *Humulus* pollen are nearly identical. Fossil pollen studies (FPSs) typically report these finds as *"C-H* pollen." Biogeographers beginning with de Candolle (1883) offer "Central Asia" as the place where *Cannabis* evolved. Wild *Cannabis* colonizes an ephemeral niche: flood-disturbed alluvial soil that incises undulating steppe. This kind of landscape was literally on the rise in Asia 27.8 mya—a time of tectonic uplift and the onset of monsoons. We analyzed FPSs from across Asia in a search for the oldest *Cannabis* fossil pollen.

METHODS: We used niche theory to separate *Cannabis* from *Humulus* pollen. *C-H* pollen was identified as *Cannabis* when it appeared in a pollen assemblage dominated by grassland pollen: *Poaceae, Artemisia, and Chenopodiaceae* (*PAC*). *C-H* pollen was identified as *Humulus* when it appeared in conjunction with pollen of trees climbed by wild hops: *Alnus, Salix,* and *Populus* (*ASP*). We analyzed 72 Asian FPSs with *C-H* pollen, dated with radiocarbon (^{14}C) or other methods.

RESULTS: FPSs dominated by *PAC* pollen rarely reported *C-H* pollen (*i.e., Cannabis*), likely because *Cannabis* was a sporadic species due to an ephemeral niche. *C-H*-with-*ASP* (*i.e., Humulus*) was more common. *C-H*-with-*PAC* first appeared in India 14 kya (thousand years ago), in Siberia 787 kya, and in southern China (Yúnnán) 3.0-2.6 mya. The oldest unequivocal *C-H*-with-*PAC* pollen dates to 19.6 mya near Gùyuán in the Loess Plateau. However, the Loess steppe was the final stage of an eastward expansion of grasslands that began earlier: Tectonic uplift and global cooling caused grasslands to replace forests in Dzüngaria *ca.* 24 mya, and in northeastern Tibetan *ca.* 34 mya, according to FPSs.

DISCUSSION: *PAC* pollen expanded through Dzüngaria and the northeastern Tibetan Plateau at the time of our calculated divergence date, 27.8 mya. We take this as the *Cannabis* center of origin. This location fits with two hypotheses regarding the evolution of cannabinoid biosynthesis: 1. Cannabinoids protect plants from ultraviolet light (UV_B) at higher altitudes, generated by the Tibetan uplift. 2. Cannabinoids deter vertebrate herbivores—the expansion of steppe led to the evolution of Central Asian animals that feed on *Cannabis* today, such as Ungulates (horses), Rodentia (some families of rats, mice, hamsters), Lagomorpha (rabbits, pikas), and Columbiformes (pigeons, doves).

A METABOLOMICS APPROACH TO THE SATIVA-INDICA DILEMMA

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The use of herbal cannabis for self-medication is rapidly increasing worldwide, despite availability of pharmaceutical drugs based on single cannabinoids such as THC or CBD. A major benefit associated with herbal cannabis is the synergy that may take place between the various cannabinoids and terpenes it contains. So far, over 100 cannabinoids and more than 120 different terpenes have been identified in various types of cannabis. With current chromatographic methods, these constituents can be accurately identified and quantified. Nevertheless, the current classification of cannabis varieties remains mainly dependent on THC and CBD content, in order to distinguish marijuana from hemp type plants for legal reasons.

Knowledge on the medicinal properties and uses of the cannabis plant does not only come from scientific studies, but increasingly so also from experienced medicinal users of cannabis in a growing number of countries. For example, the current attention for cannabis oil with high CBD content was pioneered by patients suffering from epilepsy and cancer. The non-scientific terminology used by such patients to describe their cannabis products and resulting therapeutic effects is often derived from the worldwide culture associated with recreational cannabis use.

As a result, there is an obvious disparity between the 'cultural' language used by patients, and the 'chemical' language needed by scientists to get a deeper understanding of cannabis effects by laboratory and clinical studies. The distinction between *Sativa* and *Indica* types of cannabis, and the differences between the effects associated with them, is a major example of this. Despite the widespread use of this terminology by self-medicating patients, scientific studies have yet to identify the markers that can sufficiently explain these differences. A metabolomics approach, combining detailed chemical composition data with cultural information available for a wide range of cannabis samples, would help to bridge the existing gap between scientists and patients. Such an approach could be helpful for decision making, for example when identifying which varieties of cannabis should be made legally available under national medicinal cannabis programs.

In our study we analyzed 460 cannabis accessions obtained from multiple sources in The Netherlands, including hemp and drug type cannabis. Based on the GC analysis of 44 major terpenes and cannabinoids present in these samples, followed by Principal Component Analysis (PCA) of the resulting data, we were able to identify the cannabis constituents that defined the samples into distinct groups for Indica and Sativa. This information was subsequently used to map the current chemical diversity available within the Dutch medicinal cannabis program, and to introduce a new variety missing from the existing product range. The study indicates the usefulness of a metabolomics approach for chemotaxonomic mapping of cannabis varieties.

ENDOGENOUS CANNABINOIDS LEVELS PREDICT THE POSITIVE SUBJECTIVE EFFECTS OF SMOKED CANNABIS

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Background: Chronic cannabis smokers have altered plasma levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) relative to light or non-cannabis users (Morgan et al., 2013). In non-cannabis smokers, administration of a large intravenous tetrahydrocannibinol (THC) dose initially (30 min post-THC administration) increased endocannabinoid levels, and then decreased levels below baseline for up to 48hrs (Thieme et al., 2013). The direct effect of cannabis administration on circulating endocannabinoid levels in cannabis smokers has not been characterized. The objective of this study was to assess plasma levels of endogenous cannabinoids and THC, and ratings of cannabis 'high' before and for 3h after cannabis administration to regular cannabis smokers. **Method:** Non-treatment-seeking cannabis smokers participated in a single outpatient session, beginning at 9AM to control for circadian fluctuations in endocannabinoids (Vaughn et al., 2010). Participants were instructed to not eat breakfast or use alcohol, cannabis or tobacco cigarettes the morning of a session (beginning at midnight), with compliance verified by a breathalyser and carbon monoxide levels; participants were also prohibited from other recent illicit drug use, verified by urine toxicology. A light breakfast was provided (bagel or cereal, juice, coffee), and then a nurse inserted a 20 gauge venous catheter (Quik-Cath®; Treavenol Laboratories, Deerfield, IL, USA) into the arm for repeated blood withdrawal. After collecting baseline subjective-effects ratings, cardiovascular measures and a plasma sample, participants smoked a single cannabis cigarette (5.6% THC), using a cued-smoking procedure. Once per minute, an investigator guided the participants to inhale for 5 sec and hold each puff in the lungs for 10 sec until 75% of the cigarette had been smoked. After completion of cannabis administration, blood samples (6 ml; t15, t30, t45, t60, t90, t120, 150, t180) and subjective effects ratings (t15, t30, t45, t60, t90, t120, 150, t180) were measured. Cigarette smokers were allowed a tobacco cigarette prior to catheter insertion and at t150 to prevent the onset of nicotine withdrawal symptoms. Participants passed a field sobriety test prior to discharge. **Results:** Data collection is ongoing. Twenty-three participants have completed the session, but plasma analysis is only available for a subset (3F,11M) to date. Participants reported smoking cannabis 4.7 ± 1.7 days/week, averaging 4.4 ± 4.9 cannabis cigarettes/day; 9/14 also smoked tobacco cigarettes (5.0 ± 3.9 cigarettes/day). Following cannabis administration, plasma THC and ratings of 'high' peaked at t15 and then linearly declined, returning to baseline by t180. Plasma AEA and 2-AG levels decreased following cannabis administration: Baseline levels of AEA (.34 ng/ml ± .05 ng/ml) declined after smoking and remained below baseline (.28 ± .03 ng/ml) at the end of the session (t180 post-smoking); 2-AG levels at baseline (1.23 ± .05 ng/ml) reached a nadir of 1.01 ± .05 ng/ml 30 minutes post-cannabis, and then returned to baseline level by session's end. There was a significant correlation between baseline AEA levels and peak ratings of 'high' ($r=.58$, $p<0.05$) following cannabis administration. **Discussion:** This is the first study to our knowledge to report that (1) smoking cannabis acutely decreases plasma endocannabinoid levels in current cannabis smokers; these findings are consistent with the observation that chronic cannabis smokers have lower plasma AEA levels than has been reported in studies with noncannabis smokers (e.g., Thieme, 2013; Hill et al., 2009), and (2) baseline AEA predicted cannabis intoxication. The explanation for this relationship is unknown; with further enrollment, we will determine if this correlation reflects tolerance, i.e., if heavier cannabis smokers have lower AEA and are thereby more tolerant to the intoxicating effects of a standard dose of cannabis.

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INVESTIGATING CANNABIS RELATED APPETITE CHANGES TO CHARACTERISE THE ROLE OF ENDOCANNABINOIDS IN HEDONIC HUNGER

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It is understood, predominantly from anecdotal evidence that cannabis intoxication leads to increased, often voracious, appetite and enhanced appreciation of food; a phenomenon colloquially referred to as the "munchies". This appears to reflect actions of the drug on brain systems involved in the normal regulation of appetite. In laboratory animals, cannabis-derived 'phytocannabinoids' (eg, THC) have been found to stimulate feeding, apparently through their ability to mimic the actions of endocannabinoids at brain cannabinoid receptors. Despite centuries of cannabis use, most of our knowledge about the drug's action on the physiological, psychological and behavioural aspects of appetite in people remains largely anecdotal in nature. There is little empirical evidence to substantiate users' claims, and human laboratory studies have focused principally on food intake measures rather than assessment of sensory and psychological factors affecting eating. As such, this study aims to address the shortfall in the scientific literature, as well as characterising the nature of the "munchies" in terms of alterations in motivation to eat, modulation of appetite, sensory reward and food preferences.

An online survey has been developed to directly target the cannabis user population. Survey content was based on the appetite literature as well as results from interviews with experienced cannabis users using a hybrid interview technique combining laddering questions (means-end chains) with discussion of food pictures taken by participants whilst experiencing the munchies.

Results from the interviews suggest that cannabis users experience an almost insatiable appetite. Liking of highly rewarding foods that are easy to prepare and provide instant satisfaction of hunger are reported. Sensory drivers of preference included sweet taste, as well as savoury and fatty/greasy foods and mouthfeel. A desire for flavour- and textural complexity was also reported. The online survey was completed by 760 participants. Data analysis is (at the time of writing) ongoing. First results on what food was most attractive following cannabis use indicate that the modal food preference was "anything/everything" (~40%), followed by "high sugar, sweet snack foods" (~13%), "high salt, savoury snack foods" (~12%), and "fatty savoury foods" (~10%). An exploratory Factor Analysis to identify key factors underpinning the "munchies" yielded 4 principal components: increased sensory appeal of foods (tastiness, smell), increased consumption/intake, mouthfeel (liking for temperature and textural contrasts) and desire to eat (wanting).

This study will help to elucidate the modulation of appetite by cannabis. Shifts in preferences for food types are studied as well as sensory alterations, and changes in reward and eating motivation. It is clear that the phenomenon of the "munchies" is more complex than previously understood. Hypotheses about the role of the endocannabinoids in the control of appetite can be derived from these results to guide future laboratory research.

OPTIMIZING CANNABIS VARIETIES FOR THE MANAGEMENT OF PTSD IN MILITARY VETERANS

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The use of Cannabis by military personnel and/or veterans suffering from PTSD raises questions about the medical efficacy of Cannabis over many of the current treatments. While studies involving PTSD and cannabis have often focused on "cannabis-abuse', there is evidence to support an aberrant endocannabinoid system in those suffering from PTSD which may benefit from supplemental phytocannabinoids. A few countries/states have begun to allow or even prescribe medical cannabis to Veterans suffering from PTSD. In Canada, Cannabis is financially covered by Veteran Affairs when prescribed as medicine by a physician. As such, there has been a drastic increase in medical Cannabis use over the past 3 years. Initial observations of PTSD patients using Cannabis indicated some positive results, yet there was a diverse range of experiences between veterans using different varieties of Cannabis with some even showing negative effects. As there was little to no guidance in this area, attempts were made to assess these differences by subjecting patients to blind trials that compared different Cannabis varieties to one another.

There was a clear distinction between most varieties and plants that ranked the highest for efficacy shared similar genetic background and chemotypic properties. Cannabis that exhibited these traits was typically of the "kush" family and were subsequently bred together in an attempt to increase efficacy. A very potent variety (<25% THC) was stabilized which contained a specific terpene profile that demonstrated positive results for treating PTSD. However, many users complained of the heavy sedation associated with this variety. As such, further breeding efforts aimed to reduce the sedation effect while retaining efficacy for PTSD. Crosses were made that reduced the overall content of the primary terpenoid myrcene while retaining similar chemotypic profiles to the parent. Backcrossed progeny were ranked for sedation effects, which correlated with the myrcene content of these plants. While myrcene has been reported to be primarily responsible for the sedation effect of Cannabis, other crosses were made to the original parent which increased myrcene yet at the same time reduced sedation. Continued efforts are underway to understand not only how Cannabis may benefit those suffering from PTSD, but which combinations of molecules in the plant are most effective.

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PESTICIDE CONTAMINATION OF CANNABIS IN THE LEGAL MARKET

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Introduction: Washington State legalized cannabis for medical use in 1998, where it remained largely unregulated. Legalization of recreational cannabis in the state was passed by ballot initiative in 2012, but despite recommendations, no testing for pesticide contamination was mandated. Subsequently, efforts are underway to unify the prior medical market with the legal one. No current method is available for certification of organic culture techniques, and there are no Environmental Protection Agency guidelines on acceptable pesticide levels for a smoked product. This study was prompted by informal testing demonstrated pesticide residues in 5-10% of tested cannabis inflorescence samples, and their known passage into cannabis smoke.

Methods: An initial test of 4 two-year old cannabis concentrates from legal storefronts were analyzed with 1 showing low-level presence of boscalid, diuron, piperonyl butoxide and mycobutanil. Subsequently, 26 distinct cannabis samples were purchased (24 concentrates, 2 cannabis inflorescence) from legal stores and passed via witnessed chain of command to a state certified legal licensed laboratory (Trace Analytics, Spokane, WA). Samples were homogenized, and extracted using a modified QuEChERS AOAC protocol. The supernatant was injected for LCMS-MS analysis. Detection was carried out using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer with a Shimadzu Prominence HPLC. Approximately 200 analytes were measured with over 500 MRM transitions per run.

Results: Out of the 26 samples, 22 tested positively for pesticides (84.6%). Many harbored multiple contaminants, attaining levels in the 10s of thousands of parts per billion (ppb), exceeding the upper limit of quantification. These included 45 distinct agents of every class: insecticides, miticides, fungicides, synergists and growth regulators, including organophosphates, organochlorides, etc. One single extract, a candidate for folding into the medical market in Washington, demonstrated lower levels of azoxystrobin, triflumizole, and piperonyl butoxide, with extreme levels of carbaryl, boscalid, bifenazate, pyraclostrobin, fenpyroximate and myclobutanil, with documented toxicities as carcinogens, neurotoxins, cholinesterase inhibitors, developmental and reproductive toxins, and endocrine disruptors.

Conclusions: The unregulated commerce in cannabis and lack of available organic certification have resulted in widespread abuse of the legal system. Cannabis concentrates currently account for 50% of legal sales in WA, and are also the basis for a burgeoning commerce in cannabis edibles. These products present a clear and present danger, particularly to young patients with epilepsy and other neurological conditions. Future regulation and monitoring with allowance for organic certification and employment of integrated pest management techniques without synthetic pesticides are required approaches to rectify this looming public health threat.

CHRONIC Δ^9-TETRAHYDROCANNABINOL TREATMENT DURING ADOLESCENCE IN RATS ENHANCES ACQUISITION OF COCAINE SELF-ADMINISTRATION AND ESCALATION OF DRUG INTAKE

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Cannabis continues to be the illegal drug most widely consumed by adolescents. There is epidemiological evidence suggesting that early marihuana consumption might act as a gateway to addiction disorders during adulthood. The aim of this study was to test this assumption and to further explore the possible addictive phenotype induced by such treatment on a cocaine self-administration protocol as well as some putative underlying psychological mechanisms such as alterations in pavlovian control over instrumental learning and inhibitory control over motor impulsivity. Male and female adolescent Wistar rats (PND28-42) were injected i.p. with THC (3mg/kg) or vehicle (ethanol:chremophor:saline) every other day and left undisturbed until they reached adulthood (PND90). Subsequently, they underwent cocaine self-administration (0,5 mg/kg) under different conditions to measure distinctive addiction behaviours: Acquisition of self-administration under fixed ratio 1 schedule (12 sessions), motivation for consumption under a progressive ratio schedule (6 sessions), compulsivity in a punished cocaine intake setup (1 session), escalation during extended access (10 sessions) and incubation of seeking using cue-induced reinstatements after different withdrawal periods (4 sessions). In an additional set of rats with the same adolescent treatment we studied Pavlovian to instrumental transfer (PIT) and motor impulsivity using a 2-choice serial reaction time task (2CSRTT).

THC-treated rats showed a facilitation of cocaine self-administration acquisition during the first seven sessions, but there were no clear differences during the progressive ratio phase. In addition, THC-exposed females exhibited higher cocaine intake during extended access and a different pattern of craving incubation. Preliminary analyses showed that THC-exposed females had worst instrumental extinction and impaired transfer on PIT but a mild reduction of motor impulsivity on the 2CSRTT. These results provide evidence supporting the Gateway Hypothesis of Drug Addiction and the existence of an addictive sex-dependent phenotype in rats with a chronic THC treatment during adolescence.

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TARGETING THE CB1 RECEPTOR FOR ANTI-DRUG ADDICTION THERAPEUTIC POTENTIAL: CB1R ANTAGONISTS/INVERSE AGONISTS, CB1R NEUTRAL ANTAGONISTS, OR CB1R NEGATIVE ALLOSTERIC MODULATORS (NAMS)?

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Much research over the past 20 years has shown that cannabinoid CB1 receptors (CB1Rs) are importantly involved in brain mechanisms underlying drug reward and drug addiction, including that produced by opioids, nicotine, alcohol, and psychostimulants such as cocaine and amphetamines. Accordingly, brain CB1Rs have been posited to be potential targets in medication development for the treatment of drug abuse and addiction. Indeed, preclinical animal studies have shown that CB1R orthosteric antagonists/inverse agonists show strong anti-addiction properties against a wide range of addictive drugs. However, further development of CB1R orthosteric antagonists/inverse agonists was severely impacted and – for all practical purposes – terminated in 2008 worldwide due to significant unwanted side-effects (anxiety, depression, suicidal ideation) in clinical trials and prescription use in Europe of the lead CB1R orthosteric antagonist/inverse agonist SR141716 (rimonabant; trade name: Acomplia©). It has therefore been proposed that neutral CB1R antagonists (lacking inverse agonist effects) or CB1R negative allosteric modulators (binding to transmembrane allosteric sites rather than extracellular orthosteric sites) may have therapeutic anti-addiction potential without unwanted effects. To explore this hypothesis, we evaluated the effects of these three types of CB1R ligand in animal models relating to drug addiction. We found that 1) the inverse CB1R agonist SR141716 significantly inhibited cocaine, heroin, or nicotine self-administration and cocaine-enhanced electrical brain-stimulation reward in rats; but SR141716 itself (at moderate to high doses) produced inhibition of electrical brain-stimulation reward, decreased nucleus accumbens dopamine (as measured by *in vivo* brain microdialysis), and conditioned place aversion – all of which are considered to be predictive of dysphoria at the human level; 2) the CB1R neutral antagonist AM4113 significantly inhibited nicotine or heroin self-administration; the CB1R neutral antagonist PIMSR1 significantly attenuated cocaine-enhanced brain-stimulation reward; both compounds by themselves had no effect on basal brain reward functions as assessed by electrical brain-stimulation reward; 3) the CB1R negative allosteric modulators (NAMs) GAT358 and GAT369 altered neither nicotine-enhanced brain-stimulation reward nor basal brain reward functions. Taken together, these findings suggest that neutral CB1R antagonists may be more promising than CB1R antagonists/inverse agonists or CB1R NAMs in medication development for treatment of drug abuse and addiction.

ADOLESCENT CANNABINOID EXPOSURE TRIGGERS A PERMANENT DEFICIT IN PRESYNAPTIC LONG-TERM PLASTICITY

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Cannabis abuse is considered to be the serious, if not the greatest known environmental risk for neuropsychiatric disorders. Individuals who are exposed to cannabis experience variety of psychoactive effects such as general alteration of conscious perception, euphoria, problems with social interactions, memory and learning, and occasionally anxiety and paranoia. Despite the importance of this knowledge for teenagers' well-being and their future mental health, the limited studies are incomplete, and the adolescent ontogeny of the eCB system along with its effects on network homeostasis remains elusive. To detect potential prefrontal maladaptations triggered by adolescent cannabinoid exposure, we tested multiple forms of presynaptic plasticity at the cortical layer (L)2/3 to L5 (L2/3/L5) glutamatergic synapse in the medial prefrontal cortex (mPFC) of adult female mice treated sub-chronically during adolescence with the cannabinoid agonist, WIN55,212-2 and investigated the effects of this treatment on presynaptic plasticity in the adult.

We show that mouse model of adolescent cannabis abuse shows deficits in an endocannabinoid-mediated signaling and neuroplasticity in adult prefrontal cortex, a brain region encompassing neural circuit for decision-making. Blockade of the primary gene product responsible for degrading the endogenous endocannabinoid, with the specific drug ameliorates these deficits. We also found that two types of long-term depression (LTD), LTD mediated by metabotropic glutamate receptors 2/3 (mGluR2/3) was deficient in adulthood in mice treated with WIN55,212-2. Over-activation of the CB1R during adolescence could therefore lead to permanent developmental changes in the expression of presynaptic LTD in the mPFC at excitatory synapses. In addition, we demonstrated that adolescent WIN55 exposure triggers cognitive deficiency in the novel object recognition test that is sensitive to decreased working memory, a cognitive behavior consistently impaired in schizophrenic patients. Our research indicates that abnormal interactions of neurodevelopment with the environment triggered by drugs of abuse during neonatal or adolescent periods may permanently impair brain function including the brain natural ability to alter and protect itself, i.e. endocannabinoid system (eCB)-dependent inherent neuroprotection of circuit integrity and neuroplasticity. The eCB system represents a major activity-dependent regulatory system in the central nervous system and has been implicated in multiple brain functions, including synaptic plasticity and the homeostatic regulation of network activity patterns. These observations could be linked to a maladaptation of the mPFC network during a critical period of cortical development and may underlie the alteration of gamma oscillations in adults after adolescent CB1R stimulation, as found in earlier studies (Raver et al., 2013; Sales- Carbonell et al., 2013; Skosnik et al., 2012).

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INTRACRANIAL SELF-STIMULATION RESPONSE TO INTRAVENOUS CANNABINOIDS AS A METHOD TO MODEL EXCESSIVE INTAKE

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Though an absolutely critical goal for the effective study of the abuse liability and addictive properties of cannabinoid drugs, stable self-administration models in rodents have been elusive in the scientific literature over the past 30+ years. Steve Goldberg demonstrated Δ^9-THC self-administration in squirrel monkeys 15 years ago (*Nat Neurosci.* 2000, **3**(11):1073-4), while the Fratta (*Psychopharmacology (Berl).* 2001, **156**(4):410-6) and Wiley (*Pharmacol Biochem Behav.* 2014, **118**:30-5) groups have reported on short-access stable self-administration of WIN55,212 in rats. However, expansion of the models towards models of dependence or the neurobiology of addiction have yet to be attempted, and THC self-administration in rodents remains seemingly unobtainable.

We have attempted to break down this problem, doing so in a way to examine the important parameters (dose, intermittence, timing, tolerance, vehicle) that may impact the overall outcome. After failing to achieve self-administration beyond minimal stable levels in both WIN55,212 and THC, intracranial self-stimulation (ICSS) is being used in combination with passive intravenous administration to determine optimal dosing and timelines for reducing ICSS thresholds, typically indicative of drug reward. Preliminary data is congruent with previous systemic ICSS work, and that of Goldberg's studies in monkeys, in that THC's rewarding doses fall in a narrow range (25-100 μg/kg) that quickly turns to aversion at higher dosing (200+ μg/kg). Furthermore, the persistence of these effects for hours after a single intravenous dose likely places a hard ceiling on the amount rats can effectively take without negative consequences. These data suggest shorter-acting synthetic drugs may be necessary to ultimately achieve a robust and predictable cannabinoid addiction model, and are the current focus of testing. ICSS, combined with bolus and timed-passive intravenous infusion, may provide a way of effectively measuring the optimal parameters to maintaining a stable rewarding state required to drive the initial stages of self-administration towards unstable excess.

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MAGL INHIBITION ATTENUATES Δ^9-THC SOMATIC WITHDRAWAL, BUT NOT ALTERED EMOTIONALITY-RELATED BEHAVIORS

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Cannabinoid withdrawal is commonly determined preclinically through evaluation of somatic signs, which typically include frequency of paw tremors and head twitches. Although these somatic measures are widely used in experimental animal studies, two common critiques of the validity of these models are (1) the absence of these symptoms during human withdrawal, which is characterized by drug craving, sleep disturbances, and altered emotional processing; and (2) the reliance of a CB_1 selective antagonist to precipitate withdrawal symptoms. Thus, behavioral assays that exploit emotionality-related measures of withdrawal may more closely mimic symptoms experienced in humans. In the present study, two models of anxiety-like behavior, marble burying test and light/dark box, and a model of depressive-like behavior, the tail suspension test, were used to evaluate withdrawal induced by 5.5 days of Δ^9-THC (10 or 50 mg/kg, s.c.) administration compared to vehicle-treated control animals. Withdrawal was precipitated using rimonabant (3 mg/kg, i.p.) in some instances, however, spontaneous withdrawal was also evaluated to address critiques associated with precipitated withdrawal. We hypothesized that Δ^9-THC withdrawal-induced increases and anxiety-like and depressive-like behaviors would be attenuated by pretreatment with a FAAH or MAGL inhibitor. Surprisingly, precipitated Δ^9-THC withdrawal significantly suppressed both marble burying and immobility in the tail suspension test. Neither effect was altered by pretreatment with the FAAH inhibitor PF-3845 (10 mg/kg, i.p.), the MAGL inhibitor JZL184 (8 or 40 mg/kg, i.p.), or the β-adrenergic receptor antagonist propranolol (10 mg/kg, i.p.). However, as published previously, JZL184 did significantly attenuate paw tremors and head twitches induced by precipitated Δ^9-THC withdrawal, indicating differential effects of MAGL inhibition on withdrawal behaviors. Of particular interest, spontaneous THC withdrawal also significantly altered marble burying behavior. The results of these experiments suggest that marble burying and tail suspension may be appropriate to include in test batteries of cannabinoid withdrawal with traditional tests of somatic withdrawal signs in order to increase the overall predictive validity of potential pharmacological treatments for cannabis dependence.

EARLY PHARMACOLOGICAL MODULATION OF THE ENDOCANNABINOID TONE COUNTERACTS THE LATE BEHAVIORAL AND MOLECULAR ALTERATIONS IN A RODENT DEVELOPMENTAL MODEL OF SCHIZOPHRENIA

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Epidemiological and clinical studies suggest that a neurodevelopmental dysfunction could be one of the main exploratory hypotheses of schizophrenia (SCZ), which symptoms lead to severe personal and social dysfunctions. A variety of animal and human studies found a dysregulation of the endocannabinoid system (both in term of cannabinoid receptors CB1 or CB2 and endocannabinoid ligands anandamide or 2-arachidonoylglycerol) in psychosis; thus, the pharmacological modulation of the endocannabinoid system could be a novel approach for treating SCZ. In the present study, we aimed to investigate 1) the potential effects of prenatal administration of the mitotoxin methylazoxymethanol acetate (MAM) on neurophenotypic presentations using a set of behavioral test battery, and 2) if the early pharmacological modulation of the endocannabinoid signaling could reverse the schizophrenia-like phenotype. At adulthood, prenatally MAM-exposed rats shown behavioral alterations such as social and cognitive impairment ($p<0.05$), which were correlated to changes in the endogenous cannabinoid signaling such as an increased of brain CB1 receptor expression ($p<0.05$). Interestingly, they were reversed by adolescent repeated treatment both with the non-psychotropic phytocannabinoid cannabidiol and with the CB1 antagonist AM251. These results suggest that early pharmacological modulation of the endocannabinoid signaling could be a novel potential therapeutic target to prevent the development of a SCZ-like phenotype at adulthood.

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INHIBITION OF ANANDAMIDE HYDROLYSIS REVERSES ANXIETY INDUCED BY SUSTAINED PERIPHERAL INFLAMMATION

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This study aimed to determine if chronic inflammation alters central endocannabinoid levels and if these changes related to inflammation-induced anxiety. Chronic inflammation of the colon was induced in adult male Sprague Dawley rats that were administered trinitrobenzene sulfonic acid (TNBS) intracolonically. Seven days after induction, rats were euthanized and corticolimbic brain regions were analyzed for endocannabinoids. The degree of colitis was assessed based on macroscopic tissue damage. Plasma corticosterone and cytokine levels were measured by ELISA. Anxiety behaviors were tested using an elevated plus maze in a separate cohort of animals.

Similar to what we have seen following chronic stress, sustained inflammation reduces anandamide in the amygdala (-30%), hippocampus (-14%) and medial prefrontal cortex (-30%), but increases 2-arachidonylglycerol in the hippocampus (16%) and medial prefrontal cortex (21%). The reductions in anandamide are correlated with the degree of macroscopic tissue damage, but this was not the case for the increases in 2-arachidonylglycerol, suggesting that reductions in anandamide could be proportionally related to the magnitude of systemic inflammation. Colitis led to an increase in plasma levels of corticosterone (54%) and the chemokine MCP-1 (22%), but not the cytokines IL-1β or IL-6. We observed an increase in anxiety behaviors following colitis (-50% open arm time), with no change in locomotor activity. This inflammation-induced anxiety was reversed by acute intracerebroventricular (ICV) administration of the fatty acid amide hydrolase (FAAH) inhibitor PF-4458945 (100 μg).

We show that chronic inflammation alters endocannabinoid levels in corticolimbic brain regions that are important for the regulation of stress and anxiety, in a similar manner to chronic stress exposure. Furthermore, we show that colitis leads to increased anxiety, which is then reversed by central inhibition of FAAH. Together these findings increase our understanding of the mechanisms underlying anxiety behaviors in chronic inflammatory states. They suggest that similar to stress-induced anxiety, inflammation-induced decreases in anandamide signaling are likely relevant for the change in emotional behaviors associated with chronic inflammatory states.

ENDOCANNABINOID MODULATION OF UNCONSCIOUSNESS AND CONSCIOUSNESS

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Consciousness is the most precious human attribute, but how the brain produces consciousness remains unknown. To reveal this mechanism, it is essential to probe the mystery of how consciousness recovers from general anesthesia, which is applied yearly to over 234 million patients worldwide and has been in clinical use for 170 years. The dorsomedial hypothalamic nucleus (DMH) integrates sleep, feeding and stress responses, which are regulated by *inhibitory* inputs onto DMH principle neurons. Surprisingly, here we identify that endogenous cannabinoid (or endocannabinoid, eCB) signaling at *excitatory* inputs onto DMH principle neurons significantly contributes to the recovery of consciousness after general anesthesia in rats. Our behavioral and electrophysiological experiments show that blockade of eCB signaling in DMH excitatory synapses stimulates both DMH inhibitory neurons projecting to the sleep-promoting ventrolateral preoptic nucleus of the hypothalamus (VLPO) and DMH excitatory neurons projecting to the wake-promoting perifornical area of the hypothalamus (Pef), thus accelerating recovery of consciousness after anesthesia. The concept of the unconsciousness-promoting DMH-VLPO circuitry and consciousness-promoting DMH-Pef circuitry are supported by our further studies employing a chemogenetic strategy for a selective activation or inactivation of DMH-VLPO, DMH-Pef or both circuitries. We propose that eCB activation of DMH excitatory synapses enhances unconsciousness through simultaneous deactivation of unconsciousness-promoting inhibitory DMH-VLPO circuitry and consciousness-promoting excitatory DMH-Pef circuitry.

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AN ENDOCANNABINOID MECHANISM SUPPORTING RESILIENCE TO TRAUMATIC STRESS

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Stress is the primary environmental risk factor for the development of multiple psychiatric disorders including major depression and anxiety disorders. The impact of stress on individuals is, however, highly variable with some individuals expressing resilience to stress-induced dysfunction and others expressing susceptibility. The majority of preclinical studies investigating this phenomenon have focused on chronic stress, but our data indicate that even an acute foot-shock stress exposure produces this bimodal effect on anxiety behavior. We utilized repeated novelty-induced hypophagia (NIH) testing in order to demonstrate the importance of 2-arachidonoylglycerol (2-AG), the most abundant endogenous cannabinoid, in regulating stress-resilience. Converging evidence has demonstrated that 2-AG signaling is intimately involved in the regulation of fear, anxiety, and depressive phenotypes. In this study, we demonstrate that treatment with JZL-184, an inhibitor of 2-AG degradation, significantly increases resilience of a previously stress-susceptible group of mice to the development of stress-induced persistent anxiety in NIH testing. Furthermore, natural resilience is associated with increased endocannabinoid signaling capacity at glutamatergic inputs to the basolateral amygdala (BLA). Conversely, germline genetic deletion of the primary 2-AG synthetic enzyme, DAGLα, significantly reduces resilience to acute stress. BLA-specific DAGLα deletion slightly increases basal anxiety behavior and significantly reduces resilience to repeated foot-shock stress exposure, indicating that 2-AG signaling in the BLA is important in the regulation of basal and stress-related anxiety. Based on these data we hypothesize that 2-AG signaling promotes stress-resilience via a dampening of stress-related excitability in amygdala circuitry.

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CHEMICAL PROTEOMICS MAPS BRAIN REGION DEPENDENT ACTIVITY OF ENDOCANNABINOID HYDROLASES

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The biosynthetic and metabolic enzymes of the endocannabinoids tightly regulate endocannabinoid-mediated activation of the cannabinoid CB_1 receptor. Monitoring the activity of these endocannabinoid hydrolases in different brain regions is, therefore, key to gain insight in spatiotemporal control of CB_1 receptor-mediated physiology.

We have developed a comparative chemical proteomics approach to quantitatively map the activity profile of endocannabinoid hydrolases in various mouse brain regions at the same time. To this end, we used two different activity-based probes: fluorophosphonate-biotin (FP-biotin), which quantifies FAAH, ABHD4, ABHD6 and MAG-lipase activity, and MB108 that specifically detects DAGL ✔, ABHD6 and ABHD12 amongst 37 other serine hydrolases. Both probes were applied to four different brain regions (frontal cortex, hippocampus, striatum and cerebellum).[2]

Comparison of endocannabinoid hydrolases activity in the four brain regions revealed that FAAH activity was highest in hippocampus, MAGL activity was most pronounced in the frontal cortex, whereas DAGL ✔ was most active in cerebellum. ABHD4, 6 and 12 activities were equally distributed over the brain regions. We compared our enzyme activity profile to a global proteomics dataset[1] and found pronounced differences. This could indicate that post-translational modification of the endocannabinoid hydrolases is important to regulate their activity.

Next, we studied the effect of genetic deletion of the cannabinoid CB_1 receptor on the activity of endocannabinoid hydrolases. No difference in the enzymatic activity was found in the cerebellum, striatum, frontal cortex and hippocampus of CB_1 receptor knockout animals compared to wild type mice. Our results are in line with previous reports[3] and indicate that the CB_1 receptor exerts no regulatory control over the basal production and degradation of endocannabinoids and that genetic deletion of the CB_1 receptor does not induce any compensatory mechanisms in endocannabinoid hydrolase activity.

[1] Sharma, K. Schmitt, S. Bergner, C. G. Tyanova, S. Kannaiyan, N. Manrique-Hoyos, N. Kongi, K. Cantuti, L. Hanisch, U. Philips, M. Rossner, M. J. Mann M. Simons, M. *Nature Neuroscience*, **2015**, *18*, 1819–1831

[2] Belluomo, I., Matias, I., Pernègre, C., Marsicano, G. and Chaouloff, F. *Journal of Neurochemistry*, **2015**, *133*, 26–37

[3] Di Marzo, V. Breivogel, C. S. Tao, Q. Bridgen, D. T. Razdan, R. K. Zimmer, A. M. Zimmer, A. Martin, B. R. *Journal of Neurochemistry,* **2000,** *75,* 2432-2444.

N-ACYLGLYCINE HYDROLYSIS BY RAT LIVER MICROSOMES

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A group of endocannabinoid-like molecules, the *N*-acylamino acids or lipoamino acids, epitomised by *N*-arachidonoylglycine (NAGly), have been identified in mammalian samples and are implicated in a variety of physiological and pathological conditions. In the current investigation, we have established an assay for quantification of the amino acid moiety following hydrolysis of five different *N*-acylamino acids using rat liver microsomal preparations as an enzyme source.

Hydrolysis of *N*-arachidonoylGABA, *N*-arachidonoylserine and *N*-acetylglycine could not be detected in these preparations, suggesting they might undergo metabolism via other routes. In contrast, hydrolysis of both NAGly and *N*-palmitoylglycine (PAGly) hydrolysis was detected in rat liver microsomes. K_m and V_{max} values were 7 ± 2 and 10 ± 2 μM, and 27 ± 2 and 71 ± 4 nmol/min/mg protein, respectively.

Inhibition of enzymatic activity using the FAAH-selective inhibitor URB597 showed a concentration-dependent inhibition of hydrolysis of NAGly (pIC_{50} value of 9.7 ± 0.5), PAGly (9.3 ± 0.2) and anandamide (9.6 ± 0.1), suggesting they may be substrates for FAAH. However, the URB597-evoked inhibition of anandamide hydrolysis was complete, but inhibition of NAGly and PAGly hydrolysis was incomplete with residual activities of 35 ± 9 and 28 ± 9 % control, respectively. No further inhibition was produced in the presence of the MGL-selective inhibitor JJKK48 or the non-selective inhibitors MAFP or PMSF alone or in combination with URB597.

N-ArachidonoylGABA and *N*-arachidonoylserine were investigated as potential FAAH inhibitors using anandamide as a substrate; however; no change in the hydrolytic activity of FAAH was observed.

In conclusion, these findings suggest that FAAH may, at least in part, be involved in the hydrolysis of long chain *N*-acylglycines. Further experiments are required to discover the identity of the other enzyme/s responsible for the hydrolysis of NAGly and PAGly, and the potential routes for turnover of other *N*-acylamino acids.

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MAGL INHIBITION SYNERGISTICALLY POTENTIATES THE ANTIALLODYNIC EFFECTS OF GABAPENTIN AND DICLOFENAC

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Neuropathic pain is characterized by altered nerve function that often presents as allodynia, the painful perception of non-noxious stimuli. Neuropathic pain is commonly treated with steroids, non-steroidal anti-inflammatory drugs (NSAIDs), or GABA analogues. However, in addition to the well-known side effects of steroids, NSAIDs also cause gastrointestinal inflammation and increased risk of cardiac events. GABA analogues, such as gabapentin, are commonly prescribed for nerve pain but also cause dizziness, sedation, and gait disturbance. Similarly, monoacylglycerol lipase (MAGL) inhibitors have analgesic properties but also induce sedation at high doses. In order to limit these side effects, the present study investigated the analgesic effects of coadministering a MAGL inhibitor with either an NSAID or gabapentin. Mice were subjected to the chronic constriction injury (CCI) model of neuropathic pain and then administered the MAGL inhibitor JZL184 (1-40 mg/kg, i.p.) and the NSAID diclofenac sodium (1-100 mg/kg, i.p.), separately or in combination. A second cohort of CCI-treated mice was administered the MAGL inhibitor or KML29 (1-40 mg/kg, i.p.) and the GABA analogue gabapentin (1-50 mg/kg, i.p.), separately or in combination. Mice were tested for mechanical and cold allodynia, using the von Frey and acetone tests. Dose addition analyses revealed that combined, low dose JZL184 and diclofenac synergistically attenuated mechanical allodynia and had an additive interaction in reducing cold allodynia. Similarly, the combination of KML29 and gabapentin had an additive interaction in attenuating mechanical allodynia and a synergistic interaction in reducing cold allodynia. In order to assess receptor mechanism, the CB_1 antagonist, rimonabant (3 mg/kg, i.p.) or the CB_2 antagonist, SR144528 (3 mg/kg, i.p.) was administered prior to the JZL184/diclofenac or KML29/gabapentin combination. Rimonabant, but not SR144528, blocked the analgesic effects of the JZL184/diclofenac combination and partially reversed the analgesic effects of the KML29/gabapentin combination in mechanical allodynia. Neither antagonist blocked analgesia in cold allodynia, indicating that CB_1 activation may be relatively more involved in attenuating sensitivity to mechanical stimuli. These data support the strategy of combining MAGL inhibition with commonly used analgesics as a therapeutic approach for attenuating neuropathic pain.

BRAIN-REGION DEPENDENT EFFECTS OF THC ON THE ENDOCANNABINOID AND WIDE-RANGING-RELATED LIPIDOME IN THE MOUSE BRAIN

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As the primary psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC) produces most of its behavioral effects via activation of CB_1, a receptor target for endogenous cannabinoids (eCBs) AEA and 2-AG. Linking the eCB system to broader lipid signaling, eCBs are derived from arachidonic acid, a substrate for prostaglandins (PGs). Use of THC typically induces region-specific downregulations in CB_1, but the consequences on levels of eCBs are less clear. The eCB system undergoes dynamic changes during adolescence, and therefore may be more vulnerable to challenges with exogenous cannabinoids like THC. Structural analogs of AEA and 2-AG, called lipoamines and 2-acyl glycerols, respectively, are also present in the mammalian brain. It is unknown how THC influences this wide-ranging signaling network. This study aims to measure the effects of THC on the lipoamine, 2-acyl glycerol, and PG lipidome in the mouse striatum, hippocampus, cerebellum, thalamus, cortex, hypothalamus, midbrain and brainstem and compares levels of THC and THC metabolites across brain regions. Groups of 5 mice treated with a single dose of 3mg/kg THC were each compared to 6 age (late adolescent) and sex (all female) matched vehicle treated mice from the same C57 genetic background. Animals were sacrificed 2 hours post injection, brains were removed and targeted areas were dissected and stored at -80C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS.

Varying by brain region, the concentration of THC was highest in the thalamus, followed by the hypothalamus and lowest in the cortex. Levels of THC metabolites also varied by brain region, with levels of (±)-11-nor-9-carboxyTHC being highest in the striatum and lowest in the hippocampus and levels of 11-OH-THC also being highest in the striatum and lowest in the cortex. These results suggest region-specific activity of enzymes that metabolize THC. Regarding the impact of THC on the lipidome, results here highlight those of AEA, 2-AG, and PGs; however, many differences were observed in other lipids in these THC treated mice. Interestingly, 2-AG levels were *reduced* in all brain regions except the hypothalamus of THC treated mice. In contrast, THC increased levels of AEA in the striatum and decreased levels of AEA in the hippocampus and cerebellum. AEA levels did not differ from vehicle in the thalamus, cortex, hypothalamus, midbrain or brainstem. In the published literature, there is currently no data on how a single dose of THC in adolescent female mice affects levels of eCBs. There were region-dependent increases in PGE_2 in the THC treated group, with higher levels in the striatum, thalamus, hypothalamus and brainstem. $PGF_{2\alpha}$ levels were altered by THC differently, with increases in the striatum and hypothalamus but a decrease in the hippocampus. In conclusion, in adolescent female mice, THC may drive changes in lipid metabolism in a brain-region dependent manner, with consequences for signaling. Specifically, the use of THC appears to counteract eCB signaling by altering 2-AG metabolism. The effects of THC on levels of non-eCB *N*-acyl amides, 2-acyl glycerols and PGs may have consequences for signaling outside of CB_1. Follow-up studies are warranted to determine if these effects are specific to the age and sex of mice and if they are dose-dependent.

EFFECTS OF MODULATION OF THE ENDOCANNABINOID TONE ON HUMAN SEBOCYTES

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We have previously shown that i) endocannabinoids produced in the human sebaceous glands (eCB; e.g. anandamide [AEA]) are key players in the maintenance of the homeostatic sebaceous lipid production; and ii) eCB treatment of sebocytes dramatically increase their lipid synthesis. Quite surprisingly, however, fatty acid amide hydrolase (FAAH)-inhibition-mediated intracellular elevation of AEA levels did not increase lipogenesis. Instead, it suppressed AEA-induced excessive, "acne-mimicking" sebaceous lipid synthesis. This apparent controversy led us to hypothesize that intra- and extracellular accumulation of eCBs could activate partly independent signaling pathways, thereby differentially influencing the biology of human sebaceous glands. Thus, here, we aimed to explore the effects of extracellularly accumulated eCBs on human SZ95 sebocytes by using inhibitors (i.e. VDM11, UCM707 and AM404) of the putative endocannabinoid membrane transporter (EMT).

Using [^{3}H]-AEA-uptake assay and HPLC-MS quantification of lipid extracts from sebocytes, we found that EMT is indeed functionally active in human sebocytes (10 μM UCM707; 15 min), and its inhibition (10 μM VDM11; 24 hrs) significantly increases AEA as well as oleoylethanolamide contents and tends to rise 2-arachidonoylglycerol concentrations too. Next, we found that up to 10 μM, VDM11 had no effect on the viability (MTT-assay; 48 hrs) and did not induce early apoptotic or necrotic cell death ($DilC_1(5)$-SYTOX Green labeling; 48 hrs). Of great importance, we could also demonstrate that, in a striking contrast to the effects of the FAAH-inhibitors, VDM11-mediated (most probably) extracellular elevation of eCB levels increased sebaceous lipid production (Nile Red staining; 48 hrs) – although its efficiency was substantially lower than the one usually observed upon direct AEA treatment. Intriguingly, co-administration of the most efficient pro-lipogenic concentration of VDM11 (10 μM) and AEA (30 μM) appeared to decrease the pro-lipogenic effect of the latter (Nile Red staining; 48 hrs). This effect might have been due to the increased cytotoxic potential of the combined treatment (MTT-assay; 48 hrs). Finally, by monitoring expressional alterations of various pro-inflammatory cytokines (interleukin [IL]-1α, IL-1β, IL-6, IL-8 and tumor necrosis factor-α), we found that 1 μM VDM11 was able to prevent the pro-inflammatory action of the Toll-like receptor 4 activating lipopolysaccharide (Q-PCR; 3 hrs).

Collectively, our data suggest that intra- and extracellularly increased eCB tone may activate partly independent signaling pathways, thereby differentially influencing biology of the human sebaceous glands. Thus, appropriate modulation of the eCB tone holds out the promise to be beneficial both in seborrhea- (e.g. acne) and skin dryness-accompanied cutaneous inflammatory disorders.

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RAPID AND PROFOUND REWIRING OF BRAIN LIPID SIGNALING NETWORKS BY ACUTE DIACYLGLYCEROL LIPASE INHIBITON

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Diacylglycerol lipases (DAGLα and DAGLβ) convert diacylglycerol to the endocannabinoid 2-arachidonoylglycerol. Our understanding of DAGL function has been hindered by a lack of chemical probes that can perturb these enzymes *in vivo*. Here, we report a set of centrally active DAGL inhibitors and a structurally related control probe and their use, in combination with chemical proteomics and lipidomics, to determine the impact of acute DAGL blockade on brain lipid networks in mice. Within two hours, DAGL inhibition produced a striking reorganization of bioactive lipids, including elevations in DAGs and reductions in endocannabinoids and eicosanoids. We also found that DAGLα is a short half-life protein, and the inactivation of DAGLs disrupts cannabinoid receptor-dependent synaptic plasticity and impairs neuroinflammatory responses, including lipopolysaccharide-induced anapyrexia. These findings illuminate the highly interconnected and dynamic nature of lipid signaling pathways in the brain and the central role that DAGL enzymes play in regulating this network.

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EARLY INTERVENTION USING URB597 TO REDUCE JOINT INFLAMMATION CAN ALLEVIATE END STAGE OSTEOARTHRITIS PAIN IN MICE

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Osteoarthritis (OA) is primarily a degenerative disorder of diarthroidal joints, but can be associated with intermittent bouts of inflammation. We hypothesize that these acute inflammatory flares can drive joint degeneration and chronic OA pain. It has previously been shown that joint inflammation and pain can be alleviated by inhibiting fatty acid amide hydrolase (FAAH) with URB597 [1, 2]. The aim of the present study was to determine whether reducing early onset inflammation in the sodium monoiodoacetate (MIA) model of OA could alter joint pain at later stages of the disease.

OA was induced in male C57Bl/6 mice (20-42g) by intra-articular injection of MIA (0.3mg) and animals were allowed up to 14 days for symptoms to develop. Joint inflammation was assessed by measuring articular oedema (joint diameter), leukocyte trafficking (intravital microscopy) and synovial blood flow (laser speckle contrast analysis). Joint mechanonociception was measured using von Frey hairs applied to the hindpaw. Experiments were carried out on day 1 after MIA injection (early inflammatory phase) and day 14 (joint degeneration phase). For day 1 joint inflammation studies, animals were treated with a single dose of URB597 (0.3mg/kg; topical over the exposed knee joint). For chronic pain studies, mice received 4 injections of URB597 (0.3mg/kg i.p.; days 0-3) and underwent pain assessment on day 14. Drug treatment was compared to vehicle-injected animals.

On day 1, MIA increased joint oedema, vascular conductance and leukocyte adherence compared to saline-injected sham animals ($P<0.05$; n=9-27). These inflammatory changes resolved by day 14 ($P>0.05$; n=5-6). Acute administration of URB597 significantly reduced MIA-induced leukocyte adherence and hyperaemia on day 1 ($P<0.05$; n=6-8), suggesting that endocannabinoids are anti-inflammatory in this model. On day 14, OA mice demonstrated tactile allodynia that was blocked by early treatment with URB597 ($P<0.05$; n=8-10).

Inhibition of FAAH activity reduced acute synovitis associated with early onset OA. Furthermore, early treatment of OA knees with URB597 attenuated late onset tactile allodynia. Thus, amelioration of acute joint inflammation by endocannabinoids can be protective against end stage OA pain.

[1] E. Krustev, A. Reid, J.J. McDougall, Tapping into the endocannabinoid system to ameliorate acute inflammatory flares and associated pain in mouse knee joints, Arthritis Research and Therapy 16 (2014) 437.

[2] N. Schuelert, M.P. Johnson, J.L. Oskins, K. Jassal, M.G. Chambers, J.J. McDougall, Local application of the endocannabinoid hydrolysis inhibitor URB597 reduces nociception in spontaneous and chemically induced models of osteoarthritis, Pain 152 (2011) 975-981.

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DOUBLE DISSOCIATION OF CB_1 ANTAGONISM AND MONOACYLGLYCEROL LIPASE INHIBITION IN THE CENTRAL AMYGDALA, BASOLATERAL AMYGDALA AND THE INTEROCEPTIVE INSULAR CORTEX ON THE AFFECTIVE PROPERTIES OF ACUTE NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL IN RATS

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Both CB_1 receptor antagonism and agonism**,** in particular by 2-arachidonyl glycerol (2-AG), have been shown to reduce somatic symptoms of morphine withdrawal. Here we evaluated the effects of both systemic pretreatment with the monoacylglycerol lipase (MAGL) inhibitor MJN110 (which selectively elevates 2-AG) and central administration of both MJN110 and the CB_1 antagonist (AM251) on the affective properties of morphine withdrawal. Acute morphine withdrawal induced place aversion occurs when naloxone is administered 24 hr following a single exposure to a high dose of morphine. Systemic pretreatment with the MAGL inhibitor, MJN110, prevented the aversive effects of acute morphine withdrawal by a CB_1 receptor dependent mechanism. Furthermore, in a double dissociation, AM251 infusions into the central amygdala (CeA), but MJN110 infusions into the basolateral amygdala (BLA), interfered with the naloxone-precipitated morphine withdrawal induced place aversion. As well, MJN110, but not AM251, infusions into the interoceptive insular cortex (IC, a region known to be activated in acute morphine withdrawal) also prevented the establishment of the place aversion by a CB_1 mechanism of action. These findings reveal the respective sites of action of systemically administered MJN110 and AM251 in regulating the aversive effects of morphine withdrawal.

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ENDOCANNABINOID SYSTEM RECEPTORS AND ENZYMES ARE EXPRESSED IN EARLY DEVELOPING HUMAN BRAIN ORGANOIDS

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Neither "medical marijuana" components nor "Spice" (K2) compounds have been documented for their safety in brain development or adult stem cell function. Rodent model studies have indicated that Δ^9-THC and cannabinoid agonists can alter developing brain in complex ways including neural plate aplasia in early brain development (15-19 day human embryo), and effects on progenitor proliferation, neuronal migration and differentiation of lineage-committed cells. We hypothesize that cannabinoid agonists, indirect agonists that increase endocannabinoids, and Spice compounds can cause aberrations in early stages of brain development. We developed a novel human cerebral brain organoid model to detect aberrant function in the endocannabinoid system. Construction of cerebral organoids was performed starting with an iPS line generated from human fibroblasts using a non-integrating method (WT11). Differentiation began using passage 38-day iPS cells at approximately 75% confluence. Embryoid bodies were formed in ultra-low adhesion, 96-well plates using 4,500 iPSC in embryoid body media, which after 6 days was switched to neural induction media to stimulate the formation of neuroectoderm. After a further 5 days, each developing organoid was transferred to a drop of ES qualified Matrigel and then stimulated to form expanded neuroepithelium in static culture using differentiation media. Developing brain organoids were transferred at day 15 *en masse* to a spinning bioreactor containing differentiation media with retinoic acid. After 15 days, brainoids have reached their maximum size (1-2 mm), but continue to differentiate and remain in culture for up to one year.

The 35-day brain organoids recapitulate early cerebral development, with an interior fluid-filled cavity (telencephalic vesicle or ventricle) that appears to contain a choroid plexus. Differentiated principle cell neurons (Map2+) are seen to migrate from the apical ventricular and inner/outer subventricular zones along the radially organized basilar radial glia to collect near a putative cortical plate. Brain organoids are comprised of cells characterized as GABAergic (glutamic acid decarboxylase, GAD+) and glutamatergic (mGluR1+) principle cells. Dopaminergic neurons (tyrosine hydroxylase, TH+; Nurr1 transcription factor+) appeared segregated along a cleft near the ventricle at one pole of the brainoid. CB_1 cannabinoid receptors and enzymes that synthesize and metabolize 2-AG, diacylglycerol lipase (DAGLβ and α) and monoacylglycerol lipase (MAGL), as well as N-acyl ethanolamide metabolizing fatty acid amide hydrolase (FAAH) are immunohistochemically identified in cerebral brain organoid cells. CB_2 staining appears sparse compared with CB_1 staining (utilizing multiple different antibodies for both CB_1 and CB_2 receptors). CB_2 receptors have been implicated in neuroprogenitor proliferation and survival, and neurite outgrowth and directionality in studies based upon use of pharmacological antagonists and knock-out mice. These results demonstrate the feasibility of using human cerebral organoids *in vitro* to test for effects of cannabis components and Spice compounds on receptors and enzymes in the endocannabinoid system during early brain development.

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THC AND FUNGAL IMMUNITY: HOW AN IMMUNOCOMPROMISED MOUSE'S ABILITY TO CLEAR A FUNGAL INFECTION IS AFFECTED BY Δ^9-TETRAHYDROCANNABINOL

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Δ9-tetrahydrocannabinol (THC) is known to suppress resistance to bacterial, viral and protozoan infection. However, less is known of the effects THC has on resistance to fungal infections. Recently, we found that chronic THC treatment decreased resistance to the yeast *Candida albicans (C. albicans)* in immune competent mice. However, nothing is known about how THC affects resistance to a fungal infection in an immunocompromised mouse. Our objective is to assess how chronic THC affects an immunocompromised mouse's ability to ward off systemic candidiasis. 5-fluorouracil is a commonly prescribed thymidylate synthetase inhibitor which can be useful in treating cancer and is also a potent immunosuppressor, increasing susceptibility to *C. albicans* infection. In our experiment, c57BL/6 mice will be treated via an intraperitoneal (IP) injection with vehicle (ethanol, cremophor, saline (1:1:18)) or THC (16mg/kg) 4 days a week, for three weeks (experimental days 1-18). On day 18, mice will receive an intravenous (IV) injection of 5-fluorouracil (0.1ml of 50mg/ml solution). On day 19, mice will be infected with an IV injection of $5x10^5$ *C. albicans* cells. On day 22, tissues will be harvested from some mice to assess cytokine production and tissue fungal load. Remaining mice will be observed for up to 2 weeks for survival. Our preliminary findings have shown that *C. albicans* infected mice treated with the chemotherapeutic drug, regardless of vehicle treatment, succumbed more readily to the yeast.

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THE CB_2-SELECTIVE AGONIST HU-308 ATTENUATES INFLAMMATION AND HYPERALGESIA IN CHRONIC AND ACUTE MODELS OF INFLAMMATORY ARTHRITIS

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Inflammatory arthritis is a chronic, debilitating disease that causes pain and inflammation in the joints and reduces mobility. Cannabinoid agonists and endocannabinoid enzyme inhibition have analgesic and anti-inflammatory effects. For example, genetic deletion or pharmacological inhibition of fatty acid amide hydrolase (FAAH) reduces paw swelling, joint damage, and hyperalgesia in the collagen-induced arthritis (CIA) model. The present study tested the hypothesis that selective agonism of CB_2 with HU-308 decreases inflammation and hyperalgesia that are typical of mouse models of chronic and acute inflammatory arthritis. The most extensively studied model of chronic inflammatory arthritis is the collagen induced arthritis (CIA) model. Male DBA1 mice were immunized with an emulsion of collagen and complete Freund's adjuvant (CFA) to induce CIA. Two to three weeks later, mice were given a secondary "booster" of the collagen preparation. CIA significantly increased clinical signs of arthritis (e.g., paw redness and swelling) and hyperalgesia (e.g., decreased paw withdrawal latency). Chronic administration of the selective CB_2 agonist HU-308 (3 mg/kg, ip, for eight days) significantly reduced CIA-induced paw swelling but did not significantly affect CIA-induced decreases in paw withdrawal threshold in the plantar stimulator test of hyperalgesia. In contrast to the CIA model, which is mainly used to research inflammatory arthritis immunosuppressant treatments, acute adjuvant-induced arthritis models are extensively used to characterize inflammatory joint pain. To test this hypothesis, complete Freund′s adjuvant (CFA, 20 µl) was injected into the hind paw footpad of male C57BL/6 mice. CFA significantly increased inflammatory pain and paw swelling one day after administration. Subchronic administration of HU-308 (50 mg/kg, ip, for four days) attenuated CFA-induced decreases in paw withdrawal latencies in the plantar stimulator test, but did not significantly affect CFA-induced paw edema. These results suggest that CB_2 agonism may be a promising strategy for the treatment of pain and inflammation caused by inflammatory arthritis.

THE ROLE OF DORSAL ROOT GANGLIA NEURONS INNERVATING THE RAT KNEE JOINTS DURING OSTEOARTHRITIS DEVELOPMENT: IMPLICATIONS FOR THE ENDOCANNABINOID SYSTEM

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Osteoarthritis (OA) is one of the most common chronic musculoskeletal diseases manifested by persistent joint pain. Currently, there is no cure for OA, standing treatments focus only on easing the symptoms and improving the joint functions. Furthermore, OA-related pain is still poorly managed and poses unmet medical need. Dorsal root ganglia (DRG) contain neurons that convey sensory information from the periphery to the CNS. Following nerve injury, DRG neurons may become an important source of increased nociceptive signaling. Lumbar region DRGs, namely L2-L6, are of particular interest as they are responsible for sensory innervation of the rat knee joint (Ochiai et al, *Osteoarthritis Cartilage*. 2007). More resent study proved that majority of the knee joint nociceptive fibers were detected mainly in L3 and L4 DRGs (Aso et al. *Eur J Pain.* 2014). Interestingly, two-third of sensory innervation of subchondral bone of the distal femur in rats presented its origin in L3 DRG (Aso et al. *Eur J Pain.* 2014). The constitutive expression of endocannabinoid system (ECS) components in DRGs are well established. Growing body of evidence suggest that the ECS may serve as a potentially important therapeutic target for OA pain (Porta et al. *Eur J Neurosci.* 2014). Indeed, pharmacological studies on systemic administration of ECS modulators in different animal models of OA have shown anti-nociceptive effects in the disease-affected knee (Porta et al. *Eur J Neurosci*. 2014). The development of pain behavior involves discreet changes in the molecular properties of nociceptors, including alterations in gene and protein expression. Elucidating the role of ECS functioning within DRGs innervating the osteoarthritic knee seems to be crucial for deeper understanding of mechanism underlying chronic OA pain and to propose future treatment directions. Therefore, the aim of our study was to investigate protein expression for ECS elements in L3-L6 DRGs from osteoarthritic rats in the time-course of OA development. OA was induced in male Wistar rats by intra-articular injection of sodium monoiodoacetate (MIA). Animals were sacrificed at day 2, 7, 14, 21 and 28 after OA induction and L3-L6 DRGs were isolated. Implying Western Blot technique, we determined levels of selected ECS proteins in pooled ipsilateral or contralateral L4-L6 DRGs at above described time-points. Additionally, for further evaluation of ECS expression, immunohistochemical staining has been performed using both ipsilateral and contralateral L3, L4, and L5 DRGs from control and osteoarthritic animals (at day 21 post MIA injection illustrating advance OA conditions).

All proteomics carried out on pulled DRGs revealed no significant alterations in protein expression neither of endocannabinoid receptors (CB1, CB2, TRPV1) nor of enzymes engaged in endocannabinoids' metabolism (NAPE, FAAH, COX-2). Nevertheless, based on our immunohistochemical analysis, we were able to distinguish diverse regulation of TRPV1 and FAAH protein and distinct colocalization percentage of these two targets between L3 and L4 DRGs at day 21 after OA induction (these changes were undetectable in horizontally pooled DRGs). Summarizing, development of OA in rats results in subtle molecular changes in FAAH and TRPV1 expression in DRGs important for innervation of the knee, but these alterations are DRG-level dependent and are the most profoundly manifested by the changes in the colocalization of double positive TRPV1+/FAAH+ neurons. Interestingly, intraperitoneal injection of compound being simultaneous FAAH inhibitor and TRPV1 antagonist, OMDM-198, proved to be analgesic in OA-affected rats (Malek et al, *Pain*. 2015). Moreover, it was shown that intrathecal administration of similar drug, AA-5-HT, presented anti-nociceptive effect in neuropathic pain (unpublished Author's own data). Thus, we suggest that regional interventions at the level of the peripheral nervous system (particularly lumbar L3 DRG) with utilizing double acting modulators of ECS, might be reasonable in OA pain management, but definite judgment need further pharmacological research.

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CANNABIDIOL QUINOL DERIVATIVES FOR THE TREATMENT OF SCLERODERMA

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Scleroderma is a group of rare diseases that involve the hardening and tightening of the skin and connective tissues. There are two major forms of scleroderma: localized scleroderma and systemic sclerosis (Ssc)**,** which affect up to 30% of the patients. Scleroderma is associated with early and transient inflammation and vascular injury, followed by progressive fibrosis affecting both the skin and multiple internal organs. Fibroblast activation is the hallmark of scleroderma, and disrupting the intracellular TGFβ signaling may provide a novel approach to controlling fibrosis. In addition**,** pro-inflammatory factors known to have an important role in the pathogenesis of the disease might be released or activated by immune cells such as T cells and macrophages. Because of its potential role in modulating inflammatory and fibrotic responses**,** both PPARγ and CB2 receptors represent attractive targets for the development of cannabinoid-based therapies.

We have previously found that resorcinyl-to-paraquinol oxidation of CBG (VCE-003) and CBD (HU-331) increases their PPARγ agonistic activities. However, both VCE-003 and HU-331 are unstable electrophilic compounds, with limited prospects of further development. As part of our study on the SAR of CBD we have developed a non-thiophilic and chemically stable derivative of the CBD quinol (VCE-004.3) that behaves as a dual agonist of PPARγ and CB2 receptors. VCE-004.3 inhibited TGFβ-induced Col1A2 gene transcription and collagen synthesis without interfering with SMAD2 phosphorylation. VCE-004.3 also inhibited TGFβ-mediated ✔SMA induction and myofibroblast differentiation and impaired wound-healing activity in primary fibroblasts. We also found that VCE-004.3 inhibited TCR-induced IL-17 transcriptional activity in T cells and modulated M1/M2 macrophage differentiation. The anti-inflammatory efficacy *in vivo* was investigated in an inflammatory model of dermal fibrosis induced by bleomycin. VCE-004.3 reduced dermal thickness and prevented mast cell degranulation and the infiltration of inflammatory cells. In addition, VCE-004.8 downregulated the expression of several key genes associated with fibrosis and inflammation, qualifying this semi-synthetic cannabinoid as a novel compound for the management of scleroderma and, potentially, other fibrotic diseases.

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SPECIFIC VARIATIONS BETWEEN *CANNABIS SATIVA* LINES CONFER DIFFERENT ANTI-INFLAMMATORY ACTIVITY ON INFLAMED COLON CELLS

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Inflammatory bowel diseases (IBD) are characterized by chronic intestinal inflammation and consist mainly of Crohn's disease and ulcerative colitis. For both diseases the exact etiology is unknown. Therapeutic attempts are aimed at down-regulating intestinal inflammation using b oth mediator-specific and nonspecific immune suppression, which cause considerable side eff ects. Products based on marijuana (*Cannabis sativa*) were previously shown to produce bene ficial effects for patients with IBD, and medical cannabis-based products were formerly prove n to have anti-inflammatory activity in laboratory experiments as well as in clinical tests. How ever, *C. sativa* consists of thousands of different lines, which differ in their composition of act ive compounds. Also, IBD patients are different in their genetic background and pathology. T herefore, in the process of medicalization of *C. sativa* it is important to better understand the mechanism that leads to differences in patient response to different *C. sativa* lines, in order to fine-tune *C. sativa* –based treatment to IBD patients.

Here, we have characterized the chemical composition of different *C. sativa* lines and their an ti-inflammatory activities. Extracts of *C. sativa* lines were done using various methods and ca nnabinoids, terpenoids and flavonoids content was determined. We found that different lines h ave different effects on inflamed colon cell lines, leading to changes in interleukin secretion fr om colon cells. Also we found that these differences may be derived from differences in the c omposition of the extracts. The different active compounds are now being characterized for th eir specific activity. For a better understanding of the mode-of-action of the *C. sativa* extracts we have determined their effect on various inflammation markers. We found different lines to significantly affect Matrix metalloproteinases (MMP9) expression. Additional markers as iNO S and COX2 are being tested. Also, we are determining the level of expression of the CB1 an d CB2 receptors following treatments with *C. sativa* extracts. IC50 of each of the plant extrac ts was determined, and anti-inflammatory activity was established at concentrations below the IC50. Following, clinical tests will be conducted aiming to develop cannabis–based products f rom different *C. sativa* lines, with anti-inflammatory activity that is effective and optimized fo r different IBD patient groups.

TNF INCREASES TRPA1 EXPRESSION AND FUNCTION IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS: TRPA1 AS THERAPEUTIC TARGET TO REDUCE JOINT INFLAMMATION IN RA?

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In collagen-induced arthritis, elevation of endocannabinoid levels improves clinical parameters and reduces synovial inflammation. Previous studies demonstrated that anandamide decreased TNF-induced IL-6, IL-8 and MMP-3 production by synovial fibroblasts in a transient receptor potential type ankyrin (TRPA1)-dependent manner. Joint pain and inflammation are driven by pro-inflammatory cytokines like TNF, which is also involved in sensitizing TRP-channels. Since TRPA1 activation leads to calcium influx we investigated how TNF modulates this response in isolated synovial fibroblasts and whether this can be exploited therapeutically.
TRPA1 expression after TNF exposure was analyzed by western blotting and cell based ELISA. Cell proliferation was determined by cell titer blue. Calcium flux was analyzed by staining with Fura-2. Lactate dehydrogenase release was used as marker for cell death.

Prolonged incubation (72h) with TNF (10ng/ml) significantly increased TRPA1 protein levels. TNF-induced sensitization and up-regulation of TRPA1 was confirmed by an increase in intracellular calcium in response to TRPA1 agonist allyl isothiocyanate (AITC). While synovial fibroblasts did not respond to AITC without TNF pretreatment, TNF pre-incubation (72h, 10ng/ml) elicited a robust increase in calcium in response to AITC (10µM-500µM). This was paralleled by increased lactate dehydrogenase release indicating cell death in response to TRPA1 activation. Cells pre-treated with TNF showed faster onset of LDH release after TRPA1 engagement, higher LDH levels in supernatant and a lowered activation threshold. Cell proliferation was also modulated by TRPA1 activation. While TNF naïve cells demonstrated reduced proliferation in response to 100µM AITC, TNF pre-treatment lowered the effective concentration to 12.5µM.

The observed up-regulation of TRPA1 by TNF might explain lack of effects of endocannabinoids on basal cytokine production in synovial fibroblasts. Targeting TRPA1 in inflammation might have therapeutic advantages, since it mainly affects synovial fibroblasts pre-activated by pro-inflammatory cytokines. Further experiments need to elucidate whether this is also true in other cell types involved in synovial inflammation.

CANNABIS TERPENES WITH A ROLE IN INFLAMMATION: A MOLECULAR PERSPECTIVE

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Recent data suggest that some of the terpenes found within the cannabis plant possess anti-inflammatory and analgesic activity which is additional to the effects of classical phytocannabinoids and may generate synergistic interactions [1]. Terpenes are thought to act through a variety of targets, including ion channels and immunoregulatory GPCRs [2]. In particular, β-caryophyllene, a terpene present in cannabis, can modulate CB2 receptor function and has anti-inflammatory actions, highlighting how terpenes can tone a range of molecular targets involved in nociception and inflammatory signalling pathways [2,3].

In the present study we have investigated in more detail the cellular effect of terpenes that are found in cannabis. In particular, we have evaluated cell signaling events in the RAW 264.7 macrophage cell line using calcium imaging techniques and fluorescence microscopy for analysis of pCREB signaling, under control conditions and also following activation with LPS.

Our findings suggest that the terpene β-caryophyllene can influence pCREB signaling in RAW 264.7 cells, whilst showing minimal effects on calcium signaling when compared to cannabidiol. Given the various roles that CREB plays in immune function, such activity may underlie the anti-inflammatory actions of similar sesquiterpenes. Identification of the molecular targets that mediate this effect and an evaluation of synergistic phytocannabinoid interactions are currently underway.

References

[1] Russo, E. (2011) Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British Journal of Pharmacology 163: 1344–1364

[2] Gertsch J, Leonti M, Raduner S; et al. (July 2008). Beta-caryophyllene is a dietary cannabinoid". Proceedings of the National Academy of Sciences of the United States of America 105 (26): 9099–1104.

[3] Buchbauer G (2010). Biological activities of essential oils. In: Baser KHC, Buchbauer G (eds). Handbook of Essential Oils: Science, Technology, and Applications. CRC Press: Boca Raton, FL, pp. 235– 280.

ABSTRACT WITHDRAWN

ENDOCANNABINOID SYSTEM AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE, lupus) is a chronic autoimmune disease with multifactorial etiology, in which genetic and environmental factors interplay determining disease susceptibility. Clinical manifestations of SLE, including both constitutional symptoms (fever, malaise, fatigue) and organ involvements (primarily mucocutaneous, renal, musculoskeletal, haematological and neuropsychiatric features), can vary widely from patient to patient making diagnosis and treatment a challenge. The disease is sex-related with a female/male ratios prevalence of 9:1 and the progression is non-linear and follows a relapse-remitting course. Sadly, there is still no cure for SLE but medications may decrease the rate of flares and improve expectancy and quality of life.

Here, we investigated the role of eCB system in patients with SLE and age and sex matched healthy subjects. Ten female patients with SLE (37.9±5.9 years) and 10 healthy subjects (37.8±6.2) were enrolled from outpatient clinic of Campus Bio-Medico University of Rome. Every SLE patient was positive for anti-dsDNA antibodies and/or exhibited hypocomplementemia with or without hypergammaglobulinemia, extractable nuclear antigen (ENA) or antiphospholipid antibodies (aPL) positivity. None of them was treated with biological therapy at the time of enrolment, nor with steroid bolus in the previous six months, while treatment with conventional immunosuppressants was allowed. In particular, value of the disease activity score were evaluated by the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) - SLE Disease Activity Index (SLEDAI) and by the British Isles Lupus Activity Group (BILAG) system. SELENA-SLEDAI was assessed as 6±4.7; 10% of the patients exhibited at least 1 BILAG A and 60% at least 1 BILAG B. The Severity of Disease Index (SDI) value was assessed as 0.6±0.8. Moreover, eCB was significantly altered in SLE patients. In particular, plasma levels of 2-AG were significantly increased in SLE patients compared to healthy controls ($p=0.005$), while no differences were found in AEA and PEA concentrations between the two groups. Additionally, qRT-PCR highlighted a selective down-regulation of the expression of CB_2 cannabinoid receptors in SLE patients *vs* healthy controls ($p=0.05$).

In conclusion, our results demonstrate for the first time an alteration of eCB system in SLE patients, and may help to better understand the role of lipid mediators in SLE pathogenesis.

MODULATION OF CANNABINOID RECEPTORS AND CYTOKINES EXPRESSION BY SEX HORMONE TREATMENT OF MACROPHAGES

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Sex hormones, such as testosterone and estrogen, play a major role in modulating the expression of cannabinoid receptors and pro-inflammatory responses to infections. Cannabinoid Receptor 1 (CB1R) and Cannabinoid Receptor 2 (CB2R) can be found within immune cells such as macrophages and are known to be involved in immune modulation. However, the abundance of CB1R and CB2R in macrophages in the presence of testosterone and estrogen are unknown. Furthermore, the effect of 17β-estradiol (E2) or 5α-dihydroxytestosterone (5αDHT, the stable form of testosterone) on cannabinoid receptor expression when exposed to yeast infections is unknown. *Candida albicans* (*C. albicans*) is a yeast commonly found on the skin and in the mucosa of healthy individuals. However, *C. albicans* can become pathogenic in immunocompromised individuals and is one of the most common hospital borne fungal infections.

Similar to what other have reported, we found that female mice are significantly more resistant to systemic *C. albicans* infections than male mice, and that castrated males are as resistant to the infection as female mice. To further understand the gender differences regarding resistance to *C. albicans* infection, we supplemented female or male mice with either 5αDHT or E2 and found that mouse resistance to the yeast infections decreased. Macrophages are essential in combating infections, but it is unknown how sex hormones alter macrophage immune response or cannabinoid receptor expression when the cells are challenged with yeast. Therefore, J774A.1 murine macrophages were challenged with *C. albicans* and treated with different concentrations of E2 or 5αDHT. Twenty-four hours later, cell supernatants were assessed for two pro-inflammatory markers, nitric oxide (NO) and the cytokine tumor necrosis-alpha (TNF-α). E2 at 1-100nM had no effect on NO or TNF-α. However, 1μM E2 significantly increased NO produciton. 5αDHT had no effect on either NO nor TNF-α at any concentration studied. Currently, we are investigating the effect E2 or 5αDHT on cannabinoid receptor and cytokine mRNA expression using RT-qPCR. Based on others findings involving different pathogens, we hypothesize, *C. albicans* challenged macrophages treated with either E2 of 5αDHT, will have no effect on the amount of CB1R mRNA expression. We also expect that treatment with E2 will suppress CB2R and pro-inflammatory cytokine mRNAs. Finally, we expect that 5αDHT treatment will stimulate pro inflammatory cytokine mRNA.

NEW APPROACHES TO TREATING OSTEOARTHRITIS IMPLICATIONS FOR THE ENDOCANNABINOID SYSTEM IN OSTEOBLAST METABOLISM

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Osteoarthritis (OA) is widespread joint disease characterized by chronic pain, breakdown of cartilage and underlying bone. Latest studies highlight the role of cross talk between cartilage and subchondral bone in the OA development and progression, but still it remains unclear whether cartilage changes occur before or after bone alterations. Current treatments for OA are symptomatic and limited by side effects or lack of efficacy, and despite using the available therapies, many people with OA still have significant symptoms. No drugs are available to control and modify its progression. Recently, endocannabinoids and their receptors have been reported in the skeleton, including the bone forming cells, osteoblasts. Moreover, it is known that actions of cannabinoids can be also mediated by non-classical cannabinoid receptors like TRPV1, GPR55 and GPR18. Research focused on the molecular bone pathology supports the concept that appropriate control of bone remodeling may contribute to maintenance of joint functionality and stability. We assume, the endocannabinoid system can be an emerging target in a potential disease - modifying OA therapy, focused on bone cells. We aim at pointing new molecular markers for pharmacological modulations that could slow down the disease progression. Thus, we investigate subchondral bone changes during OA *in vivo* and the influence of compounds acting on the endocannabinoid system on osteoblast activity under OA condition *in vitro*. OA was induced in male Wistar rats by single injection of 1 mg sodium monoiodoacetate (MIA) into the knee joint. Alterations in subchondral bone architecture and density were visualized by X-ray computed microtomography (XMT). Human osteoblasts were treated with 3μM of MIA to mimic animal model of OA *in vitro*. Subsequently cells were treated with compounds acting on the endocannabinoid system to investigate its influence osteoblast proliferation (BrdU incorporation assay) and migration (wound healing assay). We assessed changes in mRNA levels of receptors for cannabinoids in response to drug treatment.

XMT study revealed significant alterations in subchondral bone as consequences of disease progression; we observed dramatic changes both in bone architecture and bone density in the rat knee. Our data demonstrate impaired osteoblasts metabolism as cells displayed increased migration but decreased proliferation capacity after MIA administration. Treatment with compounds like OMDM198 (TRPV1 and FAAH dual blocker), JWH133 (CB2 agonist) and O-1602 (GPR55/GPR18 mixed agonist) restored proliferation capacity after MIA treatment. These effects were abolished after blocking the TRPV1 (SB 366,791), FAAH (URB 597), CB2 (AM630) and GPR18/55 (CID 85469571). Moreover, in MIA treated osteoblasts we observed a trend to decrease expression of both CB2 and GPR18 receptors. Interestingly upon JWH-133 treatment mRNA level of GPR18 was significantly higher than CB2. We suggest possible cross talk between CB2 and GPR18 receptors and its potential involvement in bone metabolism during cellular stress associated with osteoarthritis. These studies highlight the importance of receptors for (endo)cannabinoids in osteoblasts metabolism, thus also in subchondral bone. The effects of compounds acting on the ECS indicate its significance in maintaining bone tissue homeostasis. Disruptions in the expression of receptors for cannabinoids may contribute in the appearance and progression of OA.

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MOLECULAR UNDERSTANDINGS ON THE ACTIVATION OF CB1 AND BLOCKADE OF TRPV1 RECEPTORS: IMPLICATIONS FOR NOVEL TREATMENT STRATEGY IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a joint disease in which cartilage degenerates as a result of mechanical and biochemical disturbances. Main OA symptom is chronic pain involving both peripheral and central mechanisms of nociceptive processing. Current treatment based on symptomatic care does not always provide adequate pain relief and often lead to adverse side effects. Thus it is necessary to develop novel therapies. Recent research provides strong support for endocannabinoid system (ECS) as an important player in modulation of pain associated with joint injury. Nevertheless, molecular changes and differences between compounds used in animal studies as antinociceptive are poorly described. Presented results discuss the benefits of dual- over single-acting compounds interacting with ECS in OA treatment.

OA was induced in Wistar rats by intra-articular injection of 3 mg of monoiodoacetate (MIA). Single target compounds (URB-597 – FAAH inhibitor, 5 mg/kg; SB-366791 – TRPV1 antagonist, 2 mg/kg) and dual-acting compound OMDM-198 (FAAH inhibitor/TRPV1 antagonist, 1 mg/kg) were used in present studies. Antinociceptive potential of all drugs was measured by the means of PAM test at day 14 after OA induction. At day 21 rats were sacrificed 1h after i.p. drugs' administration (time point corresponding to drugs' highest pharmacological efficacy at day 21 after MIA treatment). Molecular alternations in expression of components of ECS after treatment with one of tested drugs were evaluated in spinal cord (SC) and dorsal root ganglia (DRG L3-L5) by RT-qPCR.

Antinociceptive action of URB-597, SB-366791 and OMDM-198 was similar in time course studies, however AUC for OMDM-198 was higher than for other compounds..MIA injection to induce OA model evoked an elevation of FAAH, CB_1 and TRPV1 transcription on the SC level whereas expression of CB_2 was not affected by this inhibitor of glycolysis administration. None of drugs used in the study altered significantly expression of ECS components on the SC level compared to the vehicle. Pain phenotype observed at day 21 after MIA administration correlated with upregulation of CB_2 mRNA expression on the DRG level. Tested compounds did not abolish elevation of CB_2 mRNA in DRG of MIA rats. URB-597 tended toward increase levels of CB_1 and TRPV1 in DRG, although had no effects on FAAH expression. Similar pattern of expression was observed after treatment with SB-366791, TRPV1 antagonist, although 1h after treatment ipsilateral elevation of FAAH mRNA was observed in comparison to vehicle group. Administration of OMDM-198 resulted in increase of bilateral expression of CB1 and TRPV1 receptors and FAAH enzyme.

Acquired data provide insight into molecular changes, particularly in DRG, after systemic treatment with mentioned compounds. Alteration of ECS components on SC and DRG level in the later stage of OA points out that modulation of that system might be of benefit in restoring endogenous balance in nervous system. OMDM-198 treatment showed most prompt changes in ECS gene expression, which correlate with highest antinociceptive potential of this compound. Obtained results suggest OMDM-198 as a suitable candidate for the OA treatment and may help to explain mechanism leading to better efficacy of dual-target compounds modifying ECS functions.

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BEHAVIORAL PROFILING OF AM1346, A HIGH AFFINITY CB1R ANANDAMIDE ANALOG, USING DRUG DISCRIMINATION

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Using a drug discrimination procedure, rats were trained to discriminate between AM1346 [N-((5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraen-1-yl)-2-methoxyacetamide] at a dose of 5.6mg/kg and vehicle injected i.p. 20 min prior to the session. Substitution studies were performed with the training drug itself, Δ^9-THC and WIN55,212-2 examined at varying doses. The potency differences among these three CB1R agonists were: Δ^9-THC=WIN55,212-2 > AM1346. Surmountable antagonism studies were performed with rimonabant in the 5.6 mg/kg AM1346 trained animals as well as with Δ^9-THC and WIN55,212-2. Antagonism challenges were performed by giving simultaneous injections of rimonabant 1 mg/kg and varying doses of AM1346, WIN55,212-2 and Δ^9-THC 20 min prior to the start of the test session. In contrast with previously published data with AM1346 (Järbe et al. 2009), we found that with this higher training dose of AM1346 (5.6 mg/kg), we did not demonstrate surmountable antagonism. A possible reason for this is another key finding, namely that the combination of rimonabant 1mg/kg and AM1346 at training dose and doses higher than training dose produced significant decreases in the rate of lever responding. This was not observed with the two other CB1R agonists tested in this assay i.e., WIN55,212-2 and Δ^9-THC, as surmountable antagonism was observed with these two drugs along with rightward parallel shifts in the dose-effect curves. A long duration of effect was previously reported for AM1346 (3 mg/kg training dose; (Järbe et al. 2009), and here we report that AM1346 at the dose of 5.6 mg/kg have a further prolonged duration of action with a functional *in vivo* half-life around 10 hours. These studies implicate that by structurally modifying endocannabinoid ligands, especially those derived from anandamide, might exhibit a different drug discrimination profile at higher doses as compared to other exogenous CB1R ligands such as e.g. THC and WIN,212-2. This is manifested mainly by their interaction with CB1R antagonists such as rimonabant and AM251. Depending on dose, such exogenous anandamide analogs might also interact with other receptors and targets in the CNS which might have different pharmacological profile as compared to other exogenous CB1R agonist and the body's endocannabinoids.

References:

Järbe TUC, Li C, Liu Q, Makriyannis A (2009) Discriminative stimulus functions in rats of AM1346, a high-affinity CB1R selective anandamide analog. Psychopharmacology (Berl) 203: 229-39

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SEX- AND REGION-DEPENDENT CONSEQUENCES OF ADOLESCENT THC EXPOSURE ON BEHAVIOR AND SYNAPTIC PLASTICITY

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Sex differences have been frequently observed in the biological and behavioral effects of substances of abuse, including cannabis. Exposure to delta-9-tetrahydrocannabinol (THC), the major psychotropic ingredient of Cannabis sativa, during adolescence results in long-term disturbances of cognitive performances and emotional reactivity in adult female rats, whereas preliminary findings suggested that this complex phenotype was not entirely present when the same treatment protocol was performed in adolescent male rats.

In this study, we fully investigated the long-term behavioral consequences of adolescent THC treatment in male rats, as compared to females.

Adolescent male Sprague-Dawley rats were treated with increasing doses of THC twice a day (PND 35-45) and, in adulthood, behavioral tests were performed to check for the presence of alterations in mood and cognition.

Adolescent THC treatment in male rats was associated with lasting cognitive impairment without alterations in the emotional sphere.

At cellular level, altered rearrangement of NMDA and AMPA receptor subunits was observed in hippocampal synaptosomal fractions from THC-exposed rats compared to controls. Changes in the levels of pre- and post-synaptic markers, synaptophysin and PSD95, were also present. Interestingly, the KCl-induced [^{3}H]D-ASP release from hippocampal synaptosomes, but not gliosomes, was significantly enhanced in THC-treated rats compared to controls. Moreover, in the same brain region, adolescent THC treatment also resulted in a persistent neuroinflammatory state, characterized by increased expression of the astrocyte, GFAP, increased levels of the pro-inflammatory markers, TNF-α, iNOS and COX-2, as well as a concomitant reduction of the anti-inflammatory cytokine, IL-10.

As a whole, these data demonstrate that the sex-dependent detrimental effects induced by adolescent THC exposure on behavior may rely on its ability to trigger different region-dependent changes in synaptic plasticity in male and female rats. The prevalence of alterations in the emotional sphere in females is associated with profound changes in the prefrontal cortex, whereas the cognitive impairment observed in males is associated with marked dysregulations in the hippocampus.

CONTEXT DEPENDENT INVOLVEMENT OF 2-ARACHIDONOYLGLYCEROL SIGNALING IN THE REGULATION OF SOCIAL CHALLENGE RESPONDING

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Previous studies showed that endocannabinoid signaling plays a role in the regulation of social behavior. However, the specific contribution of the endocannabinoid 2-arachidonoylglycerol (2-AG) in this aspect remains unclear. In the present study we aim to characterize the specific role of 2-AG in social challenges triggered by the presence of a conspecific in different contexts.

We enhanced 2-AG signaling *via* JZL184-induced inhibition of monoacilglycerol lipase (MAGL) in CD1 mice, then studied social behavior in either the sociability, social interaction or the resident-intruder paradigms. In the latter test the effects of MAGL blockade were studied in resident and intruder mice as well. Furthermore, we measured corticosterone levels to assess 2-AG effects on stress-reactivity, and studied the possible interactions between the endocrine and behavioral effects of MAGL blockade. The dependence of JZL184-induced behavioral effects on CB1 receptor activity were also assessed.

Enhanced 2-AG signaling increased social interest in the sociability paradigm, while it did not affect social anxiety in the social interaction test. 2-AG signaling dramatically attenuated both territorial and defensive aggression. Enhancement of 2-AG signaling increased the stress-reactivity of intruder mice, but its anti-aggressive effects were not secondary of this endocrine change. Behavioral effects were independent of CB1 receptor activity.

Here, we demonstrated that 2-AG signaling modulates social behavior in a context dependent manner. In a novel, neutral environment it mildly facilitates social interest, while during an aggressive conflict it blocks aggressive behavior. The latter behavioral effects occur independently of effects on stress-reactivity, *via* a yet unidentified CB1 receptor-independent mechanism.

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THE ROLE OF THE CANNABINOID RECEPTOR 1 IN STRESS-INDUCED INFLAMMATION AND BEHAVIOUR

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Stress is one of the major environmental factors that contribute to the development of mood disorders, such as depression and anxiety. In the last decades, it has become evident that also inflammatory processes play an important role in the pathology of mood disorders. The endocannabinoid system (ECS) is expressed in the central nervous and the immune system and thus enables communication between both. The ECS, especially the cannabinoid receptor 1 (CB1), is known to be implicated in stress-related disorders such as depression. The contribution of CB1 signalling to the regulation of inflammatory processes is, however, less clear. The aim of this study is therefore to analyse the role of CB1 in stress-induced inflammation and behaviour.

Male wildtype (WT) and CB1 knockout ($CB1^{ko/ko}$) mice were submitted to chronic social defeat stress for 10 days, i.e. were exposed to a new CD1 aggressor mouse for 10 min every day, and housed with visual and olfactory contact to the aggressor for the following 24h. After 10 days, anxiety and depressive-like behaviour was analysed using the open field test, sucrose preference, social avoidance, elevated O-maze, and the Porsolt forced swim test. After behavioural analyses, brain, spleen, and blood were isolated for molecular analyses of ECS components, immune cell populations and inflammatory markers.

$CB1^{ko/ko}$ mice were generally more anxious and showed lower social interaction, also in the unstressed control group. In WT mice, chronic social defeat stress induced strong social avoidance, a mild anxiety phenotype and anhedonia, indicative of depressive-like behaviour. The effects of stress on anxiety and social behaviour were even more pronounced in $CB1^{ko/ko}$ mice. Since non-stressed $CB1^{ko/ko}$ mice are already anhedonic, stress did not further decrease sucrose preference. Preliminary results of molecular analyses indicate that two weeks after the last stress exposure, ECS and inflammatory gene expression in the hippocampus is similar in stressed and unstressed mice. Compared to WT mice, $CB1^{ko/ko}$ mice show slightly altered expression of inflammatory genes, independent of stress. Flow cytometry revealed that in both genotypes, leukocyte and splenocyte subsets are still altered two weeks after the stress, with the number of T-cells being reduced in stressed mice.

In summary, first results indicate that $CB1^{ko/ko}$ mice are more sensitive to social stress. It seems that chronic social defeat induces long-lasting changes in immune cell populations in both WT and $CB1^{ko/ko}$ mice. Future experiments will be neccessary to analyse inflammatory processes during and shortly after the stress exposure to elucidate whether the high stress sensitivity of $CB1^{ko/ko}$ mice is accompanied or caused by an altered immune response.

MONOACYLGLYCEROL LIPASE INHIBITION IN THE VISCERAL INSULAR CORTEX SELECTIVELY ELEVATES 2-AG WHICH INTERFERES WITH ANTICIPATORY NAUSEA IN A RAT MODEL

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Anticipatory nausea (AN) is a conditioned nausea reaction experienced by chemotherapy patients upon returning to the clinic. Currently, there are no specific treatments for this phenomenon with the classic antiemetic treatments (e.g. ondansetron, OND) providing no relief. Elevation of the endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), by systemically administered drugs which interfere with their respective degrading enzymes, fatty acid hydrolase (FAAH) and monoacylglycerol lipase (MAGL), interfere with contextually-elicited nausea-induced conditioned gaping (a rat model of AN). The visceral insular cortex (VIC) has previously been shown to play a critical role in acute nausea-induced conditioned gaping in rats with the modulatory role of the endocannabinoid system being driven largely by 2-AG, however, the role of the VIC in AN remains unknown, as does the modulatory role of the endocannabinoid system. We investigated the potential of intra-VIC administration of both MJN110 (MAGL inhibitor) and PF3845 (FAAH inhibitor) to interfere with AN. We also evaluated the ability of intra-VIC OND to interfere with AN.

MAGL-induced elevation of 2-AG reduced AN, an effect that was reversed by coadministration of the CB1 antagonist, AM251. However, neither FAAH inhibition by PF3845 or 5HT3 receptor antagonism by OND suppressed AN. These findings suggest that the VIC plays a critical role in contextually-elicited nausea-induced conditioned gaping, likely with 2-AG modulating the anti-nausea effect via action at CB_1 receptors. As there are currently no specific therapeutics for chemotherapy patients that develop AN, MAGL inhibition by MJN110 may be a candidate treatment.

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2-OLEOYLGLYCEROL HYDROLYSIS IN THE RAT VASCULATURE BY MONOACYLGLYCEROL LIPASE ACTIVITY

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Monoacylglycerol hydrolysing activity can be detected in both the soluble and the membrane fractions of the rat brain (Ghafouri et al, 2004). Monoacylglycerols, such as 2-oleoylglycerol and 2-AG, are subject to hydrolysis by multiple lipases, including monoacylglycerol lipase (MAGL), ABHD6 and ABHD12 (Savinainen et al., 2012). Recently, JJK048 has been introduced as a selective inhibitor of MAGL (Aaltonen et al., 2013). In this study, we have investigated the use of this selective inhibitor to identify the contributions of MAGL to hydrolysis of 2OG in rat vascular preparations.

Superior mesenteric arteries, abdominal and thoracic aortae were isolated from male Wistar rats aged 8-12 weeks or 6-9 months. Following homogenisation and centrifugation, monoacylglycerol metabolism in cytosolic and membrane fractions was assessed using the hydrolysis of tritium-labelled 2-oleoylglycerol.

The yield of material from membrane fractions was too modest for biochemical characterisation using this assay. Using the soluble fractions, however, 2OG hydrolysis was measurable. Activities in the young animals were similar across the three blood vessels (1.7 ± 0.3, 1.7 ± 0.4 and 1.2 ± 0.3 nmol/min/mg protein for mesenteric artery, abdominal and thoracic aorta, respectively). In the older animals, 2OG hydrolysis in the mesenteric arteries was more variable: 0.9 ± 0.5 nmol/min/mg protein. JJKK048 caused a concentration-dependent inhibition of 2OG hydrolysis in all tissues, with identical potencies (pIC_{50} values of 9.3 ± 0.5, 9.5 ± 0.4 and 9.3 ± 0.2 for mesenteric arteries, abdominal and thoracic aortae from younger animals and 9.8 ± 0.2 for mesenteric arteries from older animals). The maximal inhibitions in the presence of JJKK048 were not different from blanks indicating that MAGL was the predominant 2OG-hydrolysing enzyme in these tissues.

In summary, this study provides evidence for functional expression of MAGL in the rat vasculature.

Aaltonen et al. (2013). *Chem Biol* PM:23521796

Ghafouri et al (2004). *Br J Pharmacol* PM:15492019

Savinainen *et al* (2012) *J Lipid Res* PM:22969151

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CANNABIDIOL-INDUCED VASORELAXATION IN ISOLATED HUMAN PULMONARY AND SMALL MESENTERIC ARTERIES OF HYPERTENSIVE RAT – PRELIMINARY STUDY

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Cannabidiol (CBD), psychoactive inactive constituent of *Cannabis sativa*, has been suggested to play a beneficial role in the cardiovascular system (Stanley et al., *Cardiovasc Res. 2015; 107: 568–578*). To date, there is a lack of data on its potential role in the vasculature in the hypertension, including pulmonary hypertension. The aim of this study was to explore the vasorelaxant properties of CBD in the human pulmonary arteries and small mesenteric arteries of hypertensive rats. Human isolated pulmonary arteries obtained from 11 patients (9 men and 2 women, mean age 63.2±4.2 years) undergoing lobectomy or pneumonectomy during resection of lung carcinoma. Small mesenteric arteries were isolated from spontaneously (SHR) or deoxycorticosterone acetate and high salt-diet treated (DOCA-salt) hypertensive rats or their appropriate normotensive controls Wistar Kyoto (WKY) or uninephrectomized (UNX) animals. Functional experiments were conducted according to Baranowska-Kuczko et al. (*NSAP 2014; 387:477-86; Life Sci. 2015, doi:10.1016/j.lfs.2016.03.014*).

CBD (0.1 -30 μM) but not its vehicle (ethanol; 0.001–0.3% v/v final concentration) relaxed concentration-dependently human pulmonary arteries pre-constricted with U-46619 (pEC_{50}=4.9±0.08, R_{max}=113.0±6.5; n=11). This effect was reduced by denudation of endothelium (R_{max}=44.5±13.7, n=4; P<0.001) and was less potent under KCl-induced tone (R_{max}=27.8±9.5, n=6; P<0.001). CBD-evoked vasorelaxation was also decreased by cyclooxygenase (COX) inhibitor, indomethacin (10 μmol/l) (pEC_{50}=4.0±0.2, R_{max}=73.6±8.4, n=3; P<0.001) and vanilloid TRPV1 receptor antagonist, capsazepine (1 μmol/l) (pEC_{50}=4.3±0.1, R_{max}=80.6±10.4, n=3; P<0.01, P<0.05). Neither antagonists of cannabinoid O-1918-sensitive and CB_1, CB_2 receptors (O-1918, 10 μmol/l; AM251, AM630, both 1 μmol/l, respectively) and or nitric oxide synthase inhibitor, L-NAME (300 μmol/l) had changed the CBD-induced vasorelaxation. In preliminary studies in hypertensive animals, we found that CBD caused a complete relaxation of the phenylephrine-pre-constricted rat mesenteric artery; compared to the respective control rats (WKY: pEC_{50}=6.1±0.2, R_{max}=98.8±0.4, n=4; UNX: pEC_{50}=5.7±0.1, R_{max}=94.2±4.2, n=8) the effect of CBD was slightly less potent in SHR (pEC_{50}=5.7±0.1, R_{max}=94.2±4.2, n=8, P<0.05) but stronger in DOCA-salt hypertensive rats (pEC_{50}=6.1±0.1, R_{max}=96.1±1.9, n=8; P<0.05).

These data demonstrated that CBD relaxes human pulmonary and rat hypertensive mesenteric arteries. In humans, its vasorelaxant effect is endothelium- and COX-dependent and may involve TRPV1 receptors and potassium channels. Potency of the CBD-mediated vascular effects in rats are dependent of experimental model of hypertension. More studies is required to explain CBD-mediated hypotensive mechanism in the light of its vascular effect in systemic and pulmonary vessels in normo- and hypertension.

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POTENTIAL INVOLVEMENT OF COX-2 MEDIATED BREAKDOWN OF ENDOCANNABINOIDS ON POST-SEIZURE ISCHEMIA/HYPOXIA

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Our laboratory has recently discovered a long-lasting period of severe hypoxia (pO_2<10mmHg for over an hour in the rat hippocampus) with concomitant constriction of local blood vessels following the termination of brief seizures. Despite known vasodilatory properties of cannabinoids, administration of the CB1 receptor agonist ACEA (5 mg/kg) prior to seizure induction had no impact on seizure-induced vasoconstriction and hypoxia. Interestingly, pre-administration, but not post-administration, of a COX-2 inhibitor, or genetic knockdown of COX-2, were able to completely prevent hypoxia following seizures. Since the vasoactive prostanoids have extremely short half-lives (less than a minute), they are unlikely to be responsible for the prolonged vasoconstriction that we observed. Indeed, blocking PGE2 and TXA2 synthesis had no effect on post-seizure ischemia/hypoxia.

COX-2 is also capable of breaking down the endocannabinoids anadamide (AEA) and 2-arachidonoylglycerol (2-AG) to the resulting prostaglandin-ethanolamides (PG-EAs) and prostaglandin glycerol esters (PG-Gs), respectively. Most importantly, the AEA-derived PG-EAs have much longer half-lives and may explain the long-lasting ischemia/hypoxia following seizures. To evaluate the importance of this pathway in mediating post-seizure hypoxia, we tested two hypotheses. (1) Administration of a FAAH inhibitor (URB597) would augment hypoxia by shifting more AEA to COX-2 degradation and (2) selective inhibition (LM-4131) of endocannabinoid degradation by COX-2, while leaving AA breakdown intact, will prevent hypoxia following seizures. Furthermore, we will investigate which PG-EAs might play a role in mediating this response and their potential receptors. These experiments will further elucidate the role COX-2 plays in post-seizure ischemia/hypoxia and potentially identify more targeted drug candidates.

CANNABINOIDS AND EXPERIMENTAL MYOCARDIAL INFARCTION: A SYSTEMATIC REVIEW AND META ANALYSIS

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The current literature suggests a cardio-protective role for cannabinoids (CB) in myocardial infarction (MI) with both CB_1 and CB_2 receptors implicated in the phenomenon of cardiac preconditioning and individual studies showing infarct reducing effect upon cannabinoid application. The current systematic review and meta-analysis intends to summarise the existing literature and data from animal studies investigating the effect of cannabinoid receptor ligands in MI.

Pubmed, Medline and Web of Knowledge were searched for publications where cannabinoids were applied in animal models of MI. Inclusion protocol included: Measurement of infarction size, a CB receptor ligand being administered, and use of either an ex vivo or in vivo animal model. CB receptor agonists and antagonists were split into seven drug 'classes' based upon origin and/or mechanism of action – CB_1 agonists, CB_2 agonists, CB_1/CB_2 agonists, phytocannabinoids, endocannabinoids, CB_1 antagonists and CB_2 antagonists, Differences in infarct volume between drug and control were compared using standardised mean difference (SMD). Pre-specified subgroup analysis included species, study quality, experimental protocol, time to administration, dose and drug class.

21 relevant publications, featuring rabbits, rats and mice with no co-morbidities (55 experiments, 684 animals), were extracted from 844 database records. Most publications tested more than one cannabinoid class. Three drug 'classes' had an overall significant beneficial effect on infarction size both *in vivo* and *ex vivo* (CB2 agonists, SMD -1.78, CI -2.44 to -1.12, $p<0.00001$; endocannabinoids, SMD -1.81, CI -2.47 to -1.15, $P<0.00001$; and phytocannabinoids, SMD -1.54, CI -2.34 to -0.74 $P=0.0001$). CB2 agonists and phytocannabinoids showed significant effect when administered post-MI. Three drug classes had significantly effect at certain doses when given pre-ischemia and had no overall effect (CB1/CB2 agonists, 3.5mg/kg, *in vivo*, SMD -4.55, CI -8.06 to -1.03, $P=0.01$; CB1 agonists, 50nM, *ex vivo*, SMD -2.22 CI -3.81 to -0.63 $p=0.006$; CB1 antagonists, 10mg/kg, *in vivo*, SMD -1.28, CI -2.25 to -0.30, $P=0.01$). CB2 antagonists had no significant effect *in vivo* or *ex vivo*.

Several cannabinoids may hold therapeutic potential in reducing infarct volume following MI in particular CB_2 agonists and phytocannabinoids. Current experimental models are largely *ex vivo* with pre-MI CB application, animals featuring no comorbidities and no co-application of relevant post-MI drugs. Therefore further research is needed into application of cannabinoids at varying dosage, post-MI, *in vivo,* with co-application of relevant drugs.

G_S COUPLING OF CB1: LIGAND BIAS AND THE INFLUENCE OF RECEPTOR NUMBER

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CB1 canonical signalling is usually understood to involve receptor coupling to Gi/o proteins and thereby affecting inhibition of cAMP production by adenylate cyclases. However, CB1 has been shown to couple to other G proteins under various experimental conditions, and the mechanisms by which such 'signalling switches' occur remains uncharacterised. Putative mediators of a switch to Gs protein-coupling include CB1 dimerisation (Bonhaus et al., J Pharmacol Exp Ther, 287 (1998) 884-8; Glass et al., J Neurosci, 17 (1997), 5327-33), and blockade of the 'primary' CB1 Gi/o-coupling phenotype_ENREF_2 (Glass et al., 1997). An additional observation in our laboratory is that heterologous transient transfection of CB1 (resulting in high receptor expression levels) also results in Gs signalling in cAMP assays. These findings led us to speculate that CB1 couples to Gs proteins under conditions when Gi is limited. In this study, we examined the role of receptor number (CB1 expression per cell) in cAMP signalling outcomes, and characterised a panel of common CB1 agonists that differentially modify these pathways. Assays for cAMP were performed on HEK cells heterologously expressing HA-tagged human CB1, both with and without a preprolactin signal sequence tag (pplss) to generate a wide range of receptor expression levels. Agonists CP55,940, WIN55,212-2, AEA, 2-AG, BAY59-3074, and Δ^9-THC were tested for the ability to stimulate or inhibit cAMP production in the presence and absence of pertussis toxin, using a cAMP BRET biosensor as previously described (Cawston et al., Br J Pharmacol, 170 (2013), 893-907). Systematic receptor knockdown was achieved using the novel covalent antagonist AM6544, and Gi protein supplementation was achieved using a GNAI1 plasmid and transient transfection protocol.

CB1 agonists inhibited forskolin-induced cAMP levels in hCB1-HEK cells. In pplss-hCB1-HEK cells, agonist treatment induced cAMP stimulation with a wide range of efficacies. THC and BAY required pertussis toxin pre-treatment in order for this Gs signalling to be unmasked. To determine the dependence of the signalling switch on receptor number, we employed pharmacological receptor knockdown using the covalent antagonist AM6544. As receptor number was progressively reduced by increasing concentrations of AM6544, *net* cAMP signalling of pplss-hCB1 HEK cells treated with CP or WIN shifted from stimulatory (Gs-like) to inhibitory (Gi/o-like), while cells treated with THC shifted from no *net* signalling to inhibitory. Similarly, supplementing Gi expression in these cells resulted in a cAMP signalling alteration in the balance of Gs and Gi signalling, with Gi transfection aligning with decreased efficacy in the Gs pathway (CP and WIN) and increased efficacy in the Gi pathway (THC). This study may therefore begin to correlate CB1 signalling phenotypes with pathologies where CB1 receptor number is a factor, including cancer (e.g. Chung et al., Eur J Cancer, 45 (2009), 174-82; Velasco et al., Prog Neuropsychopharmacol Biol Psychiatry, 64 (2016), 259-66).

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COMPARISON OF REPRESENTATIVE SYNTHETIC CANNABINOIDS TO THC

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The synthetic cannabinoid agonists JWH-073, JWH-018 and AM2201 are commonly found in synthetic cannabinoid preparations (sold as "Spice" or "K2", etc.) that are currently on the market as recreational drugs. Reports of users admitted to hospitals with serious health effects and even death indicates that these compounds may be more toxic than marijuana or Δ^9-tetrahydrocannabinol (THC), which results in relatively few hospital admissions and does not appear to have lethal effects.

The time course and dose-response relationship of JWH-073 was determined alongside THC in C57BL/6J mice by i.p. injections. Initially 15 mg/kg JWH-073 was used to determine the time course. Body temperature dropped by about 3.5°C from 30-150 min and the effect diminished to a 2°C drop at about 4 hours after injection. Latency to tail withdrawal showed a small antinociceptive effect from 30-120 min, which peaked at 75% of the Maximum Possible Effect (%MPE) at 150 min, then quickly subsided by 4 hours after injection. Next doses of 3-30 mg/kg JWH-073, and 12.5-50 mg/kg THC were tested. JWH-073 and THC decreased temperature with ED_{50} values of 19 and 22 nM, respectively. For antinociception, JWH-073 and THC gave ED_{50} values of 9.5 and 13 nM, respectively. Mice exhibited sedation and splayed hind limbs after treatment with either drug, but no other overt signs of toxicity were noted.

Next groups of 8 mice were treated chronically (daily) with each vehicle (5:1: 44 sesame oil: Tween-80: ddH_2O), 30 mg/kg JWH-073 or 50 mg/kg THC. Treatments and determination of the hypothermic and analgesic effects continued for 4 days until complete tolerance to the initial doses was observed. One day after the last dose, mice were administered the CB_1 antagonist SR141716A (rimonabant), to precipitate withdrawal. Antagonist evoked far more scratching and grooming in mice treated vehicle than either JWH-073 or THC. On the other hand, only drug-treated mice showed forepaw fluttering after antagonist.

Immediately following the withdrawal observation period mice were sacrificed. The major organs were collected for necropsy/determination of toxicity in any organs and/or muscle, and these data are pending completion of those studies.

To date JWH-073, did not appear very different from THC in effects that were assessed or even potency. Further studies will include necropsy of mice treated acutely and chronically with THC, JWH-073 and the additional synthetic cannabinoids, JWH-018 and AM2201.

A CANNABINOID LINK BETWEEN MITOCHONDRIA 1 AND MEMORY

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Brain activity critically depends on the high energetic support provided by mitochondria, the cell organelles transforming energy sources into ATP. Acute cannabinoid intoxication induces amnesia in humans and animals6-8, and the activation of type-1 cannabinoid receptors present at brain mitochondria membranes(mtCB1) can directly alter mitochondrial energetic activity9-11. Whereas thepathological impact of chronic mitochondrial dysfunctions in the brain is well established, the involvement of acute modulation of mitochondrial activity in high brain functions, including learning and memory, is unknown. Here, we show that acute cannabinoid-induced memory impairment requires activation of hippocampal mtCB1 receptors. We found that intramitochondrial inhibition of soluble-adenylyl cyclase (sAC) signaling through Gαi protein activation mediates the mtCB1 receptor dependent decrease of brain cellular respiration. Pharmacological inhibition of hippocampal sAC activity or genetic exclusion of CB1 receptors from hippocampal mitochondria prevents cannabinoid-induced reduction of mitochondrial mobility, synaptic transmission and memory formation. In addition, intramitochondrial mtCB1 receptor signaling decreases protein kinase A (PKA)-dependent phosphorylation of specific subunits of the mitochondrial electron transport system. Thus, the G protein-coupled mtCB1 receptors regulate learning processes *via* modulation of mitochondrial energy metabolism. By directly linking mitochondrial activity to memory formation, these data reveal that bioenergetic processes are primary acute regulators of cognitive functions.

MAPPING CANNABINOID RECEPTOR 1 ALLOSTERIC SITE(S): CRITICAL MOLECULAR DETERMINANT AND SIGNALING PROFILE OF GAT100 - A NOVEL, POTENT AND IRREVERSIBLY BINDING PROBE

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One of the most abundant G-protein coupled receptors (GPCRs) in brain, the cannabinoid 1 receptor (CB1R) is a tractable therapeutic target for treating diverse psychobehavioral and somatic disorders. Adverse on-target effects associated with small-molecule CB1R orthosteric agonists and inverse agonists/antagonists have plagued their translational potential. Allosteric CB1R modulators offer a potentially safer modality through which CB1R signaling may be directed for therapeutic benefit. Rational design of candidate, drug-like CB1R allosteric modulators requires greater understanding of the architecture of the CB1R allosteric endodomain(s) and the capacity of CB1R allosteric ligands to tune the receptor's information output. We have recently reported the synthesis of a focused library of rationally designed, covalent analogs of Org27569 and PSNCBAM-1, two prototypic CB1R negative allosteric modulators (NAMs). Among the novel, pharmacologically active CB1R NAMs reported, the isothiocyanate GAT100 emerged as the lead by virtue of its exceptional potency in the [^{35}S]GTPγS and β-arrestin signaling assays and its ability to label CB1R as a covalent allosteric probe with significantly reduced inverse agonism in the [^{35}S]GTPγS assay as compared to Org27569. We then performed a comprehensive functional profiling of GAT100 across an array of important downstream cell-signaling pathways and analysis of its potential orthosteric probe-dependence and signaling bias. The results demonstrate that GAT100 is a NAM of the orthosteric CB1R agonist CP55,940 and the endocannabinoids 2-arachidonylglycerol and anandamide for β-arrestin1 recruitment, PLCβ3 and ERK1/2 phosphorylation, cAMP accumulation, and CB1R internalization in HEK293A cells overexpressing CB1R and in Neuro2a and ST*Hdh*$^{Q7/Q7}$ cells endogenously expressing CB1R. Distinctively, GAT100 was a more potent and efficacious CB1R NAM than Org27569 and PSNCBAM-1 in all signaling assays and did not exhibit the inverse agonism associated with Org27569 and PSNCBAM-1. Computational docking studies implicate C7.38(382) as a key feature of GAT100's ligand-binding motif. These data help inform the engineering of newer-generation, druggable CB1R allosteric modulators and demonstrate the utility of GAT100 as a covalent probe for mapping structure-function correlates characteristic of the druggable CB1R allosteric space.

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COVALENT ANALOGS OF THE ALLOSTERIC LIGAND ORG 27569 – MOLECULAR DYNAMICS, SYNTHESIS, AND PHARMACOLOGY STUDIES DIRECTED AT IDENTIFYING THE CB1R ALLOSTERIC BINDING SITE

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With the aim of characterizing the allosteric binding site and the mechanism of action of ORG27569 on the function of the CB1R, molecular dynamics simulations were studied that afforded the hypothesis that ORG acts at a site that involves TMH6, TMH7 and the EC-3 loop. It is further proposed, that ORG binding at this site triggers an intracellular opening formed by movement of the TMH7/Hx 8 elbow-the receptor region associated with BArr signaling. In order to test this hypothesis, a crosslinking experiment was envisioned wherein isothiocyanate analogs of ORG were designed that could covalently bind to a point mutated residue (A6.53C), thus testing the hypothesis.

A description of the molecular dynamics simulation, design of the crosslinking analogs, the syntheses of these ligands, and their pharmacological characterization will be presented.

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FLUORINE-WALK ON GAT211, A POSITIVE ALLOSTERIC MODULATOR OF THE CANNABINOID 1 RECEPTOR: IDENTIFICATION OF CRITICAL SITES FOR ADVANCING STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

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Cannabinoid 1 receptor (CB1R), the most abundant class-A G protein-coupled receptor (GPCR) in the brain, governs many important physiological functions of the body and dysregulated CB1R activity has been implicated in the pathogenesis of a variety of disease states. Undesirable side effects associated with orthosteric agonists/antagonists of CB1R have greatly limited their translational potential. A promising alternative approach for therapeutically targeting CB1R is the development of positive allosteric modulators (PAMs), which, by binding to a sub-type-specific and topographically distinct site (allosteric site) from the orthosteric pocket, can modulate the action of endocannabinoids and synthetic cannabinoids, and thus act more selectively to tune CB1R signaling in a site- and event-specific fashion.

Earlier we reported a class of ligands with a 2-phenylindole scaffold which acts as PAM for the CB1R. Especially with GAT211, we identified that, by binding to the allosteric site, it enhanced CB1R activity by increasing the affinity, efficacy and potency of the CB1 orthosteric agonists in in-vitro assays. Our work also identified stereochemical requirements for PAM activity within this scaffold. In in-vivo studies, we observed that, this compound was effective in glaucoma-related intra-ocular pressure management along with retinal ganglionic cell (RGCs) preservation. This PAM also demonstrated significant therapeutic efficacy in the treatment of neuropathic pain in preclinical animal models. All these findings have sparked deep interest in understanding productive structure-activity relationship around GAT211 to identify candidates with improved efficacy, potency, solubility and oral and ocular bioavailability.

In the design of novel drugs it was observed that introduction of fluorine can be highly advantageous due to improvement of chemical properties, biological activity, metabolic stability, lipophilicity and bioavailability. Especially in the form of 'fluorine-walk', it is used to overcome a flat- SAR and understand the electronic properties of the receptor binding pocket. Based on this understanding we decided to substitute a hydrogen atom with a fluorine atom at various position on rings "A, C and D" of GAT211 (Fig. 1).

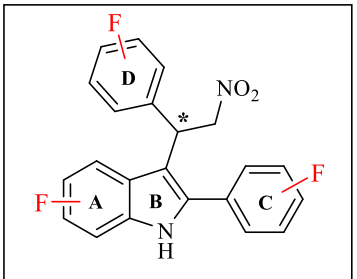

Fig. 1 Fluorine-walk on GAT211

All compounds were designed and synthesized in-house and were tested in the absence and presence of an orthosteric ligand for their functional potency and efficacy in GTPγS, cAMP and β-arrestin assays. Some of these compounds exhibited higher efficacy and potency compared to the parent ligand and also demonstrated allosteric agonism when tested alone. Overall, application of the 'fluorine-walk' strategy identified critical sites on the 2-phenylindole moiety for enhancing CB1R PAM activity.

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CANNABIS MICROBIOME SEQUENCING REVEALS *PENICILLIUM PAXILLI* AND THE POTENTIAL FOR PAXILLINE DRUG INTERACTIONS WITH CANNABIDIOL

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Utilizing next generation sequencing, we surveyed the 18S and 16S Internal Transcribed Spacer regions (ITS) in over 34 cannabis flower samples and compared these results to various culture based detection methods. Sequencing identified over 30 frequently found micro-organisms on cannabis flowers. Of the frequently found species are *Penicillium paxilli* and *Penicillium citrinum* both of which have the capacity to synthesize compounds known to interact with cannabinoid pathways in human[1].

These fungal organisms can evade culture based detection systems and may be relevant to cannabinoid based therapies because of the associated biological toxins they produce. Paxilline, produced by *P. paxilli* is a tremorgenic and ataxic potassium channel blocker and has been shown to attenuate the anti-seizure properties of cannabidiol in certain mouse models. Paxilline is reported to have tremorgenic effects at nanomolar concentrations and is responsible for Ryegrass-staggers disease. Cannabidiol is often used at micromolar concentrations for seizure reduction implying sub-percentage contamination of paxilline could present a theoretical risk. Citrinin is a mycotoxin that disrupts Ca2+ efflux in the mitochondrial permeability transition pore (mPTP). Ryan *et al*. demonstrated that cannabidiol affects this pathway also suggesting a theoretical concern for CBD-mycotoxin interaction[2]. Considering the hydrophobicity of paxilline and the recent interest in the use of cannabidiol derived from *Cannabis* flower oils for drug resistant epilepsy, more precise molecular screening of fungal toxins may be warranted.

To this end we have expanded the microbiome sampling to include 34 samples while also surveying bacterial 16S amplicons in addition to the 18S fungal amplicon sequencing. We discovered more samples with *P.citrinum*, several *Staphyloccus, Pseudomonas* and *Proprionibacterium*. These sequencing data were compared to culture based techniques that demonstrate frequent bacterial contamination in Yeast and Mold culturing systems responsible for false positive Yeast and Mold failure of Cannabis crops. Together, these data support the FDA's migration to sequencing based surveys in food born pathogen testing.

1. McKernan Cannabis microbiome sequencing reveals several mycotoxic fungi native to dispensary grade Cannabis flowers *F1000Research* **4:1422** (2015).

2. Ryan, D., Drysdale, A.J., Lafourcade, C., Pertwee, R.G. & Platt, B. Cannabidiol targets mitochondria to regulate intracellular Ca2+ levels. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **29**, 2053-2063 (2009).

SINGLE MOLECULE SEQUENCING OF THCA AND CBDA SYNTHASE REVEALS COPY NUMBER VARIATION IN MODERN DRUG-TYPE *CANNABIS SATIVA* L. AND SHEDS NEW LIGHT ON THE BT:BD ALLELE

Kevin J McKernan, Yvonne Helbert, Adrian Devitt-Lee, Vasisht Tadigotla, Stephen McLaughlin, Jessica Spangler, Lei Zhang, Ryan Lynch and Douglas Smith

It has been proposed that variation in the THCA/CBDA ratio in Cannabis is due to sequence variations at a single genetic locus (B) wherein sequence variations in the THCAS and CBDAS genes, and/or to polymorphisms in nearby regulatory elements result in THCA or CBDA ratio chemotypes. Here we explore the use of Pacific Biosciences single molecule circular consensus sequencing to better understand the B locus in over 50 chemically diverse cannabis samples. Through the generation of phased single molecule sequences of the genomic loci encoding THCAS and CBDAS, we uncovered both copy number and coding sequence variation in the THCAS and CBDAS genes. These results support the codominant model wherein the Bt (THC dominant) and Bd (CBD dominant) alelles represent functional and dysfunctional amino acid variants in both enzymes.

The results also reveal single nucleotide variants within the sequences targeted by commonly used primer pairs for analysis of Bt and Bd genotypes, described in Onofri *et al*, Weiblen *et al*. and Staginnus *et al*[1-3]. These results highlight the importance of screening primer pairs across diverse populations to avoid the potential mis-classification of Bt:Bd genotypes. For example the primers described by Staginnus *et al*. will not bind effectively A250D variants found in some Bt alleles. We also discover variants within previously described CBDAS primer binding sites that create allele drop out in previous surveys of this marker. Overall this work supports the model where a single B locus with codominant allelic series largely controls *Cannabis* THC:CBD ratio phenotypes. Additional homologous gene copies of the B locus were found to contain frameshift mutations or multiple amino acid alterations that limit or remove their protein coding potential.

1. Onofri, C., de Meijer, E.P. & Mandolino, G. Sequence heterogeneity of cannabidiolic- and tetrahydrocannabinolic acid-synthase in Cannabis sativa L. and its relationship with chemical phenotype. *Phytochemistry* **116**, 57-68 (2015).

2. Weiblen, G.D. et al. Gene duplication and divergence affecting drug content in Cannabis sativa. *The New phytologist* **208**, 1241-1250 (2015).

3. Staginnus, C., Zorntlein, S. & de Meijer, E. A PCR marker linked to a THCA synthase polymorphism is a reliable tool to discriminate potentially THC-rich plants of Cannabis sativa L. *Journal of forensic sciences* **59**, 919-926 (2014).

GENOMIC AND CHEMICAL DIVERSITY IN *CANNABIS*

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Plants of the genus *Cannabis* produce at least 113 phytocannabinoids and at least 120 terpenoids. Even though work continues to demonstrate the therapeutic potential of several cannabinoid and terpenoid compounds, our understanding of global *Cannabis* diversity and history remains quite limited. A more complete assessment of cultivated and feral *Cannabis* diversity can facilitate the development of novel and optimized varieties, with improved agronomic traits and phytochemical profiles. Templates for this process are found in other major agricultural crop complexes, such as corn or sunflowers. As a step towards these long-term goals, we are assaying the standing diversity found in *Cannabis* by building a database of genomic sequence and chemotypic data (strainSEEK^{TM}). These data will illuminate the structure of *Cannabis* diversity and drive the development of genetic markers for important phenotypic traits.

Our approach uses a restriction digest genomic DNA library preparation method, coupled to multiplexed Illumina sequencing. On average, our protocol yields at least 10x coverage of 625,000 sites of the current reference genome. These sites contain 25,000 to 30,000 informative single nucleotide polymorphisms (SNPs), which are used for downstream population genetic analyses. Chemotypic data is generated by several collaborating labs, and includes quantification of various cannabinoids and terpenoids from dried inflorescence.

After sequencing over 200 samples, and combining these with other publically available data, our analyses provide significant evidence for at least five major groups of genetic diversity and at least three groups of chemotypic diversity. Due to an extensive history of hybridization and gene flow, driven by wind pollination biology and human cultivation, the boundaries among these groups are uncertain, but will be improved through deeper sampling--particularly from European and Asian gene pools. Establishing this baseline genomic and chemotypic database provides immediate clarity for many strain name issues that plague the cannabis market and hinder clinical research efforts. Additionally, these data set the framework for the modernization of *Cannabis* breeding efforts, suggesting where heterotic groups may be found, and supporting projects such as multi-parent advanced generation intercross (MAGIC) population development.

USE OF LIVE CELL IMAGING SYSTEM TO EVALUATE BIOLOGICAL ACTIVITY OF HEMP EXTRACT

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The quality of plant products it is difficult to evaluate as a complex and not as individual compounds. The aim of this work was to find out the sensitivity of an unspecific biological test for evaluation of the complex character of the hemp extract that were obtained from different plant parts and different densities of growing.

For trials, Tiborszálási variety of industrial hemp was chosen. Plants were cultivated with density 100, 200 and 300 plants per square meter. This experiment was outdoor, annual, small-plot, established in two places in the same climatic region in the Czech Republic. The plant material was pulverized and extracted for 24 hours in growth medium. Extract was diluted in 3 ratio: 0.01, 0.001 and 0.0001. The method of Live Cell Imaging System was used for evaluation of biological effectivity of extracts, which represented time micro-cinematography collecting method. Obtained images were analyzed in order to discover the speed of growth of L929 animal tissue culture. This speed was interpreted like the average period of duplication.

The unspecific biological test of plant extracts clearly proved dependency between dilution of extract and effects on fibroblastoidal cell line L929. Specific growth rate index was 0.029 (±0.003) for dilution ratio 0.001 and 0.018 (±0.006) for dilution ratio 0.01. The expected stimulatory effect was recorded in the most dilute samples (0.0001) and exhibited 116.5 percentage of the control activity. Tests confirmed sensitivity to complex content of compounds in samples from different densities of plant growing. These significant differences were 23.3 percentage of control activity for100 plants/m^2 and 61.2 percentage of control activity for 300 plants/ m^2 by dilution ratio 0.01. It confirmed the importance of growing conditions of hemp for quality and effectivity of its extracts.

FATTY ACID AMIDE HYDROLYSE INHIBITORS CONFER ANTI-INVASIVE AND ANTIMETASTATIC EFFECTS ON LUNG CANCER CELLS

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Inhibition of endocannabinoid degradation has been suggested as tool for activation of endogenous tumor defense. One of these strategies lies in blockade of the enzyme fatty acid amide hydrolase (FAAH) which catalyzes the degradation of endocannabinoids (anandamide [AEA], 2-arachidonoylglycerol [2-AG]) and endocannabinoid-like substances (N-oleoylethanolamide [OEA], N-palmitoylethanolamide [PEA]). The present study investigates the impact of two FAAH inhibitors (arachidonoyl serotonin [AA-5HT], URB597) on A549 lung cancer cell metastasis and invasion. LC-MS analyses revealed increased levels of FAAH substrates (AEA, 2-AG, OEA, PEA) in cells incubated with either FAAH inhibitor. In athymic nude mice FAAH inhibitors were shown to elicit a dose-dependent antimetastatic action. In vitro, a concentration-dependent anti-invasive action of either FAAH inhibitor was demonstrated, accompanied with upregulation of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1). Using siRNA approaches, a causal link between the TIMP-1-upregulating and anti-invasive action of FAAH inhibitors was confirmed. Moreover, knockdown of FAAH by siRNA was shown to confer decreased cancer cell invasiveness and increased TIMP-1 expression. Inhibitor experiments point toward a decisive role of CB_2 and transient receptor potential vanilloid 1 in conferring the anti-invasive effects of FAAH inhibitors and FAAH siRNA. Finally, antimetastatic and anti-invasive effects were confirmed for all FAAH substrates. Collectively, the present study provides first-time proof for a pronounced antimetastatic action of the FAAH inhibitors AA-5HT and URB597. As underlying mechanism of its anti-invasive properties an upregulation of TIMP-1 was identified.

2-ARACHIDONOYLGLYCEROL LEVELS IN DU145 PROSTATE CANCER CELLS FOLLOWING TUMOUR NECROSIS FACTOR-α TREATMENT

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It is now well established that cyclooxygenase-2 (COX-2) metabolises the endocannabinoids AEA and 2-AG to form biologically active prostaglandin ethanolamides and prostaglandin glyceryl esters. In dorsal root ganglion cells cultured under inflammatory conditions, blockade of COX-2 increases the levels of both AEA and 2-AG (1). In contrast, in lipopolysaccharide + interferon-γ-gamma stimulated RAW264.7 macrophage cells, inhibition of COX-2 by either flurbiprofen at a dose blocking prostaglandin production has no significant effect upon 2-AG or AEA levels (2). In order to shed further light upon the functional importance of COX-2 in the regulation of endocannabinoid levels, we have investigated the effect of tumour necrosis factor-α (TNF-α) treatment of human DU145 prostate cancer cells upon the released and cellular levels of 2-AG, AEA and related N-acylethanolamines (NAEs).

DU-145 cells in six well plates were treated for 4 hours with 20 ng/ml TNF-α. This treatment protocol has been reported to produce an increased expression of COX-2 in these cells (3). At 0, 1, 2, 3 and 4 h after addition of TNF-α or vehicle, aliquots of the cell medium and cell samples for qPCR were collected. Finally, after the 4 h sampling, the remaining medium was removed and the cells were collected. Samples were extracted and analysed for endocannabinoid and NAE levels by a tandem mass spectrometry method and COX-2 and monoacylglycerol lipase (MAGL) mRNA expression were measured with qPCR (4).

TNF-α produced a greater increase in COX-2 mRNA levels than seen under control conditions. Thus, mean values (as % of the controls at t=0, n=6) in the cell lysates were: 120, 1260, 430, 570 and 320 at t= 0, 1, 2, 3 and 4 h, respectively. The corresponding values for the controls were 100, 350, 130, 130 and 100, respectively. In contrast, mRNA levels for MAGL were unchanged following TNF-α treatment. In the medium, 2-AG, PEA, OEA and SEA could be reliably measured, whereas levels of AEA were very low and not deemed to be sufficiently robust. For 2-AG median levels (in pmol / total medium, n=6) values for control and TNF-α treated cells, respectively were t=0h, 0.73 and 0.76; t=1 h, 0.62 and 0.63; t=2 h, 0.59 and 0.49; t= 3 h, 0.52 and 0.56; t=4 h, 0.45 and 0.50. P values, determined using the default settings for the functions sppba, sppbb and sppbi in the WRS2 package for the R statistical programme, for the main effects of TNF-α, time and the interaction TNF-α x time were 0.92, 0.002 and 0.69, respectively. Similar significance levels were found for PEA, SEA and OEA. In the cell extracts, no significant differences were seen between vehicle and TNF-α-treated cells for any of these lipids. We thus conclude that the treatment paradigm used does not affect 2-AG or NAE levels or release in DU145 prostate cancer cells.

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(1) Duggan et al., Nat Chem Biol 7 [20113] 803-9; (2) Gouveia-Figueira et al., PLoS ONE 10 [2015] e0139212; (3) Subbarayan et al., Cancer Res 61 [2001] 2720-6; (4) Gouveia-Figueira & Nording, Prostaglandins Other Lipid Med 121 [2015] 110-21

CANNABINOIDS AND MULTIPLE MYELOMA – TARGETING IMMUNOPROTEASOME AS NEW POTENTIAL THERAPY

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The proteasome has emerged as an important target for cancer therapy with the approval of bortezomib, a first-in-class, reversible proteasome inhibitor, for relapsed/refractory multiple myeloma (MM). However, many patients do not respond to bortezomib therapy, whereas others develop resistance, suggesting the need for other inhibitors with enhanced activity.

Two major isoforms of the proteasome have been described, including the constitutive proteasome (PS), which is present in most cells, and the immunoproteasome (iPS), which incorporates the subunits β1i, β2i, and β5i. Carfilzomib (CFZ), a new iPS inhibitor, forms an irreversible dual covalent bond with the catalitic b5i subunit of iPS. In whole-blood and peripheral-blood mononuclear cells from patients with MM, CFZ inhibites chymotrypsin-like (ChT-L) activity of the iPS. Since de-regulation of iPS activity has been described in MM, targeting iPS results in anti-tumor activity and potentiates the effects of chemotherapy in MM. Cannabidiol (CBD) has been found to reduces viability and inducing cell death in MM cell lines, but evidences about the role of CBD in regulating iPS, in MM, has not been studied. Herein, we show data about the role of CBD in regulating iPS and its effects in iPS-regulating pathways, in human MM cell lines.

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CURRENT STATE OF KNOWLEDGE OF CANNABIDIOL'S INFLUENCE ON CANCER CELLS

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Cannabidiol (CBD) is an isomer of THC which does not have psychoactive properties because of its very low affinity to CB receptors. It was demonstrated that CBD binds to a TRPV1 and GPR55 receptors and its mechanism of action relies mainly on induction of production of reactive oxygen species (ROS), which in turn activates apoptotic pathways. Despite this, details of cannabidiol's impact on cells are still not fully elucidated.

The cannabinoids are so far used in palliative medicine, but there are more and more data concerning antiproliferative and proapoptotic cannabinoids' action in cancers. Effects of cannabinoids on cancer cells are various. *In vitro* and *in vivo* studies indicate both inhibiting and inducting cancer cells growth and those effects depend on cannabinoid used, its concentration and type of neoplasm. Cannabidiol (CBD) is cannabinoid with high pharmacological potency. Because of its low affinity to the CB1 receptor, CBD does not exhibit psychoactive properties typical for THC and in the future it may be valuable supplement of classical chemotherapies.

In this work we present current state of knowledge concerning cannabidiol's influence on cancer cells and currently running clinical trials aiming to assess safety of cannabinoid preparations.

CANNABIDIOL ATTENUATES *CLOSTRIDIUM DIFFICILE* TOXIN A (TCDA) DAMAGE IN HUMAN COLON CARCINOMA CACO-2 CELL LINE

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Toxin A (TcdA) is an enterotoxin produced by *Clostridium difficile* and represents the main responsible of several gastrointestinal disorders, as pseudomembranous colitis, following extensive antibiotic treatments. In addition to intestinal inflammation and fluid secretion, TcdA is able to disrupt the intestinal mucosa through the inhibition of RhoA GTPases and the activation of p38 MAPK, both responsible for tight junctions assembly and mucosal integrity, leading to a condition known as "leaky gut". Cannabidiol (CBD) is a non-psychotropic component of *Cannabis sativa* exhibiting a large spectrum of pharmacological activities due to the interaction with different receptors. Recently the protective role of CBD has been observed in a model of intestinal permeability *in vitro* through the interaction with the CB1 receptor. In attempt to enlarge the knowledge about CBD, in this study we aim to elucidate a possible signaling pathway at the base of CBD action in a mucosal damage model *in vitro* in Caco-2 cells exposed to TcdA.

Human colon carcinoma Caco-2 cells were used for the experiments. Cells were grown until confluence to perform transepithelial electrical resistance (TEER) measurements as evaluation of intestinal mucosal integrity and immunofluorescence analysis to estimate occludin and ZO-1 protein expression. Moreover, Caco-2 cells were processed to perform immunoblot analysis to assess the levels of p-p38, RhoA, occludin, ZO-1 and β-actin proteins.

TcdA reduced in a time-dependent manner TEER values. This decrease was significantly counteracted by CBD, that improved the mucosal integrity. Similarly, TcdA markedly reduced the co-expressions of ZO-1 and occludin proteins, whereas CBD restored barrier integrity, enhancing tight junctions proteins expression. Immunoblot analysis showed a significant reduction of RhoA GTPases, β-actin, occludin and ZO-1 expression together with a marked activation of p38 MAPK induced by TcdA. Once again the effect was prevented by CBD. According to previous studies, all the effects showed by CBD occurred in a concentration-dependent manner and our preliminary data indicated that CBD events were significantly reduced in the presence of CB1 antagonist AM251.

CBD improved TcdA-induced damage, restoring the intestinal barrier integrity, through the involvement of CB1 receptor, resulting in a p38 MAPK inhibition and RhoA GTPases activation.

A NOVEL PYRIDINE INVERSE AGONIST AS A RADIOLABELED TOOL COMPOUND FOR CB2 RECEPTOR BINDING KINETIC STUDIES

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In the last decade, drug-target binding kinetics has emerged as a relevant concept in pharmaceutical research. Although often determined *in vitro*, the lifetime of the binary drug-target complex may provide an indication for the resulting *in vivo* efficacy of a potential drug candidate.

In view of the dynamic nature of CB2 receptor (CB2R) expression and its therapeutic potential, we synthesized and characterized [^{3}H]RO6957022, a CB2R inverse agonist, as a radiolabeled tool compound to further study this target. The high affinity and desirable physico-chemical properties (e.g. reduced lipophilicity) make [^{3}H]RO6957022 a superior tool for binding kinetic studies compared to the reference CB2R radioligands. Moreover, the fact that it is an inverse agonist, which targets an ample portion of the receptor population, makes this probe particularly suitable for this kind of *in vitro* studies.

For this purpose, we performed an equilibrium and binding kinetic characterization of [^{3}H]RO6957022 on CHO cells stably expressing hCB2R. Furthermore, affinity studies were carried out for a wide range of CB2 reference ligands, ranging from full, partial to inverse agonists. Finally, we used [^{3}H]RO6957022 to study the kinetic profiles of selected CB2R ligands by competition association experiments, which generated association (k_{on}) and dissociation (k_{off}) rates for these compounds. Taken together, this study shows the potential of [^{3}H]RO6957022 as a relevant new radioligand for hCBR2.

DETAILED STRUCTURE ACTIVITY RELATIONSHIP STUDIES FOR THE DESIGN OF NOVEL AND HIGHLY SELECTIVE CB2 PET TRACERS

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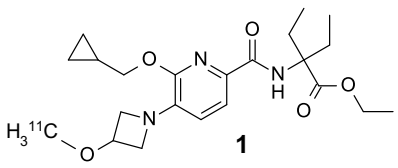
The CB_1 and CB_2 receptors are both part of the endocannabinoid system. Dysregulation in the signalling pathway of CB_2 is considered to be related to several diseases, like autoimmune diseases, allergic asthma, Parkinson's, Alzheimer's and Huntington's disease, heart failure, stroke, hepatitis as well as obesity and osteoporosis[1]. The huge variety shows certainly, that the CB_2 receptor is a very promising target to exploit.

Positron emission tomography (PET) is a powerful molecular imaging technique to investigate neuronal disorders and diseases. One of the biggest advantages of PET is the ability to study dynamic processes *in vivo*, like, for example, drug/receptor interactions[2].

2,5,6-Trisubstituted pyridines were identified to be novel, highly potent and selective CB_2 modulators. Lead optimization work led to the identification of the high affinity CB_2 imaging probe [^{11}C]RSR-056 (**1**)[3].



1

Ki hCB_2	2 nM
Ki hCB_1 / Ki hCB_2	> 1000

We report here on iterative cycles of design, synthesis and characterisation of novel 2,5,6-trisubstituted pyridines with improved absorption distribution metabolism and excretion (ADME) profiles as compared to **1**. A detailed structure activity relationship with regard to CB_2 and CB_1 binding as well as cAMP data has been established. Emphasis was put on high binding selectivity against the CB_1 receptor. Most promising ligands were further profiled with regard to properties which are required for brain penetrable PET tracers such as lipophilicity, passive membrane permeability, plasma protein binding, solubility, clearance and interaction with the P-glycoprotein transporter. A detailed discussion of these results as well as the way forward toward novel and highly selective CB_2 PET tracers will be the subject of this communication.

[1] P. Pacher, R. Mechoulam, *Prog. Lipid Res.* **2011**, *50*, 193-211.
[2] M. Honer, L. Gobbi, L. Martarello, R. A. Comley, *Drug Discovery Today* **2014**, *19*, 1936-1944.
[3] R. Slavik, U. Grether, A. Müller Herde, L. Gobbi, J. Fingerle, C. Ullmer, S. D. Krämer, R. Schibli, L. Mu, S. M. Ametamey, *J. Med. Chem.* **2015**, *58*, 4266-4277.

FUNCTIONAL CHARACTERISATION OF CANNABINOID RECEPTOR 2 SINGLE NUCLEOTIDE POLYMORPHISMS

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With continued interest in Cannabinoid Receptor 2 (CB2) as a therapeutic target, characterisation of CB2 molecular pharmacology is accelerating. The majority of studies on human CB2 (hCB2) to date have likely utilised the NCBI RefSeq / UniProt consensus sequence which specifies glutamine at position 63 in the first intracellular loop (63Q). Interestingly, an allele with arginine at this position (63R) is similarly prevalent in human populations[eg.1,2], effectively implying two CB2 "wild-type" variants exist. Differing frequencies of these relatively common single nucleotide polymorphisms (SNPs) have been associated with disease incidence (immune-related and mental illness), indicating that these variants may possess functional differences. Two further non-synonymous SNPs, L133I and H316Y (intracellular loop two and cytoplasmic tail near helix 8, respectively), have also been identified in the general population and implicated in disease[eg.3,4]_ENREF_48.

We are undertaking a detailed characterisation of the molecular pharmacology of these CB2 variants. Having utilised isogenic DNA integration (Life Technologies Flp-in) to stably express the variants under equivalent conditions in Human Embryonic Kidney cells, we initially investigated any effects of the SNPs on basal expression and subcellular distribution. mRNA levels were verified by qRT-PCR to be equivalent for the majority of cell lines; this data was used to normalise overall expression levels. 63R-containing CB2 variants were expressed at higher levels than 63Q CB2; on average expression was increased by ~50% (P=0.01). Additionally, in both 63Q and 63R backgrounds, presence of L133I enhanced expression relative to its matched wild-type (by 40-80%), whereas H316Y appeared to reduce expression only when accompanied by 63R. We have also initiated studies to investigate whether ligand affinities are influenced by the presence of these SNPs. While CP55,940 and Anandamide binding affinities were equivalent between the CB2 variants, 2-AG exhibited approximately half a log unit lower affinity at hCB2 63R than 63Q (P=0.03), while 63R/L133I also trended towards having slightly lower affinity. Interestingly, however, the 63R/H316Y 2AG Ki was equivalent to 63Q (as were 63Q/L133I and 63Q/H316Y).

These findings are indicative of subtle differences in the expression and cannabinoid binding properties of these different SNP variants which, combined with potential for direct functional heterogeneity, may well produce differing functional fingerprints. The effective presence of at least two "wild-type" CB2 variants in the human population, and potential for lower frequency non-synonymous SNPs to also influence CB2 function, seems to be under-appreciated in the field currently. Improved awareness and understanding of the functional implications of these variants will likely be important for the further development of CB2-targeted therapeutics.

1. Onaivi, E.S., H. Ishiguro, et al., PLoS ONE [Electronic Resource], 2008. 3:e1640.
2. Ishiguro, H., Y. Horiuchi, et al., Biological Psychiatry, 2010. 67:974-82.
3. Minocci, D., J. Massei, et al., Journal of Affective Disorders, 2011. 134:427-30.
4. Tong, D., S. He, et al., J Mol Neurosci, 2013.

IDENTIFICATION OF THE RESIDUES AT THE CANNABINOID CB2 RECEPTOR HOMODIMER INTERFACE

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Considerable evidence suggests that G-protein-coupled receptors (GPCRs) form homomeric and heteromeric dimers. In addition, information regarding the dimer interface has been published for a number of GPCRs. However, currently the information regarding CB2 cannabinoid homodimer and CB2 dimer interface is missing. In the current study, we sought to determine the role of TMH6 in homodimerization of CB2 cannabinoid receptor by cysteine scanning followed by crosslinking experiments. Amino acid residues of TMH6 of CB2 was mutated to cysteine one by one. The crosslinker $HgCl_2$ was applied for crosslinking the cysteines located at the dimer interface. Two residues on TMH6 were identified as part of CB2 homodimer interface, by formation of spontaneous crosslink between A6.60(270)C and formation of cysteine crossline between H6.57(267)C upon addition of $HgCl_2$. Furthermore, upon treatment with cannabinoid agonist CP55940, crosslink between A6.60(270)C was reduced, whereas crosslink between H6.57(267)C was enhanced.

The inactive (R) and active (R*) CB2 models employed in this study were taken from our previously published microsecond-long simulation of the activation of the CB2 receptor by the endogenous ligand, sn-2-arachidonoylglycerol (2-AG) via the fully hydrated lipid bilayer. The placement of CB2 receptor homodimers were based on X-ray crystal structure of chemokine CXCR4 homodimers using the Maestro module. Our model of TMH6 homodimer interface of activated CB2 receptor (R*) suggests that Cα distance between H6.57(267) is well within the crosslink formation range but same is not shown by A6.60(270). These findings suggests the involvement of A6.60(270) CB2 receptor as dimer interface residue for the inactive CB2 and H6.57(267) as dimer interface residue favoring activated CB2 receptor.

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MECHANISMS OF CROSS-TOLERANCE BETWEEN MORPHINE AND CANNABINOID RECEPTOR 2 AGONISTS

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The objective of this study was to investigate potential interaction between the cannabinoid receptor 2 (CB_2) and the mu opioid receptor (MOR) in pathological pain. The low level of side effects and lack of tolerance make CB_2 agonists an attractive pharmacotherapeutic target. The effect of a selective CB_2 agonist (JWH-133) in pathological pain was assessed in mice subjected to the formalin test of inflammatory pain. Intraplantar formalin injection (10 µl at 2.5 %) produces a biphasic nociceptive response described as acute and inflammatory phases. JWH-133 produced an antinociceptive response in both phases of the formalin test in a dose-dependent fashion, and reached maximal efficacy at 1mg/kg. Furthermore, efficacious doses of JWH-133 were not accompanied by adverse side effects (hypothermia, catalepsy, locomotor impairment). There were no differences in paw edema (microcaliper) between mice receiving JWH-133 compared to vehicle. The anti-nociceptive effect of JWH-133 was blocked by application of a CB_2 selective antagonist (SR144523).

Cross-tolerance was measured between JWH-133 and a MOR agonist (morphine) to determine whether CB_2 and MOR interact in a physiologically relevant way. Mice that were made tolerant to the effects of morphine with chronic dosing demonstrated cross-tolerance to JWH-133 relative to vehicle treated morphine-naïve animals in both phases of this inflammatory pain model. However, chronic JWH-133 administration does not appear to cause cross-tolerance for morphine, suggesting opioid and CB_2 cross-tolerance is not bidirectional. Interestingly, chronic co-administration JWH-133 and morphine appears to reduce the development of opioid tolerance compared to animals receiving morphine alone. Overall these findings suggest that CB_2 may functionally interact with MOR to modulate antinociception in the formalin test in response to inflammatory pain, as well as the development of tolerance.

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CONSTRUCTION OF A REALISTIC PHOSPHOLIPID BILAYER FOR CB2 SIMULATIONS

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Proper consideration of the solvent encompassing any chemical reaction is necessary prior to elucidating a mechanism, thus to accurately model membrane-bound cannabinoid receptors, we must accurately model a biological lipid bilayer. Rather than solely a barrier, the membrane also serves as a medium for hydrophobic ligand delivery, as the Reggio group has published on the entry of *sn*-2-arachidonoylglycerol (2-AG) into the cannabinoid CB2 receptor through the trans-membrane region (Hurst et. al., JBC 2010). Currently receptor and ligand trajectories are calculated in a single-phospholipid bilayer, however biological membranes are comprised of many lipid species. Herein we report nanosecond timescale molecular dynamics studies of various membrane compositions to lead to the development of a final model that will be utilized in future cannabinoid receptor studies.

The final composition's lipid profile reflects a neuronal bilayer; neuronal membranes contain less sphingomyelin and cholesterol, but more phosphatidylethanolamine, with respect to other cellular membranes, as well as an increased concentration of polyunsaturated acyl chains within the phospholipid constituents (Breckenridge et. al., BBA 1972). Anionic lipids like phosphatidylserine and phosphatidylinositol-4,5-biphosphate are located exclusively in the cytosolic leaflet, phosphatidylethanolamine is primary distributed in the cytosolic leaflet, and phosphatidylcholine is primarily in the extracellular leaflet (Ingólfsson et. al., JACS 2014). Phosphatidylserine and phosphatidylethanolamine are approximately 10% and 30% of the final lipid composition respectively, while sphingomyelin and phosphatidylinositol-4,5-biphosphate combined constitute less than 5%, and cholesterol makes up approximately 15% of the total bilayer (Breckenridge et. al., BBA 1972; Cotman et, al., Biochemistry 1969; Ingolfsson et. al., JACS 2014). NAMD software inputs are generated using the all atom CHARMM36 force field in the CHARMM-GUI Membrane Builder. In the lipid compositions leading up to and including the final membrane system, a small amount of endocannabinoid *N*-arachidonoyl glycine (NAGly) acts as a probe of the changing environment; by analyzing hydrogen bonding at the water interface as well as its lateral mobility, we can establish trends prior to receptor inclusion.

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HETEROMERIZATION OF GPR18 AND CANNABINOID G-PROTEIN-COUPLED RECEPTORS

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The synthetic stereoisomer of the phytocannabinoid cannabidiol (CBD), abnormal-cannabidiol (Abn-CBD), is inactive at CB_1 or CB_2 receptors, but it may activate GPR18, a recently deorphanized receptor (McHugh *et al*, 2010). *N*-Arachidonoyl glycine (NAGly), which results from anandamide (AEA) metabolism, has been reported to be the endogenous agonist of GPR18 (McHugh et al, 2010). O-1602, Abn-CBD, Δ9-THC and AEA may act as agonists at GPR18 (McHugh *et al*, 2012). GPR18 was cloned in 1997 (Gantz *et al*, 1997) and its physiological role remains still obscure although it has been implicated in cell migration, vasodilation, haemodynamic responses, apoptosis and immune regulation (McHugh *et al*, 2010). GPR18 expression is low in cortex, thalamus, adrenal tissue, colon, intestine, kidney, prostate, skin, spleen, stomach and uterus, moderate in lung, ovary, testis, thymus and striatum and strong in hypothalamus, thyroid, peripheral blood leucocytes, cerebellum and brain stem (Vassilatis et al., 2003). According to different reports GPR18 may couple to $G_{i/o}$ and promote activation of MAP kinases, to Gq, or its activation may engage noncanonical GPCR pathways. As we have identified CB_1 and CB_2 receptor heteromers (Callén *et al*, 2012), we then became interested in knowing whether GPCR18 may form heteromers with cannabinoid receptors and how heteromerization may condition GPR18-mediated signaling.

Bioluminescence Resonance Energy Transfer (BRET) assays in heterologous cells demonstrated that human GPR18 may form heteromers with human CB_2 but not with human CB_1 receptors. The GPR18-CB_2R BRET signal was neither affected by NAGly nor by CB_2R agonists. By beta-arrestin recruitment assays, we have detected that the signal induced by the CB_2R agonist, JWH133, is reduced by the presence of GPR18 as it is reduced by a CB_2 receptor antagonist, suggesting a negative cross-talk between GPR18 and CB_2 receptors. It remains to be determined whether GPCR18-containing heteromers are expressed in natural sources.

Callén et al., J Biol Chem. 2012 287:20851; Gantz et al., Genomics. 1997 Jun 15;42(3):462; McHugh et al., BMC Neurosci. 2010;11:44; McHugh et al., Br J Pharmacol. 2012;165:2414; Vassilatis et al., Proc Natl Acad Sci USA 2003;1001:4903.

EFFECTS OF NOVEL SEMI-SYNTHETIC CANNABIDIVARIN DERIVATIVES ON CB1 AND CB2 RECEPTORS THROUGH BINDING AND SIGNALLING

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Background: CB1 receptors are expressed mainly by neurons of the central and peripheral nervous system whereas CB2 receptors occur centrally and peripherally in certain non-neuronal tissues, particularly in immune cells. Selective interactions with CB1 or CB2 may lead to targeted agonistic or antagonistic effects on the cannabinoid receptor affected pathways and thus can be used in selective therapeutic concepts. Semisynthetic cannabinoids, partially derived from natural compounds, and specifically modified, can be an approach to selectively interact with CB1 and/ or CB2 as agonists or antagonist.

Objective: Three novel semi-synthetic cannabinoids (cyclohexylcannabidivarinolat (CHCBDV), hexylcannabidivarinolat (HCBDV), and 2-hydroxyethylcannabidivarinolat (HECBDV)) were tested for their effects on CB1 and CB2 binding and signaling.

Methods: The three compounds with an expected profile as CB1 and/ or CB2 receptor ligands were evaluated by competition studies that allowed to determine the affinity of these compounds (Ki values) for both receptors against a classical cannabinoid ligand ([3H]-CP55940). The competition studies were conducted with membranes transfected with either CB1 or CB2 receptors. Cytotoxicity of the compounds was determined by MTT assay. Subsequently the signalling profile of the three novel compounds was examined in CHO cells transfected with CB1 and CB2 receptors in a cAMP luciferase assay.

Results: We identified all 3 compounds to bind to CB receptors in nM doses and such in a physiological range. The three tested compounds vary in their selectivity for CB2 receptors compared to CB1 receptors. The selectivity index in favour of CB2 were CHCBDV 2.9, HCBDV 0.8 and HECBDV 33.6. From the signalling assay, HECBDV was found to be an agonist on CB2 receptor and inhibits CB1 concomitantly. CHCBDV and HCBDV both strongly bind CB1 and CB2. HCBDV show selective activation of CB1. CHCBDV is an agonist on CB1 and CB2.

Conclusion: We demonstrated, that semi-synthetic cannabdivarin-derivatives reveal diverse patterns of CB1/ CB2 agonism/ antagonism depending on the modification of the molecules. One of the novel structures could potentially be used to trigger CB2 mediated effects and concomitantly could have an inhibitory effect on CB1. Nevertheless, more structure-relationship studies are needed.

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A POSSIBLE INVOLVEMENT OF ACID CERAMIDASE IN THE DEGRADATION OF *N*-ACYLETHANOLAMINES

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Acid ceramidase (AC) is a lysosomal enzyme which hydrolyzes the amide bond of ceramide to sphingosine and fatty acid at acidic pH. AC is highly expressed in several human tumors, and its role in chemoresistance is suggested. Interestingly, AC shows a high similarity to *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) in terms of primary structures, and their catalytic mechanisms are analogous. Our earlier study showed that recombinant AC hydrolyzes *N*-acylethanolamine as well as ceramide (Tsuboi et al., J. Biol. Chem. 280 (2005) 11082-11092). However, the biological significance of this enzyme activity has not been explored. In the present study, we examined a possible involvement of AC in the degradation of *N*-acylethanolamines in mammalian cells. First, we overexpressed FLAG-tagged AC in HEK293T cells and highly purified the enzyme protein by anti-FLAG-antibody affinity chromatography. The purified AC hydrolyzed not only ceramides but also various *N*-acylethanolamines such as lauroylethanolamide, myristoylethanolamide, palmitoyl-ethanolamide, linoleoylethanolamide, stearoylethanolamide, oleoylethanolamide, and anandamide. We next metabolically labeled HEK293 cells with [^{14}C]ethanolamine. The overexpression of AC decreased the ^{14}C-labeled *N*-acylethanolamine level. Furthermore, the analysis by LC-MS/MS revealed that the overexpression of AC in HEK293 cells decreased the levels of quantitatively major *N*-acylethanolamines. These results suggest that AC participates in the intracellular degradation of *N*-acylethanolamines as a second lysosomal *N*-acylethanolamine hydrolase.

NEUROCHEMICAL AND BEHAVIOURAL TARGET ENGAGEMENT FOR MONOACYLGLYCEROL LIPASE (MAGL) INHIBITION USING TANDEM MASS SPECTROMETRY (LC-MS/MS) AND AUTOMATED BEHAVIORAL ANALYSIS IN MICE

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The role of monoacylglycerol lipase (MAGL) has been studied in various animal models to elucidate the function of endocannobinoids (eCB) in cancer, pain, psychiatric and neurodegenerative diseases. Newer findings suggest also a prominent role for MAGL in regulation of brain prostaglandin levels.

Here, we investigate the effects of the commercially available MAGL inhibitors JZL184, KML29 and MJN110 and a cyclooxygenase (COX) inhibitor rofecoxib on neurochemical target engagement markers and behavioural alterations.

A sensitive liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method was used to determine levels of 2-arachidonoylglycerol (2-AG) and anandamide (AEA) as well as arachidonic acid (AA) and prostaglandins (PGs) PGE_2, PGD_2, $PGF_{2\alpha}$ and TXB_2 out of different matrices. Male adult C57BL/6JRj mice were treated with MAGL and COX inhibitors (30 mg/kg) or vehicle. Brains and peripheral organs were removed 60 min after treatment and tissues were processed for LC-MS/MS analysis. Additionally, MAGL and COX inhibitors were analyzed in a separate study to investigate their effects on potential motor behavior impairment using an automated system to quantify rearing behavior and distance travelled in an open field by interruptions of light beam arrays.

In the brain and in peripheral tissues, MAGL inhibition significantly increased 2-AG levels and decreased AA and PGs. COX inhibition reduced PGs but had no effect on 2-AG and AA levels. Notably, MJN110, which was very potent at increasing brain 2-AG, did not show motor impairment in the behavioral observation test.

In conclusion, a LC-MS/MS biomarker method was applied to quantify changes in brain and peripheral tissue in the endocannabinoid and eicosanoid pathway. These changes induced by MAGL and COX inhibitors are indicative for target engagement and will help to elucidate the role of endocannabinoids in CNS diseases.

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PARTIAL GENETIC DELETION OF *NRG1* INFLUENCES ENDOCANNABINOID CONCENTRATIONS IN THE MOUSE BRAIN

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Neuregulin 1 (Nrg1) is a neurotrophic factor implicated in the pathophysiology of schizophrenia and drug addiction. Manipulation of the *Nrg1* gene moderates responses to stress and exogenous cannabinoid administration. Both Nrg1 and stress alone affect the endocannabinoid system. Stress influences endocannabinoid concentrations in the rodent brain. Further, exogenous Nrg1 application to hippocampal brain slices decreased 2-arachidonoylglycerol (2-AG) concentrations due to increased monoacylglycerol lipase (MAGL) expression, the major 2-AG degradative enzyme. These effects were associated with impaired long-term depression, a form of neural plasticity critical to spatial learning. The current study aims to observe whether partial genetic deletion of *Nrg1* confers vulnerability to stress-induced alterations in brain endocannabinoid concentrations. In addition, we aim to examine whether *Nrg1* hypomorphism itself alters brain endocannabinoid concentrations in vivo which would confirm the interplay between Nrg1-ErbB and endocannabinoid signalling systems found in brain slices. Adolescent *Nrg1* HET mice and wild-type (WT) were submitted to 6 h/day of restraint stress for 21 consecutive days. 24 h after the final stress exposure mice were sacrificed and brain tissue was collected for analysis of endocannabinoids. A separate cohort of WT and *Nrg1* HET mice were assessed for spatial learning performance in the Morris Water Maze. Chronic adolescent stress increased anandamide concentrations in the amygdala, however, partial genetic deletion of *Nrg1* did not influence stress-induced changes on the endocannabinoids. *Nrg1* hypomorphism increased anandamide concentrations in the amygdala and decreased 2-AG concentrations in the hypothalamus. Moreover, as predicted *Nrg1* hypomorphism increased 2-AG in the hippocampus, which correlated with impaired spatial learning performance in the Morris Water Maze. These results demonstrate for the first time in vivo interplay between Nrg1-ErbB and the endocannabinoid systems.

POLYUNSATURATED FATTY ACIDS ETHANOLAMIDES AS ENDOGENOUS CANNABINOIDS

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N-Acylethanolamines (NAEs) and their precursor phospholipids, (*N*-acyl PEs) accumulate in mammalian cells and tissues as a result of injury. With the discovery of AEA ($C_{20:4}$, ω-6) as the first endogenous cannabinoid, metabolism and functional effects of *N*-acylethanolamines received more attention. In this paper, we have investigated the hydrolysis of multiple NAEs by rat liver particulate preparations, and their effects at human recombinant CB_1 receptor.

A membrane bound amidohydrolase activity (fatty acid amide hydrolase, FAAH) from rat liver was measured using the ethanolamine methodology previously described (Alharthi et al., ICRS 2015). FAAH with a K_m of 2.6 μM and V_{max} of 183 nmol/min/mg protein. Relative to AEA, three groups of NAEs could be identified. One group, exemplified by *N*-linoleoylethanolamine ($C_{18:2}$, ω-6) had similar affinities of hydrolysis to AEA, with V_{max} values of >70 % of that of AEA. A second group, exemplified by *N*-docosahexaenoylethanolamine ($C_{22:6}$, ω-3), had similar affinities to AEA for hydrolysis, but V_{max} values of 20-40 % of that for AEA. A third group, including *N*-arachidoylethanolamine ($C_{22:0}$), had similar or lower affinities to AEA, but were hydrolysed with a V_{max} value of less than 10 % of that to AE.

CB_1 receptor activation was assessed in CHO-CB1 cells at 10 μM NAEs, monitoring ERK activation and intracellular calcium ion elevation with 10 μM HU210 as a positive control. Using the whole cell activation assay, HU210 evoked a rapid phosphorylation of ERK to levels of 213 %. The majority of NAEs failed to elicit a significant change in ERK activation (including $C_{20:0}$, $C_{18:0}$; $C_{18:1}$, ω-9; $C_{22:1}$, ω-9 and $C_{24:1}$, ω-9). AEA and *N*-docosapentaenoylethanolamine ($C_{22:5}$, ω-6) elicited responses of a similar magnitude to HU210 (181-183 % basal). Monitoring intracellular calcium ion levels with a FlexStation, 10 μM AEA and *N*-docosapentaenoylethanolamine ($C_{22:5}$, ω-6) evoked larger calcium responses compared to HU210 (21 & 18 vs 6 % of the response to 10 μM ATP). The majority of NAEs failed to elicit a significant change in $[Ca^{2+}]_i$ (including $C_{20:0}$, $C_{18:0}$; $C_{18:1}$, ω-9; $C_{22:1}$, ω-9 and $C_{24:1}$, ω-9).

In summary, different assays of CB_1 receptor activation, we have identified that *N*-docosapentaenoylethanolamine ($C_{22:5}$, ω-6) shows a similar agonist profile to AEA, but is hydrolysed at a lower maximal rate by rat liver FAAH.

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2-OLEOYLGLYCEROL HYDROLYSIS IN THE RAT BRAIN

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Monoacylglycerols, such as 2-oleoylglycerol and 2-AG, are subject to hydrolysis by multiple lipases. In the rodent CNS, monoacylglycerol lipase (MAGL), ABHD6 and ABHD12 are reported to account for about 99% hydrolysis of 2-AG (Blankman et al., 2007; Savinainen et al., 2012). MAGL is the best characterized among the monoacylglycerol hydrolysing enzymes and is detected in both the soluble and the membrane fractions (Ghafouri et al, 2004) although the primary sequence indicates a lack of a transmembrane domain. Much less is known about ABHD6 and ABHD12 regarding their biochemical characteristics, structural information and physiological function. Both are membrane-bound proteins, with the active site of ABHD6 facing the cell interior and ABHD12 facing the extracellular space (Savinainen et al., 2012). Recently, JJK048 and WWL123 have been introduced as selective inhibitors of MAGL and ABHD6, respectively (Aaltonen et al., 2013; Bachovchin et al., 2010). In this study, we have investigated the expression of MAGL and ABHD6 in rat tissues, and the use of selective inhibitors to identify the contributions of these enzymes to hydrolysis of 2-OG in rat brain preparations.

Nineteen different rat tissues were collected to investigate gene expression level by qRT-PCR of monoacylglycerol lipase (MAGL), x1 variant of MAGL and ABHD6. Three different tissues (spinal cord, prefrontal cortex, hippocampus) were used to explore monoacylglycerol metabolism in cytosolic and membrane fractions using the hydrolysis of tritium-labelled 2-oleoylglycerol.

Results from Taqman PCR showed that the highest expression level of rat MAGL was in adipose tissue, while X1 MAGL was highest in testis. In the CNS, the prefrontal cortex and hippocampus appeared to have similar levels of all three mRNAs, while ABHD6 seemed to be higher than the MAGL splice variants in the spinal cord. Results from radiometric assays showed that the total activity of 2OG hydrolysis was higher in the particulate phase compared to the soluble phase. JJKK048 caused a concentration-dependent inhibition of 2OG hydrolysis in both fractions from all tissues, with a maximal inhibition of 72-85 % of total 2OG hydrolysis. In the presence of a maximally-effective concentration of JJKK048 (1 μM), WWL123 failed to cause further inhibition of 2OG hydrolysis, with the highest concentration evoking a non-significant numerical change of 0-1.8 %.

In summary, this study provides evidence for gene expression of MAGL and ABHD6 in the rat CNS. The hydrolysis of 2OG appears to be mediated predominantly through MAGL activity and an unidentified lipase/s, with little contribution from ABHD6. Whether ABHD6 can be detected in the rat CNS using other methodologies is being investigated.

Aaltonen et al. (2013). *Chem Biol* PM:23521796
Bachovchin et al (2010). *Proc Natl Acad Sci U S A* PM:21084632
Blankman et al (2007) Chem Biol PM: 18096503.
Ghafouri et al (2004). *Br J Pharmacol* PM:15492019
Savinainen *et al* (2012) *J Lipid Res* PM:22969151

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DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL 3-CARBOXAMIDO-5-ARYL-ISOXAZOLES AS POTENTIAL FAAH INHIBITORS

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Anandamide (AEA), a lipid-derived endogenous mediator, has been identified to alleviate pain and inflammation, as well as produce anti-cancer, anxiolytic, or neuroprotective efficacies through the stimulation of cannabinoid receptors CB_1/CB_2.

Fatty acid amide hydrolase (FAAH), an integral membrane-bound enzyme, has been demonstrated to be responsible for the principal degradation of AEA. Thus, elevating AEA levels *via* the inhibition of FAAH is considered as an interesting therapeutic approach for the design of anti-nociceptive and anti-inflammatory molecules.

Previously, several molecules were synthesized and screened on an *in vitro* pharmacological model. The results indicated that 3-carboxamido-5-aryl-isoxazole is a favorable scaffold for the development of FAAH inhibitors. Especially, two compounds showed potent FAAH inhibitory effects. (Andrzejak, V et al., Bioorg. Med. Chem. 19 (2011) 3777-3786; Tourteau, A et al., Bioorg. Med. Chem. Lett. 24 (2014) 1322-1326).

Herein, 29 compounds were designed starting from this interesting scaffold. Different pharmacomodulations have been evaluated. The investigation of the *in vitro* inhibitory activity led to the identification of 5 compounds as potent FAAH inhibitors. Specifically, one of them inhibits FAAH with a better potency (IC_{50} = 0.24 μM) than the previous compound (IC_{50} = 0.46 μM) and shows a similar inhibitory potency against FAAH than the positive control URB597 (IC_{50} = 0.26 μM), one of the most potent FAAH inhibitors to date. (Tuo, W et al., Bioorg. Med. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.bmcl.2016.04.004)

DISCOVERY OF NOVEL FAAH INHIBITORS BY VIRTUAL SCREENING

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The endocannabinoid system (EC) is comprised of endocannabinoids, their receptors CB1 and CB2, transporters, and enzymes involved in their biogenesis and degradation. The two major endocannabinoids are lipids N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG). These two endocannabinoids are primarily degraded by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Apart from endocannabinoids, both CB1 and CB2 are activated by natural and synthetic cannabinoids including Δ9-Tetrahydrocannabinol (THC), a prominent psychoactive constituent of marijuana. Endocannabinoids regulate many important physiological processes including pain perception, feeding and metabolism, emotional state, learning and memory, and reward behaviors. Accordingly, regulation of endocannabinoid metabolism and degradation is an attractive strategy to treat various disorders such as cachexia, pain, and drug addiction. As such, we undertook a virtual screening approach to identify novel inhibitors of FAAH, MAGL and dual FAAH-MAGL inhibitors. To this end, we docked RTI's 22,000 compound diversity library against both FAAH and MAGL with known crystal structures. Parallelized virtual screening using Autodock VINA followed by scripted parallelized Molecular Mechanics Generalized Born Surface Area (MMGBSA) rescoring of the top 10 VINA poses yielded an approximate free-energy ranking of 220,000 poses. The top 282 ligands were selected based on their MMGBSA scores for both FAAH and MAGL. Initial duplicate screens were conducted to assess FAAH inhibition of anandamide hydrolysis at 10 μM. This effort led to the discovery of several novel FAAH inhibitor hits that decreased FAAH activity with IC_{50} <10 μM. Medicinal chemistry efforts are now underway to modify these hits to produce drug-like compounds that may be useful in treating various important disorders.

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EFFECT OF LEUKOCYTOSPERMIA ON SEMINAL LEVELS OF 2-ARACHIDONOYLGLYCEROL

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Leukocytospermia refers to the increase of white blood cell density in seminal plasma, mainly caused by a chronic inflammation of reproductive tract, that might be associated with impairment of sperm functions. On the basis that foreign antigens, as well as chronic inflammation, can lead to recruitment and immunological activation of macrophages within the male genital tract and that, in response to *in vitro* inflammatory stimuli, these immune cells can produce endocannabinoids (eCBs), we hypothesized that macrophages might be an important source of seminal eCBs in patients with leukocytospermia.

In this study, we sought to ascertain whether the levels of the two major eCBs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), were altered in seminal plasma of leukocytospermic patients compared with controls, and whether the potential modulation of these eCBs could correlate with semen concentration of both macrophages and activated macrophages.

The content of AEA and 2-AG was measured by high-performance liquid chromatography/mass spectrometry in seminal plasma of ejaculates from 18 leukocytospermic patients ($>1\times10^6$ leukocytes/mL) and 21 normozoospermic controls, selected on the basis of no or $\leq 0.2 \times 10^6$ round cells/mL. In the same ejaculates, round cells were phenotypized by flow cytometry as leukocytes (CD45+), monocytes/macrophages (CD14+) and antigen-presenting activated macrophages (HLADR+). The levels of 2-AG, but not of AEA, were significantly higher in ejaculates from leukocytospermic patients than in controls, and exhibited a significant independent association with macrophages and activated macrophages.

In conclusion, our data demonstrate that endogenous 2-AG levels in leukocytospermic ejaculates could play a pivotal role in monocytes/macrophages–mediated inflammatory response, with no significant effect on semen quality. Further studies are warranted to elucidate possible clinical relevance and potential applications of these findings for an early diagnosis and management of leukocytospermia and/or other inflammatory conditions of male genital tract.

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PROTEOMIC CHARACTERIZATION OF RECOMBINANT HUMAN α/β HYDROLASE DOMAIN 6

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Human α/β Hydrolase Domain 6 (hABHD6) is an endocannabinoid-metabolizing enzyme that inactivates 2-arachidonoylglycerol (2-AG), a potent agonist at both cannabinoid receptors. ABHD6 is tethered to the post-synaptic membrane and controls 2-AG concentrations at a different subcellular localization as compared to monoacylglyerol lipase (MGL). ABHD6 inhibitors may have therapeutic potential towards addiction, pain, inflammation, cancer, and metabolic, neuroinflammatory, and neurodegenerative disorders, but limited structural data is hindering a rational drug design. Recombinant hABHD6 overexpressed in *E. coli* and purified by single step immobilized metal affinity chromatography was able to hydrolyse 2-AG similarly to the native enzyme with comparable kinetic parameters. A novel high-throughput assay based on the fluorogenic substrate arachidonoyl, 7-hydroxy-6-methoxy-4-methylcoumarin ester (AHMMCE) was developed and used for screening of compounds to identify and characterize potent ABHD6 inhibitors. Bottom-up proteomics approach based on Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) was applied for analysis of hABHD6 inactivated with selected inhibitors. For the first time we presented experimental evidences that AM6701 and selective inhibitor WWL70 covalently modified the catalytic serine of the enzyme. A new homology model of the hABHD6 was created and used for *in-silico* experiments with AM6701 or WWL70. The probing of the binding pocket of the enzyme using different covalent ligands is critical in order to validate the model and provide Ligand Assisted Protein Structure (LAPS) characterization of ABHD6 for the development of selective inhibitors as potential therapeutic agents.

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MODULATION OF ANXIETY BY ENDOCANNABINOID SIGNALING IN THE AMYGDALA IS DEPENDENT ON AROUSAL STATE

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Evidence indicates that cannabinoids induce biphasic effects on emotionality depending on the aversiveness of the environmental context.

We examined whether the endocannabinoid anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are differentially regulated in the amygdala, depending on the level of environmental-associated emotional arousal and whether these differences in endocannabinoid levels influence the effects of endocannabinoid manipulations on anxiety.

Sprague-Dawley rats were divided in two groups and tested for anxiety in the Elevated Plus Maze (EPM). One group was not handled or habituated to the experimental room and tested under high light condition (High-Arousal group; HA); the second group was handled and habituated to the experimental room and tested under red light condition (Low-Arousal group; LA). We measured amygdalar AEA and 2-AG levels immediately after the EPM and evaluated the effects of intra-basolateral amygdala (BLA) administration of the AEA hydrolysis inhibitor URB597 (10ng/side) or the 2-AG hydrolysis inhibitor KML29 (0.2ug/side) on anxiety behavior in HA and LA rats.

The LA group exhibited significantly lower anxiety and increased AEA levels as compared to the HA group, while no changes were found in the levels of 2-AG. Both URB597 and KML29 injections further decreased the anxiety response shown by LA rats, without affecting emotional behavior in the HA group. These effects were blocked by a concurrent administration of the cannabinoid type-1 receptor antagonist AM251 (1ng/side) in the BLA.

These findings show that the endocannabinoid system is differentially activated to regulate anxiety response, depending on the level of the environmental-associated emotional arousal.

EFFECT OF Δ^9-TETRAHYDROCANNABINOL (THC) AND CANNABIDIOLIC ACID (CBDA) ON RAT MODELS OF NAUSEA AND ANXIETY

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Little research has focused on the combined effect of Δ^9-tetrahydrocannabinol (THC) and cannabidiolic acid (CBDA) in an animal models of nausea and anxiety. We have previously reported that intraperitoneal (i.p.) administration of combined subthreshold doses of THC-CBDA (0.01 mg/kg THC-0.01 μg/kg CBDA; 0.1 mg/kg THC-0.1 μg/kg CBDA) effectively reduce acute nausea-induced conditioned gaping (acute nausea), but not contextually elicited conditioned gaping (anticipatory nausea; Rock et al., 2015). Here we investigate: 1) the effects of i.p. acute and chronic administration of THC or CBDA in the light-dark emergence test of anxiety-like behaviour in rats; 2) the potential of orally administered [by intragastric (i.g.) gavage], combined subthreshold doses of THC and/or CBDA to reduce acute nausea and anticipatory nausea in rats.

In the light-dark emergence test, acute and chronic THC (10 mg/kg, i.p.) reduced anxiety-like behaviour, whereas no dose of CBDA tested (0.1 μg/kg-1 mg/kg, i.p.) modified this behaviour. These findings suggest that THC (but not CBDA) may have anti-anxiety properties at high doses in this rodent model.

For acute nausea, i.g. administration of combined subthreshold doses of THC (0.5, 1 mg/kg) and CBDA (0.5, 1 μg/kg), dramatically suppressed acute nausea-induced gaping, whereas higher individual doses of THC or CBDA were maximally effective. Combined i.g. administration of higher doses of THC (2.5, 10, 20 mg/kg) and CBDA (2.5, 10, 20 μg/kg) also enhanced positive hedonic reactions elicited by saccharin solution during conditioning, suggesting an enhancement in appetite. For anticipatory nausea, combined subthreshold i.g. doses of THC (0.1 mg/kg) and CBDA (0.1 μg/kg) suppressed contextually elicited conditioned gaping. When administered i.g., THC was effective on its own at doses ranging from 1-10 mg/kg, but CBDA was only effective at 10 μg/kg. THC alone appears to be equally effective by intraperitoneal (i.p.) and i.g. administration, whereas CBDA alone seems to be more effective by i.p. administration (Rock et al., 2015) than by i.g. administration. These findings suggest that oral administration of subthreshold doses of THC and CBDA may be an effective new treatment for both acute and anticipatory nausea in chemotherapy patients.

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FAAH INHIBITORS INDUCES ANTI-ANXIETY RESPONSES THROUGH ASTROGLIAL-MEDIATED LTD

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Pathological anxiety is the most common type of psychiatric disorders. The current first-line anti-anxiety medication produces a delayed onset of action with modest therapeutic and significant adverse effects, while long-term use of the fast-acting anti-anxiety benzodiazepines causes severe adverse effects. An increase of the brain endocannabinoid *N*-arachidonoylethanolamine (AEA) via inhibition of its degradative enzyme fatty acid amide hydrolase (FAAH) produces anti-anxiety effects without significant 'unwanted effects' of cannabinoids, but the anti-anxiety mechanism is not clear. Here we observe that the FAAH inhibitor PF3845 exerts rapid and long-lasting anti-anxiety effects in mice exposed acutely to stress or chronically to the stress hormone corticosterone. PF3845-induced anti-anxiety effects and *in vivo* long-term depression (LTD) of synaptic strength at the prefrontal cortical input onto the basolateral amygdala neurons are abolished in mutant mice without CB1 cannabinoid receptors (CB_1R) in brain astroglial cells, but are conserved in mice without CB_1R in glutamatergic neurons. Blockade of glutamate NMDA receptors and of synaptic trafficking of glutamate AMPA receptors also abolishes PF3845-induced anti-anxiety effects and LTD production. We propose that the rapid and long-lasting anti-anxiety effects of FAAH inhibition are due to AEA activation of astroglial CB_1R and subsequent basolateral amygdala LTD *in vivo*.

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THERAPEUTIC ENDOCANNABINOID AUGMENTATION FOR MOOD AND ANXIETY DISORDERS: COMPARATIVE PROFILING OF FAAH, MAGL AND DUAL INHIBITORS

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Over the past decade, pharmacological augmentation of central cannabinoid signaling has emerged as a novel non-monoamine and non-benzodiazepine based approach for the treatment of mood and anxiety disorders. Recent insights into the metabolic regulation of endogenous cannabinoids (eCB) has led to the development of pharmacological tools to augment central eCB signaling to elicit anxiolytic and antidepressant effects in animal models. Pharmacological inhibition of eCB degrading enzymes such as fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL) elicits promising anxiolytic effects in stress models without serious side effects. However, the comparative efficacy of single vs. dual enzyme inhibition has not been investigated.

In present study, we compared the effects of FAAH (PF-3845), MAGL (JZL-184), and dual FAAH/MAGL (JZL195) inhibitors on 1) brain and plasma lipid levels 2) anxiety like behavior under non-stressed and stressed conditions 3) locomotor activity and body temperature and 4) electrophysiological properties of the basolateral amygdala (BLA).

Systemic administration of PF-3845 (0.3, 1, 3 and 10 mg/kg), JZL-185 (1, 3, 5, 10 and 15 mg/kg) and JZL-195 (1, 3, 10 and 15 mg/kg) dose-dependently increased AEA, 2-AG and both AEA and 2-AG levels in the brain, respectively. Plasma levels of these inhibitors are well correlated with brain levels of respective eCB. Under non-stressed conditions, JZL-184 ($p < 0.0001$) but not PF-3845 and JZL-195 showed anxiolytic effects in the light-dark box test. Moreover, PF-3845 and JZL-184 at therapeutic dosages did not affect locomotor activity or body temperature. However, JZL-195 increased locomotor activity at higher dose and showed a trend toward decreased body temperature. Whole-cell patch-clamp recordings from BLA pyramidal neurons revealed that PF-3845 ($p < 0.01$), JZL-184 ($p < 0.05$) and JZL-195 ($p < 0.01$) decreased frequency of spontaneous excitatory postsynaptic currents (EPSCs), but had varying effects on measures of postsynaptic excitability, including action potential frequency and action potential threshold.

Under acute stress conditions, PF-3845 ($p < 0.01$) and JZL-184 ($p < 0.001$) but not JZL-195 ($p > 0.05$) treatment significantly reduced stress-induced anxiety like phenotype in the light-dark box test. Interestingly, we observed bimodal distribution in the Light-Dark behavior of eCB modulator-treated mice. Based on the % light time, mice were divided in to responders and non-responders groups. 43 % of PF3845, 47 % of JZL184 and 20 % of JZL195 treated mice showed complete reversal of stress-induced anxiety-like behavior in the light-dark box test. Brain and plasma analysis showed that differences in the Light-Dark behavior were not due to different brain eCB levels and/or plasma CORT levels. Cannabinoid receptor type 1 antagonist rimonabant blocked anxiolytic effects of PF-3845 and JZL-184, suggesting CB1 mediated effects.

Overall, these results showed that increasing either endogenous AEA or 2-AG separately produces anti-anxiety effects under acute stress conditions but same effects are not obtained from increasing both AEA and 2-AG at given dosages.

CANNABIDIOL DAMPENS THE EXPRESSION OF AUDITORY FEAR MEMORY WITHOUT AFFECTING ITS EXTINCTION IN RATS

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Anxiety disorders such as phobias and post-traumatic stress are highly prevalent and their treatment using psychological therapies or medications can be ineffective. A promising area of study involves the use of drugs to enhance exposure therapy to treat these disorders. Cannabidiol, the major non-psychotropic phytocannabinoid present in *Cannabis sativa*, is safe for use in humans due to its favourable side effect profile and shows broad therapeutic potential for treating anxiety. Emerging evidence from translationally relevant animal models indicates that cannabidiol reduces innate fear and learned fear expression induced by contextual cues while also enhancing contextual fear extinction, which is the psychological process by which exposure therapy can reduce fear memory expression. However, the effects of cannabidiol on learned fear expression and extinction related to discrete cues remains unknown.

In the present study we investigated the effects of cannabidiol on learned fear expression and its extinction using an auditory fear conditioning paradigm in male Lister hooded rats. On Day 0 the rats were habituated to contexts A and B for 10 minutes each. On Day 1 the rats underwent tone habituation (five tones presented alone: 30 sec, 80 dB, 4 kHz, 2 min inter-trial interval (ITI)) followed by auditory fear conditioning (five tones that co-terminated with foot shock: 0.5 mA, 0.5 sec) in context A. The rats were then assigned to four matched groups, such that each group showed equivalent levels of fear during late fear conditioning. On Day 2 each group was randomly assigned to receive one of four different drug treatments (0, 5, 10, or 20 mg/kg of cannabidiol, given i.p. in 2% Tween 80 / 0.9% saline vehicle) 30 min before undergoing partial extinction training (15 tones presented alone, 1 min ITI) in context B. On Day 3 the rats underwent extinction memory recall testing (two tones presented alone, 1 min ITI) drug-free in context B. Freezing during tone presentations was quantified as the measure of conditioned fear.

We found that 20 mg/kg of cannabidiol resulted in a modest but significant decrease in freezing during early extinction, compared to vehicle. However, there were no drug effects on freezing later on during extinction. Moreover, there were no differences in freezing during extinction memory recall between the groups the following day. These results indicate that 20 mg/kg of cannabidiol dampened learned fear expression at the outset of extinction without affecting extinction learning or extinction memory consolidation. Taken together, our results showing dampened learned fear expression combined with spared extinction suggest that cannabidiol might be an interesting therapeutic candidate for fear management when used as a pharmacological adjunct to reduce anxiety during exposure therapy without interfering with its benefits or having adverse side effects.

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DIFFERENTIAL INVOLVEMENT OF ENDOCANNABINOIDS ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL IN THE ACQUISITION AND EXTINCTION OF LEARNED FEAR

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Endocannabinoid signaling can affect behavioral responses to traumatic events. However, the specific roles of the two endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are poorly understood. Here we assessed the specific effects of AEA and 2-AG on acute responses to a traumatic event, as well as on the consolidation, extinction and recovery of learned fear. AEA and 2-AG signaling was enhanced in Wistar rats by the blockade of their respective degrading enzymes fatty acid amid hydrolase and monoacylglycerol lipase; treatments were administered before fear conditioning either systemically or locally to the ventral hippocampus, an area highly relevant in the regulation of responses to traumatic experiences. Fear conditioning was followed by seven fear extinction trials performed on consecutive days; spontaneous fear recovery was assessed 28 days after conditioning. Fear responses were evaluated by recording the time spent in freezing. Systemic enhancement of 2-AG signaling decreased freezing shown during the conditioning trial. Surprisingly, enhanced AEA signaling dampened these effects of 2-AG. Local enhancement of 2-AG signaling in the ventral hippocampus had no effect during fear conditioning. By contrast, locally enhanced AEA signaling decreased acute fear responses. Our findings suggest that 2-AG has a role in the regulation of acute responses to trauma but these responses can be modified by other brain sites where endocannabinoid signaling can also be observed. Systemic enhancement of 2-AG signaling accelerated fear extinction and no fear recovery occurred. In contrast, enhanced AEA signaling led to slower extinction and marked fear recovery. Similar to systemic, local enhancement of 2-AG signaling in the ventral hippocampus accelerated fear extinction and eliminated fear recovery but locally enhanced AEA signaling prevented extinction and led to fear recovery. Taken together, our findings suggest that AEA and 2-AG are differentially involved in the regulation of responses to traumatic events. Both endocannabinoids contribute to the regulation of fear memory via ventral hippocampal mechanisms: 2-AG facilitates, whereas AEA inhibits extinction.

DIFFERENTIAL EFFECTS OF IBUPROFEN AND URB937 ON FRONT AND HIND PAW MECHANICAL AND COLD SENSITIVITY IN CISPLATIN-INDUCED NEUROPATHY

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Chemotherapy-induced peripheral neuropathy is a frequent dose-dependent adverse effect associated with the commonly used antineoplastic drug cisplatin. Treatment of neuropathy is a clinical challenge; current strategies are limited and characterized by variable efficacy and severe side effects. Previous studies have focused on pain-related responses in the hind paws. However, the front paws have a greater degree of the fine sensorimotor skills that are characteristically damaged in clinical chemotherapy-induced neuropathy. Chemotherapy-induced pain responses in the front paws have not yet been assessed. Here we test the hypothesis that URB937 and clinically available ibuprofen normalize mechanical and cold sensitivity in the front and hind paws in a mouse model of cisplatin-induced neuropathy. Cold (acetone time responses) and mechanical (von Frey withdrawal thresholds) sensitivity were assessed by averaging right- and left-sided responses in the front and hind paws before and every 2 days for 28 days after beginning weekly cisplatin injections (5 mg/kg), as well as before, 30 min, and 150 min after systemic injection of different doses (0-10 mg/kg) of ibuprofen or URB937.

Cisplatin-induced neuropathy resulted in a long-lasting and stable increase in acetone withdrawal latencies and decrease in von Frey withdrawal thresholds in both the front and hind paws, corresponding to development of increased cold and mechanical sensitivity, respectively. Systemic administration of ibuprofen or URB937 resulted in a dose-dependent increase in acetone time responses with a return to normal values at the highest doses tested in both the front and hind paws. Systemic drug administration also resulted in a dose-dependent increase in von Frey withdrawal thresholds, however, thresholds returned to normal levels only in the hind paws and instead plateaued at subnormal values in the front paws. These results indicate that drug effects in chemotherapy-induced neuropathy can vary based on testing stimulus and location, and suggest that front paw responses can provide additional information about pain-related drug effects that might better predict clinical translation of preclinical findings. Future studies should be aimed at elucidating the mechanisms underlying these differential effects.

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PALMITOYLETHANOLAMIDE (PEA) IMPROVES PHARMACOLOGICAL PROFILE OF TETRAHYDROCANNABINOL (THC) IN MURINE PAIN PERCEPTION MODEL.

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For centuries, Cannabis was widely utilized as an analgesic remedy. The major cannabinoid compound, THC exerts its effects via cannabinoid receptors, mainly CB1. However, activation of this receptor is also known to produce undesired adverse effects, amongst which are cognitive deficits, anxiety and loss of movement control. These AEs are limiting the therapeutic use of THC.

PEA is a lipid messenger known to mimic several endocannabinoid-driven activities although it does not bind the classical CB receptors. An "entourage effect hypothesis" has been put forward to account for the pharmacological actions of PEA.

The objective of the current study was to evaluate the effect of predefined dose of PEA on modulating the activity of different THC doses on behavioral characteristics and pain perception in mice. The study consisted of an Open field test followed by tail pinch test to evaluate the anti-nociceptive effects of the test items. THC treatment alone led to an increased food intake and an accelerated elevation in body weight, while addition of PEA to THC treatment, in both doses, markedly reduced the THC-induced body weight gain, and resulted in a similar body weight gain as in the control group. In order to evaluate behavioral patterns, animal velocity and anxiety were assessed. An increase in animal velocity typically indicates movement loss of control. High dose THC led to both, an increase in average animal velocity, and to a significant reduction in the time spent in the center of the arena, as compared to control, while a combined treatment of PEA with high dose of THC resulted in a normalization of this effect. Furthermore, high dose THC induced anti-nociceptive effect which culminated 45 minute following the treatment. Addition of PEA to the high dose THC treatment only slightly elevated the peak latency of THC alone. However, combination of high dose THC with PEA exhibited superior latency in the short term response, during first 15 minutes, while also prolonging anti-nociceptive effect, resulting in latency to respond stay at it peak up to 90 minutes tests.

Overall, study results suggest that PEA may be efficiently utilized when applied in adjunct to THC by potentiating THC and reducing its deleterious side effects.

IMPAIRED EXPRESSION OF FEAR-CONDITIONED ANALGESIA IN THE STRESS- AND PAIN-HYPERRESPONSIVE WISTAR-KYOTO RAT STRAIN

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Fear-conditioned analgesia (FCA) is pain suppression upon re-exposure to a context previously paired with an aversive stimulus. The endocannabinoid system (ECS) plays a key role in mediating FCA. The influence of genetic background on FCA has not yet been studied. The Wistar-Kyoto (WKY) rat is an inbred strain that exhibits an anxiety/depressive-like phenotype and hypersensitivity to stress and pain, compared with Sprague-Dawley (SD) rats. Here, we compared formalin-evoked nociceptive behaviour and FCA in WKY and SD rats, and associated alterations in levels of endocannabinoids and related N-acylethanolamines in the infralimbic cortex (IL). We subsequently investigated the effects of pharmacological modulation of the ECS in the IL on formalin-evoked nociceptive behaviour in WKY versus SD rats.

Adult, male SD and WKY rats (n=9) received footshock (10x1s, 0.4mA) or no footshock (controls) in a conditioning arena. 23.5 hours later, rats received intraplantar injection of formalin (2.5%, 50µl) into the right hindpaw. 30 minutes post-formalin, rats were re-exposed to the conditioning arena for 30 minutes, during which time nociceptive and fear-related behaviour were assessed. Animals were euthanised post behavioural testing, the brain removed and liquid chromatography-tandem mass spectrometry used to determine endocannabinoid and N-acylethanolamine levels in the IL. In a subsequent experiment, adult, male SD and WKY rats (n=8-11) received intra-IL injections of either vehicle (100% DMSO), URB597 (0.1mM/0.3µL DMSO), an inhibitor of the endocannabinoid-catabolising enzyme fatty acid amide hydrolase, or methanandamide,(0.1 ug/0.3µL DMSO), an anandamide analog, via bilaterally implanted cannulae, 10 minutes prior to intra-plantar formalin injection (2.5%, 50µl). Nociceptive behaviour was assessed for 60 minutes as per the previous study. Data were analysed by two-way ANOVA (with or without repeated measures) followed by Fisher's LSD post-hoc tests or Kruskal-Wallis followed by Mann-Whitney U tests.

Robust expression of FCA was observed in SD rats, but not WKY rats. Formalin-evoked nociceptive behaviour was significantly higher and the duration of freezing significantly lower in WKY rats, compared with SD rats. There was a significant main effect of strain on levels of the endocannabinoid anandamide in the IL, with levels lower in WKY rats compared with SD counterparts. Our subsequent study revealed a significant strain effect, with WKY rats expressing higher formalin-evoked nociceptive behaviour than SD rats. Intra-IL administration of URB597 or methanandamide had no significant effect on formalin-evoked nociceptive behaviour in either WKY or SD rats.

These data provide evidence for hyperalgesia in WKY rats and suggest dysfunction of the endogenous analgesic system in this strain, as evidenced by impaired expression of FCA and increased expression of formalin-evoked nociceptive behaviour. Decreased levels of anandamide in the IL do not seem to underlie the hyperalgesia in WKY rats and further work in other brain regions involved in descending modulation of pain is warranted.

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POSITIVE ALLOSTERIC MODULATION OF CB1 RECEPTOR SIGNALING PRODUCES SYNERGISTIC ANTINOCICEPTIVE EFFECTS WITH INHIBITORS OF FATTY-ACID AMIDE HYDROLASE AND MONOACYLGLYCEROL LIPASE

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Activation of cannabinoid CB1 receptors suppresses pathological pain but also produces unwanted central side effects (e.g. psychoactivity) that constrain therapeutic dosing. We hypothesized that positive allosteric modulation of CB1 receptor signaling would suppress neuropathic pain produced by chemotherapy treatment and that these antinociceptive effects would also synergize with inhibitors of endocannabinoid deactivation. We, therefore, compared the therapeutic efficacy of GAT211, a positive allosteric modulator of CB1 receptor signaling, with URB597, an inhibitor of fatty-acid amide hydrolase (FAAH), and JZL184, an inhibitor of monoacylglycerol lipase (MGL) using a mouse model of chemotherapy-induced peripheral neuropathy. Within-subjects dose-response curves were constructed for GAT211, URB597, and JZL184; ED_{50} values were generated and used in fixed combinations to determine if the combination treatments produced antinociceptive effects that were additive or synergistic. GAT211 produced a CB1-dependent suppression of mechanical and cold allodynia induced by the taxane chemotherapeutic agent paclitaxel. Isobolographic analysis of these combinations revealed synergistic interaction of GAT211 with inhibitors of both FAAH and MGL. Moreover, GAT211 did not produce cardinal signs of cannabinoid receptor activation (hypothermia, motor ataxia, catalepsy, tail flick antinociception) in wildtype mice but produced signs of catalepsy in transgenic mice lacking either FAAH or MGL. Therapeutic efficacy of GAT211 was preserved over a chronic dosing period of 19 days with no appreciable signs of tolerance developing to its antinociceptive efficacy. By contrast, the MGL inhibitor JZL184 (16 mg/kg i.p.) initially suppressed paclitaxel-induced allodynia but tolerance developed to its antinociceptive efficacy following 8 days of repeated dosing. Thus, positive allosteric modulation of CB1 represents a promising strategy for harnessing the therapeutic potential of the endocannabinoid signaling system to suppress neuropathic pain without producing tolerance to therapeutic efficacy or detrimental CNS side-effects observed with typical cannabinoid orthosteric agonists. Moreover, our studies also suggest that CB1 positive allosteric modulators may show greater therapeutic potential compared to sustained inhibition of MGL, as manifest by absence of both tolerance and cardinal signs of CB1 intoxication (i.e. hypothermia, motor ataxia, catalepsy).

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DO CHANGES IN THE ENDOCANNABINOID SYSTEM ACCOUNT FOR HIGHER EFFICACY OF PERIPHERALLY-RESTRICTED CANNABINOIDS IN ALLEVIATING CHRONIC PAIN SYMPTOMS VERSUS THEIR ANTINOCICEPTIVE EFFECTS?

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Aim of Investigation: We developed a series of synthetic peripherally-restricted cannabinoids (PRCBs) and demonstrated the absence of central nervous system (CNS) side effects and minor antinociceptive effects (changes in latency of tail-flick responses to radiant heat) of PRCBs compared to the brain-permeant cannabinoid, HU-210 (Mulpuri et al, Soc. Neurosci. Abstr. Vol. 37:173.21, 2012). However, PRCBs are highly effective at alleviating mechanical allodynia symptoms in rodent models of cancer, sciatic nerve entrapment (SNE)-induced neuropathy, and chemotherapy (cisplatin)-induced peripheral neuropathy (CIPN). Here we tested the hypothesis that the higher efficacy of PRCBs in alleviating chronic pain symptoms versus their antinociceptive effects is due to cancer- or neuropathy-induced alterations in the endocannabinoid system.

Methods: Naïve and sham treatment animals were compared to murine models of oral cancer and rat models of SNE- and cisplatin-induced neuropathies for PRCBs' effects on withdrawal thresholds to mechanical or thermal stimuli. Lumbar (L4 and L5) dorsal root ganglia (DRG) from separate cohorts were examined using qPCR for changes in the mRNA expression of cannabinoid receptors CB1R and CB2R, endocannabinoid synthesizing enzymes N-acyl-phosphatidylethanolamine (NAPE), diacylglycerol lipase (DAGL), metabolizing enzymes monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH). Changes in CB1R expression were also examined using Western blot in DRG.

Results: Paw cancer (inoculated with human oral carcinoma cells) mechanical allodynia was ~75% suppressed by 0.6 mg/kg the systemic PRCB 4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl)methylidene]-1H-inden-3-yl]ethyl}morpholine (PrNMI). In SNE rats, 0.6 mg/kg intraperitoneal PrNMI increased ipsilateral mechanical thresholds by 29 ± 2 g, but only by 8 ±4 g in sham rats. In CIPN rats, 0.25 mg/kg intraplantar PrNMI increased mechanical withdrawal thresholds by 25 ± 4 g. CB1R immunofluorescence was previously shown to increase in L5 DRG of paw cancer mice (Guerrero et.al., Neurosci. Lett. 12:77-81, 2008). By contrast, neither the receptors nor the endocannabinoid system enzymes were significantly upregulated in DRG of SNE or CIPN rats. DAGL and MAGL were significantly downregulated in CIPN versus control rat DRG.

Conclusions: Our data suggest that in the SNE neuropathy and CIPN, increased efficacy of PRCBs is not due to increases in cannabinoid receptor expression.

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GENDER-SPECIFIC AND DOSE-DEPENDENT ANTI-INFLAMMATORY EFFECTS OF TIGECYCLINE AND ITS ADDITIVE ANTINOCICEPTIVE EFFECTS WHEN COMBINED WITH URB937

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Tetracycline compounds are broad-spectrum antibiotics with potential off-label use as anti-inflammatory agents. Investigation into the effect of tetracycline derivatives on alleviation of acute and inflammatory pain has been previously demonstrated, but studies investigating gender differences are limited. The goal of this study is to evaluate the gender specific and dose-dependent effect of a synthetic tetracycline compound, Tigecycline, alone and/or in combination with URB937, a peripheral fatty-acid amide hydrolase inhibitor in the formalin (10 µl at 2.5 % intraplantar) model. In this study, we evaluate the effect of different doses of Tigecycline (0, 2, 8, 20 and 80 mg/kg i.p.) alone or in combination with URB937 (2 mg/kg i.p.) on inflammatory pain in male and female mice. Tigecycline produced dose-dependent antinociceptive effects in male and female mice. Tigecycline lowered both acute and inflammatory pain in only the lower doses in both male and female mice. Higher doses increase nociceptive responses in female. In female, the combination of URB937 with lower doses of Tigecycline produces an additive antinociceptive effect. Future studies should investigate the mechanism of action of this additive effect and the influence of the endocannabinoid system in lowering inflammatory responses.

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AN EMERGING ROLE FOR 2-ARACHIDONYL GLYCEROL IN RETINAL FUNCTION

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Introduction

Monoacylglycerol lipase (MAGL) is an endogenous enzyme responsible for deactivation of endocannabinoid 2-arachidonyl glycerol (2-AG). Deletion of gene encoding MAGL, or MAGL inhibition, elevates 2-AG levels and has been shown to be neuroprotective. Endocannabinoid system (ECS) components, including MAGL, are expressed in ocular tissues, including retina, and suggest that the ocular ECS plays a role in the retinal function.

Objectives

(1) To determine the role of 2-AG in retinal function using wild type and MAGL knockout (KO) animals.
(2) To assess whether the absence of MAGL, and consequent increase in 2-AG, has a neuroprotective effect on the retinal ganglion cells (RGC) following injury.

Methods

Electroretinogram (ERG) was used to measure visual signalling. ERG was carried out in C57Blk/6 and MAGL KO animals. Animals were dark adapted, anesthetized, and ERGs were recorded under scotopic as well as photopic conditions.

Axotomy: Anesthetized animals, C57Blk/6 and MAGL knockout mice, were placed in a stereotaxic apparatus. The optic nerve was exposed and cut. Animals were allowed to recover, and were sacrificed 7-days post-surgery. The eyes were enucleated, retinas removed and processed for immunohistochemical staining for RGCs with anti-Brn3a antibody. The RGCs were counted and the data was analyzed.

Results

ERG results indicate that in the absence of MAGL there is a significant increase in the light response from cone driven ON bipolar cells. The a-wave, representing the signals from the rod photoreceptors, remain in the normal range. RGC numbers were increased in MAGL KO mice compared to WT, however a similar loss of RGCs was seen following axotomy, suggesting that absence of MAGL enzyme did not have a significant neuroprotective effect on RGC survival in the optic nerve injury (axotomy) model.

Conclusion

ERG data suggests that 2-AG plays an important role in the visual processing, especially in the cone-driven response. Axotomy-induced loss of RGCs was not decreased in the absence of MAGL enzyme, which suggests that there may be a compensatory mechanism responsible for 2-AG degradation.

THE EFFECT OF CANNABINOID RECEPTOR TYPE 2 DEACTIVATION ON THE RETINAL FUNCTION OF *C57BL/6J* ADULT MICE

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Introduction:

Cannabinoid receptor 2 (CB2R) plays a significant role in the regulation of the immune function, and is mainly expressed by cells of the immune system. CB2R expression has been reported on activated microglial cells and astrocytes, as well as select neuronal populations. CB2R localization has been reported in murine retinal tissue and it has been suggested that CB2R signaling may contribute to visual processing. However, the cellular mechanisms underlying a role for CB2R in visual processing is currently unknown.

Purpose:

The purpose of this study was to examine the role of CB2R in the retinal responses to light in wild type *C57BL/6J* and CB**2**R knockout (KO) mice.

Methods:

The function of CB2R in the retina was investigated by recording electroretinographic responses (ERGs) under different light conditions in CB2R KO and wild type animals. We also investigated the acute and chronic effects of CB2R antagonist AM630 on the ERG response from wild-type (WT C57BL/6J) mice.

Results:

Our results showed that the a-wave (primarily rod photoreceptors) of the ERG in scotopic conditions, as well as dark adapted cone-driven ON bipolar cells and to a lesser extent cone-driven ON bipolar cells early in light adaptation, were increased in the absence of CB2R. Chronic (7 days), but not acute block of CB2R with the antagonist AM630, mimic the results observed in the CB2R KO mice.

Conclusions:

The data obtained indicate that loss of CB2R affects vision signal modulation. Lack of acute effect of CB2R antagonist AM630 suggests that differences observed between CB2R KO mice and control are caused by developmental changes and adaptation rather than direct CB2R deactivation.

REVIEW OF CANNABIS AND THE VISUAL SYSTEM FUNCTIONAL CLINICAL FINDINGS: IN MAGNOCELLULAR PATHWAY

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Objective: The literature describes dysfunction and enhancements in visual system with the consumption of cannabis. This presentation will discuss the dysfunctions and provide examples of deficits in the magnocellular visual pathway with cannabis consumption.

Hypothesis/Background: Cannabis changes visual processing during prenatal development, during adolescent development, acute use and long term use. The acute dysfunctions have many aspects that are similar to losses in the visual system in well characterized neurodegenerative diseases. Neurodegenerative disease processes such as Alzheimer's disease (AD) and Parkinson's disease (PD) have a impact on the visual system early in the disease processes. The deficits these diseases cause impact pathways that are not readily detected during traditional tests of acuity, but none the less have repercussions for quality of life. Functions such as contrast detection, motion perception, glare recovery and light dark cycling are impacted in these diseases and these functions are critical for safe ambulation and general mobility. If functions are impaired significantly, the result can be unsafe mobility, including an inability to safely drive. Cannabis decreases function in the lateral geniculate nucleus, a primary brain nuclei involved in vision processing. AD and PD have decreases in the function of the lateral geniculate nucleus. Cannabis and PD both demonstrate depletion of dopamine within the retina. Cannabis changes visual perceptions such as depth, glare recovery, motion perception, contrast detection and light dark cycling. These are functions that are not normally measured during a conventional vision exam. These are functions that are critical for safe driving. Technologies measuring central and peripheral visual fields that have a target with fixed spatial frequency and variable temporal frequency such as alternating stripes or checkerboards can identify deficits in magnocellular visual processing and have been used to detect dysfunction in AD and PD. Such a target has been used to identify deficits with the use of tetrahydrocannabinol with functional magnetic resonance imaging.

Methods: This presentation will review the visual system as it relates to cannabinoids/cannabis and further discuss the implications of cannabis as it relates to the visual perception and neurodegenerative disease. Individual results of vision screening of patients using cannabis will be presented. The visual system was tested using a common clinical test with a ten degree visual field target of fixed spatial frequency of .25 cpd, fixed temporal frequency of 25 Hz and variable contrast. The technology is commonly used for large public health vision screenings for the detection of glaucoma or diabetes. It has two protocols; a screening test and threshold test. Both test programs were utilized and both eyes were tested.

Summary: Deficits were identified. With a clearer understanding of the impact of cannabis on the visual system we can better design tools to determine functional impairment secondary to cannabis consumption. Tests of magnocellular function are a simple noninvasive means to do this. Cannabis is being used as treatment for neurodegenerative disease and it is important to be able to determine treatment impact on daily activities including driving. Further research is warranted.

TYPE-1 AND TYPE-2 CANNABINOID RECEPTOR SIGNALING IS INVOLVED IN THE NEUROPROTECTIVE EFFECT OF SAFFRON OF RAT RETINA

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Experimental studies demonstrated that saffron (*Crocucs sativus*), administered as a dietary supplement, counteracts the effects of continuous light exposure in the albino rat retina, preserving both morphology and function, probably acting as a regulator of programmed cell death (Maccarone et al., Invest Ophthalmol Vis Sci. 49 (2008) 1254-61). Since the presence of the endocannabinoid system (ECS) in retina has been widely documented in numerous species from fishes to primates (Nucci et al., Invest Ophthalmol Vis Sci. 48 (2007) 2997-3004; Yazulla, Prog Retin Eye Res. 27 (2008) 501–26), in the present study we have investigated whether the neuroprotective effect of saffron on rat retina exposed to bright continuous light (BCL) is associated with a modulation of the ECS. At this purpose, eight experimental groups of Sprague-Dawley rats, of which six exposed to BCL for 24 hours, were used. The retinal function was evaluated by recording electroretinographic response (fERG) and, then, retinas were quickly removed for biochemical and morphological analyses. Rats were either saffron prefed or intravitreally injected with selective type-1 (CB_1) or type-2 (CB_2) cannabinoid receptor antagonists before BCL. Prefeeding and intravitreally injections were combined in two experimental groups before BCL.

Here, we provided the first evidence that BCL exposure up-regulates gene and protein expression of CB_1 and CB_2, without affecting the other major components of retinal ECS. Remarkably, this effect of BCL on CB_1 and CB_2 was reversed by saffron treatment. In addition, selective CB_1 and CB_2 antagonists (e.g., SR141716A and SR144528) reduced photoreceptor death, preserved morphology and visual function of retina, and mitigated the outer nuclear layer (ONL) damage due to BCL. Of interest, CB_2 seemed to produce a predominant effect with respect to CB_1. In conclusion, these data suggest that BCL modulates only distinct ECS elements like CB_1 and CB_2, and that saffron engages also these two receptors in order to afford retinal protection.

FUNCTIONAL ROLE OF GPR55 CANNABINOID RECEPTOR ACTIVATION IN THE REGULATION OF SUBMANDIBULAR SALIVARY GLANDS

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Endogenous cannabinoids play an important role in many different cell types. In peripheral tissues, such as salivary glands, endocannabinoids are involved in the regulation of secretory processes. Among the other salivary glands, submandibular glands (SMGs) provides a main source of unstimulated salivary fluid secretion, which is required for the moistening and disinfection of the oral cavity. In particular, at the acinar cells secretion of fluid is triggered by a complex of cytosolic Ca^{2+} signal, originating Ca^{2+} release from the endoplasmic reticulum with subsequent activation of store-operated Ca^{2+} entry, which is required for synchronized activation of separated Ca^{2+}-dependent Cl^- and K^+ channels. Previously it was found that CB1 and CB2 cannabinoid receptors (CBRs) are expressed in the submandibular salivary gland cells and their activation leads to the inhibition of the agonist-stimulated salivation as well as modification of saliva content. Also, there is a significant amount of clinical data showing that a reduction in saliva flow and consequent dryness in the mouth can be caused by the activation of CBRs. GPR55 is thought to be expressed in acinar cells of the salivary gland, however it's function to date is unknown.

Using PCR and Western blot technics we have generated preliminard data suggesting that GPR55 is expressed in SMGs. Secondly, we have found that GPR55 is involved in the regulation of SMGs function. In particular, in vivo experiments showed that the endogenous agonist of GPR55 - LPI substantially suppressed saliva flow rate, changed $[Ca^{2+}]$, $[P^{2+}]$, $[K^+]$ and concentration of total protein in concentration-dependent manner. Effects of LPI was completely eliminated by specific antagonist of GPR55 - C390-019 (10 μM) when the different GPR55 antagonist - D327-0013 (10 μM) caused a decrease in salivation by it self. In contrast, a synthetic agonist of GPR55 – SY020 (1 μM) significantly increased salivation. To determine whether GPR55 could trigger $[Ca^{2+}]_{cyt}$ signalling in salivary cells, we measured intracellular Ca^{2+} dynamics in isolated acinar cells. It was shown that both LPI and SY020 activate Ca^{2+} signalling in a concentration-dependent way and can induced store-operated Ca^{2+} entry (SOCE). In addition, we find that LPI-induced activation of GPR55 leads to increased of ERK1/2 phosphorylation. SY020, in contrast, caused decrease of ERK1/2 phosphorylation.

This data suggest that cannabinoid receptors GPR55 are present in submandibular acinar cells and are functionally coupled with $[Ca^{2+}]_{cyt}$ signalling cascades, GPR55 may represent a novel system, by regulating the secretion of fluid, concentration of protein and electrolytes, which could be used for treatment of xerostomia and antibacterial protection within the oral cavity.

N-OLEOYL-L SERINE PROMOTES CREB PHOSPHORYLATION IN A PROSTATE CANCER CELL LINE (DU145) WHICH EXPRESSES THE PUTATIVE CANNABINOID RECEPTOR, GPR55

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The putative cannabinoid receptor G-protein coupled receptor 55 (GPR55) is known to signal via multiple pathways that regulate cyclic AMP response element binding protein (CREB) phosphorylation and is thought to be expressed in a variety of different cancer cell lines. Moreover, increased expression of GPR55 correlates with aggressiveness in several different cancers. The endogenous lipid L-α-lysophosphatidylinositol (LPI) and certain cannabinoid ligands are known to activate GPR55. However GPR55 has a limited homology with the two known classical cannabinoid receptors; CB_1 and CB_2. Recently a novel subset of bioactive lipids, the N-acyl amino acids, has been gaining interest due to their structural similarities to the endocannabinoids. However, N-acyl amino acids, such as N-oleoyl-L serine (NOSer), have little or no affinity for either CB_1 or CB_2 and a biological target remains unknown at present.

In the present study we investigated GPR55 ligand-mediated CREB phosphorylation (pCREB) in a prostate cancer cell line (DU145) that is reported to express endogenous GPR55. We find that NOSer is able to promote CREB phosphorylation in DU145 cells. Furthermore, these effects were concentration dependent over the range 10 nM – 10 μM and pCREB mediated responses to NOSer were reduced in the presence of the GPR55 antagonist CID16020046 (10 μM).

This study highlights that NOSer promotes pCREB in DU145 cells through the activation of GPR55. These data further confirm GPR55 as a novel lipid sensing receptor and a potential candidate receptor for NOSer.

AN INVESTIGATION INTO THE ROLE OF THE PUTATIVE CANNABINOID RECEPTOR GPR55 IN AN *IN VITRO* MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with neuronal loss and cognitive decline. There are an estimated 46 million people worldwide currently suffering from dementia and due to an increasingly aged population this number is set to triple by 2050. A neuropathological feature of AD is the deposition of β-amyloid (Aβ) which is proposed to contribute to neuroinflammation and neuronal cell death. A therapeutic approach that can halt the actions of Aβ is attractive as this is a strategy likely to impede disease progression. The orphan G-protein coupled receptor GPR55 is responsive to cannabinoids and is widely expressed in the neurons and glia of the brain. The suggested endogenous ligand for GPR55, L-α-lysophosphatidylinositol (LPI), is activated following inflammatory responses. This evidence suggests that GPR55 may have a regulatory role in neuroinflammation. The present study aims to examine the role of GPR55 and its signalling pathways in the regulation of neuroinflammation and neuronal cell death using an in vitro model of AD.

Cultured primary rat cortical neurons were treated with LPI (1 μM, 10 μM). LPI-induced signalling effects were assessed using phospho-cAMP element binding protein (pCREB) immunocytochemical staining and confocal microscopy and imaging of intracellular calcium responses. LPI induced CREB phosphorylation in a concentration- and time-dependent manner. High LPI concentrations (10 μM) induced calcium responses. Cortical neurons were also treated with LPI (1 μM, 10 μM) in the presence or absence of Aβ (10 μM) for 72 hours. The conditioned medium was then applied to the BV2 microglial cell line and the subsequent migration of BV2 cells was assessed using a Boyden chamber assay. LPI (10 μM) downregulated microglial migration evoked by Aβ, whereas a lower micromolar concentration of LPI (1 μM) increased levels of migration evoked by Aβ. Neuronal apoptosis was assessed by caspase-3 immunocytochemistry and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). LPI (10 μM) significantly downregulated neuronal apoptosis evoked by Aβ. This study suggests that LPI can confer a neuroprotective effect and demonstrates a possible role for GPR55 in the regulation of gene expression, microglial migration and neuronal apoptosis in an *in vitro* model of Alzheimer's disease.

EVALUATION OF NOVEL GPR55 LIGANDS USING RECEPTOR TRAFFICKING AND pCREB SIGNALLING ASSAYS

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The recently deorphanised, receptor GPR55 is endogenously activated by L-α-lysophosphatidylinositol (LPI) and also by specific cannabinoid molecules. GPR55 is expressed widely in the body and has been implicated in various physiological and pathological processes. In order to evaluate the function of this receptor in more detail there is an urgent need to develop new pharmacological tools.

The aim of this study was to evaluate a range of putative GPR55 ligands including the synthetic compounds PM299, PM304 and PM328 [1] and to compare their actions with the established GPR55 agonists LPI and AM251 and the antagonist CID16020046. The assays used were GPR55 internalisation and activation of pCREB signaling, evaluated using immunocytochemical techniques and confocal microscopy in HEK293 cells stably expressing HA-tagged, human GPR55. We found that the compounds PM304 and PM328 behaved as antagonists using receptor trafficking assays. However using the pCREB assay these effects were less clear and PM328 appeared to display agonist activity.

These findings help characterise new ligands that modulate GPR55 activity that could be useful in developing new pharmacological tools to evaluate the physiological and pathological roles of this receptor.

Reference

Paula Morales, Lauren S. Whyte, Roberto Chicharro, María Gómez-Cañas, M. Ruth Pazos, Pilar Goya, Andrew J. Irving, Javier Fernández-Ruiz, Ruth A. Ross, and Nadine Jagerovic. Identification of Novel GPR55 Modulators Using Cell-Impedance-Based Label-Free Technology. J. Med. Chem., 2016, 59, 1840–1853

MODULATION OF CANNABINOID RECEPTORS AND GPR55 IN EXPERIMENTAL SEPSIS

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Sepsis is the systemic inflammatory response to an infection and severe sepsis and septic shock are associated with tissue hypoperfusion, multi-organ dysfunction and high mortality. Accumulating evidence suggests that the endocannabinoid system is up-regulated in the pathogenesis of sepsis. In particular, cannabinoid 2 receptor, CB2, and GPR55, which are highly expressed on immune cells, are activated by endocannabinoids. Therefore, the endocannabinoid system represents a potential therapeutic target in sepsis. In the present study, we used a mice model of sepsis induced by lipopolysaccharide (LPS) and investigated the impact of CB2 and GPR55 modulation on leukocyte activation during endotoxemia using intravital microscopy (IVM) of the intestinal microvasculature, a key microcirculation in sepsis.

Institutional Animal Care Committee approval was obtained for all experimental procedures used in this investigation. LPS (5 mg/kg) was administered intravenously (i.v.) to anesthetized male C57BL/6 mice (WT or $CB2^{-/-}$, 6-8 weeks old). Cannabinoid receptor and/or GPR55 modulation was studied post LPS administration using the following substances: endocannabinoid degradation enzyme (monoacylglycerol lipase, MAGL) inhibitor (JZL184, 16 mg/kg, i.v CB2 antagonist (AM630, 2.5 mg/kg, i.v.); GPR55 agonists (LPI, 5 mg/kg, O-1602, 5 mg/kg, i.v.); GPR55 antagonists (CID16020046, 20 mg/kg, O-1918, 5 mg/kg, i.v.); and cannabinoid 1 receptor (CB1) antagonist (AM281, 2.5 mg/kg, i.v.). For visualization of leukocytes and capillaries, Rhodamine-6G and FITC-labeled bovine serum albumin were administered i.v. 30 minutes prior to IVM. Intestinal leukocyte activation (rolling and adhesion) and capillary perfusion (functional capillary density – FCD) was evaluated two hours after LPS administration by IVM.

Inhibition of endocannabinoid degradation by JZL184 and antagonism of GPR55 by O-1918 or CID16020046, respectively, in WT mice showed beneficial effects on the intestinal microcirculation in experimental sepsis by significant reduction of leukocyte adhesion and improvement of FCD. In animals treated with the CB2 antagonist AM630 before JZL184 administration those effects were abolished. In contrary, inhibition of endocannabinoid degradation by JZL184 was still beneficial in $CB2^{-/-}$ mice. CB1 antagonism by AM281 was not sufficient to reverse decreased leukocyte adhesion.

Endocannabinoid degradation enzyme inhibition and GPR55 blockade reduced LPS-induced intestinal leukocyte activation and improved the microvascular blood supply in experimental sepsis in WT mice. Contradictory results found in the $CB2^{-/-}$ studies suggested alternative molecular targets of endocannabinoids to be further investigated.

EFFECTS OF CANNABIDIOL ON NEUROTRANSMITTER CHANGES INDUCED BY HYPOXIA-ISCHEMIA IN THE NEWBORN PIGLET BRAIN

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Background and aim: Changes in neurotransmitter (NT) release and/or uptake has been related to brain damage after hypoxic-ischemic (HI) insults. Modulation of such changes may account for the neuroprotective strength of novel therapeutics. Cannabidiol (CBD) has demonstrated neuroprotective effects in animal models of neonatal HI encephalopathy (NHIE). We aimed to study whether the protective effect of CBD is related to a modulation of HI-induced changes in NT.

Methods: 1 day-old piglets were studied for 72 h after a HI insult (carotid clamp and FiO_2 10% for 20 min). Thirty min after HI piglets received vehicle (HV) or CBD (GW Pharmaceuticals, Cambridge UK) 1 mg/kg (HC) single dose. Non-HI piglets (SHM) served as controls. Every 24 h, brain activity and function were assessed by amplitude-integrated EEG (aEEG) and a neurobehavioral score (NBS), respectively. Finally, brain was harvested for histological (Nissl staining –necrosis-, and TUNEL –apoptosis) studies and to determine by HPLC brain concentrations (in fmol/100 mg) of several NT and metabolites.

Results: HI led to brain damage as observed by functional and histological studies. The HI insult led to an increase of dopamine and 5-HT concentration in the brain. Such an increase was likely due to increased release rather than reduced uptake since this increase was in parallel with an increase of their main metabolites. Increases in dopamine and 5-HT were related with increased excitotoxicity. Post-insult administration of CBD reduced brain damage, restoring neurobehavioral performance and preventing HI-induced increase of dopamine and 5-HT release and excitotoxicity.

		SHM (n=4)	***HV (n=9)***	***HC (n=5)***
NEUROPROTECTION	*aEEG (mean amplitude) (μV)*	18 (0.1)	13.8 (1.4)*	22.0 (2.6)#
	NBS (points)	35.5 (0.5)	29.0 (2.1)*	35.1 (0.7)#
	% necrotic neurons (Nissl)	1.85 (0.3)	10.7 (2.6)*	2.5 (0.7)#
	Apoptotic cells (TUNEL)	2.8 (0.7)	187.3 (21.2)*	59.9 (16.3)*#
NEUROTRANSMITTERS	*Norepinephrine*	3.01 (0.2)	4.41(1.0)	4.06 (0.3)
	Glutamate	6.68 (1.8)	8.16 (1.2)	7.02 (0.3)
	GABA	3.04 (0.9)	2.7 (0.7)	3.1 (0.4)
	Glutamate/GABA	2.22 (0.1)	3.5 (0.5)	2.3 (0.2)#
	5-HT	35.6 (3.3)	69.5 (7.2)*	35.1 (7.2)#
	5-HHA	0.69 (0.1)	1.34 (0.3)*	0.43 (0.1)#
	Dopamine	0.8 (0.0)	1.53 (0.1)*	0.7 (9.2)#
	Homovanillic acid (HVA)	0,86 (0.1)	1.26(0.2)*	0.53 (0.1)#

Mean (SEM). Kruskall-Wallis (Dunne's test). (*): p<0.,05 vs. SHM; (#) p<0.05 vs. HV

Conclusions: CBD-induced neuroprotection after HI in newborn piglets as indicated by the prevention of HI-induced alterations in functional and histological indices correlates with the modulation of neurotransmitter release

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REPEATED CANNABIDIOL TREATMENT REVERSES BEHAVIORAL CHANGES IN A MODEL OF SCHIZOPHRENIA BASED ON ANTAGONISM OF NMDA RECEPTORS

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Preclinical and clinical data indicate that cannabidiol (CBD), a non-psychotomimetic compound present in the plant Cannabis sativa, induces antipsychotic-like effects. Indeed, repeated treatment with CBD prevented schizophrenia-like behavioral changes induced by repeated treatment (28 days) with MK-801, an NMDA receptor antagonist, when both drugs were administered concomitantly. Changes induced by NMDA antagonists have been observed up to 6 weeks after the end of the treatment and can be reversed by atypical but not by typical antipsychotics. In the present work, we evaluated whether a shorter repeated treatment with MK-801 (7 or 14 days) would induce long-lasting deficits in the novel object recognition (NOR) and social interaction (SI) test. We also tested if repeated CBD treatment started after the end of the repeated MK-801 treatment would reverse these deficits. Male C57BL/6J mice received twice daily intraperitoneal injections of MK-801 (0.25, 0.5 or 1 mg/kg) for 7 or 14 days. SI was performed 8 days after the end of the MK-801 treatment. Twenty-four hours later, animals were submitted to the NOR test. After that, we investigated if repeated treatment with CBD (15, 30 or 60 mg/kg; once daily, i.p.) would reverse MK-801-induced changes. CBD treatment began 24h after the end of the MK-801 treatment and lasted for 7 days. CBD effects were compared to those induced by repeated clozapine (1 mg/kg) treatment. Forty-eight hours after the last injection, animals were submitted to SI and, 1 day later, to the NOR test. Additional groups of mice received, before each CBD injection, the CB1 antagonist AM251 (0.3 mg/kg).

Behavioral impairments were observed only after the treatment with MK-801 (0.5 mg/kg) for 14 days, but not for 7 days. Repeated CBD or clozapine treatment reversed the impairment in the SI and NOR tests induced by MK-801. AM251 pre-treatment did not antagonize CBD effects, but was effective by itself. These data reinforce the proposal that CBD has antipsychotic-like properties. In addition, considering the need for new alternatives to treat the negative and cognitive symptoms of schizophrenia, they also indicate that this drug could be an interesting alternative for the treatment of this disorder.

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MONOACYLGLYCEROL LIPASE DELETION IMPAIRS FINE MOTOR COORDINATION AND TRIGGERS CEREBELLAR NEUROINFLAMMATION

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Monoacylglycerol lipase (MAGL) is the main catabolic enzyme for the most abundant endocannabinoid in the brain, 2-arachidonoylglycerol (2-AG), and its genetic deletion results in an exaggerated 2-AG signaling. We have characterized the cerebellar phenotype in mice lacking MAGL (MAGL–/–). These mice show an ostensible motor impairment in the coat-hanger, the beam-walking and the footprint tests. In the cerebellum, MAGL–/– mice show a neuroinflammatory phenotype with enhanced reactivity of microglial cells, and increased expression of cyclooxygenase 2 (COX-2) in Purkinje cells. Alterations in glutamatergic signaling in cerebellar parallel fiber–Purkinje cell synapses were accompanied by modifications in the expression of glutamate receptors involved in cerebellar long-term depression (LTD). Similar results were found in THC-withdrawn mice, suggesting that both exogenous cannabinoid administration and the enhancement of the endocannabinoid tone underlie comparable cerebellar functional alterations triggered by analogous mechanisms. These data reveal the critical role of the cerebellar endocannabinoid system in synaptic homeostasis and motor coordination, pointing to a secondary neuroinflammatory process in the motor coordination deficit resulting from endocannabinoid system dysregulation.

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USE OF ECIS TEER TECHNOLOGY TO MONITOR CANNABINOID REGULATION OF BARRIER INTEGRITY OF BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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The aim of this research was to assess the acute and long term effects of various cannabinoids (both receptor-dependent and independent effects) on the barrier function of human brain microvascular endothelial cells. The blood brain barrier (BBB) is the physical interface formed by brain microvascular endothelial cells, which separate the blood stream and the central nervous system. The endothelial cells create a selective paracellular barrier due to the high expression level of tight and adherens junctions. Electric Cell-substrate Impedance Sensing (ECIS) measures the barrier resistance to electrons continuously in real-time (referred to as Tran-Endothelial Electrical Resistance). ECIS is currently the most advanced biosensor technology available to monitor barrier integrity offering real-time continuous analysis of both acute and longer term changes in permeability to be monitored.

For these studies we used human brain microvascular endothelial cells from ABM Good USA, which we have characterised extensively in terms of inflammatory activation and barrier formation (O'Carroll et al, 2015; Wiltshire et al, 2016). Interestingly, we can reveal that these brain endothelial cells did not respond to the CB2 agonists JWH015 or JWH133 (up to 10μM) either acutely or following long term treatment with the CB2 agonists. The lack of a long term effect indicates that there were no cytotoxic effects at high concentrations. This lack of response to CB2 agonists does not agree with that reported by Ramirez et al, 2012, but is in agreement with Hind et al, 2015. Molecular analysis revealed a lack of CB2 mRNA and receptor expression, consistent with the negative pharmacological response. Work is currently on-going investigating the effects of other cannabinoid compounds on the BBB endothelial barrier function using ECIS. We suggest that where a cannabinoid mediated effect is observed, especially at very high agonist concentrations, that the presence of the receptor is proven and not assumed. The discovery of a human BBB endothelial model lacking CB2 receptors may represent a valuable tool in differentiating the involvement of CB2 in leukocyte trafficking across the BBB.

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References

O'Carroll et al, 2015. *J Neuroinflammation*; **doi:** 10.1186/s12974-015-0346-0
Wiltshire et al, 2016. *Scientific Reports*; doi:10.1038/srep19814
Ramirez et al, 2012. *J Neuroscience*; *doi: 10.1523/JNEUROSCI.4628-11.2012*
Hind et al, 2015. *British Journal of Pharmacology*; doi: 10.1111/bph.13106

MODULATION OF THE ENDOCANNABINOID SYSTEM IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the major cause of dementia among the elderly. From the pathological point of view, AD is characterized by the accumulation in the brain of senile plaques and neurofibrillary tangles, namely insoluble aggregates of amyloid-β (Aβ) peptides and hyperphosphorylated tau, respectively. These neuropathological characteristics are linked to synapse dysfunctions and progressive loss of specific neuronal populations, especially in brain regions serving memory and cognitive functions. Noteworthy, other invariant hallmarks of AD are exacerbated neuroinflammation, excitotoxicity and oxidative stress.

In the last few years, emerging evidence has been accumulated on the regulation and role of the endocannabinoid system (ECS) in AD, and on the possible targeting of this system as a therapeutic strategy to treat this disorder. Here we aimed at identifying, within the ECS, novel noninvasive biomarkers that could facilitate the clinical evaluation of AD, and at clarifying the role of endocannabinoid (eCB) signaling in the pathogenesis of AD. In order to establish a possible correlation between AD progression and the molecular changes occurring in the ECS, we studied localization, expression and activity of its major components in Tg2576 mice, a well-established murine model of AD, as well as in peripheral blood mononuclear cells obtained from individuals at risk for AD and with different cognitive impairment. We found that: (i) circulating eCBs in the blood decrease with the progression of the disease; (ii) in presymptomatic phase, type-1 cannabinoid (CB_1) receptor is re-localized on neuronal membranes, also changing its coupling to specific G proteins; and (iii) CB_1 and CB_2 receptors, as well as fatty acide amid hydrolase (FAAH), the main anandamide hydrolase, are specifically up-regulated in human peripheral monocytes isolated from AD patients. Collectively, these results demonstrate that ECS undergoes marked alterations in response to progressive synthesis, and subsequent accumulation, of Aβ peptides, highlighting the potential of selected ECS elements as valuable biomarkers for AD.

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MINOCYCLINE TREATMENT MODIFIES Aβ CLEARANCE AND CYTOKINE EXPRESSION IN 5xFAD/FAAH$^{-/-}$ MICE

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by the deposition of beta-amyloid (Aβ) in form of senile plaques, as well as by hyperphosphorylation of tau protein, leading to neurofibrillary tangles. However, AD-associated chronic neuroinflammation is a key component in the worsening of the disease. Recent reports suggest a possible role for anandamide in AD; thus, using an *in vivo* model of AD with constitutive inactivation of FAAH (5xFAD/FAAH$^{-/-}$), we found that the increase of AEA levels led to a reduction in Aβ deposition, but concomitantly caused an augmented expression of pro-inflammatory cytokines, especially relevant in the case of interleukin-1β (IL1β). Treatment of 5xFAD/FAAH$^{-/-}$ mice with minocycline (an inhibitor of IL1β synthesis) exacerbated Aβ deposition and altered the pro- and anti-inflammatory cytokine balance. These data suggest that the interplay between FAAH and IL1β may be relevant during AD development and thus become a potential target for therapeutic intervention.

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CANNABINOID RECEPTOR 2 INHIBITION PARTIALLY REVERSES IMMUNOSUPPRESSION AFTER ACUTE CNS INJURY IN MICE

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One of the most frequently occurring medical complications after acute CNS injury, such as stroke or traumatic brain injury, is infection due to the disturbance of the normally well-balanced interplay between the immune system and the CNS after the injury. This dysregulation has been termed CNS injury-induced immunodeficiency syndrome (CIDS). The underlying mechanisms that are responsible for CIDS are still not elucidated but are hypothesized to be promoted by the injured brain. The endocannabinoid system (ECS) is composed of cannabinoid receptors, endocannabinoids and enzymes - all responsible for key homeostatic functions in the CNS and the immune system. It is suggested that local upregulation of the ECS occurs following CNS injury and represents an adaptive mechanism. CB2R is expressed in higher levels on microglia and immune cells and is shown to have an immunosuppressive role, suggesting that the activation of CB2R contributes to the immunosuppression in CIDS. The present study investigated whether the immunosuppression after an acute CNS injury can be reversed by CB2R inhibition.

CNS injury was induced in C57Bl/6 mice (male, 6-8 weeks) via an intracerebral injection of the vasoconstrictor peptide, endothelin-1 (ET-1, 2µg/µl). Immune response to endotoxin challenge was assessed 24 hours later using intravital microscopy to monitor leukocyte activation within the intestinal microvasculature, a key microcirculation in systemic inflammation. Brain tissue was extracted and stained with triphenyl tetrazolium chloride (TTC) to confirm the presence of CNS injury and to calculate the volume of infarct. CB2R -/- C57Bl6 mice were used to investigate the effect of CB2R knockout on the severity of CIDS after CNS injury.

Consistent with the induction of CIDS, intravital microscopy confirmed that immunochallenged animals with CNS injury have a reduced count of activated leukocytes within the intestinal microcirculation when compared to immunochallenged animals without CNS injury. AM630 (2.5 mg/kg, i.v.) administration 15 min prior to LPS challenge, reversed this measure of suppressed immune function and did not have any detrimental impact on the infarct size. Genetic knockout of CB2R revealed that the CIDS was not induced after an acute CNS injury, confirming the involvement of the ECS in CIDS.

The findings in our study suggest that inhibition of the CB2R activity with AM630 after an acute CNS injury reverses CIDS without exacerbating the brain injury. In addition, this is the first study to link the activity of ECS with CIDS. Further studies should focus on investigating various time points throughout the onset of CIDS in order to identify the optimal treatment window for CB2R inhibition therapy.

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PALMITOYLETHANOLAMIDE CHRONIC TREATMENT REDUCES THE SENSORIAL AND COGNITIVE DISFUNCTIONS ASSOCIATED WITH MILD TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) represents a major public health problem. Traumatic brain injury (TBI) initiates a neuroinflammatory cascade that contributes to neuronal damage and behavioral impairment. Cannabinoids of all classes have the ability to protect neurons from a variety of insults that are believed to underlie delayed neuronal death after traumatic brain injury (TBI), including excitotoxicity and neuroinflammation.

We investigated the anti-neuroinflammatory properties of the palmitoylethanolamide (PEA), a commercially available compound with a pleiotropic mechanism of action.

We applied a model of mild TBI that develop sensorial and cognitive disfunctions. In particular, mice developed abnormal pain sensation (allodynia) and depression associated to repetitive, obsessive-compulsive behaviours. According to the literature, we found that TBI increased the number of proinflammatory/hypertrophic microglial cells in specific areas of the brain. We observed that PEA chronic treatment (10 mg/kg i.p.), significantly ameliorate the mechanical allodynia associated with TBI. Moreover, cognitive impairment associated with TBI such as depression and aggressiveness were reduced by PEA treatment. In particular, we measured the immobility time in sham, TBI and TBI treated animals in the tail suspension test and the results revealed that, while TBI animals showed an increased immobility time, PEA chronic treatment determined a reduction of depressive-like behaviour. Finally, we found that PEA, through a genomic mechanism PPAR-α-mediated, increased the expression level of CB2 cannabinoid receptor in primary microglial cells and, hence, could be responsible of the phenotype switch from pro to an anti-inflammatory/neuroprotective microglia.

Our results show a possible use of natural compounds such as PEA together with the already used drugs for the treatment of severe brain injury. Moreover, the discovery of new mechanisms in endogenous lipid compound could represent a new pharmacological tool to develop new molecules for the treatment of chronic neurological disorders

PROTEIN KINASE C SIGNALING MEDIATES THE SHORT-TERM MEMORY IMPAIRMENT PRODUCED BY Δ^9-TETRAHYDROCANNABINOL

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Cannabinoid agonists affect cognitive function through their interaction with the endocannabinoid system, but the molecular mechanisms involved are vaguely understood. In the present study, we found that acute delta9-tetrahydrocannabinol (THC, 3 mg/kg, i.p.) affects short-term object-recognition memory in mice through cannabinoid type-1 (CB1) receptors in a similar way as it happened when we studied long-term memory. At the molecular level, THC administration enhanced mammalian target of rapamycin complex 1 (TORC1) and protein kinase C (PKC) signaling in the hippocampus. Surprisingly, the pre-treatment with the TORC1 inhibitor temsirolimus or a protein synthesis inhibitor, which were effective in reversing the long-term memory deficits produced by THC, did not show any effect over the short-term memory impairment. Notably, pre-treatment with the PKC inhibitors chelerytrine and NPC-15437 prevented the deficit in short-term memory produced by THC. In addition, these PKC inhibitors did not affect the THC impairment of long-term memory. THC administration was found to modulate PKC preferential residues in NMDA receptor type 1 subunit and the postsynaptic calmodulin modulator neurogranin. The phosphorylation of such PKC-preferential residues by THC treatment was prevented by the pretreatment with NPC-15437 inhibitor. These results identify the crucial contribution of PKC signaling in specific cognitive effects produced by THC and reveal independent molecular mechanisms between short- and long-term declarative memories.

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INTERNATIONAL CANNABIS AND CANNABINOID INSTITUTE: UNLOCKING THE DABASES OF NATURE

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Today millions of people are finding relief for an array of medical conditions and symptoms using cannabis treatments. Pre-clinical and observational research has demonstrated promising therapeutic applications. However, the vast reach of the cannabinoid receptors in almost every homeostasis action in human body, the number of complex active compounds found in the cannabis plant, and the array of government approaches to handling cannabis use has made research in the field of the cannabis and cannabinoid treatments difficult.

The advancement of cannabis and cannabinoid treatments requires further evidence-based exploration with a modern scientific approach to refine the therapeutic applicability of cannabis-based medicines. Unlike conventional pharmaceutical development which includes a process of isolating or synthesizing new compounds, observing the effects in vitro and then in vivo before human studies can be used to discover the safety profile and therapeutic benefit. In the arena of cannabis studies, researchers have already observed the therapeutic effect and safety profile in diverse patient groups receiving cannabis in clinical settings.

These findings have been challenging to investigate due to an array of circumstances such as international criminalization of the cannabis plant, nearly a century of bias toward single compound medicines and research tools, the lack of access to technology and pathways for the proper study of multi-compound substances in current clinical trial models, sacristy of available research institutions, and the lack of funding. Despite these challenges, there is global interest in the study of these treatments that are due to patient demands and the groundwork developed by organizations such as Americans for Safe Access (ASA) in the United States (US) and KOPAC in the Czech Republic (CZ), countries are moving toward allowing the legal use.

The founders of ASA and KOPAC have come together to harness this global interest by creating the first Center of Excellence in this field of study, the International Cannabis and Cannabinoid Institute (ICCI) in CZ. ICCI identifies, coordinates and supports global research priorities for the advancement of cannabis and cannabinoid treatments through a multidiscipline evidence based approach that incorporates innovative tools and approaches. ICCI promotes shared learning, creates needed tools, offers guidance, and deploys resources in these fields. A collaborative project of non-profit, for-profit and government institutions, the ICCI is the international research and educational hub designed to meet the needs of the global market.

Unleashing the true potential of cannabis and cannabinoid treatments requires a cross discipline approach that captures the current knowledge base across the fields of biomedical research, social science, life sciences, and policy research. This cross-discipline approach will identify and solve barriers to advancements and create a platform for shared knowledge. The diverse interest in cannabis and cannabinoid research spans across academia, research institutes, private endowments, governments, medical professionals, patient advocacy organizations, health services providers and product manufactures.

The ICCI founders share a combined experience that includes clinical and basic research, direct patient advocacy, legislative efforts and studies, policy studies, government relations, advisory, and administration, medical informatics, the creation of product safety protocols such as quality control/quality assurance standards and laboratory analysis for cultivation, manufacturing, distribution of cannabis products and global entrepreneurship. Their unique positioning leverages unparalleled resources and experience to create this global resource.

EXPERIENCES AND MOTIVES OF MEDICINAL CANNABIS PATIENTS: A CROSS-SECTIONAL QUESTIONNAIRE

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Scientific knowledge surrounding cannabis for medicinal purposes keeps expanding, however the patient itself has remained a blind spot in most research. This study aims to get an insight into the experiences of Dutch patients with the medicinal use of cannabis and their motives to use it for health reasons. The main purpose was to compare behaviour and satisfaction of patients using cannabis on prescription (OP) versus patients using it with no prescription (NP).

A cross-sectional patient questionnaire was distributed via (medicinal) cannabis-related websites and patient associations. The survey consisted of a wide range of questions, e.g. asking about the different modes of delivery used, cannabis access points (legal and illicit), dosing, satisfaction with cannabis as a medicine and perceived therapeutic effects. The survey was directed specifically to Dutch inhabitants who used or had been using cannabis for medicinal purposes (either through a physician or by self-medication), and was fully completed by 590 participants at the time of data collection for this poster.

Results showed that a majority of the respondents did not use cannabis on prescription (n=492). The majority used medicinal cannabis to treat pain (n=322 for pain and n=193 for nerve pain), insomnia (n=256), stress (n=156) and depression (n=89). Cannabis oil (n=398) and smoking (n=233) were by far the most commonly used methods of intake. Smoking and vaporising was more common among OP users, while NP users largely preferred cannabis oil. In general, OP users seemed to be more satisfied with cannabis as a medicine than NP users. Respondents were furthermore asked about their preferred cannabis access point, showing home grown cannabis and the pharmacy as most popular. For a substantial amount of NP users, the pharmacy appeared to have their preference, despite the fact that they did not have a prescription. More people would prefer going to a pharmacy than those with access to it. An opposite effect was found for coffeeshops; more patients use this access point than those who would prefer it. These people probably make use of it because they see no other option in obtaining their medicine.

In conclusion, this explorative study offers interesting insights into actions and motives of Dutch patients and their medicinal usage of cannabis. A similar survey is being constructed specifically for OP users, which will be spread in pharmacies throughout the Netherlands. Results are expected at the end of 2016, after which we should be able to make a valid and thorough comparison between the two (OP and NP) groups. This will pave the way for more and deeper patient-inspired research, in order to gain more knowledge about the potential benefits and pitfalls of cannabis as a medicine.

MEDICAL CANNABIS: A DIVIDE BETWEEN DOCTOR AND PATIENT

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Unfortunately, despite growing evidence that the endocannabinoid system (eCS) is vital to human health, *cannabis sativa* remains classified under the U.S. Controlled Substances Act as a Schedule 1 Substance. Both physicians and patients interested in exploring this therapy through state-approved "Compassionate Care" programs frequently encounter resistance or confusion from the other party; particularly when it comes to the actual recommendation of cannabis. The proposed study seeks to discover how the social ramifications of the Controlled Substances Act and Schedule 1 status of *cannabis sativa* effect the doctor-patient communication experience. Dynamics such as personal belief, social stigma, lack of public and professional education, and many other factors contribute to this problem and require exploration. Considering the doctor-patient relationship, and specifically, the conversation about recommending medical cannabis, from multiple perspectives will enhance the understanding of the dynamics in play. In order to take a more comprehensive view, an Integral methodology will be employed in a phenomenological study that considers the narrative of both physicians and patients. By assessing their common and differing experiences through an Integral lens (Wilber, 2000a), while grounding the experiences in the solid AQAL theoretical frame, an accurate assessment of this particular phenomenon will emerge. A developed understanding of how doctors and patients communicate about the recommendation of medical cannabis will help guide future research and educational initiatives and assist with normalizing the use of cannabis.

CANNABIS COMPARES FAVORABLY TO CONVENTIONAL PTSD TREATMENTS

Tiffany Devitt and Adrian Devitt-Lee

Care By Design, Tufts University

A growing body of research suggests that there is a strong connection between the endocannabinoid system and PTSD, and that cannabis therapy can help mitigate the hallmark symptoms of PTSD including: impaired fear extinction, poor memory consolidation, and chronic anxiety. Care By Design, a California-based medical marijuana company, is presently surveying hundreds patients with PTSD (post-traumatic stress disorder). The survey asks what medications patients have used for PTSD-related symptoms (including cannabis), and asks patients to assess each medication in terms of its impact on the hallmark symptoms of PTSD, including anger and irritability, anxiety, depression, pain, and sleep problems.

The preliminary data, which includes responses from over 300 patients, suggests that cannabis compares favorably with conventional treatments for PTSD. Survey respondents reported taking numerous medications for PTSD with half taking at least 5 medications. Survey respondents reported that cannabis was the most likely to improve PTSD symptoms—albeit to an unknown degree—and the least likely to make symptoms worse. By comparison, the most common medication prescribed for the treatment of PTSD among survey respondents was anti-depressants (86%). Yet, only 18.1% said their depression got better on anti-depressants; and half reported that their depression got worse on anti-depressants. The majority of respondents reported that their depression worsened on anti-psychotics, tranquilizers, narcotic pain medication, mood stabilizers, and anti-convulsants. Roughly half of respondents reported they had been prescribed narcotics for PTSD. The majority of them reported that their anger and irritability, depression, and sleep problems got worse on the medication.

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OXYCODONE'S EFFECTS ON CANNABIS-INDUCED ANALGESIA AND SUBJECTIVE DRUG RATINGS

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Background: Preclinical studies have demonstrated that opioid agonists enhance cannabinoid-mediated effects, including antinociception (Maguire et al., 2013). Clinically, patients with pain report greater pain relief when cannabis is used in conjunction with opioids (Degenhardt et al., 2015). The objective of this double-blind, placebo-controlled study was to directly assess the effects of an opioid agonist on cannabis-induced analgesia using the Cold-Pressor Test (CPT), a laboratory model of pain that we have previously used to demonstrate the analgesic effects of both smoked cannabis and oral THC (dronabinol) (Cooper et al., 2013). For this outpatient, within-subject, double-blind, placebo controlled study, the effects of sub-analgesic and low therapeutic doses of oxycodone (2.5 and 5.0 mg, PO) on cannabis-induced analgesia were assessed. Subjective ratings associated with abuse liability were also assessed. **Methods:** Healthy, non-treatment-seeking cannabis smokers who had experience with an opioid at least once with no adverse effects participated in this outpatient study during which the pain-relieving and subjective effects of smoked cannabis were evaluated alone and in combination with oxycodone. Volunteers had to have had experience with an opioid at least once with no adverse effects; current use of over-the-counter medications, prescription analgesics, or opioids was exclusionary. During each session, participants smoked an inactive (0.0% THC) or active (5.6% THC) cannabis cigarette 45 minutes after ingesting a capsule containing placebo (0 mg, PO) or active oxycodone (2.5 or 5.0 mg, PO). Analgesic responses and subjective drug effect ratings were measured at multiple time points throughout each session. Analgesia was assessed using the CPT, which required participants to immerse their hand in cold water (4°C) for up to three minutes; the amount of time to report pain (pain threshold) and withdraw the hand from the water (pain tolerance) was recorded. Participants were also asked to rate the subjective 'Painfulness' and 'Bothersomeness' of the cold-water stimulus and to report the severity of the pain using the McGill Pain Questionnaire. Subjective drug effects were also measured using visual analog scales throughout the session

Results: Sixteen non-treatment-seeking cannabis smokers (10 M, 6F), 31 ± 7 years of age, who smoked an average of 11.8 ± 7.2 cannabis cigarettes per day, 6.8 ± 0.4 days per week completed this study. Participants did not have a history of opioid abuse. When administered alone, active cannabis and the 5.0 mg oxycodone dose decreased pain responses on some measures compared to placebo (placebo capsule + inactive cannabis) including pain tolerance (active cannabis, $p \leq 0.05$; 5.0 mg oxycodone, $p \leq 0.05$), pain threshold (5.0 oxycodone, $p \leq 0.05$), and subjective ratings of the 'Bothersomeness' of the cold-water stimulus (active cannabis, $p < 0.01$). Pretreatment with 5.0 mg oxycodone enhanced cannabis's analgesic effects on pain tolerance ($p \leq 0.05$). The 2.5 mg oxycodone dose alone did not affect any measures of analgesia compared to placebo. However, 2.5 mg oxycodone administered with active cannabis increased both pain threshold and tolerance, and decreased subjective ratings of pain as measured by the MPQ compared to cannabis administered alone ($p < 0.05$). Despite these effects on cannabis-induced analgesia, oxycodone (2.5 and 5.0 mg) did not affect subjective ratings of 'High,' cannabis strength, and positive drug effects including 'Take Again' and 'Good Effect.' **Discussion:** The results from this study suggest that low doses of opioids enhance the analgesic effects cannabis without increasing cannabis-induced intoxication or ratings of positive subjective effects. These findings provide evidence for the potential therapeutic use of opioid-cannabinoid combinations for the treatment of pain.

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URINARY CANNABINOID LEVELS DURING NABIXIMOLS (SATIVEX™)-MEDICATED INPATIENT CANNABIS WITHDRAWAL

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Nabiximols (Sativex™) is a buccal spray containing both Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). It shows promise as an agonist substitution therapy for treating cannabis withdrawal and dependence. Monitoring urinary cannabinoid levels during agonist substitution treatment is important to ensure medication safety and adherence. However CBD itself is not typically detected in urine, being mostly present in the form of glucoronidated secondary metabolites. We demonstrate the use of a recently described hydrolysis method to liberate CBD, and describe the trajectory of CBD, THC, and various metabolites in the urine of patients receiving 6 days of inpatient Nabiximols treatment (or placebo) during cannabis withdrawal.

Urine and plasma samples were taken before and during the 6 day treatment regime and during a 3 day drug-free washout. Urine hydrolysis was achieved with Red Abalone β-glucoronidase, and CBD, THC, THC-COOH and 11-OH-THC were quantified in daily urines using LC-MS/MS. Urinary cannabinoid levels were compared with plasma levels on days 1, 3 and 7.

Urine and plasma cannabinoid levels followed similar trajectories across the course of the treatment and reflected the dosing schedule. During nabiximols treatment, CBD levels in urine and plasma rose markedly, while concentrations of THC and its metabolites remained at or slightly above baseline levels. Following hydrolysis, urinary CBD was detected at levels approximately 50 times higher than in non-hydrolysed plasma and 200 times greater than non-hydrolysed urine. THC, THC-COOH and 11-OH-THC concentrations were also improved by urinary hydrolysis.

The hydrolysis method used here allows assessment of urinary CBD, and is remarkably more sensitive than a standard plasma CBD assay. Such analysis may prove useful in other clinical studies involving nabiximols or cannabinoid-based treatments.

PATIENT FOCUSED CERTIFICATION (PFC) – QUALITY STANDARDS OF CANNABIS PRODUCTS FOR MEDICAL USE

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Patient Focused Certification (PFC) is a 3rd party certification program focused on *Cannabis* product safety. PFC was developed by Americans for Safe Access (ASA) a medical *Cannabis* advocacy organization. ASA set the standards in the U.S. for medical *Cannabis* product safety and best practices for manufacturing, dispensing, cultivation, and laboratory operations. The PFC program is working around the world to implement existing standards and best practices for patients, providers of medical care, companies, regulatory bodies and legislators.

PFC compliance requires defined processes, documented procedures, validated methods adverse event tracking, recall plans, among other criteria, which help to ensure that *Cannabis* products have a specific composition and are free of harmful contaminants. PFC's international program is headquartered at the International Cannabis and Cannabinoids Institute (ICCI) in cooperation with ASA. The ICCI founders share a combined experience that includes clinical and basic research, direct patient advocacy, legislative efforts and studies, government relations, advisory and administration, medical informatics, the creation of product safety protocols such as quality control/quality assurance standards and laboratory analysis for the cultivation, manufacturing and distribution of *Cannabis* products and global entrepreneurship.

MARIJUANA AND MARIJUANA PRODUCTS FOR RESEARCH PROVIDED BY NIDA

Steve Gust

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Over the last few years interest in a wide variety of marijuana varieties and marijuana products has increased. This has been fueled by recent legal and policy changes regarding the use of these products for medicinal and recreational purposes. This has fueled the production and promotion of a marijuana and marijuana products with varying constituents, including cannabidiol, other cannabinoids, as well as non-cannabinoid constituents, in addition of various THC potencies. In response to this increased interest NIDA has produced a wide variety of marijuana chemotypes with large ranges of THC and CBD content, as well as several extracts and purified marijuana components for research purposes. This poster will present information on the marijuana varieties and marijuana products currently available and under development, as well as procedures and requirements for requesting these materials.

ENDOCANNABINOID MODULATION OF THE ORBITOFRONTAL CORTEX BY A CAFETERIA DIET

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The orbitofrontal cortex (OFC) plays a key role in the cognitive and emotional processing of decision-making. Dysfunction of the OFC is thought to underlie compulsive behaviours, including obsessive-compulsive disorder, drug and behavioural addictions. Previous evidence suggests that the endogenous cannabinoid (endocannabinoid) system is involved in regulating cognitive/emotional flexibility in the prefrontal cortex; however, its specific role in the OFC has yet to be confirmed. Using *in-vitro* patch clamp electrophysiology, we show that CCK-expressing GABAergic synaptic inputs onto principal layer II/III OFC pyramidal neurons are sensitive to endocannabinoids. Specifically, they exhibit depolarization-induced suppression of inhibition (DSI) that is blocked by the cannabinoid CB1 receptor inverse agonist, AM251 (3 μM), and the diacylglycerol lipase inhibitor, tetrahydrolipstatin (Orlistat, 10 μM). Since elevated endocannabinoids have been associated with consumption of a high fat diet, we examined if extended (24 hr) or restricted (1 hr) access to a cafeteria "junk food" diet altered the endocannabinoid system in the OFC. Interestingly, we found there was a reduction of inhibitory GABAergic transmission onto OFC pyramidal neurons following extended, but not restricted access to a cafeteria diet. This suppression of inhibition was partly reversed by the neutral CB1 receptor antagonist, NESS-0327 (0.5 μM). Together, our results suggest that enhanced endocannabinoid signalling and tone partially mediate a disinhibition of OFC pyramidal neurons following exposure to an extended cafeteria diet. This cannabinoid-mediated hyperexcitability of the OFC may be a mechanism for compulsive overeating.

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THE CANNABINOID QUINOL VCE-004.8 INHIBITS ADIPOGENESIS BY TARGETING ERK 1+2 ACTIVATION AND MODULATES DIET-INDUCED OBESITY

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Obesity is a growing pandemic and a major contributor to metabolic syndrome and disorders such as type-2 diabetes, cardiovascular disease, dyslipidemia, non-alcohol fatty liver and some cancers. In the past few years it has become evident that the endocannabinoid system (ECs) plays a crucial role in regulating food intake and energy metabolism. Moreover the ECs also regulate different arms of the immune system and the inflammatory process, which is closely linked to obesity and insulin resistance. Therefore, the pharmacological manipulation of the ECs is a major goal for researchers and pharmaceutical companies. We have previously found that cannabidiol (CBD) oxidation increases its PPARγ agonistic activity and we have generated a non-thiophilic CBD quinol derivative, VCE-004.8, that is a dual agonist of PPARγ and CB2 receptors (Del Río et al, Scientific Reports (2016); 6:21703), which are two attractive targets for the development of cannabinoid-based therapies.

Herein, we have investigated the effect of VCE-004.8 in different *in vitro* models of adipogenesis and in a murine model of metabolic syndrome induced by high fat diet (HFD). Using murine embryonic fibroblasts, human pre-adipocytes and human bone marrow mesenchymal stem cells, we found that VCE-004.8 inhibited both ERK1+2 phosphorylation and adipogenic differentiation measured by quantification of lipid droplets accumulation by Oil-Red staining and determination of adipogenic markers by qPCR. Adult male mice, fed for >8-wks with either HFD or the corresponding low fat, control diet (CD), were used for in vivo validation. Daily intraperitoneal administration of VCE-004.8 (20 mg/kg) for 3-wks induced a significant reduction in food intake and body weight gain, in both HFD and CD mice, with a ~10% drop of BW at the end of the treatment, irrespective of the diet. VCE-004.8 significantly ameliorated also glucose tolerance following i.p. administration of a glucose bolus, in both HFD and CD animals reflecting a better response to insulin. Moreover, VCE-004.8 reduced the plasmatic levels of leptin and the expression of phosphorylated ERK 1+2 induced by HFD in the adipose tissue. In conclusion, our studies document the potent biological actions of VCE-004.8 in adipogenesis by inhibiting ERK 1+2 activation, and highlight its potential to ameliorate inflammation associated to obesity by targeting also PPARγ and CB2 receptors.

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ROLE OF CANNABINOID-1 RECEPTORS (CB1R) IN KUPFFER CELLS IN THE REGULATION OF HEPATIC INSULIN RESISTANCE IN OBESE MICE

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Investigation into the mechanisms which make obesity a risk factor for developing insulin resistance is an area of intense research with increasing evidence for a major role of inflammation. Obesity-induced accumulation of ectopic fat in the liver is thought to contribute to the development of insulin resistance, and increased activity of hepatic CB1R has been shown to promote both processes. However, lipid accumulation in liver can be experimentally dissociated from insulin resistance under certain conditions, suggesting that other mechanisms are also involved. Obesity is also associated with proinflammatory changes such as higher production of TNF-α which, in turn, can promote insulin resistance. Kupffer cells (KCs), the liver's resident macrophages, are the major source of proinflammatory cytokines in the liver, such as TNF-α, which has been shown to inhibit insulin signaling in multiple cell types, including hepatocytes.

Using intravenously administered β-D-glucan-encapsulated siRNA to silence gene expression selectively in KCs *in vivo*, we demonstrate that an 80% knock-down of the expression of *Cnr1*, the gene encoding CB1R, results in improved glucose tolerance and insulin sensitivity in diet-induced obese mice, without affecting hepatic lipid content or body weight and adiposity. Moreover, *Cnr1* knock-down in KCs was associated with a lower concentration of anandamide and 2-arachidonoylglycerol as well as an increase in *Faah* and *Magll* gene expression in these cells. In addition, *Cnr1* knock-down led to a shift from pro-inflammatory M1 (high *Tnf*, *Tgfb* and *Nos2* expression) to anti-inflammatory M2 cytokine profile (high *Il-10*, *Arg1* expression and low *Il-6* expression), decreased NFκB activity and decreased gene expression of the oxidative stress marker p47phox, a subunit of the NADPH oxidase 2. Finally, knocking-down *Cnr1* in KCs restored insulin signaling as reflected by increased insulin-induced Akt phosphorylation.

In conclusion, these findings suggest that CB1R expressed in KCs play a critical role in hepatic insulin resistance via a pro-inflammatory mechanism and could represent a novel therapeutic target.

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MODULATION OF ACUTE STRESS-INDUCED ANOREXIA BY FATTY ACID AMIDE HYDROLASE INHIBITION IN RATS

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In rats, acute stress is known to cause a reduction in food intake. This anorectic effect is likely modulated by factors such as stressor intensity, as well as motivation to feed, i.e. hunger/satiety.

Many aspects of the stress response are regulated by the endocannabinoid system (ECS), as is food intake, but it is unclear how this system modifies stress effects on feeding. Given that fatty acid amide hydrolase (FAAH) inhibition has previously been shown to attenuate a variety of stress effects through an increase in the endocannabinoid, anandamide (AEA), we tested the hypothesis that AEA regulates stress-induced anorexia. As such, male Sprague-Dawley rats were administered vehicle or the FAAH inhibitor PF04457845 (PF; 10 mg/kg) 2hr prior to an acute psychological stressor (restraint stress). Post-stress feeding intake of regular lab diet (Prolab RMH 2500) and animal body weight were subsequently assessed over the following 24hr and 3 days, respectively.

Stress-induced anorexia was not observed when rats were fasted for 24hr prior to a 2hr stressor or with a shorter stress period of 1hr. Among non-food-deprived animals, following exposure to a 2hr restraint stressor (or a 2hr fast to control for food intake in home cage controls), stressed animals consumed significantly less chow within the first hour of testing (1.9g vs 5.4g), and feeding was significantly reduced up to 22 hr post-stress. However, the anorectic effects were not altered by systemic PF administration. Interestingly, PF was found to reduce food intake and normal body weight gain in non-stressed control animals compared to vehicle-treated rats. Taken together, these results suggest that stress-induced effects on feeding are influenced by satiety/hunger signals, which may be affected similarly by FAAH inhibition alone. Current experiments are exploring the mechanism(s) of action underlying this paradoxical effect of FAAH inhibition on stress-induced feeding behaviour.

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FATTY ACID ACYLSEROTONINS ARE PRESENT IN HUMAN COLON TISSUE

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Anandamide and other fatty acid amides are degraded by the enzyme fatty acid amide hydrolase (FAAH). Through modulating FAAH activity, the levels of endocannabinoids can be controlled. Previously, a new class of lipid metabolites known as fatty acid acylserotonins has been found to inhibit FAAH activity, increase anandamide levels, and display anti-inflammatory and anti-nociceptive properties (Bisogno et al., Biochem Biophys Res Commun. 248 (1998) 515-522; D'Argenio et al., FASEB Journal 20 (2006) 568-570). More recent work from our group demonstrated that a wide range of fatty acid acylserotonins, including arachidonoyl serotonin (AA-5-HT), docosahexaenoyl serotonin (DHA-5-HT), eicosapentaenoyl serotonin (EPA-5-HT), oleoyl serotonin (OA-5-HT), palmitoyl serotonin (PA-5-HT) and stearolyl serotonin (SA-5-HT), are present in mammalian tissues (Verhoeckx et al., Biochim Biophys Acta 1811 (2011) 578-586). To the best of our knowledge, the presence of acylserotonins has not yet been demonstrated in tissues of human origin. In the present study, the presence of these compounds in human colon tissue was investigated using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Patients between 30-60 years that were scheduled for colectomy were eligible to take part in this study, irrespective of sex, cancer stage, medication or co-morbidity. Written informed consent was obtained prior to surgery. Immediately following surgical removal of the colon, fresh tissue samples were collected and frozen at -80°C until further analysis. A solid phase extraction (SPE) procedure and LC-MS/MS method (Verhoeckx et al., Biochim Biophys Acta 1811 (2011) 578-586) were implemented that allowed for sensitive detection of fatty acid acylserotonins from colon tissue.

Six patient samples were screened for acylserotonin content. Six fatty acid acylserotonins were detected in human colon tissue. AA-5-HT and EPA-5-HT were both detected in only 2 out of 6 samples, whereas DHA-5-HT, OA-5-HT, PA-5-HT and SA-5-HT were detected in all 6 samples. The concentrations were in the pg to ng per gram wet tissue range, with the lowest concentrations for AA-5-HT and EPA-5-HT, and OA-5-HT being present in relatively higher concentrations. The concentration pattern observed in human colon resembles previously published data on murine gut acylserotonin concentrations. Generally, the recovery of the SPE method was ~30-40%, but this could be compensated for by using the appropriate deuterated internal standard. To conclude, to the best of our knowledge, this is the first report describing the presence of fatty acid acylserotonins in human tissue. Due to their FAAH-inhibitory and immune-modulating properties, fatty acid acylserotonines may have a role in human gut physiology and immunology.

THE CANNABINOID 1 RECEPTOR (*CNR1*) GENE POLYMORPHISM IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES

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The list of diseases in which medical marijuana and cannabinoid based drugs are promoted as a treatment is constantly expanding. According to scientific reports, marijuana may be effective in alleviating symptoms in patients with inflammatory bowel diseases in particular in the case of ulcerative colitis (UC) and Crohn's disease (CD). Latest data suggest a crucial role of the endocannabinoid system, including the cannabinoid receptors (CNR1), in intestinal inflammation. Accordingly, we decided to analyze the prevalence of polymorphic variant *CNR1* 1359 G/A (p.Thr453Thr; rs1049353) and its putative correlation on disease susceptibility in patients with IBD (inflammatory bowel diseases).

Group of 189 patients (93 females and 96 males) with ulcerative colitis (n=93) and Crohn's disease (n=96) and 200 healthy individuals (100 females and 100 males) from Polish population were examined. For genotyping pyrosequencing technique was used. Hardy–Weinberg equilibrium (HWE) were examined for subjected groups by chi-square distribution and Fisher exact tests. The odds ratios (ORs), 95% confidence intervals (CIs), and p-values were calculated. Statistical significance was set at $p<0.05$.

The analysis of *CNR1* gene variant showed no statistically important differences in frequencies of genotypes and allels in rs1049353 *locus* in two main subjected groups (p=0.18, OR 2.21, 95% C.I. 0.66-7.38). However, we observed a statistically significant corelation comparing UC patients with population group (**p=0.04, OR 3.47, 95% C.I. 0.97-12.32**). We did not observe this association for patients with Crohn's disease (p=0.97, OR 1.02, 95% C.I. 0.18-5.76).

The CNR1 p.Thr453Thr polymorphism seems to be important in the UC susceptibility, however further investigations need to be done. The endocannabinoid system may influence the manifestation of inflammatory bowel diseases, suggesting endocannabinoids as potential target for future therapies.

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PALMITOYLETHANOLAMIDE EXERTS ANTIPROLIFERATIVE EFFECT AND DOWNREGULATES VEGF SIGNALING IN CACO-2 HUMAN COLON CARCINOMA CELL LINE

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Angiogenesis is the formation of new blood vessels from pre-existing ones and represents the crucial step for the growth, progression and metastasis of many form of cancer, including colon cancer. It seems clear as molecular compounds able to interfere with angiogenesis may assume the role of very promising candidates to improve the therapeutic approach for colon cancer. Palmitoylethanolamide (PEA) is an endogenous N-acylethanolamide, structurally similar to anandamide (AEA) with which it shares the capability to interact with endocannabinoid receptors. PEA has been demonstrated to be a "promiscuous" molecule, acting at different cell targets exerting several activities, including anti-proliferative and anti-angiogenic effects associated to its well-known anti-inflammatory and analgesic activity. However, no evidence about these putative properties owned by PEA in human colon cancer cells has been yet provided. For this reason we aim to evaluate the anti-proliferative and anti-angiogenic effect of PEA in human colon adenocarcinoma Caco-2 cell line. Cells were exposed to increasing concentrations of PEA (0.001, 0.01 and 0.1 μM) in the presence of PPAR-α or PPAR-γ. Cell proliferation was evaluated by performing a MTT assay. VEGF release was estimated by ELISA, while the expression of VEGF receptor and the activation of the Akt/mTOR pathway were evaluated by western blot analysis.

PEA caused a significant and concentration-dependent decrease of Caco-2 cells proliferation at 48 h, VEGF secretion and VEGF-receptor expression. Inhibition of Akt phosphorylation and a downstream decrease of phospho-mTOR and of p-p70S6K were observed too. In the presence of PPAR-α, but not PPAR-γ antagonist, all effects of PEA were reverted.

PEA is able to reduce cell proliferation and angiogenesis, through the inhibition of the Akt/mTOR signaling by PPAR-α selective activation. If supported by *in vivo* models, our data pave the way to PEA co-administration to the current chemotherapeutic regimens for colon cancer.

DYSREGULATION OF GUT-BRAIN ENDOCANNABINOID SIGNALING IN MICE MAINTAINED ON A WESTERN DIET

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The gut-endocannabinoid (eCB) system is an important regulator of feeding and energy balance. Previous work from our laboratory has shown that fasting (FD) promotes production of the eCB, 2-arachidonoyl-*sn*-glycerol (2-AG), in the rat upper small intestine, when compared to free feeding controls (FF), and this signaling event drives refeeding after a fast (DiPatrizio et al, *Amer J Phys* 309 (2015) 805-13). Similarly, it is suggested that oral exposure to dietary fats is a driving force in gut eCB production, which is regulated by cholinergic signaling (DiPatrizio et al, *Proc Natl Acad Sci USA* 108 (2011) 12904-08).

Our most recent findings show that Western Diet (i.e., high levels of fats and sucrose) induced obesity (DIO, 60 day access) in mice is associated with higher levels of jejunal 2-AG in FF, similar to those of a healthy fasting mouse maintained on standard chow. 2-AG in DIO during FD remained similar to levels during FF, possibly indicating that a plateau has been reached for eCB production in the small intestine. Gene expression analysis of cannabinoid CB_1 receptors (i.e., qRT-PCR) revealed an increase in expression in response to fasting in animals fed a standard diet, an effect completely absent for those on a Western Diet. Interestingly, circulating 2-AG in blood plasma shows no response to FD in the healthy mice, but a very large increase in DIO FF and an even larger increase in FD. An analysis of feeding behaviors revealed that DIO mice consumed more calories and larger meal sizes than their lean counterparts over a 24 hour period. Collectively, this body of work suggests that eCB signaling in the gut is a general hunger signal that may be initiated under several behavioral and metabolic conditions. It is also suggested that in DIO, one or more of the pathways regulating 2-AG production and action is dysregulated. Thus, these investigations advance our understanding of gut-brain eCB signaling and suggest potential new pharmacological avenues for appetite control and the treatment of obesity.

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A POLYMORPHISM LOCATED ON CHROMOSOME 6Q16 NEAR CB1 (*CNR1*) GENE REGION IS ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE RISK AND REGULATES *CNR1* GENE EXPRESSION

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Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a chronic gastrointestinal disease that results from an excessive immune response in the gut. Several reports have suggested that the endocannabinoid system is involved in intestinal inflammation and in particular that the CB1 is involved in mediating gastrointestinal mobility in mice models (Izzo et al. Br J Pharmacol. (2009) 134:563-570 and Storr et al. J Mol Med. (2008) 86:925-936). A 6q16 polymorphism (rs13197090) located in the vicinity of *CNR1* gene region, that codes for CB1 protein, was reported to contribute to variation of *CNR1* mRNA expression levels (Schadt et al. PLoS Biol. (2008) 6:e107). Therefore, we tested the hypothesis that rs13197090 is associated with IBD risk and that rs13197090 influences *CNR1* gene expression in subjects diagnosed with IBD. We studied a case-control cohort of 293 patients with IBD and 276 controls randomly selected from the Slovene population. Gene expression was measured in PBMCs with qPCR and genotyping was performed by High Resolution Melting (HRM) analysis.

We found that according to the recessive model of genetic association, the frequency of rs13197090 CC genotype was higher in subjects with IBD (4.1%) than in the control group (0.4%, $p = 0.007$). Polymorphism rs13197090 was also significantly associated with *CNR1* mRNA expression levels. According to the recessive model of genetic association, IBD subjects with CC genotype had 1.06 ± 10.33 median relative *CNR1* mRNA levels, which was significantly higher than compared with 0.49 ± 3.03 in IBD subjects with CT or TT genotype. According to the allelic model, IBD patients carriers of rs13197090 C allele had 0.88 ± 5.54 median relative *CNR1* mRNA levels, which was significantly higher than compared with 0.46 ± 2.95 in IBD subjects carriers of rs13197090 T allele. Control subjects with rs13197090 TT genotype also had lower *CNR1* mRNA expression levels (0.82 ± 0.82) compared with subjects with CT genotype (1.27 ± 3.32, $p = 0.009$). Our study identified a polymorphism associated with *CNR1* gene expression as a novel locus contributing to the genetic risk factor of IBD.

ANANDAMIDE AND PALMITOYLETHANOLMIDE LEVELS AND METABOLISM IN T84 CELLS EXPOSED TO INTERFERON-γ

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The cytokine interferon-γ (IFN-γ) is an important mediator in inflammatory disorders of the gut. A key event in inflammatory intestinal disorders is a disruption of the mucosal barrier. Human T84 colonic epithelial cells, which grow to confluence as a polarised monolayer, are a useful *in vitro* model of the intestinal epithelium. In this model, IFN-γ produces an increased permeability, as seen as a reduced transendothelial resistance (TEER) and an increased apical to basolateral flux of an antigenic 33-mer gluten peptide, without causing cell necrosis (1,2). Given the potentially beneficial role(s) played by endocannabinoids and related *N*-acylethanolamines (NAEs) in inflammatory intestinal disorders (3,4), we have investigated these systems in T84 cells exposed to IFN-γ.

In untreated T84 cells grown in 6 well plates, 2-AG, AEA, PEA, OEA and SEA could be detected in extracts of cells + medium. Treatment of the cells with 100 U/mL of IFN-γ significantly increased the concentrations of all five lipids in the extracts. Thus, for example, mean (95% bootstrapped percentile confidence limits) concentrations of PEA with vehicle and IFN-γ, respectively, were 0.18 (0.14-0.23) and 0.23 (0.22-0.25) (8 h incubation) and 0.14 (0.12-0.16) and 0.24 (0.22-0.25) (24 h incubation) pmol/vial (data from 9 separate extractions in each case). In contrast, levels of linoleic acid-derived oxylipins and the arachidonic acid derivatives TXB_2 and 5-, 11-, 12- and 15-HETE were not significantly affected. In 24 well plates, untreated T84 cells hydrolysed both 100 nM AEA and PEA. The hydrolysis of AEA was inhibited by 94±0.2% by the FAAH inhibitor URB597 (1 μM), 6±5% by the NAAA inhibitor pentadecylamine (30 μM) and 89±2% by the combination of both compounds. For PEA, the corresponding inhibitions were 73±3 (URB597), 35±7% (pentadecylamine) and 93±1% (both compounds) (means ± s.e.m., n=5). FAAH and NAAA mRNA levels normalised to the housekeeping gene RPL19 were measured in T84 cells cultured in transwells using qPCR. Setting the mean control value in each case to 100, IFN-γ produced a small (12%) but significant decrease in FAAH mRNA levels. In contrast, NAAA levels were increased by 33%.

Permeability of the cells were assessed using TEER measurements. Under control conditions, TEER values ~1000 were seen for the T84 cells cultured in the transwells, indicative of a strong barrier function. The cells also showed, in an initial experiment, a very low permeability to apical [^{14}C]sucrose. Basolateral IFN-γ treatment produced, as expected, a ~20% decrease in the observed TEER. Concomitant incubation with either 10 μM PEA, 1 μM URB597 or 50 μM pentadecylamine did not block the effect of IFN-γ treatment on the observed TEER. It is concluded that in T84 cells, endocannabinoid and related NAE levels are increased in response to IFN-γ treatment; that the cells express both FAAH and NAAA; but that addition of either PEA or blockade of these enzymes is insufficient to counter the deleterious effects of IFN-γ treatment upon cell permeability.

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(1) Adams et al., J Immunol 150 [1993] 2356-63; (2) Bethune et al., JPET 329 [2009] 657-68;

(3) Alhouayek et al. FASEB J 25 [2011] 2711-21; (4) Capasso et al., Br J Pharm 171 [2014] 4026-37

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