25TH ANNUAL

SYMPOSIUM

OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

WOLFVILLE

NOVA SCOTIA

JUNE 28 - JULY 3, 2015

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Symposium Programming by Cortical Systematics LLC

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International Cannabinoid Research Society
Research Triangle Park, NC
USA

ISBN: 978-0-9892885-2-1

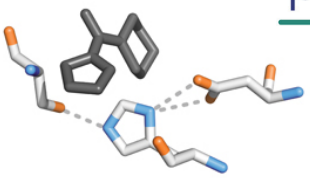
These abstracts may be cited in the scientific literature as follows:

Author(s), Abstract Title (2015) 25th Annual Symposium on the Cannabinoids, International Cannabinoid Research Society, Research Triangle Park, NC, USA, Page #.

Funding for this conference was made possible in part by grant 5R13DA016280-13 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

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Registration: June 28th, 2015, 16.00 – 18.00
Festival Theatre - Lobby

Welcome Reception: 18.30 – 20.00
KC Irving Centre

Day 1
Monday, June 29th

7.30	**Breakfast**		
8.30	Welcome and Opening Remarks **Festival Theatre**		
Oral Session 1. Novel chemical entities Moderators: Roger Pertwee, D.Phil, D.Sc. and Yossi Tam, D.M.D, Ph.D.			
9.00	Resat Cinar, Malliga Iyer, Ziyi Liu, Tony Jourdan, Katalin Erdelyi, Grzegorz Godlewski, Gergo Szanda, Jie Liu, Pal Pacher, Kenner Rice and George Kunos	PERIPHERALLY RESTRICTED, DUAL TARGET CB1R ANTAGONISTS WITH IMPROVED SAFETY AND ANTI-FIBROTIC EFFICACY	1
9.15	Brian F. Thomas, Gregory W. Endres, Jenny L. Wiley, Gerald T. Pollard, Ann M. Decker, Elaine A. Gay, Purvi R. Patel, Alexander L. Kovach and Megan Grabenauer	CHEMICAL EXPOSURES AND RISKS ASSOCIATED WITH VAPORIZATION AND INHALATION OF SYNTHETIC CANNABINOIDS	2

9.30	Jenny L. Wiley, Brian F. Thomas, Julie A. Marusich, Timothy W. Lefever and Gregory W. Endres	HIGH EFFICACY DEGRADANTS OF SYNTHETIC CANNABINOIDS EXHIBIT POTENT SUBSTITUTION IN JWH-018 DISCRIMINATION IN MICE	3
9.45	Spyros P. Nikas, Shashank Kulkarni, Rishi Sharma, Carol A. Paronis, Shan Jiang, Jimit Girish Raghav, Chandrashekhar Honrao, Srikrishnan Mallipeddi, Roger S. Gifford, Torbjörn U.C. Järbe, Jack Bergman and Alexandros Makriyannis	SAFER EFFECTIVE CANNABINOIDS THROUGH CONTROLLED DEACTIVATION	4
10.00	Pankaj Pandey, Kuldeep K. Roy and Robert J. Doerksen	CANNABIS-DERIVED COMPOUNDS AS CANNABINOID RECEPTOR MODULATORS: SYSTEMATIC ANALYSIS USING MOLECULAR DOCKING AND BINDING FREE-ENERGY STUDIES	5
10.15	Dai Lu, Hamed I. Ali, Changjiang Qiao, Kwang H. Ahn, Leepakshi Khurana and Debra A. Kendall	DESIGN AND SYNTHESIS OF PHOTOACTIVATABLE AFFINITY LIGANDS FOR THE CB1 ALLOSTERIC SITE	6
10.30	**BREAK**		
ORAL SESSION 2. PLANT STUDIES AND NON-THC CANNABINOIDS MODERATORS: ETHAN RUSSO, M.D. AND RANGAN (RONNIE) MAITRA, PH.D.			
11.00	Kazuhito Watanabe, Satoshi Yamaori, Rongrong Jiang, Yuka Kinugasa, Yoshimi Okushima and Ikuo Yamamoto	INDUCIBILITY OF CYP ENZYMES BY CANNABIDIOL	7

11.15	Justine Renard, Michael Loureiro, Laura G. Rosen, Walter J. Rushlow and Steven R. Laviolette	CANNABIDIOL'S ANTIPSYCHOTIC PROPERTIES IN THE MESOLIMBIC PATHWAY: ROLE OF MTORC/P70S6K SIGNALLING	8
11.30	Blathnaid Hughes and Caroline E. Herron	CANNABIDIOL REDUCES THE ATTENUATION OF HIPPOCAMPAL LONG-TERM POTENTIATION IN MODELS OF ALZHEIMER'S DISEASE	9
11.45	Daniel I Brierley, Joe R Harman, Natasa Giallourou, Jonathan R Swann, Ketan Patel, Benjamin J Whalley and Claire M Williams	CANNABIGEROL ATTENUATES MUSCLE CATABOLISM AND ANOREXIA IN A PRE-CLINICAL MODEL OF CHEMOTHERAPY-INDUCED CACHEXIA	10
12.00	Jahan P. Marcu, James Neal-Kababick, Melissa Wilcox and Mark Jacyno	IMPROVING QUALITY CONTROL METHODS FOR CANNABIS USING FLASH CHROMATOGRAPHY	11
12.15	N. Rielle Capler, Kim Crosby, Philippe Lucas and Zachary Walsh	THE SEED PROJECT: DEVELOPMENT OF STANDARDS AND CERTIFICATION PROGRAM FOR MEDICAL CANNABIS DISPENSARIES IN CANADA	12
12.30	Kevin McKernan, Vasisht Tadigotla, Yvonne Helbert, Jessica Spangler, Lei Zhang and Douglas Smith	SEQUENCING OF THREE MALE CANNABIS GENOMES AND DEVELOPMENT OF MULTIPLEX QPCR ASSAYS FOR RAPID MALE SEX DETERMINATION	13
12.45	Kevin McKernan, Cameron Miller, Brad Douglass, Jessica Spangler, Braden Doane, Vasisht Tadigotla, Colin Montgomery, Yvonne Helbert, Lei Zhang, Douglas Smith and Jeffrey Raber	GENOMIC, TERPENE AND CANNABINOID PROFILES OF A PUTATIVELY NOVEL CANNABIS SPECIES	14

13.00	**LUNCH** **NIDA Lunch & Mentoring** Wheelock Dining Hall		
14.00 - 16.00	**Poster Session** Fountain Commons		P1 ODD
16.00	Presidential Plenary Speaker **From Receptors to Pain:** **THE MOLECULAR DYNAMICS OF PAIN** **Michael Salter, M.D., Ph.D.** Hospital for Sick Children and University of Toronto Toronto, Canada		
17.00	**Break**		
Oral Session 3. Animal Models Moderators: Jenny Wiley, Ph.D. and James Burston, Ph.D.			
17.30	Shiran Udi, Liad Hinden, Adi Drori, Rivka Hadar, Brian Earley, Resat Cinar, Ken Mackie and Joseph Tam	ROLE OF PROXIMAL TUBULAR CANNABINOID-1 RECEPTOR IN OBESITY-INDUCED RENAL DYSFUNCTION	15
17.45	Angela M. Williams, Anthony L. Berger and Ryan J. McLaughlin	SEXUALLY DIMORPHIC EFFECTS OF ALCOHOL WITHDRAWAL ON ENDOCANNABINOID GENE EXPRESSION AND ANXIETY-LIKE BEHAVIOR	16

18.00	Sara Peñasco, Nagore Puente, Almudena Ramos, Naiara Royo, Ana Gutiérrez, Itziar Bonilla, Leire Reguero, Miren-Josune Canduela, Juan Mendizabal-Zubiaga, Fernando Rodríguez de Fonseca, Juan Suárez, Izaskun Elezgarai and Pedro Grandes	ALTERATION OF THE ENDOCANNABINOID-DEPENDENT SYNAPTIC PLASTICITY IN ADULT BRAIN AFTER ETHANOL EXPOSURE OF MICE DURING ADOLESCENCE	17
18.15	Zheng-Xiong Xi, Hai-Ying Zhang, Qing-Rong Liu, Guo-Hua Bi and Eliot L. Gardner	COCAINE SELF-ADMINISTRATION UP-REGULATES CANNABINOID CB2 GENE EXPRESSION IN MOUSE BRAIN	18
18.30	Jayme R McReynolds, Elizabeth M Doncheck, Oliver Vranjkovic, Evan N Graf, Qing-song Liu, Cecilia J Hillard and John R Mantsch	GLUCOCORTIOID-ENDOCANNABINOID INTERACTIONS IN THE PRELIMBIC CORTEX MEDIATE STRESS-POTENTIATED REINSTATEMENT OF COCAINE SEEKING	19
18.45	Sara Jane Ward, Patrick Siegele and Ronald Tuma	EFFECT OF CHRONIC CANNABINOID AGONIST TREATMENT ALONE OR IN COMBINATION WITH CANNABIDIOL ON WITHDRAWAL BEHAVIORS IN MICE	20
19.00	Martin A. Sticht, Cheryl L. Limebeer, Rehab A. Abdullah, Justin L. Poklis, Winnie Ho, Micah J. Niphakis, Keith A. Sharkey, Benjamin F. Cravatt, Aron H. Lichtman and Linda A. Parker	2-ARACHIDONOYLGLYCEROL MEDIATES THE ANTI-NAUSEA EFFECTS OF THE VISCERAL INSULAR CORTEX ENDOCANNABINOID SYSTEM IN RATS	21
19.15	Carol A. Paronis, Girish R. Chopda, Spyros P. Nikas, Vidyanand G. Shukla and Alex Makriyannis	SPONTANEOUS CANNABINOID WITHDRAWAL IN MICE: EVIDENCE FROM THREE BEHAVIORAL ASSAYS	22
19.30	**DINNER** WHEELOCK DINING HALL		

Day 2
Tuesday, June 30th

7.30	**Breakfast**		
Oral Session 4. CB2 Receptors Moderators: Andrea Hohmann, Ph.D. and Jayme McReynolds, Ph.D.			
8.30	Tomohiro Kimura, Krishna Vukoti, Diane L. Lynch, Dow P. Hurst, Alan Grossfield, Michael C. Pitman, Patricia H. Reggio, Alexei Yeliseev and Klaus Gawrisch	GLOBAL FOLD OF HUMAN CANNABINOID TYPE 2 RECEPTOR PROBED BY SOLID-STATE NMR AND MOLECULAR DYNAMICS SIMULATIONS	23
8.45	Uwe Grether, Jean-Michel Adam, Christian M. Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Fingerle, Ivan Formentini, Jürgen Funk, Sabine Grüner, Atsushi Kimbara, Matthias Nettekoven, Giorgio Ottaviani, Camille Perret, Mark Rogers-Evans, Benno Rothenhäusler, Stephan Röver, Franz Schuler, Tanja Schulz-Gasch and Christoph Ullmer	2,4,5-TRISUBSTITUTED PYRIDINES – A NOVEL CLASS OF HIGHLY POTENT, HIGHLY SELECTIVE AND IN VIVO ACTIVE CB2 AGONISTS	24
9.00	Natalia Malek, Magdalena Kostrzewa, Michal Korostynski, Julia Borowczyk, Zbigniew Madeja, Justyna Drukala and Katarzyna Starowicz	TARGETING PERIPHERAL CANNABINOID RECEPTORS CB2 AS A NOVEL THERAPY TO TREAT OSTEOARTHRITIS	25

9.15	Dan Ting Kho, Kristina Burkert, Michelle Glass, Kate Angel and E Scott Graham	DO CIRCULATING MONOCYTES IN HUMANS REPRESENT A THERAPEUTIC TARGET FOR CB2-MIMETIC DRUGS OR IS IT THEIR MACROPHAGE COUNTERPARTS?	26
9.30	Jürgen Fingerle, Christoph Ullmer, Jean-Michel Adam, Christian M. Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Funk, Sabine Grüner, Wolfgang Guba, Paul Hebeisen, Atsushi Kimbara, Matthias Nettekoven, Camille Perret, Mark Rogers-Evans, Stephan Röver, Franz Schuler and Uwe Grether	CB2 AGONISM PROTECTS FROM INFLAMMATION RELATED KIDNEY DAMAGE AND FIBROSIS	27
9.45	Ian Burkovskiy, Juan Zhou, Mel Kelly, George S Robertson and Christian Lehmann	CANNABINOID 2 RECEPTOR INHIBITION IN CNS-INJURY INDUCED IMMUNODEFICIENCY SYNDROME	28
10.00	Ming Gao, Zheng-Xiong Xi and Jie Wu	MECHANISMS OF CANNABINOID CB2 RECEPTOR-MEDIATED MODULATIONS IN MOUSE VTA DOPAMINE NEURONS	29
10.15	Julián Romero, Carmen Vázquez, RM Tolón, Alicia López, Noelia Aparicio, MªConcepción García, Javier Fernández-Ruiz, Bonnie N. Dittel and Cecilia J. Hillard	CANNABINOID CB2 RECEPTORS ARE SELECTIVELY EXPRESSED BY ACTIVATED MICROGLIAL CELLS IN ALZHEIMER'S DISEASE	30

10.30	**Break**	
11.00 - 13.00	**Poster Session** Fountain Commons	P1 EVEN
13.00	**Lunch** Wheelock Dining Hall	
14.00 - 20.30	**OUTING**	

Notes:

Day 3
Wednesday, July 1st

7.30	**Breakfast**		
Symposium in Association with NIDA **Role of Cannabinoids in Chronic Pain: An Update** Chair: Vishnudutt Purohit, Ph.D.			
8.30	Vishnudutt Purohit, Ph.D. NIDA Bethesda, MD USA	ROLE OF CANNABINOIDS IN CHRONIC PAIN SYMPOSIUM: AN UPDATE	N0
8.40	Mary Lynch, M.D. Dalhousie University Halifax, Nova Scotia Canada	CANNABINOIDS FOR THE TREATMENT OF CHRONIC NON-CANCER PAIN: AN UPDATED REVIEW OF RANDOMIZED CONTROLLED TRIALS	N1
9:05	Andrea G. Hohmann, Ph.D. Indiana University Bloomington, IN USA	CB1 AND CB2-MEDIATED STRATEGIES FOR EXPLOITING ANALGESIC EFFICACY WITHOUT DRUG ABUSE LIABILITY	N2
9:30	Aron H. Lichtman, Ph.D. Virginia Commonwealth University Richmond, VA USA	TARGETING ENDOCANNABINOID REGULATING ENZYMES TO TREAT CHRONIC PAIN	N3
9.55	Katarzyna Starowicz, Ph.D. Institute of Pharmacology Polish Academy of Sciences Krakow, Poland	ROLE OF ENDOCANNABINOID SYSTEM IN THE PATHOGENESIS OF OSTEOARTHRITIC PAIN	N4
10.20	Discussion and Future Directions		

10.30	**Break**		
Oral Session 5. EC Transport and Metabolism Moderators: Heather Bradshaw, Ph.D. and Joel Schlosburg, Ph.D.			
11.00	Kazuhito Tsuboi, Yoko Okamoto, Iffat Ara Sonia Rahman, Toru Uyama, Akira Tokumura and Natsuo Ueda	GLYCEROPHOSPHODIESTERASE GDE4 IS A NOVEL LYSOPHOSPHOLIPASE D-TYPE ENZYME GENERATING N-ACYLETHANOLAMINES	31
11.15	Kun Qian, Richard I. Duclos Jr. and Samuel John Gatley	DISPOSITION AND METABOLISM OF ANANDAMIDE IN THE MOUSE BRAIN	32
11.30	Jocelijn Meijerink, Mieke Poland, Zheng Wang, Ya Wang, Pierluigi Plastina, Michiel Balvers, Jean-Paul Vincken , Jean Paul ten Klooster and Renger Witkamp	N-3 FATTY ACID-DERIVED ENDOCANNABINOIDS, A GROWING SUBCLASS OF FATTY ACID AMIDES WITH IMMUNE MODULATING PROPERTIES	33
11.45	Matthew N. Hill, Jennifer Bialecki, Nicholas L. Wellinger and Roger J. Thompson	PANNEXIN-1 CHANNELS PERMIT POST-SYNAPTIC ANANDAMIDE TRANSPORT IN HIPPOCAMPAL PYRAMIDAL NEURONS	34
12.00	James J. Burston, Andrew J. Bennett , Micah J. Niphakis, Benjamin F. Cravatt and Victoria Chapman	MONOACYLGLYCEROL LIPASE INHIBITION REVERSES ESTABLISHED PAIN IN AN ANIMAL MODEL OF OSTEOARTHRITIS	35
12.15	Jenny L. Wilkerson, Sudeshna Ghosh, Rehab Abdullah, Benjamin F. Cravatt and Aron H. Lichtman	DUAL INHIBITION OF FATTY ACID AMIDE HYDROLASE AND MONOACYLGLYCEROL LIPASE IN MURINE MODELS OF INFLAMMATORY AND NEUROPATHIC PAIN	36

12.30	Eugene Krustev and Jason McDougall	LOCAL FATTY ACID AMIDE HYDROLASE INHIBITION BLOCKS NEUROGENIC INFLAMMATION IN MOUSE KNEE JOINTS	37
12.45	Xiaoxue Peng, Keith Studholme, Matthew W. Elmes, Yu-Han Gary Teng, Gregory Carbonetti, Dale G. Deutsch, Iwao Ojima and Martin Kaczocha	INHIBITION OF FATTY ACID BINDING PROTEINS PRODUCES ANTINOCICEPTIVE EFFECTS THROUGH PERIPHERAL AND CENTRAL MECHANISMS	38
13.00	**LUNCH** WHEELOCK DINING HALL		
14.00 - 16.00	**POSTER SESSION** FOUNTAIN COMMONS		P2 ODD
16.00	KANG TSOU MEMORIAL LECTURE **THE CANADIAN MEDICAL MARIJUANA LANDSCAPE: 1970'S TO PRESENT DAY PATIENT USE** **BRENT ZETTL** President and CEO Prarie Plant Systems Inc. Saskatoon, SK Canada		
17.00	**BREAK**		
ORAL SESSION 6. HUMAN STUDIES MODERATORS: MARY LYNCH, M.D. AND **SILVAIN DANG**			
17.30	Attila Oláh, Lídia Ambrus, Nikolett Vasas, Ágnes Angyal, Ralf Paus and Tamás Bíró	KERATINOCYTES OF ATOPIC DERMATITIS PATIENTS EXHIBIT MARKED ALTERATIONS IN GENE EXPRESSION PATTERN DURING DIFFERENTIATION – FAAH: FRIEND OR FOE?	39

17.45	Josephine M. Egan, Olga D. Carlson, Isabel Gonzalez Mariscal, Sara Santa-Cruz Calvo and Chee W. Chia	INCRETIN SECRETION IS INFLUENCED BY CANNABINOID RECEPTORS	40
18.00	Harriet de Wit, Joseph Lutz and Emma Childs	DOES DELTA-9 TETRAHYDROCANNABINOL DAMPEN RESPONSES TO SOCIAL STRESS?	41
18.15	Zach Walsh, Kam Shojania, Cheryl Koehn, Kim Crosby, Chris Carroll and Susan Holtzman	CANNABIS FOR ARTHRITIS PAIN: PATIENT CHARACTERISTICS, REASONS FOR USE, AND PERCEIVED COMPARATIVE EFFICACY	42
18.30	Ziva D. Cooper and Margaret Haney	SEX-DEPENDENT EFFECTS OF CANNABIS' ANALGESIC AND SUBJECTIVE EFFECTS IN CANNABIS SMOKERS	43
18.45	Nicholas Lintzeris, Louisa Degenhardt, Gabrielle Campbell, Michael Farrell, Raimondo Bruno and Wayne Hall	CANNABIS USE IN CHRONIC PAIN POPULATIONS: FINDINGS FROM THE POINT COHORT STUDY IN AUSTRALIA	44
19.00	Daniel Ziemianski, Lynda Balneaves, Sylvie Toupin, Fairleth McCuaig and Mark A. Ware	CANNABIS IN MEDICINE: A NATIONAL NEEDS ASSESSMENT FOR CANADIAN NURSE PRACTITIONERS	45
19.15	Ryan Vandrey, Edward J. Cone, Jeffrey C. Raber, Marcel O. Bonn-Miller, Evan S. Herrmann, George E. Bigelow, Mark Raber, Brad Douglass, Cameron Miller, John M. Mitchell, Ron Flegel and Charles LoDico	ORAL CANNABIS: PHARMACOKINETICS, PHARMACODYNAMICS AND PRODUCT EVALUATION	46

Day 4
Thursday, July 2nd

7.30	**Breakfast**		
Oral Session 7. GPR55 Moderators: Sara Jane Ward, Ph.D. and Natalia Malek			
8.30	Linda Console-Bram, Robert Zipkin and Mary E. Abood	THE EFFECTS OF ARACHIDONIC ACID AND N-ARACHIDONOYL AMINO ACIDS ON β-ARRESTIN ACTIVITY AND ERK1/2 PHOSPHORYLATION IN CANDIDATE CANNABINOID RECEPTORS	47
8.45	Carina Hasenoehrl, Johannes Haybaeck, Rufina Schuligoi, Martin Gauster and Rudolf Schicho	THE ROLE OF ATYPICAL CANNABINOID RECEPTOR GPR55 IN COLON CARCINOGENESIS	48
9.00	Nicole. A. Hofmann, Jiang Yang, Sunia A. Trauger, Hironao Nakayama, Lan Huang, Dirk Strunk, Marsha A. Moses, Michael Klagsbrun, Joyce Bischoff and Wolfgang F. Graier	THE GPR55-AGONIST L-α-LYSOPHOSPHATIDYLINOSITOL MEDIATES OVARIAN CARCINOMA INDUCED ANGIOGENESIS	49
9.15	Lauren Whyte, Aysha Khalid, Graeme Finnie, Selina Chiu, David Baker, Richard Aspden and Ruth Ross	GPR55 REGULATES STEROID HORMONE LEVELS IN MALE MICE AND OFFERS PROTECTION AGAINST AGE RELATED BONE LOSS	50

9.30	Michael Loureiro, Justine Renard and Steven R. Laviolette	GPR55 RECEPTORS CONTROL MESOLIMBIC DOPAMINE NEURONAL ACTIVITY AND EMOTIONAL PROCESSING BEHAVIOURS THROUGH A GLUTAMATERGIC MECHANISM IN THE MAMMALIAN VENTRAL HIPPOCAMPUS	51
9.45	Bright N Okine, Gemma McLoughlin, Michelle Roche and David P. Finn	ADMINISTRATION OF THE SELECTIVE GPR55 RECEPTOR ANTAGONIST, CID16020046, INTO THE ANTERIOR CINGULATE CORTEX REDUCES FORMALIN-EVOKED NOCICEPTIVE BEHAVIOR IN RATS	52
10.00	**In Memoriam**		
10.30	**Break**		
Oral Session 8. Bias and Modulation Moderators: Mary Abood, Ph.D. and Khalil Eldeeb, M.D., M.Sc., Ph.D.			
11.00	Heather B Bradshaw, Meera Manchanda and Kishan Sangani	COMBINATIONS OF N-ACYL ETHANOLAMINES HAVE HIGHER EFFICACY THAN INDIVIDUAL LIPIDS AT TRPV1 RECEPTORS: MORE LIKE LIFE?	53
11.15	Khalil Eldeeb and Allyn C. Howlett	CB1 CANNABINOID RECEPTOR AND G PROTEIN S	54

11.30	Christopher W. Cunningham, XiaoQian Liu and Cecilia J. Hillard	EVIDENCE OF BIASED AGONISM AMONG DIVERSE CANNABINOID CB1R LIGANDS	55
11.45	Alex Straiker, Danielle Yin, José Mitjavila, Anne Gibson, Jim Wager-Miller and Ken Mackie	CAMKII ACTIVATION MODULATES CANNABINOID SIGNALING BOTH PRE- AND POST-SYNAPTICALLY IN AUTAPTIC HIPPOCAMPAL NEURONS	56
12.00	Alex Straiker, Jose Mitjavila, Danielle Yin, Anne Gibson and Ken Mackie	STILL NAMBY PAMBY: FIRST GENERATION ALLOSTERIC MODULATORS OF CB1 IN A NEURONAL MODEL	57
12.15	Robert B. Laprairie, Adel Zrein, Amina M. Bagher, Pushkar M. Kulkarni, Ganesh A. Thakur, Melanie E. Kelly and Eileen M. Denovan-Wright	ENANTIOMER-SPECIFIC POSITIVE ALLOSTERIC MODULATION OF THE TYPE 1 CANNABINOID RECEPTOR FOR THE TREATMENT OF HUNTINGTON'S DISEASE	58
12.30	Elizabeth A. Cairns, Alex J. Straiker, Pushkar M. Kulkarni, Ganesh A.Thakur, William H. Badridge, and Melanie E.M. Kelly	ACTIONS OF THE AGO-PAM GAT211 AND ITS ENANTIOMER GAT229 ON INTRAOCULAR PRESSURE AND RETINAL GANGLION CELL LOSS IN THE NEE MOUSE MODEL OF OCULAR HYPERTENSION	59
12.45	Alyssa S. Laun, Pritesh P. Kumar and Zhao-Hui Song	CANNABIGEROL MODULATES THE EFFICACY OF ANANDAMIDE ON THE CB2 CANNABINOID RECEPTOR	60

13.00	**Lunch** Wheelock Dining Hall	
14.00 – 16.00	**Poster Session** Fountain Commons	P2 EVEN
16.00	**25 Years of ICRS and 50+ Years of Cannabinoids** The endocannabinoid system: looking back and ahead **Raphael Mechoulam, Ph.D.** Hebrew University of Jerusalem Jerusalem, Israel	
17.00	**Break**	
17.15	**ICRS BUSINESS MEETING**	
19.30	**AWARDS CEREMONY** **ICRS BANQUET**	

Departure: **Friday, July 3rd**

POSTER SESSION 1

ODD # - DAY 1, MONDAY, JUNE 29TH: 14:00 - 16:00
EVEN # - DAY 2, TUESDAY, JUNE 30TH: 11:00 - 13:00

Xiaoxi Ling, Shaojuan Zhang, Pin Shao, Weixia Li, Ling Yang, Ying Ding, Cong Xu, Nephi Stella and Mingfeng Bai	DEVELOPMENT AND IN VIVO IMAGING OF A NEAR INFRARED FLUORESCENT PROBE THAT PREFERENTIALLY BINDS TO CB2 OVER CB1 RECEPTORS	P1-1
Rui Liu, Chris Cayer, Diba Behzadpour, Pam Kent, John Arnason, Zul Merali and Cory Harris	INHIBITION OF MONOACYLGLYCEROL LIPASE BY ANXIOLYTIC MEDICINAL PLANT EXTRACTS AND THEIR TRITERPENES	P1-2
Willard J. Costain, Joseph S. Tauskela, Ingrid Rasquinha, Tanya Comas, Melissa Hewitt, Amy Aylsworth,Vincent Marleau and Evelyn C. Soo	PHARMACOLOGICAL CHARACTERIZATION OF EMERGING SYNTHETIC CANNABINOIDS IN CELLS AND NEURONS	P1-3
Herbert H. Seltzman, Yatendra Mulpuri and Igor Spigelman	PERIPHERALLY-RESTRICTED CBR AGONIST PRNMI SUPPRESSES CISPLATIN INDUCED PERIPHERAL NEUROPATHY CHRONIC PAIN SYMPTOMS WITHOUT TOLERANCE OR SIDE EFFECTS IN BOTH MALE AND FEMALE RATS	P1-4
Xiao-Fei Wang, Guo-Hua Bi, Yi He, Eliot L. Gardner and Zheng-Xiong Xi	CANNABINOID CB1 AND CB2 RECEPTORS MEDIATE THE CLASSICAL TETRAD EFFECTS OF DELTA9-TETRAHYDROCANNABINOL IN MICE	P1-5
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POSTER SESSION 2

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Sara R. Nass and Steven G. Kinsey	INFLAMMATORY ARTHRITIS-INDUCED HYPERALGESIA AND PAIN-SUPPRESSED BEHAVIOR ARE ATTENUATED BY THE MONOACYGLYCEROL LIPASE INHIBITOR JZL184	P2-25
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Matthew W. Elmes, Martin Kaczocha, William T. Berger, Yu-Han Gary Teng, Brian P. Ralph, Joseph M. Sweeney, Robert C. Rizzo, Iwao Ojima and Dale G. Deutsch	DEVELOPMENT OF NOVEL INHIBITORS FOR THE FATTY ACID BINDING PROTEINS TO ALTER THE ENDOCANNABINOID TONE	P2-27
Regina Nelson	THE VOICES OF WOMEN: NARRATIVES REVEALING SOCIAL STIGMA WITHIN THE MEDICAL PROFESSION	P2-28
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Nada Mahmood, Andy J Bennett, Victoria Chapman and Steve PH Alexander	EXPLORING ABHD6 EXPRESSION IN RAT TISSUES	P2-31
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Presidential Plenary Speaker

Monday, June 29, 2015
16:00 – 17:00

FROM RECEPTORS TO PAIN: THE MOLECULAR DYNAMICS OF PAIN

Michael Salter, M.D., Ph.D.

Hospital for Sick Children and
University of Toronto
Toronto, Canada

Neuron-microglial interactions are increasingly recognized as being key for physiological and pathological processes in the central nervous system. Microglia have been found to play a causal role in neuropathic pain behaviours resulting from peripheral nerve injury, and a core neuron-microglia-neuron signaling pathway has been elucidated. Within the dorsal horn, microglia suppress neuronal inhibition by a cascade involving activation of microglial P2X4 receptors causing the release of brain derived neurotrophic factor (BDNF). BDNF acts on trkB receptors which leads to a rise in intracellular chloride concentration in dorsal horn nociceptive output neurons, transforming the response properties of these neurons. In addition to suppressing inhibition, peripheral nerve injury causes activity-dependent facilitation at dorsal horn glutamatergic synapses which enhances nociceptive transmission. This enhancement is mediated by intracellular signaling networks involving serine/threonine and tyrosine kinases within nociceptive transmission neurons. Key for this enhancement is facilitation of NMDA receptor function by Src family tyrosine kinases. Recently we have discovered that microglia-to-neuron signaling is not only critical for pain hypersensitivity after peripheral nerve injury but also for the paradoxical hyperalgesic effect of morphine and other opioids. We anticipate that by targeting microglia-neuron signaling pathways new therapeutic strategies for chronic pain as well as its comorbid sequelae may be developed.

Funding: Supported by CIHR, Krembil Fdn, CRC and Anne and Max Tanenbaum Chairs.

Kang Tsou Memorial Speaker

Wednesday, July 1, 2015
16:00 – 17:00

The Canadian Medical Marijuana Landscape: 1970's to Present Day Patient Use

Brent Zettl

President and CEO
Prarie Plant Systems Inc.
Saskatoon, SK Canada

The more recent Canadian situational history to society awareness for medical use of cannabis can be traced back to the Canadian Government's interpretation of the "Le Dain Commission Report of 1972". Since that time, the combination of legal challenges and subsequent decisions by the judicial system combined with policy and regulations intended to follow those decisions initially led to the origin of the Medical Marijuana Access Regulations (MMAR) of 2004. Health Canada had a narrow range of ailments initially permitted under the MMAR. Given a series of concerns which arose, the government then established a new set of regulations, the Marijuana for Medical Purposes Regulations (MMPR) in June 2013 which eventually replaced the MMAR (April 2014). These new regulations shifted the burden for treatment with medical marijuana onto the physicians in Canada which also were not restricted in their application to treat any ailment. Since October 2013, a number of Canadian patients have sought relief from ailments by using medical marijuana produced and provided by 22 Licensed Producers (registered with Health Canada). As at April 30, 2015 (according to Health Canada) 17,000 patients have access to medical marijuana under the new program. A subset of that population (3,600) have reported their respective 1,400 conditions as patients who have self-titrated on varying ratios of THC & CBD combinations to mitigate the symptoms of those conditions. This talk will anecdotally relay the emerging patient use of cannabinoid profiles as it relates to a number of disease conditions. It will also compare this to the use of medical marijuana under the old MMAR system with a limited medical cannabis variety. Unsolicited comments of some patient's experiences with medical marijuana will also be presented as observations from their clinical participation.

PERIPHERALLY RESTRICTED, DUAL TARGET CB_1R ANTAGONISTS WITH IMPROVED SAFETY AND ANTI-FIBROTIC EFFICACY

Resat Cinar[1], Malliga Iyer[1], Ziyi Liu[1], Tony Jourdan[1], Katalin Erdelyi[1], Grzegorz Godlewski[1], Gergo Szanda[1], Jie Liu[1], Pal Pacher[1], Kenner Rice[2] and George Kunos[1]

[1] Laboratory of Physiologic Studies, [2] Chemical Biology Research Branch, NIAAA, NIH, USA

Liver fibrosis, a major contributor to liver-related mortality, currently has no effective treatment. Liver fibrosis is associated with increased activity of the endocannabinoid/CB_1R system, and the CB_1R antagonist/inverse agonist rimonabant mitigates fibrosis in animal models. However, the antifibrotic efficacy of rimonabant is low, and neuropsychiatric side effects halted its therapeutic development. Liver fibrosis is also associated with increased activity of inducible nitric oxide synthase (iNOS) and decreased activity of adenosine monophosphate kinase (AMPK), and iNOS inhibitors or AMPK activators have been shown to mitigate liver fibrosis. In order to improve the safety and antifibrotic efficacy of CB_1R antagonism, we have developed highly potent, orally bioavailable hybrid CB_1R antagonists that are behaviorally inactive due to low brain penetrance, and have additional activity either as iNOS inhibitors or AMPK activators. We have tested the antifibrotic efficacy of the two lead compounds in a murine model of liver fibrosis induced by bile-duct ligation (BDL).

The compounds, (-)MRI1867 (CB_1R antagonist/iNOS inhibitor) and (-)MRI1891 (CB_1R antagonist /AMPK activator) selectively block peripheral CB_1R (Ki 2.5 nM and 0.5 nM, respectively) due to their limited brain penetrance (plasma:brain ratio of 0.03 and 0.05, respectively). MRI1867 directly inhibits iNOS activity in vitro by 37% and MRI-1891 activates AMPK in vitro by 20%, whereas rimonabant affects neither iNOS nor AMPK activities, when tested at 1 uM. Mice were subjected BDL and were treated simultaneously with equipotent daily oral doses of rimonabant (3 mg/kg), (-)MRI1867 (3 mg/kg), (-)MRI1891 (1mg/kg) or vehicle. Both (-)MRI1867 and (-)MRI1891 were more efficacious than rimonabant in mitigating liver fibrosis as quantified by Sirius red staining and TGFβ1, Collagen 1A, TIMP1 and αSMA gene expression. Importantly, both hybrid compounds were also able to reduce fibrosis in $CB_1R^{-/-}$ mice subjected to BDL. Unlike rimonabant, neither dual-target compound elicited CNS-mediated effects such as hyperambulatory activity or anxiogenic behavior.

We conclude that dual-target CB_1R/iNOS or CB_1R/AMPK compounds can provide a novel type of pharmacotherapy for liver fibrosis with improved efficacy and safety.

Acknowledgements: Supported by intramural funds of NIAAA/NIH.

CHEMICAL EXPOSURES AND RISKS ASSOCIATED WITH VAPORIZATION AND INHALATION OF SYNTHETIC CANNABINOIDS

Brian F. Thomas[1], Gregory W. Endres[2], Jenny L. Wiley[1], Gerald T. Pollard[3], Ann M. Decker[1], Elaine A. Gay[1], Purvi R. Patel[1], Alexander L. Kovach[1] and Megan Grabenauer[4]

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Synthetic cannabinoids are often formulated in herbal cigarettes and smoked, or dissolved in propylene glycol or glycerin and vaporized, to produce intoxication while avoiding detection by forensic or law enforcement agencies. Not only are the illicitly synthesized cannabinoids in "Spice" preparations of uncertain quality and unknown toxicity, but the volatility and end products that result from heating for consumption as smoke or vapor are unknown. To investigate the volatility and thermal stability of synthetic cannabinoids, a thermolysis probe was coupled to an Agilent GC/MS for separation and identification of chemicals liberated by heating. For initial studies, samples were heated rapidly to 800 °C under ambient (zero grade) air, a condition that approximates the burning end of a cigarette. Further systematic evaluation of thermal degradation and pyrolysis pathways was done by using repeated pyrolysis to heat synthetic cannabinoids incrementally (200, 400, 600 and 800 °C). The results demonstrate that the volatility and thermal stability of synthetic cannabinoid analogs vary widely depending upon chemical class and structural constituents. For example, under isothermal conditions at 800 °C relatively high recovery was observed with JWH-018, an alkyl indole with a ketone linker to a naphthalene substituent. In contrast, alkyl indoles with a ketone linked tetramethylcyclopropyl ring system (e.g., XLR-11, UR-144) were almost completely susceptible to the thermally induced ring-opening reactions. In the case of XLR-11 and UR-144, the thermolytic degradants produced during heating retain high affinity and efficacy at the CB1 and CB2 receptors and have cannabimimetic activity in laboratory animals. Moreover, relatively modest changes in chemical structure were observed to have a profound impact on volatility and thermal stability, and in some instances potentially dangerous chemical entities are formed and delivered in the gas and vapor phase. It can therefore be concluded that whether formulated in herbal products that are combusted and inhaled, or dissolved in propylene glycol or other solvents that can be heated in electronic vaporizers and inhaled, exposure to elevated temperatures may facilitate thermal degradation of synthetic cannabinoids and generate a variety of chemical by-products to which individuals would be exposed. Due to the increased potential for exposure to chemicals of unknown toxicity, this constitutes a public health concern as well as being a matter of chemical and pharmacological interest.

Acknowledgements: Grant funding from the National Institute of Justice (NIJ-2012-3098).

HIGH EFFICACY DEGRADANTS OF SYNTHETIC CANNABINOIDS EXHIBIT POTENT SUBSTITUTION IN JWH-018 DISCRIMINATION IN MICE

Jenny L. Wiley[1], Brian F. Thomas[1], Julie A. Marusich[1],
Timothy W. Lefever[1] and Gregory W. Endres[2]

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Synthetic indole-derived cannabinoids, originally developed for use as research tools or potential medications before the turn of the century, are now best known for their inclusion in "herbal" products that are being abused for their marijuana-like subjective effects. Although JWH-018 was the first cannabinoid identified in product samples, XLR-11 and UR-144 are among the most common constituents recently. Unlike many of the previously noted synthetic naphthoyl indole cannabinoids, these two compounds are tetramethylcyclopropyl ketone indoles that contain a terminal tetramethylcycloproyl ring structure that is vulnerable to thermal degradation and/or pyrolysis. Under typical conditions of use (e.g., smoking), we have shown that the tetramethylcyclopropyl group opens (see abstract by B.F. Thomas *et al.* in this volume). Hence, primary user exposure is to compounds other than those contained in the bulk product. In this study, we evaluated the ring open degradants of XLR-11 and UR-144 in drug discrimination, an animal model of the subjective effects of psychoactive drugs.

C57/Bl6 adult male mice were trained to discriminate 0.3 mg/kg JWH-018 from vehicle in a standard drug discrimination paradigm. Since most cannabinoid discrimination studies have used D^9-tetrahydrocannabinol (THC) for training, we validated the procedure by confirming dose-dependent substitution of JWH-018 (ED_{50}=0.13 mg/kg), cross-substitution with THC (ED_{50}=1.28 mg/kg), and rimonbant reversal of JWH-018's discriminative stimulus effects. Consistent with their favorable CB_1 receptor affinities (K_i=5.0 and 11.23 nM for XLR-11 and UR-144 degradants, respectively), both compounds fully and dose-dependently substituted for JWH-018, with potencies of ED_{50}=0.18 and 0.26 mg/kg for XLR-11 and UR-144 degradants, respectively). These potencies are greater than potencies previously reported for the parent compounds, XLR-11 (ED_{50}=1.2 mg/kg) and UR-144 (ED_{50}=2.3 mg/kg), albeit the parent compounds were tested in a THC (vs. JWH-018) discrimination procedure in mice (Wiley *et al.*, 2013, *Neuropharmacology*, **75:** 145-54).

These results represent the first demonstration of JWH-018 discrimination acquisition in mice and are consistent with those obtained in rats (Wiley *et al.*, 2014, *Pharmacol Biochem Behav*, **124:** 123-8). Further, the potent substitution of XLR-11 and UR-144 degradants suggests that both would produce subjective effects similar to those produced by other synthetic cannabinoids, including their parent compounds. Because smoking results in greater exposure to the ring-open variants, cannabimimetic potency of a product containing XLR-11 or UR-144 would be predicted to be greater than their CB_1 affinities would suggest, a prediction that has received some anecdotal support (Peterson & Couper, 2014, *J Anal Toxicol*, **38:** 563-8.).

Acknowledgements: Funded by IR&D from RTI International and National Institutes of Health / National Institute on Drug Abuse Grant DA-003672.

SAFER EFFECTIVE CANNABINOIDS THROUGH CONTROLLED DEACTIVATION

Spyros P. Nikas,[1] Shashank Kulkarni,[1] Rishi Sharma,[1] Carol A. Paronis,[2] Shan Jiang,[1] Jimit Girish Raghav,[1] Chandrashekhar Honrao,[1] Srikrishnan Mallipeddi,[1] Roger S. Gifford,[1] Torbjörn U.C. Järbe,[1] Jack Bergman[2] and Alexandros Makriyannis[1]

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The plant derived (-)-Δ^9-Tetrahydrocannabinol [(-)-Δ^9-THC] and its analogs act at CB1 and CB2, two cannabinoid receptors that are currently being targeted for various conditions including pain, inflammation, CNS disorders, and cancer. Owing to the undesirable side effects associated with CB1 receptor activation/deactivation as well as poor PK/PD properties only a limited number of cannabinergic drugs have been approved to date. Thus, the development of safer THC-based medications with favorable oral bioavailability, consistent efficacy, and predictable time course of action and detoxification remains to be addressed. Toward this end, we recently reported on a controlled deactivation/detoxification approach where the "soft" analog/drug concept of enzymatic deactivation was combined with a "depot effect" that is commonly observed with Δ^9-THC and other lipophilic cannabinoids.

In the first generation ligands our design incorporates a metabolically labile ester group at strategic positions within the THC structure. In an effort to develop controlled deactivation cannabinoids with faster onset/offset and shorter duration of action than the currently existing THC analogs we have now focused on cannabinergic templates with enhanced polar characteristics that are associated with the depot effect. The novel compounds reported here exhibit high CB receptor binding affinity, in vitro and in vivo potency and efficacy, and are susceptible to enzymatic hydrolysis by plasma esterases in a controllable manner. Also, their hydrolytic metabolites are inactive at CB receptors. Importantly, one of our second generation analogs when tested in rodents and non human primates was found to be a remarkably potent and efficacious CB1 receptor agonist with relatively short duration of action.

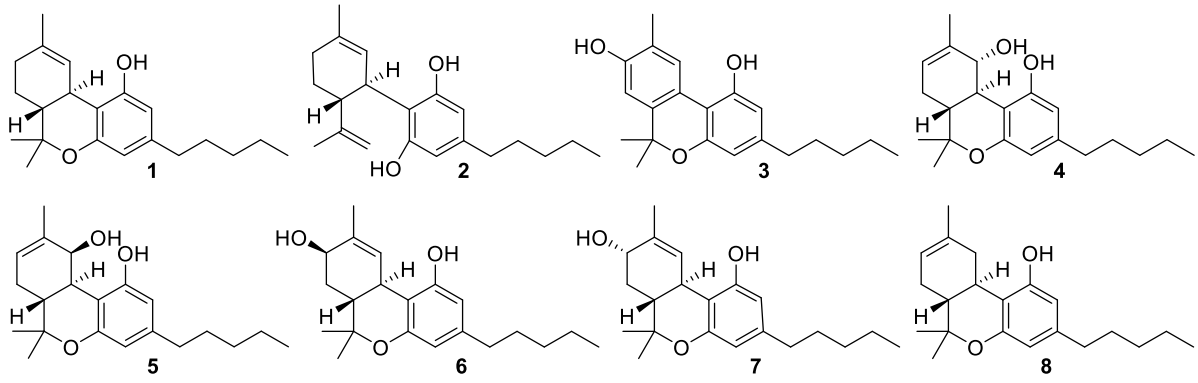
Acknowledgments: This work was supported by grants from the National Institute on Drug Abuse, DA009158, DA007215 and DA09064.

CANNABIS-DERIVED COMPOUNDS AS CANNABINOID RECEPTOR MODULATORS: SYSTEMATIC ANALYSIS USING MOLECULAR DOCKING AND BINDING FREE-ENERGY STUDIES

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Major advances in marijuana and cannabinoid (CB) research have led to the isolation of endogenous and non-classical cannabinoids, as well as functional identification of transporters and enzymes involved in the synthesis and degradation of endogenous ligands. Compounds isolated from the *Cannabis sativa* plant, such as Δ^9-tetrahydrocannabinol [Δ^9-THC (**1**)] and cannabidiol (**2**), have potential for therapeutic application in the treatment of neuroinflammation, arthritis, cancer and other metabolic disorders. However, little information is available at the molecular level about the three-dimensional structure, dynamics and conformational flexibility of the CB receptors, and their function and regulation. In order to explore the CB receptor interactions with phytochemicals, in this work we performed systematic in silico docking studies of cannabis-derived compounds to CB protein models in order to gain understanding of their modes of binding and structure-activity relationship (SAR), including identification of particular CB protein residues that aid their binding and that lead to specificity/selectivity. Our systematic docking results revealed the importance of the C-8 hydroxyl group in the cannabinol (**3**) class of compounds for better affinity and selectivity for CB2 over CB1. The C-10 α-hydroxyl group of a Δ^8-THC related compound (**4**) plays a significant role in interacting with both CB receptors, based on the extra intermolecular hydrogen bonds the compound makes that are not found for a corresponding compound (**5**) with a β-hydroxyl at that position. A compound which possesses a β-OH group at the C-8 position of Δ^9-THC (**6**) displayed better CB activity than a similar compound (**7**) which instead possesses an α-OH group at that position. Moreover, the C-3 alkyl chain and C-1 hydroxyl group substituents of Δ^8-THC (**8**) and **1** make significant contributions to the CB1 and CB2 binding via hydrophobic and H-bond interactions. This systematic study of sub-molecular structural information on binding to the CB receptors and on CB receptor subtype selectivity provides insight for design of novel compounds or analogs which could be synthesized and tested for treating various health disorders.



Acknowledgements: Funded by the US National Institutes of Health (P20GM104932 and C06RR14503).

DESIGN AND SYNTHESIS OF PHOTOACTIVATABLE AFFINITY LIGANDS FOR THE CB1 ALLOSTERIC SITE

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The recent discovery of allosteric modulators of the CB1 receptor including ORG27569, PSNCBAM-1, RTI-371 and Lipoxin A4 offer new opportunities for discovery of therapeutic agents that are distinct from orthosteric CB1 ligands. The therapeutic usefulness of CB1 allosteric modulators is emerging, which includes anorectic properties, neuroprotective effects and prevention of relapse to drug addiction. At present, development of CB1 allosteric modulators is largely based on optimization of the very few early leads. In contrast, structure-based rational design of novel CB1 allosteric modulators is difficult to carry out because of insufficient information about the allosteric binding motifs of CB1 receptor. To facilitate the structural characterization of the CB1 allosteric site, we have designed and synthesized photoactivatable covalent ligands based on the prototypical allosteric modulator ORG27569. The well-established photoactivatable groups such as azide, benzophenone and diazrine were introduced into the indole-2-carboxamide skeleton of ORG27569. Some of these ORG27569 analogs bearing photoactivatable groups exhibited a dissociation constant (K_B) and allosteric binding cooperativity factor (α) comparable to those of ORG27569. Our affinity ligands will provide valuable tools for the characterization of the binding motif of the CB1 allosteric site in future proteomic studies.

INDUCIBILITY OF CYP ENZYMES BY CANNABIDIOL

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Our previous studies have demonstrated that cannabidiol (CBD) is a potent inhibitor of CYP enzymes (Yamaori *et al*., *Biochem. Pharmacol*. 79: 1691 (2000); *Drug Metab. Dispos*. 39: 2049 (2011); *Foren. Toxicol*., 29: 117 (2011) *etc*). Many CYP inhibitors are also known to be inducers of CYP-mediated drug metabolism. The present study investigated the inducibility of CYP enzymes by CBD.

In ddY male mice (8 weeks old), CBD (120 mg/kg/day for 3 days, i.p.) induced the mRNA expression of CYP2B10 and CYP2B13 by 17- and 10-fold, respectively. In addition, the CBD-treatment also caused the induction of mRNA expression of CYP1A2, CYP2Cs (2C29, 2C37, 2C38, 2C39, 2C50) and CYP3As (3A11, 3A13, 3A25, 3A44) by 1.7 ~ 4.2 fold. None of mRNA expression of CYP2A5, CYP2Ds, CYP2E1, CYP4As and CYP4Fs was significantly induced by the CBD-treatment. The CBD-treatment resulted in the induction of 7-benzyloxyresorfin *O*-dealkylase (22 fold) and 7-benzyloxyquinoline *O*-debenzylase (2.2 fold) activities in mouse liver microsomes.

CBD concentration-dependently induced the expression of CYP1A1 mRNA in human hepatoma HepG2 cells. Among three major phytocannabinoids (tetrahydrocannabinol, CBD and cannabinol) tested, CBD was the most potent inducer of CYP1A1 expression. The induction of CYP1A1 expression by CBD was significantly attenuated by the knockdown of AhR expression with AhR siRNA-treatments. The induction of CYP1A1 by CBD was significantly suppressed by herbimycin A and omeprazole, but not by 3-methylcholanthrene. These results showed that CBD may induce human CYP1A1 expression through the activation of PTK-dependent AhR signaling. In addition, the experiments on the structural requirement for CYP1A1 induction by CBD indicated that two phenolic hydroxy groups in the pentylresorcinol moiety may be structurally important.

The present study indicates that CBD induces particular CYP enzymes by tissue- and/or species-dependent manner, which the precise mechanism need to be clarified.

Acknowledgements: This work was funded by the Ministry of Education, Culture, Sports, Science, and Technology of Japan [Grant-in-Aids for Scientific Research (C) and Young Scientists (B)].

CANNABIDIOL'S ANTIPSYCHOTIC PROPERTIES IN THE MESOLIMBIC PATHWAY: ROLE OF MTORC/P70S6K SIGNALLING

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Schizophrenia is a devastating psychiatric disorder characterized by delusions, hallucinations, cognitive disturbances and disorganized behaviour. For decades, schizophrenia has been treated with antipsychotic drugs targeting dopamine (DA) receptors. Despite the significant side effects associated with antipsychotics, no mechanistically novel or clinically effective treatment has emerged to replace them. Recent evidence points to the involvement of one specific phytochemical component of marijuana, cannabidiol (CBD) as possessing promising therapeutic properties for the treatment of schizophrenia. However, the neuronal and molecular mechanisms through which CBD may exert these effects are entirely unknown.

Here, we use amphetamine (AMPH)-induced sensitization, a classical animal model of schizophrenia-related psychosis, combined with *in vivo* neuronal electrophysiology and molecular analyses to assess the function of CBD in the nucleus accumbens shell (NASh), a brain region critically involved in the action of antipsychotics. We show that intra-NASh pre-treatment with CBD powerfully blocks amphetamine (AMPH)–induced sensitization, both in terms of DAergic neuronal activity within the ventral tegmental area (VTA) and psychotomimetic behaviours. We further identify the mTOR/p70S6K signaling pathways as functional molecular substrates underlying the effects of CBD.

Collectively, these findings demonstrate antipsychotic properties of CBD in the mesolimbic circuitry and identify a novel molecular signaling pathway through which CBD functionally reduces schizophrenia-like neuropsychopathology.

Acknowledgments: This work was supported by the Canadian Institutes of Health Research (CIHR; MOP 246144) and the National Science and Engineering Research Council of Canada (NSERC).

CANNABIDIOL REDUCES THE ATTENUATION OF HIPPOCAMPAL LONG-TERM POTENTIATION IN MODELS OF ALZHEIMER'S DISEASE

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Cannabidiol (CBD), a non-psychoactive plant derived constituent of marijuana, has become a therapeutic target for a number of diseases including Alzheimer's disease (AD). CBD has been shown to act as an anti-inflammatory agent that can protect against the neuro-toxic effects of beta amyloid peptide (Aβ) in cultured cells. Here, we have used both acute and chronic models of AD to investigate the neuroprotective effects of CBD. We have reported previously that acute application of beta amyloid peptide (Aβ) in the form of amyloid derived diffusible ligands (ADDLs) can attenuate long-term potentiation (LTP) in the CA1 region of hippocampal slices. Pretreatment of slices with CBD prior to application of Aβ can attenuate the synapto-toxic effects of this peptide (Hughes et al., 2013, 6th European Workshop on Cannabinoid Research, P020). Here we have investigated the effects of chronic CBD treatment in a mouse model of AD (APPswe/PS1dE9). In addition we have examined further the acute effects of CBD in slices to investigate the mechanism of action.

To examine the chronic effects of CBD we used the APPswe/PS1dE9 mouse model of AD. LTP was assessed in slices from 9 month old APP_{swe}/PS1dE9 mice and age-matched litter mates. Animals received either vehicle (PBS, Tween 80, EtOH 8:1:1) or Cannabidiol (10mg/kg) *i.p*, once daily for 29 days followed by a 3 day rest period prior to hippocampal slice electrophysiology. Parasagittal slices (400μm) were cut from 9 month old APP_{swe}/PS1dE9 mice and their littermate controls.We found that treatment with vehicle or CBD did not alter the levels of LTP recorded in slices from non-transgenic litter mates. In control vehicle treated non-transgenic mice LTP measured, (153.2 ± 8.4%, n=9) while following treatment with CBD, LTP measured 149.0 ± 10.7%, n=10), p=0.76. The LTP deficit in the vehicle treated APP_{swe}/PSdE9 mice (122.3 ± 9.0%, n=6) was reversed in the CBD treated group (169.8 ± 12.68, n=8), $p<0.05$ and was similar to levels recorded in the wild type vehicle group (153.2 ± 8.4%, n=9), p=0.28

As CBD has been reported to act as a $5HT_{1A}$ agonist (Ledgerwood et al., 2011, Br.J. Pharm. 162:286-94.) we have also investigated the effects of treating acute hippocampal slices from wild type mice with CBD (10μM) and the antagonist WAY100135 (300nM) prior to application of Aβ (ADDLS) (500nM). Results of these experiments will also be presented. Our data suggest that CBD is neuro-protective in our models of AD and may be a useful therapeutic agent for the treatment of AD. All experiments were conducted in accordance with the regulations from Dept of Health Ireland (86/609/EEC).

Acknowledgements: Funded by the Irish Health Research Board and University College Dublin.

CANNABIGEROL ATTENUATES MUSCLE CATABOLISM AND ANOREXIA IN A PRE-CLINICAL MODEL OF CHEMOTHERAPY-INDUCED CACHEXIA

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Cancer anorexia-cachexia syndrome (CACS) is a debilitating multifactorial wasting syndrome, primarily characterised by progressive loss of skeletal muscle, typically with concurrent anorexia and fatigue. CACS occurs in up to 80% of advanced cancer patients, resulting in reduced quality of life, diminished treatment response and a consequent increase in mortality. Indeed, cause of death is directly attributable to CACS in up to 20% of patients (Argiles et al., Nat Rev Cancer. 14 (2014) 754-62), yet no standard of care treatment can currently be recommended. Over recent decades a considerable body of evidence has emerged showing cancer induces cachexia via systemic inflammation and metabolic dysregulation, resulting in muscle catabolism and associated symptoms (Tisdale, Physiol Rev. 89 (2009) 381-410). Recently, pre-clinical research has revealed that cytotoxic chemotherapy can itself induce cachexia and anorexia via many of the same inflammatory and catabolic mechanisms as cancer (Braun et al., PLoS One. 9 (2014) e106489). Thus, identification of an effective, well-tolerated pharmacotherapy for chemotherapy-induced cachexia would prevent its dose- and compliance-limiting effects on cancer treatment, and is furthermore likely to possess at least partial efficacy for treatment of cancer-induced cachexia.

We have previously shown that the non-psychoactive phytocannabinoid cannabigerol (CBG), which has anti-inflammatory and anti-cancer properties *in vitro*, stimulated multiple feeding behaviours in rats, without exhibiting any neuromotor side-effects (Brierley et al., Appetite. 83 (2014) 344). The present study investigated whether CBG could attenuate the robust cachectic symptoms induced by a single dose of the chemotherapy agent cisplatin. Following cisplatin challenge, rats orally administered CBG (120 mg/kg, b.i.d.) exhibited significantly attenuated bodyweight loss and consumed more food than disease controls over a 72 hour timecourse. Post-mortem analyses demonstrated that cisplatin treatment induced a significant decrease in skeletal muscle mass, which was partially reversed by CBG treatment. Consistent with this, cisplatin induced selective fibre type atrophy, which was attenuated by CBG treatment. Metabonomic analysis of terminal blood samples by ^{1}H-nuclear magnetic resonance (NMR) spectroscopy indicated that the metabolic phenotype of animals treated with CBG was normalised, resembling that of cisplatin-naive control animals. Further experiments are ongoing to elucidate the mechanism(s) of this protective action, and to investigate efficacy in models of cancer-induced cachexia.

Acknowledgements: Jointly funded by GW Pharmaceuticals and the University of Reading Faculty of Life Sciences

IMPROVING QUALITY CONTROL METHODS FOR CANNABIS USING FLASH CHROMATOGRAPHY

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The American Herbal Pharmacopeia (AHP) recently published a *Cannabis* monograph, setting standards for identification, analysis, and quality control. Additionally, the American Herbal Products Association (AHPA) issued basic product safety guidelines this year for cultivation, manufacturing, dispensing, and laboratory operations for medical *Cannabis*. The recommendations from the AHP and AHPA are steadily being adopted and are implemented in US states through the 3rd party oversight program called Patient Focused Certification. However, a number of significant hurdles must be overcome in this industry to reach higher levels of product safety. Among the issues facing laboratories are access to reference standards, transportation of samples for pesticide or contaminant analysis, having flexibility to efficiently quantify several compounds from a variety of complex matrices in a high throughput manner, dependency on the use of high amounts of toxic solvents, and a shortened life span of expensive analytical equipment used in the routine analysis of viscous and particulate samples. Flash chromatography can help overcome some of the issues facing laboratories engaged in quality control of *Cannabis* medicines by offering a more efficient way to isolate cannabinoids and other compounds of interest from complex matrices, enhancing separation and identification techniques. Our data was obtained by analyzing plant and other complex matrices (i.e., Gummy bears, chocolate, vegetable oil, brownie with varying amounts of THC, CBD, and CBN) spiked with known amounts of cannabinoids and from analyzing cannabis products (i.e., concentrates, lotions/skin creams, etc). Phyto and synthetic cannabinoids were isolated from complex matrices and quantified using high performance liquid chromatography with ultra-violet, mass spectrometry, and evaporative light scattered detection (HPLC-UV/MS/ELSD). One-pass purification with the flash chromatography system generated reference standards with up to 99% purity.

*These authors contributed equally

THE SEED PROJECT: DEVELOPMENT OF STANDARDS AND CERTIFICATION PROGRAM FOR MEDICAL CANNABIS DISPENSARIES IN CANADA

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Over 100 unregulated medical cannabis dispensaries in Canada provide cannabis for medical purposes to over 50,000 patients. Although some medical cannabis dispensaries operate according to industry best practice guidelines, there remains substantial variability in practices between dispensaries. The Canadian Association of Medical Cannabis Dispensaries (CAMCD) was formed in 2011 with the goals of standardizing policies and practices across dispensaries, and developing a regulatory certification body. To this end, CAMCD partnered with researchers from UBC and Canadians for Safe Access to establish the Medical Cannabis - Standards Engagement, Evaluation, Dissemination (SEED) project, which aimed to develop, implement and evaluate standards and a certification program for medical cannabis dispensaries in Canada.

The SEED project used a Delphi process to identify key areas of dispensary operations and practices through consultations with dispensary representatives and community stakeholders. Additionally, a series of consultation meetings was organized with experts and key stakeholders including representatives from the medical community, municipal and provincial governments, law enforcement and the judicial system. In this presentation we describe the certification process and its outcomes, including an overview of the Standards, a survey of stakeholders regarding their understanding and support of dispensary practices, reports on the consultation meetings, and a summary of the baseline pilot study. Furthering the understanding of the interrelationships among medical cannabis patients, health care providers and medical cannabis dispensaries remains important as dispensaries continue to operate in Canada and are growing in number.

Acknowledgements: SEED project funded by the Peter Wall Solutions Initiative

SEQUENCING OF THREE MALE CANNABIS GENOMES AND DEVELOPMENT OF MULTIPLEX QPCR ASSAYS FOR RAPID MALE SEX DETERMINATION

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Male associated DNA Cannabis (MADC2) markers have previously been described by Mandolino *et al*[1]. These markers were reported to target the MADC2 repeat and generate many bands with gel electrophoresis of which the male cannabis plant delivered a unique band. While these markers are reported to accurately detect male plants, the use of gels and visual inspection of banding patterns could be improved with more scalable quantitative PCR (qPCR) methods. Towards this end, we cloned and sequenced these bands to over 1000x coverage using next generation sequencing. We discovered highly variable MADC2 sequences complicating qPCR assay design.

We further aligned many female whole genome sequencing reads to the published MADC2 references and found significant sequence homology and sequence coverage suggesting over 500 copies of this degenerate repeat in female genomes. Several SNPs exist in the reported Mandolino primers and suggest an allele specific amplification of male versions of MADC2.

As a result, we chose to whole genome sequence two male cannabis plants to find markers more compatible with a qPCR assay. 30X whole genome shotgun of male cultivars WIFI and Grape Stomper were aligned to existing female references. Unmapped male reads were then assembled to generate 2523 high quality contigs (1.5Mb of contigs). These contigs were *in-silico* screened for microbial contamination, extreme coverage and heterozygosity to prioritize 259 hypo-variable contigs >500bp in length. Quantitative PCR assays were designed and initially screened against 15 males and 15 females with no errors. To finalize and confirm our assay design we sequenced a distant male cultivar known as ABCh to 10X coverage and aligned those reads to the 259 hypo-variable male contigs to find adequate coverage but many polymorphisms in the contigs targeted for qPCR. Eleven percent of the male contigs had zero coverage in ABCh underscoring the value in the extensive screening required for assay design in polymorphic plants.

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GENOMIC, TERPENE AND CANNABINOID PROFILES OF A PUTATIVELY NOVEL *CANNABIS* SPECIES

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An Australian feral male cannabis strain (Australian Bastard Cannabis or ABC) was crossed with *Cannabis Sativa L*. (Purple skunk Oregon) and presented with atypical leaf patterns and stunted but hardy growth (offspring termed ABCh). Microscopy identified small trichomes and encouraged further evaluation with UV-HPLC (UV confirmed high performance liquid chromatography) that identified THCA and CBCA peaks. This cultivar was further tested for terpene profiles with GC-FID (gas chromatography-flame ionization detector) identifying many common terpenes in *Cannabis*. Thin layer chromatography (TLC) was used to confirm the cannabinoid results against Cerilliant standards and the sample was selected for genomic analysis.

Quantitative PCR for a Y chromosome *Cannabis* marker and a cannabinoid synthase gene were both positive, suggesting further analysis with whole genome sequencing. 28 million paired 251bp reads (7.5Gb, ~10X coverage) were mapped against an existing female *Cannabis* reference (CanSat3 or Purple Kush) to assess the genomic similarity to a recently sequenced *Cannabis* strain[1]. As a control, reads from several male and female *Cannabis* strains reported to be *C.sativa* or *C.indica* dominant cultivars were sequenced and mapped to the same CanSat3 reference genome to derive read mapping percentages using a single trimming and mapping algorithm. The ABCh strain had 94.6% of the reads mapping to the CanSat3 reference compared to 99.58% and 99.60% of the control reads from two respective male genomes (WIFI and Grape Stomper). The female C.*indica* cultivar 'LA Confidential' and the female hemp strain Finola mapped at 99.1% and 97.7% respectively. These data suggest ABCh is more distant to Purple Kush than Finola Hemp and a variety of other 'drug type' cultivars. Mapping Bonobo to the human genome produced 95.87% reads mapping underscoring a more distant relationship of ABCh mapped to Purple Kush than speciated mammals mapping to each other. Since mapping percentages can be influenced by microbial contamination we also performed SNP calling and principle component analysis (PCA). PCA also revealed distance albeit less extreme and perhaps more related to commonalities in the hybrid *C.Sativa* genetics.

We then analyzed the ABCh read mappings to THCA synthase reported by Sirikantaramas *et al.*[2] and found 7 SNPs in the 1635bp open reading frame. One amino acid changing variant (Ala5Thr) was in the N-terminal signal peptide but no SNPs were found in the FAD binding domain suggesting a fully functional THCA synthase enzyme with a variant of unknown significance in the N-terminal signal peptide.

In conclusion, the combination of cannabinoid and terpene profiling with genome sequencing can be a powerful toolset for cannabis classification. These methods offer synergistic perspectives to the *Cannabis Sativa L.* taxonomic debate and suggest a possible fourth *Cannabis* species or subspecies to *C.indica*, *C.sativa* and *C.ruderalis.* Sequencing of the original unhybrized genetics is required to further this debate.

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ROLE OF PROXIMAL TUBULAR CANNABINOID-1 RECEPTOR IN OBESITY-INDUCED RENAL DYSFUNCTION

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During the last few decades, there has been an epidemic increase worldwide in the prevalence of obesity and its metabolic disorders. Recently, more attention has been given to obesity-associated renal structural and functional changes, which develop early in the course of obesity. Cannabinoid-1 receptors (CB_1Rs) are expressed in various renal cells and play an important role in the onset of nephropathy. Their blockade with globally-acting as well as peripherally-restricted CB_1R antagonists improves renal function, reduces albuminuria and glomerular lesions in murine models of the metabolic syndrome. However, most of these studies failed to answer the question whether the endocannabinoid (eCB) system has also a role in obesity-associated renal pathologies? And if it does, is it mediated peripherally, via a specific cell type within the kidney?

Here, we describe the metabolic and renal phenotypes associated with high fat diet (HFD)-induced obesity in a novel mouse strain that lacks CB_1Rs in the renal proximal tubular cells (RPTCs). These cells play a crucial role in renal function and a major site of injury in a variety of metabolic and inflammatory diseases. When maintained on a HFD for 14 weeks, RPTC-$CB_1R^{-/-}$ develop obesity similar to their littermate controls. The diet-induced increase in body weight in both RPTC-$CB_1R^{-/-}$ mice and controls is associated with similar increased fat mass, fasting blood glucose, serum insulin and cholesterol levels, reduced HDL/LDL cholesterol ratio and increased intolerance to glucose. While HFD feeding results in a similar increase in renal eCB levels in both mouse strains, the deletion of CB_1R in RPTCs significantly attenuate the obesity-induced kidney dysfunction, inflammation, oxidative stress and renal fibrosis.

In conclusion, in a rodent model of obesity, targeted deletion of CB_1R in RPTCs has a key role in the pathogenesis of renal complications associated with obesity, while it does not contribute to the deleterious metabolic effects related to HFD. Thus, our findings could further support the development and clinical testing of pharmacological strategies, such as peripherally-restricted CB_1R antagonists for the treatment of not only obesity *per-se* but also to its related renal complications.

Acknowledgements: Funded by the German-Israeli Foundation (GIF) for Scientific Research and Development (Grant no. I-2345-201.2/2014)

SEXUALLY DIMORPHIC EFFECTS OF ALCOHOL WITHDRAWAL ON ENDOCANNABINOID GENE EXPRESSION AND ANXIETY-LIKE BEHAVIOR

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Alcohol withdrawal is accompanied by negative affective states that pervasively impact daily functioning and exacerbate the propensity for relapse. The endocannabinoid (ECB) system provides important negative feedback over stress and anxiety responses in the brain, which could also be relevant in the context of alcohol withdrawal. Preclinical data has revealed changes in CB1 receptor expression in the amygdala during acute alcohol withdrawal. However, changes in the expression of ECB-related genes throughout the mesocorticolimbic stress/reward circuit during different stages of alcohol withdrawal are currently unknown. Moreover, since alcohol and other drugs of abuse differentially impact males and females, it is surprising that researchers have yet to explore sexually dimorphic changes in ECB-related genes during withdrawal.

Thus, we examined whether acute and protracted alcohol withdrawal differentially affects the expression of ECB-related genes in key regions of the mesocorticolimbic stress/reward circuit in a sexually dimorphic manner. Male and female rats were subjected to chronic intermittent alcohol (CIA) vapor or air exposure (14 hr on/10 hr off, 7 days/week) for six weeks and blood alcohol levels were maintained within the range of 200-225 mg%. Six to 8 hours after the final alcohol exposure session (i.e., acute withdrawal), air-puff-induced ultrasonic vocalizations (USVs) were measured and anxiety-like behavior was assessed in the elevated plus maze (EPM) and open field test (OFT). Twenty-four hours after the final alcohol exposure session, the basolateral amygdala (BLA), infralimbic (IL) and prelimbic (PL) cortices of the medial PFC, ventral tegmental area (VTA), nucleus accumbens (NAc), and lateral habenula (LHb) were harvested for rtqPCR analysis of CNR1, CNR2, FAAH, MAGL, DAGL-α, DAGL-β, and NAPE-PLD mRNA expression.

Both male and female rats exposed to CIA vapor spent significantly less time exploring the open arms of the EPM during acute withdrawal. CIA-exposed males, but not females, made significantly more air-puff-induced 22 kHz USVs and spent significantly less time in the center quadrant of the OFT compared to control rats. Analyses mRNA expression is currently underway, but preliminary data indicate that CNR1 expression was significantly reduced in the BLA of male (but not female) rats exposed to CIA vapor compared to controls. Interestingly, both FAAH and CNR2 mRNA expression were significantly reduced in the LHb of male (but not female) rats exposed to CIA vapor. Analysis of behavioral and genetic changes during protracted (28-day) abstinence is currently being investigated in separate cohorts of male and female rats. Though still preliminary, these data suggest that the anxiogenic profile of male and female rats during acute alcohol withdrawal is subtly different, with sex-dependent changes in ECB-related genes in the BLA and LHb of CIA-exposed rats.

ALTERATION OF THE ENDOCANNABINOID-DEPENDENT SYNAPTIC PLASTICITY IN ADULT BRAIN AFTER ETHANOL EXPOSURE OF MICE DURING ADOLESCENCE

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Alcohol drinking, especially among adolescents and young adults, is a serious public health concern. Research within the past decade has made it clear that ethanol interacts with the endocannabinoid system and that the function of this system may be altered in ethanol dependence. However, how ethanol affects neuronal function and particularly synaptic neurotransmission and plasticity needs further studies.

To investigate the effect of ethanol consumption on excitatory synaptic transmission and plasticity mediated by the cannabinoid CB_1 receptor, male C57BL6 mice were exposed to intermittent ethanol intake (20% (v/v) in tap water) using a 4 days drinking-in-the-dark procedure during the adolescence period (postnatal days 30 ± 2 to 54 ± 2). An electrophysiological approach was applied to *ex vivo* slices of hippocampal dentate gyrus (DG) of ethanol-exposed and control animals.

Excitatory postsynaptic potentials (fEPSPs) were evoked after stimulation of the medial perforant path and recordings were made in the supragranular zone of the dentate molecular layer (ML) in the presence of the $GABA_A$ antagonist picrotoxin. CB_1 receptor activation by CP55,940 (10 μM) inhibited fEPSPs in controls (26.43 ± 2.77% of baseline, ** $P < 0.01$, Mann Whitney test), as was previously described. However, this effect was not observed in alcoholic mice (4.9 ± 7.47% of baseline, ns). Furthermore, synaptic stimulation applied in the dentate ML (10 min, 10 Hz) triggered a long term depression (LTD) of the excitatory synaptic transmission (about 20% of inhibition) that was absent in adult mice after ethanol consumption during adolescence (2.7 ± 3.12% of inhibition, ** $P < 0.0001$, Mann Whitney test). This excitatory plasticity was CB_1 dependent as the CB_1 receptor antagonist AM251 (4 μM) abolished LTD (8 ± 6.6% of inhibition, *** $P < 0.0001$, Mann Whitney test).

The optical density analysis revealed a significant decrease of CB_1 immunoreactivity in the dentate ML of alcoholic (87.47 ± 0.58%) versus control (100 ± 0.77%) mice (*** $P < 0.0001$, Mann Whitney test). Correspondingly, the relative mRNA and protein levels of CB_1 significantly decreased, while a significant increase of MAGL mRNA and protein was detected after ethanol exposure.

These results suggest that repetitive exposure to ethanol during adolescence leads to a deficit of endocannabinoid-dependent LTD in adult DG excitatory synapses, probably due to a down-regulation of CB_1 receptors and a reduction of the endocannabinoid tone by an increase of the 2-AG degrading enzyme MAGL.

Acknowledgements: Funded by Red de Trastornos Adictivos, RETICS, Instituto de Salud Carlos III (RD12/0028/0001; RD12/0028/0004); Ministerio de Economía y Competitividad (BFU2012-33334); Basque Government (IT764-13); University of the Basque Country UPV/EHU (UFI11/41); Instituto de Salud Carlos III (MINECO) (CP12/03109, PI13/02261); Consejería de Salud, Junta de Andalucía (SAS111224); Consejería de Economía, Innovación y Ciencia, Junta de Andalucía, UE/ERDF (PI45403, CTS-8221).

COCAINE SELF-ADMINISTRATION UP-REGULATES CANNABINOID CB_2 GENE EXPRESSION IN MOUSE BRAIN

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We have recently reported that brain cannabinoid CB_2 receptors (CB_2Rs) regulate midbrain dopamine (DA) neuronal activity, nucleus accumbens DA release and intravenous cocaine self-administration in mice and rats (Xi et al., Nature Neuroscience, 2011; Zhang et al., PNAS, 2014). These findings appear to conflict with anatomic evidence that brain CB2 gene level is very low (~50-fold) compared to that in the periphery – e.g., spleen. We hypothesized that acute or chronic use of drugs of abuse may up-regulate brain CB2 receptor expression, and produce significant effects on brain function. To test this hypothesis, we first treated animals with a single injection or repeated injections of cocaine, or chronic cocaine self-administration, respectively, and then measured brain CB2 mRNA levels using quantitative real-time PCR (qRT-PCR) and in situ hybridization (ISH) assays. We found that: 1) chronic intravenous cocaine self-administration (1 mg/kg/infusion × 50 infusions/day × 4 weeks) significantly up-regulated (4-5 fold) CB_2 mRNA expression in the prefrontal cortex (PFC) and striatum of mice compared to that observed in oral sucrose self-administration (control) mice or drug naïve mice. In contrast, a single injection (10, 20, 30 mg/kg, i.p.) or repeated injections of cocaine (10 mg/kg, i.p. per day, for 7 days) (i.e. locomotor sensitization dose regimen) failed to alter brain CB_2 mRNA expression as assessed by qRT-PCR; 2) ISH assays show similar findings - CB_2 mRNA is significantly up-regulated in cortical and striatal neurons as well as VTA DA neurons. To determine the cell types of striatal GABAergic medium-spiny neurons (MSNs) expressing CB2Rs, we used D1- versus D2-eGFP transgenic mice and fluorescence activated cell sorting (FACS) techniques, and found that CB2 mRNA is mainly expressed in D2-MSNs (3~4-fold higher in D2-MSNs than in D1-MSNs) in normal subjects. Repeated cocaine administration (20 mg/kg, i.p. per day for 7 days) significantly up-regulated CB2 mRNA expression in D1-MSNs, but not in D2-MSNs. Taken together, these findings suggest that brain CB_2 receptors are inducible and responsive to chronic cocaine abuse or repeated large doses of cocaine. Thus, these findings not only well explain the anti-addictive effects of CB2R agonists observed in cocaine self-administration mice, but also suggest that brain CB_2Rs may constitute a new target in medication development for the treatment of drug addiction and possible other CNS disorders.

Acknowledgement: Supported by NIDA IRP

GLUCOCORTIOID-ENDOCANNABINOID INTERACTIONS IN THE PRELIMBIC CORTEX MEDIATE STRESS-POTENTIATED REINSTATEMENT OF COCAINE SEEKING

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Stress is a powerful trigger for relapse as it is unavoidable in daily life and can not only induce relapse/reinstatement but can potentiate the response to other triggers for drug use. We have shown that under certain self-administration conditions, stress alone does not reinstate cocaine seeking but rather can potentiate reinstatement when paired with low dose cocaine. This effect is corticosterone-dependent and the effect of electric footshock stress (EFS) can be mimicked by systemic corticosterone suggesting that corticosterone is not only necessary but sufficient. Exactly how corticosterone potentiates reinstatement is not fully understood but may involve an interaction with the endocannabinoid (eCB) system. Stress increases eCB production in the prefrontal cortex, a region critical for reinstatement, and, like stress-potentiated reinstatement, is glucocorticoid-dependent. The present study examined the role of the cannabinoid receptor 1 (CB1R), specifically in the prelimbic cortex (PL), in stress-potentiated reinstatement of cocaine seeking. Male rats self-administered cocaine under short-access conditions (0.5 mg/kg/infusion; 2 hr/day) and then underwent extinction training followed by stress-potentiated reinstatement tests. EFS paired with low dose cocaine (2.5 mg/kg, ip) induced reinstatement whereas either low dose cocaine or EFS alone did not. An intra-PL infusion of corticosterone (0.05 μg/.03 μL) 15 min prior to low dose cocaine mimicked the effects of stress and resulted in potentiated reinstatement indicating that the PL is a likely site of corticosterone action. An intra-PL infusion of the CB1R antagonist AM251 (300 ng/0.3μL) given 15 min prior to reinstatement tests blocked both EFS- and corticosterone-potentiated reinstatement, suggesting that CB1R activation in the PL is necessary for these effects. Notably, preliminary data with intra-PL infusions of the CB1R agonist WIN 55, 212 (50 ng/0.3μL) suggest that CB1R activation is sufficient to potentiate reinstatement. Furthermore, corticosterone (1 μM) decreased the amplitude of IPSCs in layer V pyramidal neurons of the PL and this effect was attenuated by presence of AM251 (2 μM), suggesting that corticosterone effects on PL neurotransmission are CB1R-dependent. These findings support the hypothesis that corticosterone acts in the PL to potentiate reinstatement of cocaine seeking through an interaction with the eCB system likely resulting in disinhibition of PL pyramidal neuron outputs.

Acknowledgements: Funded by NIH grant DA15758 to JRM & DA038663 to JRM, CJH & QSL. Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

EFFECT OF CHRONIC CANNABINOID AGONIST TREATMENT ALONE OR IN COMBINATION WITH CANNABIDIOL ON WITHDRAWAL BEHAVIORS IN MICE

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RATIONALE. While the legalization of medical marijuana has now spread to 32 of the United States, the prevalence of marijuana dependence disorders in chronic users is on the rise. Furthermore, the Cannabis-based medication Sativex, a 1:1 mixture of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), has been fast-tracked by the FDA for entry into late stage clinical trials for indications such as pain and spasticity. A rationale given for this combination is the conception that the phytocannabinoid CBD may mitigate some of the adverse effects of THC exposure. Moreover, it is also widely believed that CBD alone is devoid of THC-associated adverse effects.

METHODS. To test these hypotheses, C57Bl/6 mice were treated with either a moderate (3.0 mg/kg WIN-55,212, 5.0 mg/kg CBD) or high (20 mg/kg THC, 20 mg/kg CBD) dose regimen (2X daily for 5 days) of cannabinoid CB agonist and CBD, alone or in combination. Mice were then treated with vehicle, the CB1 selective antagonist SR141716 (10 mg/kg), or the serotonin 5-HT1A antagonist WAY100635 (3.0 mg/kg) to test the ability of these compounds to precipitate withdrawal following chronic cannabinoid treatment. Withdrawal behaviors measured include head shakes, rearing, paw tremor, and head scratching.

RESULTS. Administration of the CB1 antagonist precipitated withdrawal in WIN-55,212 and THC treated mice, demonstrating that these dosing regimens produced physical dependence in the mice mediated through actions on the CB1 receptor. In contrast, withdrawal symptoms were not observed in CBD treated mice administered the CB1 antagonist, suggesting that chronic CBD exposure does not produce physical dependence through a CB1 receptor mechanism. Furthermore, co-administration of CBD did not mitigate the physical dependence produced by chronic CB1 agonist treatment. Because CBD is a direct agonist at the 5-HT1A receptor, we also tested whether a 5-HT1A antagonist would precipitate withdrawal in these treatment groups. Administration of the 5-HT1A antagonist did not precipitate withdrawal in the CBD or CB agonist groups, or in the combination groups.

CONCLUSION. The addition of CBD to a chronic CB agonist dosing regimen does not mitigate the development of physical withdrawal signs. Furthermore, chronic CBD alone does not appear to produce physical dependence as measured by precipitated withdrawal from CB1 or 5-HT1A antagonist administration. However, other targets of CBD administration need to be explored in the future.

ACKNOWLEDGMENTS: R03 DA03476 (SJW), R01 A1084065 (RFT, DG)

2-ARACHIDONOYLGLYCEROL MEDIATES THE ANTI-NAUSEA EFFECTS OF THE VISCERAL INSULAR CORTEX ENDOCANNABINOID SYSTEM IN RATS

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Manipulations that elevate the endogenous cannabinoids (eCBs), anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), have previously been found to interfere with nausea-induced conditioned gaping (a selective measure of nausea) in rats. Although the precise brain mechanisms underlying nausea have yet to be fully uncovered, the visceral insular cortex (VIC) appears to play a critical role in mediating its sensation. In fact, the synthetic cannabinoid agonist, HU210 (Limebeer et al. 2012), and exogenous 2-AG (Sticht et al. 2015) have both been found to disrupt the establishment of lithium chloride (LiCl)-induced conditioned gaping in rats following intra-VIC administration, suggesting an important anti-nausea role for the VIC eCB system. Therefore, to further investigate the nature of eCB suppression of nausea we assessed whether pharmacological inhibition of eCB catabolic enzymes within the VIC interferes with acute nausea-induced gaping. Moreover, we quantified VIC eCB levels following these manipulations, and assessed their effects on VIC neuronal activity using the functional activation marker, c-Fos.

Rats received bilateral intra-VIC infusions of either: 1) the dual FAAH/MAGL inhibitor, JZL195 (10μg); 2) the selective FAAH inhibitors, URB597 (0.01μg) or PF3845 (10μg); or 3) the selective MAGL inhibitor, MJN110 (2μg) prior to receiving an intraoral saccharin infusion and systemically administered 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 an acute LiCl injection resulted in a selective increase in VIC 2-AG levels relative to saline-treated control animals, whereas AEA content in the VIC remained unchanged. Furthermore, MAGL inhibition selectively increased VIC 2-AG levels following systemic or intra-VIC administration of MJN110 and suppressed conditioned gaping, as did intra-VIC infusion of the dual FAAH/MAGL inhibitor, JZL195. On the other hand, FAAH inhibition following either systemic or intra-VIC administration of URB597 or PF3845 did not elevate VIC AEA levels, and neither compound had an effect on nausea-induced gaping. Lastly, given the selective role for 2-AG in the current study we assessed the effects of MAGL inhibition on VIC neuronal activity during an episode of nausea, and found that systemically administered MJN110 reduced LiCl-induced c-Fos expression in the VIC.

Taken together, these findings suggest that VIC eCB system modulation of nausea may be driven primarily by the ligand, 2-AG. Moreover, manipulations selectively targeting 2-AG may have therapeutic potential in reducing nausea, likely by reducing neuronal activation in this brain region during an episode of nausea.

Funded by NSERC (92057) to LAP, CIHR (137122) to LAP and KAS, NIH (P30DA033934, RO1DA032933 and P01DA009789) to AHL and NIH (RO1DA932933 and DA033760) to BFC

SPONTANEOUS CANNABINOID WITHDRAWAL IN MICE: EVIDENCE FROM THREE BEHAVIORAL ASSAYS

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Spontaneous cannabinoid (CB) withdrawal following exposure to Δ^9-tetrahydrocannabinol (THC) has been described for human and nonhuman primates yet, despite several reports of antagonist-precipitated CB withdrawal, spontaneous CB withdrawal has not been well characterized in mice. To address this issue, different groups of mice were treated for 5-28 days with saline, 10-20 mg/kg/day THC or 0.03-1.0 mg/kg/day AM2389, a high efficacy CB agonist. The effects of the agonist treatments on several different measures were assessed at 4-120 hrs after the last injection. Three separate indices of drug effect - paw tremors, locomotor activity, and operant responding – yielded evidence of spontaneous cannabinoid withdrawal in mice.

In one set of studies, mice were placed in individual clear plastic chambers at 4-48 hr after 5 days of agonist injection and behavior was recorded at 30-60 min after injection of vehicle or rimonabant. Changes in observable behavior (e.g., paw tremors, scratching) were later scored from the recordings by at least two blinded observers. Other mice were implanted with emitters that transmitted information on body temperature, locomotor activity and heart rate. Behavior was recorded before (baseline), during, and after five day agonist treatment, and analyzed according to changes from baseline measures. In the final studies, mice were trained to respond (nosepoke) for 0.02 ml of sweetened milk. These mice then received 0.1 mg/kg AM2389 for 28 days and the effects of AM2389, rimonabant, or vehicle on response rate were recorded.

Results show that following five day exposure to 0.1 mg/kg/day AM2389 increases in paw tremors and locomotor activity occur. 'Spontaneous' effects on paw tremor were seen 24 hrs after AM2389 whereas rimonabant increased paw tremors at 4-24 hr after AM2389. Increases in locomotor activity were also seen at 24-72 hrs after stopping daily injection. Similarly, both rimonabant and interruption of the daily dosing regimen decreased operant responding in AM2389-treated mice; these effects were greatest at 72 hrs after stopping the AM2389 treatment. In all indices, the effects of spontaneous withdrawal were mild, yet reproducible. Furthermore, similar trends in paw tremor and locomotor activity responses were seen following exposure to THC or a lower dose of AM2389. These results indicate that daily treatment with a high efficacy CB agonist will result in dependence that can be measured via spontaneous withdrawal effects, and that cannabinoid dependence may vary with dose or efficacy of the agonist injected daily.

Acknowledgements: Funded by NIH/NIDA: DA035411

GLOBAL FOLD OF HUMAN CANNABINOID TYPE 2 RECEPTOR PROBED BY SOLID-STATE NMR AND MOLECULAR DYNAMICS SIMULATIONS

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Uniformly ^{13}C- and ^{15}N-labeled type II cannabinoid receptor, CB_2, in milligram quantities was produced by bacterial fermentation, purified and functionally reconstituted into unilamellar liposomes in the agonist-bound state. Receptor function was verified by quantitative ligand binding and G protein activation measurements. ^{13}C- and ^{15}N-NMR spectra of the labeled CB_2 were recorded by solid-state, magic-angle-spinning (MAS) NMR. For comparison with experimental results, the structure of CB_2 was obtained by homology modeling to rhodopsin, followed by energy minimization and MD simulations. The atomic coordinates of CB_2 were used for prediction of chemical shifts of resonances before and after CB_2 activation using the programs SHIFTX and SPARTA. Experimental and model-derived C_α, C_β, C=O, and N-H chemical shifts of amino acids were compared. The chemical shifts of the C_α region of the protein are in reasonable agreement between measurement and prediction from the molecular model confirming that secondary structure prediction from the model agrees reasonably well with experimental reality. Activation of CB_2 upon ligand binding is predicted to result in significant changes of chemical shifts of a small number of resonances located primarily in N-terminal domain, extracellular loop 2, the second half of intracellular loop 3, and the first half of C-terminal domain. Amino acid residues in those regions are desired targets for specific amino-acid labeling to obtain deeper insights into mechanisms of receptor activation.

Kimura, T., Vukoti, K., Lynch, D.L., Hurst, D.P., Grossfield, A., Pitman, M.C., Reggio, P.H., Yeliseev, A., Gawrisch, K. Proteins – Structure Function and Bioinformatics 82:452-465, 2014.

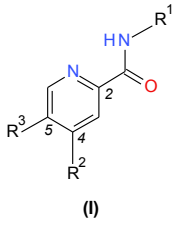
Acknowledgements: T.K., K.V., A.Y., and K.G. were supported by the Intramural Research Program of the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health. This work was supported in part by National Institutes of Health Grants RO1 DA003934 and KO5 DA021358 to P.H.R.

2,4,5-TRISUBSTITUTED PYRIDINES – A NOVEL CLASS OF HIGHLY POTENT, HIGHLY SELECTIVE AND IN VIVO ACTIVE CB2 AGONISTS

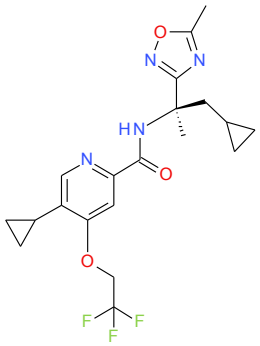
Uwe Grether, Jean-Michel Adam, Christian M. Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Fingerle, Ivan Formentini, Jürgen Funk, Sabine Grüner, Atsushi Kimbara, Matthias Nettekoven, Giorgio Ottaviani, Camille Perret, Mark Rogers-Evans, Benno Rothenhäusler, Stephan Röver, Franz Schuler, Tanja Schulz-Gasch and Christoph Ullmer

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Agonists of the cannabinoid receptor 2 (CB_2) 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] CB_2 agonists have been shown to exhibit protective effects in several animal models after inducing ischemia in organs like liver and brain. Furthermore, CB_2 agonists were also shown to protect organs such as liver from fibrosis. 2,4,5-Trisubstituted pyridines (**I**) were identified to be novel, highly potent and selective CB_2 agonists. The exploration of the different exit vectors led to the discovery of agonists with CB_2 picomolar potency in inhibiting cAMP formation. Surprisingly, a change of stereochemical information on the position 2 residue turns a potent full agonist into a potent inverse agonist. Furthermore, several 2,4,5-pyridines behaving as full agonists on the human receptor are inverse agonists on the mouse receptor. A detailed structure activity relationship for CB_2 and CB_1 binding and functional assays was elaborated.



(I)



RO6871085

hum / mouse CB_2 cAMP EC_{50} 6 nM / 2 nM

EC_{50} ratio hum CB_1 / hum CB_2 1'679

Physicochemical properties including solubility, membrane permeation and lipophilicity as well as metabolic stability and cytochrome P450 inhibition potential were optimized. Advanced compounds combined high *in vitro* potency with favorable early ADME properties and have been profiled in *in vivo* pharmacokinetic and efficacy studies. Selected compounds such as RO6871085 were found to exhibit high bioavailabilities in rodent pharmacokinetic studies and protect mouse kidneys from ischemia reperfusion injury. After a period of 25 min ischemia @ 37 °C and 24 h reperfusion, a statistically significant improvement in kidney function as measured by plasma creatinine levels was produced. Moreover, the efficacy was reflected in the reduction of relevant plasma biomarkers of kidney injury (NGAL, osteopontin). In addition, RO6871085 significantly reduced fibrosis in a rat unilateral ureter obstruction model (UUO) as measured by a reduction in collagen I deposition 8 d after UUO, thereby suggesting that CB_2 agonists might have beneficial effects in both acute and chronic kidney disease.

[1] Pacher P. & Mechoulam R., Progress in Lipid Research 50:193, 2011.

TARGETING PERIPHERAL CANNABINOID RECEPTORS CB2 AS A NOVEL THERAPY TO TREAT OSTEOARTHRITIS

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Osteoarthritis (OA) is a chronic condition widespread in the elderly population. Major OA feature is gradual degradation of articular cartilage that leads to exposure of innervated subchondral bone and in consequence to chronic pain, which impacts significantly quality of patients life. Currently, no disease-modifying drugs are available so pharmacological treatment has been aimed at reducing pain. Commonly used NSAIDs therapy does not always provide adequate pain relief, which might be due to degenerative, rather than inflammatory, nature of these disorder. There is a strong need to discover mechanism underlying development of this disease that may help to propose novel pharmacotherapies of this disorder. Endocannabinoid system is widely described as involved in modulation of pain perception and changes in cannabinoid receptors' expression were observed in the OA-affected joints. Our aim was to determine involvement of endocannabinoid system in the development of OA and to propose target(s) that may not only help to control pain, but also have an impact on cartilage regeneration.

Primary human chondrocytes treated with MIA compound were used to investigate role of endocannabinoid system in proliferation of cartilage. We examined the influence of MIA on cells viability (LDH assay), proliferation (BrdU assay) and migration (wound healing assay). Cannabinoid system components expression were measured on mRNA and protein level and compared with expression in primary human osteoblasts and fibroblasts cultures. Assessment of their antinociceptive properties was performed in rat model of OA induced by intraarticular injection MIA (1 mg). Rats were monitored for OA-related pain symptoms by means of pressure application measurements (PAM) for 28 days after OA induction. Moreover we performed von Frey's test to assess development of neuropathic component in OA. The same behavioral procedures were used for assessment of antinociceptive action of JWH-133. We also measured expression of proinflammatory cytokines (IL6, TNFα) and pain transduction factors (CGRP, NPY) in DRG and spinal cord of MIA-treated animals. For mRNA analysis we used microarrays validated by qPCR.

Cultured chondrocytes exhibit highest sensitivity to MIA compound. Moreover they were susceptible to protective action of JWH-133 after MIA treatment in LDH and BrdU assays. We observed alteration of cannabinoid receptors expression in cultured chondrocytes after MIA treatment. Pain behavior in MIA-treated rats was accompanied by elevated expression levels of IL6, TNFα, CGRP and NPY, suggesting development of neuropathic pain component. mRNA analysis of CB2 expression in animal model of OA showed upregulation of its transcript in DRG 2 days after MIA injection and then again at the late state of the disease, when it was also upregulated at the spinal cord level. Intraperitoneal administration of JWH-133 showed antinociceptive potential in PAM measurements and antiallodynic action in von Frey's test, which suggest it as a novel drug to manage pain present during development of OA.

Our studies demonstrate antinociceptive action of JWH-133, which acts on peripheral CB2 receptors. Moreover activation of CB2 receptors may be of benefit for chondrocytes' proliferation and may delay disease development. Our results propose innovative therapy, which can benefit in management of human OA, coping with more disease symptoms than currently used treatments.

Supported by OPUS UMO-2014/13/B/NZ7/02311, SONATA BIS/NCN/2012/07/E/NZ7/01269, 0044/DIA/2013/42 grants and statutory funds. Natalia Malek is a recipient of the KNOW scholarship.

DO CIRCULATING MONOCYTES IN HUMANS REPRESENT A THERAPEUTIC TARGET FOR CB2-MIMETIC DRUGS OR IS IT THEIR MACROPHAGE COUNTERPARTS?

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Circulating monocytes (i.e. in blood) in humans exist as a range of distinct functional and phenotypic subsets rather than a homogeneous population. Classically monocytes were regarded as the cells in blood expressing CD14 (classical monocytes; $CD14^{hi}/CD16^{neg}$). In humans there are also $CD14^{low}$ and $CD14^{neg}$ populations. These latter two populations express CD16, and collectively account for ~10% of monocytes in the circulation. We have shown previously that each monocyte subset expresses CB2 but at substantially different levels. The $CD16^{hi}$ subset having 7-10 fold higher CB2 than the classical CD14 monocytes. We hypothesised that the low level of CB2 expression by the classical $CD14^{hi}$ monocytes was related to their less differentiated state and that the phenotypically distinct $CD16^{+}$ monocytes, which express more CB2, were likely responsive to CB2 ligands.

Monocytes have an incredibly plastic immunological phenotype and can rapidly begin to differentiate into tissue-like macrophages. Therefore, the monocytes used in our experiments were freshly isolated from human blood; briefly PBMC were prepared then untouched monocytes were isolated using the Miltenyi MACS. The enriched monocytes were then subjected to immunological assays to assess whether the CB2 receptors were functional in these "circulating" monocyte subsets. We assessed the influence of CB2 agonists on phagocytic activity, cytokine/chemokine secretion and the expression of cell surface 'danger signal' receptors. In addition, the influence of CB2 agonists on monocyte derived macrophages was determined, interestingly these cells were considerably more responsive than the circulating monocyte subsets.

It is our contention that although the major ($CD14^{+}$) monocyte population expresses a low level of CB2, that CB2 may not be involved in regulating their function until they become more mature or begin to differentiate into macrophages. Future experiments are focused on identifying the roles of CB2 expressed by the more mature $CD16^{hi}$ monocytes. This is more challenging due to the low frequency of $CD16^{hi}$ monocytes in blood (<5% of all monocytes). Based on our data we suggest that the non-classical $CD16^{hi}$ monocytes represent a more likely target for CB2 drugs in humans even though they are very much the minority. We aim to draw attention to the growing literature on the functional heterogeneity of monocytes in humans and implications of this for the cannabinoid system's role in their regulation.

CB2 AGONISM PROTECTS FROM INFLAMMATION RELATED KIDNEY DAMAGE AND FIBROSIS

Jürgen Fingerle, Christoph Ullmer, Jean-Michel Adam, Christian M. Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Funk, Sabine Grüner, Wolfgang Guba, Paul Hebeisen, Atsushi Kimbara, Matthias Nettekoven, Camille Perret, Mark Rogers-Evans, Stephan Röver, Franz Schuler and Uwe Grether

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Pharmacological proof of in vivo efficacy has been established in multiple animal models in acute organ damage and fibrosis. One drawback of such experiments is the uncertainty of efficacy due to off target effects. Often in vivo efficacy is reached only at much higher concentrations as expected from in vitro binding assays. In order to provide further target confidence we took advantage of the availability of highly selective yet structurally divers CB2 agonists in rodent models of acute kidney injury (mouse temporary renal artery occlusion) and fibrosis (rat unilateral urether occlusion=UUO). Orally bioavailable highly potent CB2 agonists structures which were highly selective against CB1 and other targets were elaborated from high throughput screening hits. Pharmacophore modeling of four representative compounds revealed that all of them fit nicely into the CB2 model used while strongly differing in their molecular properties (structures will be disclosed at the meeting). These compounds were used to study the modulation of 5 different markers of acute kidney injury as well as deposition of collagen after UUO. For all compounds dose dependent efficacy was evident and plasma exposure verified to be above Ki of receptor ligand interaction.

%inhibition	acute kidney injury				Fibrosis (UUO)
Creatinine	Urea	Kim1	Osteopontin	NGAL	collagen I deposition
RO 6753361	25	22	91	50 64	50
RO 6806207	18	21	65	33 53	57
RO 6839828	54	32	52	86 61	51
RO 6871304	53	40	57	83 88	39

Table: Data are shown for identical dosing (10mg/kg/d) and represent a minimum of n=6-8 animals per group. For statistical analysis ANOVA of absolute values vs control was performed. With the exception of Kim1 all data were significantly inhibited p @ 0.001-0.05

The structural diversity provided ranges from a structure which is closely related to cannabinoids (RO6806207) with concomitant high liophilicity (clogP 8.8) to triazolopyrimdine (RO6871304) providing classical drug-like hydrophilicity (logD 2.8). It is thus suspected that potential off target effect should be very different. The fact that all data point into the same direction is strong evidence that efficacy is due to CB2 rather than an unspecific off target effect. We tested all cpds against 75 drug targets of general interest with no obvious sign of interactions. This however does not exclude interactions with other proteins. These data provide strong evidence that potent and selective in vivo efficacious CB2 agonists can be generated which are orally bioavailable and yet of high structural diversity. The common pattern of efficacy in animal models of acute kidney injury and fibrosis suggests, CB2 agonism to be the driving force of efficacy. These data provide great hope that the multiple opportunities to device small molecule CB2 agonists will allow to custom tune a compound to become a highly potent, selective, and safe drug to protect humans from organ damage.

CANNABINOID 2 RECEPTOR INHIBITION IN CNS-INJURY INDUCED IMMUNODEFICIENCY SYNDROME

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Central nervous system (CNS) injury, such as stroke, is known to increase susceptibility to infections that adversely affect clinical outcome. This impairment of the immune response is a common complication after stroke and is termed CNS injury-induced immune deficiency syndrome (CIDS). The activation of the cannabinoid 2 receptor (CB_2R) on immune cells has been suggested to be involved in mediation of the immune suppression that is thought to be responsible for CIDS. In the present study we have investigated whether immune suppression following hypoxic-ischemic (HI) brain injury can be reversed by the CB_2R antagonist AM630. HI brain injury was induced in C57Bl/6 mice via unilateral carotid artery occlusion followed by exposure to a low oxygen atmosphere (8%) for 50 minutes. Systemic administration of TLR-4 agonist lipopolysaccharide (LPS) 24 hrs after stroke onset was used as a model for post-stroke infection. The immune activation was assessed after the LPS challenge using intravital microscopy (IVM). Leukocyte adhesion within the intestinal microvasculature was quantified as a read out for the immune function. Functional capillary density (FCD) within the intestinal microvasculature was also evaluated. Brains were then extracted and stained with tetrazolium chloride (TTC) to confirm stroke and calculate lesion volume.
Consistent with the induction of CIDS, LPS-induced leukocyte adhesion was suppressed in animals with HI brain injury. Administration of the AM630 treatment 15 min prior to LPS challenge, partially restored the levels of leukocyte adhesion, suggesting improved immune function. The AM630 treatment did not have a detrimental impact on both the magnitude of the brain injury, as well as intestinal FCD. Our findings suggest that CB_2R-related modulation of leukocyte activation is involved in the impaired immune response following CNS injury and the inhibition of CB_2R with AM630 reverses CIDS without further exacerbation of the brain injury.

Acknowledgements: Funded by CDHA, Faculty of Medicine Bridge Funding, Canadian Foundation for Innovation.

MECHANISMS OF CANNABINOID CB2 RECEPTOR-MEDIATED MODULATIONS IN MOUSE VTA DOPAMINE NEURONS

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Growing evidence suggests that brain cannabinoid CB_2 receptors (CB_2Rs) are importantly involved in several dopamine (DA)-related behaviors and DA-related CNS disorders. We recently reported that brain CB_2Rs modulate cocaine's action, including intravenous cocaine self-administration, cocaine-enhanced locomotion and extracellular DA in the nucleus accumbens in mice. In addition, CB_2Rs are recently identified in mouse VTA DA neurons, and activation of CB_2Rs in the VTA reduces DA neuron excitability and firing rates in both in vitro and in vivo preparations. However, the cellular and molecular mechanisms underlying the CB_2R-mediated reduction in VTA DA neuronal excitability are unknown.

In the present study, we addressed this issue by using patch-clamp electrophysiological recording technology. We first recorded spontaneous excitatory or inhibitory presynaptic currents (sEPSCs, sIPSCs) in VTA DA neurons in midbrain slides. We found that that bath-applied JWH133 (a selective CB_2R agonist) significantly reduced the frequency of sEPSCs but not sIPSCs, suggesting that the activation of CB_2Rs eliminates presynaptic glutamate release probability, which may in part underlie the CB_2R-mediated reduction of VTA DA neuron firing rate. We then used perforated patch-clamp recording in single acutely-dissociated VTA DA neurons to study the effects of JWH133 on the intrinsic membrane properties of VTA DA neurons. We found that bath-applied JWH133 produced an enhancement of M- and A-type K^+ channel currents, but failed to alter G-protein coupled inwardly-rectifying K^+ (GIRK) current. This effect is mediated by activation of CB_2Rs in VTA DA neurons because co-administration of AM630, a selective CB_2R antagonist,, or genetic deletion of CB2Rs (using CB_2R knockout mice) blocked the JWH133-induced potentiation on M- and A-type K^+ currents. Collectively, the present findings suggest the important synaptic and intrinsic mechanisms, that CB_2R-mediated reduction of presynaptic glutamate release and enhancement of K^+ currents appears to play an important role in modulation of VTA DA neuron excitability, DA neuronal firing, DA release in the nucleus accumbens, and subsequent cocaine-seeking behavior.

Acknowledgements: Funded by Barrow Neuroscience Foundation and Seed fund of Barrow Neurological Institute – Basic Medical Sciences, University of Arizona Medical College, Phoenix.

CANNABINOID CB_2 RECEPTORS ARE SELECTIVELY EXPRESSED BY ACTIVATED MICROGLIAL CELLS IN ALZHEIMER'S DISEASE

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Cannabinoid CB_2 receptors are candidate targets for the development of novel therapies, mainly in the context of inflammation. However, our current knowledge of their pathophysiological roles has been limited by the lack of appropriate experimental tools. We recently reported the design and generation of a new transgenic mouse line that may help to unveil the precise pathophysiological roles of cannabinoid CB_2 receptors. The mouse model was generated by inserting an eGFP reporter gene preceded by an IRES sequence in the 3' UTR of the *Cb2* mouse gene. This approach results in the expression of the reporter gene under the control of the endogenous mouse *Cb2* promoter and transcript from the same bicistronic mRNA as the CB_2 protein. In addition, the whole exon 3, including the 3' UTR and the knocked-in reporter was flanked by *loxP* sites, allowing the conditional inactivation of the *Cb2* gene. The mouse model ($CB_2^{eGFP/f/f}$) was generated by homologous recombination in embryonic stem cells, in the C57BL/6J genetic background. These mice were then crossed with transgenic mice bearing five mutations for familial Alzheimer's Disease (5xFAD mice; Oakley et al, J Neurosci 26; 10129-10140, 2006), resulting in $CB_2^{eGFP/f/f}$/5xFAD mice. These mice produce massive amounts of beta amyloid 1-42 peptide and exhibit neuritic plaques since they are 3 months old, progressively increasing with age. Intense neuroinflammation takes place in cortical, hippocampal and thalamic areas of the $CB_2^{eGFP/f/f}$/5xFAD mouse brain, triggering glial activation. In this context, we analyzed the pattern of expression of CB_2-controlled eGFP expression. We found intense GFP signal in plaque-associated cells with morphological features of microglia in cortex, hippocampus and thalamus, while negligible in other cell types throughout the CNS. This observation was further corroborated by co-localization studies with Iba1, confirming the restricted expression of GFP to activated microglial cells in the vicinity of amyloid plaques. These data confirm and expand previous observations in human AD brains regarding the inducible nature of cannabinoid CB_2 receptors in the context of neuroinflammation.

Acknowledgements: Funded by the Research and Education Component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin, the Boynton Fund of the Greater Milwaukee Foundation, and the Ministry of Education (PR2009-0169) and Ministry of Economy (SAF2010/16706 and SAF2013-42797-R) of Spain.

Symposium in Association with NIDA

Wednesday, July 1, 2015
8:30 – 10:30

ROLE OF CANNABINOIDS IN CHRONIC PAIN SYMPOSIUM: AN UPDATE

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Chronic pain is a major public health problem affecting about 20-30% of the population worldwide. The prevalence of persistent pain is expected to rise in the near future as the incidence of associated diseases such as diabetes, cancer, and arthritis increases in the aging populations. Opioids are powerful analgesics that are commonly used and found to be effective for many types of pain. However, opioids can produce significant side effects, including constipation, nausea, and respiratory depression, which can sometimes lead to death. In addition, long-term opioid use can also result in tolerance and physical dependence. Therefore, alternative pain treatments are required, which are effective but devoid of side effects. In this regard, cannabinoids have been widely explored for the development of pain treatments in humans as well as in various experimental animal models. Δ^9 -tetrahydrocannabinol (Δ^9THC), cannabidiol, and CB2 receptor agonists are the main exogenous cannabinoids that are currently under investigation. In addition, there is a growing interest in allosteric modulators of CB1 receptors, as well as enzymes that regulate endocannabinoid levels. Inhibitors of fatty acid amide hydrolase (FAAH), which raise endogenous N-arachidonoylethanolamine (AEA, anandamide) levels, and monoacylglycerol lipase (MAG lipase) inhibitors, which raise endogenous 2-arachidonoylglycerol (2-AG) levels, reduce nociception in a variety of animal pain models. Although clinical studies have yielded mixed results, it is highly likely that in the near future safe and effective drugs targeting the endogenous cannabinoid system will be developed to treat chronic pain. The goals of this symposium are to evaluate the progress that has been made by exploiting cannabinoid system for the development of treatments for chronic pain and to discuss future directions for further research in this field.

Symposium in Association with NIDA

Wednesday, July 1, 2015
8:30 – 10:30

Cannabinoids for the Treatment of Chronic Non-cancer Pain: An Updated Review of Randomized Controlled Trials

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Introduction
There is a continuing need to review and appraise the evolving literature around cannabinoids and pain.

Methods
We conducted a systematic review of randomized controlled trials examining cannabinoids in the treatment of chronic non-cancer pain according to PRISMA guidelines for systematic reviews reporting on health care outcomes.

Results
Eleven trials published since 2010 met inclusion criteria. The quality of the trials was excellent. Eight of the trials demonstrated a significant analgesic effect. Several trials also demonstrated improvement in secondary outcomes (e.g. sleep, muscle stiffness and spasticity). There were no serious adverse events. Adverse effects most frequently reported such as fatigue and dizziness were mild to moderate in severity and generally well tolerated.

Discussion
This review adds further support that currently available cannabinoids are safe, modestly effective analgesics that provide a reasonable therapeutic option in the management of chronic non-cancer pain.

Symposium in Association with NIDA

Wednesday, July 1, 2015
8:30 – 10:30

CB1 and CB2-mediated strategies for exploiting analgesic efficacy without drug abuse liability

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Direct activation of cannabinoid CB1 receptors produces desirable therapeutic properties but also unwanted (psychoactive) side effects that limit clinical use. We present multiple pharmacological strategies that target the endocannabinoid signaling system to suppress neuropathic pain while bypassing unwanted CB1-mediated side-effects: 1) CB2 agonists, 2) CB1 positive allosteric modulators (PAMs) and 3) brain impermeant inhibitors of the anandamide hydrolyzing enzyme fatty-acid amide hydrolase (FAAH). We compared anti-allodynic effects, possible tolerance, and cannabimimetic effects (i.e., hypothermia, catalepsy, and CB_1-dependent withdrawal signs) following treatment with a cannabinoid CB_2 agonist and classical cannabinoid agonists (i.e. Δ^9-tetrahydrocannabinol (THC), CP55,940). Therapeutic efficacy was evaluated in a mouse model of toxic neuropathy produced by the chemotherapeutic agent paclitaxel. The contribution of CB_1 and CB_2 receptors to *in vivo* actions was evaluated using CB_1 knockout (CB_1KO), CB_2 knockout (CB_2KO), and wildtype (WT) mice. The discovery of an allosteric binding site on the cannabinoid CB1 receptor− a site distinct from the classical (orthosteric) binding site− has fostered drug discovery efforts to develop positive allosteric modulators (PAMs) of CB1 signaling. Such agents are hypothesized to elicit minimal cannabimimetic side effects compared to direct CB1 agonists. CB1 PAMs suppressed neuropathic pain without producing tolerance, reward, CB1-dependent withdrawal, or unwanted CB1-mediated side effects. By contrast, tolerance developed to both therapeutic and side effects of THC, and challenge with a CB1 antagonist produced robust physical withdrawal in mice treated chronically with THC, the major psychoactive ingredient in cannabis. Finally, inhibition of FAAH outside the central nervous system (CNS) was sufficient to suppress both evoked as well as spontaneous pain in model of chemotherapy-induced peripheral neuropathy with no observable tolerance or CB1-dependent withdrawal. Thus, CB2 agonists, CB1 PAMs and brain impermeant inhibitors of FAAH represent promising therapeutic strategies to suppress pathological pain in the absence of abuse liability and unwanted CB1-mediated side effects associated with direct activation of CB1 cannabinoid receptors in the CNS. These strategies offer the potential to produce a more circumscribed and beneficial spectrum of biological effects compared with direct activation of CB1 receptors.

Supported by DA037673, DA009158, and DA035068

Symposium in Association with NIDA

Wednesday, July 1, 2015
8:30 – 10:30

Targeting endocannabinoid regulating enzymes to treat chronic pain

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The endogenous cannabinoid 2-arachidonoylglyercol (2-AG), the most prevalent endocannabinoid expressed in the CNS, is regulated by the biosynthetic enzymes diacylglycerol lipase (DAGL)-α and –β and the degradative enzyme monoacylglycerol lipase (MAGL). This presentation will provide an overview of studies examining the impact of inhibiting each of these enzymes in mouse models of inflammatory and neuropathic pain. Seemingly paradoxical evidence has emerged from these studies in which inhibition of DAGL or inhibition of MAGL reverses nociceptive behavior in pathological models of pain. Whereas the antinociceptive effects of MAGL inhibitors are mediated by cannabinoid receptors, DAGL inhibitors produce antinociception through a cannabinoid receptor independent pathway. It is likely that the antinociceptive effects of DAGL inhibitors are mediated through their known inhibitory actions on the formation of arachidonic acid and concomitant formation of autocoids, such as PGE2, as well as proinflammatory cytokines. Taken together, the results of these studies suggest that inhibitors of biosynthetic enzymes and hydrolytic enzymes of 2-AG represent novel potential strategies to treat neuropathic and inflammatory pain through distinct mechanisms of action.

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Symposium in Association with NIDA

Wednesday, July 1, 2015
8:30 – 10:30

Role of endocannabinoid system in the pathogenesis of osteoarthritic pain

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Osteoarthritis (OA) is a musculoskeletal disease affecting joints. The most acute clinical symptom of OA is a chronic joint pain accompanied by functional impairment. Currently there is no disease modifying therapy for OA and the clinical strategy is focused on symptoms management. Cannabinoids and their receptors emerge as additional pharmacological key elements for the treatment of OA pain. More recently, CB1 and CB2 receptors have been isolated from chondrocytes, osteoblasts, osteoclasts and implicated in a potential disease-modifying role in OA. Additionally, analgesic effects of compounds that interact with cannabinoid system are also known to activate other targets such as the ligand gated transient receptor potential vanilloid type 1 receptor (TRPV1) and novel G protein-coupled receptors: GPR55 and GPR18. Therefore we aim at proposing the most suitable therapeutic windows for cannabinoid-based treatment in OA in order to alleviate pain and to slow down the disease progression.

This presentation will provide an overview of studies examining the impact of OA on both structural (alterations in suchondral bone architecture and density visualized by X-ray computed microtomography) and molecular changes (dysregulation of extracellular matrix metalloproteinases; signalling events in endocannabinoid systems in the spinal cord and knee joint) during disease progression. The identification and characterization of structural, transcription and proteomic data related to compounds targeting CB1 and TRPV1 will allow us to establish the right balance and the optimal conditions for the two systems to act beneficially. Aiming at two receptor systems may be an important therapeutic target for the treatment of pain and inflammation associated with OA and may evolve this research concept into the clinic in the near future. More recent studies highlight the possible role of cannabinoids in regulating and bone remodelling processes during OA. Consequently the putative role of endocannabinoid system on osteoblast migration will be addressed. In summary endocannabinoid system is an attractive target not only to control pain symptoms (modulation of pain transduction and transmission) but also outlines the advantages to regulate bone cells' metabolism.

Acknowledgements: work supported by the National Science Centre, Poland by grants SONATA BIS NCN/2012/07/E/NZ7/01269 and OPUS UMO-2014/13/B/NZ7/02311.

GLYCEROPHOSPHODIESTERASE GDE4 IS A NOVEL LYSOPHOSPHOLIPASE D-TYPE ENZYME GENERATING *N*-ACYLETHANOLAMINES

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Bioactive *N*-acylethanolamines include anandamide (endocannabinoid), palmitoylethanolamide (analgesic and anti-inflammatory substance), and oleoylethanolamide (anorexic substance). In animal tissues, they are formed from *N*-acylethanolamine phospholipids in the one-step reaction by NAPE-PLD or through multi-step routes via *N*-acylethanolamine lysophospholipids. We previously reported that glycerophosphodiesterase (GDE) 1 acts as a lysophospholipase D (lysoPLD) hydrolyzing *N*-acylethanolamine lysophospholipids to release *N*-acylethanolamines including anandamide. In the present study, we examined whether GDE4, another member of the GDE family, also has the lysoPLD activity. As overexpressed in HEK293 cells, mouse GDE4 mostly resides in the membrane fraction. We then purified recombinant FLAG-tagged GDE4 and examined its lysoPLD activity. The purified enzyme hydrolyzed *N*-acylethanolamine lysophospholipids to release *N*-acylethanolamines including anandamide. In addition, common lysophospholipids (lysophosphatidylethanolamine and lysophosphatidylcholine) also served as substrates of GDE4. In living HEK293 cells, the overexpression of GDE4 increased the [^{14}C]palmitoylethanolamide formation from *N*-[^{14}C]palmitoylethanolamine lysophospholipid, and the knockdown of endogenous GDE4 decreased the rate of this reaction. Furthermore, LC-MS/MS analysis revealed that GDE4 overexpression increased most molecular species of lysophosphatidic acid (LPA), another product of GDE4. RT-PCR analyses showed that GDE4 mRNA is widely expressed in various mouse tissues including brain, stomach, ileum, colon, and testis. These results suggested that GDE4 is involved in the generation of *N*-acylethanolamine and LPA as a novel lysoPLD-type enzyme.

DISPOSITION AND METABOLISM OF ANANDAMIDE IN THE MOUSE BRAIN

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Autoradiography of mouse brain after administration of tritiated anandamide shows a heterogeneous distribution pattern which was considered to reflect regional fatty acid amide hydrolase (FAAH) activity. (Glaser et al. J. Pharmacol. Exp. Ther. 2006, 316, 1088-97). This technique depends on the generation of radiolabeled arachidonic acid that is then locally incorporated into membrane phospholipids in the brain with hydrolysis-resynthesis turnover times of several hours, as had been previously shown by Rapoport and co-workers (J. Neurosci. Res. 1989, 24, 413-23) using labeled arachidonic acid with ex vivo autoradiography.

We found that after i.v. administration of [C-14-arachidonoyl]anandamide, the radiolabel showed a heterogeneous pattern of disposition in the brain that was similar to the pattern observed in mice given [C-14]arachidonic acid. Since FAAH is the primary enzyme hydrolyzing anandamide to release arachidonic acid, our result suggests that the pattern is not determined by FAAH and may reflect the metabolism of arachidonic acid. To examine the disposition and metabolism of anandamide without the interference of arachidonic acid, we synthesized anandamide labeled in the ethanolamine moiety instead of the acyl moiety, and used [C-14]ethanolamine in control experiments. A heterogeneous pattern of disposition was observed with [C-14-ethanolamine]anandamide but not with [C-14]ethanolamine. Radiochromatographic analyses showed that directly injected [C-14]ethanolamine or [C-14]ethanolamine released from anandamide was converted largely to phosphatidylethanolamine (PE).

To test whether the heterogeneous pattern produced with [C-14-ethanolamine]anandamide is determined by FAAH, we treated the mice with the FAAH inhibitor URB597 prior to tracer injection. Although anandamide remained intact at 15 minutes in FAAH-inhibited animals, the distribution pattern was still heterogeneous. Similar results were obtained with [C-14-arachidonoyl]anandamide. Our results indicate that i.v. administered anandamide distributes heterogeneously in the mouse brain and that the pattern is not determined mainly by local differences in rate of metabolism of anandamide, as originally anticipated. We are evaluating the extent to which local rates of blood flow and other factors determine the heterogeneous pattern.

Acknowledgements: Funded by Department of Energy (DOE DE-SC0005251).

N-3 FATTY ACID-DERIVED ENDOCANNABINOIDS, A GROWING SUBCLASS OF FATTY ACID AMIDES WITH IMMUNE MODULATING PROPERTIES

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Because of their (alleged) positive effects in health and disease, long chain poly-unsaturated fatty acids (*n*–3 LC-PUFAs) are of much interest both from a nutritional and pharmacological perspective. The fatty acid amide subclass containing conjugates of long-chain fatty acids with amino acids or neurotransmitters consists out of more than 80 members. Despite this number their potential biological roles have only recently started to be explored. Interestingly, the number of DHA (docosahexaenoic acid; 22:6n-3) and EPA (eicosapentaenoic acid; 20:5n-3) derived-endocannabinoid (like) compounds found to display immune-modulating properties is growing. Here, we compared the immune modulatory properties of several n-3 PUFA-derived endocannabinoid (like) compounds. We focussed on those which are most relevant from a physiologically and dietary point of view. Both DHA-5-HT and DHA-ethanol amide (DHEA) (Meijerink et al., Br. J. Pharm **172** (2015) 24–37) are endogenous compounds with levels influenced by diet. Experiments were performed in LPS-stimulated RAW264.7 macrophages or ConA stimulated PBMCs using Griess assay, ELISA, Q-PCR, EIA, LCMS/MS and microarray analysis.

The DHA- and EPA conjugates of ethanolamine, dopamine and serotonin (respectively called DHEA, EPEA, DHA-dopamine, EPA-dopamine and DHA-5-HT) were all found to exert potent anti-inflammatory effects on cytokine production and expression in LPS-stimulated mice macrophages. However, compound-elicited cytokine profiles varied and seemed to depend preliminary on the amine group of the compound.

DHEA and DHA-5-HT were found both to potently reduce PGE2 production. For DHEA, inhibition was found to act by competitive or non-competitive inhibition of cyclooxygenase 2 (COX-2), thereby modulating eicosanoid synthesis. Interestingly, DHA-5-HT strongly inhibited the expression of COX-2 mRNA, and exerted higher potency in reducing IL6 and levels of chemokines MCP-1 and CCl20 compared to DHEA. DHA-5-HT also dose-dependently inhibited migration of LPS-stimulated immune cells, with 500 nM evoking a 25 % reduction. Microarray analysis showed IL1b and IL1a and the matrix metallopeptidase 13 (involved in pathogenic matrix breakdown) amongst the top genes which were down-regulated by both compounds. In human PBMCs, both compounds reduced levels of the chemokine CCL20 and MCP-1 in respectively ConA- and LPS-stimulated cells.

An increasing number of DHA- and EPA-derived endocannabinoid (like) compounds have been found to exert immune-modulating properties in mice macrophages as well as in human PBMCs. Although the underlying mechanism of action of these compounds seems to differ, many of them seem to influence COX-2 activity or expression.

PANNEXIN-1 CHANNELS PERMIT POST-SYNAPTIC ANANDAMIDE TRANSPORT IN HIPPOCAMPAL PYRAMIDAL NEURONS

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The mechanisms of anandamide (AEA) transport from the synapse to the post-synaptic cell, where it is degraded by the enzyme fatty acid amide hydrolase (FAAH), have remained elusive for decades. Several lines of biochemical evidence indicate that there is a specific transport mechanism beyond passive diffusion through the membrane bilayer. Pannexin-1 is a large pore ion channel that is expressed post-synaptically in CA1 hippocampal neurons and conducts large molecules. This channel has been shown to permit transport of similar molecules, such as arachidonic acid, and so we investigated the possibility that AEA be transported through this channel. Bath application of AEA to hippocampal slices resulted in a TRPV1, but not CB1, receptor dependent increase in the frequency of asynchronous glutamate release following stimulation of Schaffer collateral inputs to the CA1. This effect was similarly seen following bath application of both PF4485 and AM404. Post-synaptic loading of the cell with AEA resulted in a similar TRPV1-dependent increase in excitatory transmitter following stimulation, an effect which has been demonstrated to occur through efflux of the AEA transporter (Ronesi et al., 2004 J Neurosci 24, 1673-9). Administration an intracellular pannexin-1 antibody, which inhibits the opening of pannexin-1 channels, prevented the ability of post-synaptic AEA to increase presynaptic glutamate release suggesting that AEA moves through pannexin channels from post-synaptic neurons to access pre-synaptic targets. Consistent with this, biochemical work indicated that blockade of pannexin-1 channels resulted in a significant increase in tissue levels of AEA in CA1 slices, with or without afferent stimulation. Finally, cell-attached patch recordings showed that both pannexin-1 currents and dye flux through open channels was reduced in the presence of AEA in the pipette, indicating that AEA competes with other molecules at transporting through the pannexin-1 channel. These data indicate that pannexin-1 is a significant mediator of post-synaptic AEA transport in hippocampal CA1 pyramidal neurons and may help to resolve the controversy surrounding mechanisms of AEA transport.

Acknowledgements: Operating grant support to RJT and MNH from Canadian Institutes of Health Research.

MONOACYLGLYCEROL LIPASE INHIBITION REVERSES ESTABLISHED PAIN IN AN ANIMAL MODEL OF OSTEOARTHRITIS

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Knee osteoarthritis (OA) is associated with chronic pain, current analgesics either do not offer adequate pain relief for OA or they are associated with serious side effects. Targeting the endocannabinoid system may offer an exciting avenue for the treatment of OA pain. Here we evaluated the effects of a potent and selective MAG lipase inhibitor MJN110 on pain behaviour in a rat model of OA. Intra-articular injection of monosodium iodoacetate (MIA) is widely used to model both OA pain behaviour and joint pathology. The effects of MJN110 on weight bearing asymmetry and hindpaw (distal site) withdrawal thresholds (PWTs) were determined. Acute MJN110 (5mg/kg i.p.) significantly reversed MIA induced weight bearing asymmetry (68 ± 6 grams in MIA + vehicle group vs. 35 ± 4 grams in MIA + MJN110 group, $p<0.001$) and ipsilateral PWTs (7 ± 0.8 grams in MIA + vehicle group vs. 11 ± 0.6 grams in MIA + MJN110 group $p<0.05$). However, repeated treatment of MJN110 (5mg/kg daily) resulted in analgesic tolerance by one week post repeated administration. However, 1 mg/kg of MJN110 produced both an initial inhibition of pain behaviour and a maintained significant inhibitory effect on both weight bearing asymmetry and hindpaw withdrawal thresholds for up to one week of repeated administration. These behavioural effects of MJN110 were associated with a significant inhibition of MPGES1 expression ($p<0.05$) in the ipsilateral dorsal horn of the spinal cord of MIA MJN110 rats, compared to MIA vehicle treated rats. Taken together, these findings support the further investigation of MAG lipase inhibitors for the treatment of OA pain.

DUAL INHIBITION OF FATTY ACID AMIDE HYDROLASE AND MONOACYLGLYCEROL LIPASE IN MURINE MODELS OF INFLAMMATORY AND NEUROPATHIC PAIN

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Chronic pain is a serious and common clinical complaint that carries a large economic burden, and has an unmet need for better therapeutics. Inhibition of either fatty acid amide hydrolase (FAAH) the chief enzyme responsible for degradation of the endogenous cannabinoid anandamide (AEA), or monoacylglycerol lipase (MAGL) the chief enzyme responsible for degradation of the endogenous cannabinoid 2-arachidonylglycerol (2-AG), produces antinociceptive effects in numerous animal models of pain. In the present study, we tested whether the dual FAAH/MAGL inhibitor SA-57 produces antinociceptive effects in inflammatory (intraplantar injection of carrageenan) and neuropathic ((constriction injury (CCI) of the sciatic nerve) mouse models of pain. In addition, because serious clinical liabilities are associated with the prescription of opiates for pain control, we examined whether SA-57 has opioid sparing effects in the CCI model.

SA-57 reversed carrageenan-induced allodynia and edema, as well as CCI-induced mechanical allodynia in dose-related fashions. The ED50 (95% confidence interval) dose of SA-57 in the CCI model was determined to be 4.03 mg/kg (3.34-4.86 mg/kg). At the lower dose of 1.25 mg/kg SA-57 only produces significant elevation of brain AEA (~11% increase of AEA vs. ~2% increase of 2-AG), however; at the dose of 2.5 mg/kg SA-57 significantly elevates both AEA and 2-AG (~11% and ~5%, respectively). SA-57 at the dose of 5 mg/kg produced further increases in both AEA and 2-AG (~14% and ~10%, respectively), but also produced partial tetrad effects and partial substitution for THC. The combination of equally effective dose combinations of SA-57 and morphine revealed enhanced anti-allodynic effects in the CCI model of neuropathic pain, which was shown to be additive via isobolographic analysis. These findings taken together suggest that dual FAAH/MAGL inhibitors may be an attractive avenue for pain therapeutics, with the promise of opiate sparing effects.

Acknowledgements: Research was supported by NIH grants: DA009789, DA017259.

LOCAL FATTY ACID AMIDE HYDROLASE INHIBITION BLOCKS NEUROGENIC INFLAMMATION IN MOUSE KNEE JOINTS

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Introduction- Arthritis is a debilitating condition, and a leading cause of disability worldwide. Although the mechanisms that underlie joint inflammation are not fully known, neurogenic factors are major contributors to the disease process. The release of immunomodulatory molecules, from the distal terminals of nociceptive nerve fibers, has been shown to induce acute joint inflammation (Levine et al., 1985, *J Immunology*). *In* vitro, anandamide has been shown to inhibit the release of pro-inflammatory molecules from these nociceptors (Ahluwalia et al., 2003, *Eur. J. Neurosci.*) The aim of this study was to investigate the influence of joint nerves on leukocyte-endothelial interactions in the articular microcirculation, and determine if fatty acid amide hydrolase (FAAH) inhibition could decrease this inflammatory response.

Methods- Deeply anaesthetised male C57Bl6 mice (24-32g) were injected with rhodamine 6G (0.05%; i.v.) to stain circulating white blood cells. The knee joint microcirculation was exposed, and intravenule leukocyte-endothelial cell interactions were measured using intravital microscopy. Leukocyte rolling (cells moving slower than the normal flow of blood) and adherence (cells that remained immobile in the venule for >30s) were measured before and after saphenous nerve electrostimulation (0.5 - 5.0Hz; 10V; 1ms pulse width; 2 or 5min stimulation period). Separate cohorts of animals were treated with selective neuropeptide antagonists (100µL bolus; topical over exposed knee joint), RP67580 (substance P antagonist; 20nmol), $CGRP_{8-37}$ (calcitonin gene related peptide antagonist; 3.2nmol) and VIP_{6-28} (vasoactive intestinal peptide antagonist; 1nmol), to assess the role of these peptides in this inflammatory response. The effects of FAAH inhibition were tested by pre-treating the knee with URB597 (26.6nmol), with or without AM251 (10.8nmol) or AM630 (11.9nmol).

Results- Saphenous nerve electrostimulation caused a frequency-dependent increase in leukocyte rolling ($p<0.05$; n=6-9), but not adherence ($p>0.05$; n=6-9). This effect was abolished by VIP_{6-28} ($p<0.05$; n=6); however, neither substance P ($p>0.05$; n=8), nor CGRP ($p>0.05$; n=6), antagonism altered leukocyte trafficking. The FAAH inhibitor URB597 successfully blocked this neurogenic inflammatory response ($p<0.05$; n=6); however, this effect was unaffected by either CB_1 or CB_2 antagonism ($p>0.05$; n=5).

Conclusions- These data provide the first evidence that electrical stimulation of the knee joint nerve supply leads to increased leukocyte rolling, and that this response is primarily mediated by VIP. Furthermore, FAAH inhibition reduced joint neurogenic inflammation which was independent of CB_1 or CB_2 receptors. These results suggest that joint afferents can influence local leukocyte-endothelial interactions, and that promoting articular endocannabinoid levels can effectively block this response.

Acknowledgements- This project was funded by the Nova Scotia Health Research Foundation. EK is the recipient of a Canadian Institute of Health Research Frederick Banting and Charles Best Scholarship, Nova Scotia Health Research Foundation Scotia Scholar Award, Dalhousie Faculty of Medicine Dean's Award and Dalhousie Medical Research Foundation Molly Appeal Traineeship.

INHIBITION OF FATTY ACID BINDING PROTEINS PRODUCES ANTINOCICEPTIVE EFFECTS THROUGH PERIPHERAL AND CENTRAL MECHANISMS

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The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are ligands for cannabinoid receptors while the structurally-related palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) activate the peroxisome proliferator-activated receptor alpha (PPARα). Activation of cannabinoid and PPARα receptors produces antinociceptive effects in rodent inflammatory pain models. Recently, our group identified fatty acid binding proteins (FABPs) as intracellular carriers for endocannabinoids and systemic administration of FABP inhibitors produced antinociceptive effects in diverse models of pain.

Here, we employed a mouse model of chronic inflammatory pain to examine whether the antinociceptive effects of FABP inhibitors are mediated peripherally or centrally. Mice received intraplantar injections of complete Freund's adjuvant (CFA) to induce thermal and mechanical hyperalgesia. Inhibition of peripheral and central FABPs was accomplished by intraplantar or intracerebroventricular administration of the FABP inhibitor SBFI26 (3-naphthalen-1-yloxycarbonyl-2,4-diphenylcyclobutane-1-carboxylate), respectively. Intraplantar administration of SBFI26 reduced CFA-associated mechanical and thermal hyperalgesia. The antihyperalgesic effects of SBFI26 were blocked by systemic administration of the cannabinoid receptor 1 antagonist rimonabant or the PPARα antagonist GW6471. Intraplantar administration of SBFI26-ME (wherein the carboxylate of SBFI26 is functionalized with a methyl ester), an analog of SBFI26 that does not inhibit FABPs (Ki >5 μM) had no effect upon mechanical hyperalgesia, indicating that inhibition of FABPs is necessary for the analgesic effects of SBFI26. Consistent with a peripheral mechanism of action, the major FABP subtype that mediates endocannabinoid transport, FABP5, was expressed in dorsal root ganglia of calcitonin gene-related peptide expressing peripheral sensory neurons. In contrast to peripherally administered inhibitor, intracerebroventricular injection of SBFI26 reduced thermal hyperalgesia but did not affect mechanical thresholds. Collectively, these results demonstrate that peripheral inhibition of FABPs produces profound analgesic effects consistent with FABP expression in nociceptive neurons and suggest that peripherally active FABP inhibitors may serve as novel analgesics.

Acknowledgements: This work was funded by NIDA grants DA035923 and DA035949.

KERATINOCYTES OF ATOPIC DERMATITIS PATIENTS EXHIBIT MARKED ALTERATIONS IN GENE EXPRESSION PATTERN DURING DIFFERENTIATION – FAAH: FRIEND OR FOE?

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Atopic dermatitis (AD) is one of the most common human skin diseases, showing ever increasing prevalence in the industrial countries. AD is characterized by the disturbance of the complex cutaneous barrier functions (i.e. physicochemical, immunological and microbiological barriers). Although both genetic (mutations in the filaggrin gene) and environmental factors ("hygiene hypothesis") were proven to play a role in its development, delicate details of the pathogenesis still remains unclear. It has already been shown that the endocannabinoid system (ECS) is able to modulate multiple elements of the cutaneous barrier functions (e.g. via the inhibition of keratinocyte proliferation and differentiation, etc.). Hence, in our current study we aimed at investigating expressional alterations of members of the ECS in AD patient-derived lesional (AD-HEK) and non-lesional keratinocytes (AD-NHEK) in comparison with cells obtained from healthy individuals (NHEK). Moreover, we also intended to identify differences in the expression patterns (EP) of selected "barrier-" or "AD-relevant" genes during the differentiation.

Epidermal keratinocytes were isolated via enzymatic digestion from skin shave biopsies. Alterations in the gene EPs during differentiation were investigated by comparing proliferating (harvested at ~70% confluence) and differentiated (harvested 2 days after confluence) cultures by RT-qPCR and Western blot. We found that EP of several genes (keratin [K]-1, K15, loricrin, filaggrin and aquaporin-3) were altered in AD-patients as compared to healthy individuals. Moreover, we showed that EPs of Toll-like receptor-2 and 3, as well as of occludin (OCLN; a key component of the barrier-forming tight junctions) and fatty acide amide hydrolase (FAAH) were different between AD-HEKs and AD-NHEKs. Further, Western blotting indicated that (despite expressing its mRNA) AD-HEKs were unable to express FAAH at the protein level, and, upon differentiation, showed less OCLN expression compared to AD-NHEKs or NHEKs. Last, but not least, we found that mimicking pharmacologically the lack of FAAH expression by using URB597, substantially decreased the expression of TLR2 and -4 in immortalized human keratinocytes, whereas TLR-activation was found to be a potent positive regulator of the FAAH expression at the protein level.

Collectively, our results argue for that in AD the inappropriate TLR-signaling and the subsequent loss of FAAH expression at the protein level can be part of a vicious circle, which, via the increased endocannabinoid tone-mediated inhibition of the physiological differentiation process (and, probably, the appropriate OCLN expression), might contribute to the development of the barrier-disruption in AD.

Acknowledgements: "Lendület" LP2011-003/2014, TÁMOP 4.2.1./B-09/1/KONV-2010-0007, TÁMOP-4.2.2./A-11/1/KONV-2012-0025, OTKA 105369, TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program – Jedlik Ányos Predoctoral Scholarship"

INCRETIN SECRETION IS INFLUENCED BY CANNABINOID RECEPTORS

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Incretins, glucose-dependent insulin secretory peptide (GIP) and glucagon-like peptide-1 (GLP-1), are hormones secreted into the circulation from K and L enteroendocrine cells, respectively, of the gut in response to food. Their ability to enhance insulin secretion has been well described in the literature over many years. The amount of insulin secreted in response to oral glucose is approximately double the amount secreted in response to a similar concentration of glucose given intravenously (IV). The difference in insulin secretion for oral versus IV glucose is due to the incretins released into the circulation in response to the oral glucose. Circulating incretin levels are increased, both fasting and after oral glucose, with increasing obesity in humans. The underlying physiological mechanism for the increased incretin levels in obesity has not been investigated before now. Based on current literature, endocannabinoid (EC) levels are increased in the circulation, gut, and liver, in the obese state. Additionally, cannabinoid 1 receptors (CB1Rs) are abundant in neurons of the parasympathetic branches of the autonomic nervous system and in the vagus nerve in mice, where they serve as key components of gastric and intestinal motility. There is also parasympathetic control over many enteroendocrine-derived hormones in mice and humans. We hypothesized that increased incretin levels in obesity may be due to parasympathetic modulation by the vagus nerve as a result of increased activity of the CB1Rs because of increased EC tone. We used oral nabilone (2mg), an FDA-approved cannabinoid receptor agonist commonly used to treat nausea during chemotherapy, as a tool to uncover CB1R effects on incretin secretion.

Twenty-three healthy men, age 38.1 ± 8.9 years, BMI 27.1 ± 2.3 kg/m^2, with normal glucose tolerance, were recruited for this randomized, double-blind, placebo-controlled, cross-over study. After an overnight 10-hour fast, they were given either nabilone 2mg or placebo. An hour later, 75 grams of oral glucose was administered. Plasma samples were collected (t = 0, 10, 20, 30, 40, 60, 90, 120, 150 and 180 minutes) and assayed for glucose, insulin and GIP levels. We found that GIP levels were significantly increased (P = 0.003) in the fasting state (one hour after receiving nabilone and before oral glucose) with nabilone compared to placebo, and remained elevated after oral glucose for the 180 minute duration. We also found that area under the curve (AUC) for insulin after oral glucose was significantly decreased (P = 0.039) with nabilone compared to placebo. The decreased insulin levels are most likely due to suppression of insulin secretion by nabilone. GLP-1 assays are currently ongoing.

We conclude that CB1Rs exert tonic control over GIP secretion, and elevated GIP levels in obesity are a consequence of increased ECs. This raises the possibility that many gut hormones, including those involved in satiety control, are under tonic control by ECs.

Acknowledgements: This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute on Aging.

DOES DELTA-9 TETRAHYDROCANNABINOL DAMPEN RESPONSES TO SOCIAL STRESS?

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Marijuana smokers often report that they use the drug to relax or to relieve stress, and there is growing preclinical evidence that endogenous cannabinoids play a stress-dampening role in the brain. However, controlled studies have not been conducted in humans to assess the effects of either marijuana or specific cannabinoid agonists on responses to social stress. In this study, healthy young adults received capsules containing placebo or delta-9-tetrahydrocannabinol (THC; 7.5 or 12.5 mg) before engaging in a public speaking social stress procedure. Healthy volunteers (N=42) were randomly assigned to one of three dose conditions: 7.5 mg THC (N=15), 12.5mg THC (N=13) or placebo (N=14). Each subject participated in two 4-hour sessions, one with a psychosocial stress test (the Trier Social Stress Test, TSST) and one with a non-stressful control task, receiving the same drug condition on both sessions. Capsules were administered under randomized, double blind conditions, 2.5h before the tasks, and dependent measures included salivary cortisol, subjective mood, heart rate and blood pressure. The effects of the drug were dose dependent but nonlinear. The lower dose of THC (7.5mg) reduced subjects' appraisals of how threatening and challenging they found the speaking task, and attenuated their ratings of subjective stress after the task. However, at the higher dose (12.5mg) THC increased subjective stress and blood pressure regardless of stress condition, and on the stress condition it impaired subjects' performance and reduced peak blood pressure. Cortisol and heart rate responses to stress were unaffected by the drug. Thus, a low dose of THC had some stress-dampening effects in this study, whereas at a higher dose the drug itself produced some anxiogenic effects and did not dampen response to the stressful speaking task. These findings provide some support for anxiolysis with a cannabinoid drug, yet highlight the dose-related complexity of the drug's effects.

Funded by the National Institute on Drug Abuse (DA02812).

CANNABIS FOR ARTHRITIS PAIN: PATIENT CHARACTERISTICS, REASONS FOR USE, AND PERCEIVED COMPARATIVE EFFICACY

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Arthritis is one of the most frequently reported conditions among applicants for authorization to possess cannabis for therapeutic purposes (CTP) in Canada, and surveys of Canadian CTP users report high levels of use to address arthritis among both authorized and unauthorized CTP users. However, extant studies that have focused exclusively on active CTP users may be subject to selection bias, and are not suited to address reasons why individuals with arthritis may choose to abstain from CTP use. The present study queried individuals with arthritis who were CTP users and non-users to provide a more complete picture of CTP users, perceived effectiveness of CTP for the treatment of arthritis pain, and barriers to CTP use.

We report results of an online survey of 264 respondents recruited through the contact lists of national arthritis patient groups. The sample was predominantly female (78.79%), and the 59 (22.35%) participants who reported using cannabis to treat pain were younger than non-users (Mean 49 y/o vs. 56 y/o, $F(1, 256) = 11.45$, $p<.01$), with no gender differences between users and non-users. Users of CTP and non-users were generally equivalent with regard to levels of pain and pain-related cognitions (i.e. pain catastrophizing and pain acceptance). Users of CTP reported using a greater number of non-cannabis medicines to treat pain ($F(1, 262) = 15.41$, $p<.01$); 51% reported also using opiates to treat pain, and 93% reported also using NSAIDs for pain. Within-subject analyses comparing the perceived effectiveness of these medications to cannabis indicated that CTP did not differ from NSAIDS and opiates with regard to *directly reducing pain intensity*. However, cannabis was rated as being more effective than NSAIDs with regard to making *pain more bearable* ($F(1, 50) = 13.35$, $p<.01$), *less irritating* ($F(1, 52) = 54.00$), $p<.01$), and facilitating a *normal life despite the pain* ($F(1, 52) = 11.28$, $p<.01$). Cannabis was also reported to be more effective at making participants feel *less depressed about pain*, relative to both NSAIDs ($F(1, 50) = 58.67$, $p<.01$), and opiates ($F(1, 28) = 7.69$, $p<.01$). The three most prominent reasons identified by non-users for not using cannabis, were concerns about lung damage (67.90%), being discriminated against (47.50%), and drowsiness (44.40%). These findings elucidate factors that may inhibit CTP use and suggest directions for further research into the analgesic mechanisms of cannabis medicines.

Acknowledgements: Funded by a grant from the UBC Institute for Healthy Living and Chronic Disease Prevention

SEX-DEPENDENT EFFECTS OF CANNABIS' ANALGESIC AND SUBJECTIVE EFFECTS IN CANNABIS SMOKERS

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Background: Preclinical laboratory studies demonstrate that male and female rodents differ in their behavioral responses to cannabinoids; it is not yet clear how these findings translate to humans. The current study sought to determine sex-dependent differences in cannabis' analgesic and subjective effects in male and female cannabis smokers matched for current levels of cannabis use. Under double-blind, placebo-controlled conditions, the subjective and analgesic effects of smoked cannabis were assessed using the Cold-Pressor Test (CPT), an experimental laboratory model of pain.

Methods: Male and female non-treatment seeking cannabis users participated in outpatient studies investigating the analgesic effects of active cannabis (3.27 or 5.60% THC) relative to placebo cannabis (0.0% THC). The subjective ratings of drug quality and drug effect were also measured. Data from equal numbers of male and female participants matched for frequency of cannabis use (days/week) and amount of cannabis smoked per day (joints/day) were pooled for this analysis. For the CPT, participants immersed their hand in cold water (4°C) for up to three minutes; the amount of time to report pain (pain sensitivity) and withdraw the hand from the water (pain tolerance) was recorded. Analgesia and subjective drug effects data were collected before (baseline) cannabis smoking and at several time-points after smoking. The time-course for cannabis' analgesic and subjective effects were analyzed according to cannabis condition (active and placebo) and sex.

Results: Male (N = 17) and female (N = 17) participants did not differ in cannabis smoking frequency (males = 6.8 ± 0.4, females = 6.4 ± 1.3 days/week), number of joints smoked per day (males = 7.3 ± 5.7, females, = 9.4 ± 8.5), or age (males = 28 ± 7 years, females = 29 ± 6 years), although males weighed significantly more than females (males = 77.5 ± 13.7 kg, females = 67.7 ± 12.2 kg, $p < 0.05$). Cannabis-induced analgesia significantly differed between men and women ($p < 0.01$): Among men, active cannabis significantly increased pain threshold and tolerance relative to placebo cannabis ($p < 0.0001$), whereas active cannabis failed to increase pain threshold or tolerance in women. Active cannabis also increased subjective ratings of cannabis 'High,' 'Liking,' and 'Take Again' relative to placebo cannabis in men and women ($p < 0.001$), an effect that did not differ between sexes.

Discussion: These findings demonstrate that when matched for current cannabis use, men exhibit significantly greater cannabis-induced analgesia relative to women. These sex-dependent differences in analgesia were present despite comparable ratings of cannabis-elicited subjective effects in men and women. Thus, sex-dependent differences in cannabis' analgesic effects are an important consideration for future investigations of the potential therapeutic effects of cannabinoids for pain relief.

Acknowledgements: This research was supported by the US National Institute on Drug Abuse Grant DA09236, DA19239, and DA02775

CANNABIS USE IN CHRONIC PAIN POPULATIONS: FINDINGS FROM THE POINT COHORT STUDY IN AUSTRALIA

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BACKGROUND: Chronic pain is one of the health conditions often cited by proponents of 'medical' cannabis use, however, there is little systematic enquiry into its use in the community for this purpose. This paper reports on baseline findings regarding cannabis use by participants in the POINT Cohort Study.

METHODS: 1514 chronic non-cancer pain patients prescribed opioid analgesics in Australia were recruited via community pharmacies and interviewed using a range of validated measures examining pain and related treatment, physical health conditions, mental health, substance use and social conditions.

FINDINGS: Whilst 649 participants (43%) reported lifetime use of cannabis, only 237 participants (16%) (37% of cannabis users) reported this had been for the management of their chronic pain. In contrast, of those reporting any cannabis use in the past month (n=126, 8.3%), the majority (n=96, 6.3%) reported this had been for pain management (76%), suggesting chronic pain may be a driver of continued cannabis use. Lifetime ICD-10 diagnosis of harmful or dependent cannabis use was identified in 12% of POINT participants, compared to 32% in those who had used cannabis for chronic pain. Of those reporting 'medical' use of cannabis for pain management, most reported it to effectively reduce their pain severity comparable to pain relief achieved with opioid analgesics. Further data regarding interactions between cannabis use, mental health, other substance use and pain treatments will be presented.

DISCUSSION: This is one of the first studies to document patterns of cannabis use in a large heterogenous chronic pain sample, suggesting cannabis use to be more common in this group than age and gender matched general populations.

CANNABIS IN MEDICINE: A NATIONAL NEEDS ASSESSMENT FOR CANADIAN NURSE PRACTITIONERS

Daniel Ziemianski, Lynda Balneaves, Sylvie Toupin, Fairleth McCuaig and Mark A. Ware

Introduction

The Canadian Marihuana for Medical Purposes Regulations (MMPR) allow nurse practitioners (NPs) and physicians to authorize patients' legal access to cannabis for therapeutic purposes. The understanding of the current and desired status of Canadian NPs' educational needs on cannabis in medicine remains to be quantified. This initiative aims to support evidence-based educational programming to address identified knowledge and practice gaps, increasing competence and patient care.

Methods

We conducted a national needs assessment of Canadian NPs to identify knowledge gaps surrounding medical cannabis and to inform educational program development. The following were the proposed objectives of this initiative:

1. Describe NPs' knowledge gaps concerning medical uses of cannabis
2. Describe NPs' experiences with cannabinoids and cannabis in clinical practice
3. Describe NPs' attitudes towards cannabinoids and cannabis in clinical practice
4. Rank barriers to the use of cannabis as a possible treatment option in clinical practice
5. Rank NPs' preferred means of education about cannabis

Results

The survey was completed by 182 subjects from August 2013 to June 2014, of whom 137 (75%) were anglophone, 96 (53%) worked in an urban setting and 155 (85%) reported working in a primary care/family practice setting. Most (157; 86%) had less than 10 years of practice. Many had experience with patients using cannabis; 109 (60%) had been approached by patients regarding medical cannabis use, and 46% had patients already using cannabis. Over half (57%) reported that they would be comfortable authorizing medical cannabis through the MMPR; this number increased to 64% if they were to receive appropriate education.

Discussion

Our data suggest that a significant proportion of nurse practitioners are prepared to consider medical cannabis for their patients. This group should be targeted for educational efforts as they are presently able to authorize cannabis under federal regulations in Canada, and that this may improve access to this approach for patients who do not have family physicians.

ORAL CANNABIS: PHARMACOKINETICS, PHARMACODYNAMICS AND PRODUCT EVALUATION

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Background. The use of cannabis (marijuana) for medical and non-medical purposes is expanding worldwide and now includes legal retail outlets for purchasing cannabis products in many areas. With the advent of retail cannabis sales, a number of unique goods have been developed, including a variety of "edible" cannabis products for oral consumption. An estimated 16-26% of medical cannabis patients consume edible products and edible products are available for sale in both medical and non-medical dispensaries in North America. However, most controlled research evaluating the pharmacokinetic and pharmacodynamic effects of cannabis has used a smoked route of administration. In addition, due to the slower and more unpredictable rate of absorption with oral administration, dose titration by users can be difficult and result in adverse side effects (e.g., panic, paranoia), highlighting the importance of evaluating oral cannabis dose effects and ensuring accurate product labeling (e.g., total dose, unit dose of cannabinoids).

Methods. Two studies were recently conducted to evaluate: (1) the pharmacodynamic and pharmacokinetic effects of oral cannabis exposure and (2) the accuracy of edible cannabis product labeling in medical cannabis dispensaries in the U.S. In Study 1, healthy adults (N=18) with a history of cannabis use, but no use for the prior 3 months, participated in a laboratory study of oral cannabis administration. Participants were randomly assigned to receive one of three doses: 100mg, 250mg, or 500mg cannabis containing approximately 10% THC baked into commercial brownies. Analysis of brownies indicated that the brownies reliably contained 10mg, 25mg, or 50mg THC respectively. Each dose was administered to 6 participants (3 male, 3 female). Blood, oral fluid, and urine specimens were obtained at baseline and for up to 9 days post-exposure to characterize the pharmacokinetic profile of each dose. Measures of subjective, cardiovascular, and cognitive performance effects were obtained at baseline and for 8 hours post-ingestion.

In Study 2, edible cannabis products with labeled THC content (N=77) were purchased from licensed medical cannabis dispensaries in the greater Los Angeles, CA, San Fransisco, CA, and Seattle, WA metropolitan areas. Products were selected to represent the range of labeled THC content within each store. Labels with content of other cannabinoids (e.g., cannabidiol; CBD) were included if THC content was also indicated. Two 1.5g samples of each product were tested for cannabinoid content via high-performance liquid chromatography **(**HPLC), with results averaged and adjusted for total product weight. If the THC concentration of duplicate tests differed by more than 10%, the entire product was analyzed. A sub-sample of randomly selected products (N=21) was tested for microbiological contamination (e.g., molds).

Results. In Study 1, the average C-Max (window of detection) for THC in whole blood specimens was 0.67 ng/mL (67.5 - 180 minutes), 3.5 ng/mL (65 – 470 minutes), and 3.3 ng/mL (45 – 410 minutes) for the 100mg, 250mg, and 500mg oral cannabis doses respectively. Average C-Max (window of detection) for THC in oral fluid specimens was 191.5 ng/mL (10 - 125 minutes), 477.5 ng/mL (13.3 – 155 minutes), and 597.5 ng/mL (10 – 570 minutes) for the 100mg, 250mg, and 500mg oral cannabis doses respectively. Analysis of the urine samples is still pending. Subjective ratings of "drug effect", heart rate, psychomotor ability (DSST number correct) and working memory (PASAT total correct) were qualitatively dose dependent. There was little difference between the 250 and 500 mg doses, but both indicated greater subjective intoxication and worse psychomotor performance compared with the 100mg dose. Ratings of "good drug effect" did not differ by dose, but ratings of "unpleasant drug effect" were higher following the 250mg and 500 mg doses compared with the 100mg dose. Peak drug effecs occurred 1.5 – 3 hours post exposure and effects typically lasted for 6-8 hours. One female experienced an intense period of anxiety following the 250mg dose and 2 females vomited 3 hours after consuming the 500mg dose.

In Study 2, only 17% of the 77 products had "accurate" labeling (content within 10% of the product label value), 22% were under-labeled (content >10% above labeled values), and 61% were over-labeled (content >10% below labeled values). Products with higher than labeled THC levels were most likely purchased in Los Angeles (X^2=14.04, p<.01), while products with lower than labeled THC levels were most likely purchased in Seattle (X^2=14.04, p<.01). Non-THC cannabinoid content was generally low. Forty-four (57%) products had detectable levels of CBD, only 9 of these had >5mg CBD. The average THC:CBD ratio of products with detectable CBD was 36:1, with seven products having ratios of <10:1, and only one product having a 1:1 ratio. Forty-six (60%) products had detectable amounts of cannabigerol (CBG), only 6 of these had >5mg CBG. Sixty-eight (88%) products contained detectable levels of cannabinol (CBN), only 8 of these had >5mg CBN (see Table 2). Insufficient decarboxylation of delta-9-tetrahydrocannabinolic-acid (THCA) to THC or cannabidiolic-acid (CBDA) to CBD during the manufacturing process was evident in 11 (14%) products. All 21 products subjected to the microbiological contamination assay passed the complete safety screen.

Summary. Edible/Oral cannabis products are increasing in popularity and represent a substantial part of the cannabis retail market. Oral administration of cannabis resulted in dose-dependent effects on most outcome measures. Quantitative levels of THC, 11-OH-THC, and THC-COOH in blood and oral fluid were generally low and were not detected for the duration of self-reported intoxication. No single blood specimen exceeded 5ng/mL, the most commonly used cut-off for roadside drug testing, despite self-reports of significant subjective intoxication and impaired performance. This study was limited by a lack of placebo condition, but a second study using the similar design is under way to address that limitation. The product surveilance study (Study 2) indicates that edible cannabis products obtained from three major metropolitan areas fail to meet basic labeling standards for modern medicine. Greater than 50% of products evaluated had significantly less THC and/or CBD than labeled, with some products containing negligible amounts of THC. Other products contained significantly more THC than labeled, which could place patients at risk of experiencing adverse effects. Findings indicate that patients are predominantly misinformed regarding cannabinoid content, and that cannabinoids are not well homogenized within many products. This suggests that obtaining a reliable and reproducible medical benefit from currently-marketed edible cannabis products is difficult. There is an urgent need for regulatory oversight of the oral cannabis market in the U.S., and for improved methods for objectively detecting acute intoxication following oral cannabis consumption.

Acknowledgement. Sources of support for this research include Substance Abuse and Mental Health Services Administration (SAMHSA), U.S. Department of Health and Human Services (HHS), NIH/NIDA training grant T32-DA07209, Johns Hopkins University School of Medicine, and testing services provided by The Werc Shop.

THE EFFECTS OF ARACHIDONIC ACID AND N-ARACHIDONOYL AMINO ACIDS ON β-ARRESTIN ACTIVITY AND ERK1/2 PHOSPHORYLATION IN CANDIDATE CANNABINOID RECEPTORS

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Interest in N-arachidonoyl conjugates of amino acids (NAAA) as endogenous modulators of G protein coupled receptors has been mounting due to their involvement in a variety of physiologic processes. In particular, a role in the endocannabinoid system for the plethora of possible NAAA has emerged. This study was designed to investigate the effects of four NAAA; N-arachidonoyl serine (NAS), N-arachidonoyl tyrosine (NATyr), N-arachidonoyl glycine (NAGly) and N-arachidonoyl glutamic acid (NAGlu) on β-arrestin and MAPK activities in CHO cells stably expressing the candidate cannabinoid receptor GPR55. The impact of arachidonic acid on these two cellular messengers was also explored, as it is the parent compound of all NAAA. Additionally the influence of fatty acid amide hydrolase (FAAH) inhibitors on the NAAA-mediated effects of β-arrestin and MAPK activities was examined.

Activation of GPR55 has previously been determined employing β-arrestin recruitment as the cellular endpoint. In the study presented here, NAAA-induced concentration dependent β-arrestin activities were different amongst the NAAA. At equimolar concentrations, the known GPR55 agonist, LPI, produced greater increases as compared to the NAAA. Maximal effects were also lower as compared to LPI. Interestingly, in the presence of the FAAH inhibitor URB597, any increase in NAAA-induced β-arrestin activity was abrogated. Alone, neither the FAAH inhibitor nor arachidonic acid induced increases in β-arrestin activity. Concentration dependent increases in MAPK activity were observed following a 5 minute incubation of each of the NAAA. At 10μM all of the NAAA significantly increased ERK1/2 phosphorylation. These increases were similar to that observed with equimolar concentrations of LPI. NAAA-induced responses remained elevated following preincubation (30 minutes) with a FAAH inhibitor (URB597, PF-622), unlike that observed with NAAA-induced β-arrestin activity. Additionally, incubation with FAAH inhibitors alone resulted in increases in ERK1/2phosphorylation. Curiously, slightly greater increases in MAPK activity were observed following exposure to 10μM arachidonic acid. Incubation of NAAA or arachidonic acid in wild-type CHO cells was without effect on MAPK activity. Findings from this study indicate that NAAA and arachidonic acid differentially contribute to GPR55-mediated β-arrestin and MAPK activities. Whereas arachidonic acid has a role in GPR55-mediated MAPK activity, it inhibits NAAA-induced β-arrestin activity. NAAA induced increases were observed in both cellular transduction pathways, although to different degrees.

Acknowledgements: Funded by NIH Grants (R01DA023204, P30DA013429)

THE ROLE OF ATYPICAL CANNABINOID RECEPTOR GPR55 IN COLON CARCINOGENESIS

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G protein-coupled receptor 55 (GPR55) has been termed the third cannabinoid receptor for its responsiveness to endo- and exogenous cannabinoids. However, GPR55 displays little homology to cannabinoid receptors 1 and 2 (CB1 and CB2) and signals through different G proteins. Since activation of CB receptors may prevent tumor growth in the intestine (Wang, D. et al. Cancer Res, 2008) we are interested in studying the role of GPR55 in the development of colon cancer and whether this receptor interacts with CB receptors as recently postulated.

To this end, an *in vivo* model of colon carcinogenesis was employed to $GPR55^{-/-}$ mice and their wildtype littermates by application of azoxymethane (AOM) and subsequent exposure to dextran sulfate sodium (DSS) supplied in the drinking water to promote tumor growth. Colon tissue was collected after 12 weeks and tumors were evaluated with a caliper under the microscope. $GPR55^{-/-}$ mice show ~40% smaller tumor areas ($p<0.05$) and ~50% lower tumor numbers ($p<0.001$) in the colon of both male and female mice (t-test, n=17-42) indicating a pro-carcinogenic role of GPR55 in the colon. In an approach to pharmacologically mimic these results C57Bl/6J mice were subjected to the same protocol of AOM and DSS treatment and additionally received 15 injections of either vehicle or 5 mg/kg/day GPR55 antagonist (CID16020046). Similar results were obtained.

In a preliminary attempt to explore what kind of factors may influence the expression of GPR55 and CB1 in colon carcinoma, we subjected colon carcinoma cells to hypoxic conditions (1% O_2). We found that only the colorectal cancer (CRC) cell line SW480 displayed high expression of GPR55 and CB1 mRNA while other cell lines (HCT116, HT29, SW620, DLD-1, CaCo-2) showed low to moderate expression. Hypoxic conditions time-dependently increased CB1 mRNA expression (20-fold) while GPR55 expression decreased (2-fold). Sections of human colon carcinoma revealed strong expression of CB1 and GPR55 in tumor cells co-localizing with hypoxia inducible factor 1 alpha (HIF-1α).

Thus, GPR55 seems to play an opposite role to CB receptors in colon carcinogenesis. Both CB1 and GPR55 are present in colon carcinomas and might be regulated by hypoxic conditions, however, in a differential manner.

THE GPR55-AGONIST L-α-LYSOPHOSPHATIDYLINOSITOL MEDIATES OVARIAN CARCINOMA INDUCED ANGIOGENESIS

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Ovarian carcinoma which is highly vascularized has been reported to secrete L-α-lysophosphatidylinositol (LPI) into the circulation. LPI is a G-protein coupled receptor 55 (GPR55) ligand and known to activate the MAPK-pathway in endothelial cells. We hypothesized that LPI could be involved in ovarian carcinoma angiogenesis. However, up to now there is very little *in vitro* and no *in vivo* evidence supporting a potential role of LPI in (tumor)-angiogenesis. We aimed to elucidate the involvement of LPI/GPR55 in (i) ovarian cancer angiogenesis *in vivo,* (ii) angiogenic capacity of human endothelial cells *in vitro,* and (iii) to explore the underlying molecular mechanisms.

We found that ovarian carcinoma conditioned medium contained LPI and stimulated angiogenesis in the *in vivo* chicken chorioallantoic membrane (CAM). Purified LPI stimulated human endothelial cell proliferation, network-formation and migration i*n vitro* and angiogenesis *in vivo*. The pharmacological GPR55-inhibitor (CID16020046) or genetic GPR55 knock-down (siRNA) blocked the LPI-induced increase in endothelial cell proliferation *in vitro* and ovarian carcinoma- and LPI-induced angiogenesis *in vivo* in the CAM. However, basal angiogenesis was unaffected by CID16020046. These pro-angiogenic effects of LPI where induced by GPR55-dependent phosphorylation of ERK1/2 and p38 kinase. We conclude that inhibiting the pro-angiogenic LPI/GPR55-pathway might be a promising anti-(tumor) angiogenesis therapy against ovarian carcinoma.

Acknowledgements: Funded by the Austrian Science Funds (FWF, P20181-B05 P21857-B18 and P22553-B18).

GPR55 REGULATES STEROID HORMONE LEVELS IN MALE MICE AND OFFERS PROTECTION AGAINST AGE RELATED BONE LOSS

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Peak bone mass is generally associated with bone mineral density in older age, therefore accrual of bone mass during childhood and adolescence is an important determinant of osteoporosis risk later in life. Previously we characterised an osteopetrotic phenotype in 3-month-old male GPR55$^{-/-}$ mice due to a decrease in osteoclast function (Whyte *et al*, 2009). It is not known whether the high bone mass phenotype is maintained with age and equates to increased bone strength. This study therefore investigated the structural and mechanical bone properties of GPR55$^{-/-}$ mice at 3, 5 and 12 months.

Micro-computed tomographic analysis of the proximal tibia and distal femur demonstrated a significant increase in trabecular bone volume in male GPR55$^{-/-}$ mice compared to wildtype mice at 3 months ($P<0.05$ tibia, $P<0.01$ femur), 5 months ($P<0.01$ tibia, $P<0.01$ femur) and 12 months ($P<0.0001$ tibia, $P<0.0001$ femur). The increased trabecular volume was associated with significantly increased trabecular number, decreased trabecular separation and increased trabecular connectivity at all ages. Additionally, a significant decrease in structural model index was noted at 12 months, indicative of a more plate like trabecular structure. Together these parameters infer increased bone strength and this was confirmed by mechanical testing of cortical bone. Three point bending demonstrated significantly increased stiffness and failure load in 3 and 12 month old GPR55$^{-/-}$ mice. Notably, the highly significant age related decrease in cortical stiffness and strength between 5 and 12 months in wildtype mice was not observed in GPR55$^{-/-}$ mice. Work to fracture was significantly increased in GPR55$^{-/-}$ mice at 3 months only.

Further phenotypic analysis of 12 month old male GPR55$^{-/-}$ mice revealed significantly augmented plasma testosterone levels (WT 3.6 ng/mL ± 1.4 and GPR55$^{-/-}$ 10.8 ng/mL ± 2.6 ($P<0.01$)), together with increased levels of Luteinizing Hormone (WT 0.205ng/ml ± 0.02 and GPR55$^{-/-}$ 0.496ng/ml ± 0.06 ($P<0.05$)) and a 2.2 fold increase in epididymal sperm counts ($P<0.05$). Low testosterone plays a role in the pathogenesis of male osteoporosis. These results suggest that GPR55 may negatively regulate testosterone levels in aged male mice, further extending the therapeutic potential of GPR55 antagonists.

Independent of changes in circulating testosterone that may contribute to the high bone mass phenotype *in vivo,* osteoclasts cultured from GPR55$^{-/-}$ mice show reduced polarisation and resorption *in vitro*, thus GPR55 also directly regulates osteoclast function. These studies therefore build upon initial research implicating GPR55 in the regulation of bone mass and further advocate the development of GPR55 antagonists for use in models of age related bone loss *in vivo*.

GPR55 RECEPTORS CONTROL MESOLIMBIC DOPAMINE NEURONAL ACTIVITY AND EMOTIONAL PROCESSING BEHAVIOURS THROUGH A GLUTAMATERGIC MECHANISM IN THE MAMMALIAN VENTRAL HIPPOCAMPUS

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The GPR55 receptor (GPR55R) is a G protein-coupled receptor that has been characterized as a novel cannabinoid receptor subtype. However, its actions within the mammalian brain are almost completely unknown. Interestingly, it has been proposed that unwanted cognitive or emotional side-effects of cannabinoids (e.g. rimonabant, cannabidiol or AM251) may be due to interactions with GPR55R. GPR55R is expressed in the hippocampus, in humans and rodents. The ventral subregion of the hippocampus (vHipp) is part of the limbic circuitry controlling mesolimbic dopamine (DA) activity and associated modulation of emotional processing. Importantly, the vHIPP is profoundly disturbed in neuropsychiatric disorders such as schizophrenia. We have recently shown that cannabinoid CB1 receptor (CB1R) stimulation in the vHIPP can potently modulate DA-dependent emotional memory processing and salience via direct modulation of DA neurons in the ventral tegmental area (VTA; Loureiro et al., *Neuropsychopharmacology*, E-pub Dec. 2014). In the present study, we tested whether pharmacological activation of intra-vHIPP GPR55R is capable of 1) modulating VTA DAergic neuronal activity and 2) able to influence emotional salience processing and social cognition. Using *in vivo* electrophysiological recordings in rats, we found that intra-vHIPP microinfusions of palmitoylethanolamide (PEA), an endogenous lipid that acts as a selective GPR55R agonist, significantly increased VTA DA neuronal firing activity and profoundly disrupted rats' natural sociability and social memory. While co-infusion of a selective GPR55R antagonist (CID16020046) blocked the effects of intra-vHipp GPR55R activation on VTA DA neuronal activity and social cognition disruption, co-infusion of a selective CB1R antagonist failed to modulate the effects of GPR55R activation, demonstrating a GPR55-selective, CB1R-independent effect directly in the vHIPP. Finally, simultaneous blockade of NMDA receptors reversed the effects of intra-vHipp GPR55R activation. Ongoing experiments are exploring the role of vHipp GPR55R in the acquisition of context-dependent fear memory. Our data add new insights into the role of the orphan GPR55R in DA-dependent behaviors and implicate the GPR55R as a potential substrate underlying the neuropsychiatric side-effects of cannabinoids.

Acknowledgements: This research is funded by the Canadian Institutes of Health Research (MOP-246144) and the National Science and Engineering Research Council of Canada (NSERC).

ADMINISTRATION OF THE SELECTIVE GPR55 RECEPTOR ANTAGONIST, CID16020046, INTO THE ANTERIOR CINGULATE CORTEX REDUCES FORMALIN-EVOKED NOCICEPTIVE BEHAVIOR IN RATS

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The G-protein coupled receptor, GPR55, modulates nociceptive processing in animal models of inflammatory and neuropathic pain [1-3]. Given the expression of GPR55 in the anterior cingulate cortex (ACC) [4], a key brain region involved in the cognitive-affective dimensions of pain [5], we hypothesised that selective blockade of GPR55 signalling in the ACC, would reduce formalin-evoked nociceptive behaviour in rats.

The aim of this study was to investigate the behavioural and molecular effects of direct administration of the selective GPR55 receptor antagonist CID16020046 into the ACC on formalin-evoked nociceptive behaviour in rats.

Adult male Sprague-Dawley rats (225-250g, n=11 per group) were bilaterally implanted with stainless steel guide cannulae just above the ACC under isofluorane anaesthesia and allowed 7-8 days to recover. On test days, rats received bilateral microinjections of either CID16020046 (10 μM /0.5μL) or vehicle (100% DMSO) into the ACC, 10 minutes prior to intraplantar injection of formalin (50μL, 2.5%). Nociceptive behaviour was assessed for 60 minutes using Ethovision XT software. Post-mortem brain tissues were harvested for histological verification of injection sites and measurement of extracellular signal regulated kinase (ERK) phosphorylation in the ACC. The ipsi- and contra-lateral dorsal horn of the spinal cord was dissected and analysed for the expression of the immediate early gene *c-fos* using qRT-PCR.

Microinjection of CID 16020046 into the ACC significantly reduced second phase formalin-evoked nociceptive behaviour compared with vehicle-treated controls. CID 16020046 treatment was associated with a marked reduction in phosphorylation of ERK, a downstream target of GPR55 activation, in the ACC. At the level of the spinal cord, intra-ACC administration of CID 16020046 abolished the formalin-induced increases in ipsilateral spinal cord expression of mRNA coding for *c-fos*, relative to the contralateral side.

These data suggest that endogenous activation of GPR55 signalling and increased ERK phosphorylation in the ACC may facilitate formalin-evoked nociceptive behaviour. The attenuation of formalin-evoked spinal *c-fos* expression by CID 16020046 suggests modulatory effects of GPR55 signalling in the ACC on the descending pain pathway.

Acknowledgements: This work was funded by Science Foundation Ireland (10/IN.1/B2976).

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COMBINATIONS OF *N*-ACYL ETHANOLAMINES HAVE HIGHER EFFICACY THAN INDIVIDUAL LIPIDS AT TRPV1 RECEPTORS: MORE LIKE LIFE?

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TRPV1 is a ligand-gated cation channel that is activated by capsaicin in chilli peppers as well as variety of phytocannabinoids. Likewise, is is activated a wide range of endogenous biological lipids. *N*-acyl amides, such as Anandamide and the *N*-acyl dopamines have been known to activate TRPV1 for over a decade; however, the additions of new endogenous lipids with activity at this receptor have continued to be elucidated throughout the last decade suggesting that specificity of TRPV1 activation is questionable at best. We recently identified 8 additional *N*-acyl amides that act as antagonists and 1 that acts as a potent antagonist at TRPV1 and bringing the number of endogenous ligands that activate TRPV1 to over 20. With so many endogenous lipids shown to interact with this receptor, pharmacologists continue to ask the question, "Will the real TRPV1 receptor, please stand up?!" Another view would be that each of these endogenous small-molecule lipids plays a role in the overall response properties of TRPV1. To test this hypothesis, we compared the relative potency of combinations of the *N*-acyl ethanolamines that have activity at the TRPV1 receptor, Anandamide, *N*-oleoyl ethanolamine (OEA), *N*-linoleoyl ethanolamine (LEA), and *N*-docosahexaenoyl ethanolamine (DEA) as well the non-TRPV1 activating *N*-strearoyl ethanolamine (SEA) for activity at TRPV1. Our prediction was that if 1 *N*-acyl ethanolamine drives calcium through TRPV1 receptors with a particular potency, then a combination of two would be additive. Interestingly, combinations of 2 *N*-acyl amides that have individual activity caused a slight decreased in potency, which appears to resemble competitive inhibition. Alternatively, combinations of 3 significantly increased the potency with a dramatic leftward shift in response curves and significant reductions in EC50s. Previous lipidomics analysis has shown that each of these N-acyl ethanolamines is regulated by deletion or pharmacological blockade of NAPE-PLD and FAAH as well as being increased as a result of inflammation. In each case, it could be argued that these decreases (NAPE-PLD) or increased (FAAH or inflammation) has the potential to change the level of activity of TRPV1 receptors. One of the primary roles hypothesized for TRPV1 is as a cellular sensor of temperature. Understanding how endogenous cannabinoids and related lipids are regulated and how that plays a role in thermoregulation will lead us to a better understanding of the many interactions of cannabinoid pharmacology.

CB1 CANNABINOID RECEPTOR AND G PROTEIN S

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The CB_1 receptor (CB_1R) stimulates cellular signalling pathways mainly through Gi/o mechanism. The link of CB_1R to Gs protein was suggested in two models of signal transduction in heterologous expression systems: 1) CB_1R-D_2R interactions (Glass and Felder 1997); and 2) a mutation in the CB_1R third intracellular loop (Abadji et al. 1999). However, these studies did not provide evidence to support a direct CB_1R-Gs interaction *in vitro* or Gs activation by receptors. The aim of the present studies was to explore CB_1R-Gs interactions using cAMP accumulation assay,[^{35}S] GTPγS binding to specific G proteins using scintillation proximity[^{35}S] GTPγS binding assays (SPAs).

To study the CB_1R-D_2R interaction, we employed MN9D cells, a fusion of embryonic ventral mesencephalic and neuroblastoma cells which endogenously express both receptors. In intact MN9D cells, both CB_1R agonist, HU210, and D_2R agonist, Quinpirole, inhibited forskolin-stimulated cAMP accumulation when used individually. In contrast, HU210 and quinpirole in combination augmented cAMP accumulation stimulated by low concentrations of forskolin only, and this augmentation was blocked by D_2R antagonist, Raclopride. In MN9D membranes, HU210 stimulated [^{35}S] GTPγS binding in a concentration-dependent manner that was blocked by CB_1R antagonist, SR141716A. Quinpirole did not affect HU210-mediated [^{35}S] GTPγS binding. In SPA assays, D_2R activation did not significantly alter HU210-stimulated [^{35}S] GTPγS binding to Gs, but was able to reduce HU210-stimulated [^{35}S] GTPγS binding to $Gi_{1/2/3}$.To study CB_1R domains proposed to signal to Gs specifically, we employed CHO cells stably expressing WT CB_1R or CB_1R in which Leu34, Ala342 was replaced with the putative Gs-coupling motif Ala341, Leu342. In cells expressing the Ala341, Leu342 motif, but not WT CB_1R, cannabinoid agonists significantly stimulated cAMP accumulation. However, there was no significant difference between the WT versus Ala341, Leu342 CB_1R in in CP55940-stimulated [^{35}S] GTPγS binding to Gs in SPA studies. On the other hand, there was greater efficacy for CP55940-stimulated [^{35}S] GTPγS binding to Gi1, Gi2 or Gi33 in WT versus Ala341, Leu342 CB_1R. In summary, we found that for two different models and using two different agonists, CB_1R coupling to Gs occurs with lower efficacy than to Gi/o. No evidence suggested regulation of Gs activation by either co-stimulation with D_2 plus CB_1 agonists, or by expression of a Gs-motif in the CB_1R. Our examination of G protein functionality suggests that the stimulation of cAMP accumulation can be explained by reduced activation of Gi/o proteins. For adenylyl cyclase isoforms (AC5, AC6) in which Gi counteracts the stimulation by Gs, the reduced Gi activation allows increased Gs-mediated cAMP production. This increased sensitivity to Gs is not readily observed under experimental conditions in which forskolin is used to augment Gs-activation of adenylyl cyclases.

Acknowledgements: Support from NIGMS IRACDA grant K12-GM102773 and NIDA grants R01-DA03690 and P50-DA006634 is appreciated. Thanks to Dr Debra Kendall for providing CHO cells.
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EVIDENCE OF BIASED AGONISM AMONG DIVERSE CANNABINOID CB1R LIGANDS

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Biased agonist probes of G protein-coupled receptors (GPCRs) facilitate study of the relationships between GPCR activation of individual intracellular signaling pathways and their influence on behavior. Such ligands also offer great pharmacologic potential, as functionally selective agonists may not produce undesired ancillary effects *in vivo*. The cannabinoid receptor CB1R is an important therapeutic target for reducing pain, stress and anxiety; however, functionally non-selective CB1R activation is associated with tolerance and dependence, learning and memory impairment, and paradoxical anxiogenic behavior. Toward developing functionally selective CB1R ligands, we have begun an investigation into the ability of endogenous and exogenous CB1R ligands to stimulate β-arrestin2 recruitment and inhibit forskolin-induced activation of cyclic AMP (cAMP) *in vitro*. Our results indicate anandamide (AEA) and CP-55,940 are unbiased CB1R agonists when the results of these assays are compared. When the Emax for CP55,940 is used as a comparator, the endocannabinoid, 2-arachidonoylglycerol (2-AG) produced a 3-fold greater recruitment of β-arrestin2 over inhibition of cAMP, whereas phytocannabinoid Δ9-tetrahydrocannabinol (Δ9-THC) produced a 4-fold greater inhibition of cAMP over β-arrestin2 recruitment. The CB1R allosteric modulator, GBR-12909 showed a dose-dependent decrease in CP-55,940-induced β-arrestin2 recruitment that was coupled with no modulation of G protein activation ([35S]GTPγS assay) or cAMP inhibition. Our results support the hypotheses that 1) functionally selective CB1R orthosteric agonists can be designed through modification of molecular structure, and 2) GBR-12909 selectively stabilizes a CB1R active state conformation that prevents β-arrestin recruitment.

Acknowledgements: Funded by R01 DA026996 (CJH) and a New Investigator Award from the American Association of Colleges of Pharmacy (AACP, CWC).

CAMKII ACTIVATION MODULATES CANNABINOID SIGNALING BOTH PRE- AND POST-SYNAPTICALLY IN AUTAPTIC HIPPOCAMPAL NEURONS

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The cannabinoid signaling system mediates several forms of neuronal plasticity; it consists of receptors, endogenous ligands and the machinery to produce and break down these endocannabinoids (eCBs). The eCB 2-arachidonoyl glycerol (2-AG) is produced by diacylglycerol lipases (DAGLs) in a calcium-sensitive manner but the question of how this calcium sensitivity is translated to DAGL activation is still unclear. One candidate is the Ca^{++}/calmodulin-dependent protein kinase (CAMK) family of proteins that have been found to be important in CNS function. One study reported that CAMKII is necessary for cerebellar endocannabinoid-mediated synaptic plasticity while another indicated that CAMKII inhibits DAGL-mediated production of eCBs in the striatum.

To further explore the relationship between CAMKII and eCB signaling we tested the effects of CAMKII blockers on eCB-mediated synaptic plasticity in autaptic hippocampal neurons. These neurons have all the machinery necessary for several forms of endocannabinoid-dependent synaptic plasticity including depolarization-induced suppression of excitation (DSE). We found that acute treatment with CAMKII blocker KN62 strongly diminished DSE but not 2-AG responses. Inclusion of autocamtide inhibitory peptide (AIP), also a CAMKII blocker, also diminished DSE. However overnight KN62 treatment or transfection with the dominant negative CAMKII A302R mutant inhibited both DSE and 2-AG responses while leaving other $G_{i/o}$–dependent synaptic plasticity intact. Taken together these results suggest that CAMKII plays an obligatory post-synaptic role in the production of 2-AG but that long-term (i.e. overnight) inhibition of CAMKII signaling selectively desensitizes cannabinoid signaling through an unknown mechanism that likely targets the cannabinoid CB_1 receptor. Interestingly, overexpression of a constitutively active form of CAMKII reduced the size of excitatory postsynaptic currents (EPSCs) in wild type but not $CB_1^{-/-}$ neurons. This suggests that the inhibition of EPSCs, may be the consequence of prolonged CB_1 activation.

In summary, our findings suggest that in autaptic hippocampal neurons brief activation of post-synaptic CAMKII promotes production and/or release of 2AG, while longer-term activation interferes with presynaptic cannabinoid signaling.

STILL NAMBY PAMBY: FIRST GENERATION ALLOSTERIC MODULATORS OF CB1 IN A NEURONAL MODEL

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Cannabinoid pharmacology has proven nettlesome with issues of promiscuity a common theme among both agonists and antagonists. One recourse is to develop allosteric ligands to modulate cannabinoid receptor signaling. Cannabinoids have come late to the allosteric table. The 'first-generation' negative and positive allosteric modulators (NAMs and PAMs) represent an important first effort. However, most studies have relied on synthetic agonists, often tested in over-expression systems rather than a defined neuronal model system that utilizes endogenously synthesized and released cannabinoids.

We have systematically examined first-generation NAMs and a PAM on endocannabinoid modulation of synaptic transmission in cultured autaptic hippocampal neurons. These neurons exhibit CB_1/endocannabinoid-mediated depolarization induced suppression of excitation (DSE) and therefore serve as a model to test CB_1 modulators in a neuronal model of endogenous cannabinoid signaling.

We find ORG27569 and PEPCAN12 attenuate DSE and do not directly inhibit CB_1 receptors. In contrast PSNCBAM offers the strongest inhibition of signaling but is also an agonist at CB_1 and is therefore not a 'pure' NAM. The reported NAMs pregnenolone and hemopressin as well as the reported PAM lipoxin A4 are without effect in this model of endocannabinoid signaling.

In summary, only two of the allosteric modulators evaluated function in a manner consistent with 'pure' allosterism in a neuronal model of endogenous cannabinoid signaling. The remaining compounds exhibit complex pharmacology but may act as allosteric modulators for other agonists and through other signaling pathways. Some of these compounds may serve as platforms for the development of improved allosteric modulators.

ENANTIOMER-SPECIFIC POSITIVE ALLOSTERIC MODULATION OF THE TYPE 1 CANNABINOID RECEPTOR FOR THE TREATMENT OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is caused by the inheritance of a single copy of the mutant *huntingtin* gene encoding mutant huntingtin protein. One of the earliest cellular changes that occur in HD is a decrease in the levels of the type 1 cannabinoid receptor (CB_1) in medium spiny projection neurons of the striatum. The decline in striatal CB_1 is thought to contribute to the pathophysiology of HD. We have previously shown that orthosteric cannabinoid ligands including anandamide and 2-AG, but not THC, increase CB_1 levels in cell culture models of HD *via* CB_1- and ERK1/2-dependent signalling. An increase in CB_1 was associated with restored cell functionality and viability in cells expressing mutant huntingtin. Depending on agent and dose, orthosteric CB_1 agonists are psychoactive and can desensitize the receptor limiting clinical utility particularly in the context of HD where CB_1 levels are reduced and patients can have prior psychotic symptoms. Positive allosteric modulators (PAM), by definition, enhance the potency and efficacy of orthosteric ligands but do not compete for receptor binding. Because PAMs lack inherent agonist activity, PAMs of CB_1 would not be expected to promote psychotropic side effects or receptor desensitization.

A series of compounds, including GAT211 and its enantiomers GAT228 and GAT229, were synthesized and biochemically evaluated. The objective of this study was to characterize these compounds as orthosteric or allosteric modulators of CB_1 by measuring defined CB_1-depending signalling pathways including β-arrestin1 recruitment, PLCβ3 and ERK1/2 phosphorylation. Signalling was assessed in cells expressing normal (ST*Hdh*$^{Q7/Q7}$) and mutant human huntingtin (ST*Hdh*$^{Q111/Q111}$). The effect of these compounds *in vivo* is being measured in wild-type and R6/2 HD mice.

In cell culture, GAT211 was a modest PAM (E_{max} 112% relative to CP55,940 alone) and a weak allosteric agonist (EC_{50} 497.3 nM) of β-arrestin1 recruitment, and PLCβ3 and ERK1/2 phosphorylation. The R-(+)-enantiomer, GAT228, was a partial CB_1 allosteric agonist (EC_{50} 732.0 nM), and the S-(+)-enantiomer, GAT229, was a potent CB_1 PAM (E_{max} 157% relative to CP55,940 alone). Importantly, GAT211 and GAT229 were able to restore ERK1/2 signalling in ST*Hdh*$^{Q111/Q111}$ to that observed in ST*Hdh*$^{Q7/Q7}$ cells

In vivo, treatment of symptomatic 7 – 10 week-old R6/2 mice with GAT211 (10 mg/kg/d i.p.) improved locomotion and reduced HD symptom severity, compared to vehicle-treated R6/2 mice. We are currently comparing the effects of GAT211, GAT228 and GAT229 in the R6/2 mouse model of HD.

We predict that PAMs of CB_1, such as GAT211 or GAT229 may improve neuronal cell function and maintain CB_1 activity in HD, whereas allosteric agonists of CB_1, such as GAT228, may be functionally antagonistic of CB_1.

Acknowledgements: Funding by NIH, CIHR, and Dalhousie University.

ACTIONS OF THE AGO-PAM GAT211 AND ITS ENANTIOMER GAT229 ON INTRAOCULAR PRESSURE AND RETINAL GANGLION CELL LOSS IN THE NEE MOUSE MODEL OF OCULAR HYPERTENSION

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Glaucoma is a multifactorial blinding eye disease involving loss of retinal ganglion cell (RGC) loss. Intraocular pressure (IOP) is the only modifiable risk factor, and is a target for therapy. However, additional therapies directly focused on RGC neuroprotection may be beneficial. Tumor necrosis factor α (TNFα) is increased in glaucoma, and strategies which reduce TNFα are neuroprotective in animal models of ocular hypertension (OH). Activation of cannabinoid receptor 1 (CB_1) in experimental models of glaucoma reduces IOP and decreases RGC loss. CB_1-mediated neuroprotection may occur independently of IOP via the reduction of TNFα. However, long-term administration of cannabinoids may be undesirable due to potential psychotropic effects and tachyphylaxis. Use of the CB_1 agonist WIN 55,212-2 produces a rapid decrease in IOP one hour after topical administration, but is not maintained beyond two hours (Hudson et al, 2011). Positive allosteric modulator (PAMs) of CB_1 may provide another mechanism to both lower IOP and decrease RGC loss, while having a reduced potential for negative side effects. Our experiments examined the effects of GAT211, a novel CB_1 ago-PAM, and its R enantiomer, GAT229, a PAM, on IOP and cell loss in the nee mouse, an experimental model of ocular OH.

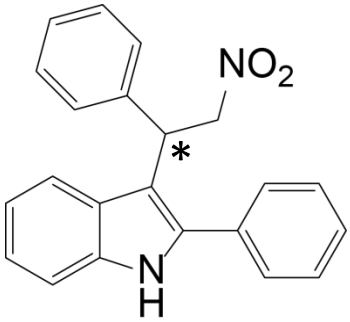


Figure 1. Structure of GAT211. GAT211 is a racemic mixture of GAT228 and GAT229. * indicates chiral centre.

IOP was measured by rebound tonometry, and RGC density was assessed post-mortem using the RGC-selective immunohistochemical Brn3a. Mice received 5 µL of either 0.2% GAT211, GAT229, or vehicle topically. 12 hours after a one-time treatment, GAT211 produced a change in IOP of -4.1 ± 2.8 mmHg from baseline measurements, compared with 2.2 ± 1.7 mmHg in contralateral eyes receiving vehicle (n=4, paired t-test). GAT229 also significantly decreased IOP compared to vehicle-treated contralateral eyes (-14.4 ± 4.3mmHg and -2.0 ± 1.9 mmHg from baseline, respectively, n=3). However, in normotensive wildtype, GAT211 or GAT229 did not reduce IOP (mean difference from baseline -0.5 ± 0.9 mmHg and 0.8 ± .07 mmHg, respectively, paired t-test, n=3). Nee mice eyes receiving topical GAT211 for 12 days had an average RGC density of 1692 ± 187 cells/mm^2, compared with 838 ± 106 cells/mm^2 in nee naïve eyes ($p<0.05$, n=4 and 8 respectively, t-test).

In conclusion, our data demonstrate that compounds with CB_1 PAM properties, such as GAT211 and GAT229, have more sustained effects on IOP compared with full CB_1 agonists, such as WIN 55,212-2, and have potential for the treatment of neurodegenerative conditions such as glaucoma.

Acknowledgments: Funded by CIHR (MEMK, Grant no: EY024717) and NEI-NIH (GAT, Grant no: MOP-97768)

Hudson BD, Beazley M, Szczesniak AM, Straiker A, Kelly ME (2011). Indirect sympatholytic actions at beta-adrenoceptors account for the ocular hypotensive actions of cannabinoid receptor agonists. J Pharmacol Exp Ther, 339(3):757-767. doi:10.1124/jpet.111.185769.

CANNABIGEROL MODULATES THE EFFICACY OF ANANDAMIDE ON THE CB2 CANNABINOID RECEPTOR

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Cannabigerol (CBG) is a non-psychoactive phytocannabinoid isolated from cannabis. The aim of this study was to measure the modulation of CBG on the effects of several synthetic and endocannabinoid agonists on the human CB2 cannabinoid receptor stably expressed in HEK293 cells. A homogeneous time-resolved fluorescence method was used to quantify cannabinoid-induced, CB2-mediated inhibition of cyclic adenosine monophosphate (cAMP) levels. At concentrations up to 10 μM, CBG by itself had no effect on forskolin-stimulated cAMP accumulation. Furthermore, CBG did not significantly modify cAMP inhibition induced by synthetic cannabinoids CP-55,940, HU-210, or endocannabinoid 2-arachidonoylglycerol (2-AG). However, CBG was found to increase the efficacy of endocannabinoid anandamide (AEA). Taken together, these results demonstrate that CBG is neither an orthosteric agonist nor an antagonist at the CB2 receptor. In addition, these data suggest that CBG possibly changes the efficacy of AEA on CB2 receptor via allosteric or metabolic modulation.

Acknowledgements: This work is partially supported by NCI training grant R25 CA134283

DEVELOPMENT AND IN VIVO IMAGING OF A NEAR INFRARED FLUORESCENT PROBE THAT PREFERENTIALLY BINDS TO CB2 OVER CB1 RECEPTORS

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The cannabinoid CB2 receptors (CB_2R) have gained much attention recently due to their important regulatory role in a host of pathophysiological processes. However, the exact biological function of CB_2R and how this function might change depending on disease progression remains unclear and could be better studied with highly sensitive and selective imaging tools for identifying the receptors. Because both CB1 receptors (CB_1R) and CB_2R are expressed by the same cells in certain physiological and pathophysiological conditions, and these receptors share high degrees of homology in their binding sites, it is critical to develop probes that preferably bind to CB_2R over CB_1R.

Here we report the first near infrared fluorescence imaging probe (NIR760-XLP6) that binds preferentially to CB_2R over CB_1R. The selectivity of the probe was demonstrated by fluorescence microscopy using DBT-CB_2 and DBT-CB_1 cells. Furthermore, in mouse tumor models, NIR760-XLP6 showed significantly higher uptake in CB_2R positive DBT-CB_2 than that in CB_1R positive DBT-CB_1 tumors. These findings indicate that NIR760-XLP6 is a promising imaging tool for studying the role of CB_2R in various diseases and physiological conditions.

Ackowledgement: This work was supported by a startup fund provided by the Department of Radiology, University of Pittsburgh. This project used the UPCI imaging facilities supported, in part, by award P30CA047904.

INHIBITION OF MONOACYLGLYCEROL LIPASE BY ANXIOLYTIC MEDICINAL PLANT EXTRACTS AND THEIR TRITERPENES

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Traditional cultures around the world use a wide variety of medicinal plants to treat anxiety and related mental health symptoms. While many of these plants demonstrate anxiolytic properties, the active constituents and mechanisms of action often remain incompletely characterized. Implicated in the regulation of mood and anxiety, the endocannabinoid (eCB) system is regarded as a promising target for drug development yet is seldom investigated as a potential mechanism of medicinal plant anxiolytic activity. Building on previous work, this study assessed the anxiolytic effects, active compounds, and potential mechanisms of Sin susto™ and its herbal components: *Souroubea sympetala,* a Central American remedy for susto (fear sickness), and *Platanus occidentalis,* an adjuvant used in the commercial formulation. Using multiple *in vivo* measures of anxiety in rodent models, we demonstrate that the plant extracts elicit distinct anxiolytic effects with significant synergistic activities observed in animals treated with both. We identified multiple bioactive triterpenes acting through multiple mechanisms, including potent monoacylglycerol lipase (MAGL) inhibition by crude extracts and pure triterpenes as well as interaction activation of the γ-amino butyric acid benzodiazepine (GABAa- BZD) receptor.

PHARMACOLOGICAL CHARACTERIZATION OF EMERGING SYNTHETIC CANNABINOIDS IN CELLS AND NEURONS

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Recently, there has been a proliferation of synthetic cannabinoids that are designed to evade drug control laws. In Canada, where cannabis is a controlled substance, synthetic cannabinoids are controlled as "similar synthetic preparations" of cannabis if they are cannabinoid receptor type 1 agonists. Unfortunately, there is a dearth of information about the physiological and pharmacological effects of emerging synthetic cannabinoids (ESCs). As a result, we aimed to characterize the pharmacological properties of ten ESCs. We used two cell based assays that enabled the determination of potency and efficacy relative to a panel of well-characterized cannabinoid drugs. Agonist-mediated inhibition in forskolin-stimulated cAMP levels were monitored in live HEK293 cells transfected with human cannabinoid receptor 1 (CNR1) and pGloSensor-22F. Pharmacological analysis of this data indicated that all of the ESCs tested were full agonists, with the following rank order of potency: 5F-PB-22 ≈ AB-PINACA ≈ EAM-2201 ≈ MAM-2201 > JWH-250 ≈ PB-22 > AKB48 N-(5FP) > AKB-48 ≈ STS-135 > XLR-11. Assessment of agonist stimulated Ca^{2+} transients was also used to confirm the efficacy of five ESCs (XLR-11, JWH-250, AB-PINACA, 5F-PB-22, and MAM 2201) in cultured primary hippocampal neurons. This is the first report concerning the pharmacological characterization of these ESCs, and adds to the body of knowledge for these emerging drugs of abuse.

PERIPHERALLY-RESTRICTED CBR AGONIST PRNMI SUPPRESSES CISPLATIN INDUCED PERIPHERAL NEUROPATHY CHRONIC PAIN SYMPTOMS WITHOUT TOLERANCE OR SIDE EFFECTS IN BOTH MALE AND FEMALE RATS

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Painful peripheral neuropathy as a consequence of cancer chemotherapy is a severe and dose limiting side effect associated with the use of antineoplastic agents such as cisplatin. Opioid medications for pain alleviation often lack long-term efficacy and exhibit undesirable side effects. While preclinical studies have demonstrated the analgesic effectiveness of brain-penetrant cannabinoids in the treatment of chemotherapy induced peripheral neuropathy (CIPN), a major impediment to the widespread use of cannabinoid analgesics is their central nervous system (CNS) mediated psychotropic side effects. The CNS effects can be circumvented (in rats) by the development of cannabinoid receptor (CBR) agonists that do not appreciably cross the blood-brain barrier. Here we report additional results that demonstrate the effectiveness of a most promising compound (4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl)methylidene]-1H-inden-3-yl]ethyl}morpholine, PrNMI) in suppressing the painful symptoms of mechanical and cold allodynia without either CNS side effects or the development of tolerance in a rat model of CIPN with both males and females.

CIPN was induced in rats by administration of cisplatin (3 mg/kg, i.p.). Oral administration of PrNMI dose-dependently suppressed mechanical and cold allodynia symptoms comparably in males and females with complete symptom suppression at 3 mg/kg. Daily oral administration at 1 mg/kg consecutively for two weeks resulted in similar daily suppression of mechanical allodynia implicating little, if any, tolerance development. PrNMI co-administration with selective CB1R or CB2R blockers revealed mainly CB1R contribution to its analgesic effects. CNS side effects assays compared the brain-permeant CB1R agonist HU-210 at doses that alleviate neuropathy symptoms to PrNMI and vehicle. While HU-210 exhibited strong CNS side effects at systemic doses that relieve neuropathy symptoms, PrNMI showed lack of side effects in the assays that test for catalepsy, hypothermia and motor incoordination.

These results suggest that the potency, peripheral selectivity, in vivo efficacy in both males and females, and absence of both CNS side effects and tolerance of this novel class of CBR agonists identify them as a potentially viable treatment for CIPN pain symptoms.

Acknowledgments: Support contributed by the Academic Senate Faculty Grant (I.S.) and UCLA School of Graduate Studies Stipend (Y.M.)

CANNABINOID CB1 AND CB2 RECEPTORS MEDIATE THE CLASSICAL TETRAD EFFECTS OF DELTA9-TETRAHYDROCANNABINOL IN MICE

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In animals, cannabinoid agonists such as delta9-THC, WIN 55,212-2, and CP 55,940 produce a characteristic combination of tetrad symptoms – hypothermia, analgesia, hypoactivity, and catalepsy. However, the receptor mechanisms underlying these actions are incompletely understood. When cannabinoid CB1 and CB2 receptors were first cloned in 1990s, CB1 receptor was found in the brain and periphery, while CB2 receptor was found only in periphery. Therefore, it is widely believed that brain CB1, not CB2, receptor mediates the tetrad effects of cannabis. This view was supported by the findings that pharmacological blockade of CB1 receptor with rimonabant or genetic deletion of CB1 receptor in CB1 receptor-knockout mice abolished the above tetrad effects produced by delta9-THC. In contrast to this view, however, growing evidence suggests that brain CB1 receptor is not the only target acted by cannabinoids, particularly, by other plant cannabinoids (phytocannabinoids) or endogenous fatty acids that were originally identified in animals (endocannabinoids), suggesting other non-CB1 receptor mechanisms are also involved. In consistent with this view, we and others have recently reported that functional CB2 receptor is expressed in the brain and in the midbrain dopamine neurons (Zhang et al., PNAS, 2014). Accordingly, systemic or local administration of CB2 receptor agonists into the brain inhibits cocaine self-administration, cocaine-induced hyperactivity, and cocaine-enhanced extracellular dopamine in the nucleus accumbens (Xi et al., Nature Neuroscience, 2011; Zhang et al Neuropsychopharmacology, 2015). These new findings inspired us to re-examine whether brain CB2 receptor is also involved in the action produced by cannabis such as delta9-THC. To address this issue, we first compared the behavioral response to delta9-THC under the same experimental conditions between wild-type (WT) and CB1 receptor-knockout (CB1-KO) or CB2 receptor-knockout mice (CB2-KO). We found that delta9-THC, at 10 or 30 mg/kg (i.p.), produced dose-dependent analgesia (as assessed by hot-plate test), hypothermia, catalepsy and rotarod performance impairment in WT mice, but not in CB1-KO mice. Surprisingly, deletion of CB2 receptor in CB2-KO mice also blunted delta9-THC-induced analgesia and rotarod performance impairment. We then observed the effects of the selective CB1 receptor agonist (ACEA) or CB2 receptor agonist (JWH133) in WT mice. We found that, systemic administration of ACEA (1-10 mg/kg, i.p.) or JWH133 (1-10 mg/kg, i.p.) alone failed to produce significant effect in the above measurements, while co-administration of ACEA and JWH133 produced significant analgesia, hypoactivity and catalepsy. These findings suggest that 1) brain CB1 receptor plays a predominant role in mediating delta9-THC-induced tetrad effects; 2) brain CB2 receptor also play an important role in mediating delta9-THC-induced analgesia and rotarod performance impairment; and 3) co-activation of brain CB1 and CB2 receptors are required in mediating the behavioral effects produced by cannabinoid ligands.

Supported by Beijing Institute of Pharmacology and Toxicology and NIDA IRP

A SELECTIVE CANNABINOID CB2 AGONIST ATTENUATES DAMAGE AND IMPROVES RETENTION FOLLOWING STROKE IN MICE

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Introduction. We have recently demonstrated that treatment with a cannabinoid CB2 agonist was protective in a mouse middle cerebral artery occlusion model of cerebral ischemia/reperfusion injury. The present study aimed to determine whether these protective effects of CB2 agonism would extend to a mouse photoinjury model of permanent ischemia and determine associated alterations in cognition and infarct size.

Methods: Mice received three injections of the CB2 selective agonist O-1966 or vehicle 1 h prior to and 2 and 5 days following induction of stroke. Infarct size was assessed at 1, 3, or 7 days post injury and learning and memory effects of injury and O-1966 treatment were assessed on days 6 and 7 using a novel object recognition task and an operant acquisition and retention procedure.

Results: O-1966 treated mice had significantly smaller infarct volumes compared with vehicle treated mice. Photoinjury was also associated with significant learning and memory impairments on days 6 and 7 post-injury, and these deficits were reversed with O-1966 treatment. Surprisingly, sham-operated mice receiving O-1966 treatment showed a significant learning *deficit* compared with vehicle treated sham mice.

Significance: We conclude that CB2 activation is protective against cognitive deficits and tissue damage following permanent ischemia, but may dysregulate glial or neuronal function of learning and memory circuits in the absence of injury and/or inflammation.

ACKNOWLEDGMENTS: R01 A1084065 (RFT, DG), R01 CA129092 (EAW), NIDA T32DA007237 (EMU)

EXAMINATION OF THE EXPRESSION OF CANNABINOID RECEPTOR 2 IN ANTIGEN PRESENTING CELLS IN HEALTHY HUMAN SKIN

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Cannabinoid receptor 2 (CB2) is a G-protein coupled receptor for cannabinoid ligands, which is expressed primarily by immune cells. Canonically CB2 is thought to have immunosuppressive effects, including impairment of cell migration, antigen processing and presentation and/or inflammatory cytokine release. The aim of this study was to identify which antigen presenting cells (APCs) in healthy human skin expressed CB2. APCs are the sentinel cells of the immune system and are ideally located in the skin to monitor for foreign pathogens like viruses or bacteria. Once APCs encounter a foreign pathogen they have the potential to orchestrate a tailored immune response against that pathogen.

We used multi-colour fluorescent immunohistochemistry to establish precisely which of the cutaneous APC subsets were recognised by the CB2 antibody. The CB2 antibody (ThermoFisher Scientific, PA1-744) used in this study has previously undergone extensive validation (Graham and Angel *et al*., *Int J Immunopathol Pharmacol.* 23:25, 2010).

Our findings demonstrate that both $CD1a^+$ dermal APCs ($CD207^-$) and $CD14^+$ dermal APCs ($CD68^{-/+}CD45^+$) expressed CB2 at varying levels. The rare $CD141^+$ APCs ($Clec9a^+CD45^+$) in the dermis were also found to express CB2. As there was pervasive CB2 expression in the epidermis, which is likely due to CB2 expression by keratinocytes (Casanova *et al, J Clin Invest*. 111:43, 2003; Zhao *et al.*, *J Cancer Res Ther.* 8:549, 2012), we were unable to establish whether the Langerhans cells in the epidermis expressed CB2; however, the Langerhans cells ($CD207^+CD1a^+$) in the dermis that are migrating from the epidermis to the afferent lymphatic vessels in the dermis were CB2 positive, although the level of expression was again variable.

Knowledge of the expression of CB2 in specific tissues is crucial to lay the foundations for therapeutic applications. The broad expression of CB2 by APCs in healthy human skin suggests the therapeutic potential of modulation of CB2 in this tissue. These results and, in time, analyses investigating the function of CB2 in cutaneous APCs may open the way for novel treatments of cutaneous inflammatory conditions and/or malignancies.

ENDOCANNABINOID-MEDIATED STRESS-POTENTIATED REINSTATEMENT OF COCAINE SEEKING

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Under conditions where stress alone cannot induce the reinstatement of cocaine seeking, it may potentiate reactivity to other triggers. However, little is currently known regarding the neurobiological processes which underlie this reinstatement-potentiating effect. Stress has recently been shown to mobilize the endocannabinoid (eCB) system, which can regulate neurotransmission, in brain regions implicated in motivated behavior. Given that, we examined the involvement of eCB signaling in the stress-potentiated reinstatement of extinguished cocaine seeking in rats. We hypothesized that cannabinoid receptor-1 (CB1R) antagonism would prevent stress-potentiated reinstatement. Following cocaine self-administration (0.5mg/kg/inf i.v., 2hr/day *x* 14days) and extinction, neither footshock stress nor low-dose cocaine (2.5mg/kg, i.p.) alone were capable of inducing reinstatement of cocaine seeking, but were capable of doing so in concert. The necessity for CB1R activation was next evaluated via pretreatment with the CB1R inverse agonist AM251 (0, 1, or 3 mg/kg, i.p.), which blocked stress-potentiated reinstatement at both doses investigated. Conversely, only 3mg/kg AM251 attenuated sucrose-reinforced lever pressing, with neither dose affecting locomotor activity. Furthermore, in contrast to stress-potentiated reinstatement, high-dose cocaine-primed (10mg/kg, i.p.) reinstatement was not blocked by AM251 pretreatment. These findings demonstrate that an underlying CB1R-dependent mechanism is unique to stress-potentiated reinstatement, though the locus for that mechanism is still under investigation. As prefrontal cortical eCB levels have been shown to elevate in response to stressful stimuli (Hill et al. 2011), we subjected drug-naïve animals to the same stress conditions that potentiate reinstatement, and then analyzed eCB concentrations via liquid chromatography-mass spectrometry (Agilent LC-MSD 1100 series) within prefrontal cortical subregions. In drug-naïve animals, footshock produced a significant increase of 2-arachidonylglycerol content within the anterior cingulate, while increased anandamide was found within the prelimbic cortex. We are extending these findings to identify alterations in prefrontal cortical eCB content that are associated with stress-potentiated reinstatement.

Acknowledgements: Funded by NIH grant DA15758 to JRM & DA038663 to JRM & CJH. Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

TO WHAT EXTENT IS THE CB2 RECEPTOR EXPRESSED BY THE VASCULATURE IN THE HUMAN BRAIN AND OTHER TISSUES

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Modulation of the endocannabinoid system via cannabinoid receptors during inflammation has been found to reduce the subsequent tissue damage in various rodent models of neuroinflammation. In this study, we investigated the role of the CB2 receptor in regulating the blood–brain-barrier (BBB) function under inflammatory condition. We have examined the expression and function of the CB2 receptor in human brain tissues and immortalized human cerebral microvascular endothelial cells (hCMVECs).

Immunohistochemistry of human brain tissues using a polyclonal CB2 antibody revealed that CB2 is largely absent in healthy human brain. However, positive staining for CB2 was observed in the brain vasculature. Interestingly vasculature in other human tissues i.e. skin, have also been shown to exhibit positive staining for CB2. Subsequently, we assessed CB2 expression by the human cerebromicrovascular brain endothelial cell line hCMVEC; hCMVECs did not express CB2 at the mRNA or protein level. In addition, the widely used skin endothelial cell line (HMEC-1) did not express CB2 either. Treatment of hCMVECs with the CB2 receptor selective agonist HU308 (up to 10μM), did not alter their secretion of inflammatory cytokines (IL-6, IL-8, IP-10, RANTES, soluble VCAM-1 and soluble ICAM-1) under steady state conditions. The secretory profile of IL-1β treated hCMVECs also remained unaffected by HU308 exposure. The lack of CB2 receptor expression and function in the brain endothelial cell line contradicts previous findings from others, where CB2 receptor agonists enhanced barrier function in human primary brain endothelial cells. However, this effect was only observed at 10μM. Pharmacologically, a CB2 agonist would need to be at least 1000-10,000 fold more selective for CB2 than other receptors to allow its use in the μM range.

Our data suggests that expression of CB2 may not be homogenous for all endothelial cells and cell lines. The hCMVEC line provides us with a valuable tool ($CB2^{neg}$) to dissect leukocyte-specific regulation by CB2 agonists in co-culture BBB models. In addition to this, on-going work aims to establish the CB2 receptor expression in endothelial cells from different brain regions and vessel types.

CANNABINOID SR141716A INDUCES HYPERACTIVITY AND VOCALS IN MICE

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Attention-deficit hyperactive disorder (ADHD) is a common disorder, affecting 3-9% of children and 2-5% of adults around the world. About 50% of the patients with Tourette syndrome are also diagnosed with ADHD. ADHD is characterised with attention deficit, hyperactivity and impulsivity. Interestingly, recent studies have shown that hyperactivity can be induced in mice by the cannabinoid SR141716A, a selective antagonist of the cannabinoid CB_1 receptor. In this study we injected mice with SR141716A and examined their locomotor activity. As alterations in the brain structure of children and adolescents with ADHD have been documented, we performed an MRI study in order to examine whether SR141716A induced changes to the brain structure of the hyperactive mice. We also recorded their Ultra Sonic Voices (USV).

Our results show that SR141716A increased the number of USV compared with vehicle. It also induced a significant increase of locomotor activity in female but not in male mice aged 1 month. A quantitative MRI measurement (T2 analysis) at this age revealed that, compared with the brain structure of vehicle-treated mice, the tissue density of several brain areas was significantly higher in the SR141716A-treated mice. The areas that were affected are related to movement (caudate putamen and thalamus), the reward system and addiction (nucleus accumbens and septal nuclei), memory (hippocampus) and impulsivity (nucleus accumbens and amygdala). At age 4 months, the female mice were still hyperactive. The SR141716A-induced hyperactivity was not reversed by Ritalin®.

Our results suggest that postnatal inhibition of the CB_1 receptor induces irreversible changes to the brain structure and increases vocalisation in young mice. The lack of response to methylphenidate suggests that dopamine transporter is not involved in the mechanism of SR141716A-induced hyperactivity. These results further support the emerging view that the endocannabinoid system is involved in hyperactive behaviour and suggest it may also be involved in vocal tic disorder.

Acknowledgments
The Research Grant Awards, Tourette Syndrome Association, USA (SAG). The Young Investigator Award and The Charles E. Smith Fellowship in honour of Professor Joel Elkes, The National Institute for Psychobiology in Israel (SAG). The Irving and Cherna Moskowitz Foundation for supporting a scholarship to TH.

CROSS TOLERANCE BETWEEN OPIOID RECEPTOR AND CANNABINOID RECEPTOR 2 AGONISTS IN INFLAMMATORY PAIN

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The objective of this study was to investigate potential interaction between the cannabinoid 2 receptor (CB2R) and the mu opioid receptor (MOR) in pathological pain. The low level of side effects and lack of tolerance make CB2R an attractive pharmacotherapeutic target. As an initial comparison, a dose response-curve for intraperitoneal injection of the CB2R agonist JWH-133, in mice was generated in the hotplate test for thermal nociceptive pain. JWH-133 induced modest thermal antinociception. The effect of JWH-133 in pathological pain was assessed in mice subjected to the formalin test of inflammatory pain. Intraplantar formalin injection (10 µl at 2.5 %) produces a biphasic nociceptive response described as acute and inflammatory phases. Compared to the hotplate test, the same dose of JWH-133 (1 mg/kg) produced a pronounced antinociceptive response in both phases of the formalin test. There was no measurable difference in paw edema (microcaliper) between mice receiving JWH-133 compared to vehicle. Cross tolerance was measured between JWH-133 and a MOR agonist (morphine) to determine whether CB2 and MOR interact in a physiologically relevant way. Mice that were made tolerant to the effects of morphine with chronic dosing demonstrated a lower response to JWH-133 relative to vehicle treated morphine-naïve animals in both phases of this inflammatory pain model. A similar finding was observed in the hotplate test. However, chronic JWH-133 administration does not appear to cause cross-tolerance for morphine, suggesting opioid and CB2R cross-tolerance is not bidirectional. Overall these findings suggest that CB2R may functionally interact with MOR to modulate antinociception in the formalin test in response to inflammatory pain.

Acknowledgements: Funded by NIH grants DA036385 (DJM), and a CURE grant to RL (S. Grigson, PI) from the Pennsylvania Department of Health using Tobacco Settlement Funds. Funded by Texas Tech University Health Sciences Center School of Medicine 121035(JG). Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

DESIGN, SYNTHESIS AND BIOCHEMICAL EVALUATION OF NOVEL ELECTROPHILIC AND PHOTOAFFINITY COVALENT PROBES TO MAP THE CB1 RECEPTOR ALLOSTERIC SITE(S)

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The pathological endocannabinoid system has been identified in the etiology of various disorders like obesity, glaucoma, post-traumatic stress disorder, metabolic syndrome, inflammation and neuropathic pain, and the cannabinoid receptor 1 (CB1) has been recognized as a major target in treating these disorders. Orthosteric CB1 receptor antagonists/inverse agonists have met with limited success as medicines due to their "undesirable CNS side effects" and such drugs have either been withdrawn from the market or their clinical development has been halted. Negative allosteric modulation of the CB1 receptor represents an alternative approach with a potential to provide safer effective medications that lack the undesirable side effects of orthosteric CB1 receptor antagonists/inverse agonists.

We report here the design, synthesis and biochemical evaluation of the first CB1 allosteric site covalent probes. Either an electrophilic isothiocyanato or a photoactivatable azido group is incorporated at the judiciously selected positions of the two well-established CB1 allosteric modulators Org27569 and PSNCBAM-1. All the novel compounds were evaluated in cyclic AMP, b-arrestin and [^{35}S]GTPgS assays. Among these ligands, the most potent analog, 3-ethyl-5-isothiocyanato-*N*-(4-(piperidin-1-yl)phenethyl)-1*H*-indole-2-carboxamide **(GAT100)** labelled the receptor covalently, inducing a robust (200+%) increase in [^{3}H]CP55940's binding at both rCB1 and hCB1 receptors. Thus this covalent probe behaved as a positive modulator of binding and negative modulator of function. These paradoxical effects on binding and function are similar to those displayed by the parent compound. Unlike Org27569, **GAT100** did not dampen the constitutive activity of the CB1 receptor in the [^{35}S]GTPgS assay refuting the possibility that it is an inverse agonist. Additionally, this study also identified 5-chloro-3-(2-isothiocyanatoethyl)-*N*-(4-(piperidin-1-yl)phenethyl)-1*H*-indole-2-carboxamide (**GAT209)** as a potent and functionally selective covalent probe.

These allosteric covalent probes of the CB1 receptor open new avenues for identifying the allosteric binding motif responsible for their modulatory effects. We are now applying proteomics and mass spectrometry techniques to accurately tap these allosteric site(s). Such evidence is vital for understanding the molecular mechanism of allosteric action on the CB1 receptor and will guide structure-based design of potentially effective and safer drugs.

Supported by DA027113 to G.A.T.

GREATER SENSITIVITY TO THE EFFECTS OF ACUTE CANNABINOID EXPOSURE ON HPA AXIS STRESS RESPONSIVITY IN ADOLESCENT MALE RATS

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Adolescence is characterized by numerous physical, neural, cognitive, and behavioural changes in the transition to adulthood. During this developmental period, the hypothalamic-pituitary-adrenal (HPA) axis and other corticolimbic structures contributing to the regulation of HPA axis stress responsivity also undergo maturational processes. Previous work has demonstrated that prepubertal male rats exhibit a protracted adrenocorticotropic hormone (ACTH) and corticosterone (CORT) response to acute stress exposure relative to adults and it has been suggested that age-dependent differences in HPA axis stress responsivity may leave adolescents particularly vulnerable to perturbations such as stress exposure. Furthermore, mechanisms underlying these age-dependent differences in HPA axis stress responsivity remain largely unknown. The endocannabinoid system is a strong candidate that may be contributing to these observed age-dependent differences, given its ability to tightly regulate the adult HPA axis. Moreover, previous work from our laboratory indicates that age-dependent differences in stress responsivity have corresponding age- and region-specific differences in endocannabinoid signaling and sub-chronic treatment of a CB1 receptor agonist (HU-210) during the adolescent period results in elevated stress-induced ACTH and CORT levels in adulthood. However, it is currently unknown whether cannabinoid exposure differentially modulates the acute HPA axis stress response during adolescent development relative to adulthood. Therefore, in the current study, 35, 45 and 75 day old male Sprague-Dawley rats were given a single intraperitoneal (IP) injection of AM-251 (3 mg/kg), HU-210 (25 ug/kg) or vehicle 30 minutes prior to a single 30 min restraint stress exposure session. ACTH and CORT plasma levels were measured at 0, 30 and 60 min following restraint stress onset. Preliminary results indicate that there were age- and drug- specific effects on the acute stress response. While adolescents and adults responded in a similar manner to AM-251 and HU-210 exposure, adolescent rats did exhibit greater stress-induced elevations of ACTH and CORT than adults. These preliminary findings are in line with previous reports suggesting that adolescents are more sensitive to the effects of cannabinoid exposure than adults.

BARIATRIC SURGERY CORRECTS PLASMA LIPID ABNORMALITIES IN PPARα KO MICE

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Oleoylethanolzmide (OEA) is a lipid amide produced in the mucosal layer of the small intestine, that regulates appetite and energy balance. OEA is increased after feeding and reduced during food deprivation, suggesting that endogenous OEA participates in the regulation of satiety. For this reason it has been targeted in treatment of obesity. Interestingly, we have found that the bariatric surgery procedure, vertical sleeve gastrectomy (VSG), which causes sustained reductions in body weight and improved lipid homeostasis, also leads to a regional increase in duodenal but not jejunal OEA levels. However, at this time the functional significance of this is unknown. The anorectic activity of OEA is thought to be due to its action at the nuclear receptor PPARα, which is a key factor in regulating lipid oxidation. Therefore, we next performed VSG or sham surgery in high fat fed weight-matched male WT or PPARα KO mice. We predicted that surgically-induced increases in OEA activate PPARα leading to improvements in lipid homeostasis. However, we found that VSG resulted in similar weight loss and increases in bile acid levels in both PPARα KO and WT mice. As demonstrated previously, PPARα KO sham animals exhibit elevated lipid responses to a meal compared to WT sham animals, yet VSG corrected this lipid abnormality. Collectively, these data suggest that PPARα is not necessary for the metabolic benefits of VSG. Moreover, if the increase in duodenal OEA is critical to the success of VSG, it is not through activation of PPARα. Future research will explore the role of other mediators of OEA action on the metabolic impact of bariatric surgery.

GLOBAL ANALYSIS OF LOCAL ENDOCANNABINOID SYSTEM EXPRESSION IN EXPERIMENTAL MODEL OF KNEE OSTEOARTHRITIS IN OA PATHOGENESIS

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Osteoarthritis (OA) is a musculoskeletal disease manifested in chronic joint pain, accompanied by their moderate inflammation, stiffness and functional disability. OA is believed to be multifactorial condition that afflicts not only cartilage but also entire joint. Currently, there are no approved disease-modifying drugs available therefore treatment of OA is mainly restricted to pain symptoms management. Growing body of evidence suggests that endocannabinoid system (ECS) may serve as pharmacological target for the treatment of OA pain. The common presence of both cannabinoid receptors on rodent and human chondrocytes has been shown. In agreement, the expression of CB1 and CB2, FAAH and presence of both AEA and 2-AG in the synovial tissue biopsies from OA patients was also demonstrated. Indeed, pharmacological studies on systemic administration of ECS modulators in different animal models of OA have shown anti-nociceptive and anti-inflammatory effects in the affected knee, implicating participation of the ECS in the pathophysiology of OA. However, local modulation of ECS has provided contradictory outcomes, taking into account reduced nociception mediated by URB597 (selective inhibitor of FAAH) and from the other hand sensitization of joint mechanoreceptors as well as increase of hindlimb incapacitance after intra-articular (i.a.) GW405833 (selective CB2 agonist). Therefore, the aim of our study was to analyze both mRNA and protein level holistic profile of ECS in rat osteoarthritic knee during 28 day of OA progression, which may underlie efficacy of local cannabinoids administration. Simultaneously, examined relationship between changes in ECS and joint pain perception. OA was induced in male Wistar rats by i.a. injection of 3 mg sodium monoiodoacetate (MIA). We used mRNA Affymetrix microarrays of joint tissue explants to evaluate all elements of the ECS at firm time points after OA induction. Selected protein levels were also determined by Western Blot technique. Additionally, rats were monitored for OA-related pain symptoms during the disease development by means of hindlimb dynamic weight bearing (DWB).

Based on the transcriptomic data, we were able to categorize four clusters of genes family, which were widely afflicted by OA processes. Due to great interest in ECS, we found highly interesting global down-regulation in genes involved in lipid metabolism and PPAR signaling. Furthermore, we observed ECS transcripts decreased from the day 2, with the exception of CB2 gene, which was significantly up-regulated already at this early time point. Surprisingly, simultaneous constant and substantial increase in CB1, CB2 and FAAH proteins was noticed. This discrepancy in levels of transcripts and corresponding amount of ECS proteins may suggest a possible feedback mechanism of expression regulation and/or significant disproportion between half-lives of transcripts vs. proteins during the pathogenesis of OA. The most significant changes in ECS proteins were seen from day 21 up to day 28, when the pain was the most severe. In our study, we confirm that ECS is heavily affected by on-going OA development in the knee and at late stage of the disease characterized by elevated pain sensation, few key ECS proteins were significantly up-regulated, despite transcripts depression. Thus, we concluded that local targeting of ECS is reasonable and may offer a therapeutic strategy for pain treatment in OA, but definite judgment needs further pharmacological tests.

Supported by Statutory Funds, SONATABIS/NCN/UMO-2012/07/E/NZ7/01269 and OPUS/NCN/UMO-2014/13/B/NZ7/02311 grants.

ENDOCANNABINOID SYSTEM IN THE MIA MODEL OF OA PAIN - IMPLICATIONS FOR CB2 RECEPTORS' AGNONISTS

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The endocannabioids (ECs) and their receptors participate in the regulation of various cerebral functions, including pain perception and modulation. They are present in pain circuits from peripheral nerve terminals up to supraspinal centres and therefore are thought to display considerable therapeutic potential. Evidence suggests that cannabinoid therapies may be useful in the treatment of pain resulting specifically from osteoarthritis a degenerative join disease associated with articular cartilage degradation. Current treatment is very limited and mainly based on symptomatic pain relief by acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs). Safety concerns of traditional pharmacotherapeutic agents used in the management of OA, such as NSAIDs have led healthcare professionals to seek other options. As some promising results have been recently obtained in support of the therapeutic value of ECs for OA management we focused our research on defining the role of spinal EC system and its potential therapeutic use at different *developmental* stages of disease in animal model of OA. Degenerative changes in male Wistar rats were induced by a single intra-articular injection of sodium monoiodoacetate (MIA). Rats were monitored for OA-related pain symptoms by pressure application measurements (PAM) and von Frey test. We assessed changes in mRNA and proteins levels of EC system (receptors and metabolic enzymes) as well as nerve injury marker (*Atf-3*) in response to OA development (0 -28 days).

The development of tactile allodynia assessed in von Frey test and increased expression of nerve injury marker, *Atf-3,* provided an evidence for the occurring neuropathy at advanced OA stages. OA development was also accompanied by an increase of the mRNA levels of *Cb1* receptor and metabolic enzymes, which highlight their contribution to OA pain. The main anandamide (AEA) synthesis pathway through hydrolysis by *Napepld* in a Ca^{2+}-sensitive manner were elevated only at day 28 while parallel Ca^{2+}-independent pathways (eg. secreted phospholipase A2 (sPLA2)—catalyzed) were strongly upregulated starting already at day 7. Remarkably we have observed an increase in *Cb2* mRNA level in spinal cord at day 7 after MIA injection, which was correlated with temporal recovery and pain alleviation approx. by day 10. This result emphasizes the potential use of CB2 receptor selective agonists with no central effects as promising pain treatment targets. These studies highlight the importance of ECs, a family of bioactive lipids in modulation of neural transmission in OA and their potential therapeutic target for treatment OA pain. The increased level of AEA synthesis pathways may suggest that EC tends to accumulate AEA possibly to counteract pain. Additionally upregulation of EC degradation enzymes indicates that inhibition of LOX and COX-mediated apart of FAAH could be valuable option for OA treatment. Interestingly upregulation of *Cb2* receptor in spinal cord correlates with reduction of joint hypersensivity indicating that application of CB2 agonist would be profitable for osteoarthritis management. Thus CB2-based agents that focus on preventing further damage to the joints have the potential to change how this disease state can be managed and will be a subject of our future studies.

Acknowledgments: Supported by the SONATABIS/NCN/2012/07/E/NZ7/01269 and OPUS UMO-2014/13/B/NZ7/02311 grants.

MOLECULAR DYNAMICS STUDY OF ORG27569 SIGNALING VIA BETA-ARRESTIN AT THE CB1 RECEPTOR

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Arrestins were initially named for their ability to arrest signaling of heterotrimeric G-proteins. Their role in G-protein coupled receptor (GPCR) desensitization and internalization has been appreciated for some time. However, we now know that arrestins can also mediate GPCR signaling that is G-protein independent (Nobles et al. Science Signaling 2011). Debra Kendall's lab has shown that the CB1 receptor also can signal via beta-arrestin (BArr), specifically beta-arrestin-1 (BArr1) and that the CB1 allosteric modulator, ORG27569 (ORG), is an agonist of this BArr1 pathway (Ahn et.al JBC 2013). The intracellular (IC) opening through which a beta-arrestin will interact with a GPCR results from receptor conformational changes induced by agonist. For beta-arrestin signaling, this conformational change has been shown to be a movement of the receptor TMH7-Hx8 IC region (Rahmeh PNAS 2012).

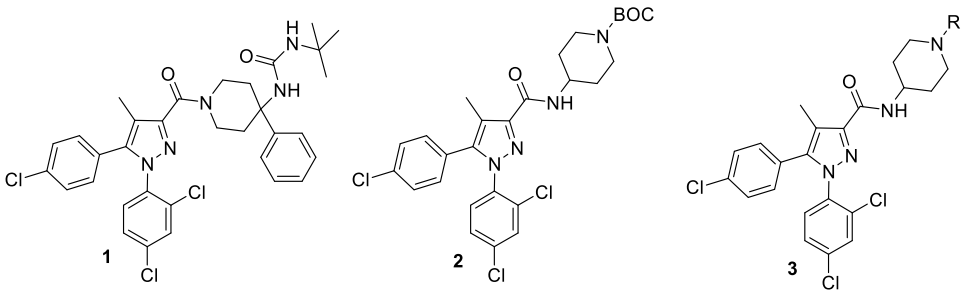
To understand the selective BArr signaling being produced by ORG, we conducted an AMBER12 molecular dynamics (MD) study of ORG interacting with the CB1 receptor model immersed in a fully hydrated POPC bilayer via microsecond time-scale all-atom MD. This study suggested not only a binding site for ORG, but also a mode of action. At 33ns into the MD production run, one ORG (in an elongated conformation) approached the receptor, interacting with residues along the lipid faces of TMH6/TMH7. Y6.57 (which faces lipid) and F7.35 (which has turned to face lipid) began interacting with ORG, acting as guides. By 250 ns, ORG assumed a bent conformation and the indole ring of ORG inserted between TMH6/TMH7 at their most extracellular (EC) turn. Y6.57 acted like a "side wall" from the TMH6 side and F7.35 rotated under ORG, acting like a "floor". By 328 ns, the CB1 EC-3 loop bent backwards and began to interact with ORG. Here, the amide oxygen of ORG formed a hydrogen bond with the backbone N-H of N372. This docking of ORG had important effects on the IC end of CB1. The distance between TMH6 and TMH7 on the IC side of the bundle (distance between centers of mass of most IC turn) showed an increase of nearly 4 Angstroms as ORG docked at the TMH6/TMH7 interface and the CB1 EC-3 loop began interacting with ORG. This distance increase occured as the TMH7-Hx8 region moved away from TMH6. Such IC changes are consistent with results reported for other BArr biased ligands in the β2-AR (carvedilol) and vasopressin V2R (SR121463) receptor fields (Liu et al. Science 2012; Rahmeh et al. PNAS 2012). The IC opening that is produced by ORG's entry into the TMH6/TMH7 EC region is large enough to permit the insertion of the fingerloop of BArr1. In contrast, the distance between TMH6/TMH7 in a control run (in which there is no ORG interaction with CB1) remains stable throughout the run. [Support: RO1 DA003934, KO5 DA021358 (PHR)]

PYRAZOLE ANTAGONISTS OF CB1 RECEPTOR WITH PREDICTED PERIPHERAL SELECTIVITY

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The CB1 receptor is a target for treatment of obesity, liver disease, metabolic syndrome, and dyslipidemias. Unfortunately due to adverse CNS-related side effects, the first clinically approved CB1 antagonist/inverse agonist rimonabant (SR141716A) was withdrawn from European markets. Currently, efforts are underway to produce peripherally selective antagonists of CB1. By targeting CB1 with antagonists that do not cross the blood-brain barrier (BBB), it is expected that CNS related adverse effects could be minimized or eliminated. A similar strategy has been successfully used to develop peripherally selective opioids. Our previous published research led to identification of peripherally selective rimonabant analogues **1** and **2** but these compounds had limited oral bioavailability. Both these compounds were found to have brain to plasma ratios that were <0.04 when dosed at 10 mg/kg ip in Sprague-Dawley rats. Efforts were undertaken to broaden SAR around **2** and improve *in vitro* pharmacological properties of these novel compounds. New analogues examined substitutions off the piperidine nitrogen of **3**. Carbamates, amides, and sulfonamides were all targeted. Structure **2** was chosen over **1** for modifications because it was hypothesized that lower molecular weight of **3** would allow introduction of larger functional groups without significantly affecting drug-like properties of these compounds. Exploration of SAR around compound **3** indicated that both linear and cyclic lipophilic substituents were tolerated at the **R** position. Carbamates, amides, and sulfonamides were all found to be potent antagonists of CB1 and selective for CB1 over CB2. Several analogues had $K_b \leq 20$ nM. Consequently, the most potent analogues were examined in an *in vitro* model of BBB penetration and found to be minimally permeable. Some of these compounds were metabolically stable upon incubation with human microsomal fractions as well. Finally, these compounds were tested in a predictive *in vitro* model of oral absorption. This iterative process led to the identification of a carbamate and amide analogue for further refinement and testing.



This research was funded by research grants AA022235 and DK100414 to RM from NIH.

CANNABINOID TRANSMISSION IN THE BASOLATERAL AMYGDALA BI-DIRECTIONALLY CONTROLS THE MOTIVATIONAL PROPERTIES OF OPIATES VIA FUNCTIONAL EXCITATORY INPUTS TO THE NUCLEUS ACCUMBENS SHELL

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The cannabinoid CB1 receptor (CB1R) system modulates glutamatergic synaptic transmission and plays a crucial role in opiate related memory recall (Domenici et al, 2006; Navarro et al., 2001). CB1Rs are found in high concentrations in areas responsible for learning, memory processing, and memory consolidation such as the prefrontal cortex (PFC), and basolateral amygdala (BLA). The BLA has functional inputs to the Nucleus Accumbens (NA), and this BLA→NAc pathway is characterized as an important mechanism controlling emotionally salient memory formation. Blockade of CB1 receptors within the BLA blocks the acquisition of associative, fear-related memory (Tan et al., 2011; McGaugh, 2000). However, how intra-BLA CB1R transmission may modulate the encoding of reward-related memories via interactions with the NAc is not currently understood. Using an unbiased conditioned place preference (CPP) procedure, we administered either a CB1 agonist or antagonist into the BLA of Sprague-Dawley rats and examined how intra-BLA modulation of CB1 transmission may influence opiate reward CPP, using either a sub (0.05 mg/kg) or supra-reward (5.0 mg/kg) threshold conditioning doses of systemic morphine. We report that intra-BLA CB1R transmission can bi-directionally control the salience of opiate-related reward signals via functional interactions with the shell region of the nucleus accumbens (NASh). Thus, intra-BLA CB1 receptor activation with WIN-55,212-2 (50-500 ng) switched normally rewarding effects of morphine into strongly aversive conditioning effects. In contrast, intra-BLA CB1R blockade with the CB1R antagonist, AM-251 (50-500 ng) strongly potentiated the reward salience of normally sub-reward threshold conditioning doses of morphine. To examine if intra-BLA CB1 modulation of opiate reward signaling depended upon functional BLA-NA projections, we blocked excitatory BLA→NA projections with an NMDA receptor antagonist (AP-5, 1mg), micro-infused directly into either the NASh or 'core' region of the accumbens (NACore). Interestingly, glutamatergic blockade in the NASh, but not NACore, prevented both intra-BLA CB1 blockade-mediated opiate reward potentiation and CB1 activation-mediated aversion effects. Furthermore, *in vivo* electrophysiological recordings from the NASh during CPP conditioning with intra-BLA CB1R activation or blockade, revealed that bi-directional CB1R activity in the BLA produces opposite effects on NASh neuronal sub-populations; thus, CB1R activation induced a decrease in fast spiking interneurons (FSI), and an increase in medium spiny neurons (MSN) activity, whereas CB1R blockade produced the opposite neuronal activity pattern.

Acknowledgements: This research is funded by the Canadian Institutes of Health Research (MOP-246144) and the National Science and Engineering Research Council of Canada (NSERC).

GENERATION AND ANALYSIS OF CNR1 AND CNR2 ZEBRAFISH MUTANTS

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In the past year, 78% of 2.4 million people who started using marijuana were aged from 12 to 20 years, a stage where the brain is still in development and vulnerable to impairment. In vertebrate animals Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient in marijuana, activates two cannabinoid receptors, CB1 and CB2, which are encoded by the *cnr1* and *cnr2* genes respectively. Here we establish viable and stable zebrafish mutant lines for *cnr1*, *cnr2* and *cnr1/cnr2*, using the CRISPR/Cas9 genome-editing tool to investigate the roles of these receptors during the early steps of brain development and the regulation of the immune response. We have designed two targets for each gene and co-injected them with Cas9 mRNA. The injected animals have been raised to adulthood without significant impairment in their development. The F1 embryos/larvae have been raised, fin clipped and genotyped to confirm the presence of a mutation in each respective gene. The confirmed founders have been outcrossed to breed the next generation. This will allow us to follow brain development and immune response in mutant and wild type animals. These mutants will also be used to test specific roles of the cannabinoid system during complex behavioral tasks. Here we will present our preliminary work on these mutant lines. Our studies will be crucial in identifying key roles of CB1 and CB2 during brain development and immune system maturation.

Acknowledgements: Funded by the National Institute of Drug Abuse (R01.DA037924), the National Science Foundation (HRD.1137725), and the NIH-RCMI Seed monies (#G12 RR03051).

PRO-INFLAMMATORY EFFCT OF WIN55, 212-2 ON CHRONIC MURINE ILEITIS

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Marijuana legalization in states such as Colorado has sparked even greater interest in the medical benefits of cannabis for inflammatory bowel diseases (IBD). A number of studies have reported therapeutic benefits of marijuana including reduced nausea, as an appetite stimulant, promoting weight gain, reducing abdominal pain and improving the overall disease activity indices. The increasing adoption of marijuana to self-medicate chronic intestinal inflammation for symptomatic relief comes despite the lack of clear understanding of the implications that this might have for a diverse patient population. Even in states that have legalized recreational use and directed some of the resultant tax income toward clinical research on potential medical benefits of cannabis, the federal legal status as a Schedule I drug have severely hampered these efforts. Additionally marijuana grown for strictly medical research purposes differs significantly in the proportions of two critical active ingredients, namely cannabidiol (CBD) and tetrahydrocannabinol (THC) from that available to the recreational user. Recreational strains strive for significantly higher THC and lower CBD levels than the medical varieties because of the associated psychoactive effects.

While a number of murine studies have described a therapeutic effect of stimulating cannabinoid receptors in models of chemically-induced acute colitis, the aim of this study was to determine the effect of cannabinoid receptor activation on spontaneous chronic murine ileitis. As such the TNFΔARE model that recapitulates many of the key features of human Crohn's disease such as discontinuous transmural spontaneous ileal inflammation and a dependence on the pro-inflammatory cytokine TNF, was employed. Mice aged either 6 weeks of age (prior to severe disease onset) or 20 weeks of age (chronically inflamed) were treated for two weeks with WIN55, 212-2, a potent cannabinoid receptor agonist by osmotic pump. The result in both cases was significantly increased inflammatory cell infiltrate, along with a worsening of histological indices of disease as determined by a blinded pathologist. While the underlying mechanism remains unclear, these studies support the need for a far greater understanding of the role of cannabinoids in chronic intestinal inflammation prior to widespread adoption by IBD patients.

SEX DIFFERENCES IN THE ROLE OF BETA-ARRESTIN2 IN THE CANNABINOID SYSTEM MAY NOT BE FOUND IN THE OPIOID SYSTEM

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Previous studies of sex differences in the responses to cannabinoids have found that generally cannabinoids produce the same effects in both sexes, but mostly minor differences in the degree of some effects exist. Many of the differences have been attributed to differences in rates of metabolism or in percent muscle mass or fat distribution between the sexes. Debra Bangasser et al. (2010) reported sex differences in the coupling of beta-arrestin2 to Corticotropin Releasing Factor Receptor-1.

We have previously reported that male beta-arrestin2 knock-out mice exhibited greater temperature depression and antinociceptive effects (via the tail flick test) than wild-type mice treated with Δ^9-tetrahydrocannabinol (THC). Other agonists tested in males, including CP55940, methanandamide and O-1812, did not show any differences between those genotypes for any of these assays performed in male mice. Studies with THC in female mice have shown very different effects from what was seen in males. The antinociceptive (but not rectal temperature) effects of THC obtained in wild-type females were nearly absent in beta-arrestin2 knock-outs. Similar to male mice, the effects of CP55940 did not differ between the genotypes in female mice.

It is of interest to determine whether or not the sex differences in the role of beta-arrestin2 found in the cannabinoid system are unique or generalize to other GPCRs. To this end, time course and dose-response studies were carried out in male and female beta-arrestin2 wild-type and knock-out mice using the mu/delta opioid agonist, morphine. Preliminary data indicated that the antinociceptive effects of morphine in the tail flick test were less in beta-arrestin2 knock-out than wild-type in both male and female mice. This is in contrast to the effects of morphine in male mice previously reported by Bohn et al. (1999), where an enhancement of the antinociceptive effects of morphine were reported in beta-arrestin2 knock-out mice using the hot plate test. No studies in female mice have been reported. Similar to results reported by Bohn et al., the temperature depressant effects of morphine were enhanced in male mice lacking beta-arrestin2, and moreover, we found similar enhancement in female knock-out mice. Thus it appears that the sex differences in the effects of beta-arrestin2 deletion previously reported for cannabinoid agonists are absent for the mu agonist morphine. This provides some evidence that these sex differences may be unique to the cannabinoid system. Future studies will investigate the effects of other opioids, and then agonists for other G-protein couple receptors.

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Acknowledgements: Beta-arrestin2-/- mice were developed by Dr. R Lefkowitz of Duke University and provided by Drs. A Lichtman and D Selley of Virginia Commonwealth University.

A GPR119-BASED SIGNALING SYSTEM IN THE MURINE EYE REGULATES INTRAOCULAR PRESSURE IN A SEX-DEPENDENT MANNER

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GPR119 is a G protein-coupled receptor that may be the endogenous target for oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). These lipids are closely related to the endocannabinoid family of neurotransmitters that act on cannabinoid CB_1 and CB_2 receptors. Interest in GPR119 has centered on its role in regulating insulin secretion, with GPR119 agonists investigated in clinical trials relating to type II diabetes. However, the role of GPR119 has not been examined in the eye. We now report evidence that GPR119 regulates intraocular pressure in a murine normotensive model.

We have detected mRNA for GPR119 in several tissues of the mammalian eye. In addition using LC-MS we have tested for the presence of the likely endogenous ligands for GPR119, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), finding that both are present in anterior murine eye. Lastly, we have found that topical OEA (but not PEA) reduces intraocular pressure (IOP) in the eye relative to the untreated contralateral eye. This effect occurs in a concentration-dependent manner and is absent in $GPR119^{-/-}$ mice. Elevated IOP is associated with most forms of glaucoma, a major cause of blindness worldwide. Importantly this depression of IOP is seen in female but not male mice, revealing an element of sex-dependence in the function of this X-linked gene. The identification of a novel approach to reduce IOP is therefore of great therapeutic interest. We also compiled a cannabinoid-related lipid expression profile for the eyes of female GPR119-/- mice relative to WT females and also relative to males. Knockouts exhibit a broad elevation of oleoyl-based lipids while only 2-OG is altered relative to males. 2-OG has been proposed to be a GPR119 agonist but does not alter IOP in this model.

In summary, we offer evidence for a functional GPR119-based signaling system in the mammalian eye, with receptors, ligands and function in the form of a reduction in intraocular pressure. This offers the prospect of a novel mechanism to lower IOP while observed sex differences have implications for the desired use of GPR119 as a therapeutic target in diabetes.

THE EFFECTS OF N-LINKED GLYCOSYLATION ON CB_1 RECEPTOR TRAFFICKING AND FUNCTION

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The CB_1 cannabinoid receptor is a glycoprotein and is mainly found on the plasma membrane of presynaptic axon terminals as well as on the outer membrane of mitochondria. The role of the sugars on glycoproteins is a topic of active investigation. It is generally observed that the addition of sugars to a protein serves as a detector for proper protein folding in the Endoplasmic Reticulum (ER) and selective trafficking to specific sites outside of the ER and Golgi, in addition to modulating function and signaling of cell surface receptors. In this study, we have investigated the functional role of CB_1 glycosylation by making several CB_1 mutants, removing either one or both of the putative amino terminus, N-linked glycosylation sites and investigating their trafficking and function. Our data suggest that glycosylation is not required to place CB_1 receptors on the plasma or mitochondrial membranes when over-expressed in HEK293 cells, but is required for proper trafficking in neurons. CB_1 wt and mutant signaling was comparable in HEK cells with regard to internalization, MAPK and cAMP assays. However, both trafficking and EPSCs were impaired when CB_1 glycosylation mutants were expressed in cultured hippocampal autaptic neurons.

MALE RAT SEXUAL BEHAVIOUR IS ATTENUATED BY INHIBITION OF MONOACYLGLYCEROL LIPASE BUT NOT FATTY ACID AMIDE HYDROLASE

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The acute effects of cannabinoid exposure on male sexual behaviour have been well characterized in the rat model. Exposure to direct CB_1 receptor agonists, such as THC or HU-210, has been consistently shown to suppress male sexual behaviour. However, further elucidation of the nature of endocannabinoid system regulation of sexual behaviour is still limited. Paradoxically, direct administration of anandamide to male rats appears to have mild facilitative effects on sexual behaviour, while exposure to fatty acid amide hydrolase (FAAH) inhibitors such as URB-597 has limited effects on sexual functioning. This suggests that the inhibitory role of CB_1 receptor activation on sexual behaviour may be endogenously mediated through a 2-AG mechanism. Therefore, in the current studies, we investigated whether enhancement of 2-AG activity via monoacylglycerol lipase (MAGL) inhibition would inhibit sexual behaviour in male rats.

In study one, adult male sexually-proficient Sprague-Dawley rats (n = 40) were given a single intraperitoneal injection (1 ml/kg) of MAGL inhibitor MJN-110 (5 mg/kg), FAAH inhibitor URB-597 (1 mg/kg), both MJN-110 and URB-957, or vehicle control. Subjects were then tested on sexual behaviour with ovarian hormone primed female conspecifics 60 minutes later. Sexual behaviour was scored on mount latency, mount frequency, intromission latency, intromission frequency, ejaculation latency, ejaculation frequency, and post-ejaculatory interval.

In study two, adult male sexually-proficient Sprague-Dawley rats (n = 50) were given a single intraperitoneal injection (1 ml/kg) of MJN-110 (1 mg/kg, 2 mg/kg, or 5 mg/kg), MJN-110 (5 mg/kg) and CB_1 receptor antagonist AM-251 (5 mg/kg), or vehicle control. Sex testing then proceeded as similarly as study one.

Results showed that MJN-110 and combined MJN-110 and URB-597 significantly inhibited male sexual behaviour compared to control, while URB-597 alone did not have a significant effect on sexual behaviour. Preliminary results also show that the effect of MJN-110 was dose-dependent and was attenuated by AM-251. These findings suggest that enhancement of 2-AG activity inhibits male sexual behaviour, and this occurs via 2-AG's binding to CB_1 receptors. Activation of CB_1 receptors by 2-AG may be the primary endogenous mechanism by which endocannabinoid system inhibition of male sexual behaviour occurs. 2-AG signalling could represent a novel area of investigation for the treatment of sexual dysfunctions in humans.

Acknowledgements: Funded by the Canadian Institutes of Health Research

TARGETING CB2 RECEPTOR FOR IN VIVO FLUORESCENCE IMAGING OF INFLAMMATION

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Chronic inflammation is associated with many types of diseases, such as cancer, rheumatoid arthritis, atherosclerosis, and neurodegenerative diseases. Currently it is not well understood how chronic inflammation affects aforementioned diseases. To reveal the role of inflammation in disease progressions, it is essential to have imaging tools that can label inflammation specifically.

Cannabinoid receptor type 2 (CB_2R) is emerging as an attractive biomarker for inflammation research. Herein, we report the first in vivo CB_2R-targeted near infrared inflammation imaging study using a synthetic fluorescent probe developed in our laboratory, NIR760-mbc94. In vitro binding assay and fluorescence microscopy study indicated that NIR760-mbc94 specifically binds towards CB_2R in mouse RAW264.7 macrophage cells. Furthermore, in vivo imaging was performed using a Complete Freund's Adjuvant (CFA)-induced inflammation mouse mode. NIR760-mbc94 successfully identified inflamed tissues and the probe uptake was blocked by a CB_2R ligand, SR144528. Lastly, immunofluorescence staining in cryosectioned tissues validated the NIR760-mbc94 uptake in inflamed tissues.

In conclusion, this study reports the first in vivo CB_2R-targeted inflammation imaging using an NIR fluorescent probe. The combined evidences indicate that NIR760-mbc94 is a promising imaging probe for inflammatory diseases.

Acknowledgements: Funded by the startup fund from the Department of Radiology, University of Pittsburgh. This project used the UPCI imaging facilities supported, in part, by award P30CA047904.

USING A ZEBRAFISH MODEL TO SCREEN THE IN-VIVO CELL-SPECIFIC ACTIONS OF CANNABINOID 2 RECEPTOR LIGANDS IN SYSTEMIC INFLAMMATION

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Background and Rationale: The zebrafish model offers the unique opportunity to study innate immunity in a transparent whole embryo in which no adaptive immunity has yet been developed. Systemic inflammation can be modeled in zebrafish through intrayolk sac injection of the bacterial endotoxin, lipopolysaccharide (LPS). LPS elicits a strong immune response in vertebrates by activation of the Toll-Like receptor-signaling pathway. Use of genetically modified zebrafish lines with fluorescently labeled immune cells, such as neutrophils (*Tg(mpx::eGFP)*) or monocytes (*Tg(mpeg1::eGFP)*) facilitates dynamic tracking of identified immune cells in response to inflammation and allows the actions of drugs on specific cells or pathways to be examined. Cannabinoid 2 receptors (CB2R) are highly expressed on immune cells and activation of CB2R is anti-inflammatory and neuroprotective in disease models. We used a zebrafish model to examine the actions of CB2R activation on the innate immune response to systemic LPS.

Methods: LPS (2nL; 5μg/mL) was injected into the yolk sac of 3 days post-fertilization zebrafish embryos to induce a systemic inflammatory response. Using both *Tg(mpx::eGFP)* and *Tg(mpeg1::eGFP)* embryos real time neutrophil and monocyte recruitment was visualized in the yolk sac, eye and brain by epifluorescence microscopy at various time points. In addition, embryos were collected at the same time points for dissociation to generate a single cell suspension to count cells *ex vivo*. Image J, an *in silico* program developed by the NIH, was used for cell quantification. The CB2R agonist, HU308 (10 μM and 100 μM), was applied in a single one-time dose dissolved in the bathing water immediately after LPS injection and animals observed for a further 2, 9 and 25 hours at which point embryos were euthanatized by bathing in high dose tricaine methanesulfonate.

Results: LPS injection into the yolk sac increases leukocyte (monocyte) recruitment to the yolk sac and other tissues such as the eye and brain. Monocyte recruitment was apparent in the yolk sac at 2 hours and was significant at 9 hours. Monocytes were also significantly increased in the eye and brain at 9 hours post-injection, with cells persisting for up to 24 hours. Exposure to the CB2R agonist, HU308, significantly reduced leukocyte recruitment in the yolk sac, eye and brain at 9 hours ($p<0.05$) but not at 2 or 25 hours after drug addition. On-going experiments are examining the actions of CB2R modulation on neutrophils in addition to monocytes and the signaling pathways involved in the immunosuppressive actions of CB2R in zebrafish embryos.

Conclusion: Zebrafish provide a unique means of studying systematic inflammation in a model that is economical and real-time. Transgenic embryos with labeled neutrophils and monocytes, the two most important cells of the innate immune response, can be used to study innate immunity in the absence of an established adaptive immune response. Further, there is the ability to knockdown individual signaling molecules and receptors to validate our target and elucidate down stream signaling.

Acknowledgments: Funded by the CIHR (MEMK, Grant no: EY024717).

CB2R MODULATION OF ENDOTOXIN-INDUCED UVEITIS AND THE MAPK PATHWAY IN OCULAR INFLAMMATION

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Introduction: Uveitis is an ocular inflammatory disease that can lead to impaired vision. Experimental endotoxin-induced uveitis (EIU) is generated with intravitreal (IVT) injection of lipopolysaccharide (LPS), a component of gram-negative bacteria. LPS activates the Toll-like receptor 4 (TLR4) on immune and endothelial cells. TLR4 signaling results in phosphorylation of mitogen-activated protein kinase (MAPK) pathways (ERK, JNK and p38 protein kinases). Activation of these MAPKs can activate transcription factor AP-1, or NF-κB increasing pro-inflammatory cytokines with resultant leukocyte recruitment and tissue damage. In the eye, the CB2R agonist, HU308, is anti-inflammatory reducing pro-inflammatory cytokines. Here, we investigate if CB2R-mediated anti-inflammatory effects are due to alterations in the phosphorylation of the MAPKs.

Methods: Five groups of BALB/c mice (n= 8-10 per group) were studied: wild-type (WT) control (saline, IVT), WT EIU (250 ng LPS, IVT), WT EIU + CB2R agonist (HU308; 1.5 μg/μL eye drop), CB2KO control (saline, IVT), and CB2KO EIU. HU308 was given 15 min after EIU induction. As a measure of inflammation, intravital microscopy was used to determine the number of leukocytes in real-time adhering to the endothelium. To determine the immune cell types contributing to the induction of uveitis, clodronate and anti-ly6g antibody were given to animals to deplete monocytes, macrophages and neutrophils, respectively. Results of these experiments demonstrated that macrophages were essential for triggering EIU. Therefore, we used the murine macrophage cell line RAW264.7 cells, to examine CB2R signalling. RAW264.7 cells were stimulated +/- LPS and +/- HU308. In-cell Westerns were performed for ERK, pERK, JNK, pJNK, p38 and pp38. Additionally, *in vivo* experiments used the JNK inhibitor, SP600125 (15mg/kg) to investigate the involvement of JNK in EIU.

Results: Induction of EIU significantly increased the number of leukocytes that adhered to the iris endothelium ($p<0.05$). Topical application of HU308 significantly reduced adherent leukocytes. CB2KO animals with EIU had a significantly increased number of leukocytes in comparison to WT EIU mice, while CB2KO control were not significantly different than WT control. Treatment of mice with clodronate to deplete monocyte/macrophages inhibited LPS-induction of ocular inflammation. HU308 decreased phosphorylation of ERK and JNK in LPS treated RAW264.7 cells. Consistent with this, the JNK inhibitor, SP600125, significantly decreased leukocyte adherence *in vivo* during EIU.

Conclusion: These data demonstrate that LPS-induced EIU inflammation involves macrophage-mediated inflammatory signalling, facilitated in part via JNK activation, leading to leukocyte recruitment in the iridial microcirculation. The anti-inflammatory actions of CB2R activation in EIU were confirmed using CB2R KO animals. These CB2R anti-inflammatory effects may involve inhibition of the phosphorylation of JNK and ERK with resultant reduction in pro-inflammatory cytokines.

PLANT REBUILD: RESEARCH WITH FDA/DEA SCHEDULE 3 (CIII) STATUS BY COMPOUNDING OR REVERSE ENGINEERING C. SATIVA'S CHEMICAL COMPONENTS

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US DEA/FDA classification of C. *sativa's* floral cannabinoids as restricted Schedule 1 (C1) substances has retarded research with this plant. A legal path was found to overcome C1 restrictions for research of the patent application, "Use of cannabinoids and terpenes for treatment of organophosphate (OP) and carbamate toxicity." (Natural plant/synthetic cannabinoids/ECS modifiers vs. man made toxins causing 300,000 deaths a year.) Solution was pharmacy compounding, from non-C. *sativa* floral sources, substances in C. *sativa* flowers. Goal is rapid approvals of cannabinoids/terpenes as neuroprotectants and to reverse miosis, respiratory, and other toxicities from OP insecticides and chemical war nerve agents. THC was sourced from FDA-approved, legally prescribed for off-label use dronabinol 10 mg capsules (CIII). Several kosher-certified food grade terpenes were bought from Sigma Aldrich. CBD was sourced from "Real Scientific Hemp Oil-Gold" = 23-24% CBD (RSHO). RSHO is food grade CBD, from industrial hemp stalk concentrates, bought on Amazon.com and Ebay. After exam of certificates of analysis, labels, sealed containers, dronabinol Rx and bottle, then obtaining IUCAC, legal, and administrative approvals, a board-certified toxicologist at Huntingdon Life Sciences (New Jersey, USA) using pharmacy compounding methods, made protocol defined mixtures of THC, terpenes, and RSHO (CBD). Test articles approximated THC, CBD, and total terpene components, defined ratios and amounts those in analyzed California regulated medical C. *sativa* floral contents and CO2 extracts.

Several such mixtures, diluted in corn oil, were given to SD rats by sniff or gastric gavage prior to SC injection of DFP. (DFP, a reference OP is a proxy for nerve agent sarin. Cannaflavins were not added but could have been.) A peer described the compounded products as "legal plant rebuilds." For quality control, a CBD:6.5::THC:1 blend in corn oil was analyzed by The Werc Shop (WS) in Orange County, CA in mg/gram as: Δ9 THC 0.48, CBD 3.24, CBG 0.11. Testing for pesticides and residual solvents could not be performed. Not detected: THCA, CBN. A specified 5 terpene blend (e.g, α pinene for bronchodilation, myrcene for neuromuscular relaxation, etc.) analyzed by WS had 20.2 mg/g terpene sum; compounding goal was dosing 40 mg/kg. (Reference medical grade *C. sativa* terpene sums were 25-30 mg/g.). α pinene was 6.4 mg/g, myrcene 3.3 mg/g, close to targeted ratio of 2:1. Small amounts (<0.5 mg/g) of unexpected terpenes, eg, humulene 0.17, were detected. Rx dronabinol (CIII THC), CBD, and other materials and compounding costs exceeded medical grade flowers/extracts. However, use of these IUCAC-approved compounded and/or similar test articles, a pseudo plant rebuild, is less restricted and legally less risky than research with *C. sativa* without a Schedule 1 permit. Under the new animal study only rule, FDA approval may be sought for Rx for nerve agent toxicity with off-label FDA approved drugs, e.g dronabinol (C III). Entourage effect aspects may possibly be explored by such compounding.

THERAPEUTIC EFFICACY OF A PERIPHERALLY RESTRICTED CB_1R ANTAGONIST/AMPK ACTIVATOR IN DIET-INDUCED OBESITY/METABOLIC SYNDROME

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Visceral obesity is associated with increased activity of the endocannabinoid/CB_1R system. Globally acting CB_1R antagonists reduce body weight and improve the metabolic complications of obesity, including insulin resistance and fatty liver, but cause neuropsychiatric side effects due to blockade of CB_1R in the CNS. The enzyme adenosine monophosphate kinase (AMPK) promotes fat oxidation and increases insulin sensitivity. In order to improve the safety and efficacy of CB_1R blockade in the treatment of high-fat diet-induced obesity (DIO), we developed a novel bivalent compound, (-)MRI1891, that selectively and potently inhibits peripheral CB_1R and also acts as a direct activator of AMPK. In male, C57Bl/6J mice with DIO, chronic treatment with 0.1–3.0 mg/kg (-)MRI1891 caused dose-dependent reductions in body weight, food intake and liver triglyceride content, improved glucose tolerance and insulin sensitivity, and reversed the hyperglycemia, hyperinsulinemia and hyperleptinemia of DIO mice, with maximal effects observed at 1 to 3 mg/kg. Unlike rimonabant, (-)MRI1891 (3 mg/kg) did not elicit hyperambulatory activity and was not anxiogenic (3-30 mg/kg) in the elevated plus maze test. Both rimonabant and (-)MRI1891 increased total energy expenditure as a result of increased fat oxidation in DIO mice, as assessed by indirect calorimetry. The effect of (-)MRI1891 but not rimonabant was maintained in lean mice, which have low endocannabinoid/CB_1R tone.

We conclude that selective inhibition of peripheral CB_1R coupled with direct activation of AMPK has therapeutic potential for the treatment of the metabolic syndrome.

Acknowledgements: Supported by intramural funds of NIAAA/NIH.

MULTIPLEX qPCR AND CANNABIS MICROBIOME SEQUENCING REVEALS SEVERAL BACTERIA AND FUNGI NATIVE TO CANNABIS FLOWERS

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The Center for Disease Control estimates 128,000 people in the U.S. are hospitalized annually due to food borne illnesses. As a result, the detection of mold and bacteria on agricultural products has become an important safety consideration. This risk extends itself to medical *Cannabis* and is of particular concern with inhaled, vaporized and even concentrated *Cannabis* products. As a result, third party microbial testing has become a regulatory requirement in the medical and recreational *Cannabis* markets.

As many regulations are beginning to mandate "heat killing" of microbial content, we must remain aware of how these drying techniques often counterfeit culture based mechanisms used to monitor colony forming units (CFU). Even though this "heat kill" process may be effective at sterilizing some of the microbial content it does not eliminate various pathogenic toxins like Aflatoxin or the DNA that encodes the Aflatoxin gene. This is of particular importance as Aflatoxin is a carcinogen. The clearance of Aflatoxin requires the liver enzyme CYP3A4 and this liver enzyme is potently inhibited by cannabinoids[1, 2]. With the publication of the *Cannabis* genome and many pathogenic microbial genomes, we designed several multiplexed quantitative PCR (qPCR) assays to detect pathogenic DNA in a background of host *Cannabis* DNA.

One of these qPCR assays is designed to detect total yeast and mold and thus targets the 18S rDNA ITS (Internal Transcribed Spacer) regions. ITS regions are routinely used to sequence the microbiomes of samples and identify and itemize the collection of microbial communities present in a given sample. The addition of next generation sequencing primer tails to this yeast and mold qPCR assay enables reflexive sequencing of samples testing positive for yeast and mold. Next generation sequencing thus reveals a digital record of the microbiological community on a given Cannabis sample. 12 Cannabis samples that tested positive for yeast and mold with qPCR and culture based techniques were sequenced to precisely identify the collection of microbes present in the failing qPCR samples. Microbes harmful to both the plant and humans were identified (Botrytis, Magneporthe, Fusarium and Aspergillus) while over 180 different microbes including endophytes were also present. This qPCR assay presents a real time tool for both quality testing and geospatial monitoring of microbial communities in cannabis production facilities.

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PROFILES OF RECENTLY IDENTIFIED SYNTHETIC CANNABINOIDS: AB-PINACA, AB-CHMINACA, FUBIMINA AND EG-018

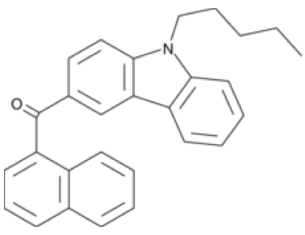
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Despite increasing legislation and public awareness about the hazards of synthetic cannabinoids, uncharacterized compounds continue to be identified in products. In this study, we evaluated four recently identified synthetic cannabinoids: AB-PINACA, AB-CHMINACA, FUBIMINA, and EG-018. All the compounds were evaluated *in vivo* in the tetrad test battery for cannabimimetic effects as well as in THC drug discrimination, an animal model of the subjective effects of psychoactive drugs. *In vitro* analysis of binding affinity and activation of CB_1 and CB_2 receptors was also carried out.

Results showed that all four compounds displaced [^{3}H]CP-55,940 at both the CB_1 and CB_2 receptor sites, with varying affinities. However, only AB-PINACA and AB-CHMINACA stimulated GTP-γ-[^{35}S] turnover with reasonable potency. They also exhibited enhanced efficacy compared to CP55,940.

In the tetrad test battery in adult male ICR mice, only AB-PINACA and AB-CHMINACA produced dose dependent cannabinoid effects: suppression of locomotor activity, antinociception, catalepsy and hypothermia. Although FUBIMINA produced a slight increase in antinociception, it did so only at very high doses. EG-018 had no effect on any measure at doses up to 100 mg/kg. In adult male C57Bl/6J mice trained to discriminate THC (5.6 mg/kg) from vehicle, both AB-PINACA and AB-CHMINACA dose-dependently substituted for THC; however, AB-PINACA only did so at a dose that also severely suppressed response rates. FUBIMINA produced partial substitution, with great variability across the mice tested. EG-018 did not substitute for THC or have any effect on response rate at any dose tested. EG-018 also did not substitute in a separate group of mice trained to discriminate 0.3 mg/kg JWH-018 from vehicle.



EG-018

Given that EG-018 had low nM affinity for the CB_1 receptor, but did not stimulate GTP-γ-[^{35}S] turnover or produce cannabimimetic effects in mice, additional tests are being conducted to more fully characterize this compound. Preliminary *in vivo* results have suggested that EG-018 does not act as an antagonist in the tetrad test battery. Alternative explanations for EG-018's unusual profile of CB_1 receptor binding without activation are currently being explored. Results of these tests will be presented at the meeting.

Acknowledgements: Funded by the U.S. Department of Justice Drug Enforcement Agency and National Institutes of Health / National Institute on Drug Abuse Grant DA-003672.

THE BEHAVIOURAL EFFECTS OF SYNTHETIC CANNABINOIDS AB-PINACA AND AB-FUBINICA IN ADOLESCENT RATS

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Synthetic cannabinoid (SC) behavioural data remains sparse due to the rapid proliferation of these compounds and various ethical and legal limitations regarding their testing in humans. *In vitro* data shows that many SCs have high affinity for CB_1 and CB_2 receptors, and user reports suggest that SCs share many of the effects of the phytocannabinoid Δ9-tetrahydrocannabinol (THC), including catalepsy, anxiety, memory impairment, and mild euphoria. However, *in vitro* data do not always directly correspond to *in vivo* behavioural data, and user reports are inconsistent and often confounded by conjoint drug use.

We therefore investigated the effects of two structurally related synthetic cannabinoids in rats. Rats were chronically dosed (i.p.) every second day with either AB-PINACA or AB-FUBINACA, first with low doses (6 x 0.2 mg/kg) and then with high doses (6 x 1 mg/kg). Acute effects on anxiety, catalepsy and place preference were measured, as were residual effects on memory and social interaction following a 2 week washout. These effects were compared to both a control group given vehicle injections, and to a positive control group given equivalent (as measured by biotelemetry and *in vitro* cell studies) doses of THC (6 x 1 mg/kg and 6 x 5 mg/kg). Rats were then sacrificed for neural and cytokine assessment (presently ongoing).

Rats showed equivalent locomotor impairment for all cannabinoid compounds at both high and low doses. Only AB-FUBINICA produced heightened anxiety at low doses, whereas all compounds increased anxiety at a high dose. AB-FUBINACA caused a mild conditioned place preference at a low dose, but no preference was observed for any compound at a high dose. Rats given AB-FUBINACA exhibited residual memory impairment in the novel object recognition task with a 2 min. inter-trial interval (ITI), and all drug pre-treatments impaired performance with a 60 min. ITI. No residual impairment in social interaction was observed except for rats dosed with THC. All cannabinoid compounds reduced weight gain compared to controls over the dosing period.

These results show that despite equivalence in physiological effects (heart rate, body temperature, rate of weight gain and locomotor activity), AB-FUBINACA produces stronger memory impairment and heightened anxiety, and possibly mild reward at a low dose, compared to both AB-PINACA and THC. Moreover, even a small change in molecular structure - in this case the substitution of a *N*-(4-flurobenzyl) group in place of a *N*-pentyl group - is sufficient to effect this increased activity. This highlights that some synthetic cannabinoids may have greater behavioural effects than indicated by either physiological or *in vitro* studies, but also that some chemical moieties may be important markers for identifying SCs with an increased risk of harm.

ENDOGENOUS 'ENDOCANNABINOID-LIKE' N-ACYL AMINO ACIDS ARE AGONISTS AT THE PUTATIVE CANNABINOID RECEPTOR, GPR55

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The orphan G-protein coupled receptor 55 (GPR55) is a novel lipid-sensing receptor that can be activated by the endogenous lipid, lysophosphatidylinositol (LPI) and also selected cannabinoids, leading to the suggestion that it could be a putative cannabinoid receptor. However GPR55 shares limited homology with the classical cannabinoid receptors (CB_1 and CB_2). Recently a family of bioactive lipids, the N-acyl amino acids, are gaining interest due to their structural similarity to endocannabinoids. However, N-acyl amino acids have little or no affinity for either CB_1 or CB_2 and many have no known biological target at present.

In the present study we have used a previously characterised HEK293 cell line stably expressing recombinant human GPR55 (hGPR55-HEK) to investigate the effects of a subset of N-acyl amino acids with either a serine or a glycine head group on GPR55-mediated Ca^{2+} mobilisation. We find that a chemical series of N-acyl serine and N-acyl glycine ligands are able to promote oscillatory Ca^{2+} transients in these cells, with some ligands generating effects at nanomolar concentrations. Interestingly, the N-acyl serine series were in general more potent and efficacious than the corresponding N-acyl glycine ligands. In control HEK293 cells no responses to the lipid ligands were observed.

This study highlights that structurally-related N-acyl serine and N-acyl glycine lipid ligands can act as agonists in hGPR55-HEK293 cells. Moreover, these data suggest that GPR55 can be activated by multiple endogenous lipid ligands, including those incorporating inositol, serine or glycine head groups.

A PRELIMINARY REVIEW OF 1200 PATIENTS REFERRED TO TWO SPECIALIZED CANNABINOID CLINICS

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The Canadian Marihuana for Medical Purposes Regulation (MMPR) is a new federal program whereby Canadian patients can legally access herbal cannabis with a Medical Document (prescription) provided by their physician.

Under the MMPR, Health Canada authorizes Licensed Producers to provide quality controlled dried marihuana to patients while following strict regulatory requirements. This new framework enables access to cannabis varieties with measured levels of THC and CBD, and potentially other cannabinoids, terpenoids, and flavonoids.

There are few Canadian physicians who are comfortable assessing a patient's suitability for herbal cannabis (less than 10% of physicians have written prescriptions for herbal cannabis) and several physicians who have an interest in cannabinoid medicine have opened specialized clinics to assess, follow, and treat these patients. Currently, there is little information about patients who have been assessed for medical cannabis under this new regulatory framework.

This review is based on a chart review of 1200 patients who have been referred, and treated by a physician at two different specialized clinics. Physicians working at these clinics incorporate all cannabinoids available in Canada into their treatment recommendations: Nabilone (Cesamet), Nabiximols (Sativex) and herbal cannabis. This review will include an analysis of patient demographics, what type of physician made the referral (family physician vs. specialist), referring diagnosis, and suggested treatments. A review of patients' previous cannabinoid use will be reported (recreational and medical) along with analysis of urine toxicology screens.

An analysis of treatment decisions will be presented, including patients who were denied cannabinoid therapy, patients who received a prescription cannabinoid, and those who received combination prescription cannabinoid and herbal cannabis therapies or solely herbal cannabis. A comparison between clinical uses of herbal cannabis varieties with specific THC and CBD profiles will be reviewed and preliminary results of assessment protocols, prescribed dosing regimes, and treatment outcomes will be discussed.

K2.60 AND E3.29: CRUCIAL CHARGED RESIDUES IN THE GPR55 BINDING POCKET

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The research presented details exploration of the GPR55 binding pocket using our recent mutation data and a revised homology model of the activated state of the receptor. Our newly updated model is based on the 1.8 Å crystal structure of the human δ-opioid receptor, with which GPR55 shares many key motifs. In the current model K2.60 and E3.29 are two charged residues in close proximity that face into the GPR55 binding pocket. To evaluate the importance of these two residues to agonist signaling, we measured the signaling produced by the GPR55 agonist ML184 (3-[4-(2,3-dimethylphenyl)piperazine-1-carbonyl]-N,N-dimethyl-4-pyrrolidin-1-ylbenzenesulfonamide, CID2440433) at GPR55 WT and the E3.29L and K2.60A mutants. The assays were run using HEK293 cells that had been transiently transfected, with hGPR55 and a luciferase reporter for serum response element (SRE), twenty-four hours prior to the addition of the GPR55 agonist. Firefly luciferase activity was then quantified six hours after induction with ML184. We found that the EC_{50} of ML184 was reduced from 54nM in WT GPR55 to 192mM in an E3.29L mutant, while ML184 lost the ability to activate a K2.60A mutant despite the presence of the mutant on the cell surface. These results suggest that both K2.60 and E3.29 are important for ML184 signaling. We have hypothesized that these two residues may have direct interaction with one another and together serve as a ligand interaction site. ML184 docking studies are currently in progress to test this hypothesis. The results of the current project will further the understanding of how ligands specifically interact with the GPR55 binding pocket which in turn will facilitate the design and evaluation of more efficacious and potent third generation analogs. [Support: NS077347-A1, DA023204, DA021358]

PHARMACOLOGICAL PROFILING OF GPR55 LIGANDS USING CELLULAR IMPEDANCE ANALYSIS

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L-α-lysophosphatidylinositol (LPI) is the endogenous ligand for GPR55, a $G\alpha_{13}$-coupled GPCR. Further studies are required to elucidate the pharmacology of GPR55 in native cells. The aim of this study was to elucidate the signaling profile of arachidonoyl-LPI (20:4 LPI) in stably expressing GPR55-HEK293 cells and human osteoclasts using cellular impedance analysis (xCELLigence) and to validate this platform as a screening tool to identify novel GPR55 ligands.

Treatment of GPR55-HEK293 cells with 20:4 LPI produced a dose dependent decrease in cellular impedance after 5 min that was absent in HEK293 cells and antagonised by GPR55 antagonists CID1261822 (CID12) and CID16020046 (CID16). The response to 20:4 LPI was completely inhibited by Y27632 after 5 min and significantly attenuated by Bisindolylmaleimide II and PP2 after 20 min, suggesting that the main pathways contributing to the decrease in impedance are Rho, PKC and Src. GPR55 mediated signaling via $G\alpha_i$, $G\alpha_s$, $G\alpha_{q/11}$ or $G_{\beta\gamma}$ were excluded due to the lack of inhibition by pre-treatment with pertussis toxin, SQ22536, U73122 and gallein respectively. Profiling of CID12 and CID16 alone in GPR55-HEK293 cells demonstrated that CID12 is an agonist at GPR55 and CID16 is an inverse agonist at GPR55. CID12 induced a Src dependant decrease in cellular impedance after 30 min that was augmented by Y27632. Similar to GPR55-HEK293 cells, treatment of human osteoclasts with 20:4 LPI produced a dose dependant decrease in cellular impedance attributed to activation of Rho, Src, PKC and ERK. Interestingly, G_i and $G_{\beta\gamma}$ subunits were also shown to partly mediate the signaling response downstream of 20:4 LPI in human osteoclasts, reflective of cell-type specific signaling. Antagonism of 20:4 LPI in human osteoclasts with 1 μM CID12 or 1 μM CID16 was donor dependant. Notably, in some donors CID12 and CID16 were agonists alone. Such studies reveal functional selectivity of compounds at GPR55.

Functional analysis of GPR55 ligands was also performed using the xCELLigence cellular impedance platform. Cell migration was significantly attenuated by 100 nM 20:4 LPI in GPR55-HEK293 cells, an effect that was antagonised by 1 μM CID12 or 1 μM CID16. Conversely, 1 μM 20:4 LPI significantly increased human osteoclast migration. Antagonism with CID12 or CID16 was again donor dependant, and in some donors CID12 and CID16 increased osteoclast migration alone. Mouse osteoclast migration assays confirmed that the increase in osteoclast migration in response to 20:4 LPI was mediated through GPR55. These studies validate the use of impedance technology to screen novel GPR55 ligands and advocate the need for further development of GPR55 antagonists by screening in native cells using functional endpoint assays.

PRO-INFLAMMATORY CYTOKINES UP-REGULATE AND SENSITIZE METABOTROPIC AND IONOTROPIC CANNABINOID RECEPTORS IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS SYNOVIAL FIBROBLASTS

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In collagen-induced arthritis, elevation of endocannabinoid levels improves clinical parameters and decreases synovial inflammation. Joint pain and inflammation are driven by pro-inflammatory cytokines like TNF, which is also involved in sensitizing transient receptor potential channels (TRP). Besides activating cannabinoid receptors type I and II (CB_1 and CB_2), endocannabinoids also bind several TRPs with TRPV1 and TRPA1 being the most important. In this study we demonstrate the influence of TNF, IL-1β and IFN-γ on the expression and function of metabotropic (CB_1 and CB_2) and ionotropic (TRPs) cannabinoid receptors.

Cannabinoid receptor expression was analyzed by western blotting. MMP-3 and cytokines were detected by ELISA. ERK 1/2 and p38 phosphorylation was assessed cell-based ELISA and western blotting. Calcium response was analyzed with Fura-2 staining.

Prolonged incubation with TNF (10ng/ml, 3-7 days) significantly increased CB_1, CB_2, TRPV1, TRPA1, FAAH and COX-2 protein levels. Similar results were obtained with IL-1β and IFN-γ (1ng/ml and 10ng/ml, respectively). TNF-induced sensitization and up-regulation of TRPA1 was confirmed by an increase in intracellular calcium in response to TRPA1 agonist polygodial. While synovial fibroblasts only responded to high doses of TRPA1 agonist (50μM) without TNF pretreatment, TNF incubation (72h, 10ng/ml) not only increased E_{max}, but also lowered the activation threshold of the receptor to 1μM. Furthermore, TNF sensitized synovial fibroblasts to the action of the endocannabinoid anandamide and the GPR55 agonist lysophosphatidylinositol (LPI). In addition, LPI reduced TNF-induced IL-6 and IL-8 production in synovial fibroblasts which was antagonized by the GPR55 antagonist O-1918.

The observed up-regulation of metabotropic and ionotropic cannabinoid receptors by TNF might explain lack of effects of endocannabinoid treatment under healthy/basal conditions. Activation of the cannabinoid receptor system might be an adaptation to the pro-inflammatory environment in rheumatoid arthritis and might help to resolve inflammation.

REPORT ON MEDICAL CANNABIS POTENCY FROM COLORADO USING *UPLC* ANALYSIS

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Quality control laboratories provide important ancillary services for the medical cannabis industry in the United States. Method development for cannabis analysis is on-going in the industry due to increasing demands on laboratories to produce accurate results in a timely and inexpensive manner. Results from analyzing cannabis with a novel method will be discussed. This method can efficiently identify the phytocannabinoids including THC, CBD, CBN, THCA, CBDA, THCV, CBDV, CBG, and CBC. The data demonstrates that Ultra-High Pressure Liquid Chromatograph (UPLC) Waters Acquity Systems are suitable for cannabinoid analysis. The method utilizes both a single wavelength for quantitative analysis and a scan of 200-400nm for spectral analysis that aids in identity confirmation of cannabinoids. This helps ensure accurate identification of cannabinoids and reduces the chances of reporting a false result. Data from analyzing cannabis samples, extracts, and concentrates will be shared.

The work was performed at CannLabs. CannLabs has been analyzing cannabis products for the State of Colorado since April 19, 2010. CannLabs currently has its flagship operation in Denver, CO, with a 2nd location in Hartford, CT and a 3rd location slated to open in North Las Vegas, NV in 2015.

Method Overview:
Instrument – Waters Acquity UPLC H-Class
Mobile Phases – (A) 100% Acetonitrile, (B) 0.1% Formic Acid in 10% Methanol, aqueous, (C) 50% methanol wash
Gradient –

Time	%A	%B
0	28	72
1	28	72
3	30	70
4.5	28	72

Elution order – CBDV, CBDA, CBG, CBD, THCV, CBN, THC, CBC, THCA
Quantitation wavelength – 242nm
Scanning wavelength – 200-400nm

BETA-ARRESTIN BIASED SIGNALING AT THE CB1 RECEPTOR: MODELING THE ORG27569 INDUCED CB1/BETA-ARRESTIN 1 COMPLEX

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The CB1 allosteric modulator, ORG27569 (ORG), is an inverse agonist of the G-protein signaling pathway and agonist of the beta-arrestin-1 pathway (Ahn et.al JBC 2013). Our recent 1.4μs molecular dynamics study of ORG interacting with CB1R via the lipid bilayer revealed that ORG binding at the TMH6-TMH7 interface produces a conformational change in the TMH7-Hx8 region at the intracellular end of CB1 that provides an opening for interaction with beta-arrestin. In the work to be presented, we report microsecond timescale molecular dynamics studies of the formation of the CB1/beta-arrestin-1 complex. The active human beta-arrestin-1 structure used for docking was derived from the phosphorylated V2R C-terminal peptide activated rat beta-arrestin-1 crystal structure (Shukla et.al. Nature 2013) via peptide removal and mutation to the human sequence. CB1 residues, T460/S462/S464/T465/T467/S468, in the distal C-terminus, important for beta-arrestin association (Daigle et.al. J Neurochem 2008), were phosphorylated and placed to interact with beta-arrestin-1 N-domain positively charged residues, including critical lysines K10/K11 (Ostermaier et.al. PNAS 2014). The arrestin finger loop residues 66-70(EDLDV) were modeled as helical, based on photoactivated rhodopsin and visual-arrestin peptide NMR studies (Feuerstein et.al. Biochemistry 2009). Two orientations of beta-arrestin-1 relative to CB1 were modeled to have the N-domain underneath the TMH7/HX8 region based on a recent visual-arrestin-1 fingerloop crystallized in Opsin (Szczepek et.al. Nature Comm 2014), or underneath the TMH5/6 IC3 loop based on a beta-arrestin-1 K77C/beta-2 adrenergic receptor TMH5 K5.78C crosslinking study (Shukla et.al. Nature 2014). MD simulations using AMBER14 with the CHARMM36 forcefield will be presented that test the stability of the two CB1/beta-arrestin-1 complexes in fully hydrated POPC bilayers.
[Support: RO1 DA003934, KO5 DA021358 (PHR)]

CHRONIC FATTY ACID AMIDE HYDROLASE INHIBITOR TREATMENT OF EXCESSIVE HEROIN TAKING AND DEPENDENCE

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The incidence of heroin use and abuse has vastly increased over the past decade in the United States, paralleling a similar trend seen in the abuse of prescription opiates. Opiate dependence is associated with altered brain stress systems and withdrawal-induced anxiety that may contribute to continued use despite efforts to stop. Based on previous evidence that chronic stressors result in upregulation of fatty acid amide hydrolase activity and lowered levels of the endocannabinoid anandamide in key brain regions, we investigated the role of enhancing fatty acid amide signaling on drug intake in dependent rats. Rats were given the fatty acid amide hydrolase (FAAH) inhibitor PF-3845, which results in accumulation of anandamide and other bioactive fatty acid amides.

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 blocked 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. This dose of intravenous PF-3845 failed to alter acute reward thresholds evaluated using an intracranial self-stimulation (ICSS) paradigm. Chronic PF-3845 is currently being evaluated for the ability to alter ICSS thresholds following moderate and excessive heroin self-administration sessions, as well as evidence of interactions on the trademark reward deficits that result after chronic drug abuse. Micropunches of key brain regions integral to heroin reward and stress systems are being analyzed for gene expression changes in response to heroin, or co-treatment with PF-3845.

Acknowledgments: Funding provided by the Pearson Center for Alcoholism and Addiction Research, along with NIH grant K99DA037344.

ENDOCANNABINOID SYSTEM DYNAMICS DURING INDUCED PLURIPOTENCY

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Induced pluripotent stem cells (iPS) provide regenerative medicine with a potentially unlimited source of clinically relevant cell types and, basic research with a powerful model to study reprogramming of cellular identity. Practically, iPS can be produced from most proliferating healthy and diseased cells, gene corrected if necessary, differentiated into any of the three somatic lineages and used for autologous transplantation. Cells and tissue derived from iPS must exhibit exquisite similarity to those they replace. Induced pluripotency results in a cellular revitalization or rejuvenation as indicated by resetting of telomere length, mitochondrial physiology, energy metabolism and epigenetic identity to levels found in embryonic stem cells. Accordingly, quality control of iPS cells is based on their biochemical, physiologic and functional similarity to embryonic stem cells.

The Endocannabinoid System, eCB, is a vital biological system active life-long in many physiological processes and described as a reporter and regulator of cell and organismal homeostasis. Classically defined through identification of the highly conserved seven trans-membrane G-protein coupled cannabinoid receptors, CB1r and CB2r, and endogenous ligands Arachidonylethanolamine (AEA) and 2-Arachidonylglycerol (2-AG), the eCB includes several lipid derived ethanolamine ligands, metabolic enzymes, ion channels and non G-protein coupled receptors such as Transient Receptor Potential Vanilloid receptor family. The eCB has been described in all stem and progenitors cells studied to date and is involved in the same biological processes reset during induced pluripotency. However, little is know about the role of the eCB in somatic cell reprogramming.

Using semi-quantitative and quantitative PCR, we assessed the changes in eCB gene transcription after cellular reprogramming to pluripotency in an inducible murine model system, comparing several fibroblast lines, the resultant iPS clones and 3 Bona Fide embryonic cell lines. The levels of FAAH, CNR1, CNR2, Magl, Dagl, Nape-pld and TRPV1 were assessed. In most iPS clones, eCB genes are variably transcribed to levels found in embryonic stem cells. However, we identified several clones that fail to reactivate individual eCB genes, although the same clones expressed standard pluripotency markers including Nanog, Oct4, Esrrb, alkaline phosphatase and readily formed embryoid bodies. Using LC-MS/MS, eCB ligands AEA, PEA, OEA, 1-AG and 2-AG were measured in iPS clones. In all cases, the eCB ligands were down regulated to levels found in bona fide ES cells. Upon induction of reprogramming, FAAH expression increases rapidly and conversely, CNR1 levels are down regulated. Pharmacological inhibition (URB597) of FAAH, which was re-expressed in all iPS clones, blocked early colony formation. However, clones arising at later time point expressed significantly lower levels of Oct4 and Nanog mRNA although FAAH levels remained unchanged. This effect was blocked neither by AM251 (CB1r antagonist) nor BCTC (TRPV1 antagonist). Cell Cycle analysis of URB597 treated cells indicates an enrichment of cells in Late G2/M phases with a decrease in S phase. We conclude that although several genes are reset during reprogramming, the eCB is not identical when comparing iPS cells with bona fide embryonic stem cells. Furthermore, modulation of specific eCB components during reprogramming may provide for higher quality reprogramming with less oncogenic potential.

INHIBITION OF ENDOCANNABINOID DEGRADATION ENZYME MAGL IMPROVES THE INTESTINAL MICROCIRCULATION IN EXPERIMENTAL SEPSIS

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Sepsis is the systemic inflammatory response to an infection and is associated with tissue hypoperfusion, multi-organ dysfunction and a high mortality rate. The endocannabinoid system has recently emerged as a potential therapeutic target in sepsis treatment due to its immune modulatory functions. We investigated the impact of endocannabinoid degradation enzyme inhibition on the intestinal microcirculation in experimental sepsis using intravital microscopy (IVM).

In the present study, experiments (in 6-8 week-old male C57BL/6 mice) were conducted in five different groups as follows: control, endotoxemia (LPS, 5 mg/kg, i.v.); endotoxemia + endocannabinoid degradation enzyme (MAGL) inhibitor (JZL184, 16 mg/kg, i.v.); endotoxemia + cannabinoid 2 receptor (CB_2R) antagonist (AM630, 2.5 mg/kg, i.v.); and endotoxemia + AM630 + JZL184. Intestinal leukocyte activation (adhesion) and capillary perfusion (functional capillary density – FCD) were studied two hours after LPS administration by IVM.

Inhibition of endocannabinoid degradation by JZL184 showed beneficial impact on the intestinal microcirculation in experimental sepsis by reduction of leukocyte adhesion and improvement of FCD. In animals treated with the CB_2R antagonist AM630 before JZL184 administration those effects were abolished.

CB_2R activation as shown in our experiments by inhibition of the endocannabinoid degradation enzyme MAGL seems to be a potential therapeutic target in sepsis.

Acknowledgements: Funded by the Nova Scotia Health Research Foundation (file no. MED-Project 2011-7475)

INHIBITION OF MONOACYLGLYCEROL LIPASE SYSTEMICALLY AND IN THE CENTRAL AMYGDALA AND VISCERAL INSULAR CORTEX PREVENTS THE AVERSIVE PROPERTIES OF ACUTE MORPHINE WITHDRAWAL IN RATS

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Activation of the endocannabinoid system, through elevation of anandamide (AEA) and 2-arachidonylglycerol (2-AG), has been shown to reduce somatic symptoms of morphine withdrawal in mice (Ramesh et al., J. Pharmacol. Exp. Ther. 339(2011) 173-185; Ramesh et al., Neuropsychopharmacology 38 (2013) 1039-1049). However, the effect of such treatments on the affective properties of morphine withdrawal has not been investigated in rats. Acute morphine withdrawal induced place aversion occurs when naloxone is administered 24 hr following a single exposure to a high dose of morphine (Parker and Joshi, Pharmacol. Biochem. Behav. 61 (1998) 331-333). Here we demonstrate that systemic pretreatment with the monoacylglycerol lipase (MAGL) inhibitor, MJN110 (which selectively elevates 2-AG), but not the fatty acid amide hydrolase (FAAH) inhibitors, URB-597 and PF-3845 (which selectively elevate AEA), prevents the aversive effects of acute morphine withdrawal. The effect of MJN110 to interfere with withdrawal was reversed with pretreatment of the CB_1 antagonist AM251, and MJN110 administration alone did not possess rewarding or aversive properties in the place conditioning paradigm. Furthermore, central administration of MJN110 to the central nucleus of the amygdala (CeA) or to the interoceptive or visceral insular cortex (VIC), regions known to be activated in acute morphine withdrawal, also prevents the establishment of the place aversion. These findings suggest that treatments that activate 2-AG may be useful in reducing the aversive effects of morphine withdrawal.

Acknowledgements: Funded by grants from the Natural Sciences and Engineering Council of Canada (NSERC: 92057), Canadian Institutes of Health Research (CIHR:137122) to LAP, National Institutes of Health (DAO32933,DAO33760) to BFC.

INHIBITION OF FATTY ACID AMIDE HYDROLASE (FAAH) REDUCES ACUTE AND ANTICIPATORY NAUSEA IN RATS AFTER ORAL DOSING

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Inhibition of fatty acid amide hydrolase (FAAH) by URB597 (0.3 mg/kg, IP) reduces nausea-induced conditioned gaping, and contextually-elicited nausea-induced conditioned gaping (rodent models of acute and anticipatory nausea, respectively) presumably through elevation of anandamide, acting at CB_1 receptors (Rock et al., Psychopharmacology (Berl). 196 (2008) 389-95). To date, oral dosing of FAAH inhibitors has not been evaluated in these models, but is an important consideration in drug development. Therefore, we investigated the potential of IW-7229, an orally active indole ketoamide FAAH inhibitor to interfere with acute and contextually-elicited nausea-induced conditioned gaping and whether the CB_1 receptor mediated these effects.

IW-7229 (10, 30, 100 mg/kg but not 3 mg/kg) significantly reduced nausea-induced conditioned gaping reactions (acute nausea), and the effect was blocked by administration of CB_1 antagonist SR141716, suggesting a CB_1 receptor mediated mechanism of action. Additionally, IW-7229 (10, 30 mg/kg, but not 100 mg/kg) reduced contextually-elicited gaping reactions (anticipatory nausea), without producing any locomotor effects at the effective doses. Administration of SR141716 blocked the effect, indicating a CB_1 receptor mediated mechanism of action.

These findings suggest that orally administered IW-7229, effectively reduces acute and anticipatory nausea in animal models, likely by enhancing endogenous anandamide through FAAH inhibition, to exert its anti-nausea effect via action at CB_1 receptors. The current non-specific benzodiazepines prescribed for chemotherapy patients with anticipatory nausea, can have debilitating sedative effects, however, FAAH inhibition by IW-7229 may be a candidate treatment, devoid of these undesirable sedative effects.

Acknowledgments: This research was funded by Ironwood Pharmaceuticals.

RESULTS FROM AUDITING MEDICAL CANNABIS OPERATIONS IN THE UNITED STATES

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Regulation is becoming mandatory in states that allow medical cannabis. The producers, manufacturers, dispensaries, and laboratories involved in this industry can operate legally in their states but function without much regulation or oversight. Due to increasing concerns over the need to standardized medicinal cannabis preparations, the American Herbal Product Association (AHPA) has created industry guidelines on manufacturing, producing, dispensing, and laboratory operation standards. Additionally, the American Herbal Pharmacopeia (AHP) completed the Cannabis monograph, a guide for the standardization of cannabis. The work of AHPA and AHP laid the foundation for a certification body called Patient Focused Certification (PFC) a project of Americans for Safe Access. AHPA and AHP guidelines are being incorporated into state level regulations as mandatory product safety standards in new state programs. PFC launched in early 2014 with facilities in several states having successfully completed the auditing process. Over a dozen operations have been certified in over 8 states. Results from over a year of auditing of medical cannabis facilities will be discussed, including data on corrective actions with research on the impact of such regulations on patients, facilities, government, universities, and neighborhoods.

NOVEL GPR55 CHROMENOPYRAZOLE LIGANDS: SYNTHESIS AND BIOLOGICAL EVALUATION

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The putative cannabinoid receptor GPR55 represents a possible target for the treatment of various diseases. Its role in inflammation, neuropathic pain, bone physiology, diabetes and cancer is currently explored. Nonetheless, the lack of potent and selective agonists and antagonists of GPR55 is delaying the exploitation of such a promising therapeutic target. Moreover, GPR55 pharmacology reveals serious inconsistencies between studies and functional outcomes.[1] Consequently, it becomes essential to focus our efforts in the discovery of novel GPR55 chemotypes that may enable the development of adequate research tools to study the biological relevance of this receptor.

In this context, we have designed and synthesized novel GPR55 ligands based on chromenopyrazoles scaffold. Structural features for GPR55 activity were determined according to structural requirements previously described in the literature[2,3] for a series of GPR55 modulators discovered from virtual screening. Appraisal of GPR55 activity of the new compounds was accomplished by using an innovative cell-impedance based assay in GPR55-HEK293 cells. The real-time impedance responses provide an integrative assessment of the cellular consequence to GPR55 stimulation. As well, the ability to antagonize the endogenous ligand LPI was evaluated. Dose-response experiments were also performed in normal HEK293 cells. Additionally, selectivity towards the classical cannabinoid receptors was explored by CB_1 and CB_2 radioligand binding experiments. From these series of chromenopyrazoles, certain derivatives are selective potent partial agonists of GPR55. Some of them are also able to inhibit LPI response when co-administered.
In summary, we have identified a novel GPR55 scaffold that may serve to develop appropriate pharmacological tools or novel drugs to continue with the challenging goal of the validation of this receptor.

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Acknowledgements: Supported by Spanish Grant SAF2012-40075-C02-02 and CANNAB-CM S2011/BMD-2308. P.M. is recipient of a fellowship JAE-Pre-2010-01119 from CSIC co-financed by FSE.

METABOLIC INTERPLAY BETWEEN ASTROCYTES AND NEURONS REGULATES ENDOCANNABINOID ACTION

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The endocannabinoid 2-arachidonoylglycerol (2-AG) is a retrograde lipid messenger that modulates synaptic function, neurophysiology, and behavior. 2-AG signaling is terminated by enzymatic hydrolysis—a reaction that is principally performed by monoacylglycerol lipase (MAGL). MAGL is broadly expressed throughout the nervous system, and the contributions of different brain cell types to regulating 2-AG activity have remained unclear. Here, we genetically dissect the cellular anatomy of MAGL-mediated 2-AG metabolism in the brain and show that neurons and astrocytes coordinately regulate 2-AG content and endocannabinoid-dependent forms of synaptic plasticity and behavior. We also find that astrocytic MAGL is mainly responsible for converting 2-AG to neuroinflammatory prostaglandins via a mechanism that may involve transcellular shuttling of lipid substrates. Astrocytic-neuronal interplay thus provides distributed oversight of 2-AG metabolism and function, and, through doing so, protects the nervous system from excessive CB_1 receptor activation and promotes endocannabinoid crosstalk with other lipid transmitter systems.

Acknowledgements: This work was supported by the National Institutes of Health grants DA033760, DA032933 (B.F.C.), GM109315 (A.V.), DA037344 (J.E.S.), DA035217 and MH101146 (Q.-S.L.), and DA030404 (L.J.S. and D.E.S.).

THE NOVEL CANNABINOID ENZYME INHIBITOR IPI-0595 BLOCKS NEUROPATHIC PAIN AND GASTRIC INFLAMMATION

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Irreversible monoacylglycerol lipase (MAGL) inhibitors increase brain levels of 2-AG and attenuate pain in many preclinical models. However, reversible enzyme inhibitors have fewer deleterious side effects than irreversible inhibitors. Thus, the therapeutic potential of MAGL inhibition has been limited because only irreversible MAGL inhibitors exist. In the present study, we tested the hypothesis that a novel reversible MAGL inhibitor, IPI-0595, would attenuate neuropathic pain and block gastric hemorrhages caused by the nonsteroidal anti-inflammatory drug diclofenac sodium. Mice were subjected to chronic-constriction injury (CCI), in which the sciatic nerve is loosely ligated to induce touch and cold sensitivity in the hind paw (termed allodynia). IPI-0595 significantly attenuated CCI-induced mechanical and cold allodynia. In line with published data from irreversible MAGL inhibitors, the CB_1 antagonist, Rimonabant, blocked the anti-allodynic effects of IPI-0595, indicating that CB_1 is required. Conversely, the CB_2 antagonist, SR144528, had no effect, indicating that CB_2 is dispensable. Another cohort of mice was fasted and administered diclofenac, which induced gastric hemorrhages. However, IPI-0595 treatment almost completely blocked these diclofenac-induced gastric hemorrhages. The results of the present study indicate that IPI-0595, the first reversible MAGL inhibitor, attenuates both pain and inflammation *in vivo*. It is noteworthy that IPI-0595 was administered orally in the present studies. Thus, reversible MAGL inhibitors may represent an effective class of gastroprotective analgesics.

THE EFFECTS OF LITHIUM CARBONATE SUPPLEMENTED WITH NITRAZEPAM ON SLEEP DISTURBANCE DURING CANNABIS ABSTINENCE

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Study Objectives: Sleep disturbance is a hallmark feature of cannabis withdrawal. In this study we explored the effects of lithium treatment supplemented with nitrazepam on objective and subjective measures of sleep quality during inpatient cannabis withdrawal.

Methods: Treatment-seeking cannabis-dependent adults (n=38) were admitted for 8 days to an inpatient withdrawal unit and randomized to either oral lithium (500 mg) or placebo, twice daily in a double blind RCT. Restricted nitrazepam (10 mg) was available on demand (in response to poor sleep) on any three of the 7 nights. Dependent outcome measures for analysis included repeated daily objective actigraphy and subjective sleep measures throughout the 8 day detox, subjective cannabis withdrawal ratings, and detoxification completion rates.

Results: Based on actigraphy, lithium resulted in less fragmented sleep compared to placebo (P=0.04), but no other objective measures were improved by lithium. Of the subjective measures, only nightmares were suppressed by lithium (P=0.04). Lithium did not significantly impact on the use of nitrazepam. Sleep bout length (P<0.0001), sleep efficiency (P<0.0001), and sleep fragmentation (P=0.05) were improved on nights in which nitrazepam was used. In contrast, only night sweats improved with nitrazepam from the subjective measures (P=0.04). A cox regression with daily repeated measures of sleep efficiency averaged across all people in the study a predictor suggests that a one unit increase in sleep efficiency (the ratio of Total Sleep Time to the total time in bed expressed as a percentage) resulted in a 14.6% increase in retention in treatment (P = 0.008, Exp(B) = 0.854, 95% CI = 0.759 – 0.960). None of the other sleep measures, nor use of lithium or nitrazepam were significantly associated with retention in treatment.

Conclusions: Lithium seems to have only limited efficacy on sleep disturbance in cannabis withdrawal. However the nitrazepam improved several actigraphy measures of sleep disturbance warranting further investigation. Discord between objective and subjective sleep indices suggest caution in evaluating treatment interventions with self-report sleep data only.

Acknowledgments: Funded by a project grant from the Australian National Health and Medical Research Council (Project Grant 556301).

INHIBITORY EFFECT OF ASPIRIN-TRIGGERED RVD1 ON SPINAL NOCICEPTIVE TRANSMISSION IN ACUTE INFLAMMATORY PAIN

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Understanding roles of endogenous bioactive lipids e.g. polyunsaturated fatty acid derivatives and arachidonic acid metabolites in the modulation of pathological pain could provide a platform for analgesic drug discovery. The present study demonstrated the inhibitory effect of aspirin-triggered RvD1 (AT-RvD_1) on spinal nociceptive processing and changes of genes expression related to both resolvin and endocannabinoid systems in acute inflammatory pain.

Single unit *in vivo* electrophysiology recording of deep dorsal horn wide dynamic range (WDR) neurons at lamina L4-L5 region was performed at >24 hour post intraplantar carrageenan or saline injection. WDR activities were recorded after electrical evoked transcutaneous stimulation delivered every 15 min to the corresponding receptive field of ipsilateral hindpaw prior to and for 1 hour post direct spinal application of 15 ng/μL AT-RvD_1 or phosphate buffer saline (PBS) as a vehicle. AT-RvD_1 significantly inhibited electrically evoked activity of WDR neurons in carrageenan-treated animals, specifically C- and Aδ fibre evoked responses when compared to PBS; the maximal inhibition (mean±S.E.M.) were; 29.33±5.33 vs 7.8±8.22, $p<0.05$ and 26.80±6.12 vs -17.00±11.76, $p<0.05$, respectively. Suppression was observed within 30 min. post application. The number of action potential per stimulus at 15 min post application were also significantly suppressed by AT-RvD1 ($p<0.01$ for slope difference vs PBS in stimulus-response curve); however such effect was not observed at the later time points. AT-RvD1 did not alter evoked responses of WDR neurones in non-inflamed animals, suggesting acute inflammation results in an alteration in this signalling pathway.

Gene expression analysis of the ipsilateral spinal dorsal horns at the similar time point of electrophysiology recording demonstrated significant changes in carrageenan-treated animals including, increased chemokine-like receptor 1 (ChemR23), a RvE_1 receptor and 5-lipoxygenase activating protein (FLAP) mRNA, and decreased 15-lipoxygenase (15-LOX).

Ongoing studies will determine the underlying mechanisms of the differential inhibition of AT-RvD_1 in this particular model and to investigate the complex changes of endogenous lipids (resolvins and endocannabinoids) and related molecules in both acute and chronic pain models.

Acknowledgements: Funded by the Ministry of Sciences and Technology, Thai Government and supported by the Arthritis Research UK Pain Centre

CB_1 ALLOSTERIC MODULATOR ORG27569 ATTENUATES CP55940 INDUCED ERK1/2 PHOSPHORYLATION IN HEK293 CELLS STABLY EXPRESSING THE HUMAN CB_1 RECEPTOR

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While the cannabinoid type-1 receptor (CB_1) is a promising pharmacological target for the treatment of numerous diseases, the psychotropic effects which occur following therapeutic doses of orthosteric agonists are a major impediment to the development of new medicines. Allosteric modulation of G-protein coupled receptors provides a means whereby alteration of the receptor's conformation may allow for functional selectivity of specific signaling pathways. This could allow for the selective activation of pathways that are more important for the therapeutic effects of cannabinoids.

While previous studies have reported increases in phospho-ERK1/2 levels following treatment with the CB_1 allosteric modulator Org27569, recently it was reported that in CHO cells, Org27569 did not increase phospho-ERK1/2 levels and also fully blocked CP55,940 stimulated ERK1/2 activation (Khajehali et al., ICRS 2014 poster P1-47). The present study sought to determine the effects of Org27569 in another cell line (HEK293) stably expressing the human CB_1 receptor.

HEK293 cells stably expressing the hCB_1 receptor were grown in Dulbecco's Modified Eagle's Medium supplemented with 5% FBS and cultured at 37° C in 5% CO2. Cells were seeded on 6-well plates at a density of 200,000 cells/well, serum starved for 24 h and were 80-90% confluent at the time of experimentation. All drug treatments were done in serum free DMEM at 37° C for 5 min in length. CP55,940 maximally increased phospho-ERK1/2 levels approximately 12-fold (EC_{50} = 1.5 nM). When tested against the EC_{80} (6.1 nM) of CP55,940, Org27569 fully antagonized (IC_{50} = 165 nM) the increase in phospho-ERK1/2.

These data support the previous report that Org27569 attenuates CP55,940 stimulated activation of ERK1/2. Future studies will examine whether these effects extend to animal models.

Acknowledgements: Research support from NIDA grants T32-DA007237 and P30-DA013429

HOW OUR MOUTH FEEDS OUR BRAIN: EFFECTS OF ENDOCANNABINOID MODULATION ON SWEET TASTE PERCEPTION AND LIKING IN HUMANS

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Everyday experience suggests that we often eat in the absence of hunger. Especially highly palatable foods can tempt us into eating even when satiated. Animal studies suggest that the endocannabinoid (eCB) system plays a major role in altering food reward, and thereby increases intake of sweet and palatable foods, but this has not been studied in humans. Therefore, the current study aimed at clarifying why sweet food intake increases by assessing if cannabinoids modulate sweet taste intensity and liking in humans.

The study had a randomized, placebo-controlled, double-blind, cross-over design. Participants came to the test location for three test sessions which were two weeks apart. During each test session participants received two doses of 250 mg Cannabis sativa, containing THC, cannabidiol (CBD) or placebo by use of a MINIVAP vaporizer. The first dosage contained 4 mg of THC or 25 mg of CBD, the second, top-up, dosage was administered 35 minutes after the first dosage aiming to maintain effects and contained 1 mg THC or 10 mg CBD.
Ten minutes after the first administration, participants received seven chocolate milk-like drinks that varied in sugar content. The order of the drinks was randomized over the participants, but kept constant over the different test sessions. Participants were asked to rate liking and sweet taste intensity of the seven drinks on respectively a labelled affective magnitude scale and a general labelled magnitude scale. Following this, a control task assessed general perception by asking participants to rate the greyness of seven shades of grey on a 100 mm visual analogue scale ranging from white to black. This was followed by a ranking task, in which participants were presented with the same seven drinks. They were asked to rank the drinks on liking. Throughout each test session, subjective effects of the administered Cannabis sativa were assessed using a 100 mm visual analogue scale.

Preliminary data of six healthy male incidental cannabis users (average age 24.3 years, *SD* = 1.0; average BMI 21.7 kg/m^2, *SD* = 1.2) showed no effect of THC or CBD on the perception of the sweet taste intensity and the liking of the different drinks. In addition, there was no effect of treatment on ranking. Perception of the different shades of grey did not differ between the three different treatments, indicating that general perception did not change when either THC or CBD was administered. Participants indicated that they felt more ‘high’ as the test session continued, but this was irrespective of treatment. This indicates that participants could not distinguish between the administered compounds. Our results so far do not provide evidence whether modulation of the eCB system has acute effects on sweet taste perception. It could be that the study so far has been underpowered. Results of more participants will be presented at ICRS2015.

EXPLORING THE 'MUNCHIES': WHAT CAN WE LEARN FROM CANNABIS USERS ABOUT THE ROLE OF THE ECS IN HEDONIC HUNGER

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The endocannabinoid system (ECS) is a key player in the control of food reward and hedonic hunger. This is reflected by a phenomenon denoted the 'munchies' in popular culture: A feeling of increased, sometimes voracious appetite experienced by cannabis (marijuana) users following intoxication, even when previously satiated. The 'munchies' is ascribed to delta9-Tetrahydrocannabinol (THC) and its actions on the ECS, and albeit a well-known phenomenon, it is poorly researched. In this study, we used a qualitative approach (in-depth interviews) to characterise the exact nature of the 'munchies' in terms of sensory alterations (taste, smell, texture), modulation of appetite and intake and shifts in food preferences, by collecting detailed information from human cannabis users.

Eight experienced cannabis users (5M/3F, aged 23-50 years) participated. First, there was a home-assignment where participants were asked to collect 5 pictures/photos of foods/dishes they typically craved for when on munchies. Second, participants came in for a 1 hour audio-recorded interview. A hybrid interview technique was used, combining laddering questions (means-end chains) and discussing the pictures brought in by the participant. Recordings were transcribed verbatim, followed by content-analysis, identification of key concepts and ladders used to create a hierarchical value map (HVM) in LadderUX©. Pictures of 'munchies-food' were explored based on their sensory characteristics (taste, texture, appearance).

Results revealed users experience an almost 'insatiable' appetite, regularly resulting in overeating till nausea. They report a liking of foods that are highly rewarding but also easy and quick to prepare/eat as they want instant satisfaction of their hunger. Sensory drivers of preference were sweet taste, but even more so savoury and fatty/greasy taste/mouthfeel. Interestingly, the munchies induce a desire for both textural and food temperature contrasts, for example a combination of both crunchy and soft texture, and warm and cold/fresh sensations, e.g. a hamburger on a crispy bun, with fresh salad, tomato and sauce. Alterations in sensory perception mainly involved increased intensity (of basically all tastes and odours), whereas taste quality shifts were hardly reported. The pictures participants selected of their favourite foods/dishes when on munchies, confirmed this strong preference for savoury fatty foods with textural and temperature contrasts (crunchy + soft, warm + cold/fresh), whereas sweet, high energy foods (e.g. candy bars, chocolate) were selected less frequently than we expected.

In conclusion, this qualitative study in human cannabis users reporting about their 'munchies' experiences resulted in several new insights in the exact manifestations in terms of sensory alterations, modulation of appetite and shifts in food preferences. This information is highly valuable as it can drive specific hypotheses regarding the modulating role of the ECS in the control of appetite and hedonic hunger in humans to guide future experimental research.

FATTY ACID HYDROLASE (*FAAH*) GENOTYPE PREDICTS BASELINE MOOD AND SUBJECTIVE RESPONSES TO *D*-AMPHETAMINE IN HEALTHY YOUNG ADULTS

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Central levels of endocannabinoids are in part controlled by fatty acid amide hydrolase (FAAH), which degrades anandamide. Preclinical and clinical studies studies suggest that a single nucleotide polymorphism in the *FAAH* gene (C385A; rs324420) predicts stress reactivity, responses to threat, and anxiety in humans and other animals. The A allele of this gene results in lower levels of the FAAH enzyme, and therefore increased endocannabinoid signaling. This variant has also been associated with reduced risk for drug addiction and obesity. However, its relationship to baseline mood states in healthy adults, or in relation to acute responses to stimulant drug have not been determined. In this study, healthy young adults (N=398) attended 3 laboratory sessions during which they received oral *d*-amphetamine (10mg, 20mg) or placebo in randomized order. They provided blood samples for genotyping, and they reported on their current mood states and subjective drug effects before and after receiving the drug. Personality measures, and cardiovascular measures of drug effects were also obtained. Carriers of the A allele reported significantly less baseline anxiety and lower negative emotionality on a personality questionnaire. They also reported significantly less 'wanting more' after *d*-amphetamine (20mg) administration. The polymorphism was unrelated to cardiovascular responses to the drug. These findings are consistent with previous studies showing that this variant is related to negative mood states, and may be protective against the development of addictive disorders, including drug abuse.

Funded by the National Institute on Drug Abuse (DA02812). Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

CANNABIS AND AGGRESSION: THE IMPORTANCE OF CANNABIS USE MOTIVES AND SUBTYPES OF AGGRESSION

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The relationship between cannabis use (CU) and aggression has been extensively researched; however, empirical findings related to this association are equivocal. These inconsistent findings may reflect heterogeneity in aggressive behavior, as past research has not differentiated among subtypes of aggression when looking at the influence of CU. The distinction between proactive and impulsive aggression has been identified as important for parsing heterogeneity in aggressive behaviour. Proactive aggression is characterised by planned and unprovoked behaviour that is a means to an end, whereas reactive aggression is characterised by impulsive and defensive responses to provocation or frustration. In the present study we examined the extent to which the consideration of heterogeneity might elucidate the relationship between CU and aggression. Specifically, we examined associations between cannabis use and cannabis use motives, and indices of reactive and proactive aggression.

A total of 315 adults completed our online survey. The sample was 73% female and were 39% cannabis users. We found that cannabis users did not differ from non-users with regard to either type of aggression. Analyses of cannabis users found that motives were distinctly associated with both forms of aggression. Proactive Aggression was associated with Coping Motives (ß = .57, SE = .15, t = 3.73, R^2 = .10, p <.01), whereas Reactive Aggression was associated with Social Motives (ß = .50, SE = .19, t = .67, R^2 = .05, p <.01), Coping Motives (ß = .66, SE = .19, t = 3.51, R^2 = .09, p <.01) and Conformity Motives (ß = .90, SE = .32, t = 2.85, R^2 = .06, p<.01). Multivariate analyses identified suppressor relationships between motives and Reactive Aggression, with an accentuated unique positive relationship with Coping Motives (ß = .99, SE = .19, t = 5.19, $R^{2\Delta}$ = .15, p <.01) and an inverse association with Enhancement Motives (ß = -.44, SE = .15, t = -3.04, $R^{2\Delta}$ = .05, p <.01) when both motives were included together in the model. This distinct pattern of association suggests that attention to cannabis use motives might help to elucidate the relationship between cannabis use and aggression.

MEASURING CANNABIS CONSUMPTION: AN INTRODUCTION TO THE FREQUENCY, AGE OF ONSET, AND QUANTITY OF CANNABIS USE INVENTORY

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Currently there are few self-report inventories available to measure frequency of cannabis use, quantity of use, and age of onset of cannabis use. While frequency and age of onset of cannabis use are rather straightforward to measure, the measurement of quantity of cannabis used is complicated by numerous factors. These include the different types of cannabis used (marijuana, hashish, concentrates), the different potencies of cannabis, the different methods of ingestion (e.g., joints, bongs, edibles, vaporizers) and the social nature of cannabis use. Moreover, while age of onset of cannabis use is increasingly being recognized as an important variable to consider, most cannabis use inventories do not contain items pertaining to age of onset.

Due to the limitations of the current inventories designed to measure frequency and quantity of cannabis use we developed the Frequency, Age of Onset, and Quantity of Cannabis Use Inventory (FAQ-CU). The FAQ-CU is the first cannabis use inventory to use pictures of different quantities of actual marijuana (1/8 gram, ¼ gram, ½ gram, ¾ gram, 1 gram) in bud form, loose form, and joint form to facilitate the identification of the quantity of cannabis typically used. The FAQ-CU also measures a variety of methods of ingesting cannabis (e.g., joints, blunts, bongs, pipes, hookahs, vaporizers, edibles) as well as the typical THC levels in the cannabis used. The FAQ-CU also contains several items designed to assess age of onset of use. Finally, to our knowledge the FAQ-CU is also the first inventory to measure the use of cannabis concentrates (oil, wax) which are becoming increasingly popular and whose effects, relative to traditional forms of marijuana, are entirely unknown.

We are currently administering the FAQ-CU along with established measures of cannabis abuse (The Cannabis Abuse Screening Test), cannabis use disorders (The Cannabis Use Disorders Identification Test), cannabis dependence (The Severity of Dependence Scale), problems associated with cannabis use (The Cannabis Use Problems Identification Test, The Marijuana Screening Inventory), and frequency and quantity of use (The Timeline Follow Back Questionnaire, The Marijuana Smoking History Questionnaire) to a student sample ($N > 1000$).

Preliminary results based on 153 respondents revealed that the frequency subscale of the FAQ-CU significantly predicts cannabis use disorders ($r = .78$), abuse ($r = .75$), dependence ($r = .29$) and problems associated with use ($r = .63$ to $r = .88$). Age of onset of use was also found to significantly predict cannabis use disorders ($r = -.34$), abuse ($r = -.40$), and problems associated with use ($r = -.32$ to $r = -.36$). Finally, the quantity subscale of the FAQ-CU also significantly predicts cannabis use disorders ($r = .54$), abuse ($r = .53$), dependence ($r = .19$) and problems associated with use ($r = .37$ to $r = .58$). Moreover, the FAQ-CU offers superior ability to predict these problems than comparable items from the Marijuana Smoking History Questionnaire (for which correlations range from $r = .06$ to $r = .64$). It also offers substantially better ability to predict each of these problems than the Timeline Follow Back Questionnaire (for which correlations range from $r = .07$ to $r = .47$).

INFLAMMATORY ARTHRITIS-INDUCED HYPERALGESIA AND PAIN-SUPPRESSED BEHAVIOR ARE ATTENUATED BY THE MONOACYGLYCEROL LIPASE INHIBITOR JZL184

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Rheumatoid arthritis (RA) is a debilitating chronic inflammatory joint disease that leads to chronic joint pain and decreased mobility. Current RA analgesic and anti-inflammatory treatments are ineffective or induce negative side effects. Cannabinoids and endocannabinoids have antihyperalgesic and anti-inflammatory properties. The most prevalent endocannabinoid, 2-arachidonoyl glycerol (2-AG), is catabolized by the enzyme monoacylglycerol lipase (MAGL). Pharmacological inhibition of MAGL increases brain levels of 2-AG and significantly decreases acute inflammatory pain. The present study tested the hypothesis that MAGL inhibition decreases hyperalgesia, and pain suppressed behavior, caused by collagen-induced arthritis (CIA), a well-established animal model of inflammatory arthritis. To induce CIA, male DBA1 mice were immunized with an emulsion of collagen and complete Freund's adjuvant. CIA caused arthritic symptoms (i.e. paw redness and swelling), and significantly decreased spontaneous locomotor activity and motor coordination, as tested in the rotarod. We investigated the antihyperalgesic effects of the selective MAGL inhibitor JZL184 in mice subjected to CIA. The hotplate and tail immersion assays were performed, to test CIA-induced thermal hyperalgesia. Thermal hyperalgesia was significantly attenuated by JZL184 (8 or 40 mg/kg, ip) on both the hotplate and tail immersion assays. CIA-induced pain suppression of locomotor activity and motor coordination were also challenged with JZL184. JZL184 (8 mg/kg, ip) reversed pain-suppressed spontaneous locomotor activity in mice subjected to CIA. In the rotarod, JZL184 (40 mg/kg) had no significant effect on motor coordination in either CIA-treated or control mice. These results suggest that MAGL inhibition may be a promising strategy for the treatment of pain caused by inflammatory arthritis.

THE ART AND SCIENCE OF INHALING - A REVIEW OF VAPORIZING AS ADMINISTRATION FORM FOR CANNABINOIDS

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Smoking of cannabis remains a major administration form for medicinal cannabis users worldwide, despite well-known health risks. Cannabis ‘‘vaporization’’ or ‘‘volatilization’’ is a technique aimed at suppressing irritating respiratory toxins by heating cannabis to a temperature where active cannabinoid vapors are formed, but below the point of combustion where pyrolytic toxic compounds are made. Vaporizing offers patients who use medicinal cannabis the advantages of the pulmonary routes of administration, i.e.: rapid delivery into the bloodstream, ease of self-titration, and concomitant minimizing the risk of over- and under-dosing, while avoiding the respiratory disadvantages of smoking. Over the years many different vaporizer devices have been developed, ranging from the medically approved Volcano Medic (Storz&Bickel) to barely legal pen-vaporizers. Despite the booming market, virtually nothing is known about most devices with regard to safety or efficacy. Some smaller vaporizers need to be used in combination with pre-filled cartridges, containing unclear quantities and compositions of cannabinoids and other chemicals. Clearly, vaporizing as medical administration form is in need of a risk-benefit analysis.

In this presentation, a full review is provided of the history and development of vaporizing. Over the last decade, our research team has added significantly to the scientific literature in this field. These studies range from the first laboratory validation studies on the Volcano vaporizer (1) and comparisons between vaporizing and smoking (2), to PKPD studies on pure THC (3) and placebo-controlled clinical trials with herbal cannabis (4). As the scientific understanding of medicinal cannabis increases, so must our demands of vaporizing grow. In this review we will look beyond THC, to include combinations of cannabinoids (e.g. THC + CBD), the role of terpenes, presence of contaminations such as pesticides, and the formation of degradation products as a result of improper heating. Finally, the presentation will focus on the very recent development of pre-dosed vaporizer systems such as the Syqe inhaler (Syqe Medical). Also, our latest research data is presented on the validation of the miniVap vaporizer (Hermes Medical Engineering). This review will provide the audience with the knowledge and means to properly apply vaporized administration forms of cannabis in their (pre)clinical research.

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2) Pomahacova et al. Inhal. Toxicol. 21 (2009) 1108–1112
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DEVELOPMENT OF NOVEL INHIBITORS FOR THE FATTY ACID BINDING PROTEINS TO ALTER THE ENDOCANNABINOID TONE

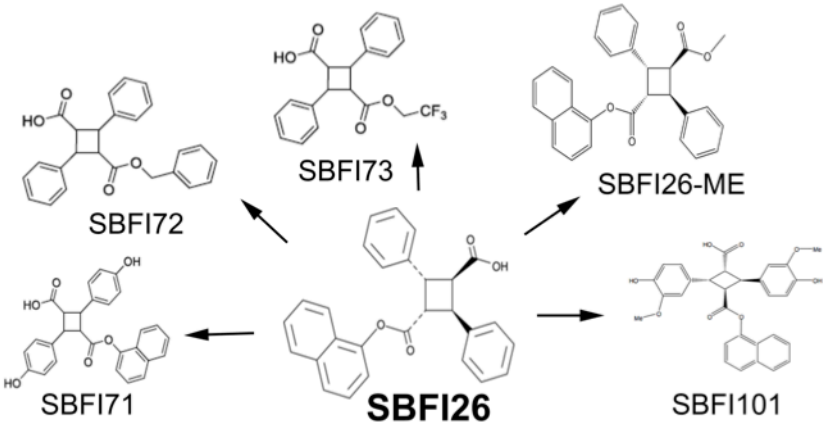
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Fatty acid binding proteins (FABPs) play an integral role in modulating metabolism of the endocannabinoid anandamide (AEA) by facilitating intracellular transport to fatty acid amide hydrolase (FAAH) for hydrolysis. Numerous studies in animal models have demonstrated that elevated levels of AEA in the brain can have beneficial effects on pain and inflammation. This has suggested the therapeutic potential of developing novel drugs that target the FABPs.

To identify new potential drug candidates we previously performed a large-scale *in silico* screen of over one million compounds obtained from the ZINC library using DOCK. The top scoring compounds were subsequently synthesized for further testing. Through computational analysis and *in vitro* testing we have identified SBFI26 ((naphthalen-1-yloxy)carbonyl-2,4-diphenylcyclobutane-1-carboxylic acid) as the most efficacious compound of the screen (Berger, *et al. PLoS ONE* 7.12 (2012):e50968).

In an effort to increase the efficacy of our lead drug, we used the chemical structure of SBFI26 as a scaffold to design and synthesize a number of derivatives of this compound. The principal goal was to develop compounds that display increased affinity for target FABPs compared to SBFI26. Functional groups to be incorporated on to the SBFI26 scaffold structure were chosen based on predicted affinity to CNS-expressed FABPs through DOCK analysis. High scoring compounds were subsequently synthesized and an *in vitro* fluorescence displacement binding assay using purified FABP3, FABP5, and FABP7 was used to assess biological efficacy for each compound. Preliminary data shows that these newly developed SBFI26 analogs typically display high to moderate affinity to FABP7, however none have yet matched the interaction strength of SBFI26 to FABP5, suggesting that SBFI26 remains the most potent compound synthesized thus far in this chemical class. Data generated from this screen will help guide further drug design to create new and more potent small-molecule inhibitors of the FABPs.



Acknowledgements: This work was funded by NIDA grants DA035923 and DA035949

THE VOICES OF WOMEN: NARRATIVES REVEALING SOCIAL STIGMA WITHIN THE MEDICAL PROFESSION

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This particular presentation will focus on the concerns of medical cannabis patients in discussing cannabis use with their personal physicians and stigmatization issues as voiced by female medical professionals. Using a phenomenological approach and qualitative methods to make problematic the standpoint of female medical cannabis patients, this project helps us understand why women choose to be recognized as medical cannabis patients (what are their reasons for obtaining a medical cannabis license/card) and the associated stigmatization (and fear thereof) that accompany this decision. The most prominent themes to emerge center on *Self*: personal health and necessary resources to use cannabis as medication; *Career*: fear of status or job loss (especially prominent among medical professionals); *Family*: familial acceptance and/or stigmatization; and *Society*: addressing the dominant perception that the use of cannabis is incompatible with the roles and responsibilities of female patients.

INVESTIGATION OF AGONIST ACTION OF N-ARACHIDONOYLGLYCINE (NAGly) AND Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC) IN GPR18-TRANSFECTED HEK293TR CELLS

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GPR18 receptor is a candidate cannabinoid receptor with the potential of being a novel therapeutic target (see McHough, 2012). NAGly has been suggested by many studies as an endogenous high potency agonist at GPR18 (see McHough, 2012) However, some studies have reported a lack of activation of GPR18 by NAGly (Lu et al., 2013; Yin et al., 2009).

The rationale of this study was to investigate the agonist effect of putative GPR18 ligands NAGly and Δ^9THC in HEK293TR heterologously expressing human GPR18.

SNAP-tagged human GPR18 under the control of a tetracycline regulated expression system was heterologously expressed in HEK293TR cells. Calcium mobilization was assessed using fluo-4 AM dye with a Flexstation. ERK phosphorylation was quantified by immunoblotting.

10μM NAGly induced significant increase in total calcium mobilization in GPR18-transfected HEK293TR cells, in both tetracycline-exposed and unexposed cells. The calcium response was slow with a gradual incline, and was readily distinguished from carbachol-evoked responses. Modification of the experimental conditions by inclusion of 1mg/ml BSA in the assay buffer did not change the result. 5 min exposure of GPR18-transfected HEK293TR cells to 10μM NAGly did not show any activation of EKR1/2 pathway in the presence and absence of 1mg/ml BSA and 2-hr serum starvation. In contrast, the positive control carbachol evoked a measurable ERK1/2 phosphorylation.

In this study, recombinant GPR18 receptor failed to respond to NAGly which indicates that GPR18 signalling may involve other pathways not examined in the current study or there is some other issue affecting GPR18-NAGly coupling in these cells. Studies are ongoing to characterize GPR18 expression in native cells.

Acknowledgment: Funded by the Higher Committee for Education Development in Iraq (HCED)

A NOVEL FLUORESCENCE-BASED ASSAY FOR AMIDE HYDROLYSIS: ANANDAMIDE AND *N*-ARACHIDONOYL-GLYCINE AS SUBSTRATES

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A number of assays of fatty acid amide hydrolase (FAAH) activity have been described. The most common makes use of *N*-arachidonoyl-[^{3}H]-ethanolamine as a substrate. We (and others) have described FAAH assays based on the detection of ammonia from primary *N*-acyl amides, which allow comparison of multiple substrates. More recently, we have generated a novel versatile FAAH activity based on ethanolamine detection. In this report, we have applied this assay to measure hydrolysis of NAGly.

Ethanolamine and glycine were detected following reaction with naphthalene-2,3-dicarboxaldehyde in 0.2 M borate buffer, pH 11.0; the resulting fluorescence was monitored with excitation at 420 nm and emission at 480 nm. We previously optimised the ethanolamine quantification for the pH of the borate buffer, the latency and stability of development of the fluorescent adduct and the choice of stopping reagent.

Rat liver microsomes hydrolysed the four *N*-acylehanolamines with similar affinities, although the maximal velocity was highest for anandamide as substrate (see Table 1). NAGly was also hydrolysed by rat liver microsomes with a K_m value higher than the *N*-acylethanolamines, and also with a higher V_{max} value. To confirm the hydrolytic activity involved, anandamide (5μM) or NAGly (25μM) hydrolysis was assessed in the presence of a range of concentrations of URB597. The potency of URB597 was identical for both substrates: pIC_{50} values of 9.2 ± 0.1 and 9.2 ± 0.1. Intriguingly, the maximal inhibition of AEA hydrolysis was -1 ± 1 % control, while that for NAGly was 32 ± 5%.
In summary, this assay has been adapted for examining the hydrolysis of NAGly as well as N-acylethanolamines. The assay will be used to investigate further the nature of *N*-acyl amino acid hydrolysis.

Table 1: Kinetics of *N*-acylethanolamines and NAGly hydrolysis

Substrate	K_m (μM)	V_{max} (nmol/min/mg protein)
NAGly (n=7)	12.3 ± 1.0	62 ± 12
PEA (C16:0) (n=2)	3.5 ± 4.1	42 ± 42
N-Stearoylethanolamine (C18:0) (n=2)	4.8 ± 5.1	24 ± 32
OEA (C18:1) (n=2)	2.7 ± 3.3	32 ± 33
Anandamide (C20:4) (n=7)	2.6 ± 0.3	49 ± 5

Acknowledgements: Funded by the Ministry of Education in the Kingdom of Saudi Arabia.

EXPLORING ABHD6 EXPRESSION IN RAT TISSUES

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Understanding of the turnover of monoacylglycerols has become more complicated in recent years with the identification of additional enzymes able to catalyse monoacylglycerol hydrolysis beyond monoacylglycerol lipase (MGL). In particular, ABHD6 (α/β hydrolase domain 6) is of interest as it represents an opportunity to fine tune levels of monoacylglycerols including 2-arachidonoylglycerol (Marrs et al., 2010). We have conducted a preliminary comparison of the expression levels of mRNA encoding MGL, an X1 variant of MGL and ABHD6.

Nineteen tissues from male Wistar rats (150 – 250g) were harvested, the RNA was extracted and real time quantitative polymerase chain reaction was performed using GAPDH, TBP and β-actin as housekeeping genes.

The range of mRNA expression of ABHD6 was more limited (range 0.20-2.04 arbitrary units) compared to MGL (0.15-8.66) and the MGL X1 variant (0.08-10.36). Analysing individual rank orders of expression indicated that adipose tissue exhibited relatively abundant expression of all three mRNAs, as did the cerebral cortex. In general, there was good correlation of the rank order of expression of mRNA encoding MGL and the X1 variant. However, there were tissues where expression of the X1 variant ranked noticeably lower than MGL itself; these were kidney cortex & medulla and liver. In comparing rank orders of expression of MGL and ABHD6, the testes and kidney cortex were tissues where MGL ranked higher than ABHD6. In contrast, large & small intestine and the hippocampus were tissues where ABHD6 expression ranked higher than MGL.

Further experiments are underway to identify whether enzyme activities follow the same distribution pattern as the mRNAs. The potential roles of these enzymes in cellular signalling and/or energy metabolism also needs clarification.

Marrs *et al.* (2010) *Nat.Neurosci.* PM:20657592

Acknowledgements: Funded by Iraqi Ministry of Higher Education.

NEUROPROCESSING, VISUAL PERCEPTION AND COGNITION: CANNABIS CONSIDERATIONS

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Neurodegenerative disease processes such as glaucoma, Alzheimer's disease, Multiple Sclerosis and Parkinson's disease have a significant impact on the visual system early in the disease process. The deficits these diseases cause impact pathways that are not readily detected during traditional tests of acuity, but none the less have repercussions for quality of life. Functions such as contrast detection, motion perception, glare recovery and light dark cycling are impacted in all these diseases and these functions are critical for safe ambulation and mobility. If functions are impaired significantly, the result can be unsafe mobility, including an inability to safely drive. Further, fine motor eye movements are deficit with Multiple Sclerosis and Parkinson's disease. Losses in visual function contribute to declines in cognitive functioning that may be present in neurodegenerative disease.

There is increasing evidence that cannabis can be a viable treatment for neurodegenerative diseases. This presentation will review the visual system as it relates to cannabinoids/cannabis and further discuss the implications of cannabis as it relates to the visual perception, eye movements, cognitive processes and neurodegenerative disease. The emphasis will be on those functions that impact mobility, including those related to driving.

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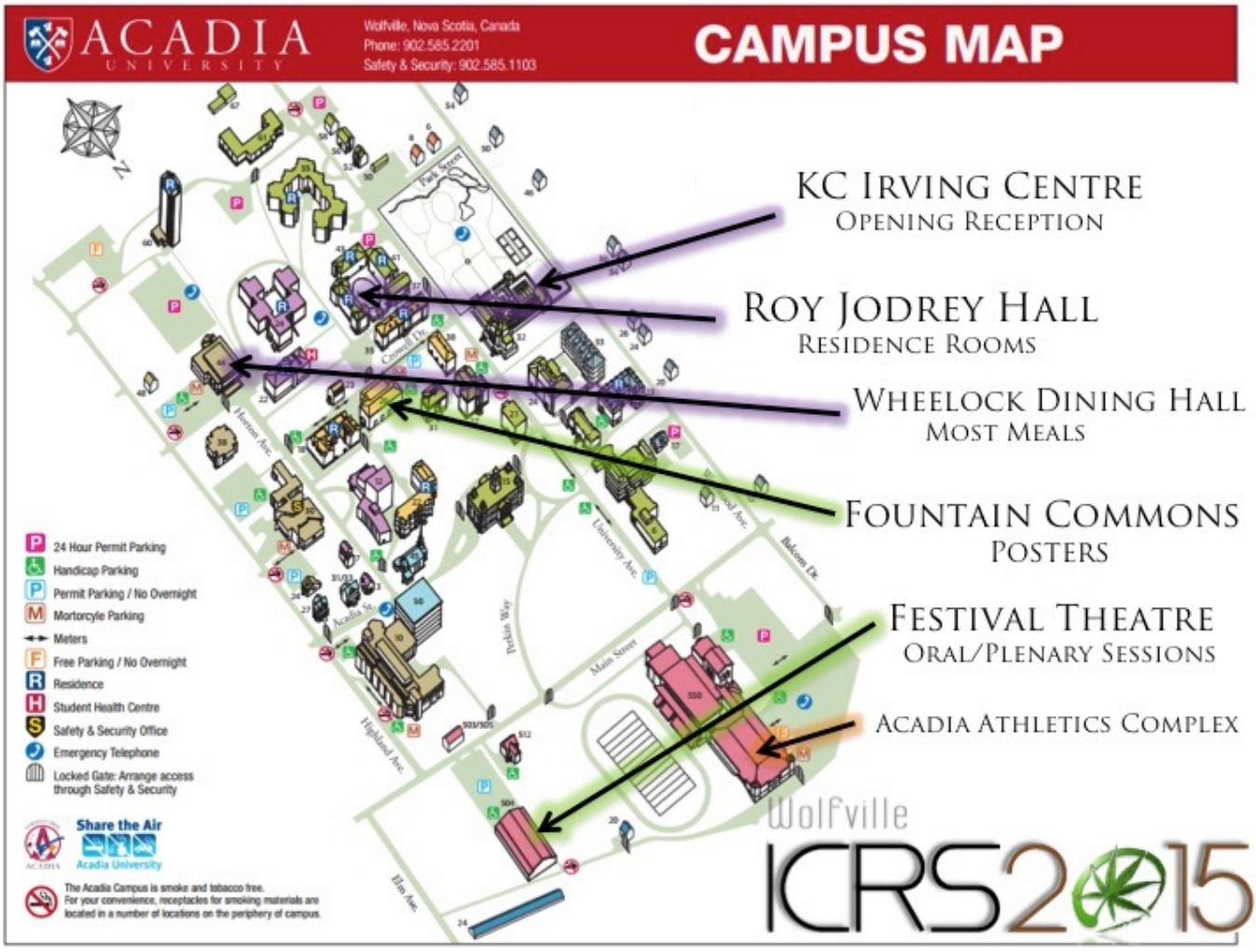
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ISBN 978-0-9892885-2-1

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	June 28, 2015 Sunday	June 29, 2015 Monday	June 30, 2015 Tuesday	July 1, 2015 Wednesday	July 2, 2015 Thursday
8:30		*Welcome & Overview*	CB2 Receptors	Session in Collaboration with NIDA	GPR55
8:45					
9:00		Novel Chemical Entities			
9:15					
9:30					
9:45					
10:00					*In Memoriam*
10:15					
10:30		*Break*	*Break*	*Break*	*Break*
10:45					
11:00		Plant Studies and Non-THC Cannabinoids	Poster Session P1 EVEN	EC Transport and Metabolism	Bias and Modulation
11:15					
11:30					
11:45					
12:00					
12:15					
12:30					
12:45					
13:00		*Lunch & NIDA Mentoring*	*Lunch*	*Lunch*	*Lunch*
13:15					
13:30					
13:45					
14:00		Poster Session P1 ODD	Outing	Poster Session P2 ODD	Poster Session P2 EVEN
14:15					
14:30					
14:45					
15:00					
15:15					
15:30					
15:45					
16:00	*Registration*	Presidential Plenary Lecture: Mike Salter		Kang Tsou Memorial Lecture: Brent Zettl	25 Years of ICRS: Raphi Mechoulam
16:15					
16:30					
16:45					
17:00		*Break*		*Break*	Break
17:15					*Business Meeting*
17:30		Animal Models		Human Studies	
17:45					
18:00					
18:15					
18:30	*Reception*				
18:45					
19:00					
19:15					
19:30		*Dinner*		On Your Own	*Banquet*
19:45					
20:00					
20:15					
20:30					


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Festival Theatre
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Acadia Athletics Complex
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Handicap Parking
Permit Parking / No Overnight
Mortorcyle Parking
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