23RD ANNUAL SYMPOSIUM OF THE INTERNATIONAL CANNABINOID RESEARCH SOCIETY

VANCOUVER
BRITISH COLUMBIA
CANADA

JUNE 21 - 26, 2013

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PROGRAMME AND ABSTRACTS

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Registration: June 21st, 2013 (16.00 – 18.00)

Walter Gage Residence Tower Lobby

Welcome Reception: 18.30 – 20.00

Museum of Anthropology

Day 1
Saturday, June 22nd

6.30 – 8.30	**Breakfast** SUB Pacific Spirit Cafeteria		
8.30	**Welcome and Opening Remarks** Earth Sciences Building		
Oral Session 1. Cannabis Use and Abuse *Chairs*: Margaret Haney and Ryan Vandrey			
8.45	John McPartland, Geoffrey W. Guy and William Hegman	DISTRIBUTION OF *CANNABIS SATIVA* IN EUROPE BASED ON FOSSIL POLLEN AND ECOLOGICAL ANALYSES	1
9.00	Zach Walsh, Robert Callaway, Lynne Belle-Isle, Rielle Capler, Robert Kay, Philippe Lucas and Susan Holtzman	THE *CANNABIS* ACCESS FOR MEDICAL PURPOSES STUDY: PATIENT CHARACTERISTICS, REASONS FOR USE AND MODES OF ACCESS	2
9.15	Christine A. Rabinak, Mohammed Milad, Israel Liberzon and K. Luan Phan	CANNABINOID FACILITATION OF EXTINCTION RECALL VIA INCREASED RECRUITMENT OF PREFRONTAL-HIPPOCAMPAL CIRCUITRY IN HEALTHY HUMANS	3

9.30	Ziva Cooper and Margaret Haney	SELF-REPORTED COGNITIVE ENHANCING EFFECTS OF MARIJUANA DO NOT PREDICT MARIJUANA-ELICITED IMPROVEMENTS IN PERFORMANCE ON TASK BATTERY IN THE LABORATORY	4
9.45	Raul Gonzalez, Randi Schuster and Natania Crane	THE IMPACT OF DECISION-MAKING PERFORMANCE AND ADHD SYMPTOMS ON *CANNABIS*-RELATED PROBLEMS AMONG EMERGING ADULTS	5
10.00	PRESENTATION OF 2012 MECHOULAM AWARD **COFFEE BREAK**		
10.45	Erin C. Hanlon, Kara Stuhr, Rachel Leproult, Esra Tasali, Harriet de Wit, Cecilia J. Hillard and Eve Van Cauter	CIRCADIAN RHYTHM OF CIRCULATING ENDOCANNABINOID (EC), 2-ARACHIDONOYLGLYCEROL (2-AG), CONCENTRATIONS FOLLOWING NORMAL AND RESTRICTED SLEEP	6
11.00	Ryan Vandrey, Michael Smith and Miral Khalil	SLEEP CHARACTERISTICS OF ADULTS SEEKING TREATMENT FOR *CANNABIS* USE DISORDERS	7
11.15	Margaret Haney, Ziva Cooper, Gillinder Bedi, Stephanie Collins Reed, Divya Ramesh and Richard Foltin	MARIJUANA WITHDRAWAL AND RELAPSE IN THE HUMAN LABORATORY: EFFECT OF ZOLPIDEM ALONE AND IN COMBINATION WITH NABILONE	8
11.30	David Allsop, Jan Copeland, Nicholas Lintzeris, Adrian Dunlop, Mark Montebello, Craig Sadler, Gonzalo Rivas, Rohan Holland, Peter Muhleisen, Melissa Norberg and Iain McGregor	CANNABINOID REPLACEMENT THERAPY FOR MANAGEMENT OF *CANNABIS* WITHDRAWAL: A RANDOMIZED CONTROLLED TRIAL OF NABIXIMOLS	9

11.45	Jenny Wiley, Timothy Lefever, Julie Marusich, Matthew Walentiny and Robert Vann	BY LAND OR BY SEA: CANNABINOID DISCRIMINATION IN MICE	10
12.00	**LUNCH** SUB BALLROOM & PARTYROOM		
13.15 - 15.15	**POSTER SESSION 1** SUB BALLROOM		P1 1 – 23
15.30 EARTH SCIENCES BUILDING	**PRESIDENTIAL PLENARY SPEAKER** "TURNING OVER A NEW LEAF: HOW THE ENDOCANNABINOID SYSTEM REGULATES GASTROINTESTINAL MOTOR FUNCTION IN HEALTH AND DISEASE" **KEITH A. SHARKEY, PH.D.** *Department of Physiology & Pharmacology* *University of Calgary, Calgary, Alberta Canada*		
16.30	**COFFEE BREAK**		
ORAL SESSION 2. NOVEL ENDO/CANNABINOID LIGANDS AND RESEARCH TOOLS *CHAIRS*: MAURO MACCARRONE AND BRIAN THOMAS			
17.00	Heather Bradshaw and Emma Leishman	EXPANDING THE RANGE OF POTENTIAL "ENDOGENOUS CANNABINOIDS" TO INCORPORATE LIPIDS IN BACTERIA, YEAST, PLANTS, WORMS, AND FLIES: CANNABIMIMETIC LIPIDS ARE NOT JUST FROM ARACHIDONIC ACID ANYMORE	11

17.15	Derek Shore, Gemma Baillie, Frank Navas, Herbert Seltzman, Ruth Ross and Patricia Reggio	RATIONAL DESIGN OF NEUTRAL ALLOSTERIC MODULATORS OF THE CB1 RECEPTOR WITH IMPROVED RECEPTOR INTERACTIONS AND UNIQUE PHARMACOLOGY	12
17.30	Gemma Baillie, Bogna Ignatowska-Jankowska, Iain Greig, Matteo Zanda, Chiara Zanato, Roger Pertwee, Aron Lichtman and Ruth Ross	*IN VITRO* EFFECTS OF ZCZ011: A POSITIVE ALLOSTERIC MODULATOR OF THE CB_1 RECEPTOR	13
17.45	Bogna Ignatowska-Jankowska, Gemma Baillie, Steven Kinsey, Molly Crowe, Sudeshna Ghosh, Scott O'Neal, Thomas Gamage, Jenny Wiley, Iain Greig, Chiara Zanato, Matteo Zanda, Ruth Ross and Aron Lichtman	*IN VIVO* EFFECTS OF ZCZ011: A POSITIVE ALLOSTERIC MODULATOR OF THE CB_1 RECEPTOR	14
18.00	Mingfeng Bai, Shaojuan Zhang, Pin Shao, Michelle Sexton, Nephi Stella and Darryl Bornhop	*IN VIVO* CANNABINOID CB2 RECEPTOR TARGETED IMAGING USING A NEAR INFRARED FLUORESCENT PROBE	15
18.15	Christian Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Fingerle, Ivan Formentini, Jürgen Funk, Uwe Grether, Sabine Grüner, Paul Hebeisen, Atsushi Kimbara, Qingping Liu, Matthias Nettekoven, Giorgio Ottaviani, Mark Rogers-Evans, Stephan Röver, Franz Schuler, Tanja Schulz-Gasch, Christoph Ullmer, Zhiwei Wang and Wulun Yang	NOVEL, HIGHLY POTENT AND SELECTIVE 2,5,6-PYRIDINE/PYRAZINE DERIVED CB2 AGONISTS PROTECT RODENT KIDNEYS FROM ACUTE INJURY AND FIBROSIS	16

18.30	Pritesh Kumar and Zhao-Hui Song	IDENTIFICATION OF RALOXIFENE AS A NOVEL CB2 INVERSE AGONIST	17
18.45	Aurelien Tourteau, Virginie Andrzejak, Mathilde Body-Malapel, Lucas Lemaire, Amelie Lemoine, Jamal El Bakali, Roxane Mansouri, Nicolas Renault, Madjid Djouina, Pierre Desreumaux, Giulio Muccioli, Didier Lambert, Philippe Chavatte, Benoit Rigo, Natascha Leleu-Chavain and Regis Millet	3-CARBOXAMIDO-5-ARYL-ISOXAZOLES: AS THE FIRST CB2 / FAAH MULTITARGET DERIVATIVES	18
19.00	**DINNER** SUB BALLROOM & PARTYROOM		

Notes:

Day 2
Sunday, June 23rd

6.30 – 8.30	**Breakfast** SUB Pacific Spirit Cafeteria		
Oral Session 3. Immunology & Autoimmune Disorders *Chairs*: Nancy Buckley and Katarzyna Starowicz			
8.30	Rebecca Robinson, Joseph Meissler, Martin Adler and Toby Eisenstein	O-1966, A CB2-SELECTIVE CANNABINOID AGONIST, BLOCKS T-CELL ACTIVATION IN THE MIXED LYMPHOCYTE REACTION	19
8.45	Nancy Buckley, Anthony Park, Gideon Blumstein, Arya Parsa and Marie Girguis	DELTA-9-TETRAHYDROCANNABINOL SUPPRESSES MURINE RESPONSE TO SYSTEMIC *CANDIDA ALBICANS* INFECTION	20
9.00	Torsten Lowin, Angelika Gräber and Rainer Straub	THE ENDOCANNABINOID ANANDAMIDE MODULATES ADHESION, PROLIFERATION AND THE PRODUCTION OF INFLAMMATORY MEDIATORS IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS BY ACTIVATING CB1, TRPV1 AND NON-CANNABINOID RECEPTOR TARGETS	21
9.15	Katarzyna Starowicz, Natalia Malek, Natalia Kolosowska and Barbara Przewlocka	PREDICTIVE PRECLINICAL ANIMAL MODEL IN OSTEOARTHRITIS AS CRITICAL FACTOR FOR IDENTIFICATION OF NOVEL PHARMACOTHERAPIES	22
9.30	Mohammad Bashashati, Catherine Keenan, Philip Kingsley, Winnie Ho, Sarah Haseen, Lawrence Marnett and Keith Sharkey	INHIBITING DIACYLGLYCEROL LIPASE (DAGL) ATTENUATES EXPERIMENTAL COLITIS IN THE MOUSE	23

9.45	Mireille Alhouayek, Pauline Bottemanne, Patrice D. Cani, Didier Lambert and Giulio Muccioli	PEA REDUCES COLON AND SYSTEMIC INFLAMMATION IN MICE MODELS OF CROHN'S DISEASE	24
10.00	**COFFEE BREAK**		
ORAL SESSION 4. CANNABINOIDS, ENDOCANNABINOIDS & CANCER *CHAIRS*: CHRISTOPHER FOWLER AND SEAN MCALLISTER			
10.30	Christopher Fowler, Johanna Winther, Mariateresa Cipriano, Jenny Häggström and Peter Hammarsten	RELATIONSHIP BETWEEN GROWTH FACTOR RECEPTORS, PHOSPHO-AKT AND CB1 RECEPTORS IN PROSTATE CANCER	25
10.45	Genevieve Laroche, Victoria Jones, Charles Okechuku and Somnath Mukhopadhyay	TARGETING ENDOCANNABINOID BREAKDOWN ENZYMES AND CB2 CANNABINOID RECEPTOR FOR REGULATION OF PROSTATE TUMOR GROWTH	26
11.00	Esther Martínez-Martínez, Irene Gómez, Paloma Martín, Antonio Sánchez, Laura Román, Félix Bonilla, Mariano Provencio and José Miguel García	PROGNOSTIC VALUE OF THE CB2 CANNABINOID RECEPTOR IN HUMAN COLORECTAL CANCER	27
11.15	Ryuichi Murase, Rumi Kawamura, Eric Singer, Arash Pakdel, Pranamee Sarma, Jonathan Judkins, Eiman Elwakeel, Sonali Dayal, Ramesh Gujjar, Anu Mahadevan, Pierre-Yves Desprez and Sean McAllister	CO-TARGETING OF TWO DISTINCT CANNABINOID ANTITUMOR PATHWAYS RESULTS IN ROBUST INHIBITION OF ADVANCED STAGES OF METASTASIS	28

11.30	Ramy Ammar, Gudrun Ulrich-Merzenich, Mona El-Azab and Yasser Moustafa	INHIBITION OF ENDOCANNABINOID REUPTAKE IS AN INNOVATIVE STRATEGY TO IMPEDE TUMOR GROWTH	29
11.45	NIDA InfoSession The Future of Research of Cannabinoid, Endocannabinoid and Phytocannabinoid Therapeutics		
12.30	**Lunch** SUB Ballroom & Partyroom		
13.30 - 15.30	**Poster Session 2** SUB Ballroom		P2 1 - 28
Oral Session 5. Pain and Nausea *Chairs*: Mary Lynch and Sara Jane Ward			
15.30	Erin Rock, Ryan Kopstick, Cheryl Limebeer and Linda Parker	CANNABIDIOLIC ACID AND TETRAHYDROCANNABINOLIC ACID REDUCE CONDITIONED GAPING (NAUSEA-INDUCED BEHAVIOUR) IN RATS AND VOMITING IN *SUNCUS MURINUS*	30
15.45	Martin Kaczocha, William Berger, William Galbavy, Youngil Kim, Brian Ralph, Sherrye Glaser, Liqun Wang, Robert Rizzo, Dale Deutsch, Mario Rebecchi and Iwao Ojima	ANTINOCICEPTIVE EFFECTS OF FATTY ACID BINDING PROTEIN INHIBITORS	31

16.00	Josée Guindon and Andrea Hohmann	ADDITIVE EFFECTS OF THE COMBINATION OF FAAH AND MGL INHIBITORS WITH CONVENTIONAL TREATMENT IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY	32
16.15	Natalia Malek, Katarzyna Popiolek-Barczyk, Barbara Przewlocka and Katarzyna Starowicz	ATTENUATION OF LPS-INDUCED PAIN-LIKE RESPONSE BY CANNABINOID RECEPTORS LIGANDS IN ACTIVATED MICROGLIA CELLS	33
16.30	Sara Jane Ward, Zachary Reichenbach and Ronald Tuma	MODULATION OF MORPHINE ANTINOCICEPTIVE EFFECTS BY A SELECTIVE CB2 RECEPTOR AGONIST IN MICE	34
16.45	**COFFEE BREAK**		
ORAL SESSION 6. INTERACTIONS WITH NON-CANNABINOID NEURAL SYSTEMS *CHAIRS*: MATTHEW HILL AND SACHIN PATEL			
17.15	Hai-Ying Zhang, Ming Gao, Guoxiang Xiong, Qing-Rong Liu, Guo-Hua Bi, Hong-Ju Yang, Xia Li, Akiva Cohen, Nancy Buckley, Eliot Gardner, Jie Wu and Zheng-Xiong Xi	FUNCTIONAL CANNABINOID CB2 RECEPTORS ARE EXPRESSED IN MIDBRAIN DOPAMINE NEURONS IN MICE	35
17.30	Marco Pistis, Sebastiano Banni, Paola Fadda, Graziella De Montis and Miriam Melis	CONTROL OF NICOTINIC CHOLINERGIC FUNCTION BY PPAR-α IN DOPAMINE NEURONS	36

17.45	Teniel Ramikie, Rita Niyles, Ken Mackie, Masahiko Watanabe, István Katona and Sachin Patel	MULTIPLE MECHANISTICALLY DISTINCT MODES OF ENDOCANNABINOID MOBILIZATION AT CENTRAL AMYGDALA GLUTAMATERGIC SYNAPSES	37
18.00	Daniel Hermanson, Nolan Hartley, Joyonna Gamble-George, Naoko Brown, Jeffrey Reese, Sachin Patel and Lawrence Marnett	SUBSTRATE-SELECTIVE INHIBITION OF COX-2 ENHANCES ENDOCANNABINOID SIGNALLING *IN VIVO*	38
18.15	Jocelijn Meijerink, Mieke Poland, Michiel G.J Balvers, Mark Boekschoten and Renger Witkamp	DHEA, AN N-3 FATTY ACID DERIVED N-ACYLAMINE AS ENDOGENOUS REGULATOR OF COX-2 ACTIVITY	39
18.30	Megan Gray, Haley Veccharelli, Alex Kim, Kowther Hassan, Daniel Hermanson, Ryan McLaughlin, Tiffany Lee, Jan Deussing, Sachin Patel and Matthew Hill	CORTICOTROPIN-RELEASING HORMONE SIGNALING DRIVES ANANDAMIDE HYDROLYSIS TO PROMOTE ANXIETY	40
18.45	Ozge Gunduz-Cinar, Shaun Flynn, Resat Cinar, Daniel Fisher, George Kunos and Andrew Holmes	SSRI FACILITATION OF FEAR EXTINCTION REQUIRES AMYGDALA ENDOCANNABINOID CB1 SIGNALING	41
19.00	**DINNER** SUB BALLROOM & PARTYROOM		

Day 3
Monday, June 24th

6.30 – 8.20	**Breakfast** SUB Pacific Spirit Cafeteria	
NIDA SYMPOSIUM CANNABINOID CB2 RECEPTORS IN THE BRAIN: ROLE IN ADDICTION AND PSYCHIATRIC DISORDER *ORGANIZERS*: VISHNUDUTT PUROHIT and RAO RAPAKA *DISCUSSANTS*: RAO RAPAKA and JENNY WILEY		
8.20	Vishnudutt Purohit *National Institute on Drug Abuse* *Bethesda, MD USA*	Introduction
8.30	Cecilia Hillard *Medical College of Wisconsin* *Milwaukee, WI USA*	CB2 receptors: problems and promises
8.55	Emmanuel Onaivi *William Paterson University* *Wayne, NJ USA*	Involvement of CB2 cannabinoid receptors in alcohol consumption: from mice to human subjects
9.20	Eliot Gardner *National Institute on Drug Abuse* *Bethesda, MD USA*	Cannabinoid CB2 receptors modulate cocaine self-administration in rats
9.45	Jorge Manzanares *Universidad Miguel Hernández* *San Juan de Alicante, Spain*	Role of cannabinoid CB2 receptor in the development of psychiatric disorders and drug addiction
10.10	Commentary and discussion	

10.35	**COFFEE BREAK**		
ORAL SESSION 7. METABOLIC REGULATION *CHAIR*: ARON LICHTMAN			
11.00	C. Silvestri, A. Martella, and Vincenzo Di Marzo	THCV AND CBD EFFECTIVELY DECREASE LIPID LEVELS IN A VARIETY OF BIOLOGICAL SYSTEMS AND THCV ALSO SENSITIZES HEPATOCYTES TO INSULIN	42
11.15	Isabel Gonzalez Mariscal, Qing-Rong Liu, Maire Doyle and Josephine Egan	DIFFERENTIAL EXPRESSION OF HUMAN SPECIFIC N-TERMINUS VARIANTS OF THE CANNABINOID RECEPTOR 1 IN HUMAN ISLETS BETA CELLS	43
11.30	Chris L. Schaich, Allyn Howlett, Brian Thomas, Megan Grabenauer, Mark Chappell and Debra Diz	CHRONIC SYSTEMIC CB1 CANNABINOID RECEPTOR BLOCKADE REDUCES WEIGHT GAIN AND LOWERS BLOOD PRESSURE IN HYPERTENSIVE (mRen2)27 RATS	44
11.45	Kayte Jenkin, Anna Simcocks, Lannie O'Keefe, Michael Mathai, Andrew McAinch and Deanne Hryciw	CARDIO-RENAL EFFECTS OF CHRONIC ADMINISTRATION WITH CB2 AGONIST AM1241 AND CB2 ANTAGONIST AM630 IN RATS WITH DIET INDUCED OBESITY	45
12.00	**LUNCH** SUB BALLROOM & PARTYROOM		
13.15 - 15.15	**POSTER SESSION 3** SUB BALLROOM		P3 1 - 26
15.15	**EXPLORE VANCOUVER ON YOUR OWN OUTING AND DINNER**		

Day 4
Tuesday, June 25th

6.30 - 8.30	**Breakfast** SUB Pacific Spirit Cafeteria		
8.30 - 10.30	**Poster Session 4** SUB Ballroom		P4 1 - 28
10.30	**Coffee Break** Earth Sciences Building		
11.00	**KANG TSOU MEMORIAL LECTURE** "Dynorphin – Still an Extraordinarily Potent Opioid Peptide" **Charles Chavkin, Ph.D.** *Department of Pharmacology* *University of Washington, Seattle, Washington U.S.A.*		
12.00	**Lunch** SUB Ballroom & Partyroom		
Oral Session 8. Cannabinoid Receptors and Signaling *Chairs:* Patricia Reggio and Alex Straiker			
13.15	Alex Straiker, Anne Gibson, José Mitjavila, Jacqueline Blankman, Sherry Shu-jung Hu, Benjamin Cravatt and Ken Mackie	ABHD12 DELETION ALTERS CANNABINOID SIGNALING AND INDUCES DESENSITIZATION IN AUTAPTIC HIPPOCAMPAL NEURONS	46

13.30	Emma Leishman, Karl Spork, Alex Straiker and Heather Bradshaw	EFFECTS OF DELETIONS IN ABHD12, MGL, AND CB2 ON THE ENDOCANNABINOID-RELATED LIPIDOME IN MOUSE STRIATUM, HIPPOCAMPUS, AND CEREBELLUM	47
13.45	Toru Uyama, Manami Inoue, Yoko Okamoto, Naoki Shinohara, Tatsuya Tai, Iffat Ara Sonia Rahman, Kazuhito Tsuboi, Takeharu Tonai, Akira Tokumura, and Natsuo Ueda	GENERATION OF N-ACYLPHOSPHATIDYLETHANOLAMINE BY PLA/AT-1 IN MAMMALIAN CELLS	48
14.00	Alex Straiker, Kyung-Tai Min and Ken Mackie	Fmr1 DELETION ENHANCES AND ULTIMATELY DESENSITIZES CB1 SIGNALING IN AUTAPTIC HIPPOCAMPAL NEURONS	49
14.15	Jagjeet Singh, Diane Lynch, Alan Grossfield, Michael Pitman and Patricia Reggio	MOLECULAR DYNAMICS SIMULATIONS OF THE 2-AG ACTIVATED CANNABINOID RECEPTOR SUBTYPE 2 / Gi PROTEIN COMPLEX	50
14.30	G. Cristina Brailoiu, Elena Deliu, Jahan Marcu, Mary Abood and Eugen Brailoiu	DIFFERENTIAL ACTIVATION OF INTRACELLULAR VS PLASMALEMMAL CB2 CANNABINOID RECEPTORS	51
14.45	Yury Morozov, Martin Dominguez, Luis Varela, Marya Shanabrough, Marco Koch, Tamas Horvath and Pasko Rakic	ANTIBODIES TO CANNABINOID TYPE 1 RECEPTOR CO-REACT WITH STOMATIN-LIKE PROTEIN 2 IN THE MOUSE BRAIN MITOCHONDRIA	52

15.00	Lawrence Blume, Khalil Eldeeb, Dana Selley and Allyn Howlett	CRIP1A REGULATES CB1R SIGNALING AND CELLULAR TRAFFICKING	53
15.15	Khalil Eldeeb, Anjali Ganjiwale, Sudha Cowsik and Allyn Howlett	CB1 CANNABINOID RECEPTOR JUXTAMEMBRANE C-TERMINAL PHOSPHORYLATIN AND CAMP ACCUMULATION IN NT18TG2 CELLS	54
15.30	**COFFEE BREAK**		
ORAL SESSION 9. DIFFERENTIATION, NEUROGENESIS & NEUROPROTECTION *CHAIRS:* BEAT LUTZ AND SOMNATH MUKHOPADHYAY			
16.00	Fabio Arturo Iannotti, Cristoforo Silvestri, Andrea Martella, Fabiana Piscitelli, Paolo Ambrosino, Stefania Petrosino, Aniello Schiano Morello, Maurizio Taglialatela and Vincenzo Di Marzo	THE ENDOCANNABINOID SYSTEM CONTROLS SKELETAL MUSCLE CELL DIFFERENTIATION VIA CB1 RECEPTOR-DEPENDENT HYDROLYSIS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP2) AND INHIBITION OF KV7 POTASSIUM CHANNELS	55
16.15	Marion Grant and Somnath Mukhopadhyay	CB1 CANNABINOID RECEPTOR-MEDIATED INCREASE IN BDNF (BRAIN DERIVED NEUROTROPHIC FACTOR) SIGNALING REGULATES DEVELOPMENTAL NEUROGENESIS IN ZEBRAFISH BRAIN	56
16.30	Yosef Sarne, Miriam Fishbein, Mikhal Gafni, Edith Hochhauser, Mayan Waldman, Ziv Ben-Ari, Michal Safran and Eylon Lahat	LONG-TERM PROTECTION OF THE BRAIN, THE HEART AND THE LIVER BY ULTRA-LOW DOSES OF TETRAHYDROCANNABINOL (THC)	57

16.45	Daniele Bolognini, Lauren Whyte, Roger Pertwee and Ruth Ross	DETRIMENTAL EFFECTS OF LINOLEOYL-LYSOPHOSPHATIDYLINOSITOL IN CORTICAL NEURONS: INVOLVEMENT OF GPR55	58
17.00	Karl Spork and Alex Straiker	MONOACYLGLYCEROL LIPASE AND ENDOCANNABINOID REGULATION OF INTRAOCULAR PRESSURE IN A MURINE MODEL	59
17.15	**ICRS BUSINESS MEETING**		
19.00	**AWARDS CEREMONY** AND **ICRS BANQUET** SUB Ballroom		

DEPARTURE: **WEDNESDAY, JUNE 26TH**

POSTER SESSION 1: TOPICS A - D

DAY 1, SATURDAY, JUNE 22ND: 13:15 - 15:15

SUB Ballroom

TOPIC A. ENDO/CANNABINOIDS IN HUMANS

Philippe Lucas and Robin Krause	PATTERNS OF THERAPEUTIC *CANNABIS* USE IN 907 PATIENTS	P1-1
Joan L. Bottorff, Lynda G. Balneaves and N. Rielle Capler	HEALTH EFFECTS OF USING *CANNABIS* FOR THERAPEUTIC PURPOSES: PATIENT PERCEPTIONS OF BENEFITS AND RISKS	P1-2
Philippe Lucas, Kim Crosby, Zachary Walsh, Robert Callaway, Lynne Belle-Isle, Rielle Capler, Susan Holtzman, Bob Kay, Jamie Marshall, Trevor Stratton and Michael Woodsworth	SUBSTANCE USE AMONG MEDICAL *CANNABIS* USERS: SUBSTITUTING *CANNABIS* FOR ALCOHOL AND OTHER PSYCHOACTIVE SUBSTANCES	P1-3
Nadia Solowij, Samantha Broyd, Erika van Hell, Lisa-Marie Greenwood, Kuna Rueb, Dave Martelozzo, Arno Hazekamp, Jessica Booth, Iain McGregor, Antonio Zuardi, Rodney J Croft and Markus Leweke	SUBJECTIVE EFFECTS OF VAPORISED THC, CBD AND THC+CBD FROM A RANDOMISED CONTROLLED TRIAL IN HUMANS	P1-4
Ilse C Schrieks, Dina Ripken, Annette Stafleu, Renger F Witkamp and Henk FJ Hendriks	EFFECT OF MODERATE ALCOHOL CONSUMPTION AND AMBIANCE DURING A MEAL ON MOOD AND PLASMA ENDOCANNABINOIDS IN HUMANS	P1-5

TOPIC B. LEARNING AND MEMORY

TOPIC C. STRESS AND ANXIETY

Vanessa Enk, Martin Häring, Alejandro Rey Aparisi, Giovanni Marsicano, Tillmann Weber, Dusan Bartsch, Beat Lutz and Krisztina Monory	CONDITIONAL MUTAGENESIS REVEALS A DIRECT CB1 RECEPTOR-MEDIATED CONTROL ON SEROTONERGIC NEURONS	P1-17
Stephan Guggenhuber, Raissa Lerner, Hector Romo-Parra, Matthias Klugmann, Hans-Christian Pape and Beat Lutz	IMPAIRED 2-AG SIGNALING IN HIPPOCAMPAL GLUTAMATERGIC NEURONS ABOLISHES DEPOLARIZATION-INDUCED SUPPRESSION OF EXCITATION AND INCREASES ANXIETY-LIKE BEHAVIOR	P1-18

TOPIC D. NOVEL LIGANDS / MOLECULAR MODELING

Rangan Maitra, Alan Fulp, Herbert Seltzman, Yanan Zhang, Timothy Fennell and Rodney Snyder	DEVELOPMENT OF PERIPHERALLY SELECTIVE ANTAGONISTS OF CANNABINOID RECEPTOR 1: RATIONAL SYNTHESIS AND CHARACTERIZATION OF RIMONABANT (SR141716A) ANALOGS	P1-19
Mark A. Tepper, Robert B. Zurier and Sumner H. Burstein	HIGHLY PURIFIED AJULEMIC ACID IS A CB2 AGONIST WITH REDUCED CB1 ACTIVITY	P1-20
William T. Berger, Brian P. Ralph, Martin Kaczocha, Jin Sun, Trent E. Balius, Robert C. Rizzo, Samir Haj-Dahmane, Raghav Tripathi, Dale G. Deutsch and Iwao Ojima	DESIGN AND SYNTHESIS OF NOVEL TRUXILLIC ACID BASED FABP INHIBITORS	P1-21
Lawrence J. Marnett, Matt Windsor, Pieter L. Valk, Shu Xu and Daniel J. Hermanson	STRUCTURAL BASIS FOR SUBSTRATE-SELECTIVE INHIBITION OF CYCLOOXYGENASE-2 BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS	P1-22
Satoshi Yamaori, Yoshimi Okushima, Kazufumi Masuda, Mika Kushihara, Takashi Katsu, Shizuo Narimatsu, Shigeru Ohmori, Ikuo Yamamoto and Kazuhito Watanabe	STRUCTURAL REQUIREMENTS FOR POTENT DIRECT INHIBITION OF HUMAN CYP1A1 BY CANNABIDIOL	P1-23

POSTER SESSION 2: TOPICS E - G

DAY 2, SUNDAY, JUNE 23RD: 13:30 - 15:30

SUB BALLROOM

TOPIC E. IMMUNOLOGY & CANCER

Daniel Austen, Christian Lehmann, Mel Kelly, Juan Zhou, Karim Wafa and George Robertson	CHANGES IN THE INTESTINAL MICROCIRCULATION IN RESPONSE TO ENDOTOXIN CHALLENGE IN CNS INJURY-INDUCED IMMUNE DEFICIENCY SYNDROME (CIDS) – EFFECTS OF CANNABINOID RECEPTOR 2 INHIBITION	P2-1
Natalia Battista, Antonio Di Sabatino, Monia Di Tommaso, Paolo Biancheri, Cinzia Rapino, Paolo Giuffrida, Cinzia Papadia, Chiara Montana, Alessandra Pasini, Alessandro Vanoli, Francesco Lanzarotto, Vincenzo Villanacci, Gino R. Corazza and Mauro Maccarrone	UP-REGULATION OF CANNABINOID RECEPTORS IN UNTREATED CELIAC DISEASE	P2-2
Kevin Wilhelmsen, Samira Khakpour, Alphonso Tran and Judith Hellman	CANNABINOID RECEPTOR MODULATION OF THE ENDOTHELIAL CELL TOLL-LIKE RECEPTOR-DEPENDENT INFLAMMATORY RESPONSE	P2-3
Jennifer M. Knight, Cecilia J. Hillard, Shi Zhao, Aniko Szabo, Jeffrey M. Lyness, Olle Jane Z. Sahler, Jane L. Liesveld and Jan A. Moynihan	PSYCHOSOCIAL FACTORS, ENDOCANNABINOIDS, AND INFLAMMATORY MOLECULES IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS	P2-4
Kristina Burkert, Erica Burns, Angela Lim, Kriebashne Moodley, Catherine E Angel, Michelle Glass and E Scott Graham	REGULATION OF HUMAN LEUKOCYTE FUNCTIONS BY CB2-RECEPTOR AGONISTS; EVIDENCE FOR DONOR-DEPENDENT RESPONSES AND IMPLICATIONS FOR THERAPEUTIC APPLICATIONS	P2-5

Valeria Gasperi, Roberta Ceci, Mirko Tantimonaco, Emanuela Talamonti, Natalia Battista, Attilio Parisi, Rita Florio, Stefania Sabatini, Antonello Rossi and Mauro Maccarrone	AN ACTIVE LIFESTYLE AFFECTS LYMPHOCYTE FATTY ACID AMIDE HYDROLASE THROUGH AN IL-6-DEPENDENT MECHANISM	P2-6
M. Valeria Catani, Daniela Evangelista, Valeria Gasperi, Mariangela Pucci, Valerio Chiurchiù, Sergio Oddi, Fulvio Florenzano, Isabella Savini, Luciana Avigliano and Mauro Maccarrone	REGULATION OF ENDOTHELIAL/LEUKOCYTE INTERACTIONS BY 2-ARACHIDONOYLGLYCEROL	P2-7
Prathibha M. Daniel, Marnie Duncan, Colin G. Stott, Roger M. Phillips and Farideh A. Javid	THE EFFECT OF GWCBG, A CANNABINOID EXTRACT ON A2780, HUMAN OVARIAN CARCINOMA CELLS	P2-8
M-J Milloy, Brandon Marshall, Thomas Kerr, Silvia Guillemi, Robert Hogg, Julio Montaner and Evan Wood	CANNABIS USE ASSOCIATED WITH LOWER PLASMA HIV-1 RNA VIRAL LOAD AMONG RECENTLY HIV-INFECTED INTRAVENOUS DRUG USERS	P2-9
Katherine A Scott, Angus G Dalgleish and Wai M Liu	Δ^9-THC AND CANNABIDIOL INCREASE THE RADIOSENSITIVITY OF GLIOMA CELL LINES *IN VITRO* WHEN USED TOGETHER	P2-10
TOPIC F. NEURODEGENERATION & NEUROTOXICOLOGY		
Carmen Vázquez, Marta Moreno, Lourdes Ruiz-Valdepeñas, Paula Fortún, Rebeca Barros, José Ramón Baldellou, Rosa María Tolón and Julián Romero	PARADOXICAL EFFECTS OF FAAH GENE DELETION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE	P2-11
Camilla Madeddu, Alberto Casti, Maria Scherma, Alessandra Porcu, Angelo Casu, Paola Fadda, M. Grazia Ennas and M. Paola Castelli	Δ^9-TETRAHYDROCANNABINOL PREVENTS METHAMPHETAMINE-INDUCED NEUROTOXICITY	P2-12

Urszula Grabiec, Marco Koch, Constanze Hobusch, Chalid Ghadban, Beat Lutz and Faramarz Dehghani	THE NEUROPROTECTIVE ENDOCANNABINOID N-ARACHIDONOYL-DOPAMINE AFFECT THE PAKT ACTIVATION IN PRIMARY MICROGLIAL AND ASTROCYTE CELL CULTURE	P2-13
Douglas McHugh, Daniel Roskowski, Sisi Xie and Heather B. Bradshaw	Δ^9-THC AND N-ARACHIDONOYL GLYCINE REGULATE THE PHENOTYPIC MORPHOLOGY OF BV-2 MICROGLIA	P2-14
Robert B. Laprairie, Jordan R. Warford, Sarah Hutchings, George S. Robertson, Melanie E.M. Kelly and Eileen M. Denovan-Wright	IN HUNTINGTON'S DISEASE, THE ENDOCANNABINOID AND CYTOKINE SYSTEMS ARE DYSREGULATED IN THE STRIATUM	P2-15
Genevieve Laroche, Dal Khatri, Victoria Jones, Marion Grant, Fulton Crews and Somnath Mukhopadhyay	ETHANOL AND CANNABINOID RECEPTOR INTERACTION IN THE REGULATION OF ADULT NEUROGENESIS	P2-16
Hamad Al-Harbi, David A Kendall and Stephen PH Alexander	EFFECTS OF CANNABIDIOL AND HU210 ON VIABILITY AND MITOCHONDRIAL MEMBRANE POTENTIAL OF SH-SY5Y HUMAN NEUROBLASTOMA CELLS	P2-17
TOPIC G. CANNABINOIDS & BEHAVIOR		
Marta Rodriguez-Arias, Francisco Navarrete, Manuel Daza-Losada, Daniela Navarro, María A. Aguilar, Pere Berbel, José Miñarro and Jorge Manzanares	CB1 CANNABINOID RECEPTOR-MEDIATED REGULATION OF AGGRESSIVE SOCIAL BEHAVIOR	P2-18
Alex Naftaly, Shimon Rabichev, Jürg Gertsch, Ruth A. Ross, Ester Fride (Z"L) and Sharon Anavi-Goffer	REDUCED LOCOMOTOR ACTIVITY BY NMDA ANTAGONIST IS REVERSED BY HU-308	P2-19

Merav Hajby, Alex Naftaly, Aviva Breuer, Raphael Mechoulam and Sharon Anavi-Goffer	MODULATION OF LOCOMOTOR ACTIVITY BY THE NOVEL CANNABINOID HU-267	P2-20
Joel E. Schlosburg, Leandro F. Vendruscolo, Marian L. Logrip, Eric P. Zorrilla, Benjamin F. Cravatt, Loren H. Parsons and George F. Koob	EFFECTS OF INHIBITION OF ENDOCANNABINOID DEGRADATION ON EXCESSIVE HEROIN AND ALCOHOL INTAKE	P2-21
Aaron E. Haas and Rebecca M. Craft	ACTIVATIONAL GONADAL HORMONE MODULATION OF THE BEHAVIORAL EFFECTS OF Δ^9 -TETRAHYDROCANNABINOL IN MALE AND FEMALE RATS	P2-22
Kiri Wills, Alan Lee, Cheryl L. Limebeer, Kiran Vemuri, Alexandros Makriyannis and Linda A. Parker	EFFECTS OF ACUTE AM251 AND AM4113 TREATMENT ON NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL-INDUCED CONDITIONED PLACE AVERSION	P2-23
Silvain S. Dang and Boris B. Gorzalka	THE INTERACTIVE EFFECTS OF ACUTE AND SUB-CHRONIC CANNABINOID EXPOSURE ON SEXUAL BEHAVIOUR IN MALE RATS	P2-24
Alexander M.H. Dodd, Silvain S. Dang and Boris B. Gorzalka	THE EFFECTS OF TAIL PINCH ON SEXUAL BEHAVIOUR IN MALE RATS FOLLOWING SUBCHRONIC HU-210 ADMINISTRATION	P2-25
Michael Chrusch and Richard Dyck	FUNCTIONAL DEFICITS IN THE BARREL CORTEX OF THE CB1 RECEPTOR KNOCKOUT MOUSE	P2-26
Francisco Navarrete, Antonio Ortega-Álvaro, Auxiliadora Aracil-Fernández, Alexander Ternianov and Jorge Manzanares	DIFFERENTIAL REGULATION OF SENSORIMOTOR-GATING DEFICIT IN CB1 KNOCKOUT MICE BY HALOPERIDOL, RISPERIDONE AND METHYLPHENIDATE	P2-27
Jesse S. Rodriguez and Lance R. McMahon	HYPOTHERMIA IN MICE AND GETTING HIGH: EVIDENCE THAT WIN 55212-2 AND JWH-018 HAVE HIGHER EFFICACY THAN Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC)	P2-28

POSTER SESSION 3: TOPICS H - K

DAY 3, MONDAY, JUNE 24TH: 13:15 - 15:15

SUB Ballroom

TOPIC H. CB2 RECEPTORS IN CNS

Emmanuel S. Onaivi, Hiroki Ishiguro, Claire M. Leonard, Susan Sgro and Qing-Rong Liu	BEHAVIORAL EFFECTS OF CB2 CANNABINOID RECEPTOR GENE VARIATIONS AND MODULATION	P3-1
Marina Nagler, Sebastian Rading, Lysann Palkowitsch, Barbara Möpps and Meliha Karsak	THE ACTIVATOR OF G-PROTEIN SIGNALLING TCTEX1 IS MODULATED BY CB2 RECEPTORS	P3-2
Francisco Navarrete, María Salud García-Gutiérrez, José Antonio Molina-Arjona, Carlos Leiva-Santana and Jorge Manzanares	CANNABINOID CB2 RECEPTOR GENE EXPRESSION DIFFERENCES IN POST-MORTEM BRAIN AND LYMPHOCYTES SAMPLES FROM PARKINSON'S DISEASE PATIENTS	P3-3
Francisco Navarrete, José Manuel Pérez-Ortiz, María Salud García-Gutiérrez, José Antonio Molina-Arjona, Carlos Leiva-Santana and Jorge Manzanares	OVEREXPRESSION OF CANNABINOID CB2 RECEPTORS ATTENUATED THE PROGRESSIVE MOTOR IMPAIRMENT AND NIGROSTRIATAL DOPAMINERGIC NEURONS LOSS IN MITOPARK MOUSE	P3-4
María S. García-Gutiérrez, Antonio Ortega-Álvaro, Arnau Busquets, Jose M. Pérez-Ortiz, Laura Caltana, María Jimena Ricatti, Alicia Brusco, Rafael Maldonado and Jorge Manzanares	SYNAPTIC PLASTICITY ALTERATIONS ASSOCIATED WITH MEMORY IMPAIRMENT INDUCED BY DELETION OF CB2 CANNABINOID RECEPTORS	P3-5
Francisco Navarrete, Antonio Ortega-Álvaro, Alexander Ternianov, Auxiliadora Aracil-Fernández, María S. García-Gutiérrez MS and Jorge Manzanares	ROLE OF CANNABINOID CB2 RECEPTOR IN THE REINFORCING ACTIONS OF ETHANOL	P3-6

Francisco Navarrete, Marta Rodríguez-Arias, Elena Martín-García, Daniela Navarro, María S. García-Gutiérrez, María A. Aguilar, Auxiliadora Aracil-Fernández, Pere Berbel, José Miñarro, Rafael Maldonado and Jorge Manzanares	ROLE OF CB2 CANNABINOID RECEPTOR IN THE REINFORCING EFFECTS OF NICOTINE	P3-7
Anna-Maria Szczesniak, Sara Nejat and Melanie E.M. Kelly	THE ROLE OF CANNABINOID RECEPTOR 2 IN THE EXPERIMENTAL PROLIFERATIVE VITREORETINOPATHY	P3-8
Qing-Rong Liu, Zheng-Xiong Xi, Haiying Zhang and Emmanuel S. Onaivi	SPECIES DIFFERENCES OF CANNABINOID RECEPTOR 2 (CB2R) BRAIN EXPRESSION AND BEHAVIORAL EFFECTS OF CB2R LIGANDS IN MICE AND RATS	P3-9
TOPIC I. ENDO/CANNABINOIDS IN PERIPHERY		
Dina Ripken, Nikkie v.d. Wielen, Heleen Wortelboer, Renger Witkamp and Henk Hendriks	THE USE OF AN *EX VIVO* PORCINE INTESTINAL TISSUE MODEL TO STUDY THE ROLE OF THE INTESTINAL ENDOCANNABINOID SYSTEM	P3-10
Tannia Uribe, Xóchitl Trujillo and Miguel Huerta	EFFECTS OF CAPSAICIN OVER TENSION IN THE PRESENCE AND ABSENCE OF CANNABINOID AND VANILLOID ANTAGONISTS IN THE FAST SKELETAL MUSCLE FIBERS OF THE FROG	P3-11
Fairouz Shermado, Kostas Tsintzas and Andrew Bennett	CANNABINOID RECEPTORS IN HUMAN, WISTAR RAT AND ZUCKER RAT SKELETAL MUSCLE TISSUES, MYOTUBES AND MYOBLASTS	P3-12
Susan Krzysik-Walker, Wook Kim, Khalea Wrensford, Jennifer Fiori, Olga Carlson and Josephine Egan	BLOCKADE OF CANNABINOID RECEPTOR 1 INCREASES PANCREATIC BETA CELL INSULIN CONTENT IN A DIABETIC MOUSE MODEL	P3-13

Ulrike Taschler, Franz P. Radner, Gabriele Schoiswohl and Robert Zimmermann	MONOGLYCERIDE LIPASE-DEFICIENCY IMPAIRS LIPOLYSIS AND LEADS TO CANNABINOID RECEPTOR DESENSITIZATION	P3-14
Lysanne Campeau, Claudius Füllhase, Norifumi Sawada, Christian Gratzke, Petter Hedlund, Allyn Howlett and Karl-Erik Andersson	INVOLVEMENT OF CANNABINOID RECEPTOR TYPE 2 IN MICTURITION IN MICE	P3-15
Molly S. Crowe, Bogna M. Ignatowska-Jankowska, Micah J. Niphakis, Benjamin F. Cravatt, Aron H. Lichtman and Steven G. Kinsey	GASTROPROTECTIVE EFFECTS OF THE MONOACYLGLYCEROL LIPASE INHIBITOR KML29 IN MICE	P3-16

TOPIC J. ENDO/CANNABINOIDS DURING DEVELOPMENT

Lindsay Silva and Diana Dow-Edwards	EARLY LIFE EXPERIENCE AFFECTS BEHAVIORAL RESPONSES TO ADOLESCENT DELTA-9-TETRAHYDROCANNABINOL EXPOSURE IN THE RAT	P3-17
Tiziana Rubino, Cristiano Nazzaro, Bruna Cuccurazzo, Erica Zamberletti, Pamela Prini, Simona Speziali, Mariagrazia Grilli, Raffaella Tonini and Daniela Parolaro	RB597 DIFFERENTLY AFFECTED EMOTIONAL AND COGNITIVE SIGNS INDUCED BY ADOLESCENT EXPOSURE TO THC: POSSIBLE CELLULAR MECHANISMS	P3-18
Erica Zamberletti, Sarah Beggiato, Pamela Prini, Marina Gabaglio, Simona Speziali, Luca Ferraro, Tiziana Rubino and Daniela Parolaro	ADOLESCENT THC EXPOSURE RESULTS IN ALTERATIONS OF GABAERGIC SIGNALLING WITHIN THE ADULT RAT PREFRONTAL CORTEX	P3-19
Anne Legler, Raissa Lerner, Laura Bindila, Beat Lutz and Krisztina Monory	CANNABINOID CB1 RECEPTOR FUNCTIONS AND ENDOCANNABINOID LEVELS IN THE PROGRESSION OF NORMAL AGING	P3-20

TOPIC J. ENDO/CANNABINOIDS & BONE

Lauren Whyte, Wendy Russell and Ruth Ross	PRODUCTION OF LYSOPHOSPHATIDYLINOSITOL SPECIES AND GPR55 MEDIATED SIGNALING IN OSTEOCLASTS	P3-21
A. Abdeldayem, M. Yousseffi, M. Genedy and M.C.T. Denyer	THE EFFECT OF DIFFERENT CONCENTRATIONS OF URB602 ON HEALING OF SCRATCHED CHONDROCYTE MONOLAYERS	P3-22
M. Genedy, M. Youseffi, A. Abdeldayem and M.C.T. Denyer	THE EFFECT OF MONOGLYCEROL LIPASE INHIBITOR (URB602) ON THE HEALING ACCELERATION OF MG-63 OSTEOBLAST SCRATCHED MONOLAYERS	P3-23
A. Abdeldayem, M. Genedy, M. Yousseffi and M.C.T. Denyer	THE EFFECT OF DIFFERENT CONCENTRATIONS OF SYNTHETIC CANNABINOID RECEPTOR 2 AGONIST (HU-308) ON HEALING OF SCRATCHED CHONDROCYTE MONOLAYERS	P3-24
M. Genedy, M. Youseffi, A. Abdeldayem and M.C.T. Denyer	THE EFFECT OF HU308/TGF-B3 COMBINATION ON MG-63 OSTEOBLAST WOUND HEALING	P3-25
Nina Hönig, M. Genedy, A. Abdeldayem, M.C.T. Denyer and M. Youseffi	THE EFFECT OF CANNABINOID ON HEALING OF SCRATCHED HACAT CELL MONOLAYERS	P3-26

POSTER SESSION 4: TOPICS L - P
DAY 4, TUESDAY, JUNE 25TH: 8:30 - 10:30

SUB BALLROOM

TOPIC L. RECEPTOR STRUCTURE & FUNCTION

Haining Liu, Ronak Y. Patel and Robert J. Doerksen	STRUCTURE OF THE CANNABINOID RECEPTOR 1: HOMOLOGY MODELING AND ENRICHMENT STUDY BASED ON CB1 ANTAGONIST DOCKING	P4-1
Amina Bagher, Melanie E.M. Kelly and Eileen M. Denovan-Wright	EXPRESSION, DIMERIZATION AND FUNCTION OF CB1 RECEPTOR CODING REGION SPLICE VARIANTS	P4-2
Lili Du, Kurt E. La Rock, Cecilia J. Hillard and Christopher W. Cunningham	ALLOSTERIC MODULATION OF THE CANNABINOID CB1 RECEPTOR BY DAT INHIBITORS DOES NOT ACT THROUGH G-PROTEIN COUPLED MECHANISMS	P4-3
Robert Brad Laprairie, Denis J. Dupré, Melanie E.M. Kelly and Eileen M. Denovan-Wright	DIFFERENT CLASSES OF CB1 LIGANDS BIAS CB1-DEPENDENT SIGNAL TRANSDUCTION	P4-4
Chris S. Breivogel, Monica Danala and Satish P. Rekulapally	BETA-ARRESTIN2 MODULATION CANNABINOID ACTIVITY IN MICE VARIES WITH ASSAY, DRUG AND GENDER	P4-5
Alex Straiker and Ken Mackie	'INTERESTING NEGATIVES' CULLED FROM TEN YEARS OF RECORDING FROM AUTAPTIC HIPPOCAMPAL NEURONS	P4-6
Shilpa Sonti, Girish Chopda, Carol A. Paronis and Samuel J. Gatley	THE EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF DELTA-9-TETRAHYDROCANNABINOL (Δ^9-THC) ON RECEPTOR DOWNREGULATION IN MICE	P4-7

Gernot F. Grabner, Ulrike Taschler, Franz P.W. Radner and Robert Zimmermann	DELETION OF MONOGLYCERIDE LIPASE IN THE CENTRAL NERVOUS SYSTEM	P4-8
Carmen Vázquez, Cristina Benito, Marta Moreno, Francisco Javier Fernández Sánchez, José Ramón Baldellou, Rebeca Barros, Julián Romero and Rosa María Tolón	GLUCOSE UPTAKE IS IMPAIRED IN FAAH-KO ASTROCYTES	P4-9
TOPIC M. PAIN		
Matthew D. Metcalf, Mary M. Lunzer and Philip S. Portoghese	SYNERGISTIC ACTION OF INHIBITORS OF ENDOCANNABINOID METABOLISM ON OPIOID ANALGESIA IN A MODEL OF ACUTE PAIN	P4-10
Herbert H. Seltzman, Craig Shiner, Erin Hirt, Anne F. Gilliam, Rangan Maitra, Rodney W. Snyder, Yatendra Mulpuri, Dmitry Ivanov, Andrei Marechek and Igor Spigelman	PERIPHERALLY RESTRICTED CB1R AGONISTS FOR TREATMENT OF CHRONIC PAIN	P4-11
Mary E Lynch, Paula Cesar-Rittenburg and Andrea Hohmann	A DOUBLE BLIND PLACEBO CONTROLLED CROSSOVER PILOT TRIAL WITH EXTENSION USING AN ORAL MUCOSAL CANNABINOID EXTRACT FOR TREATMENT OF CHEMOTHERAPY INDUCED NEUROPATHIC PAIN	P4-12
Michiel Balvers, Jocelijn Meijerink, Mieke Poland, Noortje Creemers, Kitty Verhoeckx and Renger Witkamp	DO THE N-3 N-ACYLETHANOLAMIDES DHEA AND EPEA FUNCTION AS ENDOGENOUS SUPPRESSORS OF INFLAMMATION?	P4-13

Seth M. Davis, Ram Kandasamy and Rebecca M. Craft	CB RECEPTOR MEDIATION OF THC-INDUCED ANTINOCICEPTION USING A CHRONIC INFLAMMATORY PAIN MODEL IN FEMALE VS. MALE RATS	P4-14
Alexa A. Wakley, Alisha A. McBride and Rebecca M. Craft	HORMONE MODULATION OF ANTINOCICEPTIVE BUT NOT MOTORIC EFFECTS OF I.C.V. THC IN OVARIECTOMIZED FEMALE RATS	P4-15
TOPIC N. NAUSEA		
Cecilia J. Hillard, Hershel Raff and Thangam Venkatesan	CIRCULATING ENDOCANNABINOIDS IN CYCLIC VOMITING SYNDROME	P4-16
Martin A. Sticht and Linda A. Parker	THE ROLE OF INTRA-VISCERAL INSULAR CORTEX ENDOCANNABINOIDS IN NAUSEA-INDUCED GAPING IN RATS	P4-17
Cheryl L. Limebeer, Elizabeth Imhof, Kai Wang, Rehab A. Abdullah, Aron H. Lichtman and Linda A. Parker	ATTENUATION OF ANTICIPATORY NAUSEA IN A RAT MODEL OF CONTEXTUALLY ELICITED CONDITIONED GAPING BY MANIPULATION OF THE ENDO-CANNABINOID SYSTEM	P4-18
Lesley D. O'Brien, Cheryl L. Limebeer, Giovanni Bottegoni, Daniele Piomelli and Linda A. Parker	INHIBITING ANANDAMIDE TRANSPORT: THE IMPACT OF PROLONGED ANANDAMIDE AVAILABILITY ON NAUSEA	P4-19
TOPIC O. SYSTEMS & METHODS		
Nicola Bernabò, Barbara Barboni and Mauro Maccarrone	SYSTEMS BIOLOGY ANALYSIS OF THE ENDOCANNABINOID SYSTEM	P4-20

ICRS2013 - Presidential Plenary Speaker

"Turning over a new leaf: How the endocannabinoid system regulates gastrointestinal motor function in health and disease"

Keith A. Sharkey, Ph.D.

Department of Physiology & Pharmacology
University of Calgary
Alberta, Canada

Gastrointestinal (GI) motility disorders are extremely common, but current treatments for them are very limited. The lack of treatment strategies stem in part from the complex nature of neuromuscular regulation of the gut. The predominant local control of gut motor functions is accomplished by the enteric nervous system (ENS). Factors that alter enteric neural control change patterns of motility. The endocannabinoid (EC) system is unique in its ability to rapidly regulate enteric neural functions because it primarily controls the release of other transmitters, notably acetylcholine, both tonically and in an activity-dependent manner.

The EC system of the gut consists of the cannabinoid $(CB)_1$ and CB_2 receptors, their endogenous ligands, anandamide and 2-arachidonoylglycerol (2-AG), and enzymes for the biosynthesis and hydrolysis of these ligands. The growth in our knowledge of how endocannabinoids are synthesized and regulated has allowed us to test the **hypothesis that specific components of the EC system can be targeted to normalize dysregulated gut motility.**

Enteric CB receptors provide a mechanism of maintaining network stability by suppressing synaptic strength in the ENS, specifically by inhibiting transmission between myenteric neurons through presynaptic mechanisms. Considering these findings it is therefore possible to use the EC system to *reduce* motor disturbances in models of enhanced motility. Conversely, we can modulate the EC system to normalize (*increase*) slowed GI motility, by reducing 2-AG synthesis.

Cannabinoids represent powerful potential therapeutics for motility disorders. By understanding the EC system in models of GI dysmotility, we hope to make advances that can be translated into new approaches for the treatment of motility disorders.

2013 PRESIDENTIAL PLENARY SPEAKER - SATURDAY, JUNE 22nd @ 15.30

NIDA INFOSESSION

THE FUTURE OF RESEARCH OF CANNABINOID, ENDOCANNABINOID AND PHYTOCANNABINOID THERAPEUTICS

ICRS published research has become more "translational" in recent years, deviating in a clinically relevant direction from the basic science research that brought us this far in the absence of a disease target per se. This panel discussion aims to predict the future for developing research projects in our field. What kinds of disease states are most likely to benefit from selective CB1 or CB2 agonists or antagonists, allosteric, or peripherally-restricted ligands? Will there be a major development in endocannabinoid synthesis or metabolism enzyme inhibitors? What is the role for phytocannabinoids, as the recently discovered pharmacological properties of some of these suggest that they have important potential therapeutic applications for several disorders, including drug dependence? Is there any projected future for developing classical and nonclassical cannabinoid and aminoalkylindole compounds for treatment of disease? These and other questions will be brought to the table for discussion by a panel of three notable stakeholders who can provide their insights from academic, pharmaceutical, governmental, and clinical points of view.

Chair: Rao Rapaka, Ph.D. Branch Chief, Chemistry and Physiological Systems Research Branch, National Institute on Drug Abuse, NIH.
rrapaka@nida.nih.gov

Panelist: Roger G. Pertwee MA, DPhil, DSc, FBPharmacolS, Professor of Neuropharmacology, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, Scotland, UK.
rgp@abdn.ac.uk

Panelist: Mark A. Ware, MBBS, MRCP, MSc Executive Director, Canadian Consortium for the Investigation of Cannabinoids (CCIC); Director of Clinical Research, Alan Edwards Pain Management Unit, McGill University Health Centre (MUHC); Associate Professor, Departments of Family Medicine and Anesthesia,McGill University, Montreal QC.
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NIDA Symposium

CANNABINOID CB2 RECEPTORS IN THE BRAIN: ROLE IN ADDICTION AND PSYCHIATRIC DISORDER

Until recently, CB_2 receptors were thought to be confined to the periphery, primarily in the immune system. However, more recent research has suggested that they may also be located in the central nervous system, albeit their numbers and distribution may not as extensive as CB_1 receptors. Further, whether these receptors are expressed in neurons is the subject of much debate. Complicating investigation of this issue are methodological concerns related to selection of CB_2 receptor antibodies, selectivity of pharmacological probes, and use of genetically manipulated mice. At minimum, CB_2 receptor expression in microglia has been confirmed, with the suggestion that expression may be inducible by pathological processes such as inflammation. Although not yet verified in situ, results of studies in which CB_2 receptors were expressed in neuronal cell cultures suggest that they may also exhibit constitutive activity that is regulated by basal levels of the endocannabinoid 2-arachidonoyl-glycerol. Further, CB_2 receptor ligands demonstrate functional selectivity in which signaling pathways they activate, with WIN55,212-2 showing the most selectivity and CP55,940 showing the least. In other studies, activation of CB_2 receptors has been shown to inhibit: a) firing in dorsal root ganglia and spinal cord; b) GABAergic transmission in rat cerebral cortex; c) morphine-6-glucuronide-induced emesis; and d) neuropathic pain.

In this symposium, Dr. Vishnudutt Purohit will introduce the speakers and will serve as moderator. Dr. Ken Mackie will discuss methodological issues and advances that have hindered and helped in the investigation of the nature of CB_2 receptor activity in the brain. His talk will be followed by a discussion of evidence for the presence of CB_2 receptors in neurons presented by Dr. Emmanuel Onavi. Dr. Onavi will also discuss his work on the role of CB_2 receptors in alcohol consumption. The symposium will continue with talks by Drs. Eliot Gardner and Jorge Manzanares. Dr. Gardner will present work on the role of CB_2 receptors in cocaine addiction. Dr. Manzanares will discuss the implications of his work with CB_2 transgenic mice on cocaine addiction and psychiatric disorders. Drs. Jenny Wiley and Rao Rapaka will serve as discussants and moderators of a panel question and answer session.

Organizers

Vishnudutt Purohit, DVM, Ph.D. and Rao S. Rapaka, Ph.D., FAAPS, FAAAS
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CB_2 RECEPTORS: PROBLEMS AND PROMISES

Ken Mackie, MD [1] and Cecilia Hillard, PhD [2]

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The role of CB_2 cannabinoid receptors in behaviors remains a controversial topic. In this talk, I will outline contributing factors to this situation and propose potential solutions. Three primary approaches are used to implicate specific receptors in a behavior. These are pharmacology, genetics, and anatomy. All of these approaches suffer from limitations (or can be misused!), and results from each must be interpreted with skepticism and care.

G protein coupled receptor (GPCR) agonists, particularly for lipid receptors, are notoriously non-selective. CB_2 agonists and antagonists are no exception. Often these ligands are marketed as "selective" for one receptor subtype or another with little documentation. As numerous studies have shown, almost all of these CB_2 ligands have activity at CB_1 if the concentration or dose is pushed high enough (or drug accumulates during chronic dosing). This is relevant for both agonists and antagonists. Thus, experiments conducted with high drug doses, resulting high active site concentrations, must be viewed skeptically until confirmed by multiple approaches.

Genetic manipulation (e.g., knockout, knockdown, or knockin) of CB_2 receptors has provided valuable information on the role of CB_2 receptors in behavior, but certain constraints must be kept in mind. The first is the nature of the knockout. For example, is the complete coding sequence knocked out? If not, is mRNA transcribed from the residual gene (evaluate by qPCR)? If so, is it translated to protein and does this protein alter cellular function? What compensatory changes accompany the receptor knockout? The logic of knockin experiments must also be considered. Typically, these experiments address the question: "What can CB_2 receptors do?" and they do not answer the question: "What do CB_2 receptors do?"

Unfortunately, the immunocytochemical localization of CB_2 receptors has been very problematic. For good reasons, antibodies have been called *Tools of Mass Distraction* by Ken Rhodes and Jim Trimmer. Perhaps seduced by the high abundance of CB_1 receptors in the CNS, we as a field felt that immunocytochemical localization of CB_2 was necessary to implicate it in a behavior. I propose that this is a misguided notion. Careful pharmacology and knockout studies have been sufficient to implicate other GPCRs in a variety of behaviors, simply because adequately selective antibodies for that GPCR did not exist. If insisting on the anatomical presence of CB_2 receptors, I propose that we as a field should insist on a combination of the following controls: Absence (not reduction!) of staining in knockouts (which should be stained in a strict parallel and blinded fashion with the experimental tissue), identical staining with antibodies directed against distinct epitopes, and staining that generally trends with CB_2 mRNA (regionally, by ISH or qPCR, and globally, following the very strong induction of CB_2 during certain pathological states). Experiments lacking these controls should be skeptically viewed.

Without a doubt, CB_2 receptors are involved in behavior; our challenge as a field is to determine which behaviors those are through careful and thoughtful experimentation and analysis.

INVOLVEMENT OF CB_2 CANNABINOID RECEPTORS IN ALCOHOL CONSUMPTION: FROM MICE TO HUMAN SUBJECTS

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There is abundant experimental evidence for the involvement of the endocannabinoid system (ECS) in the neurobiological effects of alcohol. The reinforcing effects of alcohol are exerted through multiple signaling systems including interaction with the ECS. In humans alcohol and marijuana-cannabinoids are known to have synergistic effect. Research using pharmacological and available genetic tools targeted at the CB1 cannabinoid receptors (CB1Rs) has revealed an important role of CB1Rs in regulating not only the rewarding properties of alcohol, but also the motivation to consume alcohol. Thus, CB1R mutant mice show a reduction in voluntary ethanol consumption and a decrease in ethanol-induced place preference. However, the functional neuronal expression of CB2Rs in the brain had been controversial and ambiguous and the role of CB2Rs in alcohol consumption and alcoholism has been much less well studied and characterized. Our discovery of two isoforms of CB2Rs, CB2ARs that are localized in the brain and testis and CB2BRs that are predominantly in immune cells in the periphery in human genome resolved the controversy and ambiguity of neuronal CB2Rs. Indeed, our studies have provided the first evidence for neuronal CNS effects of CB2Rs and its possible role in drug addiction, eating disorders, psychosis, depression and autism spectrum disorders. In this study we investigated the effects of CB2Rs and its role in alcohol abuse/dependence in an animal model and then examined an association between *CNR2* gene polymorphism and alcoholism in a human population. Alcohol consumption was enhanced in mice subjected to chronic mild stressors and treated with the CB2R agonist JWH015, whereas the CB2R antagonist AM630 prevented the development of alcohol preference. We demonstrated that there was reduced CB2R gene expression in mice showing preference to consume alcohol whereas mice that showed preference to consume less alcohol showed no changes in CB2R gene expression in the ventral mid-brain region. There was an association between Q63R polymorphism of the *CNR2* gene and alcoholism in a Japanese population. In our preliminary *CNR2* gene copy number variation (CNV) studies, we analyzed one of the CNVs located in the intron of the *CNR2* gene in Japanese alcoholics in comparison to non-alcoholic controls. The CNVs was confirmed to be relatively common in 10 out of 420 Japanese people. More alcoholic DNA samples from other ethnic populations should be analyzed to determine the nature of elevated copy numbers of *CNR2* gene in alcoholism. We also noted that CB2R gene structures differ across species, from mice and rats to human subjects. Our data provides a basis for further work on the role of CB2Rs and other components of the ECS in the development, tolerance, withdrawal, craving and relapse to alcohol dependence.

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CANNABINOID CB2 RECEPTORS MODULATE COCAINE SELF-ADMINISTRATION IN RATS

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It has been reported that activation or over-expression of brain cannabinoid CB2 receptors (CB2Rs) inhibits cocaine self-administration, cocaine-enhanced locomotion, and cocaine-enhanced nucleus accumbens (NAc) extracellular dopamine (DA) in mice (Xi et al, *Nature Neuroscience* 14:1160-1166, 2011; Aracil-Fernández et al., *Neuropsychopharmacology* 37:1749-1763, 2012). However, it is unknown if brain CB2Rs similarly modulate cocaine's action in rats. In the present study, we found that systemic administration of JWH133 (10, 20 mg/kg, i.p.), a selective CB2R agonist, fails to alter cocaine self-administration, while microinjection of JWH133 into the NAc (1, 3 μg/side) inhibits cocaine self-administration under fixed-ratio 2 (FR2) reinforcement. However, during cocaine self-administration under progressive-ratio (PR) reinforcement conditions in which work load for each cocaine infusion is progressively increased, JWH133 (10, 20 mg/kg, i.p.) dose-dependently increases break-point for cocaine self-administration, an effect that is blocked by AM630 (10 mg/kg, i.p.), a selective CB2R antagonist, suggesting CB2R mediation. To determine if this effect is mediated by activation of brain or peripheral CB2Rs, JWH133 was locally administered into the nose (a route of administration by which drugs readily enter the brain). JWH133 (50, 100 μg/nostril) significantly increased, while a higher dose (200 μg/5 μl/nostril), decreased PR break-points for cocaine self-administration. Additionally, JWH133 was micro-injected into the lateral ventricles (20, 40 μg/side) and directly into the NAc (1, 3 μg/side). We found that intra-NAc JWH133 dose-dependently decreased PR break-points for cocaine self-administration, suggesting mediation in the NAc. In vivo brain micro-dialysis experiments demonstrated that systemic JWH133 (10, 20 mg/kg, i.p.) did not alter NAc DA, while local intra-NAc JWH133 (1, 10, 100 μM) attenuated cocaine-enhanced extracellular NAc DA, suggesting that a DA mechanism may in part underlie JWH133's effects on cocaine self-administration. In sum, brain CB2R activation alters cocaine self-administration in rats. Further research is required to determine the mechanisms underlying these actions, and to reconcile the different findings produced by systemic versus intracranial JWH133 administration and by different doses of JWH133.

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ROLE OF CANNABINOID CB_2 RECEPTOR IN THE DEVELOPMENT OF PSYCHIATRIC DISORDERS AND DRUG ADDICTION

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In the last years, a number of studies have changed the concept that the CB2r were exclusively located in the spleen and lymphocytes (1). Nowadays, the results of different studies have revealed the presence of CB2r in a wide variety of brain regions suggesting their potential implications in the regulation of different neuropsychiatric disorders, including drug addiction (2,3). In addition, recent findings using confocal microscopy identified these receptors in neurons, astroglia and microglia (4). The use of genetically modified mice or pharmacological manipulations of this receptor by agonists and antagonists further evidences the important role that CB2r play in the vulnerability and treatment of anxiety, depression, schizophrenia, impulsivity like-behaviors, cognitive-related disorders and in the development or regulation of addictive behaviors induced by drugs such as alcohol, cocaine and nicotine.

Previous studies in our laboratory have shown that mice overexpressing CB2r (CB2xP) are less vulnerable to anxiety and depressive-like behaviors mainly due to a higher level of brain-derived nerve growth factor (BDNF) in the dentate gyrus (DG) of the hippocampus (3,5). Chronic blockade of CB2r acted as antidepressant in WT mice submitted to chronic mild stress (3). On the other hand, the deletion of CB2r resulted in an opposite behavioral profile including increased vulnerability to anxiety and depression, pre-attentional deficit reversed by antipsychotic administration and cognitive impairment (6). Recent unpublished observations suggest that this cognitive impairment may be due, at least in part, to decrease of MAP2, NF200 and SYN-immunoreactive fibers, reduced number of synapses, lower BDNF gene expression and increased m-TOR signaling cascade in DG. The administration of AM630 impaired whereas JWH133 enhanced aversive memory consolidation in WT mice.

The behavioral alterations depending upon deletion or overexpression of CB2r predicts distinct vulnerability to drug of abuse. In this respect, we have found that CB2xP mice presented reduced cocaine-induced motor sensitization, cocaine-induced conditioned place aversion and self-administered less cocaine than WT mice (4). Recent unpublished findings from our laboratory suggest that CB2KO mice presented increased ethanol-conditioned place preference, voluntary ethanol consumption and preference, acquisition of ethanol-self-administration and increased motivation to drink ethanol compared to WT mice. On the other hand, CB2KO mice did not show nicotine-induced place conditioning and self-administered significantly less nicotine. Somatic signs of nicotine withdrawal increased significantly in WT but were absent in CB2KO mice. Interestingly, the administration of AM630 blocked the nicotine withdrawal syndrome and failed to alter basal behavior in saline-treated WT mice.

Taken together, these results provide sufficient evidence to consider that CB2r function is closely involved in the vulnerability and development of behavioral alterations and drug abuse. Furthermore, it seems that pharmacological manipulation of this receptor may be useful as a potential new valuable target for the treatment psychiatric disorders and drug dependence.

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KANG TSOU MEMORIAL LECTURE

"DYNORPHIN – STILL AN EXTRAORDINARILY POTENT OPIOID PEPTIDE"

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In the original publication describing the purification and sequencing of dynorphin A, Avram Goldstein described this peptide as 'extraordinarily potent' ('*dyn'* from the Greek, dynamis = power and '–orphin' for endogenous morphine peptide). The name originally referred to its high affinity and great potency in the bioassay that was used to follow its activity during purification, but the name has come to have a second meaning: Studies of its physiological function in brain continue to provide powerful insights to the molecular mechanisms controlling mood disorders and drug addiction. In the 30 years since its discovery, we have learned that the dynorphin peptides are released in brain during stress exposure. Once released, they activate kappa opioid receptors distributed throughout the brain and spinal cord where they trigger cellular responses resulting in different stress responses: analgesia, dysphoria-like behaviors, anxiety-like responses, and increased addiction behaviors in experimental animals. Initial expectations were that a detailed molecular analysis of the opioid systems would someday lead to better treatments for drug addiction, and recent advances in our understanding of the dynorphin / kappa opioid system suggest that new treatments for addiction and depressive disorders may soon be in hand.

2013 KANG TSOU MEMORIAL LECTURE - TUESDAY, JUNE 25th @ 11.00

ICRS LIFETIME ACHIEVEMENT AWARD

Mahmoud A. ElSohly, Ph.D., BCFE, BCFM

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Mahmoud A. ElSohly is the recipient of the 2013 ICRS Lifetime Achievement Award. Dr. El Sohly is a founding member of the ICRS and has been a continuous member in good standing since its inception. Dr. ElSohly, via his company ELI, has routinely provided financial support for student travel to the Annual Symposium on the Cannabinoids. In addition to his role as President and Laboratory Director of ELI, Dr. ElSohly serves as Research Professor in the National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, and Professor of Pharmaceutics in the School of Pharmacy at the University of Mississippi. Dr. ElSohly is the director of the Marijuana Project at the University of Mississippi, which is funded by the National Institute on Drug Abuse. Dr. ElSohly is an internationally recognized expert in the area of forensic toxicology. Contributions to the field of cannabinoid research by Dr. ElSohly have established his expertise in the areas of analysis of a variety of drugs and their metabolites including but not limited to marijuana and Cannabinoids.

Dr. ElSohly received a B.S. in Pharmacy and Pharmaceutical Chemistry and a M.S. in Pharmacy and Pharmaceutical Sciences from Cairo University, Cairo, Egypt, and a Ph.D. in Pharmacognosy from the University of Pittsburgh. He is board certified by the American Board of Forensic Medicine (BCFM) and the American College of Forensic Examiners (BCFE).

Dr. ElSohly holds a number of patents dealing with the processing, testing, and detection of drugs of abuse along with other patents dealing with biologically active natural products and compositions for the treatment of cancer and other in the diagnostics area. He has authored over 230 scholarly articles and more than 200 presentations at scientific meetings of professional societies relative to drug discovery, analysis, and metabolism, and many of his articles deal with forensic issues of drugs of abuse. He is constantly presenting his research findings at national and international scientific conferences. He is a member of many scholarly scientific societies and was recognized by The Scientist (April 17, 1995) and Science Watch (January, 1995) as the second most cited author in forensic sciences in the world for the period 1981-1993. Dr. ElSohly is also recognized in the Journal of Analytical Toxicology (October issue, 2004) as being one of the top ten (3rd and 4th) Most Cited Authors and Most Prolific Authors in the journal between 1981 and 2003.

ElSohly Laboratories, Incorporated (ELI) is a privately held Mississippi Corporation certified by DHHS and the College of American Pathologists and registered with DEA and FDA. ELI is a small company of well-trained people who are dedicated to helping solve analytical problems in the area of drugs of abuse. ELI is a multifaceted laboratory performing testing for drugs of abuse, reference analyses for commercial and governmental clients, preparing and shipping blind quality control specimens, laboratory controls, standards and internal standards, and performing research and development activities. ElSohly Laboratories offers analytical and advisory services to the drug testing community since 1985.

DISTRIBUTION OF *CANNABIS SATIVA* IN EUROPE BASED ON FOSSIL POLLEN AND ECOLOGICAL ANALYSES

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INTRODUCTION: Advancing the science of cannabis therapeutics includes an inquiry into ethnobotany. Cultural chauvinism biases ethnobotany; people naturally seek "we-were-first" status. Scientists may overinterpret findings to support claims that *Cannabis* cultivation began in *their* culture. Many claims are supported soley by fossil pollen studies. *Cannabis* pollen has tough walls and remain identifiable for thousands of years. However, *Cannabis* pollen closely resembles *Humulus* pollen; the literature is replete with charges of misidentifications; many studies lump data as *"Cannabis-Humulus"* pollen. The present study will dissect pollen identification with a new approach: applying ecological data. Ruderal (wild) *Cannabis* thrives in grassland ecosystems alongside *Poaceae, Chenopodiaceae,* and *Artemisia* species (*PCA*); *Humulus* requires trees to climb and thrives in wet woods. Ecologists report a strong association between *Humulus* and *Alnus* tree species.

METHODS: We analyzed European fossil pollen studies that included *Cannabis*, *Humulus*, or *Cannabis-Humulus* (*C,H,C-H*) in pollen diagrams. Pollen diagrams show changes in pollen abundance (represented as areas under the curve, AUCs, that oscillate over time) dated with radiocarbon (^{14}C) methods. Pollen annotated as *C,H,C-H* whose AUCs correlated with *Alnus* AUCs were identified as *Humulus*. Pollen annotated as *C,H,C-H* lacking correlation with *Alnus* but correlating with *PCA* were identified as *Cannabis.* Sediment strata containing cereal crop pollen (*e.g., Cerealia, Triticum, Secale*) were associated with the presence of agriculture. Results were mapped using geographic information system (ArcGIS) software.

RESULTS: In most studies, oscillations in *Alnus* pollen diametrically opposed *PCA* pollen oscillations. Pollen annotated as *C,H,C-H* that pre-dated agriculture largely correlated with *Alnus* AUCs, and likely represent wild *Humulus.* Assisted by GIS mapping, evidence suggests that pre-Neolithic wild *Cannabis* was limited to southeastern Europe. Pollen AUCs were easily deduced as cultivated *Cannabis* when they spiked in strata containing cereal crop pollen, as early as 400 BCE (*Humulus* was not cultivated until the 9th century CE).

DISCUSSION: Combing pollen counts with ecological data throws the identification of *Cannabis* in many studies into question. This is particularly true in Scandanavia, the Baltic states, and northwestern Europe. Pollen verified as wild *Cannabis* by this methodology is limited to Russia and Ukraine. The oldest *Cannabis* pollen dates to 155,000 BCE at Likhvin (58º08'N, 30º17'E) situated in north-central Russia. The next study will apply this method to Asian fossil pollen data.

THE *CANNABIS* ACCESS FOR MEDICAL PURPOSES STUDY: PATIENT CHARACTERISTICS, REASONS FOR USE AND MODES OF ACCESS

Zach Walsh, Robert Callaway, Lynne Belle-Isle, Rielle Capler, Robert Kay, Philippe Lucas and Susan Holtzman

University of British Columbia (authors 1,4, & 7); University of Victoria (authors 3 & 6)

The Cannabis Access for Medical Purposes Study (CAMPS) surveyed 628 Canadians who identify as past or current consumers of cannabis for therapeutic purposes, making it the largest and most comprehensive study to date of the therapeutic use of cannabis in Canada. We present findings of CAMPS, including consumer demographics, reasons for use, substance use history, preferred modes of access, and barriers to use. The mean number of symptoms patients endorsed treating was 6.74 (SD=3.00). Sleep assistance (85%), pain relief (82%), and anxiety relief (78%) were the most frequently endorsed symptoms; 57.2% reported use to address all three symptoms, and 99% endorsed treating one or more of the three. Cannabis was perceived to provide effective symptoms relief; 72.09% reported that CTP was *always* helpful and an additional 24.14% described it as *often* helpful. The proportion of participants who described CTP as *always* helpful was relatively consistent across conditions. Over half (57%) of participants reported using other medications to address the symptoms they were treating with CTP. Of these, 78.71% described CTP as having fewer side effects than the concurrent treatment. Quantity and frequency of use were also relatively consistent across medical conditions. Most participants reported initiating non-medical use prior to use of CTP, and noted increased levels of use associated with the transition to therapeutic use.

We draw three primary conclusions from the data. First, reasons for use, patterns of use, and perceived effectiveness were generally consistent across medical conditions; respondents overwhelmingly reported using cannabis to effectively address pain, sleep disturbance, and anxiety. Second, further research is required to address the substantial disconnect between the therapeutic use of cannabis, and research on the risks and benefits of such use. This is particularly evident with regard to the anxiolytic and sedative use of cannabis; widespread use for these symptoms suggests a need for the systematic evaluation of the effectiveness and adverse effects, as well as comparisons of cannabis with the widely-used pharmaceutical products that currently represent frontline treatments. Finally, our findings highlight the apparent discrepancy in access to cannabis. Authorized and unauthorized users exhibited few meaningful differences with regard to medical conditions and patterns of use, but face substantial differences regarding access; many seriously ill Canadians risk legal sanction and other negative outcomes associated with accessing cannabis from illegal markets.

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CANNABINOID FACILITATION OF EXTINCTION RECALL VIA INCREASED RECRUITMENT OF PREFRONTAL-HIPPOCAMPAL CIRCUITRY IN HEALTHY HUMANS

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Enhancing extinction learning may optimize gains achieved by exposure therapy for anxiety disorders (e.g., maintenance of effects, hastened pace of improvement, greater generalization outside therapeutic context). Emerging evidence from animal studies suggest that enhancing cannabinoid system within the ventromedial prefrontal cortex (vmPFC) and hippocampus (HPC), brain structures critical to fear extinction, enhances fear extinction and its retention. However, the role of cannabinoids on the retention of extinction memory and its effect on the underlying neural circuits in humans remains unknown. We conducted an fMRI study using a randomized, double-blind, placebo-controlled, between-subjects design, coupled with a standard Pavlovian fear extinction paradigm and simultaneous skin conductance response (SCR) recording with an acute pharmacological challenge with oral dronabinol (synthetic Δ^9-tetrahydrocannibinol; THC, n = 15) or placebo (PBO, n = 15) 2 hours prior to extinction learning in healthy adult volunteers to assess the effects of THC on vmPFC and HPC activation when tested for recall and maintenance of extinction learning at 24 hours and 1 week after training, respectively.

Compared to subjects who received PBO, those who received THC showed increased vmPFC activation and functional coupling with the HPC, as well as low SCR to a previously extinguished CS when extinction memory recall was tested, suggesting that THC prevented the recovery of fear via increased recruitment of the vmPFC and HPC. These results advance the neurobiology of extinction learning and prompt development of novel pharmacological modulators of the cannabinoid system to maximize the potency of exposure therapy for anxiety disorders.

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SELF-REPORTED COGNITIVE ENHANCING EFFECTS OF MARIJUANA DO NOT PREDICT MARIJUANA-ELICITED IMPROVEMENTS IN PERFORMANCE ON TASK BATTERY IN THE LABORATORY

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In daily marijuana smokers, acute administration of active marijuana produces few effects on measures of attention, psychomotor ability, learning, and memory compared to placebo marijuana. Yet some individuals report that they smoke marijuana for its cognitive-enhancing effects. This retrospective preliminary analysis sought to determine if marijuana's effects on task performance varied according to participants' self-reported reason for marijuana use. Data obtained from outpatient studies investigating medication effects on marijuana in daily marijuana smokers were used for the current analysis. Preliminary data analysis was conducted (data collection is ongoing). The between-group analysis compared performance on 4 tasks between participants who reported smoking marijuana for its cognitive enhancing effects (CE; n = 7), and those who did not (NC; n = 15) after inactive marijuana (0% THC) and active marijuana (3.27% THC) was smoked. The tasks included: 1) a repeated acquisition task (RAT) that required participants to learn and then enter a 10-response sequence on a keypad as quickly as possible in a given time limit, 2) a divided attention task (DAT), which assessed attention requiring participants to track a moving target on a computer screen using a mouse while signaling when a brief stimulus appeared in one of the four corners, 3) a digit-symbol substitution task (DSST), which tested psychomotor performance and 4) a delayed and immediate recall task (DRT), which required participants to enter an 8-digit sequence that appeared on the computer screen, and again when it disappeared (immediate recall). Participants were then asked to recall and recognize one of the sequences at the end of the task battery. Subjective drug-effect ratings and cardiovascular effects of marijuana were also compared between the two groups.

For the CE group, participants reported smoking marijuana because it helped them to 'focus,' 'increased concentration,' 'enhanced experiences,' and 'increased creativity.' Participants of the NC group reported smoking marijuana for relaxation, calming effects, decreased irritability, increased appetite, and help with sleep. Between-group analysis demonstrated that the CE group showed significant decreases in RAT performance after smoking active marijuana, whereas the NC group performance did not differ between active marijuana and inactive marijuana ($p \leq 0.05$). Marijuana did not affect performance on the other tasks in either group. The CE group also exhibited greater increases in heart rate after marijuana smoking relative to the NC group ($p \leq 0.05$), and a trend for higher subjective drug effect ratings for 'High' ($p \leq 0.10$). These preliminary findings demonstrate incongruence between self-reported effects of marijuana and actual performance. However, these findings were limited to a single task. Differences between the CE and NC groups in marijuana's cardiovascular effects suggests that tolerance to marijuana's effects may have contributed to the decrements in performance observed in the CE group but not the NC group.

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THE IMPACT OF DECISION-MAKING PERFORMANCE AND ADHD SYMPTOMS ON *CANNABIS*-RELATED PROBLEMS AMONG EMERGING ADULTS

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Neurocognitive deficits and alterations in brain functioning are well documented among individuals with ADHD and in heavy, non-abstinent cannabis users. Deficits in decision-making (DM) are reported among those with ADHD, but cannabis-associated deficits in DM remain controversial, particularly as it pertains to their onset, etiology, and duration after abstinence. Recently, we reported poorer episodic memory performance in 18 to 24 year-old cannabis users with minimal other drug use and mental health problems when compared to non-users, but we found no differences on various measures of inhibitory control (including a measure of DM). Poorer DM, however, was associated with more DSM-IV symptoms of cannabis use disorders among cannabis users. We hypothesize that symptoms of ADHD, in conjunction with poor DM, may serve to worsen cannabis-related problems.

We explored the relationship among DM (as indexed by the Iowa Gambling Task [IGT]) and symptoms of ADHD (as indexed by the Wender-Utah Rating Scale [WURS]) on problems in everyday functioning from cannabis use as reported on the Marijuana Problems Scale (MPS) among a sample of young adult cannabis users (n=65). IGT performance, amount of cannabis used in the past 30 days (30dCB), WURS scores, and their interactions were independent variables in a linear regression model with MPS as the dependent variable. The omnibus model was statistically significant (R^2=.45, p< .001), with a significant WURS x 30dCB interaction (p=.04) and a trend toward a significant IGT x WURS x 30dCB interaction (p=.06). Follow-up analyses revealed that among those with less cannabis use, more ADHD symptoms were associated with greater problems from cannabis use, regardless of DM performance (p-values <.04). In contrast, among those with more cannabis use, more ADHD symptoms were related to more problems from cannabis use only among those with poorer DM performance (p=.004). Finally, we compared MPS scores among participants classified into three groups based on their IGT and WURS scores. Those with two risk factors (poor DM and high ADHD symptom report) reported significantly more cannabis-related problems than those with one risk factor (either poor DM or high ADHD symptom report), or no risk factors (good DM and low ADHD symptoms), even after controlling for amount of cannabis use (p=.01). Taken together, these preliminary findings suggest that symptoms of ADHD may exacerbate problems experienced from cannabis use, particularly in the context of poor DM.

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CIRCADIAN RHYTHM OF CIRCULATING ENDOCANNABINOID (EC), 2-ARACHIDONOYLGLYCEROL (2-AG), CONCENTRATIONS FOLLOWING NORMAL AND RESTRICTED SLEEP

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Evidence from laboratory and epidemiologic studies suggest that insufficient sleep duration may be a contributing factor to the epidemic of obesity. The EC system, a pharmacotherapeutic target for obesity treatment, mediates the hedonic control of feeding, and modulates reward mechanisms. Stimulation of the EC system could be involved in the risk in overeating associated with sleep loss. The roles of sleep and circadian rhythmicity in modulating circulating EC lipids are not known as previous studies have only assessed single time points or narrow time intervals. We examined the 24-h profile of (2-AG), and its structural analog 2-oleoylglycerol (2-OG), which does not bind cannabinoid receptors, in healthy subjects and determined the impact of recurrent partial sleep restriction.

In a randomized cross over design, nine healthy subjects (age: 23 ± 1yrs; BMI:23.6±0.7kg/m^2) were studied in the laboratory with controlled energy expenditure and caloric consumption. Blood sampling was performed for 24-h following two nights of normal sleep (2300-0730) or partial sleep restriction (0100-0530). Samples taken at 60min intervals were assayed with liquid chromatography electrospray ionization-mass spectrometry (LC-ES-MS), for detection of 2-AG and 2-OG.

Both 2-AG and 2-OG display clear circadian rhythms with a nadir around mid sleep and peak levels in the early afternoon. Following sleep restriction, the amplitude of the rhythm is significantly increased for both lipids (2-AG: 151 ±24 vs 126 ±24pmol/ml, $p = 0.01$; 2-OG 894±120 vs 709±85pmol/ml, $p = 0.005$), due to an increase in peak levels (2-AG: 386 ±60 vs 335 ±61 pmol/ml, $p = 0.008$; 2-OG: 3329±284 pmol/ml vs 3138 ±255 pmol/ml, $p = 0.08$). Mean 24-h levels of 2-AG and 2-OG were highly correlated during the normal sleep condition ($r = 0.80$, $p < 0.05$) and during the restriction condition ($r = 0.84$, $p < 0.05$). Moreover, despite the two conditions being separated by at least one month, mean 24-h levels were highly correlated between the normal and restricted sleep sessions for both 2-AG ($r = 0.98$, $p < 0.001$) and 2-OG ($r = 0.83$, $p = 0.005$), indicating within-subject reproducibility.

This study provides the first demonstration of a robust circadian rhythm of human plasma EC levels and reveals that sleep restriction results in increased amplitude of the mid-sleep to early afternoon rise of both 2-AG and 2-OG. Elevation of peak daytime levels of EC may contribute to the risk of overeating associated with sleep deprivation.

SLEEP CHARACTERISTICS OF ADULTS SEEKING TREATMENT FOR *CANNABIS* USE DISORDERS

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Sleep is important for human wellbeing. Acute use of cannabis has sleep-promoting properties and abrupt abstinence from daily cannabis use has been shown to result in clinically significant reductions in sleep continuity and altered sleep architecture. Sleep disturbance is often reported as having contributed to relapse when attempts to quit have failed. It is unclear whether sleep problems in abstinent cannabis users reflect a recurrence of pre-morbid insomnia, a pharmacologically based withdrawal effect, or a combination of the two. Sleep quality among daily cannabis users prior to a quit attempt has not been well characterized. The present study is being conducted to evaluate the role of sleep in the treatment of cannabis use disorders. Here we report baseline assessments of sleep quality among adults presenting for cannabis treatment. Measures include the Pittsburgh Sleep Quality Index (PSQI), Insomnia Severity Index (ISI), and, for a subgroup of participants eligible for study participation after an intake assessment, an overnight objective polysomnography sleep assessment was conducted.

Study volunteers (N=35; 20M, 15F) were mostly African American (N=29) with a mean (SD) age of 33 (10), had smoked cannabis frequently for 16 (10) years, and self-reported cannabis use on 28 (4) of the 30 days prior to the intake assessment. Participants self-reported an average sleep latency of 44 (43) minutes and a total sleep time of 5.5 (1.6) hours each night. Mean global PSQI score for the sample was 12 (8), and was >5, indicative of poor sleep, for 89% of participants. Total score on the ISI indicated that 43% had sub-clinical insomnia, 31% had clinical insomnia of moderate severity, and 3% had severe clinical insomnia at intake. To date, few objective sleep assessments have been formally scored, but preliminary reports are consistent with patient self-reports indicating relatively short sleep duration and poor sleep efficiency in 50% of assessments. These data suggest that disordered sleep may be common among adults seeking treatment for cannabis use disorders. Because abrupt cannabis cessation may further deteriorate sleep continuity and architecture in these individuals, incorporation of clinical interventions aimed at improving sleep may be crucial for increasing initial and long-term abstinence rates in this subpopulation of cannabis users. Future data collection will be conducted to see if intake sleep assessments predict the presence and severity of insomnia during a quit attempt, and whether the severity or type of sleep disturbance at intake or during abstinence predicts relapse.

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MARIJUANA WITHDRAWAL AND RELAPSE IN THE HUMAN LABORATORY: EFFECT OF ZOLPIDEM ALONE AND IN COMBINATION WITH NABILONE

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Disrupted sleep is a robust marijuana withdrawal symptom and one of the few factors predicting the severity of marijuana relapse as measured in the laboratory (Haney et al., 2012). These observations suggest that improving sleep during marijuana withdrawal might decrease marijuana relapse. Zolpidem, a widely used hypnotic with low abuse liability, attenuated sleep disruption during marijuana withdrawal (Vandrey et al., 2011), but it did not decrease withdrawal-related mood symptoms, and its effects on marijuana relapse are not known. The cannabinoid agonist, nabilone (4 mg BID, 6 mg once/day) robustly decreases a range of marijuana withdrawal symptoms (negative mood, marijuana craving, disrupted sleep) and decreases marijuana relapse in daily marijuana smokers relative to placebo (Haney et al., 2013), but can produce slight decrements in cognitive performance. Thus, the objective of this study was to determine if zolpidem, alone and in combination with a lower dose of nabilone than previously tested, would attenuate marijuana withdrawal and relapse compared to placebo. Male (n=11) daily marijuana smokers (10 $\pm$ 5 marijuana cigarettes/day) completed 3 inpatient phases, with each phase testing a different dose condition in counter-balanced order: placebo, zolpidem (12.5 mg once/day), and zolpidem (12.5 mg once/day) combined with nabilone (3 mg BID). On the first inpatient day of each phase, participants repeatedly smoked active marijuana (5.6% THC) under controlled conditions. For the following 3 days, they only had access to inactive marijuana (0.0% THC: marijuana withdrawal). On the subsequent 4 days, active marijuana (5.6% THC) was available for self-administration (marijuana relapse). Participants had to pay for self-administered marijuana using study earnings. Preliminary results show that the amount of marijuana self-administered during the relapse phase was significantly decreased by the nabilone/zolpidem condition relative to placebo capsules, while zolpidem alone had no effect. Data analysis assessing the effects of each medication condition on symptoms of marijuana withdrawal (mood, marijuana craving, sleep, food intake) is currently underway. These preliminary findings suggest that zolpidem alone may not be sufficient to improve marijuana treatment outcome, but that nabilone in combination with zolpidem may show promise clinically.

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CANNABINOID REPLACEMENT THERAPY FOR MANAGEMENT OF *CANNABIS* WITHDRAWAL: A RANDOMIZED CONTROLLED TRIAL OF NABIXIMOLS

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Importance: Cannabis is the most prevalent illicit drug in the world and cannabis treatment admissions have more than doubled over the last two decades. There are currently no effective medications for cannabis dependence or withdrawal. Nabiximols, a botanically derived cannabinoid medication, may support the management of cannabis withdrawal syndrome by ameliorating associated discomfort due to its agonist action.

Objective: To compare outcomes of Nabiximols to placebo for managing cannabis withdrawal syndrome.

Design, Setting, and Participants: A two-site, randomized, double-blind, placebo-controlled inpatient trial with follow-up. Specialist addiction treatment centers in New South Wales, Australia. Fifty-one DSM-IV-TR cannabis dependent treatment seekers.

Interventions: Participants were randomized to receive a 6-day regimen of either Nabiximols (n=27), or placebo (n=24), with standardized psychosocial intervention and monitoring during a 9 day inpatient admission.

Outcome measures: Primary: cannabis withdrawal severity **(**Cannabis Withdrawal Scale); retention in treatment; intoxication; adverse events. Secondary: cannabis use (self-report and urine drug screens), dependence severity (Severity of Dependence Scale) and cannabis-related problems (Cannabis Problems Questionnaire) at 28-day follow-up.

Results: The severity of cannabis withdrawal was significantly reduced [Change score mean Hedges-*g,* 0.83] and shorter [1 day vs. 7 days] in the Nabiximols group than in the placebo group (p=0.013). Retention in withdrawal treatment was significantly longer for the Nabiximols group during the six-day medication phase (p=0.004), but not after medication stopped (day-9,p=0.28). Patients could not differentiate between Nabiximols and placebo (p=0.67) and groups did not differ on intoxication ratings (p=0.97). For Adverse Events, neither the number (p=0.59) nor severity (p=0.1) was different between groups. Both groups reduced cannabis use at follow-up, but did not differ on: cannabis use (p=0.75), problems (p=0.14), or severity of dependence (p=0.9).

Conclusions: Nabiximols was more effective than placebo in achieving higher rates of withdrawal completion and reducing severity of cannabis withdrawal but this had no impact on post-withdrawal outcomes.

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BY LAND OR BY SEA: CANNABINOID DISCRIMINATION IN MICE

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Previous studies have shown considerable overlap in the discriminative stimulus effects of the major exogenous cannabinoid classes as well as correspondence between their potencies for producing these effects in rats and potencies for producing marijuana intoxication in humans [1,6]. In contrast, studies have reported that anandamide (AEA) does not substitute for Δ^9-tetrahydrocannabinol (THC) [2,3,5]. Rapid metabolism of AEA via degradation by fatty acid amide hydrolase (FAAH) is one possible explanation; however, AEA and other endocannabinoids (eCBs) also affect metabolic and endocrine function, with a primary role of facilitation of energy conservation. Through their activation of CB_1 receptors in the mesolimbic area, eCBs also enhance pleasure associated with eating [4]. Because eCB effects may affect the internal state of an animal, it is possible that differences in hunger level, hedonic value of the food, or other internal metabolic states resulting from interaction of an eCB with food intake could be part of its interoceptive effects in a food-reinforced operant procedure.

To determine whether type of reinforcement affects the discriminative stimulus effects of cannabinoids, we trained FAAH(-/-) and wildtype (C57/Bl6) mice to discriminate THC from vehicle in two types of procedures: one that used food (positive, appetitive) reinforcement and one that used escape from water immersion (negative, non-appetitive reinforcement). Mice successfully acquired both types of discrimination. In all groups of mice, THC dose-dependently substituted for itself and substitution was reversed by rimonabant. In FAAH(-/-), but not in wildtype, mice, AEA also substituted in both types of procedures, with rimonabant reversal. In contrast, a slight difference in the substitution pattern for the monoacylglycerol lipase (MAGL) inhibitor JZL184 was observed. Whereas JZL184 produced full dose-dependent substitution for THC in FAAH(-/-) mice trained in a food reinforcement procedure, it only partially substituted for THC in FAAH(-/-) mice trained in the water maze and in wildtype mice trained in either procedure. These results suggest considerable overlap in the THC-like discriminative stimulus effects of eCBs, regardless of type of reinforcement.

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EXPANDING THE RANGE OF POTENTIAL "ENDOGENOUS CANNABINOIDS" TO INCORPORATE LIPIDS IN BACTERIA, YEAST, PLANTS, WORMS, AND FLIES: CANNABIMIMETIC LIPIDS ARE NOT JUST FROM ARACHIDONIC ACID ANYMORE

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Discoveries of the endogenous lipids *N*-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG) that activate CB_1 and CB_2 receptors profoundly changed the way we have studied cannabinoid signaling systems. More recently, the discovery of the cannabinoid-like (cannabimimetic) activity of the AEA metabolite, *N*-arachidonoyl glycine (NAGly), and additional bioactive structural analogs of AEA, 2-AG, and NAGly have opened the door further to understanding those cannabimimetic signaling systems that are rooted in a myriad of endogenous signaling pathways (*e.g*. TRPVs, GPR119, GPR18, GPR92). To date, most of the focus on endocannabinoid signaling has been on mammalian systems in large part due to the fact that arachidonic acid (AA) is required for the biosynthesis of AEA, 2-AG, and NAGly, which is not synthesized in most lower organisms. Recently, the understanding that endogenous analogs to these lipids that are synthesized with more ubiquitous fatty acids, such as palmitic and oleic acids, have cannabimimetic signaling properties prompted us to investigate additional model systems for the presence of potential endogenous cannabinoids (eCBs). Here, we show that the family of *N*-acyl amides and 2-acyl glycerols which contain many cannabimimetic lipids are ubiquitous throughout the animal and plant kingdoms.

Using methanolic lipid extracts, partial purification via C18 solid phase extraction columns, and HPLC coupled to tandem mass spectrometry we have screened over 80 lipids from the *N*-acyl amide and 2-acyl glycerol families in a variety of model systems. We show that a range of these lipids are present in bacteria (*Caulobacter crescentus*), yeast (*Saccharomyces cerevisiae*), plants (*Arabidopsis thaliana*), worms (*Caenorhabditis elegans*), and insects (*Drosophila melanogaster*). Importantly, the model systems for bacteria, yeast, and worms often use "yeast media", which contains animal products such as milk. We have screened these model systems in the presence and absence of this "yeast media" and show that they incorporate AA and other PUFAs and generate these *N*-acyl amide conjugates into the organism only when fed animal products, which means that many of the current experimental paradigms using these model systems are in essence producing "mammalian" eCBs. What this means for signaling is still an unanswered question, however, it is does maintain the simple adage that "we are what we eat". That so many of these signaling lipids are ubiquitous in organisms that we humans consume also begs the question: which signaling molecules are we generating and which are we simply ingesting and how does that balance play a role in eCB signaling homeostasis?

RATIONAL DESIGN OF NEUTRAL ALLOSTERIC MODULATORS OF THE CB_1 RECEPTOR WITH IMPROVED RECEPTOR INTERACTIONS AND UNIQUE PHARMACOLOGY

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The CB_1 allosteric modulator, ORG27569, has the paradoxical effect of increasing the equilibrium binding of CP55,940 (an orthosteric agonist), while at the same time decreasing its efficacy. In addition, ORG27569 also acts as an inverse agonist at the CB_1 receptor by reducing basal activity (in G protein-mediated pathways). We have previously used computational methods combined with synthesis, mutation, and functional studies to identify the binding site of ORG27569 in the THM3/TMH6/TMH7 region (Shore et al., *ICRS*, **2012**). At this site, ORG27569 promotes an active-like conformation of the CB_1 receptor, explaining ORG27569's ability to increase CP55,940's equilibrium binding. Moreover, this site explains ORG27569's ability to antagonize CP55,940's efficacy in three complimentary ways: 1) ORG27569 sterically blocks movements of the second extracellular loop that have been linked to receptor activation, 2) ORG27569 sterically blocks a key electrostatic interaction between the third extracellular loop residue K373 and $D2.63^{176}$, and 3) ORG27569 packs against TMH6, sterically hindering movements of TMH6 that the Farrens lab have shown to be important to receptor activation. Additionally, we identified a key interaction between ORG27569's piperidine ring nitrogen and $K3.28^{192}$ that is required for ORG27569 to act as an inverse agonist.

Using our model of ORG27569 docked in our active state model (in the presence of CP55,940), we designed, synthesized, and functionally characterized 4 analogs of ORG27569 that were designed to test our model and have improved interactions with the receptor. The analogs were functionalized with 3 different goals: 1) to form electrostatic interactions with $D6.58^{366}$, 2) to form an aromatic stack with $F3.25^{189}$, or 3) to test packing with TMH6-7. **Our strategy to form new interactions with $D6.58^{366}$ was the most successful, resulting in an analog that is more potent than ORG27569.** Our strategy to form an interaction with $F3.25^{189}$ resulted in an analog that potently antagonized the efficacy of CP55,940, but did not influence CP55,940's equilibrium binding; *these results illustrate that is possible to design allosteric modulators of the CB_1 receptor that impact an orthosteric ligand's efficacy independently of its binding.* Our third strategy resulted in an analog that supports our hypothesis that ORG27569 packs tightly against TMH6-7. Interestingly, none of the analogs acted as inverse agonists, suggesting the potential therapeutic promise of CB_1's allosteric site. [Support NIDA RO1 DA003934 and KO5 DA021358]

IN VITRO EFFECTS OF ZCZ011: A POSITIVE ALLOSTERIC MODULATOR OF THE CB_1 RECEPTOR

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There has been a significant amount of interest shown in negative allosteric modulators of the cannabinoid CB_1 receptor (Price et al., 2005, Horswill et al., 2007, Baillie et al., 2012). However, positive allosteric modulation of this receptor has been little investigated. An allosteric enhancer of CB_1 receptors may provide a useful therapeutic, with fewer side-effects than a direct CB_1 receptor agonist. Thus, with an allosteric enhancer, it is hypothesized that it should be possible to increase beneficial endogenous effects of anandamide or 2-arachidonoyl glycerol without disrupting normal physiological processes, thereby circumventing the psychoactive effects associated with direct CB_1 receptor activation. We have developed a novel allosteric enhancer, ZCZ011 (Figure 1). Here we present the *in vitro* characterization of this compound; its *in vivo* characterization is described in a partner ICRS abstract (Ignatowska-Jankowska et al, 2013 ICRS abstract).

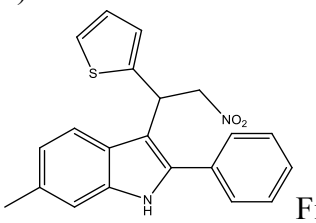

Figure 1: Structure of ZCZ011

A number of biological assays have been performed using either mouse brain membranes or cell membranes from CB_1 transfected cell lines to fully investigate the effect of ZCZ011 on (1) the affinity of [^{3}H]CP55940 for the CB_1 receptor using equilibrium and saturation binding assays and (2) the potency and efficacy of the CB_1 receptor agonists, anandamide and CP55940 in [^{35}S]GTPγS binding, β-arrestin recruitment, inhibition of cAMP and phosphorylation of ERK assays.

ZCZ011 (10nM to 1μM) caused a significant and concentration-dependent increase in the specific binding of [^{3}H]CP55940 to mouse brain membranes in equilibrium binding studies and, at 1μM, a significant increase in the B_{max} of [^{3}H]CP55940 in saturation binding assays. ZCZ011 also caused a significant enhancement of the efficacy of both anandamide and CP55940 in all of the above functional assays at concentrations between 10nM and 1μM.These results, along with the *in vivo* data presented by Ignatowska-Jankowska et al, demonstrate ZCZ011 to be a potent, positive allosteric modulator of cannabinoid CB_1 receptors which may, therefore, be both effective and highly selective as a medicine, for example for the treatment of neuropathic pain.

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IN VIVO EFFECTS OF ZCZ011: A POSITIVE ALLOSTERIC MODULATOR OF THE CB_1 RECEPTOR

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Allosteric modulators bind to a site on the receptor distinct from orthosteric ligands, and produce conformational changes of the receptor that can lead to alterations in ligand potency and/or efficacy. Positive allosteric modulators (PAMs) increase the effectiveness of endogenous ligands and/or exogenous agonists, which potentially limits side effects of administered drugs. Here, we describe the pharmacological characterization of a novel CB_1 receptor PAM, ZCZ011. *In vitro* studies indicated that ZCZ011 potentiates affinity of [^{3}H]CP55,940 for the CB_1 receptor as well as enhances CP55,940-stimulated or endogenous N-arachidonoylethanolamine (AEA)-stimulated [^{35}S]GTPγS binding in mouse brain membranes (Baillie et al., 2013 ICRS abstract).

In the present study, ZCZ011 was assessed alone and in combination with either CP55,940 or AEA in several assays indicative of cannabimimetic activity, including antinociception (tail withdrawal test), hypothermia, catalepsy (bar test), locomotor activity and in the drug discrimination paradigm. Moreover, ZCZ011 was tested in the chronic constriction nerve injury (CCI) model of neuropathic pain.

ZCZ011 (40 mg/kg) significantly enhanced the pharmacological effects of both CP55,940 in C57BL/6J mice and AEA in fatty acid amide hydrolase (FAAH) knockout mice, as well as significantly increased the potency of AEA in the drug discrimination assay. However, when given alone, ZCZ011 did not substitute for AEA in the drug discrimination paradigm or produce catalepsy, thermal antinociception, hypothermia or changes in locomotor activity. Importantly, ZCZ011 significantly reversed allodynia (i.e., nociceptive responding to a normally non-noxious stimulus) induced by mechanical (von Frey filaments) or cold (acetone) stimulation in the CCI model. These anti-allodynic effects were comparable in magnitude to those produced by the FAAH inhibitor PF-3845, and were completely antagonized by rimonabant, indicating a CB_1 receptor mechanism of action.

The results of the present study, demonstrating that ZCZ011 enhances the pharmacological effects of AEA and CP55,940 in mice, are consistent with *in vitro* data showing that it acts as a CB_1 receptor PAM. Nonetheless, it will be important to identify the putative allosteric binding site of ZCZ011 and examine its influence on other cannabinoid receptor agonists. The present data, taken together, provide proof of principle that CB_1 PAMs may offer a novel strategy to treat neuropathic pain with minimal or no cannabimimetic side effects.

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IN VIVO CANNABINOID CB2 RECEPTOR TARGETED IMAGING USING A NEAR INFRARED FLUORESCENT PROBE

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The type 2 cannabinoid CB2 receptor represents a promising therapeutic target to treat various diseases, such as cancers, neurodegenerative diseases, inflammation, pain, osteoporosis, immunological disorders and drug abuse. However, the exact role of CB2 receptor in regulation of these diseases remains poorly explored. This is largely due to the lack of reliable and specific imaging tools for CB2 receptor imaging. Although CB2 antibodies are available to label the receptor, the non-specific nature of these antibodies greatly limits their applications. As such, development of specific contrast agents for CB2 receptor imaging is critical.

We recently developed a CB2 receptor targeted near infrared (NIR) fluorescence probe, NIR-mbc94, and evaluated its specific binding to the target receptor *in vitro*. Here we report the first *in vivo* optical imaging study of CB2 receptor using NIR-mbc94. In CB2 positive mouse tumor model, we observed significantly higher fluorescence signal in the target region than free dye control, and such high fluorescence signal could be blocked by SR144528, a well-known CB2 ligand. Our results indicate that NIR-mbc94 binds to CB2 receptor specifically and has the potential to be widely used by researchers to study the role of CB2 receptor in various diseases.

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NOVEL, HIGHLY POTENT AND SELECTIVE 2,5,6-PYRIDINE/PYRAZINE DERIVED CB2 AGONISTS PROTECT RODENT KIDNEYS FROM ACUTE INJURY AND FIBROSIS

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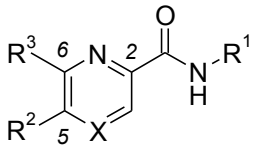
Agonists of the cannabinoid receptor 2 (CB2) positively influence a large number of pathological conditions, spanning from cardiovascular, gastrointestinal, liver, kidney, lung, neurodegenerative and psychiatric disorders to pain, cancer, bone, reproductive and skin pathologies [1]. CB2 agonists have been shown to exhibit protective effects in several animal models introducing ischemia in organs like liver and brain. Furthermore, CB2 agonists were also shown to protect from fibrosis in organs such as liver. 2,5,6-Trisubstituted pyridines and pyrazines (I) were identified to be novel, highly potent and selective CB2 agonists. The exploration of the exit vectors in positions 2, 5 and 6 led to the discovery of agonists with CB2 picomolar potency in inhibiting cAMP formation. Surprisingly, only subtle structural changes inverted the function from agonists into inverse agonists. Furthermore, species differences between human and rodent CB2 receptors have been observed. A detailed structure activity relationship for CB2 and CB1 binding and functional assays was elaborated. Furthermore, species differences between human and rodent CB2 receptors were investigated.



X = CH, N

(I)

Pyridines/pyrazines (I) were optimized not only for their *in vitro* potency on and selectivity for the target receptor but also for their physicochemical properties including solubility, membrane permeation and lipophilicity as well as for their metabolic stability and cytochrome P450 inhibition potential. Representative compounds combining high *in vitro* potency with favorable early ADME properties have been profiled in *in vivo* pharmacokinetic studies. Advanced compounds including 2,5,6-pyrazine RO6839828 protected mouse kidneys from warm ischemia reperfusion injury. After a period of 25 min ischemia and 24 h reperfusion (n=6 per group) RO6839828 produced a statistical significant improvement of kidney function as measured by plasma creatinine (50% improvement; $p<0.001$). Moreover, efficacy of RO6839828 was reflected in improvement of relevant plasma biomarkers of kidney injury (reduction of plasma biomarkers: NGAL 62%, $p<0.003$; osteopontin 87%, $p<0.001$; KIM1 50%, $p<0.002$). In addition, RO6839828 significantly reduced fibrosis in a rat unilateral ureter obstruction model (UUO) as measured by a 50% reduction in collagen I deposition 8 d after UUO (n=8, $p<0.001$), thereby suggesting that CB2 agonists might have beneficial effects in both acute and chronic kidney disease.

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IDENTIFICATION OF RALOXIFENE AS A NOVEL CB2 INVERSE AGONIST

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The purpose of the current study was to apply a high throughput assay to systematically screen a library of FDA-approved drugs as potential ligands for the cannabinoid receptor 2 (CB2). A cell-based, homogenous time resolved fluorescence (HTRF) method for measuring changes in intracellular cAMP levels was validated and found to be suitable for screening ligands that may act on CB2. Among the 640 FDA-approved drugs tested, raloxifene, a drug used to treat/prevent osteoporosis, was identified for the first time to be a novel CB2 inverse agonist. Our results demonstrated that raloxifene enhances forskolin-stimulated cAMP accumulation in a concentration-dependant manner. Furthermore, our data showed that raloxifene competes concentration-dependently for specific [^{3}H]CP-55,940 binding. In addition, raloxifene pretreatment caused a rightward shift of the concentration-response curves of the cannabinoid agonists CP-55,940, HU-210, and WIN-55,212-2. Raloxifene antagonism is most likely competitive in nature, as these rightward shifts were parallel and were not associated with any changes in the efficacy of cannabinoid agonists. Our discovery that raloxfiene behaves as an inverse agonist for CB2 suggests that it might be possible to repurpose this FDA-approved drug for novel therapeutic indications for which CB2 is a target. Furthermore, identifying raloxifene as a CB2 inverse agonist also provides important novel mechanisms of actions to explain the known therapeutic effects of raloxifene for treating/preventing post-menopausal osteoporosis.

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3-CARBOXAMIDO-5-ARYL-ISOXAZOLES: AS THE FIRST CB_2 / FAAH MULTITARGET DERIVATIVES

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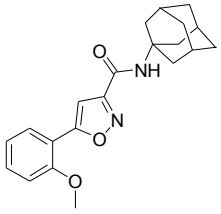
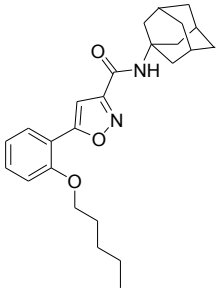
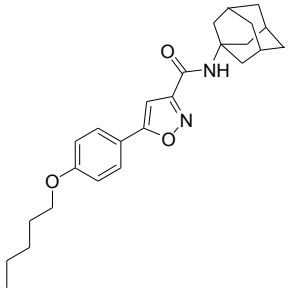
Recent investigations showed that anandamide, the main endogenous ligand of CB_1 and CB_2 cannabinoid receptors, possesses analgesic, antidepressant and anti-inflammatory effects. In the perspective to treat inflammatory bowel disease (IBD), our approach was to develop new selective CB_2 receptor agonists without psychotropic side effects associated to CB_1 receptors. In this purpose, a new series of 3-carboxamido-5-aryl-isoxazoles, never described previously as CB_2 receptor agonists, was designed, synthesized and evaluated for their biological activity. The pharmacological results have identified great selective CB_2 agonists with *in vivo* anti-inflammatory activity in a colitis mice model. Some of these new molecules appeared to have inhibitory effect on FAAH activity.



47
IC_{50} (FAAH) = 956 ± 44 nM
K_i (CB_2) = 70.1 ± 5.4 nM



51
IC_{50} (FAAH) > 1000 nM
K_i (CB_2) = 36.0 ± 3.4 nM



56
IC_{50} (FAAH) = 8.0 ± 0.5 nM
K_i (CB_2) > 1000 nM

Herein, we describe the first multitarget FAAH inhibitor / selective CB_2 agonist reported so far in the literature. This finding represents a promising starting point for the discovery of dual activity compounds targeting CB_2 receptors and FAAH.

O-1966, A CB_2-SELECTIVE CANNABINOID AGONIST, BLOCKS T-CELL ACTIVATION IN THE MIXED LYMPHOCYTE REACTION

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We have previously shown that a CB_2-selective agonist, O-1966, inhibits the Mixed Lymphocyte Reaction (MLR), an *in vitro* correlate of organ graft rejection, via CB_2 receptors. The cannabinoid directly affected T-cells and significantly decreased expression of mRNA for several markers of immune activation and for molecules involved in cell division. In addition, there was an increase in mRNA expression of molecules involved in immunosuppressive pathways, including IL-10. The increase in IL-10 was confirmed by measuring IL-10 protein. Furthermore, there was an increase in regulatory T-cells (T-regs) in MLR cell cultures. Current studies explored further the mechanisms for the suppressive properties of this class of cannabinoids. The importance of increased IL-10 levels in the inhibition of proliferation and increase in T-regs in O-1966 treated cells in the MLR was studied using an anti-IL-10 antibody in MLR cultures. Pretreatment with anti-IL-10 resulted in a partial reversal of inhibition of proliferation and blocked the increase of T-regs. The effect of O-1966 on T-cells was further examined by measuring the activation of several transcription factors in T-cells activated with anti-CD3 and anti-CD28. O-1966 treatment resulted in a dose-dependent decrease in the active nuclear forms of NF-κB and NFAT. Further, T-cells in the MLR treated with O-1966 also had decreased expression of CD4, a co-receptor with the T-cell receptor. Together the results show that this CB_2 agonist is immunosuppressive by at least 3 different mechanisms. These data support the potential of this class of compounds as useful therapies to prolong graft survival in transplant patients.

Acknowledgements: This work was supported by NIDA grants DA13429, DA06650, and T32-DA07237, and a Temple University Tobacco Settlement Grant.

DELTA-9-TETRAHYDROCANNABINOL SUPPRESSES MURINE RESPONSE TO SYSTEMIC *CANDIDA ALBICANS* INFECTION

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Candida albicans (*C. albicans*) is a major opportunistic fungal pathogen, especially in immune suppressed hosts. Delta-9-tetrahydrocannabinol (THC), the psychoactive compound of marijuana, has been shown to suppress the immune response to bacterial, viral and protozoan infections, but less is known of the effects of THC on fungal infections. We used an infection model in which we investigated the role of THC and the peripheral cannabinoid receptor (CB2R) on the immune response to a systemic *C. albicans* infection. C57BL/6 mice were given vehicle or THC (4, 8, 16, 32 or 64mg/kg) on days 1-4, 8-11 and 15-18 and infected with $7.5x10^4$ *C. albicans* cells/mouse on day 2 and with $5x10^5$ *C. albicans* cells/mouse on day 19. Mice were observed for survival. Tissue fungal load and serum cytokine production was determined for infected animals treated with vehicle or THC (8 or 16mg/kg). THC (16-64mg/kg) dose-dependently decreased the survival of mice systemically infected with *C. albicans.* THC (8 or 16mg/kg) suppressed interferon-gamma (IFN-γ) and interleukin (IL-12) serum levels and increased kidney, liver, spleen and brain yeast burden in wild type mice. THC (8 or 16mg/kg) also decreased IFN-γ and IL-12 serum levels and increased tissue fungal load in CB2R knockout mice. Interestingly, in vehicle treated mice, IFN-γ, but not IL-12, serum levels were decreased in CB2R knockout mice compared to wild type mice. We propose that chronic THC use, independently of CB2R, enhances the susceptibility to *C. albicans* infection by suppressing immune function. Furthermore, we suggest that CB2R plays a functional role in IFN-γ production in this experimental paradigm, an observation we are exploring further.

Acknowledgements: Funded by the National Institutes of Health (Grant No. 5SC3GM087220-04). Authors also acknowledge NIDA for supplying the THC.

THE ENDOCANNABINOID ANANDAMIDE MODULATES ADHESION, PROLIFERATION AND THE PRODUCTION OF INFLAMMATORY MEDIATORS IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS BY ACTIVATING CB_1, TRPV1 AND NON-CANNABINOID RECEPTOR TARGETS

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In rheumatoid arthritis (RA), synovial fibroblasts (SF) secrete large amounts of IL-6, IL-8 and matrix metalloproteinases (MMPs) which are crucial for cartilage destruction. RASFs are sensitive to the action of cannabinoids and they express cannabinoid receptors type I and II (CB_1 and CB_2), the vanilloid receptor (TRPV1) as well as endocannabinoid degrading enzymes. Cannabinoids are regarded as antiinflammatory and since anandamide (AEA) is found in RA synovial fluid we investigated how this endocannabinoid affects adhesion, proliferation, and production of inflammatory mediators of RASF.

Adhesion was assessed by the XCELL*igence* system. Proliferation was quantified by the amount of incorporated fluorescent dye into cellular DNA. MMP-3 and cytokines were detected by ELISA.

In OASF, AEA dose-dependently decreased the IL-1β induced production of MMP-3 (by 23%) in a TRPV1-mediated manner. IL-6 and IL-8 levels were only weakly modulated. In RASF however, AEA decreased IL-1β induced production of IL-6 (23 %), IL-8 (18 %) and MMP-3 (20 %). The effects of AEA were not inhibited by CB_1, CB_2 or GPR55 antagonists but were blocked by the TRPV1 antagonist capsazepine. The inhibitory capacity of AEA was enhanced by cyclooxygenase-2 inhibition in RASFs and OASFs, but was unaltered or even slightly reduced by FAAH inhibition. AEA was even more potent in reducing above mentioned mediators when RASFs but not OASFs were incubated under hypoxic conditions and treated with TNF. Furthermore AEA increased adhesion of OASFs and RASFs to fibronectin. Adhesion was modulated by CB_1, GPR55, and TRPV1 antagonists. Combined FAAH and cyclooxygenase-2 blocked the stimulatory effect of AEA on adhesion. Proliferation was decreased by AEA in RASFs and OASFs via a cyclooxygenase-2 but not via CB_1, CB_2 or TRPV1 dependent mechanism.

In conclusion, AEA promotes an antiinflammatory phenotype of RASFs and OASFs by activating TRPV1. CB_1, TRPV1, and GPR55 act in concert to modulate adhesion of SFs and this is highly dependent on the intracellular concentration of AEA. Additionally, cyclooxygenase-2 metabolites of AEA exert their anti-proliferative effects independent of CB_1 and CB_2.

PREDICTIVE PRECLINICAL ANIMAL MODEL IN OSTEOARTHRITIS AS CRITICAL FACTOR FOR IDENTIFICATION OF NOVEL PHARMACOTHERAPIES

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Osteoarthritis (OA) is one of the most prevalent sources of chronic pain, affecting around 10% of men and 20% of women aged 60+ worldwide. Current analgesics are relatively effective, but associated with various gastrointestinal, cardiac and renal adverse effects. Thus the use of valid predictive preclinical animal models is critical for identification of novel pharmacotherapies. The monoiodoacetate model of OA, in which a single injection of the irreversible NADPH inhibitor, sodium monoiodoacetate (MIA), is made into the joint space, provides a model of the painful and structural components of OA in rodents. However the available data suggests that there are different pathophysiological sequellae that follow different doses of MIA, which are a matter of the utility of MIA model for translational study of OA pain. Therefore in the present studies we have investigated the pathophysiological consequences of MIA used in a range of doses from 1 to 4.8 mg. We have compared the MIA-evoked mechanical pain-related hypersensitivity of the hindpaw (Pressure Application Measurements, PAM) at all doses. We have compared the DRG pain molecules: HCN2 gene, present in pain-sensitive nerve endings and ATF-3, a sensitive marker for peripheral neuron stress/injury. Our previous studies revealed that dual targeting of peripheral FAAH or CB1 and TRPV1 yields more successful results in OA treatment those obtained so far with selective FAAH inhibitors thus we have also studied the molecular *components* of the *endocannabinoid and endovanilloid system.* Modulators that regulate TRPV1 activity (phosphorylation and dephosphorylation processes) were also investigated.

Significant decrease in mechanical pain threshold in joint hypersensitivity was seen following 1, 3 and 4.8 mg MIA injection. There was no hypersensitivity on the contralateral hindpaw. The time course was biphasic, with asymmetry vaguely correcting at the day 7 time point but returning at day 14. The percentage of animals that did not develop OA pain phenotype was as follows: 20%, 10% and 0% in 1 mg-, 3mg- and 4.8 mg-MIA treated animals, receptively. Dose-depended increase in spinal microgliosis (Iba-1 immunofluorescent staining) was observed in a time-course following intra-articular MIA treatment. OA was accompanied by a gradual increase of the mRNA levels of HCN2 and ATF-3 matching the MIA dose used. CB1 and TRPV1 mRNA expression and protein levels significantly increased while CB2 expression levels decreased following the differential MIA dosage used. Protein kinase A (PKA), protein kinase C (PKC), Ca2+/calmodulin- dependent kinase II (CaMK II), and cyclin-dependent kinase 5 (Cdk5) that phosphorylate and sensitize TRPV1 were upregulated. Finally the neuronal phosphatase calcineurin, a major player in TRPV1 dephospholylation was not significantly affected by MIA-treatment.
We demonstrated significant increase in expression of HCN2 and ATF-3 after intraarticular injection of increasing MIA doses, reflecting mild through moderate to advanced OA. Changes in DRG chronic pain markers were associated with spinal microgliosis and greater hindpaw mechanical hypersensitivity related to the increasing MIA doses. Our data suggest that compounds targeting both FAAH and TRPV1 downstream signaling targets may enable development of improved treatments for patients with severe OA pain.

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INHIBITING DIACYLGLYCEROL LIPASE (DAGL) ATTENUATES EXPERIMENTAL COLITIS IN THE MOUSE

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Endocannabinoid (EC) tone is generally believed to be enhanced during gastrointestinal inflammation with EC's having a protective role through the activation of cannabinoid receptors. Studies on the role of ECs in colitis are primarily based on inhibiting the degradation of anandamide (AEA) with fatty acid amide hydrolase (FAAH) blockers or 2-arachidonoylglycerol (2-AG) with blockers of monoacyl glycerol lipase (MAGL). AEA, but not 2-AG, levels are elevated during colitis suggesting potentially different roles for these EC ligands. While systemically administered AEA attenuates trinitrobenzene sulfonic acid (TNBS) colitis in mice, to our knowledge, no study has addressed the direct role of 2-AG in colitis. We hypothesize that 2-AG may augment inflammation through its proinflammatory metabolites (e.g. arachidonic acid and prostaglandins). Consequently, another therapeutic approach for the treatment of intestinal inflammation could be through the inhibition of the production of 2-AG and its pro-inflammatory metabolites. Therefore, we designed the current study to assess whether (a) inhibiting DAGL has an anti-inflammatory role in colitis, and (b) if so whether this effect is through an alteration in the levels of intestinal ECs.

Male CD1 mice with experimentally-induced colitis (TNBS model) were treated with the DAGL inhibitor Orlistat (1 and 5mg/kg, i.p., b.i.d.) or vehicle. The mice were sacrificed on day 4 or 7 post inflammation. Colitis was scored macroscopically and tissue levels of myeloperoxidase (MPO), ECs and their substrates and metabolites were measured. Tumor necrosis factor (TNF) and chemokine (C-X-C motif) ligand 1 (CXCL1) mRNA expressions (RQ values) were assessed with real-time PCR.

Orlistat treatment attenuated TNBS-induced colitis. 7 days post colitis the macroscopic score was reduced from 11.6±0.8 in vehicle treated animals to 8.2±0.4 and 7.7±0.5 after treatment with 1 and 5mg/kg Orlistat, respectively ($p<0.01$). MPO levels tended to decrease in Orlistat treated mice. Colonic CXCL1 mRNA levels which were elevated in colitis (5 fold) were decreased by Orlistat treatment (Veh: 1.5±0.3 vs. 1mg/kg: 0.3±0.1 and 5mg/kg: 0.4±0.2; $p<0.05$). There was a tendency toward increases in prostaglandin D2 (PGD2) and decreases in prostaglandin F2α (PGF2α) levels in the colon of Orlistat treated animals. Orlistat (1 and 5 mg/kg) significantly decreased ratios of colonic 2-AG to 1-stearoyl, 2-arachidonyl glycerol (SAG) from 0.90±0.15 to 0.38±0.06 and 0.50±0.08, respectively ($p<0.05$), suggesting it effectively inhibited DAGL and altered the balance of ECs in the gut wall.

Inhibiting DAGL attenuates colitis in the mouse, possibly by decreasing endogenous 2-AG biosynthesis and its proinflammatory metabolites. The mechanism may be through inhibition of chemotaxis by Orlistat, but further investigations are necessary.

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PEA REDUCES COLON AND SYSTEMIC INFLAMMATION IN MICE MODELS OF CROHN'S DISEASE

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N-acylethanolamines (NAEs) play numerous roles in the gastrointestinal (GI) tract, regulating food intake and energy balance (e.g. *N*-oleoylethanolamine, *N*-arachidonoylethanolamine) as well as pain and inflammation (*N*-arachidonoylethanolamine). The activity of these bioactive lipids is controlled through their hydrolysis by two main enzymes, Fatty Acid Amide Hydrolase (FAAH) and *N*-acylethanolamine hydrolyzing acid amidase (NAAA).

N-palmitoylethanolamine (PEA) is known to exert anti-inflammatory, analgesic and neuroprotective actions, and has extensively been studied in neurological diseases or inflammatory neuropathic pain, but its effects on the GI tract are less well documented. Here we thought to assess the anti-inflammatory potential of PEA in two murine models of inflammatory bowel diseases (IBD), chronic inflammatory disorders of the GI tract with an undeniable need for new therapeutic approaches.

In the TNBS-induced colitis murine model, mice received either PEA, the FAAH inhibitor PF-3845 or a combination of both. Administration of PEA (10mg/kg, i.p.) reduced the macroscopic alterations observed in colitis, as well as the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-12) and other markers of inflammation and cellular infiltration (myeloperoxidase activity, COX-2, F4-80). Interestingly, the FAAH inhibitor (PF-3845, 10mg/kg, i.p.) reduced some, but not all, of the parameters affected by PEA. This could be explained by the fact that FAAH inhibition led to a general increase in NAEs levels in the colon (AEA, OEA, SEA), but did not increase PEA levels, as measured by HPLC-MS. The increased AEA levels could be responsible for the effects of PF-3845 since AEA has been shown to possess anti-inflammatory actions in the colon. PEA and PF-3845 administration both decreased the colitis-associated inflammation observed in the liver and the brain. Moreover, FAAH inhibition also led to an increase in NAEs levels in the liver, including PEA levels. This would point to a differential regulation of PEA levels in the colon and the liver, and seems to implicate NAAA rather than FAAH in the control of intestinal PEA levels.

In order to further confirm its therapeutic potential, PEA was also tested in the DSS-induced colitis mouse model, and showed similar anti-inflammatory effects, even when administered after the colitis was established.

In conclusion, we show that PEA administration results in a reduction of colitis as well as the related systemic and central inflammation. This could offer a novel pharmacological approach for the treatment of IBD. Moreover, this shows the need for potent and systemically active NAAA inhibitors that could be used *in vivo* in order to assess the contributions of both NAAA and FAAH on NAEs levels, which seem to be tissue specific.

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RELATIONSHIP BETWEEN GROWTH FACTOR RECEPTORS, PHOSPHO-AKT AND CB_1 RECEPTORS IN PROSTATE CANCER

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Introduction*:* The expression of tumour CB_1 receptors and phosphorylated epidermal growth factor receptors (pEGFR) in prostate cancer tumour tissue are positively correlated and are additive prognostic factors for disease outcome (Fowler *et al., PLoS One* 5 [2010] e15205). It is not known, however, whether other growth factor receptors associate with the endocannabinoid system in prostate cancer. Here we have determined whether the expression of the related growth factor ErbB2 (Her2) is also associated with CB_1 receptor expression.

Method: Formalin-fixed, paraffin-embedded specimens of tumour tissue (1-8 cores/patient) obtained at diagnosis from patients who underwent transurethral resection surgery for prostatic enlargement were stained using an ErbB2 monoclonal antibody cocktail (c-erbB-2, Biocare Medical, San Francisco, USA). Tumour epithelial immunoreactive (IR) intensity and distribution were scored in the range 1-4. In cell culture experiments, PC3 prostate cancer cells were incubated with EGF for three weeks, with endocannabinoid modulators being added for the last week.

Results*:* Tumour tissue from 357 cases was scored for ErbB2-IR. The ErbB2-IR score was significantly correlated with CB_1IR and with the downstream signalling molecule pAkt, and these correlations remained when controlled for the mutual correlation with the cell proliferation marker Ki67. ErbB2 did not correlate with EGFR or, in the first-order correlations, with pEGFR. A model with pEGFR, ErbB2 and CB_1 receptors as modulators of pAkt expression better fitted the data than a model with pEGFR, ErbB2 and pAkt as modulators of CB_1 receptor expression, as assessed by repeated (1000) non-parametric multiple regressions of randomly chosen portions (80%) of the dataset. In cases followed by expectancy, ErbB2 had prognostic value in that a high expression was associated with a poor disease-specific survival (Exp(B) value 2.02, p=0.006). The prognostic value was additive to that provided by CB_1IR or by pEGFR-IR but not both biomarkers, or with pAkt-IR. The association between EGFR and the endocannabinoid system was also investigated in PC3 cells. For untreated PC3 cells, incubation with the CB receptor agonist CP55,940 (100 nM) did not significantly affect cell proliferation whereas the MGL inhibitor JZL184 (1 μM) increased the number of cells. EGF treatment produced a mitogenic response, and the two endocannabinoid modulatory agents both reduced EGF-induced cell proliferation.

Conclusion*:* Taken together, the data are consistent with the hypothesis that in prostate cancer both EGFR and ErbB2 systems associate with the endocannabinoid system and that the survival factor pAkt is a downstream effector.

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TARGETING ENDOCANNABINOID BREAKDOWN ENZYMES AND CB2 CANNABINOID RECEPTOR FOR REGULATION OF PROSTATE TUMOR GROWTH

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Prostate cancer is a major health problem and a significant cause of mortality in men worldwide. Family history and race are the two major risk factors for this disease. The growth of prostate cancer is androgen-sensitive, and therefore hormonal therapy is very common for this metastatic disease. However, while most prostate cancer patients initially respond very well to hormonal therapy, the majority develops substantial resistance over time. The mechanism responsible for this transition to lack of hormone responsiveness is usually explained by clonal selection and androgen receptor (AR) downregulation/desensitization. Thus, an understanding of the molecular mechanism responsible for the development and progression of prostate cancer is extremely important to the development of more effective therapeutic strategies. Previously we showed that CB2 cannabinoid receptor (CB2R) expression is higher in androgen-sensitive (LNCaP and LAPC4) and androgen-insensitive (DU145 and PC3) human prostate cancer cells compared to non-malignant prostate epithelial cells (PrEC) and activation of CB2R [JWH-015(1-10 μM)] significantly inhibited prostate cancer cell proliferation. In the current study we investigate the role of endocannabinoid system regulating enzymes in the regulation of prostate cancer growth.

In the current study we first showed that FAAH-inhibitor URB597 (10 μM) treatment significantly inhibited cell proliferation, migration and prostate-specific antigen (PSA) expression in androgen-sensitive (LNCaP and LAPC4) and androgen-insensitive (DU145 and PC3) human prostate cancer cells compared to non-malignant prostate epithelial cells (PrEC) cells in CB2R antagonist SR144528-senstive manner. Using short-hairpin RNA (shRNA) against two endocannabinoid inactivation enzyme, FAAH and MAGL in human prostate cancer cells (LNCaP) we developed the stable cell lines (shFAAH-LNCaP and shMAGL-LNCaP). We found that basal cell proliferation rate and cell migration in the shFAAH-LNCaP or shMAGL-LNCaP cells were significantly decreased compared with the shControl-LNCaP cells. We further showed that in orthotopic xenograft model for prostate tumor growth, CB2R agonist JWH 015 (3 mg/kg/day, ip) or FAAH inhibitor URB 597 (3 mg/kg/day, ip) treatment for 4 weeks significantly reduced androgen-sensitive (LAPC4) and androgen-insensitive (DU145) human prostate tumor growth in SCID mice. We also found that CB2R activation significantly reduced cyclinD1, cyclin D2 and c-Myc expression in prostate cancer cells and JWH-015 treated tumor tissue samples.

Collectively, these results suggest that i) a decrease in that endocannabinoid signaling triggers the proliferation of prostate tumor and ii) an exogenous or endogenous activation of endocannabinoid signaling via CB2R inhibits or reverse the cancerous growth. This also suggests that an activator of endocannabinoids and/or CB2R has the therapeutic potential for prostate tumor.

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PROGNOSTIC VALUE OF THE CB_2 CANNABINOID RECEPTOR IN HUMAN COLORECTAL CANCER

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Colorectal cancer (CRC) is a major cause of morbidity and mortality in Western countries and a leading cause of death for cancer. A large amount of studies has demonstrated that during cancer progression the endocannabinoid system suffers alterations in different types of tumor. Particularly, in colon cancer is described down-regulation of CB_1 receptor, up-regulation of CB_2 receptor or increasing amounts of endocannabinoids levels. However, little is known about the clinical relevance of these alterations in patients with CRC. Moreover, there is controversy whether this system prevents tumorigenesis or, if conversely, promotes tumor progression. In the present study we have analyzed the expression of the CB_2 receptor in a large series of CRC and its correlation with clinico-pathological data.

The immunohistochemical analysis performed confirmed that CB_2 is expressed at high levels in tumor epithelial cells but it is not detected, or at very low levels, in normal epithelial cells. Interestingly, the samples with high CB_2 expression were the ones with more Ki67 staining, indicating that those tumors with higher amounts of CB_2 have greater proliferation. On the other hand, we analyzed CB_2 expression in a series of 175 patients with CRC and it was detected in a 28.6% of the cases by rt-qPCR. This expression correlated with lymph node metastasis (LNM) ($p=0.016$), an established poor prognostic marker, as well as with worse disease free survival (DFS) ($p=0.014$) and overall survival (OS) ($p<0.001$). *In vitro* experiments with colon cancer derived cell line HT29 treated with CB_2 agonist JWH-133 showed a possible activation of the epithelial-mesenchymal transition program because a concentration-dependent increase of SNAIL1, one of the critical transcription factors in the EMT process, was observed. Moreover, we observed a positive correlation between CB_2 and SNAIL1 expression in 128 samples analyzed ($p=0.007$), corroborating *in vitro* results and suggesting that CB_2 is an active molecule in the tumor samples. Collectively, these results shown that CB2 receptor expression is a poor prognostic marker in colon cancer patients, and it suggests that its activation could be collaborating with tumor progression. From a clinical point of view, these results suggest that inactivation instead of activation of this receptor would be appropriate for the treatment of this type of tumor.

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CO-TARGETING OF TWO DISTINCT CANNABINOID ANTITUMOR PATHWAYS RESULTS IN ROBUST INHIBITION OF ADVANCED STAGES OF METASTASIS

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A majority of breast cancer-related deaths are the result of tumor metastasis. The cannabinoids cannabidiol (CBD) and D^9-tetrahydrocannabinol (THC) are effective at inhibiting breast cancer progression, however, they each target unique pathways. In mouse models of metastasis, a detailed pharmacological assessment of CBD revealed the ability of the cannabinoid to significantly extend survival. In addition, we discovered that CBD could reduce the number and size of metastatic foci in advanced stages of breast cancer metastasis resulting in moderate increases in survival suggesting the need to develop more active analogs based on CBD. We therefore took an approach where we screened for analogs that were more effective at inhibiting breast cancer proliferation, invasion, expression of the pro-metastatic gene Id1, and in addition were active at targeting CB_2 receptors; activation of latter was more specific to THC, while inhibition of Id1 was more specific to CBD. We reasoned that dual targeting of each unique antitumor pathway with a single compound would result in a more robust inhibition of more advanced stages of metastasis. We found that this approach is a viable strategy and will present mechanistic data unique to our lead compound. Co-targeting of two distinct cannabinoid antitumor pathways may therefore represent a promising approach for the treatment of breast cancer patients with advanced stages of breast cancer metastasis.

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INHIBITION OF ENDOCANNABINOID REUPTAKE IS AN INNOVATIVE STRATEGY TO IMPEDE TUMOR GROWTH

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Endocannabinoids are endogenous compounds known to mediate psychotropic effects by stimulating the cannabinoid receptor type-1 (CB-1). Endocannabinoid signalling has been shown to be enhanced in several cancer tissues and malignant cells. Studies in transformed cell lines propose that this up-regulation contributes to the inhibition of their proliferation. Here, we investigated the effect of the endocannabinoid reuptake inhibitor, OMDM-2, on the growth of Ehrlich solid tumor induced in mice. Three different sets of experiment were carried out: (I) Mice (n= 70) treated either with OMDM-2 (5 mg/kg, i.p.) (A), R-Methanandamide (R-Met) (0.5 mg/kg, i.p.), a direct cannaboid agonist (B), NIDA 41020 (0.7 mg/kg, i.p.), CB-1 receptor blocker (C), R-Met + NIDA 41020 (D), OMDM-2 + NIDA 41020 (E), or Carboplatin (5 mg/kg, i.p.) (F) were evaluated for tumor volume, mean survival time, increase in the life span and hematological parameters. (II) All groups (n= 70) were assessed for the angiostatic activity using Evans blue method (% of control). (III) Time course measurements of tumor weight, serum transforming growth factor-β_1 (TGF-β_1), and the intra-tumoral receptor (CD-105) on days 7, 14, and 21 post-inoculation (n= 147, 21/ group).

OMDM-2 and R-Met, both significantly impeded tumor weight and volume ($p<0.05$) at all time points. Further, OMDM-2 significantly reduced angiogenesis as efficaciously as R-Met. On day 7 significant increments in the serum concentrations of TGF-β_1 and significant down regulation of the expression of CD-105 receptor were observed. Furthermore, OMDM-2 significantly increased the survival time and also increased the life span of treated animals by 59.5% compared to the control group. After 14 days of inoculation, OMDM-2 was able to reverse changes observed in the hematological parameters after tumor inoculation. The combination of OMDM-2 or R-Meth with NIDA 41020 counteracted all previous effects in the R-Met-treated group but not in the OMDM-2 group.

In conclusion, we provide for the first time data on the possible *in vivo* use of OMDM-2 as an anti-cancer drug. Endocannabinoid reuptake inhibitors may have the potential to be developed as alternative to direct cannabinoid receptor agonists to avoid CB1-mediated psychotropic side effects.

Keywords: Anti-cancer, Anti-angiogenesis, OMDM-2, R-Methanandamide, NIDA41020, TGF-β_1, CD-105.

CANNABIDIOLIC ACID AND TETRAHYDROCANNABINOLIC ACID REDUCE CONDITIONED GAPING (NAUSEA-INDUCED BEHAVIOUR) IN RATS AND VOMITING IN *SUNCUS MURINUS*

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The phytocannabinoids Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) effectively reduce nausea and vomiting, however little is known about their acidic precursors. Previous research from our group indicates that cannabidiolic acid (CBDA), CBD's acidic precursor, potently suppresses toxin-induced vomiting in *Suncus murinus* and conditioned gaping reactions to a lithium chloride (LiCl)-paired flavour (a measure of nausea-induced behavior in rats) and to a LiCl-paired context (a rodent model of anticipatory nausea); each effect mediated by the 5-hydroxytryptamine 1A ($5\text{-}HT_{1A}$) receptor (Bolognini et al., Br J Pharmacol. (2013) 168:1456-70). In the present set of studies, we assessed the minimally effective dose of CBDA to reduce LiCl-induced conditioned gaping reactions in rats, and whether a subthreshold dose of CBDA could enhance the anti-nausea effects of low doses of ondansetron (OND, a classic $5\text{-}HT_3$ receptor antagonist). In addition, we also examined the anti-emetic and anti-nausea properties of tetrahydrocannabinolic acid (THCA), the acidic precursor of THC, in our models and determined its mechanism of action.

CBDA (at doses as low as 0.5 μg kg^{-1}) suppressed nausea-induced conditioned gaping reactions. A low dose of OND (1.0 μg kg^{-1}) alone reduced nausea-induced conditioned gaping, but when combined with a subthreshold dose of CBDA (0.1 μg kg^{-1}), enhanced the suppression of conditioned gaping. CBDA potently reduced conditioned gaping in rats, as well as enhancing the anti-nausea effect of a low dose of OND. These findings suggest that combination low doses of CBDA and OND could more effectively manage acute nausea in chemotherapy patients.

THCA (0.05 and 0.5 mg kg^{-1}) suppressed conditioned gaping to a flavour and THCA (0.05 mg kg^{-1}) reduced conditioned gaping to a context; the latter effect was blocked by the CB_1 receptor antagonist, SR141617. In *S. murinus*, THCA (0.05 and 0.5 mg kg^{-1}) reduced LiCl-induced vomiting; an effect that was also reversed with SR141716. THCA potently reduced conditioned gaping in rats and vomiting in *S. murinus*; effects that seem to be CB_1 receptor mediated. These data suggest that THCA may be a more potent non-psychoactive alternative to THC in managing nausea and vomiting.

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ANTINOCICEPTIVE EFFECTS OF FATTY ACID BINDING PROTEIN INHIBITORS

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The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) serve as ligands for cannabinoid receptors and regulate a plethora of physiological processes including appetite, pain, inflammation, and reproduction. Inhibition of endocannabinoid inactivating enzymes potentiates endocannabinoid signaling and produces antinociceptive and anti-inflammatory effects. Recently, our group identified fatty acid binding proteins (FABPs) as intracellular carriers that deliver N-acylethanolamines, including AEA, to fatty acid amide hydrolase (FAAH) for hydrolysis. Inhibition of FABPs reduced AEA inactivation *in vitro* and produced endocannabinoid-mediated antinociceptive effects *in vivo*.

Here, we examined the antinociceptive effects of three FABP inhibitors, SBFI26 (α-truxillic acid mono-1-napthyl ester), SBFI50 (α-truxillic acid mono-2-napthyl ester), and SBFI52 (α-truxillic acid mono-1-napthyl amide). The binding of the three compounds to neurally expressed FABPs was examined. Of the three compounds, SBFI26 bound to FABPs with highest affinity while SBFI52 was least potent. The antinociceptive properties of the compounds were subsequently examined. In the carrageenan model of inflammatory pain, SBFI26 and SBFI50 reduced paw edema and thermal hyperalgesia while SBFI52 was without effect. In the acetic acid model of visceral pain, SBFI26 reduced acetic acid induced writhing while SBFI50 and SBFI52 were without effect. These antinociceptive effects of SBFI26 were blocked by pretreatment with SR141716, a cannabinoid receptor 1 antagonist. Importantly, SBFI26 did not activate cannabinoid receptor 1 nor inhibit AEA or 2-AG hydrolysis, indicating that its analgesic effects stem from potentiation of endocannabinoid signaling. SBFI26 also reduced thermal hyperalgesia in the chronic constriction injury model of neuropathic pain in rats but did not affect mechanical allodynia. Taken together, our results demonstrate that pharmacological inhibition of FABPs produces analgesia in multiple models of pain and suggest that FABPs may represent targets for the development of novel antinociceptive drugs.

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ADDITIVE EFFECTS OF THE COMBINATION OF FAAH AND MGL INHIBITORS WITH CONVENTIONAL TREATMENT IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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Cisplatin, a platinum-derived chemotherapeutic agent, produces mechanical and cold allodynia in rodents that is reminiscent of chemotherapy-induced neuropathy observed in humans. Enzymes such as fatty-acid amide hydrolase (e.g. FAAH) and monoacylglycerol lipase (MGL) break down the body's own endocannabinoid ligands (e.g. anandamide) and 2-arachidonoyl glycerol. They represent targets for analgesic drug development. We compared antinociceptive effects of FAAH (URB597, URB937) and MGL (JZL184) inhibitors on mechanical and cold allodynia induced by cisplatin treatment. Anti-allodynic efficacy of each endocannabinoid modulator was compared with agents used clinically to treat neuropathy (i.e. the tricyclic antidepressant amitriptyline). Groups received intraperitoneal (i.p.) injections of either amitriptyline, URB597, URB937, JZL184 or vehicle. FAAH (URB597, URB937) and MGL (JZL184) inhibitors administered alone at lower dose partially reversed mechanical and cold allodynia. However, the combination of FAAH or MGL inhibitors with amitriptyline, reversed cisplatin-evoked mechanical and cold allodynia to pre-cisplatin levels. These observations suggest the presence of antinociceptive synergism between mechanistically distinct treatments for neuropathic pain. Our results suggest that both FAAH and MGL inhibitors attenuate mechanical and cold allodynia in a model of chemotherapy-induced peripheral neuropathy. Moreover, the combination of amitriptyline with FAAH or MGL inhibitors represents a useful therapeutic option to conventional treatment to alleviate neuropathic pain. Strikingly, both FAAH and MGL inhibitors combined with amitriptyline were both efficacious in suppressing cisplatin-evoked mechanical and cold allodynia. Our studies suggest that the endocannabinoid system combined with conventional treatment represents a promising target for suppressing chemotherapy-induced neuropathic pain.

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ATTENUATION OF LPS-INDUCED PAIN-LIKE RESPONSE BY CANNABINOID RECEPTORS LIGANDS IN ACTIVATED MICROGLIA CELLS

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Introduction: Neuropathic pain represents a large unmet clinical need and despite many potential pharmacological therapies, few have demonstrated more than moderate efficacy. The most important targets may not be present before injury. Attention now is focused on the unique long-term consequences of nerve injury involving glial cell activation (Mika et al. 2009), indeed microglial cells contribute to the development of neuropathic pain (Tsuda et al. 2013). There is considerable evidence supporting a role for endocannabinoids (ECs) in the modulation of pain. Anandamide (AEA), the best studies endocannabinoid activates primarily cannabinoid receptors: CB1 and CB2. Cells lacking cannabinoid receptors are more vulnerable to damage (Marsicano et al. 2003), suggesting the involvement of cannabinoid system in neuronal protection, through microglial cells.
Aim: The aim of our study was to determine the effect of the cannabinoid receptor inhibition on cell inflammation during microglia activation present during injury-induced changes. We examined the influence of AEA and cannabinoid receptors antagonists on activated primary cultured rat microglia by using NO assay, as well as mRNA.
Methods: Primary cultures of microglial cells were prepared from 2-days-old Wistar rat pups. Adherent cells were incubated for 48 h in culture medium before being used for the treatments. Phenotype was confirmed with C1q gene expression and astrocyte contamination was excluded by measuring amount of GFAP transcript. Cells (both resting and activated) were treated with AEA and AEA in the presence of CB1 antagonist - AM-251. Results were assessed to control (no LPS) treated with vehicle. The Griess reagent was used to determine level of NO released from microglial cells. RNA was isolated from cells placed onto 24-wells plates to perform gene expression assay. Isolated RNA has been used for reverse transcription reaction. Quantitative PCR were performed using Assay-On-Demand Taqman probes. Genes expression levels were assessed against housekeeping gene.
Results & Conclusions: LPS activates NO production in microglia cells through Toll-like 4 receptors. These effects were slightly attenuated after pre-treatment with AEA. LPS-activation of cells was significantly lower after administration of AEA in the presence of AM-251, rather than antagonist alone as shown in NO assay. This result correlates with inhibition of iNOS expression in microglial samples. Although AEA exhibits lower affinity for CB2 than for CB1 (and there was no expression of gene coding TRPV1 receptor in tested cell cultures), we postulate that the cytoprotective effect of AEA is mediated by CB2 (rather than CB1) receptor. We demonstrated that when CB1 was blocked the observed effect of NO production was even more inhibited. Still other AEA molecular targets should be considered (and will be studied in future experiments). A similar pattern of changes in chemokines level was observed in spinal cord samples from animals with developed neuropathy in CCI (chronic construction injury) model.
Our results support the idea that targeting microglial activation represents clinically promising method for enhancing protective effects of cannabinoid receptor ligands in neuropathic pain cause by nerve damage.

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MODULATION OF MORPHINE ANTINOCICEPTIVE EFFECTS BY A SELECTIVE CB2 RECEPTOR AGONIST IN MICE

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We and others have previously demonstrated that CB1 receptor activation interacts with morphine antinociception. It is unclear however whether modulation of the CB2 receptor affects morphine antinociception or the development of morphine antinociceptive tolerance. This is important to determine as CB2 agonists have also shown promise preclinically as neuroprotective agents and could potentially be given alongside opioid treatment following CNS injury.

In the first set of experiments, increasing doses of morphine and a selective CB2 agonist O-1966 were administered alone and in combination and their ability to alter thermal sensitivity on the hot plate was compared. Combination dosing was administered as either 1) morphine + O- 1966 given simultaneously, 2) O-1966 given 15 min prior to morphine, 3) morphine given 15 min prior to O-1966, or 4) morphine + O-1966 + SR144528 simultaneously. Latency to attend to the hind paw on the hotplate was then assessed 30 min post last injection. In the second set of experiments, the effect of chronic morphine and O-1966 administration alone and in combination on the development of morphine antinociceptive tolerance was assessed. During the morphine tolerance dosing regimen, mice were treated with a twice daily dosing regimen for 5 days of one of the following treatments: 1) saline, 2) cremophor vehicle, 3) O-1966, 4) O-1966 followed 15 min by morphine, or 5) morphine followed 15 min by O-1966.

Morphine (0.3 – 32 mg/kg IP) produced dose dependent antinociception, while O-1966 was without effect. Simultaneously injected morphine + O-1966 produced a 4-fold rightward shift in the morphine dose response curve (a decrease in morphine's antinociceptive effect) and was blocked by co-administration of the selective CB2 antagonist SR144528. Surprisingly, this shift was not observed when morphine was administered 15 min prior to O-1966 administration. Chronic administration of morphine produced antinociceptive tolerance, and co-administration of O-1966 during the morphine tolerance dosing regimen could potentiate the development of tolerance. Again the nature of this interaction was dependent on the order of drug administration.

These data suggest that activation of CB2 receptors has the potential to modulate morphine signaling at the mu-opioid receptor, and that the functional consequences of this interaction on antinociception and tolerance are dependent upon whether morphine or the CB2 agonist is administered first. Possible mechanisms underlying these effects are currently being explored, including whether O-1966 alters morphine binding or and functional activation of the mu-opioid receptor or whether O-1966 alters the proinflammatory effects of morphine administration.

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FUNCTIONAL CANNABINOID CB_2 RECEPTORS ARE EXPRESSED IN MIDBRAIN DOPAMINE NEURONS IN MICE

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The presence of neuronal cannabinoid CB_2 receptors (CB_2Rs) in the brain is highly controversial. It is generally believed that brain CB_2Rs are expressed and up-regulated in activated microglia during neuroinflammation, but not present in neurons. Here, we report that functional CB_2Rs are highly expressed in dopamine (DA) neurons in the midbrain ventral tegmental area (VTA) in normal mice. Using quantitative RT-PCR with three different Taqman probes, we detected two mouse CB_2 mRNA isoforms in the brain with CB_{2A} being ~20-fold higher than CB_{2B}. To determine mouse CB_2 specificity of the detected mRNA signal, we used a commonly used CB_2-knockout mouse strain as controls, in which the gene sequence that encodes parts of the intracellular and extracellular 3rd loops, trans-membrane regions 6 and 7, and the intracellular C-terminus region is deleted (Buckley et al., 2000). When using a specific mouse CB_2-ko probe that recognizes the deleted sequence, we detected CB_2 mRNA only in wild-type (WT) and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice. Then, we used *in situ* hybridization to detect CB_2 mRNA in VTA DA neurons with a mouse CB_2-specific riboprobe that targets the same gene sequence as the above-mentioned CB_2-ko Taqman probe. We detected CB_2 mRNA in VTA DA neurons in WT and $CB_1^{-/-}$, but not $CB_2^{-/-}$ mice. Next, we used double-label fluorescent immunohistochemistry assays and found that CB_2R-immunostaining was co-localized in TH-positive VTA DA neurons with density ~70% lower in $CB_2^{-/-}$ mice than in WT mice.

Given concerns regarding antibody specificity, we further used electrophysiological methods to study VTA DA neuron activities at three levels – single dissociated VTA DA neurons, midbrain VTA slices, and intact anesthetized mice. We found that systemic or local (bath) administration of JWH133, a selective CB_2R agonist, significantly inhibited VTA DA neuronal firing in WT mice, but not in $CB_2^{-/-}$ mice. This inhibitory effect was reversed by co-administration of AM630, a selective CB_2R antagonist, suggesting a CB_2R-mediated effect. Finally, we observed the effects of microinjections of JWH133 into the VTA on DA-regulated behavior. We found that intra-VTA microinjections of JWH133 significantly inhibited cocaine self-administration in WT mice, but not in $CB_2^{-/-}$ mice. Taken as a whole, all these findings suggest that brain CB_2Rs are expressed in VTA DA neurons where they modulate DA neuronal activity and, consequently, DA-regulated behavior.

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CONTROL OF NICOTINIC CHOLINERGIC FUNCTION BY PPAR-α IN DOPAMINE NEURONS

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Nicotinic acetylcholine receptors on dopamine neurons, particularly those containing the β2 subunits (β2*nAChRs), contribute to firing properties of VTA DA cells and mediate the addicting and motor-activating effects of nicotine. These receptors are targets for peroxisome proliferator-activated receptors-α (PPARα). PPARα, nuclear receptors activated by endogenous lipids of the endocannabinoid family, namely oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), affect discharge rate of DA cells and their response to nicotine by modulating β2*nAChRs.

How PPARα endogenous ligands are synthesized by dopamine cells is currently unknown. Using *ex vivo* and *in vivo* electrophysiological techniques combined with biochemical and behavioral analysis, we show that activation of α7nAChRs on dopamine neurons increases levels of PPARα endogenous ligands in a Ca^{2+}-dependent manner. Accordingly, *in vivo* production of OEA and PEA, triggered by α7nAChR activation, blocks nicotine-induced excitation of dopamine neurons and hyperlocomotion and displays antidepressant-like properties. Furthermore, tyrosine phosphorylation of the β2 subunits of nAChR was increased following *in vivo* PPARα activation. These data demonstrate that PPARα ligands are effectors of α7nAChRs and that their neuromodulatory properties depend upon phosphorylation of β2*nAChRs. This pathway is part of a homeostatic short loop feedback mechanism driven by nAChRs through of PPARα ligand synthesis and autocrine activation of PPARα.

Our results unveil important physiological functions of nAChR/PPARα signaling pathway in dopamine neurons. Overall, the present study suggests new therapeutic targets for disorders associated with unbalanced dopamine-acetylcholine systems.

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MULTIPLE MECHANISTICALLY DISTINCT MODES OF ENDOCANNABINOID MOBILIZATION AT CENTRAL AMYGDALA GLUTAMATERGIC SYNAPSES

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The central amygdala (CeA) is a nodal structure at the limbic-motor interface regulating aversive emotional learning. Furthermore, the endocannabinoid (eCB) system is heavily implicated in the regulation of emotional learning processes, which alludes to its potential role in CeA functionality. However, very little is known about the role eCB signaling plays in the modulation of CeA synaptic efficacy. In these studies we: 1) describe the subcellular localization of CB1 receptors and eCB synthetic machinery at glutamatergic synapses in the lateral CeA (CeAL) and 2) determine the role of eCB signaling at these excitatory synapses. To carry out this study, *ex vivo* electrophysiological recordings were performed in the CeAL of male, ICR mice in the presence of the $GABA_A$ receptor antagonist, picrotoxin. Local stimulation was used to evoke excitatory post-synaptic currents (eEPSCs)

CB1 expression was observed in presynaptic elements forming asymmetric synapses in the CeAL, while DAGLα was expressed within postsynaptic dendritic spines, with strong staining observed at the post-synaptic density. Functionally, activation of CB1 receptors decreased eEPSC amplitudes via a presynaptic mechanism. Moreover, CeAL glutamatergic synapses exhibit multiple, mechanistically- distinct modes of eCB-mediated synaptic plasticity. These excitatory synapses expressed depolarization-induced suppression of excitatory transmission, which was enhanced by muscarinic receptor activation. Additionally, relatively brief and continuous muscarinic receptor activation induced 2-AG release via calcium and DAGL-independent and dependent mechanisms, respectively. Conversely, effects of CB1 receptor activation on GABAergic transmission in the CeAL were more variable, with only a subset of neurons showing synaptic depression. These data support a prominent role for eCBs in the regulation of CeA synaptic physiology and provide insight into the mechanisms by which eCB signaling could modulate emotional learning processes.

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SUBSTRATE-SELECTIVE INHIBITION OF COX-2 ENHANCES ENDOCANNABINOID SIGNALLING *IN VIVO*

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Augmentation of endogenous cannabinoid (eCB) signaling represents an emerging approach to the treatment of affective disorders. Development of eCB degradation inhibitors has significantly advanced the therapeutic potential of eCB signaling for a variety of pathological conditions including mood and anxiety disorders. Cyclooxygenase-2 (COX-2) oxygenates arachidonic acid (AA) to form prostaglandins, but also inactivates eCBs *in vitro*. Inhibition of COX-2 elevates eCB levels *in vitro* but the potential of COX-2 as a target for *in vivo* eCB augmentation has not been fully explored.

We utilized *in vivo* analytical and behavioral pharmacology approaches to demonstrate a role for COX-2 in the regulation of brain endocannabinoid (eCB) levels through the novel pharmacological strategy of "substrate-selective" inhibition of COX-2 (SSIC). SSIC augments eCB levels without increasing AA and related non-eCB lipid levels or inhibiting prostaglandin synthesis. The SSIC by VU0359681 increases brain and peripheral AEA levels 2 hours after i.p. injection without affecting other NAEs, AA, or prostaglandins in any tissue. In contrast, the FAAH inhibitor PF-3845 increases AEA and other non-eCB ethanolamides 2 hours after i.p. injection. The biochemical effects of VU0359681 are mediated through COX-2 as it has no effect on AEA levels in COX-2(-/-) mice and increases AEA in FAAH(-/-) mice. VU0359681 also increases brain, but not peripheral, 2-AG levels to a small degree via a COX-2-dependent mechanism, suggesting COX-2 can also play a role in 2-AG metabolism.

To assess the therapeutic potential of SSIC, we tested the effects of VU0359681 in several models of anxiety. VU0359681 significantly increases center distance and time in the novel open-field test. VU0359681 also significantly increases light zone entries, time, and distance in the light-dark box. The anxiolytic-like effects of VU0359681 in the novel open-field and light-dark box assays are mediated by signaling through CB_1 receptors as co-treatment of VU0359681 with rimonabant or treatment of CB_1(-/-) mice with VU0359681 did not produce significant behavioral effects. VU0359681 also significantly decreased open arm entry latency in the elevated plus maze. Similar behavioral effects are observed with the FAAH inhibitor, PF-3845. These data indicate that SSIC is a viable strategy for *in vivo* augmentation of eCB signaling, and that VU0359681 has anxiolytic-like actions in animal models mediated by eCBs.

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DHEA, AN *N*-3 FATTY ACID DERIVED N-ACYLAMINE AS ENDOGENOUS REGULATOR OF COX-2 ACTIVITY

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Increasing evidence shows that DHEA (docosahexaenoylethanolamide), an endogenous metabolite of the *n*-3 fatty acid DHA (docosahexaenoic; 22 : 6*n*-3), possesses a diverse spectrum of biological activities (Meijerink et al., Br. J. Pharmacol. (2012), in press). Interestingly, DHEA concentrations in animal tissues and in human plasma have been found to parallel dietary intake levels of DHA, and recently we provided evidence for the anti-inflammatory effects of DHEA in macrophages and adipocytes. DHEA belongs to the group of *N*-acyl ethanolamines (NAEs), which contain a number of important mediators including AEA, PEA and OEA. To elucidate the immune modulatory activities of DHEA, the underlying mechanisms of action were further studied. RAW264.7 macrophages were pre-incubated with DHEA and stimulated with LPS. Immune-modulation of key inflammatory mediators and pathways (COX-2, NF-κB, IFNβ, eicosanoids, TLR4-, TLR3-pathways) was assessed using different methods, like LC-MS/MS, Q-PCR, ELISA, Microarray and Ingenuity Pathways analysis.

Upstream regulator analysis (Ingenuity pathways analysis) of genes regulated by DHEA in macrophages showed that DHEA-induced inflammatory gene expression profiles were the opposite of TNF-, TLR4-, TLR3- and PGE2-induced profiles. This suggests that DHEA inhibits the activity of these signalling pathways, likely by affecting a key inflammatory mediator or an upstream regulator. Out of several important players from these pathways, activity of NF-κB and IFNβ was not altered by DHEA. However, analysis of eicosanoids (LC-MS/MS) derived from various enzymatic pathways established that predominantly cyclooxygenase 2 (COX-2) generated prostaglandins and thromboxane B levels were highly reduced by DHEA (10 μM). Dose-response effects assessed for PGE2 showed 35% inhibition in response to 1 μM DHEA. As gene and protein expression of COX-2 were not altered by DHEA we hypothesize that DHEA acts at the level of the enzyme. This could be by substrate competition or via other forms of inhibition of COX-2 activity. Concluding, our data suggest that DHEA functions as an endogenous regulator of COX-2 activity. It would be interesting to further explore the pharmacological properties of DHEA in inflammation models.

CORTICOTROPIN-RELEASING HORMONE SIGNALING DRIVES ANANDAMIDE HYDROLYSIS TO PROMOTE ANXIETY

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Corticotropin releasing hormone (CRH) and endocannabinoids (eCBs) are neuromodulators influencing how the brain perceives and responds to stress. CRH activation of the R1 receptor (CRH-R1) acts throughout the brain and pituitary to facilitate endocrine and behavioral stress responses, while activation of the eCB CB1 receptor has an opposite effect on these outputs. Although these receptor systems have overlapping distribution, few studies have investigated if these neurotransmitter systems are capable of interacting. Using adult male Sprague Dawley rats, we have found that icv administration of CRH decreases eCB levels (anandamide) and increases FAAH activity in the amygdala, but not the PFC, at both 10 and 30 min post-CRH administration. Subsequent tests using agonist and antagonist approaches revealed these effects are mediated by CRH-R1 activation and not CRH-R2. Consistent with these findings, antagonism of CRH-R1 attenuated stress-induced activation of FAAH and reduction in AEA content within the amygdala. Employment of mice lacking CRH-R1 exclusively on glutamatergic forebrain neurons similarly revealed that the absence of CRH-R1 on glutamate neurons prevents stress-induced changes in amygdalar FAAH activity and AEA levels. Functionally, we have also determined that CRH-R1 activation of FAAH shapes both stress-endocrine and behavioral responses. Using the elevated plus maze, icv CRH increased behavioral indices of anxiety (less time in open arms) and plasma corticosterone. Both CRH-driven effects were attenuated by pretreatment with the FAAH inhibitor URB597, indicating CRH-R1 signaling during stress events plays a pivotal role in launching the spectrum of stress responses, in part through its direct effects on FAAH. These data suggest that stress-induced CRH-R1 activation on glutamatergic neurons removes the 'tonic inhibitory brake' normally exerted by anandamide within the amygdala, through a mechanism whereby increases in FAAH activity reduce anandamide levels within the amygdala; this model likely accounts for why FAAH inhibitors only exert anxiolytic actions in aversive or stressful environments as they simply block the effects of CRH signaling. As such, this supports the hypothesis that inhibition of FAAH represents a putative target for stress-related anxiety disorders.

SSRI FACILITATION OF FEAR EXTINCTION REQUIRES AMYGDALA ENDOCANNABINOID CB1 SIGNALING

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There is growing evidence supporting a major role for endocannabinoids in modulating protection and recovery from stress and traumatic experiences. Previous work has shown that augmenting anandamide, via inhibition of the catabolic enzyme fatty acid amide hydrolase (FAAH) facilitates fear extinction in mice. This effect is mediated via CB1 receptors in basolateral amygdala and is associated with facilitation of synaptic plasticity (long-term depression of inhibitory transmission). Interestingly, recent studies demonstrate that chronic treatment with the SSRI fluoxetine, a first-line therapeutic treatment for many anxiety and stress-related neuropsychiatric conditions, facilitates fear extinction and promotes amygdala plasticity in an analogous manner to FAAH inhibitors. However, it is currently unclear whether fluoxetine's effects on fear extinction involve functional interactions with endocannabinoids. To test this, we first assessed changes in endocannabinoids in amygdala and other extinction-mediating brain regions following chronic fluoxetine. This revealed a significant elevation in anandamide levels in the basolateral amygdala, dorsal striatum and dorsal hippocampus after chronic fluoxetine treatment. Next, we tested whether the increased endocannabinoid tone after fluoxetine mediated the drug's extinction-facilitation effects by blocking CB1 receptors, via Rimonabant, either systemically or only in the basolateral amygdala. Rimonabant administration fully abolished the behavioral effects of fluoxetine. Ongoing experiments seek to elucidate the key CB1-mediated synaptic plasticity and molecular signaling pathways involved, for example by quantifying the effects of chronic fluoxetine on amygdala calcineurin activity and on CB1-expressing perineuronal nets, key molecular stuctures for extinction, and by analyzing the interaction between SSRI and endocannabinoid metabolism. Taken together, these findings demonstrate a novel, obligatory role for amygdala endocannabinoids in the extinction-promoting effects of a major pharmacotherapy for anxiety disorders.

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THCV AND CBD EFFECTIVELY DECREASE LIPID LEVELS IN A VARIETY OF BIOLOGICAL SYSTEMS AND THCV ALSO SENSITIZES HEPATOCYTES TO INSULIN

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The endocannabinoid system (ECS) has quickly emerged as a key player in the regulation of energy homeostasis and its overactivity especially, though not exclusively, within the liver is associated with the development of insulin-insensitivity and metabolic syndrome. The accumulation of triglycerides in the liver is linked to obesity, dyslipidemia, type 2 diabetes and metabolic syndrome in general, thus strategies to decrease hepatic triglyceride levels hold significant therapeutic potential. We have assayed the potential of THCV and CBD (CB1 antagonists, though the former behaves as CB1/CB2 agonists at higher concentrations) to decrease lipid levels in hepatocytes and in a fish model of lipid mobilization as well as testing ability of THCV to regulate glucose homeostasis/insulin sensitivity in murine models of obesity. We have found that, in hepatocytes treated with oleic acid to up-regulate triglyceride accumulation, to mimic hepatosteatosis, THCV rapidly decreased lipid stores in a concentration dependent manner, possibly through increased lipolysis. The dependence of THCV on cannabinoid receptor CB1 for this effect appears to be minimal based on pharmacological and knockdown studies. Nuclear magnetic resonance analysis of lipophilic extracts from treated hepatocytes confirmed that oleic acid increased intracellular triglyceride levels and that these were reduced by THCV and CBD concomitant with an increase in free fatty acids. *In vivo* studies utilizing zebrafish embyos treated with THCV or CBD had showed an increased rate of yolk absorption in a time- and dose-dependent manner and in *ob*/*ob* mice THCV significantly decreased liver triglyceride levels. Furthermore, *in vitro* models of hepatic insulin resistance showed that THCV, in a manner more efficacious than a CB1 antagonist, dose-dependently sensitized cells to insulin-dependent activation of AKT, a key modulator of glucose homeostasis. And while in mice THCV did not modulate food intake or weight, it reduced glucose intolerance in *ob*/*ob* mice while it increased insulin sensitivity in diet-induced obese mice. These data indicate that THCV has significant positive, and partly CB1 receptor-independent, effects on lipid metabolism and may hold therapeutic potential for the treatment of the metabolic syndrome.

DIFFERENTIAL EXPRESSION OF HUMAN SPECIFIC N-TERMINUS VARIANTS OF THE CANNABINOID RECEPTOR 1 IN HUMAN ISLETS BETA CELLS.

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The cannabinoid receptor 1 (CB1R) controls many metabolic parameters in peripheral tissues, including lipogenesis, insulin release, glucose uptake and insulin sensitivity. These functions of the CB1R in the periphery are independent from its activation in the central nervous system, where it has other important roles such as control of appetite, anxiety, pain, and is responsible for the psychotropic effect of Δ^9-tetrahydrocannabinol. The peripheral antagonism of the CB1R is able to improve the metabolic parameters in obesity. Nevertheless, specifics antagonists developed for the treatment of obesity and diabetes, such as rimonabant, have been withdrawn from market due to their blood brain barrier penetrance and subsequent psychological side effects. Three different isoforms of the receptor have been described specifically in humans, which differ in their affinity for diverse ligands and in potency of action. A more thorough identification and understanding of these isoforms within the pancreas may be useful in the development of novel therapeutics to treat diabetes.

We have shown previously the presence of the endocannabinoid system in islets of Langerhans in rodent and human pancreas. We have also reported the control that the CB1R exerts on insulin release from pancreatic beta cells. In this study we now show the main isoforms expressed in human islets of Langerhans. We demonstrate that human islet of Langerhans express three isoforms in a different proportion compared to the central nervous system. The relative abundance of a specific isoform in the endocrine pancreas might allow a new approach for the development of a second generation of CB1R antagonists. A periphery-restricted antagonist specific to the most abundant CB1R isoform in beta cells would preserve their insulin-secreting function while eliminating the adverse physiologic side effects of earlier compounds.

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CHRONIC SYSTEMIC CB_1 CANNABINOID RECEPTOR BLOCKADE REDUCES WEIGHT GAIN AND LOWERS BLOOD PRESSURE IN HYPERTENSIVE (mRen2)27 RATS

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There is growing evidence for a strong signaling interaction between the endocannabinoid system (ECS) and the renin-angiotensin system (RAS), which may enhance the pathogenic properties of the pressor peptide hormone angiotensin (Ang) II in hypertension and the metabolic syndrome. Hypertension is associated with impaired baroreflex sensitivity (BRS) for control of heart rate, an important index of vagus nerve function. BRS, measured as the bradycardia evoked in response to transient increases in blood pressure, is mediated by the solitary tract nucleus (NTS) of the dorsal medulla. Our model system is the transgenic (mRen2)27 rat, a monogenetic Ang II-dependent model of hypertension in which the mouse Ren2 renin gene was transfected into the genome of the Sprague-Dawley rat, resulting in a phenotype of chronic hypertension with impaired BRS. Previous microinjection studies demonstrated that CB_1 cannabinoid receptor blockade by SR141716A (0.36 and 36 pmol) in the NTS of hypertensive (mRen2)27 rats dose-dependently improves BRS for control of heart rate ($P < 0.001$), whereas the CB_1 antagonist had no effect on BRS in normotensive Sprague-Dawley control rats. Biochemical studies reveal a trend for elevated levels of the endocannabinoid 2-AG and higher expression of CB_1 receptors in the dorsal medulla of (mRen2)27 rats relative to controls. Together, we interpret these data as evidence for the chronic upregulation of the ECS in the brain of this RAS-dependent model of hypertension.

Data from acute studies prompted us to study the effects of chronic systemic CB_1 receptor blockade in the (mRen2)27 strain. Beginning at 16 weeks of age animals were administered daily oral injections of SR141716A (10 mg/kg/day) for 28 days. In comparison to rats receiving vehicle (0.1% Tween-80 in dH_2O), daily dosing of SR141716A significantly reduced systolic blood pressure (172 ± 1 vs. 151 ± 5 mmHg, $P < 0.01$), and decreased cumulative weight gain and adiposity index without long-term changes in food or water consumption. These beneficial actions by SR141716A in (mRen2)27 animals are consistent with the interpretation that an upregulated ECS contributes to the maintenance of hypertension in this RAS-dependent model. We conclude that blockade of CB_1 receptors is an effective approach to reduce blood pressure and influence cardiovagal reflexes in a positive manner in certain forms of hypertension.

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CARDIO-RENAL EFFECTS OF CHRONIC ADMINISTRATION WITH CB2 AGONIST AM1241 AND CB2 ANTAGONIST AM630 IN RATS WITH DIET INDUCED OBESITY

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It is widely recognised that obesity elicits a number of comorbidities including heart and kidney disease. The progression of these diseases is frequently exacerbated by other clinical factors associated with obesity such as hypertension and metabolic imbalance. Obesity associated pathology includes cardiac and renal hypertrophy, which can lead to an increased risk of developing ischemic heart disease, stroke and hypertension, in addition to a decreased glomerular filtration rate and increased urinary protein excretion. Previous research has indicated that chronic activation of the cannabinoid receptor 2 (CB2) may have a protective role in ameliorating renal function decline associated with diabetic nephropathy and a range of cardiomyopathies. It is still unclear however what role the CB2 receptor may play in modulating cardiac and renal tissue in the obese state. The aim of the current study was to investigate whether activation or inhibition of the CB2 receptor in an obese rat model altered cardiac and renal tissue in diet induced obesity.

Following nine weeks of a high fat diet, male Sprague Dawley rats were injected with either 3 mg/kg body weight of the CB2 agonist AM1241, or 0.3 mg/kg body weight of the CB2 antagonist AM630, or saline for six weeks, while continuing on the high fat diet. Diet induced obesity was confirmed via comparison to animals fed standard chow and treated with saline. Urine was collected and blood pressure measured throughout the treatment period. Following the 6 weeks of treatment the heart and kidneys were harvested and weighed.

The CB2 antagonist group showed significant changes to both the heart and kidneys, with organ weight relative to body weight ratio significantly higher than the high fat saline control group ($p<0.05$). There were no changes to heart and renal size for the CB2 agonist group. Analysis of urine samples and blood pressure will further elucidate the role of the CB2 receptor in an obese model of cardio-renal function.

Future studies should investigate the structural modifications and functional outcomes underlying these changes in obese animals.

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ABHD12 DELETION ALTERS CANNABINOID SIGNALING AND INDUCES DESENSITIZATION IN AUTAPTIC HIPPOCAMPAL NEURONS

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Cannabinoid receptors are the target of the chief psychoactive ingredients of marijuana and hashish but are also part of an endogenous cannabinoid signaling system. This includes the machinery to produce and break down endogenous cannabinoids, arachidonic acid-based lipids such as 2-arachidonoyl glycerol (2-AG), which have been shown to serve as retrograde feedback messengers in numerous brain regions. Monoacylglycerol lipase (MGL) is responsible for the bulk of 2-AG breakdown in brain homogenate, but other enzymes have been found to also metabolize 2-AG, including ABHD12. Notably, deleterious mutations of ABHD12 have recently been found to be responsible for PHARC syndrome, a disorder that includes several neuron-related pathologies. To investigate the role of ABHD12 in neuronal cannabinoid signaling, we have made use of autaptic neurons from ABHD12-/- mice. Autaptic hippocampal neurons possess the machinery required to produce a strong cannabinoid retrograde signal. This signaling has been extensively characterized and includes the retrograde depolarization induced suppression of excitation (DSE).

In examining the expression of ABHD12 protein in cultured hippocampal neurons we have found that it is expressed in the soma of neurons in a pattern that is consistent with Golgi expression. Functionally we find that ABHD12 deletion does not appreciably alter the DSE profile in young autaptic cultures. However as the cultures age, maximal responses decline relative to younger cultures and to wild type controls. Because we see a similar shift in 2-AG dose responses, we interpret this to mean that CB_1 receptors desensitize over the course of several days in ABHD12-/- neurons. This is presumably due to a low level of residual 2-AG loitering at the synapse.

In summary we find that ABHD12 is expressed in neuronal Golgi where it regulates basal levels of endocannabinoids. The net effect of ABHD12 deletion is ultimately an enhancement of neurotransmission as CB1 inhibition diminishes as a result of desensitization.

EFFECTS OF DELETIONS IN ABHD12, MGL, AND CB_2 ON THE ENDOCANNABINOID-RELATED LIPIDOME IN MOUSE STRIATUM, HIPPOCAMPUS, AND CEREBELLUM

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The enzymes monoacylglycerol lipase (MGL) and α,β-hydrolase domain 12 (ABHD12) are responsible for approximately 85% and 10% of the endogenous cannabinoid (eCB) 2-arachidonoyl glycerol's (2-AG) hydrolysis, respectively, in the rodent brain. The eCBs 2-AG, *N*-arachidonoyl ethanolamine (AEA), and the AEA metabolite *N*-arachidonoyl glycine (NAGly) are all derivatives of arachidonic acid, a polyunsaturated fatty acid (PUFA) that is also a substrate for the production of prostaglandins. However, other FAs and PUFAs undergo similar enzymatic reactions as arachidonic acid to make structural analogs (*e.g.* 2-linoleoyl glycerol); therefore, the effects of enzyme deletion or blockade may have broader effects than these three more studied lipids. This study aims to elucidate the effects of genetic deletion of MGL and ABHD12 and the eCB receptor CB_2 on the *N*-acyl amide and 2-acyl glycerol lipidome in the mouse striatum, hippocampus, and cerebellum. 6 MGL, 6 CB_2, and 6 ABHD12 knockout (KO) mice were compared to 12 wild-type (WT) mice from the same genetic background. Animals were sacrificed, 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.

A wide range of *N*-acyl amides and 2-acyl glycerols were detected in all groups, though differential production was demonstrated across brain areas. Results here highlight those of AEA, 2-AG and NAGly; however, many differences were observed in other lipids in these KO mice. Levels of AEA increased relative to WT in the hippocampus and cerebellum of the ABHD12 and CB_2 KO mice with no change in striatum. In contrast, levels of AEA decreased in the striatum and cerebellum and remained the same as wild-type in the hippocampus of the MGL KO mice. 2-AG levels were significantly higher in all areas in the MGL KO mice; however, 2-AG was only increased in the cerebellum of ABHD12 KO mice. Importantly, no changes in 2-AG levels in any brain region were observed in CB_2 KO mice. NAGly levels were significantly higher in all areas analyzed in the ABHD12 and CB_2 KO mice; whereas, NAGly levels were significantly lower in the striatum and cerebellum of the MGL KO mice. Like AEA, NAGly concentrations remained unaltered in the hippocampus of MGL KO mice. Levels of the prostaglandins PGE_2 and $PGF_{2\alpha}$ increased in all areas for the ABHD12 and CB_2 KO mice. However, levels of the same prostaglandins decreased in all areas for the MGL KO mice. These data replicate and greatly extend the finding that deletion of MGL drives changes in a range of AA metabolites and extends this to *N*-acyl amides. Additional *N*-acyl amides and 2-acyl glycerols not discussed here, likewise, changed production levels either up or down in each of the KO mice demonstrating far-reaching effects on lipid metabolism, and therefore lipid signaling as a result of deletion of each of these important eCB proteins.

GENERATION OF *N*-ACYLPHOSPHATIDYLETHANOLAMINE BY PLA/AT-1 IN MAMMALIAN CELLS

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N-Acylphosphatidylethanolamines (NAPEs) serve as precursors of anandamide and other bioactive *N*-acylethanolamines (NAEs). The HRAS-like suppressor (HRASLS) family, consisting of five proteins (HRASLS1–5), were originally discovered as tumor suppressors negatively regulating the activity of oncogene Ras. Recently, we demonstrated that all HRASLS1–5 proteins possess phospholipid-metabolizing activities including NAPE-forming *N*-acyltransferase activity, and proposed to call these proteins phospholipase A/acyltransferase (PLA/AT)-1–5, respectively. PLA/AT-1 attracts attention by its expression in all of human, mouse and rat, in contrast to PLA/AT-2, which has a potent *N*-acyltransferase but is absent from rodents. Moreover, the predominant expression of PLA/AT-1 in testis, skeletal muscle, brain and heart suggests a role in the NAPE generation upon degeneration and inflammation in these tissues. Therefore, we examined whether the expression of PLA/AT-1 causes the formation of NAPE and NAE in living cells. As analyzed by metabolic labeling with [^{14}C]ethanolamine, the transient expression of human, mouse and rat PLA/AT-1s in COS-7 cells as well as the stable expression of human PLA/AT-1 in HEK293 cells significantly increased the formation of NAPE and NAE. These results were confirmed with liquid chromatography-tandem mass spectrometry. Furthermore, knockdown of PLA/AT-1, which was endogenously expressed in mouse ATDC5 cells, decreased intracellular NAPE levels. These results suggest that PLA/AT-1 is an enzyme responsible for the generation of NAPE in mammalian cells.

Fmr1 DELETION ENHANCES AND ULTIMATELY DESENSITIZES CB_1 SIGNALING IN AUTAPTIC HIPPOCAMPAL NEURONS.

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Fragile X Syndrome (FXS) is a heritable form of mental retardation caused by a non-coding trinucleotide expansion of the FMR1 gene leading to loss of expression of this RNA binding protein. Mutations in this gene are linked to enhanced Group I metabotropic glutamate receptor (mGluR) signaling. A recent report found that mGluR5-dependent endogenous cannabinoid signaling is enhanced in hippocampal slices from *fmr1* knockout mice, suggesting a link between FXS and cannabinoid signaling. Alterations in cannabinoid signaling have an impact on learning and memory and may therefore be linked to some aspects of the FXS phenotype.

We have used autaptic hippocampal neurons cultured from *fmr1* knockout mice to further explore the interaction between endocannabinoid signaling and FMRP. These neurons express several robust forms of retrograde endocannabinoid signaling including depolarization induced suppression of excitation (DSE) and a metabotropic form (MSE) that results from Group I mGluR activation.

We now report that young *fmr1* neurons exhibit considerably enhanced DSE, likely via increased production of 2-AG, rather than enhanced mGluR-MSE. We find that depolarizations as brief as 50ms, which do not ordinarily produce DSE, routinely inhibited glutamate release. Furthermore, as neuronal cultures mature, CB_1-receptor signaling strongly desensitizes. Our results suggest that loss of FMRP broadly affects the endocannabinoid signaling system, possibly through local 2-AG over production. Furthermore, the net effect of the loss of FMRP may actually be diminished cannabinoid signaling due to receptor desensitization as an adaptation to 2-AG overproduction.

MOLECULAR DYNAMICS SIMULATIONS OF THE 2-AG ACTIVATED CANNABINOID RECEPTOR SUBTYPE 2 / G_i PROTEIN COMPLEX

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The CB2 signaling cascade begins with agonist binding to an inactive CB2 receptor, causing conformational changes that activate the CB2 receptor. In previous work, we showed the activation of the CB2 receptor, by the endogenous ligand, 2-arachidonylglycerol (2-AG) via the lipid bilayer using molecular dynamics simulations (*Hurst et al., 2010*). Here, 2-AG entered the receptor from the lipid bilayer between TMH6 - TMH7 and activated the CB2 receptor by causing the key ionic interaction between TMH3 (R3.55) and TMH6 (D6.30) to break.

We are currently studying the next step in the CB2 signaling process which involves the coupling of the Gi protein to an activated CB2 and the subsequent release of GDP. To probe the structural determinants for this next step, we used our 2-AG activated CB2 model to produce an initial 2-AG/CB2/Gαi1β1γ2 assembly based on the crystal structure of β2 adrenoreceptor in complex with Gαsβ1γ2 (*Rasmussen et al., 2011*). The 2-AG/CB2/Gαi1β1γ2 assembly was immersed in a fully hydrated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer and NPT NAMD (*Phillips et al., 2005*) molecular dynamics simulations were initiated for this system. In two separate runs, now near 1 μs each, we have seen very similar results. (1) In the first 300 ns, Gαi1β1γ2 adjusts its position to be in "register" with the receptor. Here, the G protein rotated under the receptor to permit an interaction between P139 on the CB2 IC-2 loop and the Switch 1 region of the G protein (including Gαi1 α5 helix). (2) The C-terminus of Gαi1 (last 11 residues on α5 helix I344 to F354) inserts into the intracellular opening of the CB2 activated receptor but at an altered angle compared to the β2 adrenoreceptor/Gαsβ1γ2 complex. (3) This insertion causes the distance between the α3 helix (Gαi1) and α5 helix (Gαi1) to widen, allowing increased hydration of GDP. (4) Key salt bridges such as the R90/E238 salt bridge between the helical and ras-like domain on Gαi1 break. Taken together, these effects all weaken GDPs interactions with Gαi1, however, GDP has not yet left the complex.

Effects of complex formation are also evident on the extracellular side of the complex. The head group of 2-AG stays in the binding pocket between TMH6 - TMH7 interacting with K3.28 and D275 during the period when the G protein adjusts its position to facilitate the IC-2 loop/ Switch I region interaction. After 500ns, 2-AG begins its exit by interacting with residues C6.47, L7.43 and E1.49. After 1 μs, 2-AG exits the CB2 receptor between TMH6-TMH7 to reach the outer leaflet of the lipid bilayer. [Support: RO1 DA003934 and KO5 DA021358 (PHR)]

DIFFERENTIAL ACTIVATION OF INTRACELLULAR VS PLASMALEMMAL CB2 CANNABINOID RECEPTORS

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The CB_2 cannabinoid receptor has emerged as an important therapeutic target in several pathologies, as it mediates beneficial effects of cannabinoids, while having little, if any psychotropic activity. Difficulties in the development of CB_2-based therapeutic agents have been related to its intricate pharmacology, including the species-specificity and functional selectivity of the CB_2-initiated responses. Plasmalemmal or subcellular location of the receptor may also affect the signaling pathways initiated by its activation. To dissociate between these two, we used extracellular and intracellular administration of CB_2 ligands and concurrent calcium imaging in CB_2-expressing U2OS cells. We found that extracellular administration of anandamide was ineffective, whereas WIN55,212-2 triggered a delayed, CB_2-dependent Ca^{2+} response, that was Gq protein-mediated. When microinjected, both agonists elicited a fast, transient and dose-dependent elevation in intracellular Ca^{2+} concentration upon activation of Gq-coupled CB_2 receptors. The CB_2-dependency was confirmed by sensitivity to AM630, a selective CB_2 antagonist and by unresponsiveness of untransfected U2OS cells to anandamide or WIN55,212-2. Our results support the functionality of intracellular CB_2 receptors and their ability to Gq and elicit Ca^{2+} signaling. Our results add further complexity to the CB_2 receptor pharmacology and argue for careful consideration of receptor localization in the development of CB_2-based therapeutic agents.

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ANTIBODIES TO CANNABINOID TYPE 1 RECEPTOR CO-REACT WITH STOMATIN-LIKE PROTEIN 2 IN THE MOUSE BRAIN MITOCHONDRIA

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Application of antibodies for immunocytochemical identification of cannabinoid type 1 receptor (CB_1) played a significant role in advances made in research of endocannabinoid signaling in mammalian brain. Nevertheless, as is often the case in studies involving antibodies (Mayrose et al., 2007 Bioinformatics, 23, 3244-3246), the molecular construct of the CB_1 antibody-binding site (epitope; which might be composed of discontinuous sections of the antigen's amino acid sequence) was not fully characterized. As a result, serological identification of CB_1 in some organelles might be uncertain and prone to misidentification. During our light and electron microscopic analyses of the distribution of CB_1 in the developing mouse brain (e.g., Morozov et al., 2009 Cerebral Cortex, Suppl. 1, i78-89), we detected mitochondrial binding of anti-CB_1 (C-terminus) sera. However, we observed that anti-CB_1 sera reveal a non-CB_1 peptide in the mitochondria, as indicated by equivalent immunolabeling patterns in wild type and CB_1-KO mouse brain. Using Western blots, immunoprecipitation and mass spectrometric protein identification we found that anti-CB_1 antibodies, in addition to CB_1, also recognize the mitochondrial protein stomatin-like protein 2 (SLP-2). Finally, we show that the effect of synthetic cannabinoid WIN 55,212-2 on mitochondrial complex III respiration is not detectable in purified mitochondrial preparations. Thus, our ultrastructural and biochemical findings, together with *in vitro* analyses of mitochondrial oxygen consumption, indicate that direct relationship between CB_1 and mitochondrial functions in the cerebral cortex is highly unlikely. We do not deny the validity of most studies that use CB_1 antibodies; however, we emphasize the need for additional controls and conscious interpretation of unusual immunolabeling.

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CRIP1A REGULATES CB_1R SIGNALING AND CELLULAR TRAFFICKING

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The CB_1 receptor (CB_1R) is one of the most abundantly expressed GPCR's in the CNS and has been targeted therapeutically for multiple diseases; however side effects profiles for CB_1R molecules have posed difficulty in the development of clinically successful CB_1R drugs. The identification of GPCR Interacting Proteins (GIPs) has provided additional insight into the fine-tuning and regulation of numerous GPCR's. The Cannabinoid Receptor Interacting Protein 1a (CRIP1a) binds to the last 9 residues of CB_1R and was initially characterized for its ability to reverse CB_1-mediated tonic inhibition of voltage-gated Ca^{2+} channels (Neihaus et al., 2008). The mechanisms by which CRIP1a regulates CB_1R activity and trafficking are poorly understood, and therefore the focus of this study was to investigate the effects of CRIP1a on CB_1R expression, trafficking and activity.

CB_1R was co-immunoprecipitated from membranes and identified histochemically using antibodies developed against a non-CB_1R binding domain of CRIP1a. Analysis of stable CRIP1a overexpression or knock-down in N18TG2 neuronal cells revealed that CRIP1a did not alter the total levels of CB_1R mRNA or protein. However, CB_1R plasma membrane (PM) density was significantly reduced in CRIP1a overexpressing clones, but increased in CRIP1a knock-downs. Pre-treatment with tetrahydrolipstatin (THL) to block 2-arachidonoyl glycerol production, followed by the CB_1R agonist WIN55212 led to CB_1R internalization in WT and CRIP1a knock-down cells, but a loss of internalization and a net increase in receptor externalization in CRIP1a overexpressors.

To identify the role that CRIP1a plays in regulating CB_1R-mediated signal transduction, CRIP1a overexpressing or knock-down WT and CRIP1a overexpressing or knock-down clones were treated with THL, and phosphoERK and cAMP levels were determined. CB_1-mediated ERK phosphorylation in WT N18TG2 cells was reduced by rimonabant, consistent with "constitutive activation" of signal transduction. When compared to WT cells, CRIP1a overexpressing clones displayed a reduction in basal phosphoERK levels, whereas CRIP1a knock-down clones showed a significant increase in basal pERK levels. Stimulation of ERK phosphorylation by the CB_1 agonist WIN55212-2 was unaltered in CRIP1a over-expressing clones compared with WT. Basal cAMP levels were similar in WT and CRIP1a overexpressing and knock-down clones. However agonist-mediated CB_1R inhibition of forskolin-stimulated cAMP production was enhanced in CRIP1a knock-down clones, but unaltered in CRIP1a overexpressing clones when compared to WT cells. These findings suggest that the function of CRIP1a may be to modulate internalization associated with CB_1R constitutive signal transduction.
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CB_1 CANNABINOID RECEPTOR JUXTAMEMBRANE C-TERMINAL PHOSPHORYLATIN AND cAMP ACCUMULATION IN NT18TG2 CELLS

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The CB_1 cannabinoid receptor carboxyl terminus possesses critical structures that are important for CB_1 activity and regulation. The aim of the study is to investigate the effect of phosphorylation of the juxtamembrane C-terminal domain of the CB_1 structure and signal transduction via Gi/o proteins. Nuclear Magnetic Resonance (NMR) analyses demonstrated that phosphorylation on the three serine residues within the juxtamembrane domain differentially influenced the helical structure and altered the conformation proximal to the cysteine residue. Phosphorylation of Ser402 disrupted the helix, whereas phosphorylation of Ser411 and Ser415 extended the helix but modified the conformation distal to the helix. To investigate functional changes, we examined cAMP accumulation in N18TG2 neuronal cells. C-terminal peptides were incubated with N18TG2 cells in media containing phosphodiesterase inhibitors for 15 min at 37 °C. Cells were then incubated with forskolin (1μM) for 4 min and the reaction was terminated by dropping the pH to 4.5 and boiling. Cells were disrupted by a freeze-thaw cycle, sedimented, and cAMP accumulation was quantitated in the cell-free supernatants using a radioligand displacement assay based upon [^{3}H]-cAMP binding to protein kinase A regulatory proteins. Bound proteins were aggregated, harvested on a UniFilter and quantitated using a Top Count scintillation counter (Packard). The data for each assay were normalized to forskolin-stimulated cAMP accumulation as 100%, and statistical differences were determined by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison post hoc test.

The unphosphorylated peptide (Arg401 to Glt420) was able to inhibit forskolin-stimulated cAMP accumulation in a dose-dependent fashion (10-100 μM), with a decrease to 44± 8.4% of maximal. Phosphorylated peptides (Ser402, Ser411, or Ser415) (10-100μM) were also able inhibit forskolin-stimulated cAMP accumulation in a dose-dependent fashion, with maximal attenuations at 100μM reaching Ser402: 22± 7%; Ser411: 15.6± 8%; and Ser415; 7.6± 11%. Co-incubation with the CB_1 antagonist, SR141716A (1μM) was not able to reverse the response to either unphosphorylated or phosphorylated peptides. We determined whether phosphoylation by protein kinase C (PKC) could regulate CB_1 -mediated attenuation of cAMP accumulation in intact N18TG2 cells by prior exposure of cells to bradykinin, which activates a Gq-coupled receptor leading to PKC activation. Preincubation with bradykinin augmented the CP55940-stimulated attenuation. Co-incubation with the PKC inhibitor bisindolylmaleimide I (100nM) was able to reverse the effect of bradykinin. These data show that the C-terminal peptides were able to modulate signal transduction in N18TG2 cells, and this could be augmented by structural modifications imposed by phosphorylation of Ser residues. We further provide evidence that stimulating a Gq-coupled receptor could augment the CB_1-mediated signaling, consistent with the suggestion that PKC might promote phosphorylation of these residues and thereby alter 8th helix structure and modulate function.

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THE ENDOCANNABINOID SYSTEM CONTROLS SKELETAL MUSCLE CELL DIFFERENTIATION VIA CB1 RECEPTOR-DEPENDENT HYDROLYSIS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP_2) AND INHIBITION OF KV7 POTASSIUM CHANNELS

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Myogenesis is a tightly regulated process in which proliferating myoblasts withdraw from the cell cycle and fuse to form multinucleated myotube (1). The purpose of this study was to investigate the expression profile and functional role of the endocannabinoid system (ECS) during both skeletal muscle cell proliferation and differentiation.

Using murine C_2C_{12} cells as an experimental paradigm, we found a significant decrease in 2-AG, but not AEA levels during myotube formation. These changes were also corroborated by the expression of all the genes known to be involved in 2-AG and AEA metabolism. Q-PCR and western blot analysis also showed that *Cnr1* and *Cnr2* gene expression was increased during differentiation in C2C12 cells and in primary human myoblasts, with *Cnr1* showing the highest degree of up-regulation. In C_2C_{12} cells, 2-AG (1-3 μM) and ACEA (1-3 μM) inhibited myoblast differentiation and prevented myotube formation; 2-AG and ACEA, as well as AEA (1μM), stimulated cell proliferation. All these effects were shown to be mediated by CB1 receptors through pharmacological and molecular approaches.
In C_2C_{12} myoblasts, CB1 stimulation by ACEA reduced PtdIns(4,5)P2 (PIP_2) levels and increased $[Ca^{2+}]_i$ in a PLC-dependent manner. Finally, since neuronal K_v7 potassium channels, and Kv7.4 channels in particular, are regulated by PIP2 (2), and control myoblast proliferation and myogenesis (3,4) we tested for their involvement in CB1R-induced inhibition of myogenic differentiation. In C_2C_{12} cells, co-immunoprecipitation analysis revealed that CB_1 receptor stimulation by ACEA reduced PIP_2 binding to $K_v7.4$ and inhibited $K_v7.4$ activation in transfected CHO cells in a PLC-mediated manner. Indeed, Retigabine, a selective activator of the K_v7 subfamily, reversed the effects of ACEA on C_2C_{12} myoblast proliferation and myogenic gene expression. These data indicate a novel role for ECs in controlling skeletal muscle differentiation, and highlight Kv7.4 channels as potential targets for EC-induced regulation of myogenesis.

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CB1 CANNABINOID RECEPTOR-MEDIATED INCREASE IN BDNF (BRAIN DERIVED NEUROTROPHIC FACTOR) SIGNALING REGULATES DEVELOPMENTAL NEUROGENESIS IN ZEBRAFISH BRAIN

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CB1 cannabinoid receptor (CB1R) has been shown regulate developmental and adult neurogenesis. Recently it was found that that zebrafish brain express CB1R (zCB1R) with 70% amino acid sequence homology with mammalian CB1R. Previously we showed that zCB1R mRNA transcript was expressed in the different regions of developing brain and CB1R agonist treatment [6hpf (hour post fertilization) -72 hpf] significantly increased zCB1R expression and motor neurogenesis in developing zebrafish brain. We further showed knocking down CB1R using morpholino or blockade of CB1R by CB1R antagonist SR141716 significantly attenuated CB1R-mediated increase in neurogenesis. The objective of the present study is to determine the molecular mechanism of zCB1R-mediated regulation of developmental neurogenesis.

In the present study we have used wild type and green fluorescence protein (GFP)-expressing transgenic zebrafish (ISLET-1) zebrafish embryos to determine zCB1R expression (in situ) and developmental neurogenesis (confocal microscopy) along with pharmacological tools for activation or inhibition of the receptors. We found that CB1R agonist methanandamide or ACEA (both at 1-10 μM) treatment from 6hpf- upto 72 hpf significantly increased BDNF transcript and concurrent neurogenesis in developing zebrafish brain. CB1R agonist-mediated increase in BDNF expression is significantly blocked CB1R antagonist SR141716 (1μM). We further showed that knocking down BDNF receptor (using morpholino) or blocking BDNF receptor significantly attenuated CB1R agonist-mediated increase in neurogenesis without reducing BDNF expression. Together, results from this study showed that zCB1R-mediated increase in BDNF signaling plays a vital role in the regulation developmental neurogenesis. We are currently engaged in a) identifying the signaling determinant downstream of CB1R-BDNF signaling axis that regulates endocannabinoid-mediated developmental neurogenesis and b) the role of endocannabinoid system in the regulation of drug-abuse (ethanol)-mediated inhibition of developmental neurogenesis.

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LONG-TERM PROTECTION OF THE BRAIN, THE HEART AND THE LIVER BY ULTRA-LOW DOSES OF TETRAHYDROCANNABINOL (THC)

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We have previously shown that a single ultra-low dose of tetrahydrocannabinol (THC) protected the mice brain from a variety of insults including PTZ-induced seizures, CO-hypoxia, pentobarbital deep anesthesia and MDMA neurotoxicity. THC (0.002mg/kg, a dose that is 3-4 orders of magnitude lower than the doses that elicit the known acute effects of the drug), injected either 7 days before or 3 days after the insult, prevented the deteriorating effects of the insult as measured by a battery of cognitive assays 3-7 weeks following the insult. The same dose of THC induced long-lasting (7 weeks) biochemical effects in the brain, including the activation of ERK, CREB and BDNF in the hippocampus and frontal cortex.

A common feature of the various insults is the secondary activation of the innate immune system in the brain in response to the primary neuronal damage. We tested, therefore, whether THC is able to protect the brain from LPS activation of the innate immune response. Intraperitoneal injection of the bacterial lipopolysaccharide (LPS) induced systemic inflammation that dissipated in 2-3 days; nevertheless, cognitive deficits were observed even 7 weeks later. A single injection of THC 0.002mg/kg 48 hrs before LPS did not affect the systemic acute inflammation but prevented the long-lasting cognitive damage. THC was similarly effective when injected 7 days after LPS, a time when the mice already recovered from the systemic inflammation. The protective effect of THC was blocked by SR141716A, but not by SR144528, indicating the involvement of CB1 receptors. THC also attenuated the induction of cyclooxygenase-2 (COX-2) in the hippocampus and frontal cortex 1-7 weeks following the application of LPS.

We then tested whether the same ultra-low dose of THC protected the mice heart from ischemic damage. Myocardial infarction (MI) was produced by coronary artery ligation and its morphological, functional and biochemical outcomes were studied 24 hrs later. MI induced a necrotic area (TTC staining) that was infiltrated by neutrophils (immunohistology), deteriorated functional shortening of the heart (echocardiography) and elevated the level of troponin T in the serum. All these parameters were significantly improved by THC 0.002mg/kg injected either 2 or 48 hours before MI.

The ultra-low dose of THC was similarly effective in protecting the liver from ischemia-reperfusion (I/R) damage. A single injection of THC attenuated the necrotic area, suppressed caspase-3 activation and reduced the level of liver enzymes in the serum.

Current studies are aimed at revealing whether THC-induced neuroprotection, cardioprotection and hepatoprotection share similar molecular mechanisms.

DETRIMENTAL EFFECTS OF LINOLEOYLLYSOPHOSPHATIDYLINOSITOL IN CORTICAL NEURONS: INVOLVEMENT OF GPR55

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Lysophosphatidylinositol (LPI) has been identified as the endogenous ligand of the orphan receptor GPR55 (Henstrindge *et al*., 2009). Data in literature have shown the ability of LPI to induce an increase in intracellular calcium and neurite retraction in neuronal-like cells, through a GPR55-mediated mechanism (Lauckner *et al*., 2007; Obara *et al*., 2011). Moreover, a more recent publication has shown the ability of LPI to induce a GPR55-mediated glutamate release in hippocampal cultures, pointing out a role of this orphan receptor in neurotransmitters release (Sylantyev *et al*., 2013). To further characterize the pharmacology of LPI and GPR55 in neurotransmission, we evaluated the effect of soybean LPI, and in particular one of its species, linoleoyl-LPI (18:2 LPI), on the cell viability of E18-derived cortical primary cultures. Cell viability was determined by immunocytochemistry with neuronal or glial markers, and alamar blue assay.

Results revealed that short (30 minutes) and long (24 hours)-term exposure of cortical primary cultures to soybean LPI (Sigma) reduced the cell viability of neurons in a dose-dependent manner, at concentrations above 1 μM. Surprisingly, when cortical primary cultures were challenged with a different batch of soybean LPI (Sigma), none of the concentrations tested significantly affected the cell viability. Thus, as soybean LPI is composed of different LPI species, saturated and unsaturated, we started investigating the percentage of each species contained in the two different batches. Using LC-MS analyses we found that the batch with neurotoxic properties on cortical cultures contained a double amount of unsaturated LPI species. This prompted us to investigate the effect of the single species contained in soybean LPI on cortical primary cultures in cell viability assay. Results revealed that 18:2 LPI, which was contained approximately in double amount in the active batch of soybean LPI (7.4%), was the more neurotoxic LPI species, suggesting it to be the main active component of the original active batch of soybean LPI. Next, as GPR55 has been shown to be the main biological target of LPI, we tested the effect of 18:2 LPI in both GPR55 (+/+) and (-/-) mice-derived cortical primary cultures. Results revealed that the neurotoxicity induced by this compound was significantly attenuated in GPR55 (-/-) neuronal cells. Thus, as this orphan receptor has been shown to induce glutamate release in hippocampal cultures, and as an excess of glutamate release can induce excitotoxicity and affect neuronal viability, we further evaluated the possible implication of glutamate release in 18:2 LPI-induced neurotoxicity. For this, cortical primary cultures were challenged with 18:2 LPI together with methyl-β-cyclodextrin or riluzole, which can both block the neurotransmitters release, or NMDA receptor antagonists (DL-AP5 and CGS-19755), which block the glutamate-induced excitotoxicity. Results showed that the neurotoxic effect of 18:2-LPI was significantly attenuated by treatment with each of these compounds, suggesting that this lysophospholipid can induce activation of NMDA receptors through glutamate release.

Together these data suggest that low micromolar concentrations of 18:2 LPI can have detrimental effects on neuronal viability *in vitro*, through a mechanism involving GPR55-mediated glutamate release. Thus, considering that PLA2 activity is up-regulated in neurological disorders (Farooqui *et al.*, 2006), evaluation of LPI levels could lead to new insight the role of 18:2 and other lysophosphatidylinositol species in brain-related diseases.

MONOACYLGLYCEROL LIPASE AND ENDOCANNABINOID REGULATION OF INTRAOCULAR PRESSURE IN A MURINE MODEL

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Cannabinoids are part of an endogenous signaling system that is present in the vertebrate eye and that has been shown to reduce IOP via CB_1 receptors. The disposition and role of endogenous cannabinoids (eCBs) are still poorly understood. The objectives of this research are: 1) to test the candidate eCB 2-AG in a normotensive mouse model, 2) to investigate the enzymatic regulation of eCBs with a view to exploring the therapeutic potential of harnessing eCBs for the purpose of reducing intraocular pressure (IOP). We have used a murine model to take advantage of assorted available knockout mice.

IOP was measured in mice following topical corneal application of the eCB 2-AG and/or an assortment of blockers of serine hydrolases that are known or suspected to play a role in eCB metabolism. We found that topically applied 2-AG reliably reduced IOP in a concentration-dependent manner. 2-AG lowered IOP in CB_2-/- but not CB_1-/- mice. A subthreshold concentration of 2-AG reduced IOP if animals were pretreated topically with MGL blocker JZL184. This indicates that MGL plays a role in 2-AG metabolism in the anterior eye. Topical JZL184 did not however lower IOP on its own, suggesting that in this normotensive model endogenous production of 2-AG is relatively modest or that other enzymes contribute to 2-AG breakdown. Notably however, 2-AG did not reduce IOP in MGL-/- mice, raising the possibility that a degree of desensitization occurs in the absence of MGL.

We present evidence that 2-AG serves as an endogenous cannabinoid regulator of IOP in a normotensive mouse model. We additionally demonstrate that MGL plays a substantial role in metabolizing 2-AG in the anterior eye. These findings suggest that 2-AG and MGL may serve as desirable targets for the development of novel ocular hypotensive medications.

PATTERNS OF THERAPEUTIC CANNABIS USE IN 907 PATIENTS

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Background

Research has shown that cannabis can be useful in treating a number of conditions, and although there is some evidence that individual cannabis strains and specific modes of ingestion have different subjective affects on the symptoms and conditions of medical patients, large-scale tracking of patient strain/symptom preferences and patterns of use has been challenging due to regulatory restrictions on the use of medical cannabis in North America. This study is an examination of 12 months of patient purchasing data for 907 members of the Vancouver Island Compassion Society, with a focus on strain/symptom correlations and modes of ingestion.

Methods

A composite VICS active member profile (n=907) was created by averaging all purchases made between April 1st 2011 and March 31st, 2012; this was then compared to composite profiles of the top 10 primary conditions and/or symptoms affecting active VICS members: chronic pain/pain, arthritis/osteoarthritis, hep-c, fibromyalgia, anxiety, Crohn's/IBD/IBS, HIV/AIDS, MS, cancer, and depression. These conditions account for 617 (or 71%) of 907 active members, and for 66.2% of raw cannabis purchases (or 73721 of 111359 total grams) and 65.8% total cannabis-based medicine purchases for that period.

Findings

This data is equivalent to 907 patient years of medical cannabis purchases, and significant differences in strain preferences, methods of ingestion, and amounts purchased were identified between primary condition and/or symptom groups. Average amount of cannabis purchased per annum is 122.8 gr. (or 0.34 gr. per day); however, cancer patients only purchased 68.8 gr. per year (or 44% less than the average), while those suffering from depression purchased 156 gr. per year (27.3% more than the average patient). Significant differences in methods of ingestion were also noted. For example, those affected by HIV/AIDS (n=44) purchasing 78.4 ml of oromucosal spray per year, which is nearly triple the 28.7 ml average.

Discussion

Having a better understanding of how patients benefit from different strains or methods of ingestion would be useful in designing more symptom-specific treatments and dosages for primary indications. These findings suggest that more research needs to be conducted to identify the interplay between the >400 chemicals in the cannabis plant, different methods of ingestion, and the many conditions or symptoms that might benefit from cannabis for therapeutic purposes.

HEALTH EFFECTS OF USING *CANNABIS* FOR THERAPEUTIC PURPOSES: PATIENT PERCEPTIONS OF BENEFITS AND RISKS

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While cannabis remains an illegal substance in Canada, the Canadian government has created a regulatory framework for therapeutic use to provide a mechanism for legal access to cannabis for individuals with diagnosed medical conditions and debilitating symptoms. To date, there has been a remarkable lack of research into how individuals make sense of diverse and seemingly conflicting messages describing the health benefits and risks of using cannabis for therapeutic purposes (CTP). Such insights are essential to understanding why CTP is utilized, what benefits and risks are perceived to be associated with CTP, and how public health messages need to be framed to best meet the needs and contextual realities of potential and current users.

This qualitative study of 23 individuals who self-report therapeutic use of cannabis describes how they perceive its health effects. Descriptions of the health benefits of cannabis for therapeutic purposes included cannabis as life preserving, a disease therapy, a medicine for the mind, a means for self-management, and a way to manage addiction. Self-management of risks focused on the potential effects of excessive use, smoking-related risks, and purchasing precautions.

On the basis of these finding, CTP users would benefit from the following information about CTP: how to safely titrate dosage, how to manage potential health risks, how to assess for dependency, and the importance of communicating to one's primary care provider the use of CTP and its perceived impact on health and conventional treatment protocols. As evidence related to the health effects of cannabis in the treatment and management of select illnesses emerges, harm reduction messages will need to be balanced with information regarding the potential health benefits of cannabis to support informed decision making.

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SUBSTANCE USE AMONG MEDICAL *CANNABIS* USERS: SUBSTITUTING *CANNABIS* FOR ALCOHOL AND OTHER PSYCHOACTIVE SUBSTANCES

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Background: With over 628 responses so far, the Cannabis Access for Medical Purposes Survey (CAMPS) is the largest polling of Canadian medical cannabis patients to date. This study examined the use of cannabis as a substitute for alcohol and other psychoactive substances among medical cannabis users with and without histories of substance use treatment.

Methods: Participants were 476 self-selected Canadian adults who endorsed current medical cannabis use and reported substituting cannabis for alcohol and/or psychoactive substances in a 414 question survey that queried attitudes and behaviors associated with medical cannabis use, substance use treatment history, and cannabis substitution effect.

Results: Past or current treatment for substance use was reported by 16% (n=74) of the sample, of whom 65% (n=48) reported substituting cannabis for alcohol and/or psychoactive substances 49% (n=36) reported substituting for alcohol, 53% (n=39) reported substituting for psychoactive substances, and 36% (n=27) reported substituting cannabis for both. In general, rates of endorsement substitution did not differ greatly between individuals with and without treatment histories; 54% (n=215) of individuals with no history of treatment reported substituting cannabis for alcohol and/or psychoactive substances. Patients with cancer, HIV/AIDS, gastrointestinal disorders, epilepsy, mental health, arthritis and chronic pain reported similar rates of substitution, however only 30% of MS patients used cannabis as a substitute for alcohol and/or psychoactive substances (n=6).

Conclusions: The findings of this cross-sectional study suggest that cannabis is perceived to play an important role in reducing use of alcohol and psychoactive substances among medical cannabis patients with and without histories of treatment for problematic substance use. These findings have implications for treatment programs that encourage abstinence from cannabis in the process of reducing the use of other substances. The finding that cannabis substitution was consistent across illnesses and generalized to individuals with no history of substance use treatment suggests that cannabis use may play a role in moderating substance use across a broad range of adults, and highlights the importance of conducting further research on the complex interaction between cannabis use and the use of other psychoactive substances.

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SUBJECTIVE EFFECTS OF VAPORISED THC, CBD AND THC+CBD FROM A RANDOMISED CONTROLLED TRIAL IN HUMANS

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Introduction: The acute effects on brain function of two primary constituents of cannabis, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), have gained interest in recent years. Most previous administration studies in humans have focused on the independent effects of THC and CBD; few have examined combined effects, some administering THC via a vaporiser route, while CBD has been administered in oral form only. Here we report preliminary data from a clinical randomised controlled trial in humans investigating the acute effects of vaporised THC and CBD on subjective intoxication and psychotic-like symptoms in regular cannabis users and non-naïve non-user controls. We have developed protocols to vaporise CBD and examine the effects of each compound alone and in combination (THC + low CBD and THC + high CBD) in a double blind crossover, placebo-controlled design.

Method: In this ongoing study, regular cannabis users (use > 2x/month for at least 2 years; n=20) will be compared to non-naïve healthy controls (lifetime use < 20x and ≥ 5x; n=20). Participants complete 1 baseline (non-drug) session and 5 drug administration sessions in a randomised order: 1. Placebo (ethanol vapour); 2. THC alone, 8 mg; 3. High CBD alone, 400 mg; 4. THC 8 mg + Low CBD 4 mg; and 5. THC 12 mg + High CBD 400 mg. Drugs are dissolved in ethanol solution and vaporised using a Volcano® Vaporiser. To maintain intoxication across the experiment duration, two top up doses are administered approximately 1 and 2 hours after the main dose (1. Placebo; 2. THC 2 mg; CBD 100 mg; 4. THC 2 mg + CBD 1 mg; 5. THC 2 mg + CBD 100 mg). Subjective effects are measured using a Visual Analogue Scale (VAS; Bowdle et al. 1998; including a 1-10 scale of intoxication), the Clinician Administered Dissociative States Scale (CADSS), and the Psychotomimetic States Inventory (PSI) at different time points until at least 3 hours after first drug administration. Preliminary data on a portion of the sample are reported here.

Results: THC induced significant intoxication (M: 7.0, SD: 2.4, VAS scale 1-10), modest changes in Internal and External Perception as measured by the VAS, perceptual distortion as measured by the PSI and feelings of derealisation as measured by the CADSS. Preliminary results suggest that low dose CBD may potentiate some of these effects when combined with THC, while the effects of high dose CBD when combined with THC were less clear. Interestingly, high dose CBD alone appeared to induce moderate feelings of intoxication relative to placebo, but few perceptual changes. Results of blood assays for level of each compound and metabolites will also be reported in relation to these subjective effects measures. Data from n=12 will be reported.

Discussion: The preliminary results of this ongoing study show that 8 mg THC induces considerable intoxication and perceptual distortion which may be differentially altered by co-administration of CBD at low versus high doses. High dose CBD alone also caused moderate feelings of intoxication, albeit at much lower levels than THC. Implications for understanding the psychoactive interaction of these compounds will be discussed.

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EFFECT OF MODERATE ALCOHOL CONSUMPTION AND AMBIANCE DURING A MEAL ON MOOD AND PLASMA ENDOCANNABINOIDS IN HUMANS

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The endocannabinoid system plays an important role in mood and stress. However, data are derived from animal studies mainly, whereas human observations are scarce. The current study examined associations of plasma levels of the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) and the related acylethanolamides (NAEs) after consumption of a meal with or without moderate amounts of alcohol in either a pleasant or an unpleasant ambiance. Twenty-eight healthy women participated in a randomized cross-over study. They consumed sparkling white wine (340 mL; ~30 g alcohol) or alcohol-free sparkling white wine (340 mL; <0.5 g alcohol) as part of a standard evening meal in a room with a created pleasant or an unpleasant ambiance.

Subjects scored higher on the happiness scale and lower on the depression scale in the pleasant ambiance as compared to the unpleasant ambiance. Alcohol consumption increased happiness in the pleasant ambiance, and even more in the unpleasant ambiance. Plasma levels of 2-AG and AEA were not influenced by ambiance or alcohol, whereas palmitoylethanolamide (PEA) and stearoylethanolamide (SEA) increased more after the meal in the unpleasant ambiance than in the pleasant ambiance. Changes over time in NAEs correlated with changes in serum free fatty acids (FFA). These are the first data to demonstrate that plasma NAEs are responsive to an unpleasant ambiance. Although this study shows an effect of ambiance on both NAEs and mood states, a consistent pattern of associations between endocannabinoids, NAEs and mood states could not be observed.

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ENDOCANNABINOIDS ENHANCE MEMORY CONSOLIDATION OF EMOTIONALLY AROUSING EXPERIENCES: KEY ROLE OF THE BASOLATERAL COMPLEX OF THE AMYGDALA

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Extensive evidence indicates that cannabinoids, either administered exogenously or released from endogenous sites, have pronounced effects on learning and memory (Marsicano and Lafenetre, Curr Top Behav Neurosci 1 (2009) 201-230; Campolongo et al., Proc Natl Acad Sci U S A 106 (2009) 4888-4893; Wilson and Nicoll, Science 296 (2002) 678-682). It is well known that Cannabinoid type 1 receptors (CB1) are highly expressed in the basolateral complex of the amygdala (BLA), the medial prefrontal cortex (mPFC) and the hippocampus (Hipp) (Tsou et al., Neuroscience 83 (1998) 393-411), three brain regions crucially involved in the modulation of memory for emotionally arousing experiences (Roozendaal and McGaugh, Behav Neurosci 125 (2011) 797-824).

In this study we investigated the role of the endocannabinoid system in the BLA, Hipp and mPFC during memory consolidation of an aversive training experience. To examine whether the endocannabinoids anandamide and 2-arachidonoylglycerol are released within these brain regions after a stressful event, male adult Sprague-Dawley rats were trained on an inhibitory avoidance task at different footshock intensities (no, low, mild footshock) and tissue of these three brain areas was collected at different time points (10, 30, 60 min) after training. We found that rats trained with a mild footshock intensity presented higher levels of anandamide in the amygdala, Hipp and mPFC than rats exposed to the context in the absence of any footshock. In a second set of experiments we investigated whether exogenous manipulation of the endocannabinoid system could modulate memory consolidation. To this aim, the anandamide hydrolysis inhibitor URB597 was infused bilaterally into the BLA, Hipp or mPFC immediately after inhibitory avoidance training. We found that URB597 administered in any of the three brain regions enhanced memory consolidation. Posttraining infusion of a non-impairing dose of the cannabinoid receptor antagonist AM251 blocked the memory-enhancing effects induced by URB597, thus indicating that URB597 enhances memory consolidation *via* indirect activation of cannabinoid receptors. In the last set of experiments we investigated the role of the BLA in modulating the endocannabinoid response to this aversive training experience. To this aim we first measured endocannabinoid levels in the Hipp and mPFC of rats with bilateral lesions of the BLA trained on the inhibitory avoidance task. Furthermore, in different experimental groups, we infused post-training URB597 into the Hipp or mPFC of bilaterally BLA-lesioned rats subjected to the inhibitory avoidance task. We found that in BLA-lesioned rats there was neither an increase of anandamide levels nor an enhancement of memory consolidation when URB597 was administered in the Hipp or mPFC. Our findings provide evidence that the endocannabinoid system modulates the consolidation of memory for emotionally arousing experiences and, interestingly, that the BLA plays a crucial role in modulating endocannabinoid responses after a stressful event.

ENDOCANNABINOIDS AND MEMORY: PROLONGING ACTIVATION OF CB1 RECEPTORS IMPAIRS ENCODING AND MNEMONIC PERFORMANCE

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Activation of the brain cannabinoid (CB1) receptor produces a clear modulation of memory in rats performing a Delayed-Nonmatch-to-Sample (DNMS) task. Exogenously applied CB1R agonists reduced behavioral performance and suppress the neural response which encodes task-critical information [Hampson & Deadwyler, J. Neurosci. 2000]; however, identifying the precise role of endocannabinoids either via CB1R antagonism [Deadwyler et al. Behav. Pharmacol. 2007] or via direct manipulation of endocannabinoid metabolism [Hampson & Deadwyler ICRS 2010] has proven to be much more difficult. Given the rapid metabolism and low concentrations of endocannabinoids, the best means of studying endocannabinoid actions is to suppress metabolism/reuptake of endocannabinoids, thus prolonging activation of CB1Rs. However, results from other laboratories have had mixed success – both positive [Robinson et al. Psychopharmacology 2008] and negative [Falenski et al. Neuropsychopharmacology 2010] – with mimicking exogenous cannabinoid effects at CB1Rs via manipulation of endocannabinoids.

This study shows that inhibition of anandamide metabolism by inhibition of fatty-acid-amide-hydrolase via URB597, or by inhibition of MAG-lipase via URB602, produces mnemonic impairment in two ways: (A) DNMS performance is reduced at long delays by both URB597 and URB602. (B) Hippocampal neuronal encoding of Sample phase information in the DNMS task is also suppressed by both URB597 and URB602. Pharmacological suppression of endocannabinoid degradation, prolonging CB1R receptor activation, impairs the hippocampal encoding of task-relevant information the Sample. This impairment is consistent not only with effects of exogenous cannabinoid activation of CB1R on hippocampal neural activity, but also consistent with effects of both exogenous cannabinoids and URB suppressing DNMS behavior at long delays. These results will be discussed in terms of a role for endocannabinoids and CB1Rs in the processing of memory under both normal and abnormal circumstances.

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REPEATED EXPOSURE TO Δ9-TETRAHYDROCANNABINOL (THC) IMPAIRS EXTINCTION LEARNING IN MICE

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Extinction is an active learning process that requires the suppression a learned response when reinforcement is withheld. Disruption of cannabinoid CB1 receptor signaling, through CB1 receptor antagonism or genetic deletion of the CB1 receptor, has been shown to impair extinction learning in conditioned fear paradigms. Repeated administration of cannabinoid agonists, including the primary active constituent of marijuana Δ9-tetrahydrocannabinol, produces changes in cannabinoid receptor signaling, including CB1 receptor desensitization and downregulation. This suggests that repeated administration of cannabinoid agonists disrupts normal functioning of the endogenous cannabinoid system and may impair extinction processes. Given that CB1 receptor antagonism impairs consolidation of extinction learning in the contextual conditioned freezing paradigm, we evaluated whether repeated THC administration would also impair extinction learning in this paradigm. We first established that contextual conditioned freezing occurs 7 days after 3 pairings of shock with the context and that conditioned freezing could be extinguished in a 30 min session in mice. Additionally, consolidation of extinction in a test session 24 h after the extinction session was observed. We then tested whether different doses of THC given repeatedly would impair extinction learning or the consolidation of extinction learning. We found that higher doses of THC administered repeatedly delay extinction learning and disrupt consolidation of extinction learning as compared to vehicle-treated mice. In contrast, lower doses of THC administered repeatedly produce vehicle-like performance in the 30 min extinction session but disrupt consolidation of extinction learning. The present data indicate that repeated use of cannabinoid agonists, such as marijuana, may lead to disrupted extinction learning. Moreover, stress-related disorders, such as post-traumatic stress disorder (PTSD), depression, and anxiety disorders are characterized by deficits in extinction learning, suggesting that cannabinoid use may exacerbate or contribute to these disease states.

PARADOXICAL IMPAIRMENT IN FEAR EXTINCTION AFTER AUGMENTATION OF 2-ARACHIDONOYLGLYCEROL-MEDIATED ENDOCANNABINOID SIGNALING

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Impairments in fear extinction are thought to be central to the psychopathology of posttraumatic stress disorder (PTSD). The endogenous cannabinoid (eCB) anandamide has been heavily implicated in the fear extinction learning leading to the suggestion that augmentation of brain anandamide levels could represent a viable treatment strategy for PTSD. However, exogenous cannabinoids at high doses can worsen anxiety and impair fear extinction suggesting a more comprehensive evaluation of the role of eCB signaling, including the effects of the most abundant brain eCB 2-arachodonoylglycerol (2-AG), could clarify role of eCB signaling in fear modulation. Here we utilized the monoacylglycerol lipase inhibitor JZL-184 to selectively augment brain 2-AG levels, combined with and an auditory fear-conditioning paradigm to test the hypothesis that 2-AG-mediated eCB signaling modulates fear extinction learning in mice. In contrast to anandamide signaling, we show that JZL-184 robustly *prevents* fear extinction in a CB1 receptor-dependent manner without affecting non-specific freezing behavior or the acquisition of conditioned fear responses. This effect was also observed in over-conditioned mice environmentally manipulated to re-acquire fear extinction, and using a pure contextual fear-conditioning paradigm. Moreover, we elucidate a short ~3 day temporal window during which 2-AG augmentation impairs extinction behavior. These data highlight the emerging complexity of eCB signaling in the regulation of emotional learning processes and elucidate paradoxical extinction-impairing effects of pharmacological 2-AG augmentation. Ongoing studies are aimed at determining the synaptic mechanisms by which 2-AG augmentation modulates conditioned fear extinction.

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CANNABINOIDS AND STRESS: A MODEL FOR THE COMORBIDITY OF DRUG ABUSE AND POST-TRAUMATIC STRESS DISORDER

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Traumatic stress has been previously demonstrated to have a deleterious effect on learning and memory [Harvey et al, Brain Research 2003, Kohda et al, Neuroscience 2007, Wang et al, Physiology Research 2010]. Exposure to an animal model of traumatic stress - the Single Prolonged Stress (SPS) procedure [Liberzon and Young, Psychoneuroendocrinology 1997] intense stress has also been demonstrated to cause physiological and structural changes in the hippocampus, an area critical to these cognitive faculties [Eagle et al, Neuroscience Research 2012, Knox et al, Neuroscience 2012, Villareale et al, Biological Psychiatry 2002]. Abuse of marijuana and synthetic cannabinoids is becoming increasingly prevalent in populations which have experienced post-traumatic stress [Loeffler et al, Military Medicine 2012]. This pronounced comorbidity of drug abuse with history of traumatic stress suggests that, aside from escapism, abused drugs such as marijuana may activate compensatory neural mechanisms which alleviate the deleterious effects of the stress experience. This suggests that it may be possible to intervene in the effects of an intense stressor by altering cannabinoid receptor functions shortly after the stress. Previous studies have successfully blocked some of the effects of SPS by administering WIN 55,212-2 one day after the procedure [Ganon-Elazar and Akirav Neuropsychopharmacology 2012]. Further, we have demonstrated that animals exposed to SPS stress show deficits in learning and memory, and hypothesize that these effects can be blocked by the administration of a cannabinoid receptor agonist shortly after exposure.

Preliminary data collected in this lab suggests that rodent performance on, the Delayed Non-Match to Sample (DNMS) task is sensitive to intense stress. Exposure to a single session of forced swim (partial SPS) caused deficits in performance on the DNMS task, relative to non-exposed controls. Further, rats exposed to the full SPS procedure show delayed progression through the various stages of training on the task, relative to non-stress exposed controls. A separate group of animals was exposed to the SPS and administered a cannabinoid receptor agonist 24 hours later, to assess whether cannabinoid receptor activation alters effects of SPS on acquisition and performance of the DNMS task. Results will be discussed in terms of characterizing the role of cannabinoids to modulate effects of traumatic stress on learning and memory.

CIRCULATING ENDOCANNABINOIDS IN HEALTHY PROBANDS AFTER INTRAVENOUS CRH INJECTION

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Preclinical evidence indicates that stress activates the endocannabinoid system, which is thought to negatively modulate stress-induced HPA-axis activation during stress-recovery. The interaction mechanisms between HPA-axis activity, noradrenergic activity and the endocannabinoid (eCB) system in humans are not completely understood. This study aimed to investigate the effects of intravenous corticotropin-releasing hormone (CRH) as a selective HPA-axis activator on circulating endocannabinoids using a double-blind between-subjects design.

156 young adults (23.66±0.47 years) participated in a single-session design study, in which they intravenously received either 100 µg CRH or a placebo. Blood samples for eCBs, cortisol assays and interleukin 6 and TNF alpha analyses as well as cardiovascular and subjective measures were obtained before and at regular intervals after CRH or placebo infusion. 70 minutes after injection, probands underwent a startle paradigm, in which they were exposed to aversive tones via headphones for six minutes. Probands were also subjected to a cognitive testing 100 minutes after CRH or placebo injection. Serum levels of the endocannabinoids arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are currently determined using isotope-dilution liquid chromatography/mass spectrometry.

To date, endocannabinoid concentrations of 20 CRH subjects and 12 placebo subjects are available for analysis. 2-AG and AEA concentrations did not significantly differ between the groups. Anxiety levels (Visual Analogue Scale) were significantly negatively correlated with 2-AG concentrations in all subjects. In women (N=12), levels of cortisol were significantly negatively correlated with 2-AG concentrations.

These preliminary results indicate that components of the HPA-axis might directly interact with the eCB system and that 2-AG concentrations possibly reduce feelings of anxiety in humans. These pilot data will be followed up upon in the total sample, which is hoped to elucidate the function and potential therapeutic benefit of endocannabinoid mediators in stress-associated psychiatric disorders.

SEX-DEPENDENT EFFECTS OF STRESS ON LIMBIC ENDOCANNABINOID FUNCTION

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Several studies have demonstrated that there are sex- and hormone-dependent differences in the endocannabinoid system between males and females. For example, CB1 receptor binding has been shown to be lower in the hippocampus and hypothalamus of female rodents relative to males, while there is increased CB1 receptor densities in the amygdala of adult females versus males. To date, although there have been relatively few studies focusing on sex differences in the endocannabinoid system, there is a relative paucity of studies looking at sex differences in the limbic endocannabinoid system after acute and repeated stressors. As endocannabinoid activity is known to be important for the regulation of several aspects of acute and repeated stress, such as HPA axis activity, anxiety and dendritic plasticity—the determination of sex differences in the effects of stress on endocannabinoid function is of particular interest given that there are profound sex differences in the effects of stress on all of these domains.

Given the basal differences in the endocannabinoid system components between males and females, we sought to investigate if there were differences in the endocannabinoid system between males and females after acute and repeated restraint stress. Approximately 70 day old male and female Sprague Dawley rats were used and they were exposed to either one or ten repeated 30 minute restraint sessions. After the final restraint, animals were rapidly decapitated and limbic regions were extracted to perform CB1 receptor radioligand binding assays and FAAH activity assays. Within the hippocampus, the maximal hydrolytic activity (Vmax) of FAAH was increased in both males and females in response to acute stress, although this response habituated back to baseline levels following repeated stress. Interestingly, overall females had lower levels of FAAH activity in the hippocampus relative to males. Within the amygdala, females exhibited a similar shift in FAAH activity, such that it was increased by acute stress, but this effect habituated following repeated stress. Males, interestingly, exhibited a small, but non-significant increase in FAAH activity in the amygdala following acute stress, but an increase following repeated stress. Consistent to previous reports, under basal conditions females had lower CB1 receptor binding sites in the hippocampus, and higher CB1 receptor binding sites in the amygdala, relative to males. In response to repeated stress, male rats exhibited a reduction in CB1 receptor binding within the hippocampus, but an increase in CB1 receptor binding within the amygdala. Female rats, on the other hand, exhibited an upregulation of CB1 receptor binding in the hippocampus following repeated stress, and no change in CB1 receptor binding sites within the amygdala. Ongoing research is determining sex-dependent changes in endocannabinoid content following acute and repeated stress, but these data indicate that limbic endocannabinoid function is sexually dimorphic and responds differently to stress exposure.

ENHANCED ANANDAMIDE SIGNALING BY FATTY ACID AMIDE HYDROLASE BLOCKADE PROMOTES ACTIVE COPING WITH ENVIRONMENTAL CHALLENGES

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We investigated the effects of enhanced endocannabinoid signaling in behavioral tests and under conditions relevant to coping styles. The fatty acid amide (FAAH) inhibitor URB597, which enhances brain levels of the endocannabinoid anandamide, made rats unresponsive to environmental challenges in the elevated plus-maze test, and abolished temperature-dependent behavioral changes in mice submitted to the forced swimming test. These changes reflect an active coping style, as active copers base their behavior on routines that are weakly influenced by environmental stimuli. URB597 also increased active coping behaviors in the rat tail-pinch test and the mouse back-test, providing further evidence that enhanced anandamide signaling shifts the behavior of rodents towards more active coping styles. In addition, URB597 administration, coincident with uncontrollable electric shocks, promoted more active behavioral responses to the shocks and reduced long-term emotional disturbances induced by contextual reminders. These findings suggest a new way endocannabinoids can modulate behavior. In addition to, or instead of, controlling particular behaviors, endocannabinoids appear to shape the general strategy individuals adopt to cope with challenging situations. Our data also suggests how the endocannabinoid system can be targeted for therapeutic intervention in stress-related diseases.

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A ROLE FOR ENDOCANNABINOID SIGNALING IN THE STRESS RESPONSE OF WILD BIRDS

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The acute stress response is generally assumed to be adaptive – a physiological response to a noxious stimulus that enables survival in the wild. Endocannabinoid (eCB) signaling in the mammalian brain modulates the release of glucocorticoids, a critical component of the acute stress response. Despite our assumption of the adaptive value of the glucocorticoid response to stress and our knowledge of the role of eCBs, very little is known about how the eCB signaling regulates the stress response system in wild animals. Further, there has been no described role for eCB signaling in the stress response of avian species. Here we will describe evidence for eCB signaling in the neural stress response system in a wild bird species, the European starling (*Sturnus vulgaris*). We have cloned several components of eCB signaling including the type-1 cannabinoid receptor (CB1), fatty acid hydrolase (FAAH), and diacylglycerol lipase (DAGL) from brain tissue. The gene sequences show relatively high homology (e.g. greater than 81% for CB1) with mammalian models (including humans). In addition we have mapped CB1 mRNA expression in the brain using in situ hybridization, demonstrating localization of CB1 expression to the paraventricular nucleus of the hypothalamus, the hippocampus, and the avian amygdala. The high homology of the gene sequences and the expression patterns suggest a role for eCBs in the avian stress response, consistent with the eCB system and its function being evolutionarily conserved. To determine the functional capacity of this signaling pathway, we injected increasing doses of AM251, a CB1 antagonist. Higher doses of this compound increased circulating glucocorticoid concentrations, suggesting that eCBs may play a predominantly inhibitory role on glucocorticoid release, similar to that, which has been demonstrated in mammals. Overall, our data provide the first evidence that eCB signaling may play an important role in the avian stress response; further demonstrating the conserved nature of this signaling pathway in the physiological stress response.

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ADOLESCENT CANNABINOID EXPOSURE SEX DEPENDENTLY ALTERS ADULT STRESSS RESPONSIVITY AND AMPHETAMINE SENSITIZATION

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Evidence indicates adverse effects of cannabinoid exposure during adolescence on neuroplasticity, emotional behaviour, cognition and reward sensitivity in adult rats. We investigated whether escalating doses of the cannabinoid CB_1 receptor (CB_1R) agonist, HU-210, in adolescence would affect adult stress responsivity and amphetamine sensitization in male and female Sprague-Dawley rats. Escalating doses of HU-210 (25, 50, and 100 μg/kg), or vehicle were administered from postnatal day (PND) 35 to 46. Animals were left undisturbed until PND 75, in which hypothalamic-pituitary-adrenal (HPA) axis reactivity to an acute restraint stress (30 min; PND 75) and behavioural sensitization to d-amphetamine sulfate (1-2 mg/kg; PND 105-134) were assessed. Adolescent HU-210 administration induced significantly higher peak corticosterone levels despite comparable basal levels. This effect was more pronounced in males than females. Furthermore, adolescent cannabinoid treatment resulted in significantly higher stereotypy scores, indicating that adolescent HU-210 treated animals exhibited greater propensity to amphetamine sensitization. This effect was more prominent in the female than male rats. Thus, increased CB_1 receptor activation during adolescence results in long-term, sex dependent alterations to HPA axis stress responsivity and amphetamine sensitization.

ENHANCED 2-ARACHIDONOYL-GLYCEROL SIGNALING AFFECTS HYPOTHALAMO-HYPOPHYSIS-ADRENAL-AXIS-REGULATED BEHAVIORAL RESPONSES TO CHANGES IN ENVIRONMENTAL AVERSIVENESS

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Previous studies showed that the behavioral effects of enhanced 2-arachidonoyl-glycerol (2-AG) signaling induced by monoacilglycerol-lipase (MAGL) blockade highly depend on the environmental context. Furthermore, it is intriguing that biochemical effects of the newly developed MAGL inhibitor, JZL184, were reported to develop rapidly while the behavioral effects of the compound were only showed much later. Investigating the context-dependency and temporal dynamics of the behavioral effects of MAGL blockade, we found, that CD1 mice receiving MAGL inhibitor treatment showed hypermotility and decreased anxiety in unfamiliar environment, however, these changes were only developed at least 80 min after treatment. In contrast, we showed that MAGL treatment rapidly and transiently dampened injection-induced increases in body temperature and changes in locomotion patterns in familiar environment. Since environmental stressfulness affected MAGL blockade-induced behavioral changes, we investigated the possible role of the hypothalamus-hypophysis-adrenal-axis (HPA-axis) in these effects. We showed for the first time, that MAGL blockade rapidly increased basal cortciosterone (CORT) levels in CD1 mice, while leaving stress-induced levels unaltered. We also found that MAGL blockade-induced increases in basal HPA-axis activity decreased anxiety, as CORT-synthesis inhibitor Metyrapone partly abolished anxiolytic actions of MAGL blockade. In contrast, locomotor activity-increasing effects of MAGL blockade were independent of CORT synthesis. Based on these findings, we can assume that 2-AG signaling represents a highly sensitive, fine tuning mechanism in the central nervous system, which interacts with the HPA-axis in the control of how environmental changes affect behavioral responses.

CONDITIONAL MUTAGENESIS REVEALS A DIRECT CB1 RECEPTOR-MEDIATED CONTROL ON SEROTONERGIC NEURONS

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Serotonergic projections are sent from the brainstem raphe nuclei to almost all other brain regions. In a previous study, we have shown that 7-22% of these neurons express the cannabinoid receptor type 1 (CB1). Presynaptic CB1 receptor influences physiological functions by decreasing the release of various neurotransmitters. Accordingly, we proposed that CB1 receptor on serotonergic cells might play a role in serotonin-regulated physiological functions by decreasing the release of serotonin. To this end, we generated an inducible conditional CB1 receptor knock-out mouse lacking the receptor specifically in serotonergic cells. In detail, we exploited the Cre/loxP system by crossing mice carrying a tamoxifen-inducible Cre recombinase under the control of regulatory elements of the tryptophan hydroxylase type 2 (TPH2) gene with mice possessing a floxed CB1 receptor gene.

To avoid possible developmental consequences of early gene deletion, Cre recombinase expression was activated by injecting 10 week old $CB1^{f/f;TPH2\text{-}CreERT2}$ mice and their control littermates ($CB1^{f/f}$) with 1 mg tamoxifen/day for five days. Three weeks after the last injection, mice were subjected to a battery of behavioral assays. The lack of CB1 receptor on serotonergic neurons induced an anxiogenic-like phenotype under stronger aversive conditions and caused a decrease in social interaction: Thus, mutants took longer to accustom to higher light conditions in the novelty suppressed feeding test and displayed less social interaction in the resident/intruder and sociability test as compared to control animals. Furthermore, $CB1^{f/f;TPH2\text{-}CreERT2}$ animals weigh significantly less than their control littermates. Taken together, we could show for the first time that serotonergic neurons are under direct control of the CB1 receptor and that its expression is of physiological importance. A behavioral effect of the Cre recombinase can be excluded, as additional control experiments with $CB1^{+/+;TPH2\text{-}CreERT2}$ animals did not cause any behavioral changes as compared to their $CB1^{+/+}$ littermates.

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IMPAIRED 2-AG SIGNALING IN HIPPOCAMPAL GLUTAMATERGIC NEURONS ABOLISHES DEPOLARIZATION-INDUCED SUPPRESSION OF EXCITATION AND INCREASES ANXIETY-LIKE BEHAVIOR

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Endocannabinoids are produced and released on demand and thus endocannabinoid degrading enzymes control endocannabinoid signaling. Monoacylglycerol lipase (MAGL) is the primary degrading enzyme of the endocannabinoid 2-arachidonoylglycerol (2-AG) and abundantly expressed in the hippocampus. In the present study, we overexpressed MAGL specifically in glutamatergic pyramidal neurons of the mouse hippocampus by adeno-associated virus-mediated gene transfer and analyzed concomitant effects at the cellular and behavioral level. This genetic modification resulted in highly increased MAGL activity accompanied by a 50% decrease in 2-AG levels in the hippocampus without affecting the content of arachidonic acid and other endocannabinoids. Elevated MAGL protein levels at glutamatergic terminals eliminated depolarization-induced suppression of excitation (DSE) in hippocampal CA1 pyramidal neurons, while depolarization-induced suppression of inhibition (DSI) was not affected. This result suggests that presynaptic MAGL precisely controls the activation of CB1 receptor located on the same synaptic site by quenching postsynaptically synthesized and released 2-AG molecules prior to 2-AG-mediated stimulation of perisynaptic CB1 receptor. At the behavioral level, the absence of 2-AG-mediated hippocampal DSE manifested in an increased anxiety-like behavior. Surprisingly, mice overexpressing MAGL exclusively in hippocampal pyramidal neurons showed no changes in aversive memory formation and in seizure susceptibility. These results suggest that 2-AG-mediated hippocampal DSE is essential for adapting to aversive situations, but not required to form aversive memory and to protect against kainic acid-induced seizures.

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DEVELOPMENT OF PERIPHERALLY SELECTIVE ANTAGONISTS OF CANNABINOID RECEPTOR 1: RATIONAL SYNTHESIS AND CHARACTERIZATION OF RIMONABANT (SR141716A) ANALOGS

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Cannabinoid receptor 1 (CB1R) antagonists are potential therapeutics for several important diseases such as obesity, drug addiction, diabetes, and liver disease. Regrettably, central nervous system (CNS) related adverse effects including depression and suicidal ideation were reported with non-tissue selective first generation antagonists of CB1R. However, recent studies indicate that targeting peripherally expressed CB1R with non-CNS permeable compounds is an attractive strategy that can lead to drugs that are devoid of adverse CNS-related side-effects. Previously, we have reported the development of peripherally selective CB1R antagonists based on rimonabant (Bioorg Med Chem Lett. 21:5711-4; J Med Chem. 55:2820-34). Here we report results of our latest efforts at developing peripherally selective CB1R antagonists based on the rimonabant scaffold. Because our previous data indicated that compounds with high topological polar surface areas (TPSA) do not cross the blood brain barrier (BBB), these compounds were designed to have TPSAs similar to our previous compounds. However, these new compounds were designed to explore SAR in a region not thoroughly explored in our previous work. A calcium mobilization assay was used to measure CB1R activity. Radioligand displacement at CB1R and CB2R was used to determine binding affinities and receptor selectivity of selected compounds. Highly potent (Ke ranging between 0.3-30 nM at CB1R) and selective (>100-fold selective for CB1R versus CB2R) analogues with high TPSAs (TPSA>90) were identified. An *in vitro* model of BBB permeability was used to characterize these compounds further. Several compounds with limited BBB permeability were identified. *In vivo* efficacy studies are planned with some of these compounds.

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HIGHLY PURIFIED AJULEMIC ACID IS A CB2 AGONIST WITH REDUCED CB1 ACTIVITY

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Background. Ajulemic acid, a synthetic analog of the THC metabolite 11-carboxy-THC, was designed to be a potent cannabinoid agonist free of the psychotropic effects typical of most cannabinoid agonists. However, a number of studies have yielded conflicting findings on the occurrence of these psychotropic effects. To help resolve this issue, we have prepared highly purified ajulemic acid (>99%) using a new synthetic method which has a step in which the 11-methyl is completely oxidized to the carboxylic acid. We compared the cannabinoid receptor binding affinity of the new highly purified ajulemic acid to those obtained from previous synthetic methods.

Results. While CB2 binding did not vary greatly among the different ajulemic acid samples, the CB1 binding showed a wide range of affinities. The highly purified product (JBT-101) had the lowest affinity for CB1 while the original preparation obtained from The Hebrew University (HU-239) showed the strongest affinity for CB1.

Conclusions. The CB1 binding activity of ajulemic acid is dependent on its purity and the synthetic method used. This is best explained by ajulemic acid made with prior synthetic methods containing potent, low abundance impurities that contribute to the increased CB1 binding activity. The most likely candidate for this impurity is HU-210 the 11-OH intermediate of ajulemic acid reported in the literature to be generated using the older synthetic methods. Based on these new results, we conclude that all reports on ajulemic acid to date must take into account the presence of CB1 activity due to potent, low abundance impurities in the material used.

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DESIGN AND SYNTHESIS OF NOVEL TRUXILLIC ACID BASED FABP INHIBITORS

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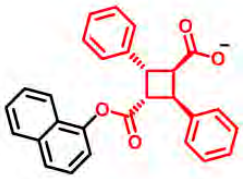
The endocannabinoid anandamide (AEA) and 2-arachidonoylglycerol (2-AG) serve as endogenous regulators of cannabinoid receptor potentiation in numerous physiological processes including: appetite, pain, inflammation, and reproduction. Regulation of endocannabinoid metabolism is largely regulated by fatty acid amide hydrolase (FAAH) an enzyme located in the cytosol. Furthermore, inhibition of endocannabinoid inactivating enzymes potentiates endocannabinoid signaling and produces antinociceptive and anti-inflammatory effects. Thus, a significant amount of attention has been placed on the discovery of selective inhibitors of FAAH.

Interestingly, upstream from FAAH, transport of endocannabinoids through the cytosol has become in promising new target for drug discovery. We have shown that selective inhibition of fatty acid binding proteins (FABPs) can bottleneck endocannabinoid transport further preventing endocannabinoid breakdown. Furthermore, unlike FAAH which is largely distributed throughout the body indiscriminately, FABPs are very tissue specific giving a promising therapeutic window in which to develop inhibitors. We have shown previously truxillic acid based mono esters such as (SB-FI-26) reduced AEA inactivation *in vitro* and produced endocannabinoid-mediated antinociceptive effects *in vivo*. Interesting this core truxillic acid structure is the prominent structural core of the natural product (-)-Incarvillateine known to produce graded inhibition of pain and inflammation *in vivo*.

The design and synthesis of mono-functionalized truxillic acid analogues is described. Additionally described is our current biological evaluation of several of these analogues against various FABPs (FABP5, FABP7, FABP3).

(-)-Incarvillateine



SB-FI-26

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STRUCTURAL BASIS FOR SUBSTRATE-SELECTIVE INHIBITION OF CYCLOOXYGENASE-2 BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Cyclooxygenase-2 oxygenates arachidonic acid (AA) and the endocannabinoids, 2-arachidonoylglycerol (2-AG) and arachidonoylethanolamide (AEA). Previous work has shown that some, but not all, non-steroidal anti-inflammatory drugs (NSAIDs) selectively inhibit the oxygenation of 2-AG and AEA without inhibiting AA oxygenation. This phenomenon is termed substrate-selective inhibition of COX-2 (SSIC) and it results in the elevation of endocannabinoids at sites where COX-2 is induced by pro-inflammatory or other stimuli. We have surveyed the major classes of NSAIDs to identify scaffolds that exhibit SSIC. Some, but not all, of the profens, fenamates, arylacetic acids, diarylheterocycles and oxicams selectively inhibit endocannabinoid oxygenation by COX-2.

We used a combination of structure-activity analysis, site-directed mutagenesis and X-ray crystallography to explore the molecular determinants of SSIC by the profens and arylacetic acids. The (*S*)- or (*R*)-enantiomers of the profens bind in an orthodox configuration in the COX-2 active site with their carboxyl groups ion-paired to Arg-120 at the base of the active site. Mutation of Arg-120 to Gln eliminates inhibition of endocannabinoid oxidation by the profens. The arylacetic acid, lumiracoxib, exhibits powerful SSIC. Lumiracoxib binds in an inverted fashion in COX-2 with its carboxyl group chelated by Tyr-385 and Ser-530 near the top of the active site. Mutation of Ser-530 to Ala abolishes inhibition by lumiracoxib. The *m*-methyl group of lumiracoxib causes Leu-384 to rotate out of the COX-2 active site, which creates a small hydrophobic pocket that increases binding of the methyl group. Structural modifications indicate that at least one halogen atom is required on the anilino ring of lumiracoxib analogs to effect SSIC.

Our structural and functional analysis provides a basis for the design and synthesis of more potent and selective inhibitors of endocannabinoid oxygenation by COX-2.

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STRUCTURAL REQUIREMENTS FOR POTENT DIRECT INHIBITION OF HUMAN CYP1A1 BY CANNABIDIOL

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Our recent study has shown that cannabidiol (CBD) exhibits the most potent direct inhibition of human cytochrome P450 1A1 (CYP1A1) among the CYP enzymes examined (Yamaori et al., Biochem. Pharmacol. 79 (2010) 1691-1698). However, the mechanism underlying this CBD inhibition remains to be clarified. Thus, to elucidate the structural requirements for the potent inhibition by CBD, the effects of CBD and its structurally related compounds on CYP1A1 activity were investigated with recombinant human CYP1A1. Olivetol, which corresponds to the pentylresorcinol moiety of CBD, inhibited the 7-ethoxyresorufin *O*-deethylase activity of CYP1A1; its inhibitory effect (IC_{50} = 13.8 μM) was less potent than that of CBD (IC_{50} = 0.355 μM). In contrast, *d*-limonene, which corresponds to the terpene moiety of CBD, only slightly inhibited CYP1A1 activity. CBD-2′-monomethyl ether (CBDM) and CBD-2′,6′-dimethyl ether inhibited CYP1A1 activity with IC_{50} values of 4.07 and 23.0 μM, respectively, indicating that their inhibitory effects attenuated depending on the levels of methylation on the free phenolic hydroxyl groups in the pentylresorcinol moiety of CBD. Cannabidivarin, a CBD analogue having a propyl side chain, inhibited CYP1A1 activity, although its inhibitory potency (IC_{50} = 1.85 μM) was lower than that of CBD possessing a pentyl side chain. The inhibitory effects of Δ^9-tetrahydrocannabinol and cannabielsoin (IC_{50}s ≈ 10 μM), which contain a free phenolic hydroxyl group and are structurally constrained, were less potent than that of CBDM, which contains a free phenolic hydroxyl group and is rotatable between the pentylresorcinol and terpene moieties. These results suggest that the pentylresorcinol structure in CBD may have structurally important roles in direct CYP1A1 inhibition, although the whole structure of CBD is required for overall inhibition.

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CHANGES IN THE INTESTINAL MICROCIRCULATION IN RESPONSE TO ENDOTOXIN CHALLENGE IN CNS INJURY-INDUCED IMMUNE DEFICIENCY SYNDROME (CIDS) – EFFECTS OF CANNABINOID RECEPTOR 2 INHIBITION

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Background: The intestine represents a physiological reservoir of pathogens. Disturbances of the intestinal microcirculation during severe infections/sepsis, as seen in CIDS, can contribute to morbidity and mortality by increased bacterial translocation. Aim of the experimental study was to evaluate the changes in the intestinal microcirculation during endotoxemia as model of CIDS-induced sepsis in an animal model of CIDS and the therapeutic effect of cannabinoid 2 receptor (CB2R) inhibition.

Methods: CNS injury was induced in mice by occlusion of the right common carotid artery and subsequent hypoxia (35 minutes). 24 hours after induction of CNS injury we administered intraperitoneally 5 mg/kg lipopolysaccharide (LPS). CB2R antagonist (2.5 mg/kg AM630) was administered before endotoxin challenge. Intestinal microcirculation was studied by intravital microscopy (IVM). Release of inflammatory mediators was measured by multiplex cytokine assay (TNF-alpha, IL-1,2,6,10,12, IFN-gamma, GM-CSF).

Results: In comparison to endotoxin challenged animals without CNS injury, the microcirculatory response in CIDS animals was significantly diminished (reduced leukocyte adhesion, reduced decrease in functional capillary density). Release of pro-inflammatory mediators was significantly decreased. Administration of the CB2R antagonist, AM630, further reduced the inflammatory response within the intestinal microcirculation and inflammatory mediator release in endotoxemic CIDS animals.

Conclusion: Our results obtained in the intestinal microcirculation in response to endotoxin confirm the impaired immune response after severe CNS injury (i.e. CIDS). Surprisingly, CB2R inhibition further reduced inflammatory changes in the gut in CIDS.

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UP-REGULATION OF CANNABINOID RECEPTORS IN UNTREATED CELIAC DISEASE

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Cannabinoid receptors (CBR) belong to the large superfamily of heptahelical Gi/o protein coupled receptors. Type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors act as main molecular targets of anandamide (AEA) The presence of CBR has been demonstrated in various sections of the gastrointestinal tract and a dysregulation of their expression has been reported in several gut pathologies. Here, we investigate CBR mRNA and protein as well as functional activity levels in the duodenal mucosa of untreated celiac patients (UCD), celiac patients on a gluten-free diet for at least 12 months (TCD) and control subjects (CS). Moreover, we explore the effect of the peptic-tryptic digest of gliadin on CBR expression in organ culture biopsies taken from TCD patients.

Our *in vivo* data show that mRNA and protein levels of both CB_1 and CB_2 receptors are remarkably increased in UCD mucosa compared to TCD mucosa and normal mucosa. Moreover, e*x vivo* experiments on organ culture confirm that gluten-induced damage is responsible for this increase, at least at the protein level.

In conclusion, we demonstrate an altered expression of CB_1 and CB_2, that points to the therapeutic potential of targeting CBR in patients with celiac disease.

CANNABINOID RECEPTOR MODULATION OF THE ENDOTHELIAL CELL TOLL-LIKE RECEPTOR-DEPENDENT INFLAMMATORY RESPONSE

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The cannabinoid receptor (CBR) family is composed of the G-protein coupled receptors, CB_1R and CB_2R, and potentially GPR18 and GPR55, and the transient receptor potential ion channel, $TRPV_1$. Recent reports suggest that cannabinoids may have the ability to modulate the activation of the endothelium in response to inflammatory mediators; however, it is not clear which receptors and what mechanisms are responsible for this modulation. Our initial studies aim to: 1) identify the repertoire of CBRs expressed by different human endothelial cell (EC) niches; 2) establish the cannabinoid receptors responsible for modulating EC activation by different toll-like receptor (TLR) agonists; and 3) delineate the cellular mechanisms by which CBRs modulate the endothelial TLR inflammatory response. Through qPCR analysis, we have thus far found that different human primary EC types express a very similar subset of CBRs. Additionally, by utilizing a panel of diverse cannabinoids, we discovered that the endocannabinoid N-arachidonoyl dopamine and the synthetic agonist WIN55,212-2 both diminish the TLR2- and TLR4-dependent inflammatory activation of endothelial cells, as measured by pro-inflammatory cytokine secretion. These results implicate a role for the cannabinoid system in regulating the inflammatory response to bacterial infection in the vasculature.

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PSYCHOSOCIAL FACTORS, ENDOCANNABINOIDS, AND INFLAMMATORY MOLECULES IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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The endocannabinoid system modulates neural processes and down-regulates pro-inflammatory, stress-related signals. Inflammatory-mediated processes significantly contribute to increased morbidity and mortality in hematopoietic stem cell transplantation (HCT). The relationship between inflammatory molecules and the endocannabinoid system is poorly understood, and its activity has never been examined in HCT recipients. We conducted a pilot study evaluating 19 individuals undergoing HCT for any reason and collected psychosocial data and blood samples at 3 timepoints: 1) prior to transplant, 2) hospital discharge (within days of neutrophil engraftment), and 3) Day +100. Serum was analyzed for inflammatory (TNF-a, IL-6, IL-10, IL-8, VEGF, CRP) and endocannabinoid molecules. Psychosocial measures included depressive symptoms, anxiety, and social support. Canonical correlation analysis indicated a significant association between change in inflammatory molecules and change in psychosocial scores between the first and second time points ($r=1.0$, $p<.0001$), but no significant association between change in inflammatory molecules and endocannabinoids, or change in psychosocial scores and endocannabinoids. Pearson correlation analyses at individual timepoints demonstrated a significant positive association of the endocannabinoid 2-arachidonoylglycerol (2-AG) with IL-6 and CRP at time points 1 (IL-6, $p=.02$; CRP, $p<.01$) and 3 (IL-6, $p=.01$; CRP, $p=.03$). These data suggest that circulating 2-AG concentrations are related to critical immune modulators, IL-6 and CRP. Future research should address whether the endocannabinoid system may buffer the adverse physiological effects of negative psychosocial factors post-transplant.

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REGULATION OF HUMAN LEUKOCYTE FUNCTIONS BY CB2-RECEPTOR AGONISTS; EVIDENCE FOR DONOR-DEPENDENT RESPONSES AND IMPLICATIONS FOR THERAPEUTIC APPLICATIONS

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Aim. CB2 receptor selective agonists have anti-inflammatory properties in models of stroke and multiple sclerosis, and appear to improve the neurological deficits caused in part by the detrimental neuroinflammation in these debilitating diseases (often fatal). The aim of this study is to better understand the function of the cannabinoid type 2 receptor (CB2) expressed by specific human immune cells circulating in blood. The ultimate objective of this research is to ascertain the therapeutic suitability of CB2 intervention for the treatment of neuroinflammatory conditions in humans.

Methods. The effect of several CB2 receptor agonists (HU308 and JWH015) on various functions of human leukocytes was investigated. Peripheral blood mononuclear cells (PBMC) were prepared from young healthy donors (fully consented with ethical approval). Human PBMC or monocytes were used for all studies. Functional assays were conducted to measure the effects of the cannabinoid ligands on leukocyte survival, cytokine secretion, cell migration and phagocytic activity of monocyte-derived macrophages.

Results. The CB2 agonists we tested were not cytotoxic towards human leukocytes (n=6 donors) at any concentration. Human leukocytes secrete a variety of cytokines and chemokines, which are readily detectible basally. These include IL-8, MCP-1, MIP-1α, MIP-1β, RANTES and IP-10. None of these chemokines were affected by either HU308 or JWH015 (10 μM to 1nM inclusive). This was surprising as monocytes and macrophages, which are the major sources of these cytokines in PBMC, have an abundance of CB2 receptors. Monocyte derived macrophages were regulated by HU308, which substantially reduced phagocytic activity.

Leukocyte migration was also influenced by JWH015 and HU308, however this effect was highly donor-dependent. Basal migration of leukocytes in PBMC was relatively low and unaffected by CB2 receptor activation. In contrast, SDF-1 a potent inflammatory chemokine increased migration approximately 20 fold. We next investigated the ability of CB2 agonists to suppress the SDF-1 induced migration. The response was highly donor dependent. In some donors, the CB2 agonists abrogated the SDF-1 migration, whereas in other donors there was no suppression of SDF-1 induced migration.

Conclusions. Intriguingly, we observed pronounced effects of CB2 agonists on macrophage phagocytosis, but not on cytokine secretion. The donor heterogeneity is clinically very relevant for the application of these drugs in humans and requires more detailed studies to better understand the cellular and molecular basis to the donor variation.

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AN ACTIVE LIFESTYLE AFFECTS LYMPHOCYTE FATTY ACID AMIDE HYDROLASE THROUGH AN IL-6-DEPENDENT MECHANISM

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Both endocannabinoids (eCBs) and interleukin (IL)-6 levels change during and following exercise, thus suggesting their involvement in modulation of exercise-related biological processes, including metabolism homeostasis, and inflammation. In order to investigate whether a regular physical exercise might affect the activity of the eCBs-degrading enzyme fatty acid amide hydrolase (FAAH), eight subjects, performing aerobic exercise for 8.1 ± 3.4 hours/week (39.3 ± 2.9 yrs; BMI=21.1 ± 0.4), and eight sedentary subjects (38.8 ± 3.7 yrs, BMI=23.1 ± 0.8).were enrolled.

Basal circulating IL-6 levels (2.74 ± 0.73 pg/mL) and lymphocyte FAAH activity (215.7 ± 38.5 pmol/min per mg protein) were significantly higher in the active group confronting to those measured in the sedentary group (0.20 ± 0.02 pg/mL, and 42.0 ± 4.2 pmol/min per mg protein). *In vitro* treatment of lymphocytes from sedentary individuals with 10 ng/mL IL-6 for 48 hours significantly increased FAAH activity and expression. Moreover, transient transfection experiments showed that IL-6 induced the expression of a reporter gene under the control of a CRE (cAMP response element)-like region in the human *faah* promoter. Coherently, a mutation in the same element abolished IL-6 upregulation, thus indicating that the cytokine modulates FAAH activity at the transcriptional level.

Collectively, these findings demonstrate that increased IL-6 levels lead to a CRE-like element-dependent activation of the *faah* promoter, thus enhancing FAAH activity that modulates eCB homeostasis in physically active people.

REGULATION OF ENDOTHELIAL/LEUKOCYTE INTERACTIONS BY 2-ARACHIDONOYLGLYCEROL

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Accumulated evidence points to an immuno-modulatory role for endocannabinoids, although the exact mechanism of action remains not fully elucidated. Several data support an anti-inflammatory effect through modulation of chemokines and cytokines, but a pro-inflammatory role can be sustained as well. Furthermore, the knowledge gained to date presents some shortcomings, since literature data concern single cell types or analysis of late stages of the inflammatory process. Therefore, we aimed at characterizing the role of endocannabinoids in early events of endothelium/leukocyte interactions.

We found that 2-arachidonoylglycerol (2-AG) was able to initiate and complete the leukocyte adhesion cascade, by modulating the expression of selectins and their ligands. A short exposure of endothelial cells to 2-AG was sufficient to prime them towards a pro-inflammatory state: within 1 hour of treatment, endothelial cells showed time-dependent plasma membrane expression of P- and E-selectins, which trigger the initial steps (namely capture and rolling) of inflammation. Commitment to inflammation was permanent, since activated endothelial cells released the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) for up to 24 hours, despite the removal of 2-AG after 1 hour of incubation. TNF-α-containing medium is, then, able to promote leukocyte recruitment; Jurkat cells grown in the conditioned medium derived from 2-AG-treated endothelial cells showed enhanced L-selectin and P-selectin glycoprotein ligand-1 (PSGL1, the specific ligand for selectins) expression, as well as increased efficiency of adhesion and trans-migration.

In conclusion, 2-AG indirectly promotes leukocyte recruitment into inflamed sites by acting on endothelial cells, thus representing a potential therapeutic target for treatment of inflammatory diseases.

THE EFFECT OF GWCBG, A CANNABINOID EXTRACT ON A2780, HUMAN OVARIAN CARCINOMA CELLS

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In recent years, the anti-tumour potential of cannabinoids has highlighted the importance of this system in the generation of new anti-cancer therapies (Freimuth et al., 2010; Patsos et al., 2005). The aim of the present study was to investigate the potential anti-tumour activity of a cannabinoid extract rich in cannabigerol on ovarian tumour cells.

A2780 cells were grown and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37^{o}C, 5% CO_2 .The cells were plated in 96-well culture plates at a density of 1x10^{4} cells/well and allowed to adhere at 37^{o}C for 24 hours. The following day, various doses of extract in the absence and presence of AM251, AM630, T0070907 and capsazepine, were added to the cells and further incubated for 3 days. Then the supernatant was removed and MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added for 4 hours. The ability of cells to form formazan crystals by active mitochondrial respiration was determined by using a Microplate reader after dissolving the crystals in DMSO. Cytotoxicity was expressed as a relative percentage of the absorbance measured at 540 nm in the control and extract-treated cells. Data were presented as the mean $\pm$ s.e.mean and analysed using ANOVA followed by Dunnet's t-test; n=4.

The extract induced dose-dependent cytotoxic effects on A2780 cells with an IC50 of 9.6±0.12 μM. Interestingly, the cytotoxicity was potentiated by the application of AM251, AM630, T0070907 and capsazepine with an IC50 of 0.08±0.014 μM, 0.16±0.068 μM, 1.56±0.81 μM and 4±0.22 μM respectively. Single application of antagonists alone did affect the survival rate of the A2780 cells but not the vehicle.

The data confirms that the cannabinoid system is involved in the apoptosis of A2780 tumour cells. Further experiments are required to investigate the receptor type/subtypes involvement and the mechanism of cell death.

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CANNABIS USE ASSOCIATED WITH LOWER PLASMA HIV-1 RNA VIRAL LOAD AMONG RECENTLY HIV-INFECTED INTRAVENOUS DRUG USERS

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A recent study of tetrahydrocannabinol (THC) experimentally administered to simian immunodeficiency virus-infected rhesus macaques identified an antiviral effect for THC, supporting a hypothesized immunomodulatory pathway for cannabinoids. However, the possible effect of exposure to cannabinoids on plasma HIV-1 RNA levels among individuals living with HIV/AIDS (PLWHA) has not been evaluated. We used data from the Vancouver Injection Drug User Study (VIDUS), an ongoing community-recruited prospective cohort of injection drug users in Vancouver, Canada. We included individuals who were HIV-seronegative at baseline who seroconverted to HIV infection during follow-up. The outcome of interest was plasma HIV-1 RNA copies/mL (log10 transformed) in the first 365 days following the estimated date of seroconversion.

Between May 1996 and March 2012, 149 antiretroviral (ART)-naïve individuals acquired HIV infection and were included in these analyses. Median pVL among at least daily cannabis use was 0.55 log10 c/mL lower compared to others (4.73 vs. 4.18, $p = 0.003$) (Figure 1). In a multivariate linear mixed effects model, cannabis use remained associated with lower pVL ($\beta = -0.47$, $p = 0.007$). Although cannabis is commonly used by PLWHA to manage the symptoms of HIV infection or ameliorate the side-effects of HIV treatment, we believe this is the first study to identify a potentially beneficial impact of cannabis on HIV disease state among ART-naïve individuals. Our results support the need for the prospective study of the antiviral effect of THC in humans.

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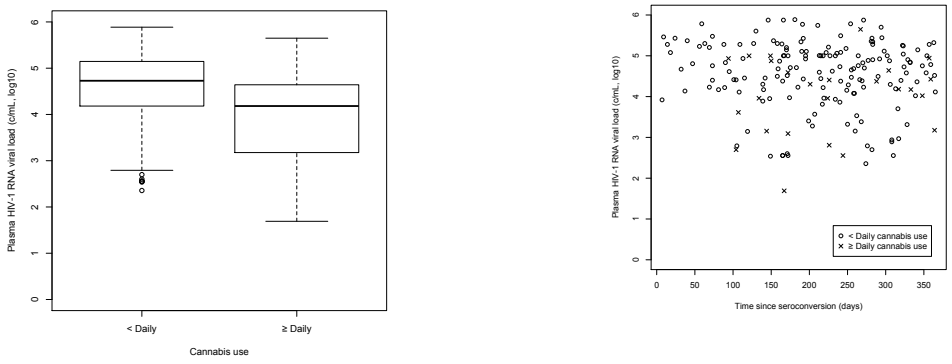

Figure 1. Boxplot of plasma HIV-1 RNA (PVL) observations stratified by cannabis use (left); scatterplot of PVL observations by days since seroconversion (right) among newly-infected HIV-positive IDU.

Δ^9-THC AND CANNABIDIOL INCREASE THE RADIOSENSITIVITY OF GLIOMA CELL LINES *IN VITRO* WHEN USED TOGETHER

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Cannabinoids have been shown to directly inhibit the growth of a number of tumour types as well as neutralising oncogenic processes such as angiogenesis. Importantly, these effects appear to be tumour specific. High-grade glioma is one of the most aggressive cancers in adult humans and long term survival rates are very low. Standard treatment for glioma remains largely unsuccessful, yet glioma is a cancer type in which cannabinoids have recently shown good activity. In an attempt to improve further the treatment outcome for glioma, we have investigated the effect of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) on glioma cells, both alone and in combination with radiotherapy. Our principal aim was to investigate the role of these compounds in priming glioma cells to respond to radiotherapy. To this aim, we have evaluated the differences between the pure (P) and botanical drug substance (BDS) forms of the compounds in two human glioma cell lines (T98G and U87MG), and also studied the effects that different ratios of these compounds may have on activity. Results demonstrated a duration- and dose-dependent reduction in cell viability with each cannabinoid and suggested that THC-BDS is more efficacious than THC-P. Conversely, CBD-P was more efficacious than CBD-BDS (48h IC_{50} in T98G: 16 ± 1.7 μM vs. 21 ± 3.2 μM for THC-BDS and THC-P; 22 ± 5.6 μM vs. 13 ± 3.6 μM for CBD-BDS and CBD-P ($p \leq 0.05$)). When comparing THC and CBD in combination, those combinations containing CBD-P were most effective at reducing cell viability, and subsequent median effect analysis revealed all combinations to be additive or synergistic (CI at FU50 at 48h in T98G: 0.77-1.09). Similarly, clonogenic survival assays showed that pre-treating cells with THC-P and CBD-P together for 24h before irradiation increased their radiosensitivity when compared to pre-treating with either of the cannabinoids individually (combination SER_{30} in T98G: 1.6 vs. 1.3 and 1.1 for THC and CBD alone, respectively). Results also showed both THC and CBD reduced the rate at which double-strand breaks in DNA caused by irradiation were repaired, as indicated by the prolonged phosphorylation of γH2AX . Taken together, our data shows that combining THC and CBD is preferential for reducing cell viability compared to using the compounds individually, and that when used together these cannabinoids can prime glioma cells to respond better to ionising radiation. These data suggest a potential clinical benefit for glioma patients, resulting from combining THC and CBD with radiotherapy.

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PARADOXICAL EFFECTS OF FAAH GENE DELETION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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The role of the fatty acid amide hydrolase (FAAH) in neurodegeneration and neuroprotection requires further study. While neuroprotective effects derived of FAAH blockade have been reported, we have recently shown that astrocytes obtained from FAAH-null mice exhibit an exacerbated inflammatory response after exposure to the pathogenic form of beta-amyloid. The aim of the present study was to analyze the impact of FAAH gene deletion on disease progress in an animal model of Alzheimer's disease (AD). We chose the 5xFAD model based on the rapid development of the pathology in these mice as well as on the inflammatory phenotype triggered by the amyloid peptide. Two strains of mice were generated (5xFAD/FAAH$^{+/+}$ and 5xFAD/FAAH$^{-/-}$) and we followed the development of the disease at two different ages (3 and 6 months). With this approach, we could analyze the impact of FAAH gene deletion at pre- and fully-symptomatic phases of the disease. At the behavioral level (studied with the water morris maze test), no differences were found between 5xFAD/FAAH$^{+/+}$ and 5xFAD/FAAH$^{-/-}$ mice at 3mo of age. At 6mo of age, however, a significant improvement in memory was found for 5xFAD/FAAH$^{-/-}$ mice, as compared to 5xFAD/FAAH$^{+/+}$. At the molecular level, the hippocampal expression level of iNOS, COX-2 and TNF-alpha was not modified by the disease at 3mo of age, either in 5xFAD/FAAH$^{+/+}$ or in 5xFAD/FAAH$^{-/-}$ mice. IL1b mRNA levels were significantly higher in 5xFAD/FAAH$^{+/+}$ as well as in FAAH$^{-/-}$ mice at this age. Remarkably, significant increases in those inflammatory parameters were evident at 6mo of age, being also significantly higher in mice lacking FAAH. These data confirm our previous findings in astrocytes in culture, indicating that the long-term absence of FAAH boosts amyloid-induced inflammation. Paradoxically, FAAH-null mice performed better in a memory test. This apparent discrepancy could be explained by the recently described regulatory role of IL1b on amyloid accumulation.

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Δ9-TETRAHYDROCANNABINOL PREVENTS METHAMPHETAMINE-INDUCED NEUROTOXICITY

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Methamphetamine (METH) abuse is known to increase activation of neuronal nitric oxide synthase (nNOS), production of peroxynitrite, microglia stimulation, hyperthermia and to induce anorectic effect. Notably, the majority of METH recreational users also consume Cannabis. Many *in vivo* and *in vitro* preclinical studies have shown that both natural (Δ^9-tetrahydrocannabinol, Δ^9-THC) and synthetic cannabinoid CB1 receptor agonists exert neuroprotective effects in different model of cerebral damage.
In this study we investigated the Δ^9-THC neuroprotective effect on METH-induced neurotoxicity by examining its ability to prevent anorectic and hyperthermic effects and reduce glial activation and nNOS over-expression in selected brain areas. To this aim, rats receiving a neurotoxic regimen of METH (4x10 mg/kg, s.c., 2 hours apart) were either pre-treated or post-treated with Δ^9-THC (1 or 3 mg/kg i.p.) at room temperature, and killed 3 days after METH administration. Δ^9-THC-induced neuroprotective effects were analyzed by monitoring body weight and core body temperature, and by semi-quantitative immunohistochemistry by using antibodies against nNOS and Glial Fibrillar Acidic Protein (GFAP), a well-known marker of cerebral toxic insults. Data showed that METH significantly decreased body weight, increased body temperature, and enhanced the expression of GFAP and nNOS in the CPu, PFC and CPu, respectively. Two-way repeated measures ANOVA (treatment x time) revealed that Δ^9-THC (1 or 3 mg/kg) failed to revert anorectic and hyperthermic effects of METH. Two-way ANOVA revealed that post-treatment with 1 mg/kg of Δ^9-THC significantly attenuated METH-induced nNOS overexpression in the CPu, while no differences were observed in the number of nNOS positive neurons in rats either pre-treated or post-treated with the highest dose of Δ^9-THC. As regards GFAP-immunoreactivity (IR), Bonferroni *post-hoc* comparisons showed that GFAP-IR was significantly lower in the CPu of Δ^9-THC1 (-40%) and Δ^9-THC3 (-37%) post-treated rats, as well as Δ^9-THC3 (-56%) pretreated rats as compared to controls. In the PFC, Δ^9-THC 1 mg/kg pre- or post-treatment significantly decrease METH-induced GFAP-IR (-32% and -38%, respectively) with respect to their controls, whereas Δ^9-THC 3 mg/kg only administered before METH reduced GFAP-IR (-26%). In order to determine if Δ^9-THC acts through a CB1 receptor-mediated mechanism, a group of Δ^9-THC (1 mg/kg) post-treated animals were pre-treated with the CB1 receptor antagonist SR141716A (SR, 1 mg/kg, i.p.). Dunnet *post-hoc* analysis revealed that nNOS staining in the CPu of METH-Δ^9-THC was reduced compared to METH-vehicle treated rats, being this effect reverted by SR. In the CPu, both Δ^9-THC and SR treatment alone or in combination reduce METH-induced GFAP-IR, while in the PFC GFAP-IR was lower in METHΔ^9-THC treated rats compared to their control, with SR reverting this effect only partially. Finally, SR by itself significantly reduced GFAP-IR in METH treated rats. Our findings indicate that Δ^9-THC protects against METH neurotoxicity, through both CB1 and not-CB1-dependent mechanisms involving the suppression of nNOS expression and glial activation.

THE NEUROPROTECTIVE ENDOCANNABINOID N-ARACHIDONOYL-DOPAMINE AFFECT THE PAKT ACTIVATION IN PRIMARY MICROGLIAL AND ASTROCYTE CELL CULTURE

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After trauma or stroke different inflammatory processes become strongly activated, leading to neurodegeneration. Classical endocannabinoids like 2-AG are known to exert neuroprotective effects after brain injuries. Recently we have reported that N-arachidonyl-dopamine (NADA), a novel endocannabinoid with anti-oxidative and anti-inflammatory properties reduced the amount of microglial cells and degenerating neurons within the dentate gyrus in organotypic hippocampal slice cultures (OHSC). Neuroprotective properties of low (1 nM) but not high (10 μM) NADA concentrations were solely blocked by AM251 and were absent in CB1-/- mice.

To figure out the intracellular pathway responsible for NADA effects we looked at the MAP kinase signal transduction pathway. For this purpose neuronal hippocampal cell line HT22, microglial cell line BV2, primary astrocytes and astrocyte/microglia cocultures were analyzed. NADA did not influence the activation of p38 and p44/42 MAPK in investigated cells after 30 min, 2 h, 6 h, 24 h. Nevertheless, NADA reduced ATP mediated activation of pAkt. It is well known that ATP acts on glial cells and represents a central player in glial cell pathology.

Our findings demonstrate that NADA protects dentate gyrus granule cells by acting upon CB1. NADA reduced the number of microglial cells at distinct concentrations. p44/42 and p38 seems not to be involved in NADA mediated actions.

In conclusion, our findings show that NADA protects dentate gyrus granule cells after excitotoxic lesion. NADA-mediated neuroprotection is driven by CB1 receptors. The results strongly suggest the involvement of P2X or P2Y purinoceptors signaling in NADA mediated actions.

Δ^9-THC AND *N*-ARACHIDONOYL GLYCINE REGULATE THE PHENOTYPIC MORPHOLOGY OF BV-2 MICROGLIA

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Microglial cells are extremely plastic, and undergo a variety of shape changes based on their location and current role. "Resting" microglia possess a characteristic form composed of long branching processes that are very sensitive to small changes in physiological condition. "Active" amoeboid microglia scavenge CNS plaque and foreign agents and mediate cytotoxic and inflammatory signalling. They are especially prevalent during rewiring of the brain, when there are large amounts of extracellular debris and apoptotic cells to remove. Once the phagocytic task of amoeboid microglia is complete, a return to a multi-branched, surveillant form is usually observed. However, during chronic neuroinflammation amoeboid cells remain activated for an extended period and the production of neurotoxic mediators is sustained longer than usual, contributing to neuronal death.

Walter *et al* (2003) reported that pathological stimulation of both neurons and microglia triggered microglial cell migration by engaging CB_2 and abnormal cannabidiol (Abn-CBD) receptors. *N*-arachidonoyl glycine (NAGly) signalling via GPR18 has been introduced as an important new target in microglial-neuronal communication (see McHugh *et al*., 2012a and McHugh *et al*., 2012b for review). NAGly is synthesized primarily from AEA via a fatty acid amide hydrolase (FAAH) dependent pathway and can be prevented by URB597, an irreversible inhibitor of FAAH. It is ineffective as an agonist at either CB_1 or CB_2 receptors despite the obvious structural overlap with AEA. Our hypothesis is that NAGly-GPR18 signalling regulates phenotypic change in BV-2 microglia.

BV-2 microglia were plated on 25x75 mm glass microscope slides and exposed for 12 hours to Vh control (0.1% DMSO), 10 nM NAGly or 10 nM Δ^9-THC in the presence and absence of 100 mM CBD. Phorbol 12- myristate 13- acetate (PMA) was used as control. Cells were then fixed and images from 10 random fields of view were collected at x40 magnification using a light microscope. Cell morphology was categorized by multiple investigators. The microglial population exposed to NAGly and Δ^9-THC was significantly different compared to control (one-way ANOVA; $p<0.05$); the number of amoeboid and multi-branched cells decreased and increased, respectively. CBD was effective at antagonizing the effects of NAGly, but not those of THC.

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IN HUNTINGTON'S DISEASE, THE ENDOCANNABINOID AND CYTOKINE SYSTEMS ARE DYSREGULATED IN THE STRIATUM

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Proponents of cannabinoid-based therapies have suggested that cannabinoids might be useful for hyperkinetic and neurodegenerative movement disorders such as Huntington's disease (HD). However, it is well known that levels of the type 1 cannabinoid receptor (CB_1) are decreased in the brain of individuals suffering from HD prior to motor symptom onset. Moreover, cannabinoid signaling affects the inflammatory response by CB_1-dependent NF-κB activation, and CB_2-dependent inhibition of pro-inflammatory cytokine release. We sought to determine whether components of the endocannabinoid and cytokine systems were dysregulated in HD. We found that mRNA levels of the type 2 cannabinoid receptor (CB_2) and fatty acid amide hydrolase (FAAH) were increased in tissue isolated from advanced-stage HD patients, while monoacyl glycrol lipase (MAGL) levels were unchanged. Additionally, NF-κB p65 mRNA and protein levels were lower in the late-stage human HD brain than in age-matched healthy control tissue. Levels of the cytokines CCL5, IL-1β, IL-8, and TNF-α, all of which are regulated by NF-κB, were reduced in the human HD brain, which contrasts with the observation that these cytokines are up-regulated in the plasma of HD patients. While peripheral changes in cytokine levels may be useful as markers of disease progression, they do not reflect the immunosuppressed state of the central nervous system in late-stage HD. Therefore, cannabinoids that enhance NF-κB activity, such as endogenous CB_1 agonists or FAAH inhibitors, may normalize cytokine levels. In contrast, cytokine suppression *via* CB_2 agonists may not be beneficial for these patients.

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ETHANOL AND CANNABINOID RECEPTOR INTERACTION IN THE REGULATION OF ADULT NEUROGENESIS

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Ethanol consumption reduces brain developmental and adult neurogenesis. Although CB1cannabinoid receptor has been implicated in alcohol-addiction, the effect of cannabinoid receptor (CB1R and CB2R) signaling on neurogenesis and ethanol-mediated changes in adult neurogenesis are poorly understood. The goal of this study was: a) To determine the effect of CB1R and CB2R receptor activation on adult rat brain hippocampal neurogenesis and b) to determine the impact of CB1R receptor vs. CB2R activation on acute binge ethanol-mediated inhibition of adult neurogenesis and c) the effect of ethanol treatment on Ca+2 regulating TRP channels in relation to adult neurogenesis in dentate gyrus.

Adult male wistar rats (body wt 280-320 gm) were treated with vehicle or CB1R agonist ACEA (3 mg/kg, ip) or CB2 receptor agonist HU308 (15 mg/kg, ip) or CB1R or CB2R antagonist (3 mg/kg, ip) alone or in combination with ethanol (5 g/kg, ig). For combined treatment cannabinoid drugs were administered 30 min prior to ethanol treatment and animals were sacrificed 3 or 24 hr following ethanol treatment. The effects of the drug treatments on neurogenesis and cell proliferation in dentate gyrus were assessed immunohistochemically by DCX+IR (doublecortin positive) and Ki67+IR staining in brain slices respectively as well as biochemically using RT-PCR and Western blot analysis.

We found that acute ethanol or CB1R agonist ACEA treatment significantly reduced DCX+IR (doublecortin positive) in dentate gyrus, but CB2R agonist HU-308 did not produce any significant change in DCX+IR compared to vehicle treated animals. Co-treatment with CB1R agonist and ethanol produced a larger reduction in DCX+IR that was attenuated by pretreatment with CB1R antagonist SR141716. Interestingly, neither CB1R agonist ACEA nor CB2R agonist HU308 produced any alteration in Ki67+IR. Preliminary study also suggest that acute ethanol treatment significantly increased TRPV1 and TRPC5 expression in in dentate gyrus but CB1R agonist or Cb2R agonist treatment did not produced any change. Together, these findings suggest that acute CB1R activation and/or acute binge level ethanol treatment reduces neurogenesis in adult rat brain and CB1R plays a pivotal role in the ethanol reduce adult neurogenesis. These studies indicate acute ethanol and CB1 agonists reduce neurogenesis. The ability of CB1 antagonist to reduce ethanol inhibition of neurogenesis is consistent with CB1 receptors contributing to ethanol inhibition of neurogenesis.

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EFFECTS OF CANNABIDIOL AND HU210 ON VIABILITY AND MITOCHONDRIAL MEMBRANE POTENTIAL OF SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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SH-SY5Y cells represent a useful model to investigate neurotoxicity *in vitro*. After SH-SY5Y cells were differentiated into a neuronal phenotype over six days in the presence of 10 μM retinoic acid, we examined the sensitivity of these cells to cannabidiol (CBD) and HU210 on cell viability using the MTT assay. Following differentiation, 48 hrs exposure to 10 μM CBD or HU210 in the presence of 10 % fetal calf serum resulted in cell viability levels which were modestly reduced (94 ± 1 and 94 ± 2 %, respectively, both $P<0.05$). However, reducing the fetal calf serum concentration to 5 % resulted in cell viability levels of 99 ± 1 and 82 ± 1 % ($P<0.001$) control, in the presence of 1 and 10 μM CBD, respectively. In comparison, cell viability levels were 97 ± 10 and 77 ± 3 ($P<0.001$) over the same period of exposure to 1 and 10 μM HU210, respectively. The inhibitory effects of 10 μM CBD or HU210 were unaffected in the presence of the CB_1 cannabinoid receptor antagonist AM251 (300 nM, 107 ± 3 %) with levels of 74 ± 2 and 77 ± 9 %, respectively. The structurally-distinct CB_1 receptor antagonist LY320135 (10 μM) was also ineffective (control 108 ± 2; +CBD 82 ± 2; +HU210 77 ± 9 %).

90 min exposure to cannabinoids failed to alter mitochondrial membrane potential (assessed in intact cells using rhodamine 123 with at least three different passages), although 3 μM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) reduced rhodamine 123 fluorescence to 26 ± 1 % ($P<0.001$). However, investigating differentiated cells grown in the absence of serum for 24h, 90 min exposure to 10 μM CBD increased the membrane potential to 166 ± 29 % control ($P<0.001$) while 10 μM HU210 did not affect mitochondrial membrane potential. Application of FCCP (2μM) for 30min led to a drop in mitochondrial membrane potential to 45 ± 9%; preincubation with 10 μM CBD prevented this reduction (103 ± 2%). Increasing the exposure time of 2 μM FCCP to 90min resulted in a drop to 24 ± 6 % but a loss of the protective effect with simultaneous exposure to 10μM CBD (40 ± 15 %) or 60 min preincubation with 10 μM CBD (54 ± 15 %).

We conclude that the mechanism of damage appears to be independent of CB_1 cannabinoid receptors, while higher concentrations of CBD appear to alter mitochondrial function, but only in serum-free medium.

CB1 CANNABINOID RECEPTOR-MEDIATED REGULATION OF AGGRESSIVE SOCIAL BEHAVIOR

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The present study was aimed to examine the role of cannabinoid CB1 receptors (CB1r) on social and aggressive behavior. For this purpose, social encounters experiments were performed in grouped and isolated knock-out mice lacking CB1r (CB1KO) and in wild-type (WT) littermates. In addition, the cognitive impulsivity level was also evaluated in the delayed reinforcement task (DRT). Gene expression analyses of Monoaminooxidase-A (MAO-A), catechol-o-methyl-transferase (COMT), 5-hydroxytriptamine transporter (5-HTT) and 5-HT1B receptor in the medial and dorsal raphe nuclei (MnR and DR, respectively), and in the amygdala (AMY), were carried out by real time-PCR. Double immunohistochemistry studies were aimed to evaluate COMT and CB1r co-localization in the rostral lineal nucleus of raphe (NLR) and in the cortical (ACo), basomedial (BMA) and basolateral (BLA) amygdaloid nuclei. The behavioral effects of the CB1r agonist ACEA (1 and 2 mg·Kg-1) on social activity and aggression were also evaluated.

When grouped, CB1KO mice showed more aggression than their WT mice, spending more time in threat and attack during the social interaction test. CB1KO mice needed shorter time to perform the first aggressive behavior and the mean time in each aggressive interaction was longer. In addition, CB1KO mice showed an increased cognitive impulsivity level, in comparison with their WT mice, especially when elevated times of delay were imposed. Gene expression analyses revealed that: 1) 5-HTT was unaltered, COMT was increased and MAO-A was decreased in the MnR of CB1KO mice; 2) 5-HTT was unaltered, COMT was increased and MAO-A was unaltered in the DR of CB1KO mice; and 3) 5HT1B, COMT and MAO-A gene expression levels were significantly higher in the AMY of CB1KO mice. COMT and CB1r double-immunohistochemistry showed cytoplasmic labeled COMT(+) cells either in the NLR and in the ACo, BMA and BLA. CB1r immunolabeling was only observed in ACo, BMA and BLA, and it was localized in axons and buttons; however, the density of labeled processes increased in BLA. Acute administration of the CB1 agonist ACEA (2 mg·Kg-1) significantly decreased the aggressive level of CB1KO mice, with a significant reduction in threat, the mean time of each threat interaction and the mean time of each attack.

These results suggest that CB1r play a relevant role in the social interaction and aggressive behavior. Pharmacological manipulation of this receptor deserves further consideration as a potential new valuable target for the management of aggressive-related psychiatric disorders.

REDUCED LOCOMOTOR ACTIVITY BY NMDA ANTAGONIST IS REVERSED BY HU-308

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Core evidence from laboratory and clinical research points to the involvement of the endocannabinoid system in the development of schizophrenia. Specifically, recent studies have highlighted a role for the cannabinoid CB_2 receptor in schizophrenia. This has led us to investigate the effects of CB_2 receptor selective ligands in a mouse model for schizophrenia. In this study the effect of HU-308 was investigated in a mouse model for NMDA antagonist-induced inhibition of locomotor activity.

Phencyclidine (PCP; 5 mg/kg), an NMDA antagonist, was injected after birth to Sabra mice. The effect of HU-308, a CB_2 receptor selective agonist, was studied in a paradigm for locomotor activity. Injections of PCP significantly reduced the body weight of mice (vehicle vs. PCP, $p<0.001$, two-way ANOVA). Chronic treatment with HU-308 (5 mg/kg) had no effect on body weight and it did not reverse the effect of PCP on postnatal body weight. We then tested the locomotor activity with the open-field test. Compared with the control group, PCP significantly decreased the exploration activity and the number of rears ($p<0.01$). Treatment with HU-308 significantly reversed both effects of PCP ($p<0.01$). Thus, following treatment with HU-308 the ambulation and rearing activities of PCP-induced mice were not significantly different from that of the control group. These results suggest that under certain conditions, such as the inhibition of the NMDA receptors, there is a role for CB_2 receptor in the modulation of motor activity.

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MODULATION OF LOCOMOTOR ACTIVITY BY THE NOVEL CANNABINOID HU-267

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ADHD is one of the most common childhood behavioural disorders, which affects 5-10% of children worldwide. This disorder is characterised by inattention, hyperactivity and impulsivity. The increased use of cannabis by ADHD patients is well documented, and motivated us to investigate the relative contribution of cannabinoids to these behaviours. HU 267 is a novel synthetic compound whose structure resembles that of cannabidiol, a ”non-psychotropic” phytocannabinoid. Our results show that compared with vehicle treated-mice, there was no significant change in the body weight of pups following a postnatal injection of HU-267 (20 mg/kg). At the age of 5-20 weeks, females that had been treated with HU-267 were hyperactive compared with the control group. The ambulation behaviour was increased by 96% and the number of rears was increased by 91% in HU-267-treated females at 20 weeks of age. On the other hand, the behaviour of male mice in the open field was not affected by HU-267. Characterizing the pharmacological profile of HU-267 will enhance the study of mechanisms which may contribute to the development of ADHD in girls.

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EFFECTS OF INHIBITION OF ENDOCANNABINOID DEGRADATION ON EXCESSIVE HEROIN AND ALCOHOL INTAKE

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Both opiate and alcohol dependence are associated with great social costs and detrimental personal health consequences. Furthermore, both drugs of abuse are associated with altered brain stress systems and withdrawal-induced anxiety that may contribute to continued use despite efforts to stop. Given the emerging evidence on the interaction between endocannabinoid function and the modulation of stress responsivity, we investigated the role of enhancing endocannabinoid signaling on drug intake in dependent rats. Rats were given the fatty acid amide hydrolase (FAAH) inhibitor PF-3845, which causes anandamide to accumulate, and the monoacylglycerol (MAGL) inhibitor MJN110, that increase 2-AG levels, then tested for alcohol or heroin self-administration.

Heroin intake was evaluated using an extended access 12 h self-administration paradigm, which closely models the escalating heroin intake associated with the transition to drug dependence. We found that acute FAAH inhibition failed to suppress heroin intake in dependent rats, whereas prolonged daily PF-3845 (1 mg/kg, i.v.) prevented the escalation of heroin intake under free-access conditions, and the increased motivation for heroin infusions under a progressive ratio schedule. Long-term FAAH inhibition also resulted in reduced release of the stress hormone corticosterone during opiate withdrawal.

Rats trained to orally self-administer alcohol (10% w/v) were exposed to 14 h daily ethanol vapor exposure to induce dependence. Following one month exposure, dependent rats drink twice as much alcohol in a 30 min period compared to pre-vapor, or non-dependent air-exposed, self-administration. PF3845 (0-10 mg/kg, i.p.) failed to reduce alcohol self-administration; however, MJN100 (0-20 mg/kg, i.p.) dose-dependently reduced alcohol intake selectively within the dependent alcohol group without altering non-dependent responses. These results suggest that both anandamide and 2-arachidonoylglycerol signaling are functionally implicated in the transition to alcohol and heroin dependence possibly via interactions with brain stress systems.

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ACTIVATIONAL GONADAL HORMONE MODULATION OF THE BEHAVIORAL EFFECTS OF Δ^9 -TETRAHYDROCANNABINOL IN MALE AND FEMALE RATS

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Our lab has previously reported that estradiol (E2) significantly enhanced antinociceptive sensitivity to Δ^9-tetrahydrocannabinol (THC) in ovariectomized female rats, whereas testosterone (T) significantly decreased THC's motoric effects in castrated males, suggesting that sex differences in THC-induced behavioral effects could be attributed to the activational effects of T in males and E2 in females (Craft & Leitl, 2008). However, it is still unknown whether organizational hormone effects also contribute to adults' sexually differentiated response to THC's antinociceptive and motoric effects. The purpose of the present study was to test this hypothesis in adults by determining whether T would decrease THC's motoric effects in females, and whether E2 would enhance THC's antinociceptive effects in males. THC-induced antinociception (tail withdrawal, paw pressure tests) and motoric effects (horizontal locomotion, catalepsy) were compared in male and female Sprague-Dawley rats that were gonadectomized as adults and chronically treated with the "opposite-sex" hormone (E2 in males, T in females) vs. those that were gonadectomized and received no hormone treatment. Three weeks after gonadectomy/hormone treatment, baseline latencies were determined on the warm water tail withdrawal and mechanical paw pressure tests. Approximately 24 hr later, rats were injected with vehicle or 5 mg/kg THC i.p. and tested for antinociception on tail withdrawal and paw pressure tests at 15, 30, 60, 120, and 240 min post-injection. Following the paw pressure test at 30, 60, 120, and 240 min post-injection, rats were placed into locomotor chambers to assess horizontal locomotor activity (# of photobeam breaks) for 5 min. Following the locomotor test at 30 and 60 min post-injection, rats were assessed for catalepsy via placement of the forepaws onto a raised bar. E2 significantly enhanced the antinociceptive effects of THC in males, on the tail withdrawal and paw pressure tests. T slightly suppressed the antinociceptive effects of THC in females on the tail withdrawal and paw pressure tests while slightly decreasing THC's motoric effects in the locomotor test. THC's motoric effects on the catalepsy test did not differ between hormone and non-hormone treated rats in either males or females. Taken together with our previous study demonstrating activation effects of E2 in females (antinociception) and T in males (motoric effects) (Craft & Leitl, 2008), the present results suggest that the behavioral effects of THC are modulated similarly in BOTH sexes via gonadal hormones in adulthood.

EFFECTS OF ACUTE AM251 AND AM4113 TREATMENT ON NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL-INDUCED CONDITIONED PLACE AVERSION

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Of growing interest has been the involvement of the cannabinoid system in the etiology and maintenance of opiate addiction. Although an ability of this system to modulate the positive reinforcing effects of opiates has been characterized, an elucidation of the cannabinoid system in ameliorating the negative reinforcing and affective motivational state of opiates is warranted.

Administration of naloxone (1 mg/kg, sc) produces a one cycle conditioned place aversion (CPA) in rats having been injected with morphine (20 mg/kg, sc) 24 hr earlier (Parker & Joshi, 1998). This paradigm provides a sensitive measure of the motivational effects of acute morphine dependence. The present experiments investigated the ability of the CB_1 inverse agonist (AM251) and the CB_1 neutral antagonist (AM4113) to interfere with the establishment (Experiment 1) and reinstatement (Experiment 2) of previously extinguished naloxone-precipitated morphine withdrawal-induced conditioned place aversion.

AM251 and AM4113 prevented the establishment, but not the reinstatement, of the CPA. CB_1 blockade may alleviate the affective, motivationally aversive effects of naloxone-precipitated morphine withdrawal, but does not appear to prevent the ability of such withdrawal to reinstate the place aversion.

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THE INTERACTIVE EFFECTS OF ACUTE AND SUB-CHRONIC CANNABINOID EXPOSURE ON SEXUAL BEHAVIOUR IN MALE RATS

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There is substantial evidence to suggest that endocannabinoid system activation has an inhibitory effect on male sexual behaviour. Sub-chronic exposure to exogenous cannabinoid agonists, such as HU-210, has been shown to produce lasting deficits in sexual functioning in male rats (Ferrari et al., Physiol. Behav. 69 (2000) 547-554). This finding does not appear to be due to a direct effect of drug withdrawal (Riebe et al., Neurosci. Lett. 472 (2010) 171-174), but potentially neuroadaptive changes in CB_1 receptor-mediated signaling. However, the nature of these potential changes remains unclear. It is hypothesized that sub-chronic exposure to exogenous cannabinoids may cause a dysfunctional down-regulation of endocannabinoid activity, with reduced sexual functioning being secondary to this disruption. Alternatively, sub-chronic administration of cannabinoids may lead to persistently elevated CB_1 receptor activity. The current study seeks to investigate these potential mechanisms by examining the interaction of sub-chronic and acute cannabinoid exposure on male sexual behaviour.

Forty-two sexually proficient adult male Sprague-Dawley rats were divided into six experimental groups: 1) sub-chronic HU-210 and acute HU-210, 2) sub-chronic HU-210 and acute CB_1 receptor antagonist AM-251, 3) sub-chronic HU-210 and acute vehicle, 4) sub-chronic vehicle and acute HU-210, 5) sub-chronic vehicle and acute AM-251, and 6) sub-chronic vehicle and acute vehicle. Sub-chronic intraperitoneal injections of 100 ug/kg HU-210 or vehicle was administered daily for 14 days. Subsequently, the subjects received two weeks of drug abstinence, followed by acute intraperitoneal injections of 25 ug/kg HU-210, 1 mg/kg AM-251, or vehicle. Sex testing with sexually receptive female conspecifics occurred one hour after acute drug administration. Sexual behaviour was scored by trained observers blind to the experimental condition.

The study is currently in progress, though results collected so far indicate sub-chronic HU-210 treatment has caused decreased male sexual behaviour, and that this effect may be attenuated by subsequent acute HU-210 administration. This result suggests that a disruptive down-regulation of endocannabinoid activity may be implicated in the sexually inhibitory effects of sub-chronic cannabinoid exposure. Findings from this study will contribute to an understanding of how the endocannabinoid system regulates sexual behaviour, and how alterations to this system can contribute to sexual dysfunction.

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THE EFFECTS OF TAIL PINCH ON SEXUAL BEHAVIOUR IN MALE RATS FOLLOWING SUBCHRONIC HU-210 ADMINISTRATION

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Research regarding the effects of the CB1 receptor agonist HU-210 [(-) 11-OH-Δ^8-THC-DMH] on sexual behaviour indicates that it is a potent inhibitor of male rat copulatory behaviour following both acute and subchronic administration (Ferrari et al., Physiol. Behav. 69 (2000) 547-554; Gorzalka et al., Neurosci. Lett. 472 (2010) 171-174). The exact mechanisms by which this inhibition occurs remain unclear, although impairments in locomotor function and sexual motivation have been suggested as possibilities. Ferrari et al. (2000) showed that subchronic HU-210 administration (0.1 mg/kg) caused a deficit in intromission latency, a measure most associated with sexual motivation, which persisted despite a 14-day abstinence period during which time ejaculatory functioning returned to baseline levels. This prolonged effect on intromission latency cannot be entirely attributed to locomotor impairments since rats rapidly develop tolerance to the sedative effects of HU-210 (Ferrari et al., Life Sci. 65 (1999) 823-831). In the present study a tail pinch paradigm was used to investigate the possible involvement of a dopaminergic motivation-based mechanism in the continued sexual impairment observed during abstinence following subchronic HU-210 exposure. Tail pinch is known to increase sexual motivation and dopamine influx in the Nucleus Accumbens (NAc) (Leyton & Stewart, Physiol. Behav. 60 (1996) 77-85).

Twenty eight sexually proficient adult male Sprague-Dawley rats were examined using four subject groups (N= 7/group): 1) tail pinch following subchronic HU-210 administration, 2) tail pinch following subchronic vehicle administration, 3) subchronic HU-210 administration alone, and 4) subchronic vehicle administration alone. Subjects either received 0.1 mg/ml/kg intraperitoneal injections of HU-210, or 0.1ml/kg vehicle once a day for 14 days. Following drug administration and another 14 days of drug abstinence, males were placed in testing chambers with sexually receptive females and scored on measures of sexual performance by trained observers blind to the drug/vehicle manipulation. Rats in the tail pinch condition had a clothespin applied to their tail at the beginning of sex testing, and it remained attached until the end of testing.

Although this study is ongoing, preliminary tail pinch data combined with the findings of Ferrari et al. (2000) suggest that tail pinch attenuates the inhibitory effects of subchronic HU-210 on sexual behaviour. This result suggests that dopaminergic signaling is implicated in the prolonged sexual inhibition following subchronic HU-210 exposure. Ultimately, the results of this study will provide further insight into the neurobiological mechanisms of sexual function and motivation.

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FUNCTIONAL DEFICITS IN THE BARREL CORTEX OF THE CB1 RECEPTOR KNOCKOUT MOUSE

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Sensory deprivation can lead to cortical reorganization of the somatosensory map through a variety of mechanisms. The immediate result of this manipulation is a loss of activity in the cortical area of the sensory deprived region. Yet, over time this sensory deprived area will become more responsive to neighbouring, sensory intact, regions Sensory deprivation weakens the Layer (L) 4-L2/3 synapse through long-term depression (LTD)-like processes. Endocannabinoid (eCB) signalling has been implicated in this process, as this synaptic weakening was found to be mediated in part by Cannabinoid Receptor 1 (CB1R)-dependent LTD. Yet, very little research has been done to examine the role of eCB signalling in mediating cortical reorganization. In this study, we utilize the rodent vibrissae sensory system to study the role of the CB1R in experience-dependent plasticity of somatosensory (barrel) cortex, and vibrissae-dependent behaviour.

Using the immediate early gene c-Fos (a marker of neuronal activity), we compared experience-dependent changes in barrel cortex activity, following sensory deprivation induced by whisker plucking, between CB1R knockout and wildtype (WT) mice. While one hour of sensory deprivation completely abolished c-Fos activity in the sensory deprived area, within one week this area became more active in response to stimulation of intact neighbouring whiskers. This change was even greater in CB1R KO mice when compared to WT controls, suggesting that eCB signalling is integral to experience-dependent reorganization following sensory deprivation. The altered plasticity evident in the barrel cortex of the CB1 KO mice suggested that they might also have abnormal sensory processing. To that end, we examined the ability of the CB1 KO and WT mouse to process sensory information using a novel texture discrimination task. While CB1 WT mice could successfully distinguish between even small differences in textures, CB1 KO mice were unable to do so, even when the difference in textures was large.

Taken together, these findings implicate CB1 receptor signaling in normal functioning and experience-dependent plasticity of the somatosensory cortex.

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DIFFERENTIAL REGULATION OF SENSORIMOTOR-GATING DEFICIT IN CB1 KNOCKOUT MICE BY HALOPERIDOL, RISPERIDONE AND METHYLPHENIDATE

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The purpose of this study was to examine if deletion of CB1 receptors induced sensorimotor-gating deficit and how this behavioral alteration may be regulated with different treatment strategies.

Evaluation of sensorimotor gating was carried out in male mice lacking CB1 receptor (CB1KO) and their wildtype (WT) littermates by using the prepulse inhibition (PPI) of the acoustic startle response. Five types of trials were used: pulse alone (120dB), three with prepulse (68, 71 and 77dB) and no stimulus trial. After a baseline determination of PPI response, the effects of chronic haloperidol, risperidone and methylphenidate oral administration were tested in PPI at 4, 8 and 12 days of treatment. Three doses of haloperidol (0.01, 0.03 and 0.09 mg/kg), risperidone (0.015, 0.03 and 0.06 mg/kg) and methylphenidate (0.5, 1 and 2 mg/kg) were administered twice a day. The 4, 8 and 12 days sessions were performed between 3 and 5 hours after the last dose administered. Furthermore, gene expression analyses were aimed to evaluate adrenergic alpha-2C receptor (alpha2Cr) in the prefrontal cortex (PFC), and tyrosine hydroxylase (TH) and dopamine transporter (DAT) in the ventral tegmental area (VTA) of CB1KO and WT mice.

CB1KO mice showed significantly decreased baseline PPI compared to WT mice. Chronic oral treatment with haloperidol significantly reduced PPI in WT mice only, whereas chronic oral treatment with risperidone significantly reduced PPI in both genotypes. On the other hand, chronic oral treatment with methylphenidate significantly reduced PPI levels in WT mice, but produced an improvement in CB1KO mice at 71 and 77dB prepulse sound level. On the other hand, gene expression analyses showed that methylphenidate (2 mg/Kg) treatment induced an up-regulation of alpha2Cr in the PFC, and TH and DTA in the VTA of CB1KO mice, but not in WT mice.

These results suggest that mice lacking CB1 receptor display a PPI deficit resistant to antipsychotic treatment which responds to psychostimulant treatment with methylphenidate. Therefore, CB1KO mice may be considered a new model for evaluation of new potential therapeutic agents for treating attention deficit and hyperactivity disorder (ADHD).

HYPOTHERMIA IN MICE AND GETTING HIGH: EVIDENCE THAT WIN 55212-2 AND JWH-018 HAVE HIGHER EFFICACY THAN Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC)

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Many CB_1 receptor agonists (e.g., WIN 55212-2 and JWH-018) are reported to have higher efficacy than Δ^9-THC, as evidenced by stimulation of G-proteins *in vitro*. However, the extent to which in vivo effects vary as a function of efficacy is less clear. Hypothermia in male C57BL/6J mice was used to examine whether drug interactions and changes in sensitivity after chronic treatment vary as a function of efficacy at CB_1 receptors. Rank order potency determined from the subcutaneous dose that decreased rectal temperature from control (37°C) to 33°C was JWH-018 (0.6 mg/kg), WIN-55212-2 (1.4 mg/kg), and Δ^9-THC (15.5 mg/kg). The maximum hypothermia produced by Δ^9-THC (up to a dose of 100 mg/kg) was -6°C, which was significantly lower than that produced by JWH-018 (-9°C up to 10 mg/kg) and WIN 55212-2 (-9.5°C up to 32 mg/kg). To examine potential antagonism of WIN 55212-2 and JWH-018, dose-effect functions were re-determined after a dose of 32 mg/kg of Δ^9-THC. The hypothermic effects of WIN-55212-2 were reduced to the level of Δ^9-THC alone, and increasing the dose of WIN 55212-2 resulted in surmountable antagonism, i.e., a 2-fold rightward shift in the WIN 55212-2 dose-effect function. In contrast, Δ^9-THC (32 mg/kg) did not antagonize the hypothermic effects of JWH-018. After 7 days of Δ^9-THC treatment (3.2 or 32 mg/kg once daily), tolerance was evidenced by failure of Δ^9-THC (up to 320 mg/kg) to decrease temperature to below 33°C for up to 15 days after discontinuation of daily treatment. Daily Δ^9-THC treatment produced dose-related cross-tolerance to JWH-018 and WIN 55212-2. The magnitude of cross-tolerance to WIN 55212-2 was less than JWH-018, and cross-tolerance to both was less than tolerance. In addition, the return to control sensitivity after discontinuation of Δ^9-THC treatment occurred more rapidly for WIN 55212-2 as compared with JWH-018. Collectively, these data are highly consistent with Δ^9-THC having lower efficacy than JWH-018 in vivo, which in turn has lower efficacy than WIN 55212-2. While individuals tolerant to cannabis might retain sensitivity to higher efficacy synthetic cannabinoids, simultaneous use might also result in a reduction of the effects of the synthetic cannabinoid by Δ^9-THC. Supported by USPHS grant DA19222.

BEHAVIORAL EFFECTS OF CB2 CANNABINOID RECEPTOR GENE VARIATIONS AND MODULATION

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Significant advances have been achieved in marijuana and cannabinoid research, with the discovery of endocannabinoids (eCBs) in animals and humans that are components of an elaborate and ubiquitous endocannabinoid system (ECS). The ECS consists of eCBs, their synthesizing and degrading enzymes, and the genes encoding cannabinoid receptors (CBRs) - CB1Rs and CB2Rs. CB1Rs have been well characterized and are known to be abundantly distributed in mammalian CNS and peripheral tissues. However, the functional neuronal expression of CB2Rs in the brain has been much less well studied and characterized. The CNS effects of CB2Rs from mice to human subjects have been evaluated. Multidisciplinary approaches including RT-PCR, genotyping, immunoblotting, hippocampal cell cultures, immunoelectron microscopy, and stereotaxic techniques with behavioral assays were used to determine the CNS effects of CB2Rs. CB2Rs and their gene transcripts are expressed in the brains of mice and rats and are modulated following exposure to stressors and administration of drugs of abuse. Cannabinoid induced behavioral changes and CBR gene structures differ across species notably in motor function and emotionality tests in rats and mice. We discovered two isoforms of CB2Rs; CB2ARs are localized in the brain and testis whereas CB2BRs are in immune cells in the periphery in human genome.

In our preliminary *CNR2* gene copy number variation (CNV) studies, we analyzed one of the CNV regions located in intron of the *CNR2* gene in a human population of Japanese alcoholic DNA samples in comparison to non-alcoholic controls. The CNVs in *CNR2* gene region was confirmed to be relatively common in 10 out of 420 Japanese people. More alcoholic DNA samples and samples from other neuropsychiatric disorders and in other ethnic populations should be analyzed to determine the nature of elevated copy numbers of *CNR2* gene in neuropsychiatric disease risk. There was high incidence of Q63R polymorphism in the *CNR2* gene in psychosis, eating disorders, depression and alcoholics in the human population investigated. The genes encoding the components of the ECS and their gene products are major therapeutic targets in many disorders and in drug addiction and our data provide a basis for further studies.

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THE ACTIVATOR OF G-PROTEIN SIGNALLING TCTEX1 IS MODULATED BY CB2 RECEPTORS

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The dynein light chain protein tctex1, which is identical to the activator of G protein signalling 2 (AGS2), has been shown to bind to the beta subunit of GTP-binding protein (Gβ) (Takesono et al. 1999) and to GPCRs like rhodopsin and parathyroid hormone 1 receptor (Tai et al. 1999; Sugai et al. 2003). A putative consensus sequence of tctex1 interacting proteins is also present in the C-terminus of human CB2 receptors. However, we could not identify a direct interaction of CB2 receptors with tctex1 so far.

In the present study we wanted to test whether tctex1 co-immunoprecipitates with Gβ in transiently transfected HEK293 cells. Because we observed a modulatory role of CB2 receptor on tctex1 protein levels, we systematically studied this effect in co-transfection experiments. Additionally, we studied effects of transiently transfected Gβ/γ-proteins on tctex1 protein amounts and analysed if endogenous Gβ proteins co-localize in cells with tctex1 and CB2 receptors. Therefore we performed immunocytochemistry in the mouse monocytic RAW 264.7 cell line, which we analysed by laser-scanning microscopy.

Our results corroborate previous findings that tctex1 interacts with Gβ/γ proteins in co-immunoprecipitations. These results are strengthened by our immunocytochemistry experiments indicating a cellular co-localization of endogenous Gβ proteins with tctex1 and CB2 receptors in mouse monocytic cells. Furthermore, we identified that tctex1 is strongly down regulated by CB2 receptor and up regulated by Gβ/γ co-expression indicating a stabilizing effect of Gβ on tctex1. Interestingly, the tctex1 degradation induced by CB2 over-expression could be partially blocked by a proteasome but not by a lysosome inhibitor.

In summary, these results suggest that the CB2 receptor over-expression leads to a proteasomal degradation of the activator of G protein signalling tctex1 and that Gβ might act as a „scavenger“ rescuing tctex1 from this pathway.

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CANNABINOID CB2 RECEPTOR GENE EXPRESSION DIFFERENCES IN POST-MORTEM BRAIN AND LYMPHOCYTES SAMPLES FROM PARKINSON'S DISEASE PATIENTS

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CB2 cannabinoid receptor is differentially expressed between Parkinson's and controls patients in the cerebellum and hippocampus (Grunblatt et al., 2007). In addition, increased CB2r gene expression has shown a promising neuroprotective role in a rodent model of PD (Ternianov et al., 2009). The aim of this study was to analyze central (brain) and peripheral (lymphocytes) CB2r gene expression differences between PD and control patients.

Post-mortem human brain samples from Parkinson's (n=28) and appropriate controls (n=23) cases (substantia nigra, putamen, globus pallidus -internal and external parts-), and blood samples from recently diagnosed and non-treated Parkinson's disease patients (n=8) and appropriate controls (n=8) were analyzed. Gene expression studies of the specific A (central-brain) and B (peripheral-lymphocytes) isoforms of the CB2r were carried out by quantitative real time-PCR.

Gene expression analyses in brain samples showed that the A isoform is significantly reduced in putamen (-40%) and globus pallidus (internal, -47% and external, -31% parts). Interestingly CB2r gene expression dramatically increased (388%) in the substantia nigra. On the other hand, in lymphocytes from non-treated PD patients CB2r gene expression of B isoform gene expression is significantly down-regulated (-30%).

The results of this study revealed significant CB2r gene expression differences between PD and controls in key brain regions of the basal ganglia suggesting that this receptor may play a relevant role in the neurodegenerative process of PD. Preliminary alterations of peripheral CB2r gene expression in lymphocytes suggest the potential value of this receptor as a biomarker of this neurodegenerative process.

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OVEREXPRESSION OF CANNABINOID CB2 RECEPTORS ATTENUATED THE PROGRESSIVE MOTOR IMPAIRMENT AND NIGROSTRIATAL DOPAMINERGIC NEURONS LOSS IN MITOPARK MOUSE

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The purpose of this study was to evaluate if the increase of cannabinoid CB2 receptor (CB2r) gene expression in MitoPark mice (Ekstrand et al, 2009) result neuroprotective on motor impairment and loss of nigrostriatal dopaminergic neurons. MitoPark mice and their control littermates (30-32 weeks of age) were used for behavioral and neurochemical analysis. Double heterozygous mice were crossed with mice overexpressing CB2r to obtain MitoPark-CB2 mice. The open field test was carried out to evaluate motor activity. Real time-PCR and immunohistochemical analyses were performed to measure tyrosine hydroxylase (TH) and CB2r gene and protein expression in the substantia nigra (SN).

Traveled distance in the open field test was significantly reduced in MitoPark mice (-69%), although it was higher in MitoPark-CB2 mice (-55%) compared with MitoPark control littermates. TH gene expression was dramatically decreased in the SN of MitoPark mice (-93%), but this reduction was lower in MitoPark-CB2 mice (-80%). CB2r gene expression was significantly increased in MitoPark mice (+100%) and even more in MitoPark-CB2 mice (+489%). Stereological studies suggest that there were 55% and 36% less TH-positive neurons in the SN of MitoPark and MitoPark-CB2 mice, respectively.

These preliminary results suggest that CB2r plays a relevant role in the progression of motor impairment and loss of TH-positive neurons.

Reference: Ekstrand, MI, Galter, D. The MitoPark Mouse: An animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons. Parkinsonism and Related Disorders 15S3 (2009) S185–S188

SYNAPTIC PLASTICITY ALTERATIONS ASSOCIATED WITH MEMORY IMPAIRMENT INDUCED BY DELETION OF CB2 CANNABINOID RECEPTORS

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The identification of CB_2r in areas involved in learning and memory, such as the HIP, the close interaction between CB_2r and the GABAergic system, and the cognitive alterations observed in CB2KO mice, suggest that CB_2r might be an important neurobiological substrate for cognitive processes. In this study, the role of CB_2r on aversive memory consolidation was further evaluated. Mice lacking CB_2r (CB2KO) and their corresponding littermates (WT) were exposed to the step-down inhibitory avoidance test (SDIA). MAP2, NF200, and synaptophysin (SYN)-immunoreactive fibers were studied in the hippocampus (HIP) of both genotypes. The number of synapses, postsynaptic density thickness, and the relation between the synaptic length across the synaptic cleft and the distance between the synaptic ends were evaluated in the HIP (dentate gyrus (DG) and CA1 fields) by electron microscopy. Brain-derived neurotrophic factor (BDNF), glucocorticoid receptor (NR3C1) gene expressions and mTOR/p70S6K signaling cascade were evaluated in the HIP and prefrontal cortex (PFC). Finally, the effects of acute administration of CB_2r-agonist JWH133 or CB_2r-antagonist AM630 on memory consolidation were evaluated in WT mice by using the SDIA.

The lack of CB_2r impaired aversive memory consolidation and reduced MAP2, NF200 and SYN-inmunoreactive fibers and the number of synapses in DG of CB2KO mice. BDNF and NR3C1 gene expression were reduced in the HIP of CB2KO mice. An increase of p-p70S6K (T389 and S424) and p-AKT protein expression was observed in HIP and PFC of CB2KO mice. Interestingly, administration of AM630 impaired whereas JWH133 enhanced aversive memory consolidation. Further functional and molecular assessments would have been helpful to further support our conclusions.

These results revealed that CB_2r are involved in memory consolidation, suggesting that this receptor could be a promising target to develop novel treatments for different cognitive impairments-related disorders.

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ROLE OF CANNABINOID CB_2 RECEPTOR IN THE REINFORCING ACTIONS OF ETHANOL

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This study examines the role of the cannabinoid CB_2 receptor (CB_2r) on the vulnerability to ethanol consumption. The time-related and dose-response effects of ethanol on rectal temperature, handling-induced convulsions (HIC) and blood ethanol concentrations (BEC) were evaluated in CB_2KO and WT mice. Reinforcing properties of ethanol were evaluated in conditioned place preference (CPP), preference and voluntary ethanol consumption and oral ethanol-self-administration. Water maintained behavior schedule was performed to evaluate the degree of motivation induced by a natural stimulus. Preference for non-alcohol tastants assay was performed to evaluate differences in taste sensitivity. Tyrosine hydroxylase (TH) and μ-opioid receptor gene expressions were also measured in ventral tegmental area (VTA) and nucleus accumbens (NAcc), respectively.

CB_2KO mice presented increased HIC score, ethanol-CPP, voluntary ethanol consumption and preference, acquisition of ethanol-self-administration and significant motivation for ethanol-seeking behavior compared to WT mice. No differences were found between genotypes in water maintained behavior schedule and preference for non-alcohol tastants. Naïve CB_2KO mice presented increased μ -opioid receptor gene expression in NAcc. Acute ethanol administration (1-2 g/kg) increased TH and μ-opioid receptor gene expressions in CB_2KO mice whereas the lower dose of ethanol decreased TH gene expression in WT mice.

These results suggest that deletion of CB_2r gene increased the preference and vulnerability for ethanol consumption, at least in part, by ethanol-induced increase sensitivity on TH and μ-opioid receptor gene expressions in mesolimbic neurons. Future studies will determine the role of CB_2r as a target for the treatment of problems related with alcohol consumption.

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ROLE OF CB2 CANNABINOID RECEPTOR IN THE REINFORCING EFFECTS OF NICOTINE

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The present study was aimed to evaluate the involvement of CB2 cannabinoid receptors (CB2r) in the reinforcing and motivational effects of nicotine. For this purpose, conditioned place preference (CPP) and intravenous self-administration experiments were carried out in knock-out mice lacking CB2r (CB2KO) and in wild-type (WT) littermates. Gene expression analyses of tyrosine hydroxylase (TH), α3- and α4-nicotinic acetylcholine receptor subunits (nAChRs) in the ventral tegmental area (VTA), and immunohistochemical studies to elucidate if CB2r co-localized with α3- and α4-nAChRs in the nucleus accumbens (NAcc) and VTA were performed. Mecamylamine-precipitated withdrawal syndrome after chronic nicotine exposure was evaluated in CB2KO mice and in WT mice treated with the CB2r antagonist AM630 (1 and 3 mg/Kg).

CB2KO mice did not show nicotine-induced place conditioning and self-administered significantly less nicotine. Under baseline conditions, TH, α3- and α4-nAChRs mRNA levels in the VTA of CB2KO mice were significantly lower compared with WT mice. Confocal microscopy images revealed that CB2r co-localized with α3- and α4-nAChRs. Somatic signs of nicotine withdrawal (rearings, groomings, scratches, teeth chattering and body tremors) increased significantly in WT but were absent in CB2KO mice. Interestingly, the administration of AM630 blocked the nicotine withdrawal syndrome and failed to alter basal behavior in saline-treated WT mice.

These results suggest that CB2r play a relevant role in the reinforcing and motivational effects of nicotine. Pharmacological manipulation of this receptor deserves further consideration as a potential new valuable target for the treatment of nicotine dependence.

THE ROLE OF CANNABINOID RECEPTOR 2 IN THE EXPERIMENTAL PROLIFERATIVE VITREORETINOPATHY

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Introduction: Proliferative vitreoretinopathy (PVR) is an experimental model of a sight-threatening complication of retinal detachment, retinal tear, severe ocular trauma or inflammation. PVR is characterized by the proliferation and migration of retinal pigmented epithelial (RPE) cells to form contractile membranes over the inner and outer surfaces of the retina, as well as immune cell activation and infiltration of ocular tissues. Microglia, a resident population of macrophages in the central nervous system (CNS), play an important role in host defense and tissue repair. Paradoxically, microglia activation also contributes to neuronal damage in a number of CNS diseases, and may contribute to pathology seen in PVR. The endocannabinoid system is a potent regulator of immune response. Cannabinoid receptor 2 (CBR2) is expressed on all immune cells including the microglia and activation of this receptor by cannabinoid agonists has been shown to attenuate the inflammatory response and consequently reduce tissue damage. However, the mechanisms of action of cannabinoid ligands in mitigating disease severity in the eye are not fully understood. The aim of the current study was to examine the role of CBR2 in retinal microglia activation and pro-inflammatory cytokine production in PVR pathology, using a genetic knock-out of CBR2.

Methods: PVR was induced in $CBR2^{-/-}$ and C57BL/6 mice by intravitreal injections of dispase (0.1U; 2μl). The morphology of the eyes was evaluated under the light microscope at 7, 14, and 21 days after immunization, and the clinical scores were recorded. One or three weeks following the induction of PVR, animals were sacrificed; the eyes were enucleated and processed for histological grading and immunohistochemical staining for activated microglia. Pro-inflammatory cytokine expression profile in the eyes from $CBR2^{-/-}$ and C57BL/6 mice was evaluated by ELISA.

Results: The induction of the PVR resulted in pathological changes in retinas of $CB2R^{-/-}$ mice. In contrast, only mild, or absence of pathology, was observed in C57BL/6 animals treated with the same dose of dispase. In the $CB2R^{-/-}$ mice, there was evidence of retinal folds, retinal detachment, choroiditis (inflammation of the choroid), increases in inflammatory markers and exudation. In addition, we found a significant increase ($p<0.05$) in the number of activated microglia in the $CB2R^{-/-}$ animals, as compared to control, C57Bl/6 mice following induction of PVR.

Conclusion: Mice lacking CB2R showed increased susceptibility to retinal damage together with increased numbers of activated MG in an experimental model of PVR. This data is supportive of an immunosuppressive role for CB2R in inflammatory ocular conditions.

SPECIES DIFFERENCES OF CANNABINOID RECEPTOR 2 (CB2R) BRAIN EXPRESSION AND BEHAVIORAL EFFECTS OF CB2R LIGANDS IN MICE AND RATS

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The discovery of brain functional cannabinoid receptor 2 (CB2R) suggests a potential new therapeutic target for neurological and psychiatric disorders. However, species differences in CB2R gene structure and function confound the pharmacological and behavioral interpretations. We found that there are striking differences in expression and splicing of CB2R isoforms in mouse, rat and human brain regions (Liu QR et al., 2009). In comparison with human and rat CB2R genes, an evolutionally occurred premature stop codon in mouse mCB2R gene truncates 13 amino acids in the carboxyl-terminal motif containing an autophosphorylation site (Ser352). The phosphorylation of Ser352 was reported to induce human hCB2R cellular internalization, an effect that could be blocked by the CB2R inverse agonist SR144528 (Bouaboula M. et al., 1999). We previously found that mouse brain expresses mCB2A and mCB2B isoforms, with mRNA level of mCB2A higher than that of mCB2B in different mouse brain regions. In the current study, we discovered novel rat-specific isoforms of rCB2C and 2D that are produced by inter- and intra-exonal splicing events, respectively, without altering the protein coding sequence. Rat rCB2C is a brain enriched isoform with mRNA levels lower in spleen and higher in striatum and amygdala in comparison with those of rCB2A, rCB2B and rCB2D.

The pharmacological effects of JWH133 (a selective CB2R agonist) or WIN55212-2 (a dual CB1/CB2 receptor agonist) are also significantly different between mice and rats. Systemic administration of JWH133 (10, 20 mg/kg) significantly and dose-dependently inhibited ***mouse*** intravenous cocaine self-administration under both fixed-ratio (FR) and progressive-ratio (PR) reinforcements (Xi ZX et al., 2011), but had little effect on ***rat*** intravenous cocaine self-administration under FR reinforcements (while increased PR break-point for rat cocaine self-administration). Systemic administration of WIN55212-2 (1 and 2 mg/kg) significantly reduced spontaneous wheel running activity in mice, but not in rats. In the elevated plus-maze test the rats were more sensitive to WIN55212-2 than mice, in producing anxiety-like behaviors. These findings suggest significant species differences in both brain CB2R gene expression and the pharmacological action of CB-R agonists (including CB2R-selective) in mice and rats.

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THE USE OF AN *EX VIVO* PORCINE INTESTINAL TISSUE MODEL TO STUDY THE ROLE OF THE INTESTINAL ENDOCANNABINOID SYSTEM

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Introduction: Nutrient sensing may be defined as the ability to sense available nutrients and to generate a physiologically adequate regulatory response to these nutrients involving release of satiety hormones such as glucagon like peptide 1 (GLP-1). Endocannabinoids (ECs) and N-acyl ethanolamines (NAEs) are known to play a role in food intake regulation, however their function in the gut is not yet understood. It is hypothesized that ECs and NAEs may regulate satiety and appetite by controlling satiety hormone secretion.

Methods: *Ex vivo* porcine intestinal segments from healthy adult male pigs were stimulated with carbohydrate (sucrose), protein (casein), and fatty acid (safflower oil) in various concentrations. Tissue levels of ECs and NAEs were analyzed using a LC-MS method. Incubation media was analyzed for GLP-1 and lactate dehydrogenase (LDH) content. Leakage of intracellular LDH was included as viability marker**.**

Results: All the analyzed ECs and NAEs were found in pig duodenum, jejunum and ileum. Stimulation of intestinal tissue segments with nutrients resulted in changed tissue levels of ECs and NAEs (AEA, 2-AG, PEA, OEA, SEA, DLE, SHEA and EPEA), coinciding with an increased release of GLP-1.

Conclusion: *Ex vivo* intestinal pig tissue can be used as a physiologically relevant model to study the interaction of nutrients, GLP-1 release and intracellular levels of ECs and NAEs. Preliminary data show that that ECs and NAEs may be involved in nutrient sensing by mediating GLP-1 production and regulation of food intake.

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EFFECTS OF CAPSAICIN OVER TENSION IN THE PRESENCE AND ABSENCE OF CANNABINOID AND VANILLOID ANTAGONISTS IN THE FAST SKELETAL MUSCLE FIBERS OF THE FROG

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Skeletal muscle possesses two types of muscle fibers: 1) fast and 2) slow or tonic (Gilly and Hui, 1980; Huerta et al. 1986). In these muscle fibers exists evidence of cannabinoid receptors presence (Sánchez-Pastor et al., 2007; Huerta et al. 2009).

It is well known that action of CB1 receptor agonist, as ACPA (Anandamide synthetic-derived), decreases tension in slow muscle fibers of the frog (Huerta et al., 2009). This effect is partially reverted by the antagonist AM281, suggesting the participation of more than one pathway in the action of cannabinoids over tension. On the other hand, is also known that vanilloid receptor TRPV1 is activated by capsaicin, which has been described in skeletal muscle (Xin et al. 2005; Huerta et al. 2009). The activation of the vanilloid receptor inhibits the Ca^{2+} channel, which implies a possible pathway to explicate the action of cannabinoids in the skeletal muscle fibers.

By the use of isometric recording in the fast skeletal muscle fibers of the frog, the effect of capsaicin (10 μM) over caffeine (6 mM) evoked contractures was measured in the presence and absence of the antagonists AM281 (5 μM) and capsazepine (1 μM), for CB1 and TRPV1, respectively. Capsaicin diminished tension in the fast skeletal muscle fibers until 42.71% respecting to control, an effect that was partially reversible in the presence of capsazepine (32.9%). The application of the TRPV1 antagonists (CAPZ) and CB1 (AM281), reverted the diminishment over tension caused by capsaicin (94.90%, $p<0.05$) emulating the control conditions. These results suggest that in addition to activate TRPV1, capsaicin is also capable to activate the CB1 cannabinoid receptor.

CANNABINOID RECEPTORS IN HUMAN, WISTAR RAT AND ZUCKER RAT SKELETAL MUSCLE TISSUES, MYOTUBES AND MYOBLASTS

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Skeletal muscle is one of the major insulin sensitive tissues and is responsible for 80% of insulin stimulated glucose disposal. Elevated circulating levels of endocannabinoids have been reported in obese and diabetic individuals, the tissue expression level and the link between cannabinoid receptors and the insulin signalling in skeletal muscle (skm) are contradictory [1, 2,& 3]. The aim of this study was to characterize the cannabinoid receptors and identify their role in cell signalling in human and rat skm tissue and primary cultured skm cells. The expression of other cannabinoid-related receptors, GPR 119 and GPR55 was also investigated. Skm tissues (fast and slow fibres) were collected from rat (Wistar and Zucker lean and fat) and human skm biopsies were obtained from the vastus lateralis of healthy volunteers using needle biopsy. Satellite cells were isolated and cultured as previously described [4]. Gene expression was measured using Taqman real time PCR and Agilent one colour microarray. Protein expression was determined by western blot.

The microarray analysis indicated the expression of CB1 , CB2, GPR55 & GPR119 as well as metabolic enzymes FAAH, MGLL, DAGLA, DAGLB and NAPE-PLD in human skeletal muscle tissue and primary cultured myoblasts and myotubes. RT-PCR analysis confirmed the expression of CB1, CB2 and GPR119 but not GPR55 in both human and rat muscle tissue and cultured cells. Expression levels of CB1, CB2 and GPR119 in muscle tissues were similar at 12weeks for Zucker Fat Rat (ZFR) and Zucker Lean Rat (ZLR)($p>0.05$), however at 20 weeks ZFR had a significantly ($p<0.01$) higher expression of CB1 than ZLR. In cultured human and rat myotubes treatment with endogenous (10µM AEA) and synthetic (10nM ACEA) CB1 agonists increased ERK1/ERK2 and MAPK p38 phosphorylation ($P<0.05$). Treatment with a CB1 selective antagonist (100nM Rimonabant) attenuated ERK activation in cells obtained from Wistar and ZLR ($P<0.05$) but not ZFR. In contrast to previously published work,, neither 100nM ACEA nor 100nM Rimonabant affected insulin-stimulated phosphorylation of Akt or GSK3α/b ($P>0.05$) (Figure 1). In conclusion, cannabinoid receptors as well as the enzymes involved in their synthesis and degradation are expressed in skm Our data indicates that CB1 receptors arte elevated in the ZFR model of obesity and that cultured cells from obese animals were refractory to the inhibition of CB1 by Rimonobant. The lack of effects of CB1 activation upon insulin signalling is in contrast to previous studies which indicate that CB1 activation diminishes the response to insulin.

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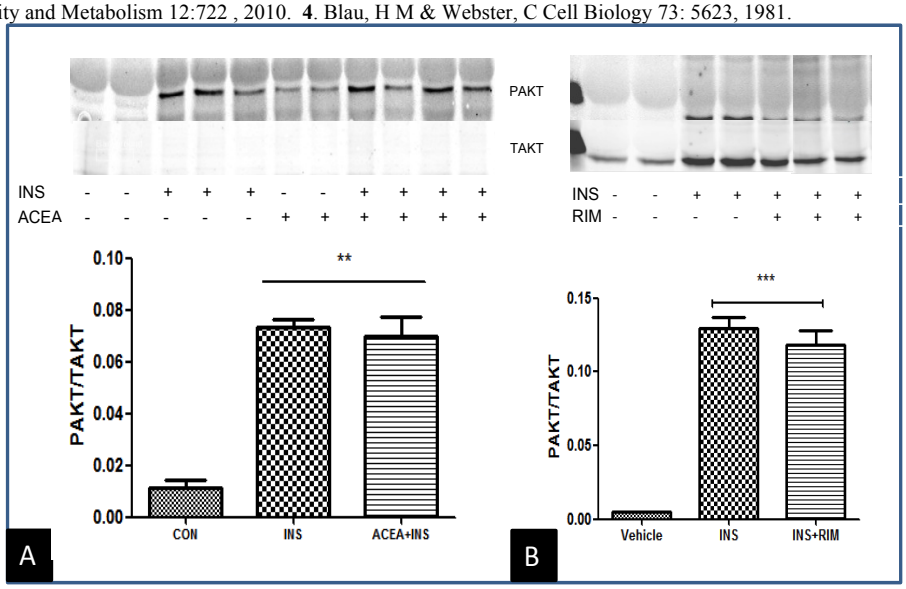

Effect of ACEA and RIM on insulin stimulated AKT phosphorylation in Wister rat myotubes. A) Pre-treatment (24 hours) of myotubes with 100nM ACEA did not alter the acute (10min) stimulatory effect of 100 nM INS (insulin) on AKT phosphorylation. ** denotes $P<0.01$ compared to vehicle (0.01%). B) Pre-treatment (24 hours) of myotubes with 100nM RIM did not alter the acute (10min) stimulatory effect of 100 nM INS (insulin) on AKT phosphorylation. * ** denotes compared to vehicle ($P<0.001$). Protein expression (above) and Densitometric (bellow) of PAKT and TAKT. Data were analysed by one way ANOVA with Bonferroni post-hoc test.

BLOCKADE OF CANNABINOID RECEPTOR 1 INCREASES PANCREATIC BETA CELL INSULIN CONTENT IN A DIABETIC MOUSE MODEL

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Type 2 diabetes mellitus is characterized by progressive loss of pancreatic beta (β)-cell function, resulting in decreased insulin secretion and uncontrolled hyperglycemia. With more than 25.8 million individuals suffering from diabetes in the U.S. alone and another 79 million with pre-diabetes, there is an urgent need to develop novel therapies to treat this rapidly expanding metabolic disease. The endocannabinoid system, composed of both lipid-derived ligands and their receptors, is an attractive target since overactivation of this system has been linked with obesity, insulin resistance and impaired glucose homeostasis. The cannabinoid receptor 1 (CB1R) is of particular interest as blockade of this receptor by inverse agonists such as rimonabant have induced potent weight-loss effects. Interestingly, our previous studies have demonstrated that both rodent and human pancreatic β-cells express CB1R and that inhibition of this receptor increases insulin secretion, β-cell proliferation and insulin sensitivity. However, whether such beneficial effects also occur in the islets of Langerhans of a diabetic model has yet to be elucidated.

The objective of the current study was to determine if CB1R inverse agonists would alter β-cell function in the *db/db* diabetic mouse model. Chronic treatment with either rimonabant or a novel, peripherally-specific CB1R inverse agonist (JD-5037) significantly decreased body weight and blood glucose levels compared to vehicle-treated mice. Importantly, both compounds also increased insulin content and total β-cell area compared with their vehicle counterparts. Collectively, these results suggest that such peripherally-specific CB1R inverse agonists may be a useful therapeutic to protect β-cell function in diabetic individuals.

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MONOGLYCERIDE LIPASE-DEFICIENCY IMPAIRS LIPOLYSIS AND LEADS TO CANNABINOID RECEPTOR DESENSITIZATION

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Monoglyceride lipase (MGL) influences energy metabolism by at least two mechanisms: First, it hydrolyzes monoglycerides (MG) into glycerol and fatty acids. These products may consequently be used for energy production or synthetic reactions. Second, MGL degrades 2-arachidonoyl glycerol (2-AG), the most abundant endogenous ligand of cannabinoid receptors (CBR). Activation of CBRs affects energy homeostasis by central orexigenic stimuli, by promoting lipid storage, and by reducing energy expenditure. To characterize the metabolic role of MGL *in vivo*, we generated a MGL-deficient mouse model (MGL-ko). These mice exhibit a reduction in MG hydrolase activity and a concomitant increase in MG levels in brain, liver and white adipose tissue. Fasted MGL-ko mice exhibit reduced plasma glycerol, plasma triglyceride, and liver triglyceride levels indicative for impaired lipolysis. Interestingly, MGL-ko mice receiving a high-fat diet exhibit significantly improved glucose tolerance and insulin sensitivity in comparison to wild-type controls despite equal weight gain. Despite a strong elevation of 2-AG levels in brain and peripheral tissues, MGL-ko mice exhibit normal food intake, liver TG content, fat mass, and energy expenditure. Yet, mice lacking MGL show a pharmacological tolerance to the CBR agonist CP 55,940 suggesting that the elevated 2-AG levels are functionally antagonized by desensitization of CBRs. In conclusion, our observations implicate that MGL-deficiency impairs lipolysis and attenuates diet-induced insulin resistance. Defective degradation of 2-AG does not provoke cannabinoid-like effects on feeding behavior, lipid storage, and energy expenditure which may be explained by desensitization of CBRs.

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INVOLVEMENT OF CANNABINOID RECEPTOR TYPE 2 IN MICTURITION IN MICE

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Introduction: Systemic administration of cannabinoid (CB) receptor agonists affects bladder function, but the contribution of individual CB receptors during normal micturition has not been clearly defined. Our goal was to study if differences in urodynamic endpoints or *in vitro* bladder contractility exist between CB receptor type 2 knockout (CB2 -/-) and C57BL/6J control mice following CB2 receptor modulators.
Methods: Female C57BL/6J (n=15) and CB2 -/- mice (n=15) underwent bladder catheterization three days prior to cystometry (approved by IACUC). Cystometry was performed in awake animals at baseline, and after sequential administration of HU308 (CB2 agonist) followed by AM630 (CB2 antagonist). Comparisons of effects were made with ANOVA of repeated measures. Bladders were extracted for *in vitro* assessment of contractility to carbachol and electrical field stimulation (EFS).
Results: Bladder capacity (BC) in C57BL/6J mice was increased from 0.0322 ± 0.0047 (baseline) to 0.0397 ± 0.0053 mL by HU308, and then decreased to 0.0324 ± 0.0058 mL after AM630 ($p<0.05$). Similarly, intercontraction interval (ICI) increased from 77.2 ± 11.4 sec to 95.2 ± 12.7 sec with HU308, and then decreased to 77.8 ± 14.0 sec with AM630.
CB2 -/- mice had at baseline a lower maximal pressure (MP) (26.7 ± 1.4 vs 32.7 ± 1.4 cm H_2O), basal pressure (BP) (12.0 ± 1.7 vs 17.3 ± 1.8 cm H_2O), and area under the curve (AUC) (16.9 ± 1.6 vs 22.7 ± 1.9), and a higher ICI (139.9 ± 19.6 vs 77.2 ± 11.4 sec), BC (0.0583 ± 0.00819 vs 0.0322 ± 0.0048 mL) and compliance (0.0058 ± 0.0008 vs 0.0037 ± 0.0004 mL/cm H_2O) than controls ($p<0.05$).
There were no differences in contractility after carbachol or EFS between both groups.
Conclusion: Indicating a role for the CB2 receptor in afferent micturition signals in normal mice, CB2 receptor agonism modified ICI and BC. CB2 -/- mice had lower pressures, along with a longer ICI and larger BC and compliance than C57BL/6J. These differences may be explained by compensatory up-regulation of other signals due to the knockout of CB2.

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GASTROPROTECTIVE EFFECTS OF THE MONOACYLGLYCEROL LIPASEINHIBITOR KML29 IN MICE

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used analgesics that paradoxically cause gastrointestinal irritation and bleeding. These side effects of NSAIDs are so well characterized that NSAID use has now replaced bacterial infection as the leading cause of gastritis. Cannabinoids are a class of analgesic compounds that are also known to have anti-inflammatory effects. The endogenous cannabinoid 2-arachidonoylglycerol (2-AG) binds to and activates cannabinoid receptors CB_1 and CB_2. 2-AG is primarily metabolized *in vivo* by the enzyme monoacylglycerol lipase (MAGL). The highly selective MAGL inhibitor KML29 irreversibly blocks MAGL from metabolizing 2-AG, thereby indirectly increasing brain levels of 2-AG. Published reports indicate that MAGL is a potential therapeutic target for attenuating NSAID-induced gastric hemorrhages. However, the gastroprotective effects of KML29 are unknown. Moreover, previous studies used the less selective MAGL inhibitor JZL184, which also inhibits fatty acid amide hydrolase and increases brain levels of anandamide and other fatty acid amides. Thus, in the present study, we tested whether a range of doses of KML29 (1 - 40 mg/kg, ip) would block gastric hemorrhages induced by the NSAID diclofenac sodium (100 mg/kg, po) in fasted mice. Mice that received only diclofenac exhibited gastric hemorrhages, while mice that were pretreated with KML29 ($\geq$ 5 mg/kg) showed a statistically significant decrease in gastric hemorrhages. Pretreatment with the CB_1 antagonist rimonabant (3 mg/kg, ip) blocked the gastroprotective effects of KML29, indicating the necessity of CB_1 receptors. These data suggest that MAGL represents a promising target for the development of new treatments of gastritis.

EARLY LIFE EXPERIENCE AFFECTS BEHAVIORAL RESPONSES TO ADOLESCENT DELTA-9-TETRAHYDROCANNABINOL EXPOSURE IN THE RAT

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Marijuana is the most commonly abused illicit substance in the United States. Additionally, almost 60% of all first time marijuana users were under the age of 18 in 2010. Adolescents who use marijuana are more likely to exhibit anxiety, depression and other mood disorders including psychotic-like symptoms. However, our study on the effects of early adolescent (P29-38) Δ-9-tetrahydrocannabinol (THC) exposure in rats shipped on P14 showed no effect on a measure known to be altered in clinical and pre-clinical models of psychosis, the pre-pulse inhibition (PPI) task. PPI is a measure of sensorimotor gating and reduced response to the pre-pulse can indicate abnormal sensory information and motor integration and processing. Since early postnatal stress is known to alter the function of the endocannabinoid system (ECS), we repeated the study in rats born in our vivarium and exposed to our standard rearing/handling protocol (weekly weighing starting at P1). Preliminary data suggest that THC dampens the PPI response (a pro-psychotic effect) in animals raised in our vivarium, with a stronger effect in males than females. In contrast, our study examining early adolescent THC effects on elevated plus maze (EPM) behavior (a measure of anxiety) showed no effects in male animals born in our vivarium, but significant effects in males shipped at P14. Similar effects were found in males in the forced swim test (FST) (a measure of depressive-like behavior). Males shipped at P14 tended to show less anxiety and less depressive-like behavior in the EPM and FST, respectively, following THC administration. Therefore, early life stress increases responsiveness to THC in some behavioral modalities (EPM and FST) and decreases responsiveness to THC in others (PPI). These data indicate that early life experience can alter the effects of adolescent THC exposure on aspects of rodent emotional and psychotic-like behavior which could be due to unique alterations in ECS function in brain regions mediating these different behaviors.

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URB597 DIFFERENTLY AFFECTED EMOTIONAL AND COGNITIVE SIGNS INDUCED BY ADOLESCENT EXPOSURE TO THC: POSSIBLE CELLULAR MECHANISMS

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We recently demonstrated that chronic administration of THC to adolescent female rats induces subtle but long-lasting alterations in emotional behaviours and cognition still present in adult animals. Chronic treatment with the inhibitor of the fatty acid amide hydrolase URB597 in adult animals was able to reverse most depressive-like symptoms induced by adolescent THC exposure, such as the passive coping strategy observed in the forced swim test as well as anhedonia and the reduced social activity. However URB597 treatment was not able to improve the cognitive impairment induced by adolescent THC exposure.

In order to investigate the possible cellular mechanisms of URB597 effects we focused our attention on the cerebral areas most involved in emotion and cognition, the prefrontal cortex and hippocampus, and performed a series of morphological, electrophysiological and biochemical analyses. Thus adolescent female rats were exposed to increasing doses of THC for 11 days, and when they reached adulthood, the age where the behavioural deficits were observed, they were submitted to URB597 treatment (0.3 mg/kg daily for 3/4 weeks). In the prefrontal cortex chronic URB597 treatment was able to recover the decrease in CB1 receptor density induced by THC pre-treatment as well as the impairment in the endocannabinoid-mediated LTD. In the hippocampus, adolescent THC exposure decreased both the number and dendritic length of doublecortin positive cells, and URB597 was able to fully recover these parameters. URB597 per se was able to significantly increase both the number and the dendritic length of newborn neurons, thus suggesting that it could exert a trophic action. Regarding newborn neurons functionality, in control animals high frequency stimulation of the medial perforant path in the absence of GABAA receptor blockers was able to induce newborn neuron-dependent LTP that was instead blocked in THC pre-exposed rat hippocampal slices, and recovered after URB597 treatment. However, when we checked the survival of these newborn neurons by monitoring BrdU/NeuN positive cells, we found that both adolescent THC exposure and URB597 treatment per se decreased cell survival and URB597 treatment in THC-pre-exposed rats only partially recovered it. This could explain, at least in part, why URB597 treatment was not able to recover the cognitive impairment induced by adolescent THC exposure.

As a whole these data suggest that the recovery of the prefrontal cortex functionality may play a role in the URB597 ability to improve the emotional deficits induced by adolescent exposure to THC, whereas the still altered picture in the hippocampus could account for the lack of URB597 effect on cognition.

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ADOLESCENT THC EXPOSURE RESULTS IN ALTERATIONS OF GABAergic SIGNALLING WITHIN THE ADULT RAT PREFRONTAL CORTEX

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Exposure to delta-9-tetrahydrocannabinol (THC) in female Sprague-Dawley rats during adolescence leads to long-term disturbances of cognitive performances and emotional reactivity, and sensitizes to the locomotor activating effects of acute PCP, when administered in adulthood. Interestingly, no behavioural abnormalities are observed when THC is administered in adulthood, suggesting a specific vulnerability of the adolescent brain to the long-term adverse effects of cannabinoids.

However, the neurobiological processes underlying this vulnerability are unknown. Based on the observation that cortical GABAergic deficits contribute to both the pathophysiology and symptomatology of schizophrenia, in the present study we investigated whether adolescent THC exposure could affect the maturation of the GABAergic system in the adult prefrontal cortex (PFC), possibly leading to abnormal behavioral responses in adulthood.

Biochemical analyses showed that adolescent THC exposure results in lower GAD67 levels, the enzyme responsible for most GABA synthesis, as well as in reduced basal GABA release within the adult PFC. Double immunofluorescence staining revealed that GAD67 expression is reduced both in parvalbumin (PV)- and cholecystokinin (CCK)-containing interneurons.

To investigate whether reduced GAD67 expression is sufficient in determining the behavioral alterations observed in adult rats that underwent adolescent THC exposure, we silenced GAD67 expression selectively in the PFC of adult naïve rats and tested them for behavior. Silencing GAD67 expression in the PFC was sufficient to impact rats' behavior in the FST, suggesting that this biochemical alteration may be directly related to the altered emotional reactivity triggered by adolescent THC.

These results suggest for the first time that alterations in the GABAergic system within the PFC could play a role in the development of some signs of the THC-induced psychotic-like phenotype.

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CANNABINOID CB1 RECEPTOR FUNCTIONS AND ENDOCANNABINOID LEVELS IN THE PROGRESSION OF NORMAL AGING

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The endocannabinoid (eCB) system has emerged as a multifunctional neuromodulatory system, implicated in a plethora of physiological and pathophysiological processes. Among others, it plays important roles in memory processing, neuroprotection and synaptic transmission. The most prominent receptor that mediates the effects of the two major eCBs, 2-arachidonoyl glycerol and anandamide, in the brain is the cannabinoid type 1 (CB1) receptor.

The dichotomy of CB1 receptor functions in the two major neurotransmitter systems of the brain, glutamatergic and GABAergic neurons, has been the focus of numerous investigations. Furthermore, several studies indicate that impaired eCB signaling contributes to some features that are also typically observed in aging, e.g. memory impairment and neuroinflammation.

In the present study, we aimed at understanding the dynamics of the activity status of the eCB system in the wild-type mouse brain during the progression of normal aging, ranging from young age (6 weeks) via middle age (6 months) to old age (18 months). In the light of the dichotomic functions of the CB1 receptor in glutamatergic and GABAergic neurons, the activity status of the eCB system was also determined in conditional CB1 receptor mutant mice, lacking the receptor in either of these two neuronal populations. A gene expression study of the eCB components via quantitative PCR, and the determination of eCB levels in different brain regions elucidated age-related variations of the eCB system and also allowed us to address the question whether or not conditional CB1 receptor mutant mice display compensatory processes affecting the eCB system. Lately, by testing hippocampus-dependent behavior in these two conditional mutants, we aimed at unraveling the roles of the eCB system in age-dependent cognitive performance.

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PRODUCTION OF LYSOPHOSPHATIDYLINOSITOL SPECIES AND GPR55 MEDIATED SIGNALING IN OSTEOCLASTS

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A role for GPR55 in bone physiology was the first study to demonstrate a non-neuronal physiological role for GPR55 *in vivo* (Whyte *et al*, 2009). In summary, male mice lacking GPR55 display a marked high bone mass phenotype due to impairment in osteoclast function. Consistent with this, the GPR55 agonist, L-α-Lysophosphatidylinositol (soy LPI), stimulates osteoclast function *in vitro*. Soy LPI contains 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoinositol (16:0 LPI) and 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphoinositol (18:0 LPI), but does not contain 1-arachidonoyl-2-hydroxy-*sn*-glycero-3-phosphoinositol (20:4 LPI), thought to be the endogenous GPR55 agonist. Given that osteoclasts express GPR55 and are subject to regulation by LPI, it is not known whether osteoclasts produce LPI and whether levels are subject to regulation by GPR55 agonists or by cytokines known to stimulate osteoclastic resorption. Using LC-MS, levels of 16:0 LPI, 18:0 LPI and 20:4 LPI were measured in human osteoclasts treated with O-1602, the synthetic GPR55 agonist, or the pro-inflammatory cytokines IL-1β or TNFα. Upon treatment of human osteoclasts with 1μM O-1602, levels of all 3 species of LPI were significantly increased relative to control. These results suggest that activation of GPR55 results in an increase in production of the endogenous ligand. Treatment of osteoclasts with 10U/ml IL-1β, but not TNFα, significantly increased 16:0 LPI and 18:0 LPI. These results suggest that IL-1β may increase osteoclast resorption through local production of LPI species.

Previously, we demonstrated soy LPI activates RhoA in human osteoclasts *in vitro.* Having shown that human osteoclasts produce 20:4 LPI, we sought to determine whether there are differences in the ability of individual LPI species to signal via GPR55 to RhoA. To do this we utilised cellular impedance technology [xCELLigence]. Initial experiments in HEK293 and GPR55-HEK293 cells revealed that all 3 species of LPI produce a GPR55 mediated, dose dependant decrease in cellular impedance with a rank order of potency as follows: 20:4 > 16:0 > soy LPI > 18:0 LPI. These effects were significantly inhibited by the ROCK inhibitor Y27632, suggesting that the GPR55 mediated decrease in cellular impedance is attributed to downstream activation of RhoA. Experiments in human osteoclasts also demonstrated that all species of LPI significantly decreased cellular impedance with a rank order of efficacy as follows: 20:4 > 18:0 > 16:0. The effects of 18:0 LPI and 20:4 LPI were attenuated by Y27632. These studies advocate the use of impedance technology to study GPR55 mediated signaling in primary cells *in vitro*. Further studies are necessary in order to determine whether the difference in species efficacy identified in this study translates to their ability to stimulate osteoclast function *in vitro.*

THE EFFECT OF DIFFERENT CONCENTRATIONS OF URB602 ON HEALING OF SCRATCHED CHONDROCYTE MONOLAYERS

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Monoglycerol lipase (MGL) terminates the biological ability of endogenous cannabinoid receptor 1 (CB1), i.e. MGL hydrolyzes 2-Arachidonoyl Glycerol (2-AG) to arachidonic acid and glycerol, thereby terminating its biological actions. On the other hand, URB602 is a selective inhibitor of MGL, thus increasing the effects of 2-AG on CB1 receptors. Our previous studies showed that treating chondrocytes with 1µM of the synthetic non-specific CB1 and CB2 agonist WIN55, 212-2 (WIN-2) increased the rate of wound closure in a simple scratch assay model. In this study, the role of two different concentrations of URB602 with and without the synthetic CB1 antagonist LY-320,135 on the rate of chondrocyte wound closure has been investigated. Chondrocytes were treated with 1µM and 2µM of URB602, and 1µM of URB602 + 500nM of LY-320,135. The rate of chondrocytes wound closure has been measured and compared to those of untreated chondrocytes. Although treating chondrocytes with 1µM of URB602 increased the rate of wound closure, it was not as significant as treating them with 1µM of WIN55, 212-2 (WIN-2). Furthermore, adding 500nM of the CB1 antagonist LY-320,135 did not affect the rate of wound closure, suggesting that URB602 did not act via CB1 receptor pathway. On the other hand, treating chondrocytes with 2µM of URB602 lead to a significant decrease in cell proliferation and rate of wound closure.

THE EFFECT OF MONOGLYCEROL LIPASE INHIBITOR (URB602) ON THE HEALING ACCELERATION OF MG-63 OSTEOBLAST SCRATCHED MONOLAYERS

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Endocannabinoids are known to play an important role in the regulation of bone mass and bone remodelling (Idris and Ralston, 2010). One of the main endocannabinoids in bone is 2-arachidonoylglycerol (2-AG). Bone osteoblast cells have been reported to express type 1 cannabinoid receptor (CB1), type 2 cannabinoid receptor (CB2), and orphan G protein coupled receptor 55 (GPR55) which has been found to be activated by some cannabinoid receptor ligands (Ross, 2009). However, GPR55 does not appear to play a role in regulating bone formation (Whyte et al., 2009). Interestingly, CB1 expression in osteoblasts and their precursors is expressed in low levels (Tam et al., 2006) and the 2-AG synthesizing enzymes DAGLα and DAGLβ are expressed in bone cells since blood 2-AG level is negligible. In the current study the effect of monoglycerol lipase (MGL) inhibiter URB602 was investigated on MG-63 osteoblast scratched monolayers. It is known that MGL hydrolyzes 2-AG to arachidonic acid and glycerol, thereby terminating its biological actions. Our results showed that MGL inhibitor URB602 accelerated wound healing with concentration of 1μM and increased proliferation rate. In contrast, cell migration as indicated by the percentage of cell surface reduction, was highest for concentrations of 500nM and 2μM and decreased for concentration of 2μM. Immunofluorescence revealed fibronectin and collagen type I expressions which are related to the mechanisms of wound healing, thus indicating the potential role of URB602 in regulation of bone formation.

THE EFFECT OF DIFFERENT CONCENTRATIONS OF SYNTHETIC CANNABINOID RECEPTOR 2 AGONIST (HU-308) ON HEALING OF SCRATCHED CHONDROCYTE MONOLAYERS

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Cannabinoids have anti-inflammatory effects with production of eicosanoids promoting the resolution of inflammation. Cannabinoid receptor type-2 (CB2) appears to be responsible for the anti-inflammatory and possibly other therapeutic effects of cannabis. Furthermore, CB2 has been identified on chondrocyte membrane. Our previous studies showed that treatment of chondrocytes with 1μM of the synthetic non-specific CB1 and CB2 agonist WIN55, 212-2 (WIN-2) increased the rate of wound closure in a simple scratch assay model. In this study, the effect of three different concentrations of HU-308 with and without the synthetic CB2 antagonist AM630 on the rate of chondrocyte wound closure has been investigated. Chondrocytes were treated with 500nM, 1μM and 2μM of HU-308, and 500nM of HU-308 + 500nM of AM630. The rate of chondrocytes wound closure was measured and compared to those of untreated chondrocytes. Unlike WIN-2, the concentraion that gave the higest wound closure rate was at 500nM. Furthermore, adding 500nM of the CB2 antagonist AM630 to this concentration reduced the rate of wound closure suggesting that HU-308 may have acted via CB2 receptor pathway. On the other hand, the 1μM of HU-308 treatment decreased the rate of wound closure, and the 2μM treatment significantly decreased both cell proliferation and migration.

THE EFFECT OF HU308/TGF-B3 COMBINATION ON MG-63 OSTEOBLAST WOUND HEALING

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Endocannabinoids (ligands) and their receptors have been identified in the skeleton system. It has also been indicated that their functions in bone are physiologically regulated by CB2 receptors. The purpose of this investigation was to study the rate of wound healing using a scratch assay wound model created on MG63 osteoblast bone cell-line monolayers and to investigate their proliferation and migration using CB2 agonist HU308 in combination with TGF-β3. It is well established that the process of osteoblast cell wound healing occurs in three distinct but overlapping stages: (1) The early inflammatory stage; (2) The repair stage; and (3) The late remodelling stage, thus the use of Inflammatory or cytotoxic medication during the 1st stage of injury may delay the inflammatory response which is termed the reactive phase that is responsible for the recruitment of various cytokines to the site of injury. One such cytokine is transforming growth factor-Beta3 (TGF-β3) which is most abundant in the regulation of bone osteoblast cells. Our finding suggested that proliferation rate with 500nM HU308 was significantly higher than control and TGF-β3/HU308 combination groups ($P<0.005$). Interestingly, percentage of wound remained open after 15 hours for combination groups was 17.6%±1.32 whereas HU308 treatment groups had 20%±2.25 indicating that the combination groups took the lead throughout wound healing. It was also observed that bridge formation in treatment groups was taking place between 15 to 20 hour period whereas within control groups bridge formation started to take place after 25 hours. So far, this is the only investigation with CB2 HU308 selective synthetic cannabinoid in combination with TGF-β3 suggesting its synergistic role as a combination in the process of MG-63 osteoblast monolayer wound healing.

THE EFFECT OF CANNABINOID ON HEALING OF SCRATCHED HACAT CELL MONOLAYERS

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Cannabinoids are compounds from the marijuana plant and their receptors CB1 and CB2 have been found in epidermal keratinocytes of normal skin and can be modulated through specific ligands [Ständer S, et al. 2005]. Damaged cells secret nitric oxide (NO) into the wound area and excess NO inhibits the important proteoglycan synthesis necessary for efficient wound repair [Mbvundula EC, et al. 2005]. It was also found that excessive amount of NO mediates inhibition of epithelial wound closure, especially by decreasing cell migration. This study showed that synthetic WIN55,212-2 cannabinoid agonist inhibited NO production or at least reduced its negative effects. Addition of a cannabinoid antagonist was also found to affect the impact of WIN55,212-2, leading to further improved wound closure [Mbvundula EC, et al. 2005 and Pertwee RG, 1997].
In this work the effect of WIN55,212-2 additions in two different concentrations of 1.0µg/ml and 1.5µg/ml using DMSO and ethanol as solvents on healing of scratched HaCaT cell monolayers have been investigated. Comparison was therefore made between the two solvents, i.e. DMSO and ethanol in terms of the wound healing process. The effect of antagonist LY320135 additive (1.5µg/ml in ethanol) in the presence of agonist WIN55,212-2 on the healing of scratched HaCaT cell monolayers was also investigated. Measurement of wound width was carried out every 2 hrs over a period of 12 hrs for the above mentioned experiments.

The first 2 hour interval showed slight decrease or even an increase in wound width and wound closure after the first 2 hour was regarded as almost linear. DMSO appeared to reduce wound closure and ethanol did not seem to affect wound closure significantly. The cannabinoid agonist WIN55,212-2 showed less wound closure after 12 hours than the control even in the presence of antagonist LY320135. It was therefore observed that reduction in contact inhibition lead to migration and proliferation of cells and that in the first 2 hours after scratching, there is an adjustment to the environment with removal of damaged border cells. DMSO was found to be an inefficient solvent due to its toxic impact on cells.

STRUCTURE OF THE CANNABINOID RECEPTOR 1: HOMOLOGY MODELING AND ENRICHMENT STUDY BASED ON CB1 ANTAGONIST DOCKING

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Cannabinoid receptor 1 (CB1) antagonists have potential to be used clinically to treat obesity, but there are currently no such drugs on the market. Since no X-ray structure is available for CB1, considerable attempts have been made to prepare CB1 protein models, but in this work we propose a new CB1 model which is specific to CB1 antagonists as validated by its enrichment performance. We first built multiple CB1 homology models. The enrichment performance of these models was then systematically examined using two datasets. A small dataset that contains 72 highly active CB1 antagonists was docked into these models. Only one of the models was able to dock all the compounds. After minimization and followed by redocking, two more models were able to dock all the 72 compounds. Next, a large dataset that contains 181 active CB1 antagonists and 3439 inactive CB1 antagonists/decoy compounds was used to assess the enrichment performance of the 3 models. One of the models was found to have much better enrichment performance than the other 2 models. This best CB1 model will be used in future virtual screening studies.

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EXPRESSION, DIMERIZATION AND FUNCTION OF CB1 RECEPTOR CODING REGION SPLICE VARIANTS

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The pharmacological functions of the type 1 human cannabinoid receptor (hCB_1) are thought to be modulated through the isoform encoded by the fourth exon of the CNR1 gene. Two other mRNA variants of the coding region of this receptor have been described. These mRNAs variants encode receptors with a small deletion in the amino terminus of the coding region (hCB_{1b}) or a different amino terminus (hCB_{1a}). The contribution of these variants to endocannabinoid physiology and pharmacology remains unclear. The present study aimed to determine the relative abundance and distribution of mRNAs encoding the three coding region hCB_1 variants in human, to examine whether the hCB_{1a} and hCB_{1b} are expressed as proteins in the monkey (*Macaca fascicularis*) brain, to determine whether the variants can physically interact and to determine if co-expression of variants alters trafficking and signaling of hCB_1.

The three hCB_1 coding region variants were expressed in all human brain regions examined. Western blot analysis of homogenates from different regions of the monkey brain and antibodies against the C- terminus of CB_1 demonstrated that proteins with the expected molecular weights of CB_1, CB_{1a} and CB_{1b} receptors were present throughout the brain. hCB_1, hCB_{1a} and hCB_{1b} could each form homodimers as determined by BRET saturation curves of homogenates isolated from transiently transfected HEK293A cells. hCB_1 and either hCB_{1a} or hCB_{1b} could form heterdimers. Heterodimerization of hCB_1 and each of the splice variants increased cell surface expression of hCB_1 receptors and increased $G\alpha_i$-dependent ERK phosphorylation in response to cannabinoid agonists. Our results suggested that the hCB_1 coding region splice variants play an important physiological role in the activity of the endocannabinoid system.

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ALLOSTERIC MODULATION OF THE CANNABINOID CB1 RECEPTOR BY DAT INHIBITORS DOES NOT ACT THROUGH G-PROTEIN COUPLED MECHANISMS

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Despite the diverse therapeutic potential of cannabinoid CB1 receptor activation, orthosteric agonists produce psychotomimetic side effects. Preclinical success of fatty acid amide hydrolase inhibitors in treatment of depression and anxiety has sparked interest in exploring alternatative indirect mechanisms of CB1 activation. A 2009 report indicated an intriguing CB1-positive allosteric modulator (PAM) effect of a series of dopamine transporter (DAT) inhibitors when administered *in vitro* in CB1-transfected Chinese hamster ovary (CHO) cells (*Br. J. Pharmacol.*, 2009, 156, 1178-1184). This effect has not been replicated in *ex-vivo* assays. Using mouse brain membranes, we tested the ability of the previously reported CB1-PAMs, GBR 12909 and JHW 007, and close analogue GBR 12935, to modulate CP-55,940 binding and efficacy to induce GDP/GTP exchange. GBR 12909 dose-dependently enhanced the Bmax for [^{3}H]CP55,940 at concentrations up to 10 μM; the other compounds did not significantly affect the Bmax. Concentration response relationships with GBR 12909 and JHW 007 found that both decreased [^{3}H]CP55,940 specific binding with pK_B values of 5.746 and 5.203, respectively. None of the compounds tested were capable of enhancing CP55,940 (10 μM)-mediated stimulation of [^{35}S]GTPγS binding over 5 log unit concentration-effect curve, with JHW 007 diminishing activity at approximately 10^{-5}M. In the [^{35}S]GTPγS functional assay, GBR 12909 (1 μM) produced an insignificant enhancement in CP55,940 stimulation. This is in contrast with previous reports showing significant enhancement of Emax for both GBR 12909 and JHW 007 in calcium mobilization assays. Our results indicate that CB1-PAM activity of this ligand series is not mediated by G-protein coupled signaling pathways in brain membranes.

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DIFFERENT CLASSES OF CB_1 LIGANDS BIAS CB_1-DEPENDENT SIGNAL TRANSDUCTION

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Biased agonism describes the ligand-dependent selectivity of different signal transduction pathways by molecules that act as agonists at the same receptor. This functional selectivity may be exploited to alter the balance between signaling pathways downstream of a receptor. Agonists of the type 1 cannabinoid receptor (CB_1) are structurally diverse and include the aminoalkylindole WIN55,212-2, the endocannabinoid *N*-arachidonoyl ethanolamine (anadamide), and the Δ^9-tetrahydrocannabinol (THC)-like, CP55,940. Given the differences in potency and efficacy of these agonists, we sought to determine whether activation of CB_1 by these ligands resulted in biased signal transduction in a cell culture model of striatal neurons. We found that the THC-like compound CP55,940 enhanced the interaction between CB_1 and β-arrestin2 compared to vehicle, anandamide, and WIN55,212-2, which resulted in CB_1 internalization and persistent pERK signaling (30 min). In contrast, the endocannaibnoid anandamide and the aminoalkylindole WIN55,212-2 enhanced the interaction between CB_1 and $G_{\alpha i}$, and promoted a rapid and transient increase in pERK signaling (5 – 10 min) compared to vehicle or CP55,940. Based on these data, the therapeutic efficacy of cannabinoid agonists may be maximized by selecting agents with specific potency, affinity, and signaling bias.

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BETA-ARRESTIN2 MODULATION CANNABINOID ACTIVITY IN MICE VARIES WITH ASSAY, DRUG AND GENDER

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Beta-arrestin1 and 2 have been reported to couple G-protein couple receptors (GPCRs) causing: 1) uncoupling from G-proteins, 2) internalization of GPCRs, and/or 3) activation of non-G-protein-mediated signaling pathways. Previous studies in male beta-arrestin2+/+ and -/- mice found that the absence of beta-arrestin2 resulted in enhanced effects of 50 mg/kg THC in the depression of rectal temperature and latency to tail withdrawal assay (for antinociception). The same was not true of 0.5-2.0 mg/kg CP55940, as the effects of that drug were not different between genotypes.

In the present studies, the same experiments were performed in female mice. In beta-arrestin2-/- females, the effect of THC was less than in +/+ mice in the latency to tail withdrawal assay (2-way ANOVA, $p < 0.05$), and there was no difference between the genotypes in the depression of rectal temperature. Spontaneous activity in an open field was also performed, and in that assay the effect of THC was not significantly different between genotypes. Experiments on the effects of CP55940 in female mice are planned.

The forced swim test was also performed in male mice. In this assay, 0.06 mg/kg CP55940 showed an anti-depressant effect in beta-arrestin2-/- mice in that it decreased time spent immobile. The same dose did not show this effect in male beta-arrestin2+/+ mice. Moreover, THC at 2.5 or 4 mg/kg failed to significantly alter time spent immobile in either genotype. These same experiments in female mice are planned.

Thus, beta-arrestin2 appears to selectively modulate effects of cannabinoids in a manner that varies with the effect being measured, the drug assayed and the gender of the mouse.

‘INTERESTING NEGATIVES’ CULLED FROM TEN YEARS OF RECORDING FROM AUTAPTIC HIPPOCAMPAL NEURONS

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The autaptic hippocampal neurons are an architecturally simple model of neurotransmission. They also possess all the machinery required for several forms of retrograde cannabinoid signaling. Over the past ten years we have used these neurons to study the components of the cannabinoid signaling system in detail. Experiments using these cultures have ranged from investigating enzymatic control of endocannabinoid production and breakdown, to CB_1 mutants, species and splice variants, to CB_2 signaling, ‘spice’ pharmacology, and more. It is in the nature of scientific research to publish positive, but not negative, results. However in the course of studying cannabinoid signaling in these neurons we have occasionally encountered what one might call ‘interesting negatives’. The current abstract is an opportunity to share these negative or perplexing findings, in the hope that this will prove beneficial to other laboratories. Perhaps it will also elicit some discussions regarding the relevance and significance of these negative findings.

In autaptic hippocampal neurons we have found that:

1) The CB_1 antagonist hemopressin does not alter cannabinoid signaling.
2) DHEA does not alter neurotransmission.
3) LOX5/12 blockers do not alter cannabinoid signaling.
4) NAPE-PLD-/- neurons signal normally.
5) CAMKII blocker KN62 diminishes cannabinoid signaling but CAMKII blocker autocamtide does not.
6) Lipopolysaccharide treatment (overnight) does not alter the time course of cannabinoid signaling.

The challenge with negative findings is determining whether they are negative due to operator error or because these manipulations really don’t affect the functioning of the endocannabinoid system in autaptic neurons. Because of our long experience with these neurons we believe that these failures are not due to the preparation itself though there remain other potential sources of error (e.g. drug handling/preparation/storage/ administration).

THE EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF DELTA-9-TETRAHYDROCANNABINOL (Δ^9-THC) ON RECEPTOR DOWNREGULATION IN MICE

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Delta-9-tetrahydrocannabinol (Δ^9-THC), known for its psychoactive effects, is the principle active compound of the plant, Cannabis sativa. This compound acts as an agonist at the G-protein coupled cannabinoid receptors – CB1 and CB2 receptors. The central effects including analgesia and euphoria of Δ^9-THC are mediated by the CB1 receptors. These receptors are activated endogenously by endocannabinoids such as anandamide and 2-arachidonylglycerol. The distribution of CB1 receptors is restricted mainly to the nerve terminals. Endocannabinoids are synthesized on demand and they act on pre and the post synaptic receptors and are metabolized rapidly by membrane bound enzymes such as FAAH and MAGL. CB1 receptors modulate other neurotransmitter systems.

AM 281 is a well-validated cannabinoid receptor inverse agonist and its radiolabeled form, I-125 AM281, is an excellent tool for radioligand binding assays. By using the above tracer and using cold AM281 as a competitive inhibitor, we performed a cannabinoid receptor binding assay and established that acute administration of Δ^9-THC to mice (1, 3, 10 and 30 mg/kg) did not cause any significant receptor downregulation. In contrast to this observation, chronic administration (mice administered with 10 mg/kg Δ^9-THC for 7 consecutive days) showed a significant receptor downregulation ($p<0.05$). Studies in progress are evaluating the extent to which receptor down regulation contributes to tolerance after periods of intermittent administration of Δ^9-THC.

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*both the authors have contributed equally to this study.

DELETION OF MONOGLYCERIDE LIPASE IN THE CENTRAL NERVOUS SYSTEM

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Monoglyceride lipase (MGL) plays a crucial role in the endocannabinoid (EC) system by degrading 2-arachidonoylglycerol (2-AG), the most abundant EC in the body. Previous studies demonstrate that global deletion of MGL in mice (MGL -/-) does not provoke cannabimimetic effects despite strongly elevated brain 2-AG levels. This was explained by cannabinoid receptor (CBR) desensitization causing functional antagonism. Since MGL is expressed in neuronal and non-neuronal cells of the central nervous system, we investigated how cell-type-specific deletion of MGL in mice affects 2-AG catabolism and CBR sensitivity.

In this study we present mouse models with deletion of MGL using the Cre-LoxP system under the control of neuron and astrocyte specific promoters (Nestin and GFAP, respectively). Compared to MGL -/- mice, cell-type-specific deletion of MGL in mice leads to moderate increase in brain 2-AG levels. Furthermore, global but not partial deletion results in reduced sensitivity to CBR agonist treatment (CP 55,940). Together, our observations suggest that neuronal and non-neuronal cells are involved in 2-AG catabolism but global MGL-deficiency is required for CBR desensitization.

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GLUCOSE UPTAKE IS IMPAIRED IN FAAH-KO ASTROCYTES

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Fatty acid amide hydrolase (FAAH) activity is critical for the metabolism of anandamide and other N-acylethanolamines. We have recently found that the genetic deletion of FAAH induces profound changes in the phenotype of astrocytes, both *in vivo* and in vitro. These changes included decreases (PPAR-alpha and -gamma) and increases (TRPV1) in gene expression level, exacerbation of the proinflammatory response after challenge with a pathological stimulus (i.e. beta amyloid), and enhancement of hemichannel activity *in vivo*. As hemichannels are known to modulate glucose uptake by astrocytes and glucose metabolism in these glial cells is critical for brain function, we studied whether FAAH-ko astrocytes exhibit differences in their ability to uptake glucose. To that end, primary astrocytes from FAAH-ko and wildtype (WT) newborn mice were exposed to the fluorescent glucose analog 2-NBDG for eight minutes, and the amount of fluorescence inside the cells measured using a fluorimeter (Victor3, Perkin Elmer). We found that 2-NBDG uptake was lower in FAAH-null astrocytes. In addition, these cells produced significant lower amounts of lactate as compared to WT astrocytes. Furthermore, gene expression analysis by RT-PCR revealed that mRNA levels of the astrocyte-specific glucose transporter GLUT-1 and of connexin-43 (Cx43) were also significantly lower than those in WT astrocytes. As these membrane structures are known to play a determinant role in glucose uptake and in other cellular functions, we used selective blockers to corroborate this critical role. As expected, cytochalasin-B and phloretin (for GLUT-1) and flufenamic acid and carbenoxolone (for Cx43) dramatically decreased glucose uptake by astrocytes. These data indicate that the genetic deletion of FAAH diminishes glucose uptake by astrocytes, by decreasing the gene expression of GLUT-1 and Cx43. Ongoing experiments will try to unveil the functional implications of these observations.

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SYNERGISTIC ACTION OF INHIBITORS OF ENDOCANNABINOID METABOLISM ON OPIOID ANALGESIA IN A MODEL OF ACUTE PAIN

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The synergistic antinociceptive activity of cannabinoid-opioid agonist combinations has been well documented over the past few decades. The potential for decreased side effects such as dependence and tolerance of such combinations has spurred the investigation of opioid combinations with inhibitors of endocannabinoid metabolism. Literature reports of FAAH inhibitor-opioid combinations suggest that the without the application of exogenous anandamide, the combination is not efficacious in producing significant antinociceptive activity in models of acute pain such as the hot-plate and tail flick mouse models. These reports have resulted in subsequent investigations focusing on alternative pain state models for the combination. However under-explored are tissue and temporal factors of FAAH inhibitor-opioid combinations in acute pain states. To test the hypothesis that antinociceptive synergy from opioid-endocannabinoid interactions is tissue and temporally dependent, combinations of endocannabinoid metabolism inhibitors and morphine were tested in the tail flick model of acute pain in mice. The combination of an acute, sub-efficacious dose of morphine and a single dose of endocannabinoid metabolism inhibitor (inactive alone) were tested via various routes of administration. The combinations trended towards ineffective antinociception acutely, however at extended durations the combinations displayed synergistic antinociceptive activity. For example the intrathecal administration of a single dose of URB597 (0.5 nmol/mouse, t = 0) and acute morphine (3.12 pmol/mouse, t = hrs) produced significant synergistic antinociceptive activity which increased with time, a peak effect at 48 hours, and gradually declined to baseline by 96 hours. The combination was reversed by pretreatment with either naloxone or rimonabant, and abolished by co-pretreatment with both antagonists. We report the results of these studies, and discuss the implications of endocannabinoid metabolism inhibitor type, site of administration, and time dependent factors on the display of synergistic antinociceptive activity. The results indicate there is a synergistic interaction of opioids and endocannabinoids when their metabolism is inhibited that is both tissue and temporally specific.

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PERIPHERALLY RESTRICTED CB1R AGONISTS FOR TREATMENT OF CHRONIC PAIN

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Cannabinoid receptor (CBR) agonists are effective in alleviating chronic pain symptoms in humans even after prolonged repeated treatment in contrast to opioids, which have only limited effectiveness. However, psychotropic side effects of the majority of cannabimimetics mediated by central nervous system (CNS) CB1R activation have precluded their general application to widespread use in establishment medicine. We report further studies on the development of peripherally restricted CBR agonists of the substituted naphthylidene morpholinoethylindene family (XNMI) directed towards ameliorating chronic neuropathic pain while preventing CNS-mediated psychotropic side effects.

Candidate analogs were screened for high binding affinity at CB1Rs and CB2Rs in a displacement assay, and for functional activity in a Ca^{2+} flux assay at hCB1Rs. The MDCK model of blood-brain barrier (BBB) penetration was employed to identify analogs that exhibited non-penetration. Compounds with good affinity, activity and with no penetration in the BBB model were tested for metabolic stability in rat plasma and S9 liver fractions. Stable compounds were advanced to testing in the unilateral sciatic nerve entrapment (SNE) model of neuropathy and in the Complete Freund's Adjuvant (CFA)-induced model of unilateral chronic inflammation, in male SD rats. Compounds that relieved chronic pain symptoms in the SNE and CFA models were tested in the tetrad of behavioral assays wherein they showed no CNS effects. Collectively, these tests identified compounds that relieve chronic pain without CNS behavioral effects in rats.

The new work that we present here reveals the mechanism of pain reduction to be predominantly peripheral and CB1R mediated by co-administration of the candidate agonists with the CB1R antagonist SR141716, the CB2R antagonist SR144528, or a peripherally restricted CB1R antagonist RTI-18A in the SNE model. Further, *in vivo* (SD rats) pharmacokinetics demonstrated the limited brain penetration of the agonists tested by analysis of their plasma, brain and CSF levels. Oral activity for a potent and effective analog was also demonstrated.

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A DOUBLE BLIND PLACEBO CONTROLLED CROSSOVER PILOT TRIAL WITH EXTENSION USING AN ORAL MUCOSAL CANNABINOID EXTRACT FOR TREATMENT OF CHEMOTHERAPY INDUCED NEUROPATHIC PAIN

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Neuropathic pain caused by chemotherapy limits dosing and duration of potentially lifesaving anti-cancer treatment and impairs quality of life. Chemotherapeutic neuropathy responds poorly to conventional treatments and there is an urgent medical need for new treatments. Recent pre-clinical studies demonstrate that cannabinoid agonists suppress established-chemotherapy-evoked neuropathy. This was a pilot trial to begin to investigate a currently available cannabinoid agent (oral mucosal spray containing cannabinoids) in treatment of chemotherapy induced neuropathic pain.

A randomized double blind placebo controlled crossover pilot study was done in 16 patients with established chemotherapy induced neuropathic pain. A 0-10 point numeric rating scale for pain intensity (NRS-PI) was used as the primary outcome measure. When examining the whole group there was no statistically significant difference between the treatment and the placebo groups on the (NRS-PI). A responder analysis demonstrated there were 5 participants who reported a 2-point or greater reduction in pain that trended toward statistical significance and the NNT was 5.

Chemotherapy induced neuropathic pain is particularly resistant to currently available treatments. This pilot trial found a NNT=5 and an average decrease of 2.6 on a 10 point numeric rating scale for pain in 5 “responders” (as compared with a decrease of 0.6 with placebo) and supports that it is worthwhile to study nabiximols in a full randomized placebo controlled trial of chemotherapy induced neuropathic pain.

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DO THE N-3 N-ACYLETHANOLAMIDES DHEA AND EPEA FUNCTION AS ENDOGENOUS SUPPRESSORS OF INFLAMMATION?

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Dietary n-3 fatty acids have been linked to an attenuation of inflammatory processes, but the underlying mechanisms are not completely understood. Previous work from our group has shown that dietary n-3 fatty acids increase endogenous concentrations of the n-3 fatty acid derived N-acylethanolamides (NAEs) docosahexaenoylethanolamide (DHEA) and eicosapentaenoylethanolamide (EPEA) (Balvers MGJ et al, Metabolomics 8 (2012) 1130-1147; Balvers MGJ et al, Biochim Biophys Acta 1801 (2010) 1107-1114). These molecules have recently gained interest for their potential as endogenous bioactive since potent anti-inflammatory effects were found in *in vitro* experiments using murine macrophages (Meijerink J et al, Br J Nutr 105 (2011) 1798-1807). However, it is not known if inflammatory processes affect *in vivo* DHEA and EPEA levels, and whether DHEA and EPEA also have anti-inflammatory properties in human-derived primary immune cells . The present work aimed to address these issues by (1) investigating if *in vivo* inflammation affects DHEA and EPEA levels in mice, and (2) to study the effects of these newly discovered n-3 NAEs on inflammation using human peripheral blood mononuclear cell (PBMC) cultures. In the first study, C57BL/6 mice which had been fed a fish-oil rich diet, received 3 mg/kg of lipopolysaccharide (LPS) or sterile saline by *i.p.* injection. After 2, 4, 8 or 24 hrs, 4 mice from each group were sacrificed, and plasma, liver, ileum and adipose tissue DHEA and EPEA levels were determined using LC-MS/MS (Balvers MGJ et al, Int Immunopharmacol 13 (2012) 204-214). In the second study, human PBMCs were stimulated with 1 ng/ml LPS and 0.01-10 μM DHEA or its precursor docosahexaenoic acid (DHA) for 24 hrs, after which medium monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) concentrations were determined using ELISA.

Inflammation caused time- and tissue dependent effects on *in vivo* DHEA and EPEA levels. DHEA levels were increased from 2 to 24 hrs after LPS administration in plasma and liver, but no changes were observed in ileum and adipose tissue levels. EPEA concentrations were increased in plasma and liver only at 4 and 8 hrs following LPS, whereas ileum concentrations were increased from 2 to 24 hrs. Adipose tissue EPEA levels were not affected. In LPS-stimulated human PBMCs, DHEA reduced MCP-1 concentrations at 5-10 μM with a maximum reduction of 55% compared to the vehicle control, whereas DHA was ineffective at similar concentrations. IL-6 levels were not affected by DHEA.
In conclusion, LPS caused time- and tissue dependent increases in DHEA and EPEA concentrations *in vivo*, which supports the point of view that the endocannabinoid system is to be considered as a dynamic system interacting with different other pathways in fatty acid metabolism. In addition, DHEA displayed anti-inflammatory properties in human PBMCs *in vitro* by reducing MCP-1 concentrations without affecting IL-6. Together, these data suggest that DHEA and EPEA might play a role as endogenous immune-modulating compounds during inflammation in vivo.

CB RECEPTOR MEDIATION OF THC-INDUCED ANTINOCICEPTION USING A CHRONIC INFLAMMATORY PAIN MODEL IN FEMALE VS. MALE RATS

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Previous studies have shown that Δ^9-tetrahydrocannabinol (THC) produces antinociceptive effects in both acute and chronic pain models, and is more effective in female than male rats in tests of acute pain. The question of which cannabinoid (CB) receptor sub-type mediates the sex differences remains unclear. *Experiment 1:* Male and female rats underwent baseline testing on the von Frey measure of mechanical allodynia, the Hargreaves measure of thermal hyperalgesia, and horizontal locomotor activity, and then complete Freund's adjuvant (CFA) was injected into the right hindpaw. Three days later, a systemic (*i.p.*) injection of THC (0, 0.32, 1.0 or 3.2 mg/kg) was administered, and rats were reassessed on the same measures of pain. THC produced dose-dependent anti-allodynia, anti-hyperalgesia, and locomotor suppression in both sexes, but had significantly greater anti-hyperalgesic and locomotor suppressant effects in females compared to males. *Experiment 2:* On Day 3 after CFA injection, a systemic injection of a CB1 or CB2 receptor-selective antagonist (vehicle, or 1 mg/kg SR141716A or SR144528) was given, followed by i.p. THC (vehicle or 3.2 mg/kg). THC alone produced anti-hyperalgesic effects in males and females, while producing anti-allodynia and locomotor suppression in females alone. The CB1 antagonist SR141716A reversed THC-induced anti-hyperalgesia in both sexes, and locomotor suppression (but not anti-allodynia) in females. In contrast, the CB2 antagonist SR144528 failed to block any of THC's effects in either sex. This study extends previous findings using acute pain models to a chronic inflammatory pain model, suggesting that THC may be more effective in females than males against chronic inflammatory pain. Furthermore, the current findings suggest that the antinociceptive effects of THC against inflammatory pain are mediated primarily by the CB1 receptor in both sexes, when THC is given systemically.

HORMONE MODULATION OF ANTINOCICEPTIVE BUT NOT MOTORIC EFFECTS OF I.C.V. THC IN OVARIECTOMIZED FEMALE RATS

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Estrous cycle-related fluctuations in THC-induced antinociception have been found (Craft & Leitl, 2008; Wakley & Craft, 2011). It is not clear which ovarian hormone (estradiol (E2), progesterone (P4) or both) produces these changes (Craft & Leitl, 2008; Kalbasi Anaraki et al., 2008). Ovarian hormones also modulate supraspinal cannabinoid receptors (CBr), depending on the brain region examined (Rodriguez de Fonseca et al., 1994; Riebe et al., 2010). The aim of this study was to examine which ovarian hormone modulates the antinociceptive and motoric effects of supraspinally administered THC and whether changes in brain CBr are associated with hormone modulation of THC's behavioral effects.

Female rats were ovariectomized (OVX) and implanted with a cannula guide into the right lateral ventricle. Vehicle (oil) or hormones (2 μg E2, or 500 μg P4, or both) were administered on Days 3 and 7 after surgery. On the morning or late afternoon of Day 8 or Day 9, vehicle (1:1:8 ethanol:cremaphor:saline) or THC (100 μg) was administered *i.c.v.* (N=12-16/day/hormone condition/dose). Antinociception was measured using paw pressure and tail withdrawal tests at 5-180 min post-injection. Horizontal locomotion was examined in 5-min periods at 15-180 min post-injection. Brain tissue was taken from separate groups of oil+oil- and E2+P4-treated females for [^{3}H]SR141716A binding, to examine hormone effects on CBr in structures known to mediate THC's effects.

THC produced antinociception on both tests (THC: $P<0.001$). E2 with or without P4 enhanced THC-induced paw pressure antinociception (E2 x THC: $P<0.05$). THC also reduced locomotor activity (THC: $P<0.001$). THC's effects did not differ significantly among the various hormone-treated groups on the tail withdrawal or locomotor tests and also did not differ across test days. CBr density in the caudate putamen (CPu) and periaqueductal gray was increased and decreased, respectively, in oil-treated females on Day 9 compared to E2+P4-treated females on either day (Day x Hormone: $P<0.05$). Within the CPu, CBr affinity was increased in Day 8 females compared to Day 9 females (Day: $P<0.05$) and hormone treatment also increased affinity compared to oil-treated females (Hormone: $P<0.05$). Hormone treatment did not significantly alter CBr density or affinity in hypothalamus, amygdala or cerebellum.

These results suggest that the ovarian hormone E2 (alone and in combination with P4) enhances supraspinal THC-induced antinociception against mechanical pain in OVX females. Neither ovarian hormone appears to modulate the motoric effects of THC. Ovarian hormone enhancement of THC's antinociceptive effect cannot be explained by changes in CBr density or affinity in the brain areas examined.

CIRCULATING ENDOCANNABINOIDS IN CYCLIC VOMITING SYNDROME

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Cyclic vomiting syndrome (CVS) is characterized by episodic vomiting often triggered by stress. Although patients with CVS frequently use cannabis to control symptoms, chronic cannabis use can induce a similar hyperemesis syndrome. Since acute cannabinoid receptor activation produces anti-emetic effects, but chronic cannabinoid exposure can down-regulate CB1R signaling, these clinical observations lead us to hypothesize that the activity of the endocannabinoid system (ECS) is reduced in patients with CVS.

We measured the concentrations of endocannabinoids and related lipids in sera, salivary cortisol (sCort) concentrations (index of HPA axis activity), and salivary alpha amylase (sAA) levels (a surrogate for sympathetic activity) in patients with CVS and age-matched controls. These outcomes were measured in CVS patients in both well and sick phases, and both morning and evening samples were collected in each phase in most patients. The Rhodes Index of nausea, vomiting and retching (INVR), a validated and reliable tool, was used to study the severity of vomiting episodes.

Eleven CVS patients (mean age 37±12, female=8) and 6 controls (mean age 39±13, female=5) were studied. Concentrations of *N*-arachidonylethanolamine (AEA) and two other *N*-acylethanolamines (NAEs), *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) were significantly increased during an episode vs the well phase in patients. NAE concentrations were not different between patients in the well phase and controls. A second endocannabinoid, 2-arachidonoylglycerol (2-AG) was also measured; although 2-AG serum concentrations tended to be higher in the sick phase, they were variable and not statistically significantly different. Median sCort concentrations were significantly higher in patients during an episode compared to the well phase with no differences between controls and the well phase in patients. Patients also had higher median sAA levels during an episode compared to the well phase with a trend towards significance. The mean INVR score during an episode was 21.9 ± 7.2; there were no significant correlations between the INVR score and 2-AG, AEA, OEA and PEA concentrations.

This pilot study demonstrates that serum NAE concentrations (AEA, OEA and PEA) and sCort are significantly increased during CVS episodes in comparison to the well phase in CVS patients. However, contrary to our hypothesis, there were no differences in serum endocannabinoids between control and CVS patients in the well phase. This study contributes to our understanding of the pathophysiological mechanisms in CVS; further studies to explore their role in nausea and vomiting are warranted.

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THE ROLE OF INTRA-VISCERAL INSULAR CORTEX ENDOCANNABINOIDS IN NAUSEA-INDUCED GAPING IN RATS

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Manipulations that elevate the endogenous cannabinoids (eCBs), anandamide (AEA) and 2-arachidonoyl glycerol (2AG), have previously been found to interfere with the establishment of lithium chloride (LiCl)-induced conditioned gaping in rats (a selective measure of conditioned nausea; Cross-Mellor *et al.,* 2007; Sticht *et al.,* 2012). Although the precise brain mechanisms underlying nausea have yet to be fully uncovered, the visceral insular cortex (VIC) appears to be a key structure in mediating its sensation such that administration of the anti-emetic drug, ondansetron or the synthetic cannabinoid, HU210, disrupts the establishment of nausea-induced conditioned gaping in rats (Tuerke *et al*., 2012; Limebeer *et al*., 2012). However, at present, the precise role of the VIC in mediating eCB-suppression of nausea remains unknown. Therefore, the current study investigated the potential of intra-VIC eCB manipulations to interfere with establishment of conditioned gaping in rats.

A series of experiments evaluated the effects of VIC eCB manipulations prior to administration of illness-inducing LiCl. Rats received an intraoral infusion of 0.1 % saccharin (3 min) that was preceded or followed by an intra-VIC infusion of either exogenous AEA (0.4, 4 μg) or 2AG (0.5, 1 μg), the FAAH inhibitor, URB597 (0.01 μg), or the dual FAAH/MAGL inhibitor, JZL195 (1.0 μg), and an injection of LiCl. Rats were subsequently re-exposed to LiCl-paired saccharin 72 hr later in a drug-free taste reactivity test, in which conditioned gaping was assessed. It was found that bilateral intra-VIC infusions of 2AG or JZL195 dose-dependently suppressed conditioned gaping in rats, whereas exogenous AEA and URB597 were without effect. On the other hand, conditioned taste avoidance of saccharin was unaffected by any of the eCB manipulations, as measured by a two-bottle consumption test. The ability of intra-VIC 2AG administration to interfere with nausea-induced conditioned gaping does not appear to be mediated by CB_1 receptors, as pretreatment with the CB_1 antagonist, AM251 (1 μg, intra-VIC), did not reverse the suppressive effects of 2AG.

These findings suggest that manipulations that elevate intra-VIC eCBs may have anti-nausea potential, and that, consistent with the effects of systemic administration (Sticht *et al.,* 2012) downstream metabolites of 2AG may be partially responsible for mediating the anti-nausea effects of exogenous administration. Future studies aim to assess the mechanism of action underlying the suppressive effects of JZL195, as well as eCB levels following intra-VIC JZL195 and LiCl.

ATTENUATION OF ANTICIPATORY NAUSEA IN A RAT MODEL OF CONTEXTUALLY ELICITED CONDITIONED GAPING BY MANIPULATION OF THE ENDOCANNABINOID SYSTEM

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Following one or more chemotherapy treatments, many patients report that they experience anticipatory nausea (AN), a classically conditioned response where a conditional association develops between the contextual clinic cues and the nausea and/or vomiting that developed following treatment. Once AN develops it is resistant to standard anti-nausea treatments. Although rats do not vomit in response to a toxin challenge, they express distinctive conditioned gaping reactions when exposed to a context previously paired with an emetic agent. This conditioned gaping reaction serves as a rodent model of nausea-like behavior. Both the fatty acid amide hydrolase (FAAH) inhibitor, URB597, and the monoacylglycerol lipase (MAGL) inhibitor, JZL184, suppress vomiting in emetic species and conditioned gaping in rats. Here, the potential of the dual FAAH/MAGL inhibitor, JZL195 on its own and combined with anandamide (AEA) or 2-arachidonoyl glycerol (2-AG) to reduce conditioned gaping in rats was evaluated.

In each experiment, rats received 4 conditioning trials in which they were injected with Lithium Chloride (LiCl) immediately prior to placement in a distinctive context for 30 min. The rats then received a 5 min test for AN. Rats were injected with vehicle (VEH) or JZL195 120 min prior to placement in the context previously paired with nausea. As well, additional groups were injected with AEA or 2-AG 15 min prior to placement in the chamber. Finally, the potential of the CB1 antagonist/inverse agonist SR141716 to reverse the suppression of AN was evaluated. Following the test, the rats' brains were removed and frozen at -80^0 for whole brain tissue analysis of AEA and 2-AG levels.
When administered prior to the AN test, JZL195 suppressed conditioned gaping and this suppression was reversed by SR141716. The suppressive effect of JZL195 on conditioned gaping was amplified by pretreatment with either AEA or 2-AG. As well, on its own AEA, but not 2-AG, suppressed conditioned gaping, and this suppressant effect was prevented by pretreatment with SR141716. Evaluation of whole brain levels of AEA and 2AG following JZL195 treatment revealed an increase in AEA levels but not 2-AG levels. The JZL195-induced increase in AEA levels was not reversed by SR141716. Manipulations of the endocannabinoid system may have therapeutic potential in the treatment of AN.

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INHIBITING ANANDAMIDE TRANSPORT: THE IMPACT OF PROLONGED ANANDAMIDE AVAILABILITY ON NAUSEA

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Considerable evidence supports anandamide as an important mediator in the regulation of nausea. The effects of prolonging anandamide presence within the synapse through anandamide transport inhibition are however, yet to be understood. Recently reported was a protein that mediates the cellular transport of anandamide, FLAT (FAAH-1-like anandamide transporter). Here we present evidence that inhibiting FLAT activity with ARN272 (ARN) produces a type of indirect agonism to attenuate nausea-induced responding in rats through elevated anandamide tone. We used a Lithium Chloride (LiCl)-induced conditioned gaping model of nausea in rats. In Experiment 1, rats were injected with the anandamide transport inhibitor, ARN (0.1 mg/kg, 1.0 mg/kg, or 3.0 mg/kg, i.p.) or vehicle, 2 hrs prior to behavioral conditioning. Two conditioning sessions separated by 72 hours were followed by a drug free test day, at which time gaping reactions were recorded. Experiment 2, evaluated the potential of the CB_1 antagonist SR141716 (1.0 mg/kg, 2.5 mg/kg), given 30 min prior to conditioning, to reverse the ARN suppressed gaping.

Administration of ARN produced a dose-dependent reduction of LiCl-induced conditioned gaping, with 3 mg/kg being the optimally effective dose. The suppression of conditioned gaping by ARN was reversed partially by SR141716 at 2.5 mg/kg, and fully at 1.0 mg/kg. The reversal of ARN's effects by SR141716 suggests a CB_1 receptor mechanism of action. Administration of SR141716 alone did not enhance LiCl-induced conditioned gaping reactions. The results suggest that inhibiting the transport of anandamide, tonically activates CB_1 receptors to regulate toxin-induced nausea. As well, ARN272 attenuated LiCl-induced vomiting in the *Suncus murinus* (house musk shrew). On-going is the investigation into how anandamide transport mediates nausea centrally within forebrain regions, specifically the visceral insular cortex.

SYSTEMS BIOLOGY ANALYSIS OF THE ENDOCANNABINOID SYSTEM

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The last 25 years have witnessed an amazing explosion of experimental data concerning the endocannabinoid system (ECS). Here, for the first time, we adopted a systems biology approach to try to dissect the complexity of the ECS. To this aim, we built a biological network-based computational model where ECS elements were included as nodes and the interactions among them as links.

The statistical analysis of the network showed that it follows a scale-free topology, that offers the advantage of being robust against random damages, easily navigable and controllable. Network topological parameters such as clustering coefficient (i.e., a measure of how the nodes tend to form clusters) of 0.0009, a network diameter (i.e., the largest distance between two nodes) of 12, an averaged number of neighbours (i.e., the mean number of connections of each node) of 3.073, and a characteristic path length (i.e., the expected distance between two connected nodes) of 4.715 suggested that the ECS is suitable to elaborate and transfer molecular messages in a fast and specific manner. Interestingly, □75% of nodes were found to be directly located on, or to exert their biological activity at the level of, the cell membrane.

The most linked molecules (called hubs) of the ECS network were shown to be anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which accounted for more than one third of all the network links. Their central role in ECS-dependent signaling was also demonstrated by the value of their betweeness centrality (the highest scored), and by the finding that network integrity was completely destroyed by their removal. Noteworthy, the two molecules seem to have distinct roles: AEA acts as an ubiquitous player while 2-AG plays more restricted actions. Instead, the product of AEA and 2-AG degradation, arachidonic acid, seemed to have a marginal impact on ECS-dependent signaling, because its removal did not significantly affect ECS network architecture.

TARGETED METABOLOMICS UPLC-ESI/MSMS METHOD FOR QUANTIFYING OXYLIPINS AND ENDOCANNABINOIDS IN HUMAN PLASMA

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Oxylipins are oxygenated metabolites derived from polyunsaturated fatty acids (PUFAs) such as arachidonic acid, linoleic acid, eicosapentaenoic acid produced primarily *via* three metabolic pathways, cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP), as well as by non-enzymatic oxidation processes. Endocannabinoids are bioactive lipid-related signaling molecules derived from membrane phospholipids and the most common are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG).

These two classes of compounds despite being structurally diverse are known to play an important role in a number of physiological and pathological processes in particular in inflammation. Hereby, we present a work flow to quantify 14 endocannabinoids and 15 oxylipins in human plasma (Figure **1**).

Oxylipins and endocannabinoids were extracted by SPE and quantified by independent UPLC-ESI/MSMS validated, sensitive and specific methods using Waters BEH C_{18} column (2.1 mmx150 mm, 2.5 μm). Endocannabinoids were analyzed in ESI-positive mode using 10mM CH_3COONH_4 (A) and 10mM methanol CH_3COONH_4 (B) as mobile phases. The gradient of elution was 0.0–9.0 min (79%B), 9–9.5 min (79–90%B), 9.5-10.5 min 90%B, 10.5-14.0 min 79%B with a flow rate of 0.4 mL/min.

For oxylipins negative mode was selected and mobile phase consisted of 0.1% acetic acid (C) and acetonitrile:methanol (85:15) with 0.1% acetic acid (D) with a gradient of 0.0-0.75 min (15%B), 0.75-1.5 min (15-30%B), 1.5-3.50 min (30-47%B), 3.50-5.0 min (47-54%B), 5.0-6.0 min (54-55%B), 6.0-10.50 min (55-60%B), 10.50-15.0min (60-70%B), 15.0-16.0 min (70-80%B), 16.0-17.0 min (80-100%B), 17-19 min (100%B), 19.30-22.0 min (15%B) with a flow rate of 0.3 mL/min.

For each standard the MS/MS parameters (mass transitions, CAP, CE and CV) were optimized. Intra-day and inter-day precisions were < 15% and accuracy was > 80% for all compounds. Recovery for internal standards was established in PBS and plasma using recovery standards (CUDA for oxylipins and DHEA-d_4 for endocannabinoids). The values obtained gave recovery to be between 50 and 109 % both for oxylipins and endocannabinoids with exception of 12(13)-EpOME-d_4 (recovery around 30%).

The LOQ values for endocannabinoids were in the range 0.61 pg-146.1 pg with exception of 2-LG (1.17 ng). For oxylipins higher LOQ values were found (1.35 pg – 0.91 ng) which is expected due to the lowest sensitivity of ESI in negative mode.

These values compared well to profiling methods dealing with a large array of endocannabinoids. Analysis of human plasma is under way.

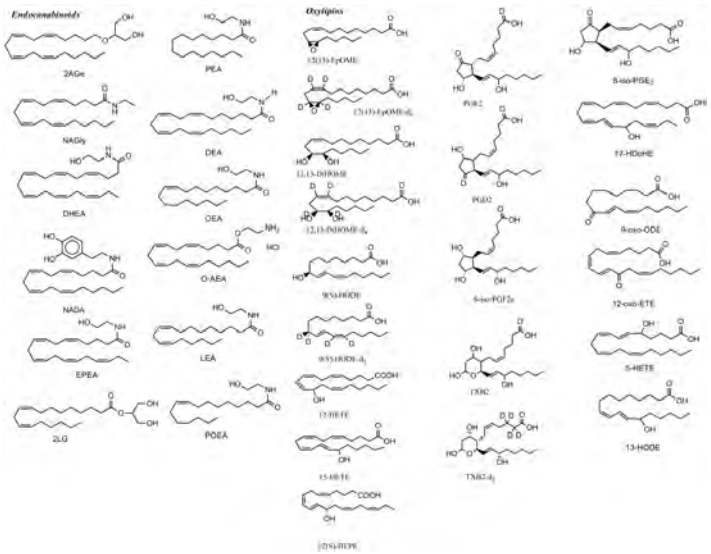

Figure 1. Chemical structures of oxylipins and endocannabinoids analyzed.

GROUP-ASSISTED PURIFICATION (GAP) CHEMISTRY WITHOUT USING CHROMATOGRAPHY AND RECRYSTALLIZATION

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The solution phase synthesis of *N*-protected amino acids and peptides has been achieved through the Group-Assisted Purification (GAP) chemistry without using traditional purification protocols such as chromatography and recrystallization. Pure product of each peptide synthesis step can be obtained simply by washing the crude mixtures with inexpensive petroleum solvents or co-solvents. This GAP synthesis strategy can avoid disadvantages of the solid-phase-peptide synthesis (SPPS) and liquid-phase-peptide synthesis (LPPS), and can reduce the use of solvents, silica gels, energy and manpower. In addition, the GAP auxiliary can be conveniently recovered for re-use. This environmentally friendly benign strategy can substantially reduce the generation of wastes during academic and industrial synthesis.

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DETERMINATION OF PESTICIDES IN CANNABIS SMOKE

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Before sensible regulations pertaining to pesticides can be established we must first quantify to what extent cannabis consumers may be exposed to pesticide and other chemical residues through inhaled cannabis smoke. Three different smoking devices were evaluated with different chemicals on cannabis in order to provide a generalized data set representative of pesticide exposures possible for medical cannabis users. Three different pesticides, Bifenthrin, Diazinon, and Permethrin, along with the plant growth regulator Paclobutrazol, which are readily available to cultivators in commercial products, were investigated in the experiment. Smoke generated from the smoking devices was condensed in tandem chilled gas traps and analyzed with GC-MS. Recoveries of residues were as high as 69.5% depending upon the device used and the component investigated, suggesting that the potential of pesticide and chemical residue exposures to cannabis users is substantial and may pose a significant toxicological threat in the absence of adequate regulatory frameworks. This insight should prove helpful in designing adequate toxicity studies for cannabis in the future, and raises questions pertaining to the validity of conclusions of previous studies that did not first screen the cannabis being consumed for the presence of pesticides or other chemical residues.

ANALYSIS OF SMOKE CONDENSATE FROM COMBUSTION OF SYNTHETIC CANNABINOIDS IN HERBAL PRODUCTS

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Illicit use of synthetic cannabinoids has become increasingly prevalent in recent years given their widespread availability via the internet. As legislation is passed to ban specific cannabinoids and targeted analytical methods are developed to detect illicit substances, manufacturers have diversified the cannabinoids used to evade detection and prosecution. This diversification has created a significant analytical challenge. While several research efforts have focused on identifying which cannabinoids are present in herbal formulations, the pyrolytic fate of these cannabinoids, and hence their human exposure, have yet to be adequately determined. Given the increasing number of emergency-room related reports relating to herbal product exposure including acute kidney injury and psychiatric disturbance, understanding the pyrolytic and metabolic pathways of these products is vital. The aim of this work was to identify pyrolytic and combustion products found in the smoke of an herbal matrix spiked with selected synthetic cannabinoids. Recovery was determined for the spiked ingredient in the mainstream smoke, side-stream smoke, and in the butt.

Many of the most prevalent synthetic cannabinoids in use in 2012 and early 2013 contain a single fluorine atom, therefore, in the work described here herbal cigarettes were prepared from marshmallow leaf herbal material laced with a series of fluorinated synthetic cannabinoids and their non-fluorinated analogs: AM-2201 and JWH-018, UR-144 and XLR-11, 5-fluoro-PB-22 and PB-22. The cigarettes were smoked using a Borgwaldt KC smoking machine. Mainstream and sidestream smoke condensates were collected and remaining un-smoked butts were also recovered and extracted. All samples were analyzed by LC/MS using a Waters Synapt G2 Q-TOF high-resolution mass spectrometer and GC/MS using an Agilent 7001B triple quadrupole system. Smoke from control cigarettes containing only marshmallow leaf was also collected for comparison to the laced cigarette samples. Appropriate reference standards were prepared and analyzed to confirm identity and determine recovery.

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CANNABIDIOL FROM *CANNABIS SATIVA* IS A POTENT INDUCER OF CYTOPROTECTIVE PHASE 2 ENZYMES

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Cannabidiolic acid (CBDA), the main phytocannabinoid present in hemp varieties of *Cannabis sativa*, rapidly decarboxylates to cannabidiol (CBD) upon heating. CBD has garnered attention for its neuroprotective effects in animal models of oxidative stress and ischemia. However the mechanism of this protection is not completely understood. We found that CBD and CBDA are potent inducers of the cytoprotective phase 2 enzyme NAD(P)H:quinone oxidoreductase-1 (NQO1) in mouse hepatoma cells (Hepa-1c1c7). NQO1 plays multiple protective roles in animal cells including preventing the redox-cycling of quinones, maintaining vitamin E and coenzyme Q10 in their reduced forms, and scavenging superoxide radicals. The concentrations required to double the activity of NQO1 (CD values) were 1.6 and 20.8 μM for CBD and CBDA, respectively. The CD value for CBD compares favourably with that of the known phase 2 inducing phytochemical sulforaphane which had a CD value of 0.27 μM. The maximal induction levels achieved by THC and THCA were 1.4 and 1.7-fold, respectively. We also provide evidence that induction of NQO1 by CBD is bifunctionally mediated through both transcription factors Nuclear factor (erythroid derived 2)-related factor 2 (Nrf2) and the aryl hydrocarbon receptor (AhR). These results may provide an explanation for some of the neuroprotective activity of CBD and suggest other phytocannabinoids should be investigated for their cytoprotective activity.

IN VIVO CHARACTERIZATION OF THE EFFECT OF CANNABIDIOL ON DORSAL RAPHE 5-HT NEURONAL ACTIVITY

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Cannabidiol (CBD) is the primary non-psychotropic compound of cannabis, comprising approximately 40% of its extract. The therapeutic potential of CBD is currently being explored in domains such as chronic pain, inflammation, nausea, epilepsy, psychosis, sleep, and anxiety, with particularly promising effects (see Campos et al., 2012 for review). However, CBD is an enigma of sorts in that its primary mechanism of action is currently unknown. In addition to displaying low to modest affinity for CB_1/CB_2 receptors as well as $TRPV_1$ channels, CBD has also been shown to display partial agonist affinity for the $5\text{-}HT_{1A}$ receptor in *vitro* (Russo et al., 2005). Moreover, Parker and colleagues have recently revealed that CBD dose-dependently enhances $5\text{-}HT_{1A}$ receptor agonist-stimulated GTPγS binding in brainstem slices (Rock et al., 2012). Interestingly, the anxiolytic and antidepressant effects of CBD in preclinical models can be prevented by pretreatment with a $5\text{-}HT_{1A}$ receptor antagonist (Zanelati et al., 2010).

Despite this evidence, no studies to date have directly evaluated the effects of CBD on DRN 5-HT neurotransmission in *vivo*. Thus, we performed single-unit extracellular recordings of DRN 5-HT neurons in rats in response to increasing cumulative doses of CBD (0.05-1 mg/kg, i.v.). The mean firing rate of DRN 5-HT neurons was progressively decreased with increasing doses of CBD (ED_{50} = 0.20 mg/kg). To probe the potential mechanism of action, we next examined whether pretreatment with antagonists targeted to $5\text{-}HT_{1A}$ receptors, $TRPV_1$ channels, or CB_1 receptors are able to prevent the suppressant effect of CBD on 5-HT firing. Pretreatment with the $5\text{-}HT_{1A}$ receptor antagonist WAY100635 (0.3 mg/kg, i.v.) immediately prior to CBD administration completely prevented the ability of CBD to decrease DRN 5-HT firing. Similarly, pretreatment with the $TRPV_1$ antagonist capsazepine (0.02 mg/kg, i.v.) prevented the decrease in DRN 5-HT firing elicited by CBD administration. Pretreatment with the CB_1 receptor antagonist AM251 (1 mg/kg, i.v.) prevented the ability of low doses (0.1 mg/kg) of CBD to decrease 5-HT firing, but failed to prevent the decrease in firing elicited by higher doses of CBD (0.25-1 mg/kg). These data are the first to demonstrate that CBD modulates 5-HT firing in *vivo*, and suggest that this likely occurs via activation of $5\text{-}HT_{1A}$ receptors and/or $TRPV_1$ receptors. Ongoing experiments are testing whether chronic CBD administration elicits a recovery of DRN 5-HT firing and desensitizes DRN somatodendritic $5\text{-}HT_{1A}$ autoreceptors in a manner similar to conventional antidepressants.

HEMPSEED IS AN ADOPTOGEN!

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Cannabis sativa L. and its byproducts like marijuana, hashish, hempseed as single or as a part of herbal formulation, have been employed for the treatment of several psychological disorders throughout history. However, it is used as a functional food like hempseed or abused as a recreational remedy like hashish in Middle East. The aim of this study was to investigate psychopharmacological effects of whole hempseed in rats.

Adult rats (n=14) divided to two equal groups. The control group (NC) received basal diet while hempseed-treated group (HS) had free access to whole hempseed plus basal diet throughout 60 days to mimic human's recreational usage. On 46, 50 and 60 days of study, anxiety, depression and stress were recorded according to the elevated plus maze (EPM), forced swimming (FST) and tail pinch (TPT) paradigms, respectively. Data were reported as mean±SEM. All parameters were analyzed using one-way *ANOVA* with SPSS *ver.*16 software. Statements of significance were based on $p<0.05$.

In EPM paradigm, spent time (sec) in open arms in HS group (21±7) was longer than in NC group (16±6; p=0.638). In FST paradigm, swimming time (sec) in HS group (27±8) increased in comparison to NC (16±5; p=0.255). In TPT paradigm, gnawing/biting time (sec) in HS group (68±21) decreased in comparison to NC (158±18; p=0.008).

The results of this study shown that hempseed may reduce depression and anxiety but has considerable anti-stress effect and could be recognized as an adoptogen. Its adoptogenic effects may be indebted to its high levels of essential fatty acids or its phytocannabinoids.

THE EFFECTS OF HEMPSEED (*CANNABIS SATIVA* L.) ON LIPID, LIPOPROTEIN, AND APOLIPOPROTEIN PROFILES OF MALE RATS

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Cannabis sativa L. is a natural factory of many bioactive phytochemical compounds. Anecdotal evidence indicated that hempseed, as a rich and balanced source of omega-6 and omega-3 polyunsaturated fatty acids, phytocannabinoids, and phytoestrogens might be useful in various ailments but its effects on normal physiology are still obscured!. We examined the effect of various dietary fatty acid composition derived from sole source: Hempseed, on serum lipid, lipoprotein and apolipoprotein profiles in rats.

Male adult Wistar rats were divided in to five groups. One group received the control diet (modified AIN93M, namely (SCD), whereas the remaining for group received two types of diets: one type received isoenergetic diets in comparison to SCD with 2% (HST2) and 4% (HST4) of hempseed , the other type received high energy, high fat diet contained 100% (WHS) and 50% (MD) whole hempseed. After 35 days, following overnight food deprivation, blood was obtained and related biochemical parameters (mg/dl) were determined by Autoanalyzer using commercial kits.

Triacylglycerols (TGs) was significantly ($p<0.05$) lower in WHS group (28.1±3.83) and insignificantly higher in HST2 group (43.3±2.86) compared to SCD (40.2±2.18). Serum total cholesterol (TC; 35.3±2.35) was significantly lower in WHS group compared with the rest groups and also isoenergetic diet type did not exert any significant difference compared with control group. The serum high-density lipoprotein cholesterol (HDL-C) concentration did not differ among all groups while low-density lipoprotein cholesterol (LDL) significantly was lower (12.7±0.51) in WHS and nonsignificantly lower among all other groups, compared with MD (15.1±0.79). Serum apolipoprotein A-1 (apo A-1) was significantly higher (0.47±0.11) in HST4 group compared with the rest of groups and generally consuming of hempseed at all levels (100, 50, 2, and 4 percent) significantly increased apo A-1 concentration compared with SCD (0.14±0.014). Serum apolipoprotein B (apo B) was significantly higher (51.2±1.082) in HST2, compared with other groups and isoenergetic diet types (HST2, HST4) significantly increased apoB among all diet types.

Hempseed as a sole nutritional resource modified positively not only profiles of lipid and lipoprotein but also improved cardiovascular risk factors. However, these changes are affected in a high extent by type of the diet.

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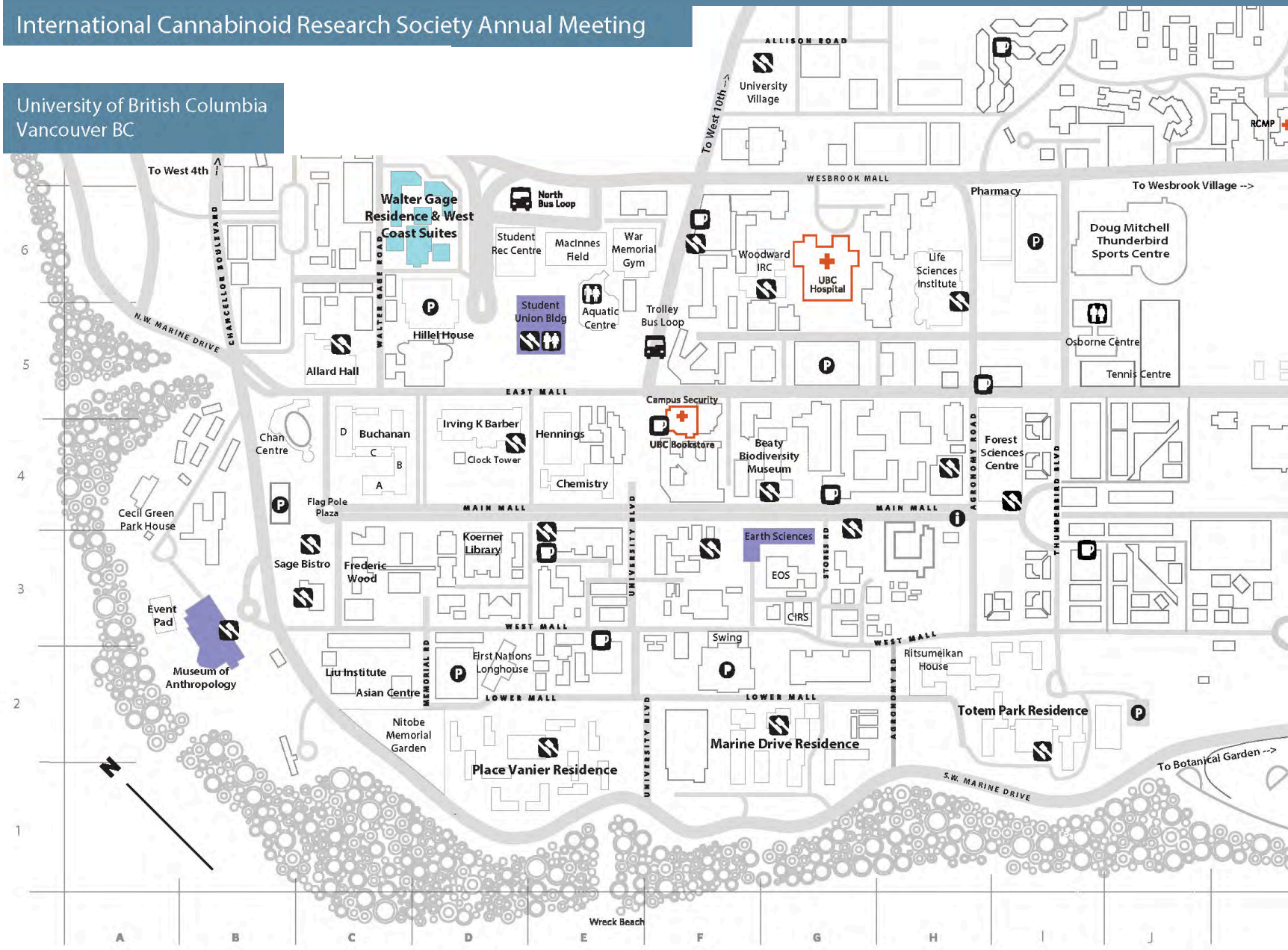

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B 3 Museum of Anthropology

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G6 UBC Hospital
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