INTERNATIONAL CANNABINOID RESEARCH SOCIETY

17th ANNUAL SYMPOSIUM ON THE CANNABINOIDS

June 26 - 30, 2007
Saint-Sauveur, Québec
Canada

PROGRAM AND ABSTRACTS

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

17th ANNUAL SYMPOSIUM ON THE CANNABINOIDS

Program and Abstracts

June 26 - 30, 2007
Manoir Saint-Sauveur
Saint-Sauveur, Québec
Canada

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University of Vermont
Burlington, VT 05405
USA

World Wide Web Site: http://www.cannabinoidsociety.org

Logo by Natalia Realini

Produced by Cortical Systematics LLC

ISBN: 0-9786040-1-6

These abstracts may be cited in the scientific literature.
The citation format is as follows:

Author(s), Abstract Title (2007) 17th Annual Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, Page #.

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2007 Symposium on the Cannabinoids

June 26 – 30, Manoir Saint-Sauveur
Saint-Sauveur, Québec, Canada

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17TH ANNUAL SYMPOSIUM ON THE CANNABINOIDS

2007 PROGRAM

Registration: June 26, 2007 (16.00 – 20.00)

Day 1
Wednesday, June 27th

8.20 – 8.30	**Opening Remarks**		
Session 1. SAR Studies and New Synthetic Molecules ➢ Chairs*: Drs. Brian Thomas and Giulio Muccioli*			**Page #**
8.30	Ying Pei, Richard W Mercier, Jenine Sanzari, Arpad Kastal, Ganesh A Thakur, Dai Lu, Lakshmipathi Pandarinathan, Alexander M Zvonok, Dow Hurst, Patricia H Reggio and Alexandros Makriyannis	UTILIZING A GLOBAL SET OF CYSTEINE SUBSTITUTION STRATEGY TO ELUCIDATE LIGAND BINDING MOTIFS IN HUMAN AND MOUSE CB2: LIGAND-BASED STRUCTURAL BIOLOGY	1
8.45	Karin Worm, Q Jean Zhou, Gabriel J Stabley, Robert N DeHaven, Nathalie Conway-James, Christopher J LaBuda, Michael Koblish, Patrick J Little and Roland E Dolle	NON-PSYCHOTROPIC BIARYL CANNABINOID AGONISTS	2
9.00	John Huffman, Jianhong Chen, Ross Mabon, Jianzhong Lu, Jenny Wiley and Billy Martin	GEM-DIMETHYLHEPTYL ANALOGS OF DELTA-8-THC, 1-DEOXY AND 1-METHOXY-DELTA-8-THC	3
Session 2. Receptor Structure and Signal Transduction ➢ Chairs*: Drs. Michelle Glass and Monica Bari*			
9.15	Liang Nong, Tracy Sherwood, Marisela Agudelo, Shilpa Mikkilineni, Cathy Newton and Thomas W Klein	EXPRESSION OF CANNABINOID RECEPTOR 2 (CB2) IN THE MOUSE B CELL LINES K46U AND 18.81	4
9.30	E Scott Graham, Natasha L Grimsey, Catherine E Goodfellow, Emma L Scotter, Megan J Dowie and Michelle Glass	CANNABINOID CB1 RECEPTOR ANTIBODIES ARE NOT ALL CREATED EQUAL!	5
9.45 – 10.15	**Coffee Break**		

Session 2 (cont.)			
10.15	Brian Thomas, Yanan Zhang, Brian Gilmour, Hernan Navarro, Anne Gilliam, Marcus Brackeen and Herbert Seltzman	BIVALENT LIGANDS TARGETING CANNABINOID RECEPTOR OLIGOMERS	6
10.30	Brian Hudson and Melanie Kelly	CB1 CANNABINOID RECEPTORS DIMERIZE WITH BETA2 ADRENERGIC RECEPTORS TO INFLUENCE RECEPTOR TRAFFICKING	7
10.45	Gemma Baillie, Graeme Finnie, Paul Macbeath, John Miskelly, Sonia Watson, Lesley Stevenson, Tesmol George, Anu Mahadevan, Raj Razdan, Roger Pertwee and Ruth Ross	ALLOSTERIC MODULATION OF THE CANNABINOID CB1 RECEPTOR: NOVEL ALLOSTERIC MODULATORS	8
11.00	Patricia Reggio, Dow Hurst and Diane Lynch	CAN THE LIPID BILAYER CONTRIBUTE TO CANNABINOID CB1 RECEPTOR CONSTITUTIVE ACTIVITY?	9
11.15	Chris Breivogel and Vanita Puri	BETA-ARRESTIN2 AFFECTS CB1 LOCALIZATION & DOWNREGULATION	10
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13.00 – 15.30	**Poster Sessions 1 - 3**		
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15.45	Natsuo Ueda, Xing-Hua Jin, Yasuo Okamoto, Jun Morishita, Kazuhito Tsuboi and Takeharu Tonai	DISCOVERY OF A CALCIUM-INDEPENDENT PHOSPHATIDYLETHANOLAMINE N-ACYLTRANSFERASE (INAT)	12
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16.15	Takayuki Sugiura, Keisuke Nakajima, Seishi Kishimoto and Saori Oka	AN ENDOGENOUS LIGAND FOR GPR55, A G PROTEIN-COUPLED RECEPTOR	14

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17.45	Jose Inacio Carvalho, Alline Campos, Frederico Ferreira and Francisco Guimarães	OPPOSITE EFFECTS OF AM404, AN INHIBITOR OF ENDOCANNABINOID UPTAKE INJECTED INTO THE VENTRAL HIPPOCAMPUS OF STRESSED OR NON-STRESSED RATS SUBMITTED TO THE ELEVATED PLUS MAZE	18
18.00 – 19.00	**Kang Tsou Memorial Lecture** **Dr. Jean-Pierre Després** Québec Heart Institute, Hŏpital Laval Department of Social and Preventative Medicine, Université Laval *Visceral adiposity: A new therapeutic target for the optimal management of the risk of type-2 diabetes and cardiovascular disease* Page P1		
19.30	**Dinner**		

Notes:

Day 2
Thursday, June 28th

Session 4. Immune System ➢ Chairs*: Drs. Stephen Alexander and Heather Bradshaw*			
8.30	Xavier Thuru, Mathias Chamaillard, Meliha Karsak, Caroline Dubuquoy, Edmone Erdual, Emilie Gantier, David Philippe, Christel Rousseaux, Pierre Dubus, Jacek Stalewski, Frederique Menzaghi, Andreas Zimmer, Pierre Riviere and Pierre Desreumaux	CANNABINOID RECEPTOR 2 IS REQUIRED FOR HOMEOSTATIC CONTROL OF INTESTINAL INFLAMMATION	19
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9.30	Matthew Hill, Ryan McLaughlin, Victor Viau, Boris Gorzalka and Cecilia Hillard	STRESS-INDUCED ALTERATIONS IN LIMBIC ENDOCANNABINOID CONTENT IN THE RAT: CORRELATIONS TO HPA AXIS ACTIVATION	23
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11.30 – 12.30	**Lunch**		
12.30 – 15.00	**Poster Sessions 4 – 9**		
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15.00	Sharon Chung, Naheed Hossain, Tejas Shah, Nancy MacFarlane, Adam Blackman and Colin Shapiro	USE OF THE CANNABINOID NABILONE FOR THE PROMOTION OF SLEEP IN CHRONIC, NON-MALIGNANT PAIN PATIENTS: A PLACEBO-CONTROLLED, RANDOMIZED, CROSSOVER INSOMNIA PILOT STUDY	29
15.15	Vincent Maida	CANNABINOIDS IN THE MANAGEMENT OF CANCER PAIN: A COHORT STUDY USING PROPENSITY SCORING	30
15.30	Mark Ware, Tongtong Wang, Stan Shapiro, Thierry Ducruet, Ann Robinson, Ann Gamsa, Gary Bennett and Jean-Paul Collet	SMOKED CANNABIS FOR CHRONIC NEUROPATHIC PAIN: RESULTS OF A PILOT STUDY	31
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17.00	Alessia Ligresti, Daniele De Filippis, Piero Orlando, Maurizio Guida, Santosh Nigam, Stefania Petrosino, Vincenzo Di Marzo and Teresa Iuvone	OVERACTIVATION OF THE ENDOCANNABINOID SYSTEM IN HUMAN ENDOMETRIAL CARCINOMA	35
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17.30	Linda Parker, Erin Rock, Cheryl Limebeer, Daniele Piomelli and Raphael Mechoulam	THE EFFECT OF URB597 AND CANNABIDIOL ON THE EXPRESSION OF ANTICIPATORY NAUSEA IN A RAT MODEL	37
17.45	**Career Achievement Award** **Harald Schmid and Patricia Schmid** Presenter: ICRS President Christopher Fowler		
18.00 – 22.00	**Dinner / Water Park** Bus Transport begins at 18.00 To swim and/or ride the water slides, bring your swimming suits Changing rooms will be provided		

Notes:

Day 3
Friday, June 29th

Session 8. Food Intake and Energy Balance ➢ Chairs*: Drs. Raphael Mechoulam and Jenny Wiley*			
8.30	Ester Fride, Hilit Braun, Hila Matan, Shachar Steinberg, Patricia H Reggio and Herbert H Seltzman	INHIBITED DEVELOPMENT AFTER A NEUTRAL CANNABINOID CB1 RECEPTOR ANTAGONIST IN NEONATAL MICE: IMPORTANCE OF AN ENDOCANNABINOID TONE FOR MILK INTAKE AND GROWTH	38
8.45	Andreas Artmann, Gitte Petersen, Lars I Hellgren, Christian Skonberg, Steen Honoré Hansen and Harald S Hansen	INFLUENCE OF DIETARY FATTY ACIDS ON ENDOCANNABINOID AND N-ACYLETHANOLAMINE LEVELS IN RAT BRAIN, SMALL INTESTINE AND LIVER	39
9.00	Hodaya Dahan, Aron Weller, David Branski, Raphael Mechoulam and Ester Fride	NEONATAL BLOCKADE OF THE CB1 RECEPTOR: FURTHER SUPPORT FOR ENDOCANNABINOID-CB1 DEFICIENCY AS THE BIOLOGICAL BASIS OF 'NON-ORGANIC FAILURE-TO-THRIVE' IN INFANTS	40
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9.30	Vincenzo Di Marzo, Isabel Matias, Katarzyna Starowicz, Francesca Borrelli, Gabriella Aviello, Raffaele Capasso, Stefania Petrosino and Angelo Izzo	ALTERATIONS OF ENDOCANNABINOID AND ACYLETHANOLAMIDE LEVELS IN PERIPHERAL TISSUES OF MICE ON A HIGH FAT DIET: IMPLICATIONS FOR GASTRIC EMPTYING AND METABOLISM	42
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10.30	María-Paz Viveros, Ricardo Llorente, Stefania Petrosino, Tiziana Bisogno, Alvaro Llorente, Eva-María Marco, Jorge Serrano, Carmen Guaza, Vincenzo Di Marzo and Meritxell López-Gallardo	NEONATAL STRESS-INDUCED NEURODEGENERATION: A SUITABLE ANIMAL MODEL TO EVALUATE NEUROPROTECTIVE AND MODULATORY PROPERTIES OF CANNABINOIDS	44

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11.00	Tiziana Bisogno, Alberto Martire, Stefania Petrosino, Patrizia Popoli and Vincenzo Di Marzo	IMPAIRED ENDOCANNABINOID LEVELS IN SELECTED BRAIN AREAS OF TRANSGENIC R6/2 MICE, AN EXPERIMENTAL MODEL OF HUNTINGTON'S DISEASE	46
11.15	Fabian Docagne, Frida Loría, Fernando Correa, Diego Clemente, Miriam Hernangómez, Leyre Mestre and Carmen Guaza	THE ENDOCANNABINOID SYSTEM LIMITS AXONAL DAMAGE AND EXCITOTOXICITY	47
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17.30	Aron Lichtman, Pattipati Naidu, Billy Martin and Benjamin Cravatt	ANANDAMIDE REGULATES EDEMA AND PAIN THROUGH DISTINCT PERIPHERAL AND CENTRAL PATHWAYS	54
17.45	Katarzyna Starowicz, Sabatino Maione, Luigia Cristino, Enza Palazzo, Ida Marabese and Vincenzo Di Marzo	TONIC ENDOVANILLOID FACILITATION OF GLUTAMATE RELEASE IN BRAINSTEM DESCENDING ANTINOCICEPTIVE PATHWAYS	55
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19.30	**Dinner**		

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Day 4
Saturday, June 30th

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8.45	W-S Vanessa Ho and Sheila M Gardiner	INFLUENCE OF ACUTE HYPERTENSION AND FATTY ACID AMIDE HYDROLASE ON HAEMODYNAMIC EFFECTS OF ANANDAMIDE IN CONSCIOUS RATS	57
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9.15	Lauren Whyte, Susan Ridge, Michael Rogers and Ruth Ross	CP55940, A NON-SELECTIVE CB1/CB2 AGONIST, STIMULATES BONE RESORPTION BY HUMAN OSTEOCLASTS IN VITRO	59
9.30	Lorna Ford, Susan Ridge, Gary Cameron, Michael Rogers and Ruth Ross	ENDOCANNABINOIDS ARE PRODUCED BY BONE CELLS AND STIMULATE BONE RESORPTION IN VITRO	60
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10.30	Partha Mukhopadhyay, Sandor Batkai, Mohamraj Rajesh, Judith Harvey-White, George Kunos and Pal Pacher	PHARMACOLOGICAL INHIBITION OF CANNABINOID-1 RECEPTOR PROTECTS AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY	62
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13.30	Marina Rubio, Heather Bradshaw, Douglas McHugh, Rosario de Miguel, Javier Fernandez-Ruiz, Jose A Ramos and J Michael Walker	BACLOFEN EFFECTS IN ALCOHOL-ABSTINENT RATS ARE ASSOCIATED WITH CHANGES IN THE LEVELS OF ENDOCANNABINOIDS AND RELATED N-ACYLETHANOLAMINES IN SPECIFIC BRAIN REGIONS	65
13.45	Robert Vann, Thomas Gamage, Billy Martin and Jenny Wiley	PMSF POTENTIATES THE DISCRIMINATIVE STIMULUS PROPERTIES AND MOUSE TETRAD EFFECTS OF CANNABINOIDS	66
14.00	M Jerry Wright Jr. and Jenny L Wiley	THE MOTIVATION TO WORK FOR FOOD AFTER SUSTAINED CB1 BLOCKADE DURING ADOLESCENCE	67
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14.30	Tiziana Rubino, Daniela Vigano, Natalia Realini, Daniela Braida, Valeria Capurro, Mariaelvina Sala and Daniela Parolaro	NEUROCHEMICAL AND BEHAVIORAL EVIDENCES THAT Δ^9-THC EXPOSURE IN ADOLESCENCE PROVOKES MOOD AND COGNITIVE ALTERATIONS IN ADULTHOOD	69
14.45	Dana Selley, Vanna Zachariou, Linda Perrotti, Stephen Gold, Michael Cassidy, Billy Martin, Eric Nestler and Laura Sim-Selley	SUBCHRONIC Δ^9-THC ADMINISTRATION MODULATES EXPRESSION OF MOLECULAR REGULATORS OF REWARDING DRUG ACTION IN THE STRIATUM	70
15.00	Stephen Varvel, Billy Martin and Aron Lichtman	COGNITIVE IMPAIRMENTS ASSOCIATED WITH PRECIPITATED WITHDRAWAL FROM THC IN MICE	71

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15.45 – 16.30	**Young Investigator Lecture** **Dr. Andrea Hohmann** Department of Psychology, Neuroscience and Behavior University of Georgia *Cannabinoid mechanisms of pain suppression* Page P2
16.30 – 18.00	**Poster Sessions 13 - 15**
19.30	**Awards Dinner**

Notes:

13.00 – 15.30	**Day 1 – Wednesday, June 27th** **Poster Sessions 1 – 3**	
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Plenary Lecture

18.00 – 19.00
Wednesday, June 27th, 2007

Kang Tsou Memorial Lecture

VISCERAL ADIPOSITY: A NEW THERAPEUTIC TARGET FOR THE OPTIMAL MANAGEMENT OF THE RISK OF TYPE 2 DIABETES AND CARVIOVASCULAR DISEASE

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The prevalence of type 2 diabetes is showing a spectacular progression worldwide, a phenomenon largely resulting from the epidemic proportions reached by obesity in various populations of the world. It is well established that there is a greater prevalence of chronic metabolic diseases such as diabetes and cardiovascular diseases in obese patients than among normal weight individuals. However, obesity is heterogeneous both in terms of its etiology and its metabolic complications. Body fat distribution, especially visceral adipose tissue accumulation, has been found to be a major correlate of a cluster of diabetogenic, atherogenic, prothrombotic and inflammatory metabolic abnormalities referred to as the metabolic syndrome. This dysmetabolic profile is predictive of a substantially increased risk of coronary heart disease even in the absence of hyperglycemia, elevated LDL-cholesterol or hypertension. Results of the recently reported analysis of the 13-year follow-up of men of the Québec Cardiovascular Study have shown that the cluster of abnormalities of the metabolic syndrome observed in subjects with visceral obesity is associated with a substantially increased risk of coronary heart disease. Additional evidence also suggests that the hyperglycemic state observed in subjects with the metabolic syndrome only represents the tip of a dysmetabolic iceberg largely explained by the high prevalence of abdominal obesity in our population. It has been shown that irrespective of the therapeutic approach used (diet, exercise, pharmacotherapy) to induce weight loss in these high-risk patients, a preferential mobilisation of visceral fat is observed leading to improvements in the metabolic risk profile predictive of a reduced coronary heart disease risk. In that regard, the recent discovery of the endocannabinoid-CB_1 receptor system (EC system) and of its impact on the regulation of energy metabolism represents a significant advance which has paved the way to the development of new pharmacological approaches which could fill an unmet clinical need: targeting excess visceral adiposity and its related metabolic complications. Therefore, blocking the CB_1 receptor could represent an interesting approach for the management of high-risk abdominal obesity and related cardiometabolic risk. Evidence derived from recently completed phase III trials in overweight/obese patients suggests that such an approach could yield substantial clinical benefits.

Plenary Lecture

15.45 – 16.30
Saturday, June 30th, 2007

Young Investigator Lecture

ENDOCANNABINOID MECHANISMS OF PAIN SUPPRESSION

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The discovery of cannabinoid receptors and identification of brain constituents that act at these receptors established the existence of an endocannabinoid transmitter system in the nervous system. In the mid 1990's, the lack of information on the functions of endocannabinoids in behavior represented a major gap in our understanding of the chemical control of neural function. In an attempt to narrow this gap, our research has focused on identifying whether the antinociceptive effects of cannabinoids are indicative of a novel, nonopiate system that modulates pain. Cannabinoids are antinociceptive in animal models of acute, tissue and nerve injury-induced nociception. Here, we provide behavioral, neurophysiological and neuroanatomical evidence to demonstrate that cannabinoids suppress nociceptive processing through activation of both CB_1 and CB_2 receptor subtypes. Behavioral pharmacological approaches, in conjunction with high performance liquid chromatography/mass spectrometry, are used to document a physiological role for endocannabinoids in pain modulation. These studies provide evidence that exposure to an environmental stressor mobilizes endocannabinoids, such as 2-arachidonoylglycerol (2-AG) and anandamide, to dampen sensitivity to pain. The mechanisms governing *in vivo* synthesis and deactivation of 2-AG remain poorly understood. Our results are consistent with the hypothesis that exposure to an environmental stressor stimulates biosynthesis of 2-AG through the consecutive activation of two distinctive enzymes– phospholipase C and diacylglycerol lipase– to induce stress-induced analgesia. Our data further suggest that this process may be initiated by activation of group I metabotropic glutamate receptors. Our collaborative studies also identify an enzyme (i.e. monoacylglycerol lipase), which degrades 2-AG as a novel therapeutic target. The present studies provide a functional framework with which to understand the physiological roles of endocannabinoids in modulating nociceptive processing at supraspinal, spinal and peripheral levels.

Acknowledgments: Supported by: DA021644, DA022478, DA014022, DA14265

UTILIZING A GLOBAL SET OF CYSTEINE SUBSTITUTION STRATEGY TO ELUCIDATE LIGAND BINDING MOTIFS IN HUMAN AND MOUSE CB2: LIGAND-BASED STRUCTURAL BIOLOGY.

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The cannabinoid CB2 receptor is an increasingly important therapeutic target for neuropathic pain, inflammation, cancer treatment, as well as a variety of other physiological disorders. A target-based approach for the design and synthesis of novel CB2 selective compounds requires structural data on the manner with which ligands interact with this GPCR. To characterize the ligand binding motifs for CB2, we have designed and synthesized high affinity cannabinergic compounds representing the major chemical classes of CB1/CB2 ligands and carrying suitable groups capable of forming covalent attachments with different residues within the binding domain.

Our approach which we named "Ligand Based Structural Biology" involves a combination of biochemical and chemical methods aimed at identifying the ligand binding motifs at the CB2 receptor. We shall present our results with the covalent ligand AM841 ((-)-7'-isothiocyanato-11-hydroxy-1', 1'-dimethylheptylhexahydrocannabino), a classical cannabinoid with high affinity for the CB2 receptor carrying an electrophilic isothiocyanate group at the terminal carbon of the side chain. Using a global set of cysteine substitution (cysteine to serine and/or alanine) mutant transgenic cell lines, we have identified C6.47 cysteine as the residue to which AM841 is covalently attached. No covalent attachment is observed when C6.47 is mutated to alternate amino acids. In addition, mutation of other candidate cysteine residues in TMH 7 does not eliminate or diminish covalent attachment of AM841 to hCB2 receptor. The irreversible interaction between C6.47 residue and AM841 has been confirmed by LC-MS experiments performed in our lab. We have also shown that this covalent interaction results in exceptionally potent CB2 receptor activation. Molecular modeling approaches allow us to speculate on the nature of this activation.

This work is supported by NIDA Training Grant P32-DA7312 and NIDA P01-DA09158.

NON-PSYCHOTROPIC BIARYL CANNABINOID AGONISTS

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Robert N. DeHaven,[b] Nathalie Conway-James, [b] Christopher J. LaBuda, [b]
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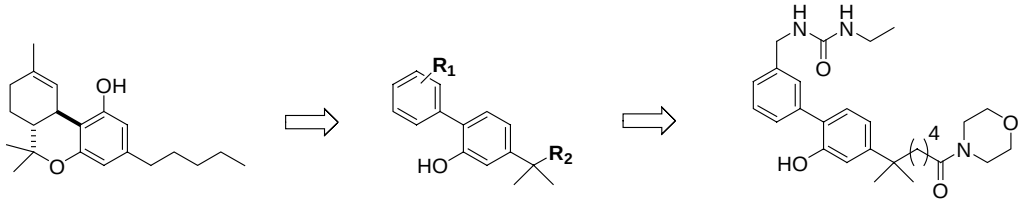
Cannabinoid receptor agonists, such as CP55,940 and WIN 55,212-2, produce potent antinociception with equivalent efficacy to morphine in animal models of acute pain, persistent inflammatory pain and neuropathic pain. They also induce a number of unwanted CNS side effects, which are accounted for by the central distribution pattern of CB1 receptors. Catalepsy in mice is indicative of CB1 activation and predictive of cannabinoid psychoactivity.

A separation between therapeutic effects and undesirable CNS side effects could be accomplished by increasing the selectivity for the CB2 receptor over the CB1 receptor or by preventing the cannabinoid from crossing the blood brain barrier.

Lipinski introduced a set of rules imposing limitations on logP, molecular weight, number of hydrogen bond donors and acceptors. This "rule of 5" is now well established to predict absorption and bioavailability of novel compounds. Similar rules were proposed for potential CNS penetration. We reversed the argument from the literature and applied the resulting criteria to design a cannabinoid drug unlikely to penetrate the blood brain barrier thereby preventing it from interacting with CB1 receptors in the brain. These compounds should exhibit the following properties: MWT > 400, logP < 4, H-bond acceptors > 6, H-bond donors ≥ 3, PSA (TPSA) > 80, rotatable bonds > 7. We report here that it is possible to peripheralize CB ligands, using a unique combination of polar substituents on a cannabinomimetic biaryl core **1**, retaining the binding affinity to the receptors (K_i < 10 nM) but preventing CNS side effects from occurring as demonstrated by the absence of catalepsy in the ring test at doses up to 100 mg/kg i.p..

Examples illustrate that several conditions had to be fulfilled and the right combination of substituents had to be chosen to arrive at a peripheral CB agonist **2.**



THC, TPSA = 29 **1** **2**, TPSA = 91

To retain good binding to the receptors the position of the R_1-substituent in the top aryl ring and the position and length of the R_2-substituent in the bottom aryl are critical.

Furthermore, the combination of R_1 and R_2 substituents is critical to reach a high enough polar surface area (TPSA) and a urea substitution on the top aryl is essential to reach a sufficient amount of H-bond donors necessary to avoid CNS side effects.

Synthesis, SAR and initial biological evaluation in animal models of pain will be presented.

GEM-DIMETHYLHEPTYL ANALOGS OF Δ^8-THC, 1-DEOXY AND 1-METHOXY-Δ^8-THC

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It is known that a *gem*-dimethyl group appended to C-1 of the alkyl side chain of a traditional cannabinoid, such as Δ^8-THC, enhances CB_1 receptor affinity and *in vivo* potency. This effect is mirrored in the CB_1 and CB_2 receptor affinities of 1-methoxy and 1-deoxy-Δ^8-THC analogs (Huffman *et al. Bioorg. Med. Chem.* **1999**, *7*, 2905; **2002**, *10*, 4119). Several years ago we reported the synthesis and pharmacology of the methylheptyl analogs of Δ^8-THC and found that a methyl substituent at C-1' to C-3' enhanced CB_1 receptor affinity relative to 3-heptyl-Δ^8-THC, while substitution at C-4' or C-6' had little effect, but a C-5'-methyl group attenuated CB_1 receptor affinity (Huffman *et al. Bioorg. Med. Chem.* **1998**, *6*, 2383). To continue the investigation of the effect of side chain substitution on CB_1 receptor affinity in the Δ^8-THC series and the possible development of new CB_2 selective ligands we have instituted a program to prepare 2', 2'- to 6', 6'-dimethylheptyl-Δ^8-THC and the corresponding 1-methoxy and 1-deoxy analogs.

Currently, the synthesis of 2', 2'- and 6', 6'-dimethylheptyl-Δ^8-THC and their 1-methoxy and 1-deoxy analogs have been completed and the receptor affinities have been determined. Both 2', 2'- and 6', 6'- dimethylheptyl-Δ^8-THC have high affinity for the CB_1 receptor (K_i = 1.5 ± 0.3 and 9.4 ± 1.2 nM, respectively) and the 1-methoxy-2', 2'- and 6', 6'-dimethyl compounds have no measurable affinity for the CB_1 receptor. The 1-methoxy-2', 2'-dimethyl analog has modest affinity for the CB_2 receptor (K_i = 120 ± 12 nM), but the 6', 6'- isomer has little CB_2 receptor affinity (K_i = 1368 ± 53 nM). Neither of the 1-deoxy analogs have significant affinity for the CB_1 receptor, however 1-deoxy-2', 2'-dimethylheptyl-Δ^8-THC has moderate affinity for the CB_2 receptor (K_i = 76 ± 1 nM). The 6', 6'-dimethyl isomer has little affinity for the CB_2 receptor (K_i = 479 ± 42 nM).

Because 1-methoxy and 1-deoxy-6', 6'-dimethylheptyl-Δ^8-THC have little affinity for the CB_2 receptor the corresponding 6'-methylheptyl analogs were prepared. Both 1-methoxy and 1-deoxy-6'-methylheptyl-Δ^8-THC have no measurable affinity for the CB_1 receptor. The 1-deoxy compound has moderate affinity for the CB_2 receptor with K_i = 61 ± 7 nM; however, the 1-methoxy compound has little affinity for the CB_2 receptor (K_i = 310 ± 9 nM).

The method of synthesis of these compounds and their SAR will be discussed.

Acknowledgements: Supported by NIDA grants DA03590 and DA03672.

EXPRESSION OF CANNABINOID RECEPTOR 2 (CB_2) IN THE MOUSE B CELL LINES K46μ AND 18.81

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Cannabinoids have been shown to modulate immune function and the immune system contains elements of the endocannabinoid system such as cannabinoid receptors and ligands. CB_2 is reported to be expressed most abundantly on B lymphocytes suggesting that these receptors may play an important role in B cell biology and immune function. In order to study CB_2 expression in B cells, we have examined receptor expression in two B cell lines, K46μ and 18.81. These cell lines are reported to represent different stages in B cell maturation and we wanted to see if they expressed different basal levels of CB_2. Cells were isolated in mid-log phase growth and analyzed for CB_2 at both the mRNA and protein levels by real-time RT-PCR and flow cytometry, respectively. Results examining mRNA levels showed both cell lines expressed equal amounts of message. However, flow cytometry data using antibodies to the carboxy end of the receptor showed a significantly increased expression of CB_2 in K46μ. Both cell lines were also transfected by electroporation with pGL3 Luciferase reporter vectors (Promega) containing either the SV40 enhancer sequence only or both the enhancer and promoter sequences. Results from these studies showed that reporter gene activity was 5 to 10 fold higher with the vector containing both promoter and enhancer elements. These results suggest that both B cell lines support transcription and translation of the CB_2 gene as well as support the expression of reporter constructs with and without promoter elements. Therefore, it is possible that these cell lines represent good models for future studies designed to examine CB_2 gene expression.

Supported by DA019824 from NIDA.

CANNABINOID CB1 RECEPTOR ANTIBODIES ARE NOT ALL CREATED EQUAL!

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The ability to study endogenous CB1 receptor proteins in neuronal tissues and cells relies on the availability of robust highly-specific antibodies. A lack of good antibodies may force the use of transfected proteins and various epitope tags such as HA and FLAG. However, where possible the biochemistry of these recombinant receptors i.e. their synthesis, trafficking, pharmacology and signal transduction must be validated against the behaviour of wild-type receptors.

We have screened a series of CB1-specific antibodies using a combination of immunological methods to detect endogenous receptors in tissues and cells, as well as epitope tagged (HA) recombinant receptors in cell lines. The antibodies we tested were from Sigma (C1108, two different lots tested), Affinity BioReagents (PA1-745), Cayman (#101500), Biosource International (44-310, two different lots) and from the Mackie Lab (L15). Initially we compared the staining patterns of several CB1 antibodies to receptor autoradiography generated with [3H]CP55,940 in mouse brain. Only the L15 antibody generated a pattern of staining consistent with ligand binding.

Next we generated HEK lines expressing HA-tagged rat and human CB1 receptors to compare cellular staining and protein detection of each CB1-specific antibody to the anti-HA staining. Surprisingly the anti-HA and L15 were the only antibodies to detect CB1, with all of the commercial antibodies binding non-specifically to untransfected HEK cells, and failing to detect transfected CB1.

Finally we screened all antibodies by Western blotting against the HEK cell lines generated above, and tissue from rat and mouse brain. The HA and L15 antibodies detected proteins of the correct molecular weight in transfected cell lines. Only the L15 antibody detected proteins of the correct weight in brain homogenates, while all other antibodies failed to distinguish between transfected and untransfected cell lines, and recognised multiple proteins in all tissues.

It is possible that the differences we have observed compared to those published by others are due to different antibody lots thus different antibody titres, indeed we have previously observed apparently specific staining with an earlier batch of the Biosource antibody (Park et al, 2003). This would highlight the requirement of better quality control between antibody batches to guarantee antibody specificity and efficacy. We conclude that caution should be used when interpreting data generated using these antibodies. Most importantly that the appropriate negative control tissue and cell line controls are essential to exclude non-specific detection of other proteins that we have observed.

BIVALENT LIGANDS TARGETING CANNABINOID RECEPTOR OLIGOMERS

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Cannabinoid receptor (CB1) antagonists can attenuate the abuse and relapse liability of a wide variety of drugs. One plausible mechanism through which CB1 receptors can modulate the pharmacological properties of drugs of abuse involves physical interaction with G-protein coupled receptors (GPCRs) or other membrane components. Oligomeric interactions linking G protein-coupled receptors and other signaling molecules together in a receptor mosaic may provide the cell membrane with additional signal integration capacity and a molecular basis for complex behaviors, including the affective processes involved in drug addiction. With respect to the CB1 receptor, evidence exists for a direct physical interaction between CB1 receptor monomers (homodimers), CB1 and orexin A receptors, and CB1 and opioid receptors (heterodimers). Because of the increased appreciation for the pharmacological relevance of receptor oligomerization, the development of single chemical entities that target receptor oligomers, with the aim of enhancing efficacy and/or improving safety relative to drugs that address only a single target, has recently become more prevalent. Such a goal can be achieved through bivalent ligands that possess novel pharmacological properties compared to their individual monomers.

Bivalent ligands containing two SR141716 pharmacophores (targeting CB1 receptor homomers), as well as mixed bivalent ligands containing antagonist pharmacophores for CB1 and opioid receptors (to probe for functional receptor heteromers), have been synthesized and tested. Bivalent ligands containing two SR141716 pharmacophores coupled by alkyl linkers have been shown to retain nM affinity at the cannabinoid receptor and potently shift the dose response curve of CP55940. However, when a similar approach was used to synthesize a mixed bivalent ligand containing both SR141716 and naltrexamine pharmacophores, CB1 receptor affinity was lost. Nevertheless, this compound had improved functional antagonist activity at opioid receptors (with an apparent Ke of 0.18 ± 0.06 against DAMGO at the μ receptor, an apparent Ke of 1.6 ± 1.0 nM against U69,593 at the κ-receptor, and partial agonist activity at the δ receptor (EC_{50}: 1.4 ± 0.4 nM, Emax: 71±3%). In addition, cells stably transfected with CFP and YFP receptor fusion proteins for fluorescent resonance energy transfer studies have been generated that enable receptor oligomerization to be characterized. It is anticipated that further synthesis and testing in these systems will 1) identify unique antagonists/inverse agonists; 2) further our knowledge of ligand and receptor structure-activity relationships; 3) facilitate our understanding of neurochemical systems; and, 4) provide biochemical tools and potential medicinal agents relevant to drug abuse and to brain dysfunction.

Acknowledgements: Funded by the National Institute on Drug Abuse (R01 DA019217).

CB_1 CANNABINOID RECEPTORS DIMERIZE WITH β_2 ADRENERGIC RECEPTORS TO INFLUENCE RECEPTOR TRAFFICKING

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A number of family A G-protein coupled receptors (GPCRs) have been shown to interact with each other as dimers or higher order oligomers. The CB_1 receptor has been found to interact with itself as well as other GPCRs, including the D_2 dopamine, the orexin-1, and the opioid receptors. However, since the CB_1 receptor shows wide spread expression both within the central nervous system and the periphery, it is likely that CB_1 interacts with additional GPCRs. The objective of the present study was, 1) to identify novel interactions of the CB_1 receptor and, 2) to elucidate the importance of these complexes in receptor function.

A bioluminescence resonance energy transfer (BRET) approach was employed to identify GPCRs that interact with the CB_1 receptor. CB_1 receptor constructs with the BRET donor (Renilla Luciferase) or acceptor (GFP^2) fused to the C-terminus were expressed in 293H cells with BRET donor or acceptor constructs of other GPCRs in order to identify interacting partners. Through this approach, the β_2 adrenergic receptor was identified as a novel interacting partner of the CB_1 receptor. The specificity of this interaction was confirmed by the observations that the BRET efficiency ($BRET_{Eff}$) for this interaction was saturable and that the $BRET_{Eff}$ was competed by the co-expression of untagged β_2 receptors.

To address the functional importance of the CB_1-β_2 interaction, we used confocal microscopy and a quantitative near-infrared fluorescence assay (On-Cell Western) to measure surface expression and internalization of GFP^2 tagged CB_1 (CB_1-GFP) and HA tagged β_2 (HA-β_2) receptors expressed in 293H cells. We found that cells stably expressing only CB_1-GFP possessed ligand-independent CB_1 receptor activity, resulting in substantial constitutive internalization of CB_1-GFP. In contrast, cells expressing only HA-β_2 showed primarily cell surface expression of the receptor. Transient expression of HA-β_2 in the CB_1-GFP cell line resulted in a significant increase in the basal surface expression of CB_1-GFP, suggesting that the CB_1-β_2 interaction facilitates the surface expression of CB_1 receptors. Having demonstrated the impact of this interaction on the basal surface expression of CB_1-GFP, the influence of this interaction on agonist induced receptor internalization was examined. When cells co-expressing CB_1-GFP and HA-β_2 were treated with WIN 55,212-2, internalization of not only CB_1-GFP but also of HA-β_2 was observed. Similarly, when these cells were treated with the β adrenergic agonist isoproternol, internalization of both HA-β_2 and CB_1-GFP were observed, demonstrating that this interaction has implications for both CB_1 and β_2 receptor internalization. Together, these data demonstrate the ability of the CB_1 and β_2 receptors to physically interact with each other and that this interaction influences receptor surface expression and trafficking.

Acknowledgements: CIHR (MEMK); NSERC and Killam Trust studentships (BDH)

ALLOSTERIC MODULATION OF THE CANNABINOID CB_1 RECEPTOR: NOVEL ALLOSTERIC MODULATORS

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In a recent publication (Price et al, 2005) we identified three novel allosteric modulators of the cannabinoid CB_1 receptor. Interestingly, these compounds are allosteric *enhancers* of agonist binding *affinity*, but allosteric *inhibitors* of agonist signalling *efficacy*. To date, this is the most striking example in the GPCR field of dissimilitude between modulator effects on orthosteric ligand affinity versus efficacy, and provides us with a unique opportunity to dissect the mechanistic basis of this effect. The compounds that we have identified display a number of characteristics commonly associated with allosteric modulators, including (a) enhancement of the binding of the orthosteric ligand binding of [^{3}H]CP55950 to mouse brain membranes, (b) a slowing of the dissociation rate constant(s) for [^{3}H]CPP55940 from the occupied CB_1 receptor and (c) a non-competitive inhibition of orthosteric agonist efficacy, as demonstrated, for example, by the effect of the compounds on stimulation of [^{35}S]GTPγS binding to mouse brain membranes and inhibition of electrically-evoked contractions of the mouse vas deferens by CB_1 receptor agonists. We have extended our study by synthesising novel structural analogues of these compounds.

In equilibrium binding assays in mouse brain membranes, all three compounds inhibited the binding of the CB_1 receptor agonist, [^{3}H]CP55940. The Ki values were 360nM, 1000nM and 200nM for O-4722, O-5430 and O-5379 respectively. In contrast to the parent compounds, these analogues produced an apparent 100% displacement of the radiolabelled cannabinoid. In functional assays, the compounds behaved as insurmountable antagonists of receptor activation; in the mouse vas deferens assay they elicited a reduction in the E_{max} value for the CB_1 receptor agonists CP55940. A significant reduction in the E_{max} was observed at 10 nM for O-4722 and at 0.1 nM of O-5430 and O-5379. The compounds also modulated the stimulation of [^{35}S]GTPγS binding by CP55940 in mouse brain membranes. The data presented confirm that the cannabinoid CB_1 receptor contains an allosteric binding site that can be recognized by synthetic small molecule ligands.

References: Price M.R. et al (2005) Mol Pharmacol 68: 1484-1495

Acknowledgements: Funded by BBSRC CASE award with Organon Research, NIDA and GW Pharmaceuticals.

CAN THE LIPID BILAYER CONTRIBUTE TO CANNABINOID CB1 RECEPTOR CONSTITUTIVE ACTIVITY?

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Our recent molecular dynamics (MD) study of the interaction between anandamide and CB1 transmembrane helix (TMH) 6 in a lipid bilayer has suggested that CB1 might have been designed to recognize endocannabinoids from the lipid bilayer. One interesting feature of CB1 is that it is highly constitutively active in recombinant systems as well as in native tissue. In work to be presented, we test the hypothesis that this constitutive activity may be due in part to the lipid bilayer itself.

The CB1 receptor is a Class A G protein-coupled receptor (GPCR). Biophysical studies of another Class A GPCR, rhodopsin, have suggested that the conformation of W6.48(265) when rhodopsin is in its inactive / ground state (R; χ_1 = g+) changes during activation (i.e., W6.48(265) χ1 g+ $\rightarrow$ trans). We have shown that a W6.48/F3.36 interaction may act as the "toggle switch" for CB1 activation, with W6.48 χ1 g+/F3.36 χ1 trans representing the inactive (R) and W6.48 χ1 trans/F3.36 χ1 g+ representing the active (R*) state of CB1. Therefore, the change in W6.48 χ1 can be used as an indication of GPCR activation.

Multi-nanosecond (100 ns) molecular dynamics calculations were undertaken on the full CB1 bundle embedded in a high hydration palmitoyl-oleoyl-phosphatidylcholine (POPC) bilayer using NAMD2 with the CHARMM27 parameter set and the TIP3P model for water. Multi-nanosecond (100 ns) molecular dynamics calculations were also undertaken on the full CB1 bundle in an implicit membrane model, IMM1.

Simulations in explicit lipid revealed that a lipd acyl chain can intrude into CB1 from the TMH5-6 interface or from the TMH6-TMH7 interface (but never both simultaneously). Simulations in which lipid intruded from the TMH5-6 interface had a low incidence of the W6.48 χ1 g+ $\rightarrow$ trans transition. In this trajectory, a specific lipid appeared to block movement of W6.48. Simulations in which lipid intruded from the TMH6-7 interface had a high incidence of the W6.48 χ1 g+ $\rightarrow$ trans transition. In this trajectory, a specific lipid appeared to promote the transition. In the absence of specific lipids (i.e., in an implicit membrane model, IMM1), there was a low incidence of the W6.48 χ1 g+ $\rightarrow$ trans transition. These results suggest that lipid may be capable of promoting activation of CB1. The frequency of this promotion appears to be dependent on the ability of lipid to specifically intrude at the TMH6-7 interface. [Support: NIDA Grants DA03934 and DA000489].

BETA-ARRESTIN2 AFFECTS CB_1 LOCALIZATION & DOWNREGULATION

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Beta-arrestins are believed to regulate G-protein coupled receptors, but little is known of the role of the two specific subtypes in regulating CB_1 receptors. Previous studies have demonstrated that chronic cannabinoid treatment results in down-regulation and desensitization of CB_1 receptors. Recently, we have shown that the effects of acute Δ^9-tetrahydrocannabinol (THC), but not other cannabinoid agonists, were greater in beta-arrestin2-/- than in wild type mice. This implied that beta-arrestin2 selectively regulates the effects of THC. Chronic administration of THC or CP55940 resulted in rapid tolerance at rates that did not differ between wild-type and beta-arrestin2-/- mice. This implied that beta-arrestin2 does not affect tolerance to cannabinoids. The present study examines the role of beta-arrestin2 in the localization and down-regulation of CB_1.

The effect of deletion of beta-arrestin2 on the subcellular localization of brain CB_1 was examined. Brain from drug-naïve wild-type and beta-arrestin2-/- mice were separated into P1, P2, P3 and S3 fractions by differential centrifugation. [^{3}H]SR141716A binding was used to determine the density of CB_1 receptors. It was found that beta-arrestin2-/- mouse brain exhibited lower density of CB_1 in the P1 (nuclear) fraction, and greater levels of CB_1 in the P2 (lysosomal and synaptosomal) fraction compared to wild-type. There were no differences between genotypes in the P3 (cell body membrane) fraction, and there was no binding in the S3 (soluble) fraction. Finding high levels of CB_1 in the P1 fractions was of note, since there is little previous mention of CB_1 receptors associated with the nucleus. Yet the density of CB_1 in the P1 fraction was as great as in the P2, and greater than in the P3 fraction. These results indicate that beta-arrestin2 affects localization or trafficking of CB_1 receptors between synapses and the cell nucleus.

To examine the effects of chronic cannabinoids on brain cannabinoid receptor levels, separate groups of mice were injected daily with 50 mg/kg THC, 0.5 mg/kg CP55940 or vehicle (1:1:1:17, sesame oil: ethanol: emulphor: ddH20). Prior to the first and after each injection, tail withdrawal latency and/or rectal temperature were measured. Daily treatments were repeated until mice were completely tolerant to the effects of these drugs. One day after the last injection, brains were removed and whole brain homogenates assayed for brain CB_1 levels by antagonist radioligand saturation analysis. Treatment with 50 mg/kg THC decreased brain CB_1 density in wild-type brain by ~40%, but there was no change in beta-arrestin2-/- brain. This finding indicates that beta-arrestin2 affects CB_1 down-regulation by THC, but leaves an interesting question: What is the mechanism of tolerance to THC if no down-regulation of CB_1 occurs. To address this, we will measure activation of G-proteins in drug-naïve vs. drug-treated mouse brain.

Treatment with 0.5 mg/kg CP55940 produced no change in CB_1 levels in either genotype. These treatments are being repeated with 2 mg/kg CP55940, a concentration demonstrated to decrease wild-type mouse brain CB_1 levels (F Fan, Q Tao, M Abood and BR Martin, 1990, Brain Res **706**(1), 13-20). Brain levels of CB_1 receptor and G-protein activation in drug-naïve vs. drug-treated mouse brain will be compared.

THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ AGONIST CIGLITAZONE INHIBITS FATTY ACID AMIDE HYDROLASE

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The peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors are ligand-activated transcription factors that are involved in the regulation of inflammation and cell proliferation. Recent studies have demonstrated that the *N*-acyl ethanolamines AEA, palmitoylethanolamide and oleoylethanolamide can activate PPARs (Fu et al., *Nature* 425 [2003] 90-3; Lo Verme et al., *Mol Pharmacol* 67 [2005] 15-19; Bouaboula *et al., Eur J Pharmacol* 517 [2005] 174-81). The present study was designed to determine whether the reverse is true, namely whether PPARγ ligands interact with the endocannabinoid system in general, and with FAAH in particular.

FAAH activity was measured in rat brain membranes using AEA as substrate. Five thiazolidinedione PPARγ agonists were tested and found to inhibit AEA hydrolysis with an order of potency MCC-555 > ciglitazone ≈ pioglitazone > rosiglitazone > troglitazone. This differs from their reported potencies towards PPARγ where rosiglitazone > pioglitazone ≈ troglitazone > MCC-555 > ciglitazone. Ciglitazone was chosen for further investigation and found to inhibit FAAH in a competitive manner. The inhibition was pH sensitive, with IC_{50} values (0.5 μM AEA) of 16, 22, 37, 62 and 110 μM being found at assay pH values of 6.0, 6.6, 7.2, 7.8 and 8.4, respectively. In RBL2H3 cells, 30 and 100 μM ciglitazone significantly reduced the uptake of AEA, but this was not seen for cells treated with the FAAH inhibitor URB597, suggesting that the effect upon uptake is secondary to an action upon this enzyme. The pattern of FAAH inhibition is similar to that seen by the NSAID (R)-ibuprofen, which inhibits FAAH with IC_{50} values of 55 and 210 μM at assay pH values of 6.0 and 8.0, respectively, and which inhibits AEA uptake into C6 glioma cells in a manner not seen following an FAAH inhibitor (Fowler *et al., J Enzym Inhib Med Chem* 18 [2003] 55-58; Holt & Fowler, *Naunyn-Schmiedeberg's Arch Pharmacol* 367 [2003] 237-4). However, in contrast to ibuprofen, ciglitazone inhibited 2-OG hydrolysis by human recombinant monoacylglycerol lipase at pH 7.2 with an IC_{50} value of 100 μM.

In 2004, Perés-Ortiz *et a*l. (*J Biol Chem* 279 [2004] 8976-85) reported that ciglitazone was more efficacious than rosiglitazone in reducing C6 glioma cell viability. Using calcein-AM to assess cell viability (for details of the methodology used, see De Lago *et al., J Neurochem* 99 [2006] 677-88), we found that ciglitazone inhibited C6 glioma cell viability with IC_{50} values of 94, 68 and 23 μM following incubations for 3, 6 and 24 h. The effects of ciglitazone (24 h) were not blocked by either PPARγ antagonists (TOO 70907, GW9662), or CB receptor antagonists (AM251, AM630).

From these studies, it can be concluded that a) the thiazolidinediones interact with FAAH; b) ciglitazone may be a useful template for the design of FAAH-inhibitory, PPARγ agonist dual action compounds; and b) the inhibition of C6 glioma cell viability by ciglitazone does not involve activation of PPARγ or CB receptors.

DISCOVERY OF A CALCIUM-INDEPENDENT PHOSPHATIDYLETHANOLAMINE *N*-ACYLTRANSFERASE (iNAT)

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Animal tissues produce anandamide and other bioactive N-acylethanolamines from membrane phospholipids in two consecutive reactions catalyzed by N-acyltransferase (NAT) and N-acylphosphatidylethanolamine (NAPE)-hydrolyzing phospholipase D (NAPE-PLD). NAT is a membrane-bound, Ca2+-dependent enzyme which transfers sn-1 fatty acyl group of various glycerophospholipid molecules to phosphatidylethanolamine (PE) leading to the formation of NAPE. However, its cDNA has not yet been cloned. Lecithin retinol acyltransferase is an enzyme to transfer sn-1 acyl group of phosphatidylcholine (PC) to retinol generating retinyl ester. Considering functional similarity of NAT to LRAT, we investigated a possible NAT-like activity of proteins with structural homology to LRAT. We cloned cDNA encoding a rat LRAT homologous protein tentatively named rat LRAT-like protein (RLP)-1 and overexpressed it in COS-7 cells. When the recombinant RLP-1 was allowed to react with 1, 2-[14C]dipalmitoyl-PC and non-radioactive PE as acyl donor and acceptor, respectively, the formation of [14C]NAPE was observed. Further treatment of [14C]NAPE with NAPE-PLD and fatty acid amide hydrolase resulted in the release of [14C]palmitic acid, confirming that the N-acyl group of NAPE is radiolabeled. However, RLP-1 utilized both of sn-1 and sn-2 acyl groups of PC to produce NAPE, and therefore the anandamide precursor N-arachidonoyl-PE could be formed from 2-arachidonoyl-PC. Moreover, RLP-1 was a soluble protein, and the activity was little stimulated by Ca2+. In addition, mRNA level of RLP-1 and the cytosolic, Ca2+-independent NAPE-forming activity were by far the highest in testis among various rat organs, which was different from Ca2+-dependent NAT with the highest activity in brain. Taken together, RLP-1 was discovered as the first cloned protein which has ability to catalyze PE N-acylation, but clearly differed from the known NAT. To distinguish RLP-1 from the Ca2+-dependent NAT (Ca-NAT), RLP-1 may be referred to as Ca2+-independent NAT (iNAT).

EVALUATION OF N-ARACHIDONYL MALEIMIDE (NAM) AS A POSSIBLE INHIBITOR OF 2-AG DEGRADATION

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Developing selective and potent compounds that inhibit endocannbinoid degradation promises to unmask the biological functions of the various endocannabinoids and potentially to lead to treatments for disease and pathological conditions. Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) are the two primary enzymes involved in degradation of anandamide and 2-arachidonyl glycerol (2-AG), respectively. URB 597, the first potent and selective inhibitor of fatty acid amide hydrolase (FAAH), helped to unmask a potential role for anandamide in the treatment of chronic pain. However, despite this success with FAAH inhibition, the development of inhibitors of monoacylglycerol lipase (MAGL) has been less successful. Thus far, development of two compounds (URB 602 and URB 759) has been reported. Unfortunately, recent work has shown that neither compound is a selective or potent inhibitor of MAGL. Other reports have also shown that URB 759 is not effective in vivo. Recently, N-arachidonyl maleimide (NAM) has been proposed as a very potent inhibitor of MGL; as yet, however, there is no published research investigating use of NAM as an inhibitor of 2-AG degradation. Therefore, the aim of this project was to examine if NAM inhibits 2-AG degradation. To accomplish this, we have utilized both in vivo (THC tetrad of behavioral effects and a feeding protocol) and in vitro tests (radiolabeled SR141716A displacement assay as well as GTPγS autoradiography). Our initial in vivo results show that at doses lower than 3mg/kg NAM alone does not cause hypothermia, locomotor inhibition, catelepsy or antinociception and does not impact feeding. Our molecular results show that at concentrations less than 0.5 μM, NAM does not displace SR141716A from the cannabinoid CB_1 receptor, although it does so at higher concentrations. At this 0.5 μM concentration, NAM enhances the ability of 2-AG to stimulate CB_1 G-proteins in brain sections. These initial findings suggest that NAM attenuates degradation of 2-AG and may be a useful tool for unmasking the biological function of the endocannabinoid 2-AG. Further work is needed to validate these initial findings; therefore, the future directions of this project are (1) to determine the effect of 2-AG in the presence and absence of NAM in our behavioral paradigms and (2) to conduct GTPγS assays in order to ascertain if NAM by itself activates CB_1 receptor coupled G-proteins. This work was supported by NIH grants MH-64771 and DA-016644 (JLW), DA-05274 (DES), and DA-14277 (LJS).

AN ENDOGENOUS LIGAND FOR GPR55, A G PROTEIN-COUPLED RECEPTOR

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GPR55 is a seven transmembrane, G protein-coupled receptor. Recently, several investigators reported that GPR55 is a putative novel receptor for cannabinoids (Brown et al., 2005 Symposium on the Cannabinoids Abstract; 2005, p. 16; Sjogren et al., 2005 Symposium on the Cannabinoids Abstract; 2005, p. 106). These reports are exciting because GPR55 may be the "CB_3" receptor. However, not much information is thus far available concerning this unique G protein-coupled receptor. In fact, it remains unclear whether GPR55 actually acts as an intrinsic receptor for cannabinoids. Moreover, the endogenous ligand for GPR55 is not yet identified. Needless to say, the identification of the endogenous ligand is essential for the elucidation of the physiological significance of an orphan receptor. In this study, we decided to explore possible endogenous ligand for GPR55. We isolated several candidates from rat brain and examined whether these compounds interact with GPR55-expressing cells. We found a remarkable compound. This compound interacted with GPR55 and stimulated the cells which expressed GPR55. The EC_{50} was around 200 nM. This compound did not stimulate mock-tranefected cells which lack GPR55 at all. On the other hand, 2-arachidonoylglycerol (2-AG) and anandamide failed to stimulate the cells which expressed GPR55. Virodhamine and abnormal cannabidiol also did not elicit cellular responses. The agonistic activities of other synthetic cannabinoids were also very low if any. These results suggest that this novel compound acts as an endogenous ligand for GPR55 and that GPR55 is a specific receptor for this molecule.

FURTHER EVIDENCE THAT THE ENDOCYTOSIS INHIBITOR GENISTEIN REDUCES ANANDAMIDE UPTAKE SECONDARY TO INHIBITION OF FATTY ACID AMIDE HYDROLASE

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Cells are capable of accumulating anandamide (AEA), but the exact mechanisms involved in this accumulation are a matter of current debate. In 2004, McFarland *et al.* (*J Biol Chem* 279 [2004] 41991-7) showed that the uptake of AEA by RBL2H3 cells was reduced by about 50% by four different treatments, all of which affect caveola-dependent endocytosis: reduction of the assay temperature to 18 °C, *N*-ethyl-maleimide, a combination of nystatin and progesterone, and genistein. However, a reduction in assay temperature affects the AEA concentration available for uptake (Thors & Fowler, *Br J Pharmacol* 149 [2006] 173-81), *N*-ethylmaleimide is an inhibitor of fatty acid amide hydrolase (Schmid *et al., J Biol Chem* 260 [1985] 14145-9), and the combination of nystatin and progesterone was as effective at inhibiting AEA retention by wells alone as it was the accumulation of AEA (Thors *et al., Br J Pharmacol*, in press). Genistein was studied in detail and found to reduce the uptake to the same extent and over a similar time frame as the FAAH inhibitor URB597. In addition, the combination of the two compounds was not additive. Similar results were seen in C6 glioma cells. In rat brain homogenates, genistein was found to act as a competitive inhibitor of FAAH, with a K_i value of 2.8 μM (Thors *et al., ibid.*).

Although the experiments described above would suggest that actions of genistein upon FAAH rather than caveola-dependent endocytosis account for its effects upon AEA uptake, the possibility that endocytotic effects are masked in the RBL2H3 (and C6) cells due to the large FAAH-driven component of the uptake. 3T3-L1 preadipocyte cells are rich in caveolae (Huo *et al., J Biol Chem* 278 [2003] 11561-9) and have been shown to accumulate AEA in a manner that is increased when the cells are differentiated into adipocytes (Gasperi *et al., Cell Mol Life Sci* 67 [2007] 219-229). In our hands, homogenates from undifferentiated 3T3-L1 cells hydrolysed AEA very slowly, the activity [4.3±1.0 fmol.mg protein^{-1}.min^{-1} at pH 7.4 with 0.5 μM AEA as substrate], being two order of magnitude lower than that seen for RBL2H3 cell homogenates assayed concomitantly. This makes them ideal for the study of genistein effects upon AEA uptake in a cell with low FAAH activity. The sensitivity of undifferentiated 3T3-L1 cells to URB597 and genistein were compared with RBL2H3 cells. Over a concentration range of 0.01-3 μM, URB597 inhibited the accumulation of 100 nM AEA by RBL2H3 cells by ~60%. In contrast, the uptake of AEA into 3T3-L1 cells was not affected at all by these concentrations of URB597, indicating that the low FAAH activity present in the cells is not sufficient to "drive" the uptake under the conditions used. Initial experiments with genistein over a concentration range of 1-100 μM indicated that a 30 min preincubation with this compound reduced the subsequent uptake of AEA (4 min incubation time) into RBL2H3 cells in a concentration-dependent manner, whereas the uptake of AEA into 3T3-L1 cells was not affected at all. This would suggest that inhibition of FAAH by genistein is the primary determinant of its effects upon AEA uptake.

BOTH AGONISTS AND ANTAGONISTS OF CANNABINOIDS RECEPTORS CAN ACTIVATE PPAR ALPHA

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Although most of the physiological responses to cannabinoids are generally thought to be due to the action on the CB receptors, the PPAR (peroxisome-proliferator-activated receptor) family of nuclear receptor transcription factors have recently been found to be targets of some cannabinoids. In this work agonists and antagonists of CB receptors have been demonstrated to show diversified effects on PPARα in fluorescence-based ligand binding assay and reporter-gene based transcriptional transactivation assay. The phytocannabinoid, THC, neither binds nor acts on PPARα although it has been demonstrated to act on PPARγ. Endocannabinoids, OEA (pEC_{50} = 4.34), anandamide (pEC_{50} = 4.61), noladin ether (pEC_{50} = 4.78) and virodhamine (pEC_{50} = 4.36), were found to bind the ligand binding domain (LBD) of PPARα as determined by fluorescent ligand displacement assay. All the endocannabinoids tested induced a significant 2-fold or greater activation of PPARα transcriptional activity when added to HeLa cells at concentrations between 100nM and 10μM. Interestingly, whilst PEA was shown to be able to stimulate transcriptional activation by PPARα, we were unable to detect its binding to the PPARα LBD. The synthetic cannabinoid Win 55212-2 was found to bind PPARα with relatively high affinity (pEC_{50} = 4.74) and increase PPARα transcriptional activation at low concentration (100nM). OEA and Win 55212-2 were found to induce lipolysis both *in vivo* and *in vitro*, a robust physiological property of PPARα agonists. Interestingly, the CB_1 antagonists SR141716A and AM251 have also been found to significantly increase transcriptional activation of PPARα in transiently transfected CHO cells. They also showed small but significant lipolysis effects in differentiated 3T3-L1 cells (adipocytes). Ligand binding assay to determine whether SR141716A or AM251 binds to the PPARα LBD was inconclusive due to the high background fluorescence of these compounds in this assay. Conclusions: endocannabinoids and synthetic cannabinoid Win 55212-2 were shown to be capable of increasing PPARα transcriptional activation. Many appear to act as classical agonists of PPARα whereas PEA may act via an indirect mechanism. The mechanism of SR141716A and AM251 mediated PPARα activation and its functional relevancies remain to be determined.

ENDOCANNABINOID-SIGNALING REGULATES DEVELOPMENT OF SEA URCHIN EMBRYOS

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Development of pre-implantation mouse embryos is negatively impacted by prenatal exposure to exogenous cannabinoids, such as THC, and by treatments that perturb the endogenous "anandamide (AEA) tone". AEA is an agonist for mammalian CB_1, CB_2, and TRPV1 receptors. We now report evidence that AEA-signaling modulates early embryonic development in the sea urchin *L. variegatus*. In developing embryos, Western blots detected immunoreactive (IR) bands with CB_1 and CB_2 polyclonal antibodies. However, only the CB_2 IR band was detected in sperm. In embryos, CB_1 bands were weakly IR, even at the pluteus stage, while CB_2 bands were quite intense from the 2-cell stage on. Interestingly, CB_1- and CB_2-like IR bands exhibited opposite developmental dynamics, such that CB_1-IR bands increased progressively in intensity during development, whereas intensity of CB_2-like bands was highest at the 2-cell stage, then declined to towards the pluteus stage. Immunocytochemical studies localized CB_2-like IR in developing embryos, which was consistently seen in cells at the tip of the archenteron in gastrula stage embryos, suggesting localization either to secondary mesenchymal cells or to small micromeres, which give rise to primary germ cells. Specific binding of the non-selective CB agonist, [^{3}H]CP 55,940 was detected in *L. variegatus* embryos and sperm, consistent with previous studies showing specific binding of this ligand in *S. purpuratus* sperm. Also consistent with previous studies on *P. lividus* ovaries, AEA was detected in extracts of developing embryos analyzed by LC/MS: 8-16 cell embryos contained 1.33±0.12 pmole/mg protein, which increased more than 5X to 6.8±3.5 pmole/mg protein at the mid-blastula stage. Embryos cultured in exogenous AEA developed normally until the beginning of gastrulation, but became malformed thereafter. Similar effects were produced by AEA-reuptake inhibitors, other cannabinergic agonists [CP 55940, WIN 55212-2], CB_1- and CB_2-selective antagonists, together with rescue by drugs like the vanilloid-receptor agonist, arvanil. Results of perturb and rescue experiments suggest that a tightly regulated "AEA-tone" modulates differentiation during development of sea urchin embryos. AEA-signaling in sea urchin embryos may be mediated by CB_1-, CB_2-, TRP-like receptors, and/or by interactions with receptors for other neurotransmitters. However, orthologs of mammalian CB_1 and CB_2 have not been annotated in the current *S. purpuratus* sea urchin genome. Results of the present study may be explained in one of two ways: either: 1) genes encoding ancestral members of the endocannabinoid-signaling system have low homology to chordate cannabinoid receptors; or 2) AEA-signaling in sea urchins is mediated by non-cannabinoid receptor pathways. Supported by grants from NIDA # R21-DA018103 (JML) and # F32-DA16502 (MEF), The Center for Alternatives to Animal Testing (CAAT) Johns Hopkins University, and a University of NC Research Council grant (JML).

OPPOSITE EFFECTS OF AM404, AN INHIBITOR OF ENDOCANNABINOID UPTAKE INJECTED INTO THE VENTRAL HIPPOCAMPUS OF STRESSED OR NON-STRESSED RATS SUBMITTED TO THE ELEVATED PLUS MAZE

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INTRODUCTION: Several studies have suggested that the endocannabinoid system modulates anxiety and stress adaptation. For example, anxiolytic-like effects of AM404, an inhibitor of endocannabinoid transport, have been previously reported after systemic administration (Bortolato *et al.*, Neuropsychopharmacology, 2006). There is, however, a paucity of data regarding the involvement of endocannabinoids on brain sites related to these effects. The ventral hippocampus (VHC) has been related to anxiety behaviors and has a high expression of cannabinoid-1 receptors. Moreover, endocannabinoid signaling on hippocampus is proposed to regulate stress adaptation (Hill *et al.*, Neuropsychopharmacology, 2005). Thus, the aim of this study was to investigate the effects of AM404 microinjected into the VHC of stressed and non-stressed rats.

METHODS: Male Wistar rats (240-270g) with cannulae aimed at the VHC were forced immobilized for 2 h. Twenty four hours later they received bilateral injections of vehicle (V, 0.5 microL) or AM-404 (AM, 5 or 50 pmoles, N=8–11/ group) and were tested in the elevated plus maze (EPM).

RESULTS: Restraint induced a significant decrease in the percent of entries and time spent in the open arms. Microinjection of AM-404 (5 pmol) into the VHC reduced the percentage of entries and time spent in the open arms in non-stressed animals. On the other hand, the same dose of AM404 increased these behaviors in previously stressed animals.

DISCUSSION: The results suggest that the endocannabinoid system on the VHC modulates anxiety-like behavior depending on previous stressful experiences of the animal. FINANCIAL SUPPORT: FAPESP, CNPq, CAPES

CANNABINOID RECEPTOR 2 IS REQUIRED FOR HOMEOSTATIC CONTROL OF INTESTINAL INFLAMMATION

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Introduction: The phytocannabinoids from *Cannabis sativa* showed various medicinal properties, including in chronic pain disorders and cancer. Broad cellular and immunocyte responsiveness to cannabinoids are conferred by the cannabinoid receptor 1 (CB1) and 2 (CB2), which are two members of the G-protein coupled receptors (GPCRs). However, their respective physiological role in the gut remains elusive.

Aim: Given the regulatory role of endocannabinoids in inflammatory and nociceptive disorders, we hypothesized that abnormal enteric sensing by the cannabinoid receptors, CB1 and/or CB2, might predispose to inflammatory bowel diseases (IBD).

Methods: Expression of cannabinoid receptors in colonic resection specimens of IBD patients (40 Crohn Disease (CD), 30 Ulcerative Colitis (UC) and 30 non-IBD subjects) was evaluated by RT-PCR and immunohistochemistry. The effect of Fe 200859, a specific CB2 ligand, was investigated in different models of acute or chronic induced colitis (Trinitrobenzene sulfonic acid (TNBS), Dextran sodium sulfate (DSS)). The mechanism of action of this compound was assessed in vitro in two murine cell lines (RAW macrophage and MEF myofibroblast cell lines).

Results: We found abnormal expression of cannabinoid receptors expression in both colonic resection specimens of IBD patients. CB2 was significantly more expressed in non-inflammed resection specimens of CD patients, but not in UC. Consistently, by using pharmacological and genetic inhibition of Cnr2, we unraveled physiological anti-inflammatory and anti-fibrotic properties of CB2 in three preclinical models of IBD. These beneficial effects were reflected by increased survival rates, an improvement of macroscopic and histologic scores, a decrease in colon TNF-alpha and IL-1beta mRNA levels. Furthermore, CB2 blockade inhibited cell proliferation in a p65- and ERK-dependent manner, indicating that CB2 controls cell proliferation through NF-kB and MAPK signaling pathways.

Conclusion: CB2 was also required to limit immunocytes recruitment in vivo and chemokine production, providing an explanation for its anti-inflammatory and anti-fibrotic function in the intestine. Hence, since CB2 is required for homeostasis of intestinal inflammation, CB2-targeting compound should be considered to treat intestinal inflammation and visceral pain.

CANNABINOID-2 RECEPTOR MEDIATES PROTECTION AGAINST HEPATIC ISCHEMIA/REPERFUSION INJURY

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Hepatic ischemia-reperfusion (I/R) injury continues to be a fatal complication that can follow liver surgery or transplantation. We have investigated the involvement of the endocannabinoid system in hepatic I/R injury using an *in vivo* mouse model. Here we report that I/R triggers several-fold increases in the hepatic levels of the endocannabinoids anandamide and 2-arachidonoylglycerol, which originate from hepatocytes, Kupffer and endothelial cells. The I/R-induced increased tissue endocannabinoid levels positively correlate with the degree of hepatic damage and serum TNF-α, MIP-1α and MIP-2 levels. Furthermore, a brief exposure of hepatocytes to various oxidants (H_2O_2 and peroxynitrite) or inflammatory stimuli (endotoxin and TNF-α) also increases endocannabinoid levels. Activation of CB_2 cannabinoid receptors by JWH133 or HU308 protects against I/R damage by decreasing inflammatory cell infiltration, tissue and serum TNFα, MIP-1α and MIP-2 levels, tissue lipid peroxidation and expression of adhesion molecule ICAM-1 *in vivo*. JWH133 and HU308 also attenuates the TNF-α-induced ICAM-1 and VCAM-1 expression in human liver sinusoidal endothelial cells (HLSECs) and the adhesion of human neutrophils to HLSECs *in vitro*. Consistent with the protective role of CB_2 receptor activation, $CB_2^{-/-}$ mice develop increased I/R-induced tissue damage and pro-inflammatory phenotype. These findings suggest that oxidative/nitrosative stress and inflammatory stimuli may trigger endocannabinoid production, and indicate that targeting CB_2 cannabinoid receptors may represent a novel protective strategy against I/R injury.

DELTA-9-TETRAHYDROCANNABINOL-INDUCED PERITONEAL INFILTRATION OF NEUTROPHILS IS MAST-CELL DEPENDENT

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Delta-9-tetraydrocannabinol (THC), the major psychoactive constituent of marijuana, possesses significant immunomodulatory properties. We observed that i.p. administration of THC into C57BL/6 (WT) mice resulted in a dramatic dose-dependent accumulation of cells (THC $9\pm3.5x10^6$; vehicle $1.8\pm0.6x10^6$) in the peritoneal cavity, which peaked at 12-24 hrs. FACS analysis for markers CD3, F4/80 (macrophage) and Gr-1 showed that the infiltrating cells were predominantly (>80%) granulocytes. Also, majority of these granulocytes (>90%) were found to be neutrophils. A significant increase in Gr-1^+ cells was also observed in liver, but not in blood. Similar results were obtained in C3H mice, ruling out effects due to possible contaminating LPS. Chronic nasal challenge of WT mice also resulted in significant increase in granulocyte number in lungs. Cannabinoids mediate their effect through G-protein-coupled cannabinoid receptors. CB1 receptors are expressed in the brain and immune cells, whereas, CB2 receptors are found primarily on immune cells. Both CB1 and CB2 selective agonists (JWH133 & ACEA, respectively) independently induced intraperitoneal granulocyte infiltration comparable to THC. Moreover, pretreatment of animals with CB1 or CB2 select antagonists resulted in partial blocking, whereas, a combination completely blocked THC-induced granulocyte infiltration. Mast cells release inflammatory mediators upon activation, which can lead to leukocyte recruitment and inflammation. To test whether THC-induced granulocyte infiltration is caused by activation and degranulation of tissue mast cells, we used mast cell-deficient W/W(v) mice . Unlike WT mice, W/W(v) mice did not show significant granulocyte accumulation upon THC injection. We found bone marrow derived mast cells expressed both CB1 and CB2 mRNA. In conclusion, THC can activate tissue mast cells leading to dramatic neutrophil-specific chemotaxis.

(Support: NIH R01AI053703, R01ES09098, R01AI058300, R01DA016545, R01HL058641).

MICE LACKING THE CB1 RECEPTOR ARE RESISTANT TO FEVER INDUCED BY THE ENDOTOXIN LIPOPOLYSACCARIDE

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The anti-inflammatory actions of cannabinoids have been well documented. There is confusion, however, as to the role of CB_1 receptors in the neuroimmune response to lipopolysaccharide (LPS), in that both the cannabinoid agonist WIN55,212, and the CB_1 receptor antagonist SR141716A were reported attenuate the LPS-fever response in rats (Benamar *et al,* JPET; 320:1127-1133, 2006). The aim of the present study was to clarify the role of cannabinoid receptors in the febrile response to LPS by examining temperature responses to LPS in CB_1 knockout mice.

Wild type and CB_1 knockout mice (C57Bl6 background) were implanted with temperature data loggers (SubCue Dataloggers, Calgary.). Animals were then left for 7 days to recover, then were acclimatized to 27° C for 18 hours prior to experiment. Animals were then given an injection of either saline or LPS (100µg/kg, i.p.) and temperature was monitored for a further 7 hours.

Circadian temperature rhythms were not significantly different between wildtype and CB_1 knockout mice at either 21 or 27°C. Both wildtype and knockout mice treated with saline had no change in core body temperature compared with baseline. Wildtype mice treated with LPS displayed a typical biphasic fever response, with an initial peak in core body temperature at around 60 mins after injection and subsequent second peak that remained elevated for up to 5 hours. In contrast, CB_1 knockout mice had no febrile response to LPS in that their temperature responses were not significantly different to the saline controls or to their own baseline temperatures.

Our findings that CB_1 knockout mice are unable to mount a febrile response to an LPS challenge suggest that CB_1 receptors play an important role in the febrile response, but it is unclear if peripheral or central CB_1 receptors are involved. Further investigations will reveal whether endogenous cannabinoids, acting at CB_1 receptors, are involved either in thermogenesis required for the elevation of body temperature or in the peripheral immune responses to LPS.

Acknowledgments: Alberta Heritage Foundation for Medical Research, Canadian Association of Gastroenterology, Canadian Institute of Health Research and Janssen Ortho.

STRESS-INDUCED ALTERATIONS IN LIMBIC ENDOCANNABINOID CONTENT IN THE RAT: CORRELATIONS TO HPA AXIS ACTIVATION

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Several studies reflect an inhibitory role for the endocannabinoid system on the stress-related activation of the hypothalamic-pituitary-adrenal (HPA) axis. However, systematic examination of how these systems concurrently respond to acute stress exposure has yet to be performed. To this extent we obtained blood and brain tissue in adult male rats under basal conditions, immediately following a single 30 min episode of restraint, or 1hr thereafter. To correlate the relationship between restraint-induced changes in endocannabinoid and HPA activity, we measured 2-AG and AEA content in the prefrontal cortex, amygdala, and hypothalamus as a function of plasma corticosterone concentrations within individual animals. Rats displayed a significant increase in 2-AG levels in the hypothalamus and prefrontal cortex at 30 min of restraint, and these values were no different than pre-stress levels at 1 hr. We observed no significant difference in 2-AG content in the amygdala. In contrast, restraint caused a sustained decrease in AEA content, but only in the amygdala. With respect to the adrenal, restraint-induced changes in 2-AG content did not associate with the magnitude of corticosterone response. However, AEA content in the amygdala varied strongly and negatively with corticosterone response. Based on these findings in the amygdala, we then examined the corticosterone response to acute restraint following microinjection of the cannabinoid CB_1 receptor agonist HU-210 into the basolateral amygdala. Relative to vehicle-injected controls, the corticosterone response to restraint was significantly reduced in HU-210-injected rats. These data support the hypothesis that endocannabinoids act to dampen the stress-induced activation of the HPA axis, and reveal an important role for the basolateral amygdala in mediating the endocannabinoid response to restraint.

DELTA-9-TETRAHYDROCANNABINOL TREATMENT AMELIORATES T CELL-MEDIATED HEPATITIS BY SUPPRESSING IMMUNE RESPONSE IN A CB1 RECEPTOR-SPECIFIC MECHANISM

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Immune-mediated liver diseases including autoimmune and viral hepatitis are a major health problem worldwide. Natural cannabinoids such as delta-9-tetrahydrocannabinol (THC) effectively modulate immune cell function and have shown therapeutic potential in treating inflammatory diseases. We investigated the effects of THC in a murine model of Concanavalin A-induced hepatitis. Intraperitoneal administration of THC after ConA challenge inhibited hepatitis as shown by significant decrease in liver enzymes and less severe liver tissue injury. Furthermore, THC treatment resulted in significant suppression of crucial inflammatory cytokines in ConA-hepatitis. Surprisingly, T and NKT cell numbers were higher in ConA-injected mice treated with THC. However, intrahepatic $CD4^+CD25^+$ cells were increased by more than two fold upon THC treatment, and Foxp3 mRNA was significantly upregulated both in spleen and liver. We observed that CB1 but not CB2 receptor agonist was able to block hepatitis similar to THC. Moreover, THC mediated immunomodulation in ConA-induced hepatitis was blocked by a specific CB1 receptor antagonist. Our data demonstrate that early treatment with THC inhibits development of liver inflammation in ConA-induced activated T cell-mediated hepatitis model by inducing immune suppression mediated through CB1 receptors. Thus, THC or cannabinoids signaling at the CB1 receptor hold great therapeutic potential for treating T cell-mediated inflammatory liver diseases.

(Support: NIH R01AI053703, R01ES09098, R01AI058300, R01DA016545, R01HL058641).

CHRONIC EFFECTS OF EXOGENOUS CANNABINOID AGONIST/ANTAGONIST ON SHORT-TERM MEMORY PERFORMANCE IN RATS

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Systemic administration of many cannabinoid agonists (Δ^9-THC, CP 55940, anandamide) have revealed dose/delay dependent deficits in working/short-term memory performance in spatial learning and memory paradigms such as the radial-arm maze, water maze and delayed non-matching to sample (DNMS) tasks. Here we assess the chronic exposure of the potent CB1 receptor agonist, WIN 55212-2 (WIN-2) and CB1 receptor antagonist, SR141716A (SR) on DNMS performance using subcutaneous osmotic mini-pumps to deliver the drug uniformly in an effort to better mimic the persistent effects of these exogenous cannabinoid compounds. Concomitant neuronal ensemble recordings from hippocampal CA1/CA3 sub-fields were carried out in an attempt to correlate their activity with performance of this hippocampal dependent task.

9 Adult, male Long Evans rats were pre-trained to perform the DNMS (i.e. short-term memory) task and implanted with 16 micro-wires to CA3/CA1 regions of the hippocampus. Following cell isolations, all subjects were implanted with 14 day mini-pumps containing 10% DMSO + 0.1% Tween-80 in distilled water (vehicle), WIN-2 (3.75 mg/kg/day) and SR (5.0 mg/kg/day) according to a randomly assigned schedule. The initially identified CA3/CA1 neurons in all subjects were "tracked" from day 3-12 following each treatment. 4 animals were challenged with 0.5 mg/kg of WIN-2 (i.p) on day 12 following each treatment

Continual effects of WIN-2 produced a delay-dependent deficit in DNMS performance from day 3 to 6 but, showed behavioral adaptation/tolerance from day 7 onwards. SR did not have any effect on DNMS performance. WIN-2 induced an overall suppression in hippocampal activity around the sample (i.e. encoding) phase as opposed to the non-match (i.e. recall) phase. This suppression was absent on day 7, 11 and even after challenging with WIN-2 on day 12.

These results confirm that chronic WIN-2 exposure impairs working/short-term memory by distrupting (i.e. suppressing) hippocampal firing during the encoding phase but, not the recall/retrieval phase of the DNMS task. The key difference from what was observed previously with daily bolus injections, is the faster development of tolerance/adaptation to this cannabinoid agonist (i.e. 7 days as opposed to 30 days).Furthermore, a failure of SR to produce any effect on DNMS performance, suggests that endocannabinoids (anandamide and/or 2-AG) probably do not play a role in executing this short-term memory task via CB1 receptor activation.

EFFECT OF CB1 RECEPTOR ANTAGONISM AND EXTINCTION LEARNING ON RELAPSE BEHAVIOR IN MICE

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Introduction: In humans, relapse remains a hindrance to successful treatment of compulsive behaviors such as drug use and uncontrollable food intake. Behavioral therapies focused on extinguishing food or drug cravings elicited by the presence of reward-associated cues may enhance the effectiveness of pharmacological interventions to prevent relapse. Recently, CB1 receptor antagonism has emerged as a promising therapeutic approach to relapse prevention, but the combined effect of behavioral extinction and CB1 receptor antagonism is unknown. Therefore, we conducted experiments to assess the effects of CB1 receptor antagonism and extinction learning, both alone and in combination, on subsequent motivation to respond for a food reward. Importantly, several phenomena arising during extinction learning may actually provide mechanisms for relapse, and pharmacotherapies that attenuate these mechanisms and enhance extinction learning may also be beneficial to relapse prevention. Interestingly, the CB1 receptor system has also been implicated in extinction learning, so we have also conducted experiments to assess the effect of CB1 receptor antagonism throughout extinction learning.

Methods: Male C57Bl/6 mice were trained to self-administer a 32% corn oil solution. In Experiment 1, we assessed the effect of 1) the CB1 receptor antagonist SR141716A (SR) versus vehicle 2) exposure to extinction learning versus passive abstinence and 3) combined extinction learning and subsequent SR treatment on the subsequent motivation to self-administer corn oil. In Experiment 2, we assessed the effect of SR pretreatments throughout extinction learning on 1) initial burst responding (first extinction session) 2) spontaneous recovery of responding (second extinction session), and 3) reinstatement of responding elicited by a food prime (third extinction session).

Results: Experiment 1: Both SR and extinction learning decreased motivation to self-administer corn oil, and the largest effect was seen in SR-pretreated mice that underwent extinction learning.
Experiment 2: SR pretreatment significantly attenuated extinction burst responding, spontaneous recovery, and food prime-induced reinstatement of corn-oil seeking as compared to vehicle.

Conclusion: Taken together, results suggest that extinction learning and CB1 receptor antagonism attenuate corn oil self-administration, with a combination of the two treatments being most effective. Furthermore, administration of a CB1 antagonist over the course extinction learning decreased behaviors that may provide mechanisms of relapse in humans. Therefore, a combined behavioral/pharmacological treatment strategy consisting of concurrent extinction therapy and CB1 receptor antagonism may be an effective treatment for relapse prevention.

THE FATTY ACID AMIDE HYDROLASE (FAAH) INHIBITOR URB597 PRODUCES IMPROVEMENTS IN LONG-TERM MEMORY RETENTION THAT ARE MEDIATED BY α-TYPE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARα) AND NOT BY CANNABINOID CB_1 RECEPTORS

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Fatty acid amide hydrolase (FAAH) is an intracellular enzyme catalyzing hydrolysis of endogenous lipid mediators, such as anandamide (an endogenous cannabinoid CB_1 receptor agonist), as well as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), which are endogenous agonists for the peroxisome proliferator-activated receptor-α (PPARα). URB597 selectively inhibits intracellular FAAH activity, resulting in increased levels of anandamide, OEA and PEA in several brain areas, including regions mediating learning and memory. Here we studied effects of FAAH inhibition and PPARα activation on learning and memory using a passive-avoidance task in rats. During a single learning trial, rats were placed in the lighted compartment of a shuttle box, and received foot shocks when they entered the dark compartment. During subsequent testing, latency to enter the dark side of the box was recorded during retention tests conducted 1 and 7 days after the learning trial. In an acute study, separate groups of rats were injected at different times to assess the effects of URB597 on acquisition, consolidation, retrieval and extinction of avoidance learning (40 min prior to the learning trial, 30 min after the l.t., 30 min prior to the first retention test, and 30 min prior to the second r.t, respectively). URB597 did not impair learning or memory at any time of injection, compared to vehicle-injected controls. In contrast, scopolamine (0.5 mg/kg) significantly impaired acquisition, consolidation, retrieval and extinction, confirming that the procedure was sensitive to disruptions in learning and memory processes (positive control). During the first retention test 24 hr after the learning trial (short-term memory), rats that had received 0.1 mg/kg URB597, but not 0.3 or 1 mg/kg, before the learning trial showed small but significant increases in latency to enter the dark compartment, indicating improvements in learning by FAAH inhibition. During the second retention trial 7 days later (long-term memory), rats that had received all three doses of URB597 before the learning trial showed marked and significant increases in latency to enter the dark compartment. This effect of URB597 on long-term memory retention was not blocked by the CB_1 receptor antagonist SR141716 (1 or 3 mg/kg, i.p., 1 h before the learning trial), but was blocked by the PPARα antagonist MK886 (1 mg/kg, i.p., 1 h before the learning trial). When the synthetic PPARα agonist WY14643 (40 mg/kg) was given i.p. 10 min before the learning trial, the latency to enter the dark compartment during the 7-day retention test was also markedly and significantly increased and this effect was reversed by MK886. These results suggest that improvements in long-term memory retention produced by FAAH inhibition are not cannabinoid-receptor dependent and, instead, are mediated by PPARα activation. *The research was supported in part by NIDA, IRP, NIH.*

CANNABINOID$_1$ RECEPTORS IN THE HIPPOCAMPUS ARE NECESSARY FOR THE MEMORY DISRUPTING EFFECTS OF CANNABINOIDS

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It is well established that the administration marijuana as well as a variety of cannabinoid agonists impair memory. Additionally, there is considerable evidence suggesting that the stimulation of CB_1 receptors in the hippocampus may underlie this effect on memory. Thus, the present study was designed to test the hypothesis that the disruption of spatial working memory following cannabinoid administration is mediated by CB_1 receptors in the hippocampus. We have previously demonstrated that intrahippocampal administration of the cannabinoid agonist CP 55,940 led to spatial working memory deficits in a rat eight-arm radial maze task (Lichtman and Martin, Psychopharmacology (1995) 119:282-90). In the present study, we confirmed that intrahippocampal CP55,940 (10 μg/rat) disrupts memory in the radial arm maze and further tested whether this effect was blocked by intrahippocampal administration of the CB_1 antagonist rimonabant (0.06 μg/rat). We then assessed whether intrahippocampal rimonabant would block the memory disruptive effects of systemically administered (i.p.) CP 55, 940 (0.05 mg/kg) or THC (5.6 mg/kg) in the radial arm maze. Additionally, we sought to determine if the antagonistic effects of intrahippocampal rimonabant were selective for cannabinoid-induced memory impairment; thus, we tested the ability of intrahippocampal rimonabant to block the effects of systemic CP 55,940 (0.5 mg/kg) in the tetrad (body temperature, locomotor activity, analgesia, and catalepsy). Intrahippocampal administration of rimonabant significantly reduced the number of errors of re-entry in the maze observed following intrahippocampal (VEH-CP 55, 940: mean ± SEM = 3.00 ± 0.69; Rimonabant-CP 55, 940: mean ± SEM = 0.44 ± 0.24) or systemic (VEH-CP 55, 940: mean ± SEM = 3.7 ± 0.76; Rimonabant -CP 55, 940: mean ± SEM = 0.81 ± 0.26) CP 55,940 administration. Most importantly, intrahippocampal administration of rimonabant significantly blocked the spatial memory deficits observed following systemic THC administration (VEH-THC: mean ± SEM = 2.63 ± 0.63; Rimonabant -THC: mean ± SEM = 0.87 ± 0.22). In contrast, intrahippocampal rimonabant did not the block the effects of CP 55,940 in the tetrad, indicating that hippocampal CB_1 receptors play a specific role in mediating cannabinoids' memory disruptive effects. These findings strongly support the hypothesis that hippocampal CB_1 receptors are necessary for the memory impairing effects of systemically administered cannabinoids.

Acknowledgements: This research was supported by the National Institute on Drug Abuse (DA015683 and T23DA07027)

USE OF THE CANNABINOID NABILONE FOR THE PROMOTION OF SLEEP IN CHRONIC, NON-MALIGNANT PAIN PATIENTS: A PLACEBO-CONTROLLED, RANDOMIZED, CROSSOVER INSOMNIA PILOT STUDY

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Objectives: Evidence suggests that THC can alleviate pain and improve sleep but there are no randomized, controlled studies evaluating the effect of synthetic THC analogues such as nabilone (Cesamet). The objectives of this study were, in patients with insomnia and chronic, non-malignant pain, (1) to evaluate the effect of nabilone (1mg) on sleep efficiency (SE), total sleep time (TST), sleep onset latency (SOL), arousal index (AI) and patient-reported pain measures; and (2) to determine if nabilone treatment causes daytime sleepiness. Methods: This was a double-blinded, placebo-controlled, randomized, crossover clinical pilot study with consecutive subject enrollment. Following baseline assessment, subjects were randomly assigned to either Group 1 or 2. Group 1 received nabilone for the first four weeks followed by four weeks of placebo administration. Group 2 received placebo for the first four weeks followed by four weeks of nabilone treatment. Overnight sleep assessment and tests of daytime sleepiness and alertness (Multiple Sleep Latency Test – MSLT and Maintenance of Wakefulness Test - MWT) were conducted at the start of the study and at the end of the first and second four-week periods. Results: A total of 11 of 12 patients (10F, 1M; median age 54.7 yrs, range 33-65 yrs) completed the study. With nabilone treatment, there was a 2.6% average increase in SE (68.1% ± 22.8 baseline vs. 70.7% ± 15.2 with treatment), an average TST increase of 18.2 minutes (329.8 ± 108.5 min baseline vs. 348.0 ± 76.6 min with treatment) and a decrease in the AI (number of arousals from sleep per hour; 7.4 ± 5.1 baseline vs. 5.6 ± 5.3 with treatment). There appeared to be a significant increase ($p= 0.03$) in SOL with nabilone treatment (29.6 ± 25.5 min baseline vs. 61.4 ± 45.2 min with treatment). The improvements in SE, TST and AI did not reach significance ($p=0.69$, $p=0.55$ and $p=0.5$, respectively) but in 5 of the 11 patients there was a consistent and significant ($p=0.05$) improvement in both SE and TST with nabilone treatment. Further, in all study patients there was a significant reduction in pain symptoms (assessed by the McGill Pain Questionnaire) with nabilone treatment ($p=0.005$; 18.5 ± 7.9 baseline vs. 13.8 ± 7.3 with treatment). Nabilone treatment did not result in daytime sleepiness or diminished alertness in the study patients (MSLT: 12.9 ± 5.8 mins baseline vs. 13.3 ± 6.7 with treatment, $p=0.85$; MWT: 22.2 ± 6.2 mins baseline vs. 23.2 ± 7.3 with treatment, $p=0.70$). Conclusions: Nabilone treatment decreased pain symptoms in all study patients and, overall, resulted in modest improvements in sleep efficiency and total sleep time. Despite its clear analgesic effect, nabilone did not cause daytime sleepiness or impaired alertness. About half the study patients responded very favourably to nabilone treatment and had substantial improvements in their sleep. These patients were given prescriptions for nabilone as part of their subsequent clinical management. The distinction between primary insomnia and secondary insomnia caused by chronic pain may help explain the findings of this study. In patients with secondary insomnia, amelioration of pain symptoms with nabilone treatment would result in improved sleep.

Acknowledgement: Funding for this study was provided by Valeant Canada.

CANNABINOIDS IN THE MANAGEMENT OF CANCER PAIN: A COHORT STUDY USING PROPENSITY ANALYSIS

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It is estimated that approximately 80 percent of cancer patients experience significant pain and that up to 50% die in a state of poor pain control. The use of drugs such as opioids, anti-epileptics, corticosteroids, and tricyclic anti-depressants, is often associated with less than optimal analgesia as well as significant side effect burden. Anecdotal and clinical evidence support the role of cannabinoids as adjunctive therapy in pain management. A retrospective study was carried out to analyse the effects of the synthetic cannabinoid nabilone on pain and concomitant use of analgesic medications in a case series of out-patient palliative medicine consultations. Patients referred to a specialist out-patient consultative palliative medicine program from July 1, 2005 to June 30, 2006 were stratified according to whether or not they received nabilone therapy on their initial consultation. All patients completed the Edmonton Symptom Assessment System (ESAS) questionnaire at baseline and at least one other time thereafter. The differences in ESAS pain scores and medication use between non-nabilone and nabilone patients as well as within the two groups were analysed using propensity scoring. Results: A total of 122 patients with advanced-stage cancer met criteria for inclusion in the analyses. Of the patients, 65 received nabilone, while 57 were not prescribed the medication. The mean duration from baseline to last ESAS score was 44.9 days (+60.7) in the non-nabilone group compared with 58.0 days (+83.6) in the nabilone group. All patients were then subjected to propensity scoring in order to arrive at two groups that were similar at baseline. From baseline to last measurement, cancer patients receiving nabilone experienced a significant reduction in pain. The utilization of opioids and other adjuvants was reduced in nabilone-treated patients. The results of this cohort analysis correlate with emerging data supporting the use of cannabinoids to optimize pain management in cancer patients.

SMOKED CANNABIS FOR CHRONIC NEUROPATHIC PAIN: RESULTS OF A PILOT STUDY

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Introduction
Chronic neuropathic pain affects an estimated 5-10% of adults and is refractory to many pharmacological treatments. Surveys suggest that 10-15% of patients attending pain clinics self-medicate with smoked cannabis to relive pain, improve sleep, reduce stress and improve mood. Here we present the results of a pilot study to explore the safety and efficacy of smoked cannabis for chronic neuropathic pain.
Methods
We conducted a pilot randomized controlled crossover trial comparing four potencies of herbal cannabis (0%, 2.5%, 6% and 9.5% THC). Adult subjects with chronic neuropathic pain due to trauma or surgery with allodynia or hyperalgesia who were not current cannabis users were randomized to receive the four potencies in four five-day periods separated by nine-day washout periods. Subjects smoked a single inhalation of 25mg of cannabis using a pipe device three times daily for each five-day period. The first exposure of each period was done under direct supervision in a clinical laboratory; the remaining doses were taken at home. The primary outcome was the difference in the 5-day average pain intensity score, obtained by daily pain intensity measurements using an 11-point numerical rating scale administered by telephone during the exposure period between the 9.5% THC period and the 0% THC period. Effects on mood (POMS), sleep (LSEQ) and quality of life (EuroQOL-5D) were measured as well as adverse events.
Results
Twenty-three subjects were recruited, with a mean age of 45.4y (SD 12.3); 12 were female (52%). The median duration of pain was 2.9 years (range 1.1-41y). Two subjects dropped out in the first week, and 21 completed the trial. The average daily pain intensity was lower during the 9.5% THC period compared to the 0% THC period (5.4 (SD 1.7) vs. 6.1 (1.6); p=0.02). No significant differences in mood were observed. Subjects reported significantly improved ability to fall asleep (easier; p=0.001; faster, p<0.001; more drowsy, p=0.003) and improved quality of sleep (less wakefulness, p=0.01) on the 9.5%THC period than the 0% THC period. Overall quality of life was not different between the two groups. At the end of the study, 76% of subjects correctly guessed which was the 9.5%THC period and 62% correctly guessed the 0% THC period. Compliance was excellent. No serious adverse events were observed. The most common (6 or fewer episodes) drug-related adverse events during the 9.5% THC period were headache, dry eyes, burning sensation, dizziness and numbness and cough.
Conclusion
This pilot study has shown that smoking 25mg (one puff) of 9.5% THC herbal cannabis three times daily for five days has a modest analgesic effect on chronic neuropathic pain and improves sleep. The drug was well-tolerated. Further long-term safety and efficacy studies are needed to evaluate the duration of these effects

FUNCTIONAL ACTIVITY ASSOCIATED WITH SUCCESSFUL AND UNSUCCESSFUL DECISION MAKING STRATEGIES IN CHRONIC MARIJUANA USERS

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The majority of chronic marijuana (MJ) users have an inability to develop successful decision making strategies while performing a modified version of the Iowa Gambling Task (IGT; Whitlow et al., 2004). This is evident when chronic MJ users continuously select cards from disadvantageous decks once healthy controls have learned to shift their selections from disadvantageous to advantageous decks. Though it is not common, some chronic marijuana users develop successful decision making strategies while performing the IGT. The purpose of this study was to use fMRI to determine the neurofunctional correlates associated with **1)** controls' ability to develop and maintain advantageous strategies and **2)** chronic MJ users' ability or inability to develop advantageous strategies on the IGT. Subjects selected 10 cards at a time from one of four decks (2 advantageous and 2 disadvantageous) with rest blocks in between, for a total of 100 card selections. Using standard linear regression techniques, the fMRI data collected during the early card selections (developing strategies) were compared to late card selections (maintaining the strategies) both within and between the groups. When comparing early to late card choices in controls, we found that controls relied on increased activity in the left medial prefrontal cortex (MPFC, BA10/32), the middle temporal gyrus (MTG,BA 21), the precuneus (BA 7) and the thalamus in order to develop successful decision making strategies (early > late). In contrast, they relied on increased activity in the right dorsal lateral prefrontal cortex (DLPFC, BA 9) to maintain those strategies (late > early). When comparing controls and MJ users during early card selections, we saw that MJ users who did not develop successful decision making strategies showed increased activity in the superior frontal gyrus (SFG,BA 8), the right PFC (BA 9), the anterior cingulate (ACC, BA 24), the left angular gyrus (BA 39), the left cuneus (BA 18/19) and the cerebellum (Cbl). Furthermore, MJ users had decreased activity in the left DLPFC, thalamus and bilateral anterior insular cortex (BA 13), compared to controls. We then divided the MJ users into groups based on whether they did not (MJNLs) or did (MJLs) develop successful decision making strategies. Comparing the groups based on early card selections revealed that MJNLs had increased activity in the right medial PFC (BA 9), the left lateral PFC (BA 9), the anterior cingulate (ACC, BA 24), bilateral anterior insular cortex (BA 14) and the cerebellum (Cbl). In contrast, MJLs had a discrete increase in activity in the left mid cingulate (BA 24). Demographically, MJNLs and MJLs differed in their duration of marijuana use with MJNLs using 13.2 years on average and MJLs using 6.5 years.

These data suggest that developing successful strategies for performing the IGT involves increased functional activity in a distinct neural network comprised of the medial PFC, precuneus and DLPFC. In contrast, the inability to develop successful strategies is related to diffuse and inefficient changes in brain activity. Furthermore, whether or not chronic marijuana users develop successful decision making strategies to perform the IGT may be related to the duration of marijuana use.

CHARACTERISTICS OF ADULT, NON-TREATMENT-SEEKING CANNABIS SMOKERS

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Cannabis is the most widely used illegal psychoactive drug in the world, with more than 160 million current users. Relatively little is known about those who have tried to quit use without formal treatment. We have previously reported on the characteristics of a convenience sample of 104 non-treatment-seeking adult cannabis smokers recruited from other research studies (Boyd et al., *Am J Addict* 14:35-42, 2005; Copersino et al., *Am J Addict* 15:8-14, 2006; Copersino et al., *Am J Addict* 15:297-302, 2006). We here extend these findings with data from a separate convenience sample of 235 adult cannabis smokers (mean [SD] age = 29.2 [10.0] years, range 18-64 years, 57.9% male, 79.6% African-American, 15.7% white, 3.8% other, 47.7% unemployed) recruited from the community who reported at least one "serious" (self-defined) quit attempt without formal treatment. Subjects reported their first cannabis use at age 14.9 [3.4] years and first regular (at least weekly) use at age 16.8 [3.9] years. One hundred and fifty-one subjects (64.3%) reported experiencing at least one problem associated with their cannabis use, at age 19.0 [5.2] years. Common experiences were needing to use more cannabis than at first to get the same “high” (81.3% of subjects), suggestive of tolerance, and experiencing symptoms when quitting cannabis use (41.0%), suggestive of withdrawal symptoms. Eighty-six percent of these subjects resumed cannabis use to reduce or avoid symptoms from quitting, suggesting that cannabis withdrawal can serve as a negative reinforcer for cannabis use. The influence of age (younger than or the same vs. older than median age of 26 years), gender, and race on cannabis use characteristics was evaluated by 2-tailed t-test for quantitative variables and chi-square test for categorical variables. Older subjects reported an older age than younger subjects for first using cannabis (15.8 [4.1] vs. 14.2 [2.3 years, $p < 0.0001$, 95% CI for difference -2.5 to -0.7 years), first regular cannabis use (18.0 [4.7] vs. 15.8 [2.5] years, $p < 0.0001$, 95% CI -3.2 to -1.2 years), and first cannabis-related problem (20.3 [6.6] vs. 17.8 [3.0] years, $p = 0.004$, 95% CI -4.3 to -0.8 years). Older subjects also reported a trend towards longer latency between first use and first regular use (2.2 [3.2] vs. 1.6 [1.6] years, $p = 0.06$, 95% CI -1.3 to 0.02 years) and between first regular use and first cannabis-related problem (2.9 [5.1] vs. 1.8 [2.2] years, $p = 0.10$, 95% CI -2.5 to 0.2 years). Women reported an older age than men for first using cannabis (15.8 [3.7] vs. 14.3 [3.0] years, $p < 0.001$, 95% CI -2.4 to -0.6 years) and first regular cannabis use (17.8 [4.4] vs. 16.1 [3.3] years, $p < 0.001$, 95% CI -2.8 to – 0.8 years), and were more likely to report using cannabis to reduce or avoid withdrawal symptoms (95.4% vs. 78.0%, $p = 0.03$). There was no significant gender difference in age (29.8 [11.0] vs. 28.8 [9.3] years for women and men, respectively, $p = 0.50$, 95% CI -3.6 to 1.7 years). There was no significant effect of race on any cannabis use characteristic. These findings suggest that there are significant age and gender differences in the cannabis use histories of adult, non-treatment-seeking users, although the clinical significance of these differences remains unclear.

Supported by the Intramural Research Program of the US National Institutes of Health, National Institute on Drug Abuse.

MULTIDRUG TRANSPORTERS MRP1 AND BCRP ARE INHIBITED BY PLANT-DERIVED CANNABINOIDS

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Cannabis is often used by cancer patients to alleviate the side-effects of chemotherapeutic treatment. We have recently shown that cannabinoids might modulate the effectiveness of anti-cancer drugs by interacting with the multidrug resistance (MDR) transporter, P-glycoprotein. Other ATP-binding cassette transporters, such as multidrug resistance protein 1 (MRP1) and breast cancer resistance protein (BCRP) also influence both the cytotoxicity and pharmacokinetics of anticancer drugs. In this study we examined whether three well-characterized compounds found in cannabis and also in other cannabinoid pharmaceutical preparations (e.g. Marinol™, Sativex™), interact with either MRP1 or BCRP. Using transfected mammalian cell lines we found that Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) (10-50 μM) increase the accumulation of known MRP1 and BCRP substrates as assayed using fluorescent activated cell sorting (FACs). Analysis of the vanadate-sensitive ATPase activity of MRP1 and BCRP demonstrated an inhibition of substrate stimulated ATPase activity for both MRP1 and BCRP by all compounds, with no affect on basal ATPase activity for MRP1 and a slight inhibition of activity for BCRP. Together, these results indicate that THC, CBD and CBN interact directly with both MRP1 and BCRP to inhibit transporter activity. As predicted, MRP1 and BCRP transfected cell lines were less sensitive to the cytotoxic actions of substrate anticancer drugs, however, no reduction in sensitivity to the cytotoxic actions of THC, CBD or CBN was observed. The expression of cannnabinoid receptors (CB1, CB2) was not detected in these cell lines. Therefore, the cytotoxicity of THC, CBD and CBN appears cannabinoid receptor independent, presumably involving an intracellular target. This finding provides evidence that THC, CBD and CBN, while acting as inhibitors of MRP1 and BCRP, are not themselves effectively transported by these MDR transporters.

OVERACTIVATION OF THE ENDOCANNABINOID SYSTEM IN HUMAN ENDOMETRIAL CARCINOMA

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The womb, anatomically distinguished in cervix and corpus (myometrium and endometrium), is the major female reproductive organ of most mammals, with the main function to accept the fertilized ovum. Uterine neoplasias represent the most frequent feminine pathologies in oncology. In particular, endometrial carcinoma occurs in both pre-menopausal (25%) and postmenopausal women (75%) and is the third most common cause of gynecologic cancer deaths (behind ovarian and cervical cancer). The development of this cancer arises from a series of genetic alterations that transform the normal endometrium through the stages of hyperplasia, dysplasia and finally overt carcinoma. The endocannabinoid (EC) system has a role in the regulation of female reproduction. In fact, anandamide (AEA) regulates fertility, embryo implantation and pregnancy progression. Furthermore the EC system plays a protective function against the growth and spreading of several types of carcinomas. Yet, the involvement of the endocannabinoid system in uterine and ovarian neoplasias is still poorly investigated. To date, only one report has been published indicating a pro-apoptotic effect of AEA in uterine cervix cancer cells via activation of aberrantly expressed vanilloid TRPV1 receptor [Contassot E. *et al*. (2004) *Gynecol Oncol*. 93:182-8]. Aim of the present study was to asses the occurrence and regulation of the endocannabinoid system in uterine tumors with particular interest towards endometrial carcinoma.

We focused our attention on possible differences in AEA and 2-arachidonoyl-glycerol (2-AG) levels and cannabinoid receptors (CBRs) expression in endometrial tissues at different stages of malignancy. CBRs expression was also evaluated in cell lines derived from endometrial carcinomas, where the potential anti-proliferative action of selective CBRs and TRPV1 agonists was assessed. LC-MS analyses of endometrial carcinomas biopsies in comparison with bioptic tissues from healthy patients indicated a significant elevation of 2-AG levels in carcinomas. Furthermore, western immunoblotting analyses revealed a selective up-regulation of CB_2 receptors.

Regarding cell lines, results obtained using quantitative RT-PCR analyses clearly indicate high expression of TRPV1, but not of CB_1 and CB_2. Surprisingly, however, not only, as expected, CBRs agonists, but also TRPV1 agonists did not significantly affect endometrial carcinoma cell proliferation. Nevertheless, overexpression of CB_2 receptors in a human undifferentiated endometrial cancer cell line (AN3Ca) resulted in a significant reduction of vitality, suggesting that the upregulated CB_2 receptors found here in biopsies of human endometrial carcinomas might play a possible tumor-suppressing action.

In conclusion, our data support the hypothesis of an overactive endocannabinoid system in human uterine neoplasias, although the exact role of ECs and CBRs in these types of cancer still needs to be further investigated.

CANNABIDIOL AS A NOVEL INHIBITOR OF ID-1 GENE EXPRESSION IN AGGRESSIVE BREAST CANCER CELLS

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Invasion and metastasis of aggressive breast cancer cells to other tissues of the body is the final and fatal step during cancer progression, and is the least understood genetically. Id-1 has recently been shown to be a key regulator of the metastatic potential of breast and additional cancers (Fong et al., 2003; Minn et al., 2005). Id proteins are inhibitors of DNA binding that modulate the function of basic helix-loop-helix transcription factors. Using a mouse model, we previously determined that metastatic breast cancer cells became significantly less invasive in vitro and less metastatic in vivo when Id-1 was down-regulated by stable transduction with antisense Id-1. Reducing Id-1 expression could provide a rational therapeutic strategy for the treatment of aggressive breast cancers. It is not possible at this point, however, to use antisense technology to reduce Id-1 expression in patients with metastatic breast cancer. Cannabinoids have been shown to act as tumor inhibitors in a variety of cancer models (Bifulco and Di Marzo, 2002; Guzman, 2003). Therefore, multiple classes of cannabinoid compounds were tested for their ability to reduce the expression of Id-1. Here we report that cannabidiol (CBD) was the most effective inhibitor of Id-1 expression in aggressive hormone-independent breast cancer cells. The concentrations of CBD that reduced Id-1 expression correlated with those used to inhibit the proliferative and invasive phenotype of breast cancer cells. CBD was able to inhibit Id-1 expression at the mRNA and protein level in a concentration-dependent fashion. These effects appeared to occur as the result of a direct inhibition of the Id-1 promoter. CBD represents the first non-toxic exogenous agent that can significantly decrease Id-1 expression in metastatic breast cancer cells leading to down-regulation of tumor aggressiveness. CBD has recently been shown to inhibit the metastasis of aggressive human breast cancer cancers in vivo (Ligresti et al., 2006), and has been shown to inhibit the invasive characteristics of other highly aggressive cancers (Vaccani et al., 2005). We propose that down-regulation of Id-1 may be one of the primary mechanism through which CBD inhibits the metastasis of aggressive human breast cancers. Since CBD inhibits Id-1 expression, a rational drug design strategy could be used to potentially create more potent and efficacious analogs.

This work was supported by grants from the National Institutes of Health (CA102412, CA111723 and DA09978), the Department of Defense (PC041013), the California Breast Cancer Research Program (12IB-0116) and CPMCRI.

THE EFFECT OF URB597 AND CANNABIDIOL ON THE EXPRESSION OF ANTICIPATORY NAUSEA IN A RAT MODEL

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Chemotherapy patients often experience nausea when they return to the environment in which they experienced their treatment. This classically conditioned response is called anticipatory nausea. Although the acute phase of chemotherapy- induced vomiting can be controlled by currently available anti-emetic treatment, acute nausea and anticipatory nausea are less well controlled. Here we present a rat model of anticipatory nausea and demonstrate that the fatty acid amide hydroxylase (FAAH) inhibitor, URB597, as well as cannabidiol, but not ondansetron, are effective in attenuating AN. Rats were injected with the emetic agent, Lithium Chloride (LiCl; 127 mg/kg) immediately prior to placement in a distinctive chamber permeated with vanilla odor on each of 4 trials. In a subsequent test of AN, the rats displayed conditioned gaping reactions (reflective of nausea) in the chamber previously paired with illness. The conditioned gaping reactions were suppressed by pretreatment with URB597 (0.1 and 0.3 mg/kg, ip) and cannabidiol (5 mg/kg, ip), but not by pretreatment with the classic anti-emetic agent ondansetron (0.1 mg/kg, sc). These result support previous findings that cannabinoid compounds may be an effective treatment for anticipatory nausea in chemotherapy patients.

INHIBITED DEVELOPMENT AFTER A NEUTRAL CANNABINOID CB_1 RECEPTOR ANTAGONIST IN NEONATAL MICE: IMPORTANCE OF AN ENDOCANNABINOID TONE FOR MILK INTAKE AND GROWTH

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We have shown in previous studies that a single exposure to the cannabinoid CB_1 receptor antagonist/inverse agonist rimonabant (SR141716) resulted in impaired milk suckling and severe growth failure. From these observations it was not clear, whether the developmental deficiencies were induced by neutral CB_1 receptor blockade, thereby inhibiting endogenous cannabinoid tone or by inverse agonist activation of the CB_1 receptor. The first mechanism supports our hypothesis that a CB1 receptor deficiency and/or reduced endocannabinoid availability underlie infant "non-organic failure-to-thrive" (NOFTT). If the latter mechanism was responsible for the growth stunting effects we observed, an endocannabinoid CB_1 receptor-based model for NOFTT would be difficult to justify. In the present study we injected the neutral CB1 receptor antagonist 5-(4-chlorophenyl)-3-[(E)-2-cyclohexylethenyl]-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole (VCHSR1) to one day old mouse pups and recorded weight gain, gastric milk (‘milkbands’), axillary temperature and survival between age 1 and 10 days. The results showed a significant, dose-related interference with milk ingestion, weight gain, body temperature regulation and survival.

We conclude that 1) the growth stunting effects we found previously are not unique to rimonabant, but can be ascribed more generally to CB_1 receptor inactivation and that 2) an endocannabinoid tone is essential for normal milk ingestion, growth and survival of newborn mice.

Acknowledgements: EF was supported by an intramural grant from CJS; HM and HBwere recipients of fellowships for outstanding students granted by CJS.

INFLUENCE OF DIETARY FATTY ACIDS ON ENDOCANNABINOID AND N-ACYLETHANOLAMINE LEVELS IN RAT BRAIN, SMALL INTESTINE AND LIVER

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The endocannabinoid system and *N*-acylethanolamines have since the discovery of their biological importance been correlated to a number of physiological and pathophysiological conditions[1]. Presently nobody has in detail been looking at the influence of dietary fatty acids on the level of endocannabinoids and *N*-acylethanolamines. Here we present the results from a study where 48 rats were fasted 24 hours and divided into 6 groups:

Group	Diet Composition	Energy % from total fat	Energy % specific fatty acid
Control	Altromin rat diet	15%	-
16:0	Altromin rat diet + Palm oil (38% Palmetic acid)	45%	15%
18:1	Altromin rat diet + Olive oil (71% oleic acid)	45%	27%
18:2	Altromin rat diet + Safflower oil (72% linoleic acid)	45%	30%
20:4	Altromin rat diet + Olive oil + arachidonic acid (6.6%)	45%	2.4%
20:5 + 22:6	Altromin rat diet + Eskimo-3 fish oil (19% eicosapentaenoic acid and 14% docosahexaenoic acid)	45%	3.5% (EPA) 2.4% (DHA)

The rats were given free access to the specific diets for one week. One part of the tissues was analyzed for fatty acid composition in phospholipids and triacylglycerols. The other part was analyzed for quantitative levels of endocannabinoids and *N*-acylethanolamines using LC-ESI-MS and LC-ESI-MS/MS. Analysis of the brain samples showed a significant increase in AEA in the 18:1 and 20:4 groups relative to the control group. OEA was significantly increased in the 18:1 and 20:4 groups relative to the 16:0 group. 2-AG was increased in the 20:4 group relative to the control group. Fish oil had no effect on AEA and 2-AG levels. LEA was significantly increased in the 18:1 and 18:2 groups relative to the control and 16:0 groups.

1. Hansen HS, Petersen G, Artmann A, Madsen AN (2006) Endocannabinoids. European Journal of Lipid Science and Technology 108: 877-889.

NEONATAL BLOCKADE OF THE CB1 RECEPTOR: FURTHER SUPPORT FOR ENDOCANNABINOID-CB1 DEFICIENCY AS THE BIOLOGICAL BASIS OF 'NON-ORGANIC FAILURE-TO-THRIVE' IN INFANTS

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We have shown in previous studies that a single exposure to the cannabinoid CB1 receptor antagonist/inverse agonist rimonabant (SR141716) resulted in impaired milk suckling and severe growth failure. We further showed that the growth failure which is due to an inability to ingest maternal milk, does not result from a motivational deficiency, but from an (oral)-motor impairment. The similarities between the SR141716-treated mice and the symptoms which characterize the enigmatic "non-organic failure-to-thrive" (NOFTT), which appears in 2-4% of infants, spurred us to suggest neonatal CB1 blockade-induced growth failure as the first animal model for NOFTT. In the present work we performed additional experiments (using ICR mice) to establish a deficient endocannabinoid-CB1 receptor system as the biological basis for NOFTT.

General Methods: Neonatal (ICR) mice were injected with SR141716 (10-20mg/kg) at several time interval after birth. Parameters including body weight, milk ingestion and body temperature were measured throughout the first 10 days of life.

Three studies were performed:

1. We studied the correlation between the timing of SR141716 administration with the severity of its effect. 2. We allowed SR141716- and vehicle-treated pups to lick a mixture of milk/cream from a dish ('lapping'), on each of the first 3 postnatal days. Successful food ingestion by the SR141716-treated pups would indicate that SR141716 indeed selectively impairs oral-motor strength required to suck milk from the maternal nipple, rather than the motivation and ability to ingest and assimilate food. 3. We raised mice in very small *vs* very large litters and thus established an undernourished state without SR141716. The pups were treated daily with THC (1-5 mg/kg) or 2AG (1 mg/kg-5 mg/kg) for the first 5 days of life.

Results: 1) Our findings suggest that as long as the pups were treated within 24 h of birth, the exact timing was not critical for the SR141716-induced effects. 2) The pups were able to ingest by lapping, significant amounts of the milk/cream mixture from the first day of life; lapping within the first week of life has not been shown previously in mice. Moreover, the SR141716-treated pups were able to ingest the mixture to the same degree as their vehicle-treated littermates. 3) Finally, whereas injection of 5 mg/kg of 2AG did not improve weight gain consistently, 1 mg/kg significantly enhanced weight gain. THC injections had no effect on the growth curve.

We conclude from these observations that we have now solid evidence that endocannabinoid and/or CB1 receptor insufficiency underlies the enigmatic infant condition NOFTT and that cannabinoid-based treatment should be considered to improve food intake and weight gain in NOFTT infants.

Acknowledgements: supported by an intramural grant from CJS

CRITICAL ASSESSMENT OF THE MECHANISM OF ACTION OF LH-21

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LH-21 has been reported as a peripherally acting CB1R neutral antagonist with weight loss efficacy in rats. However, the reported in vitro data indicated this compound is not potent compared to other published compounds (J Med Chem 47: 2939, 2004; Neuropharmacology 51:358, 2006). Here we have evaluated the pharmacological properties of LH-21 both in vitro and in vivo. Consistent with the previous report, LH-21 inhibited the binding of [^{3}H]CP55940 to the rat or human CB1R with an IC50 of 630-690 nM. LH-21 exhibited inverse agonist properties in a whole cell Gi-cAMP assay analogous to known inverse agonists such as AM251. While LH-21 did inhibit food intake and reduce body weight acutely in mice at high doses, it was also effective in CB1R-deficient mice. In contrast, other selective and potent CB1R inverse agonists were effective only in the WT mice but not in the CB1R-deficient mice. Furthermore, LH-21 was readily detected in rat brains, with a brain/plasma ratio of 1 when LH-21 was dosed to rats intravenously. These data demonstrate that LH-21 acts on non-CB1R targets to cause food intake inhibition in mice, and it apparently crosses the blood-brain barrier in rats. These studies illustrate the importance of combining knockout mice with pharmacological treatment to ascertain the mechanism of action of newly discovered compounds.

ALTERATIONS OF ENDOCANNABINOID AND ACYLETHANOLAMIDE LEVELS IN PERIPHERAL TISSUES OF MICE ON A HIGH FAT DIET: IMPLICATIONS FOR GASTRIC EMPTYING AND METABOLISM

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Endocannabinoids (ECs) control ingestive behaviour and metabolism, and the CB_1 receptor antagonist rimonabant (Acomplia^{TM}) is being marketed in Europe for the treatment of obesity and metabolic co-morbidities. Oleoylethanolamide (OEA), an anandamide congener and fatty acid amide hydrolase (FAAH) substrate, exerts anorexic properties via peroxisome proliferator-activated receptor-α (PPAR-α) and transient receptor potential vanilloid type-1 (TRPV1) channels. Here, we investigated: *i*) the possible dysregulation of the levels of cannabinoid CB_1 receptors, ECs, OEA and palmitoylethanolamide (PEA) in the pancreas, adipose tissue and stomach of mice on a high fat diet (HFD) for up to 14 weeks, as compared to a standard diet (STD); and *ii*) the involvement of these molecules in the regulation of gastric emptying, which plays a crucial role in the regulation of food intake and of nutrient digestion and assimilation.

EC, OEA and PEA levels were measured by isotope-dilution liquid chromatography-mass spectrometry. The expression of CB_1 receptors, fatty acid amide hydrolase (FAAH), *N*-acylphosphatidylethanolamine-specific PLD (NAPE-PLD), diacylglycerol lipase α (DAGL) and monoacylglycerol lipase (MAGL) was analyzed by quantitative reverse-transcription polymerase chain reaction and/or immunoistochemistry and/or biochemical assays. The effect on gastric emptying was evaluated by measuring the amount of phenol red recovered in the stomach after oral challenge.

Following HFD and the subsequent increase of body weight and fasting glycemia levels, a strong up-regulation of biosynthetic enzymes and a decrease of FAAH levels were observed in pancreatic β-cells, together with an increase of EC, but not PEA or OEA, pancreatic levels, but no changes in CB_1 levels. In the subcutaneous, but not visceral, fat a decrease in EC, OEA and PEA concentrations was accompanied by down- and up-regulation of biosynthesising enzymes and FAAH, respectively, with no changes in CB_1 levels. In STD mice, gastric emptying was reduced by anandamide and OEA, only the former effect being counteracted by rimonabant, which *per se* enhanced emptying. The FAAH inhibitor, *N*-arachidonoyl-serotonin (AA-5-HT), reduced gastric emptying in a way only partly reduced by rimonabant. Compared to STD mice, HFD mice showed: *i*) delayed gastric emptying; *ii*) only slightly decreased anandamide levels with no changes in 2-arachidonoyl-glycerol levels; *iii*) ~2-fold higher levels of OEA and PEA; *iv*) increased NAPE-PLD, and decreased FAAH and CB_1 expression; and *v*) higher sensitivity of gastric emptying to the effect of OEA and, particularly, AA-5-HT, but not to that of rimonabant.

Whereas the dysregulation of EC levels in the pancreas and adipose tissue following a HFD might have important consequences on the regulation of insulin and adipokines, our data in the stomach suggest that gastric emptying might be reduced during HFD because of elevation of OEA, and not of EC, activity. The potent inhibitory effect of OEA on gastric emptying might also underlie part of the anorexic action of this compound in lean and obese rodents.

IN VIVO MICROPET MAPPING OF CANNABINOID, DOPAMINERGIC AND METABOLIC MARKERS IN THE QA RAT MODEL OF HUNTINGTON'S DISEASE

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Introduction: It has been suggested that the endocannabinoid system (ECS) may be altered in Huntington's disease (HD). Using microPET and a novel ^{18}F-labeled high-affinity, subtype-selective CB1 Receptor (CB1R) radioligand (^{18}F-MK9470), we explored in vivo CB1R alterations in the quinolinic acid (QA) rat model of HD, in correlation to glucose metabolism and dopamine D2 receptor binding, as well as amphetamine-induced turning behavior.

Methods: 21 Wistar rats (female; 11 QA and 10 sham phosphate buffered saline (PBS) animals; age 16-18 wk) were investigated. QA (240 nmol/µl) and PBS were stereotactically injected in the left caudate-putamen (CPu). MicroPET acquisitions were conducted on a FOCUS 220 system at 11-26 weeks post lesioning after 50 mg/kg pentobarbital IP anaesthesia, and using 18 MBq ^{18}F-MK9470 (n=21; 60min dynamic), ^{18}F-FDG (n=20; 40min, 60min p.i.) and ^{11}C-Raclopride (n=20; 60min dynamic). Parametric maps ^{18}F-MK9470 (SUV, 40-60 min p.i.) and ^{11}C-Raclopride (cerebellar reference tissue model) were generated and ^{18}F-FDG was normalized to whole-brain uptake. Data were spatially normalized to Paxinos space and analyzed using SPM2.

Results: ^{18}F-MK9470 binding, energy metabolism and D2 receptor binding were reduced in the ipsilateral CPu by 7%, 35% and 77% respectively (all $p_{height}<2.10^{-5}$), while an increase for these markers was seen in the contralateral CPu (>5%, all $p_{height}<7.10^{-4}$). ^{18}F-MK9470 binding was also increased in the cerebellum ($p_{height}=2.10^{-5}$), where it was inversely correlated to the number of ipsiversive turnings ($p_{height}=7.10^{-6}$), suggesting that overexpression of CB1R in the cerebellum was related to a better functional outcome. In addition, relative metabolism was increased in the contralateral hippocampus, thalamus and sensory-motor cortex ($p_{height}=1.10^{-6}$).

Conclusion: In vivo cerebral microPET mapping in QA rats shows little change in ^{18}F-MK9470 binding in the CPu in contrast to D2 receptor binding. The data is consistent with the destruction of interneurons and output neurons in the CPu that are known to have high levels of D2 receptors. The behavioural effects observed were also consistent with the loss of D2 receptors. The translational validity of this acute model of HD and the involvement of the ECS needs further study in other HD models.

Research Support: Research Council of the K.U.Leuven (OT/05/58), the Flemish Fund for Scientific Research (FWO/G.0548.06). This work is performed under European Commission FP6-project DiMI, LSHB-CT-2005-512146.

NEONATAL STRESS-INDUCED NEURODEGENERATION: A SUITABLE ANIMAL MODEL TO EVALUATE NEUROPROTECTIVE AND MODULATORY PROPERTIES OF CANNABINOIDS

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The endocannabinoid system appears to play a role in neuroprotection (van der Stelt & Di Marzo 2005, Neuromolecular Med 7:37–50.). In the present study we have used a rat model of schizophrenia based on the neurodevelopmental hypothesis [24 h maternal deprivation (MD), postnatal day (PND) 9-10] (Ellenbroek & Cools 2002, Pharmacol Biochem Behav 73:177-84) to study its detrimental effects (neurodegeneration-gliodegeneration) on hippocampus and the possible protective action of two compounds that modulate the endocannabinoid system, the fatty acid amide hydrolase inhibitor *N*-arachidonoyl-serotonin, AA-5-HT, and the endocannabinoid reuptake inhibitor, OMDM-2. Pharmacological treatment consisted of daily subcutaneous injections during the period PND 7-12 and the animals were sacrificed at PND 13. MD induced significant increases in corticosterone levels and morphological modifications of the apical pyramidal cell dendrites in *stratum radiatum* of area CA1 (more modest in CA3 area). Morphological changes were manifested as irregular and waved dendritic aspect. We did not observe signs of cell death (Tunel and caspase-3 assays). In addition, MD induced hippocampal astrogliosis [more glial fibrillary acidic protein (GFAP) positive cells] in CA1 and CA3 areas, concomitantly with a significant increase in 2-AG levels. In general, all these effects were more marked in males. The two endocannabinoid system modulators, AA-5-HT and OMDM-2, attenuated endocrine and cellular effects of MD, thus suggesting that up-regulation of endocannabinoids during early postnatal development might represent an adaptive response aimed at counteracting the consequences of this condition. Interestingly, however, data obtained with isolectin-B4 revealed that the two drugs increased the number of labelled vessels and their staining intensity in CA1 and CA3 areas of control animals, thus suggesting that endocannabinoids might also contribute to angiogenesis. At any rate, the effects of AA-5-HT and OMDM-2 on hippocampal endocannabinoid (anandamide and 2-AG) levels, as measured at PND 13, showed a correlation with their endocrine and cellular effects at this age only in some cases. Therefore, it is possible that these effects are partly attributable to endocannabinoid level modifications that: *i*) occur at any time from day 7 to day 12 (duration of the pharmacological treatment) and *ii*) are subsequently not necessarily detectable at the time point of sacrifice. Marked and intriguing sex dimorphisms were observed throughout the study, notably with regard to neuronal and astroglial changes (more marked in males) as well as in endocannabinoid levels.

Acknowledgements: Supported by Ministerio de Educación y Ciencia ref: SAF2006-07523

REPEATED ANTIPSYCHOTIC TREATMENT ALTERS *IN VIVO* EFFECTS OF Δ^9-THC IN RATS: IMPLICATIONS FOR SCHIZOPHRENIA

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Co-localization of CB_1 and dopamine (DA) receptors in striatum and cortex strongly suggest the possibility of interaction(s) between endocannabinoid and DA systems in regulation of neural functioning in these areas. Confirmatory evidence includes reports of Δ^9-THC-induced increases in DA levels in the NAc and increases in burst activity of DA neurons in the VTA. Regulation of DA activity by endocannabinoids may be bi-directional, as DA activity affects AEA and/or 2-AG levels in limbic and prefrontal areas through intermediate glutamatergic mechanisms. Our previous research has shown that repeated dosing with haloperidol (HAL) or with the atypical antipsychotic clozapine (CLZ) attenuated maximal stimulation of CB_1-mediated G-protein activity in the prefrontal cortex of adult female rats. CLZ (but not HAL) also decreased CB_1-mediated G-protein activity in the striatum of these rats as well. Agonist-stimulated [^{35}S]GTPγS binding in brains of adult male rats was not affected by antipsychotic treatment. Further, B_{max} values of [^{3}H]SR141716A were not altered in any of the rats. Subsequently, we determined that adolescent male and female rats did not exhibit any antipsychotic-induced changes in CB_1 receptor number or function.

In a continuation of this study, we examined the effects of repeated dosing with HAL or CLZ on subsequent *in vivo* response to Δ^9-THC in adult and adolescent rats of both sexes. Male and female Long-Evans rats were injected with saline, 0.3 mg/kg HAL, or 10 mg/kg CLZ twice daily for 9.5 days (for the adolescents, postnatal day 30-39, inclusive). On the 11th day (PN40 for adolescents), rats were injected with vehicle and with cumulative doses of Δ^9-THC and assessed for changes in locomotor activity, rectal temperature, and catalepsy after each injection. Total cumulative Δ^9-THC doses were 0 (vehicle), 3, 10, 30 and 100 mg/kg. Δ^9-THC produced dose-dependent suppression of activity, hypothermia and catalepsy in saline-treated rats of both ages and sexes. No significant alteration of this pattern was observed for antipsychotic-treated adolescent rats. In contrast, Δ^9-THC-induced hypomobility and hypothermia were potentiated in adult male HAL-treated rats, but none of the measures was affected by CLZ treatment. In CLZ-treated female adult rats, Δ^9-THC-induced suppression of locomotor activity was significantly attenuated without effect on the other measures. HAL did not affect female response to Δ^9-THC. These results suggest that sex differences in antipsychotic effects on CB_1 receptor function and Δ^9-THC-induced *in vivo* effects are age-dependent and do not appear during adolescence. Second, attenuation of Δ^9-THC-induced suppression of activity in CLZ-treated adult female rats corresponds with the CLZ-induced desensitization of CB_1 receptors in the striatum observed in adult females. The finding that HAL-induced potentiation of Δ^9-THC's effects in adult males is not associated with changes in CB_1 receptor function suggests that it may be related to alterations in another receptor system affected by HAL (e.g., DA).

Acknowledgements: Research supported by NIH grants MH-64771, DA-016644, DA-14277, and DA-05274.

IMPAIRED ENDOCANNABINOID LEVELS IN SELECTED BRAIN AREAS OF TRANSGENIC R6/2 MICE, AND EXPERIMENTAL MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by motor disturbances, dementia, psychiatric symptoms and early death, and caused by a mutation (a CAG trinucleotide expansion) in exon 1 of the IT15 gene. The expanded CAG repeat is translated into a polyglutamine (poliQ) expansion in the protein huntingtin (htt), that in this mutated version becomes very toxic for neurons, especially for GABAergic, medium-sized spiny neurons (MSNs) of the striatum, thus eventually leading to degeneration of striatopallidal fibers, impairment of the "indirect" pathway of movement and, among other things, uncontrolled choreic movements. Previous studies have shown that in animal models of HD the endocannabinoid system is impaired at the level of either CB_1 receptor expression or endocannabinoid levels (Glass et al., *Prog Neuropsychopharmacol Biol Psychiatry*, 2001; Maccarrone et al., *Ann. Neurol.* 2007, for reviews). The transgenic R6/2 model, created by inserting exon 1 of the human *IT15* mutant gene into the mouse genome (Mangiarini et al., *Cell*, 1996), and exhibiting 150 CAG repeats, develops a severe and progressive neurological phenotype starting from approximately 7-8 weeks of age. In these mice, a progressive decline of CB_1 receptor expression and abnormal sensitivity to CB_1 receptor stimulation have been reported (McCaw et al., *Eur. J. Biochem*., 2004; Centonze et al., *Biol. Psychiatry*, 2005). The aim of the present work was to establish if endocannabinoid levels are also altered in different brain areas of transgenic R6/2 *versus* wild-type (WT) mice at different disease phases.

A colony of R6/2 mice and littermate controls was established at Charles River, Italy. Animal use and care followed the European Communities Council Directives (86/609/EEC). Pre-symptomatic (aged 4 and a half weeks, n=4) or overtly symptomatic (aged 10 weeks, n=4, 2 males and 2 females) R6/2 mice and age- and gender-matched WT mice (n=4/group) were used. Animals were decapitated, the brains removed, and brain areas (striatum, cortex and hippocampus) rapidly dissected and immediately frozen in liquid nitrogen. Lipids were extracted and endocannabinoids and palmitoylethanolamide analysed by isotope-dilution LC-MS, as described previously.

Except for a ~25% decrease in 2-arachidonoylglycerol (2-AG) levels in the cortex, no significant changes in endocannabinoid and PEA levels were observed in pre-symptomatic R6/2 mice *vs.* WT mice. By contrast, the levels of all three compounds significantly decreased by ~30% in the striatum of symptomatic R6/2 mice. In symptomatic mice, no changes were observed in the hippocampus, and a ~25% decrease of 2-AG levels, accompanied by a ~25% increase of anandamide levels, was observed in the cortex.

These findings indicate that endocannabinoid levels in the brain of R6/2 *vs.* WT mice: 1) become progressively reduced in the striatum in parallel with down-regulation of CB_1 receptors; 2) are reduced in the cortex in the pre-symptomatic but not necessarily in the symptomatic phase; 3) are unchanged in the hippocampus. Our study confirms that an impaired endocannabinoid system is a hallmark of symptomatic HD.

THE ENDOCANNABINOID SYSTEM LIMITS AXONAL DAMAGE AND EXCITOTOXICITY

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The mechanisms sustaining axonal damage in chronic diseases such as MS are still poorly understood. Over-activation of glutamatergic ionotropic receptors (excitotoxicity) is considered as one of the potential mechanisms responsible for damage to axons in demyelinating diseases. Cannabinoids exert beneficial effects in animal models of MS and clinical trials are beeing performed to assess their therapeutic potential. However, the mechanisms sustaining these effects are still controversial, and the therapeutic potential of the activation of the endocannabinoid (EC) system has been less documented. The aim of this study was to estimate, in vitro, the ability of the EC system to act on neuronal death and axonal damage.

We developed a model of axonal damage in vitro, using low doses of the calcium ionophore A23187. We observed that the genetic or pharmacological blockade of CB1 worsened neuronal death in this model, and that Win 55212,2 induced a neuroprotective effect. As axonal damage in the spinal cord may be related to excitotoxic processes, we studied the ability of the endocannabinoid (EC) system to counteract AMPA-induced excitotoxicity in vitro. We observed that inhibiting EC recapture or degradation was beneficial in this model. Furthermore, cultures performed from CB1-/- mice were more susceptible to AMPA-induced excitotoxicity. Finally, treatment with the endocannabinoids, anandamide or 2-AG, promoted neuroprotection.

Taken together, these data suggest that after A23187 or AMPA treatment, the endocannabinoid system is activated and limits neuronal cell death and axonal damage in our cultures.

MODULATING THE BALANCE BETWEEN CB1 AND CB2 RECEPTORS ACTIVATION CAN PROTECT THE BRAIN FROM ISCHEMIC DAMAGE

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There are conflicting reports in the literature describing the influence of cannabinoid receptor activation on ischemia/reperfusion injury. We have previously reported that a selective CB2 agonist (O-1966) provides protection for the brain in a mouse model of stroke. The goal of this investigation was to evaluate whether endogenous cannabinoid production influences outcome following ischemia/reperfusion injury and how changing the balance between CB1 and CB2 receptor activation influences this outcome. Transient cerebral ischemia was induced in the male C57BL/6 mouse by intravascular occlusion of the middle cerebral artery for 60 minutes. Infarct size and motor function were evaluated 24 hours after reperfusion. A comparison of these parameters was made following administration of a CB1 antagonist alone, a CB2 antagonist alone and following administration of a CB1 antagonist with a CB2 agonist and a CB2 agonist with a CB2 antagonist. The results of this investigation demonstrated that administration of the CB1 antagonist alone reduced the mean infarct size compared to untreated animals (48.5 ±4.2 mm^3 vs. 91.1± 4.3 mm^3) while administration of the CB2 antagonist increased infarct size (121.2± 9.07 mm^3 vs 91.1± 4.3 mm^3). The greatest degree of protection from injury was provided when the CB1 antagonist was combined with the CB2 agonist, reducing infarct size to a mean of 6.87±3.67 mm^3 .The results of this investigation therefore provide evidence that activation of the CB1 receptor by endogenous cannabinoids may contribute to injury while activation of the CB2 receptor is protective.

This project is funded, in part, under a grant with the Pennsylvania Department of Health.

CANNABINOID RECEPTOR STIMULATION IS ANTI-INFLAMMATORY AND IMPROVES MEMORY IN OLD RATS

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The number of activated microglia increase during normal aging. Stimulation of endocannabinoid receptors can reduce the number of activated microglia, particularly in the hippocampus, of young rats infused chronically with lipopolysaccharide (LPS). In the current study we demonstrate that endocannabinoid receptor stimulation by administration of WIN-55212-2 (2 mg/kg/day) can reduce the number of activated microglia detected using immunohistochemistry in hippocampus of aged rats (45% reduction in the CA3 region, $p<0.05$) and attenuate the spatial memory impairment in the water pool task (Day 2, 3 and 4, $p<0.05$ compared to untreated aged animals). Our results suggest that the action of WIN-55212-2 does not depend upon a direct effect upon microglia or astrocytes, as no colocalization was found between CB1 and GFAP (astrocytes), OX-6 (activated microglia) or OX-42 (resting microglia), but seems dependent upon stimulation of neuronal cannabinoid receptors, as a strong colocalization was found with NeuN (neuronal marker) and NMDA-R1 (neuronal glutamate receptor). Aging significantly reduced cannabinoid type 1 receptor binding (about 33% reduction in the hippocampus, $p<0.05$) but had no effect on cannabinoid receptor protein levels in the hippocampus. We are currently exploring which subtype of cannabinoid receptor is involved in this anti-inflammatory effect. Overall, stimulation of cannabinoid receptors may provide clinical benefits in age-related diseases that are associated with brain inflammation, such as Alzheimer's disease.

IDENTIFICATION AND CHARACTERIZATION OF ENDOGENOUS PROSTAGLANDIN E2 GLYCEROL ESTER (PGE_2-G), A COX-2 METABOLITE OF 2-ARACHIDONOYLGLYCEROL (2-AG), IN PAIN AND INFLAMMATION

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Previous research showed that the endocannabinoid 2-arachidonoylglycerol (2-AG) can be oxygenated by COX-2 to produce prostaglandin glycerol esters (PG-Gs) *in vitro*. One of the oxygenated 2-AG metabolites of this *in vitro* model, PGE_2-G, but not PGE_2 or glycerol, triggered intracellular Ca^{2+} mobilization via the IP_3 and PKC pathways in RAW264.7 macrophage cells. However, the oxygenated products of 2-AG by COX have not been found *in vivo* to date. Therefore, the aim of the present study was to isolate and identify endogenous PGE_2-G from mammalian tissues and to examine its role in pain and inflammation. To identify endogenous PGE_2-G in the mammalian periphery, rat hindpaws were homogenized in 100% methanol and centrifuged for 20 min. The paw extracts were partially purified by solid phase extraction methods and analyzed with triple quadrupole mass spectrometry (LC/MS/MS) and quadrupole time of flight mass spectrometry (qq-TOF LC/MS/MS). To test if PGE_2-G affects pain sensitivity, PGE_2-G, PGE_2-G combined with the prostanoid receptor antagonists or vehicle was injected intraplantarly (i.pl.) and the animals were tested with the Hargreaves and von Frey tasks. Finally, mouse macrophage-like cells (RAW264.7) were transiently transfected with an NFκB-luciferase plasmid and activated with LPS. PGE_2-G or vehicle was added for 30 min and followed by the substrate for luciferase. The emitted light was measured by a scintillation counter.

Our results showed that the constituent found in the rat hindpaw has the same exact mass, HPLC retention time, fragmentation pattern, and structural components as synthetic PGE_2-G. Therefore, we concluded that PGE_2-G is present in the rat hindpaw. PGE_2-G caused thermal hyperalgesia and mechanical allodynia, and its effects were only partially mediated by PGE_2, a metabolic product of PGE_2-G. PGE_2-G caused an apparent bell-shaped dose-response curve in the activation of NFκB activity in the immune cells. In conclusion, it is shown for the first time that PGE_2-G, a COX-2 metabolite of 2-AG, is a naturally occurring molecule in the rat hindpaw. Our behavioral data suggest that PGE_2-G binds to a unique receptor on the skin cells to induce pain. It appears that PGE_2-G is a member of a new class of lipid signaling molecules with a role in pain and inflammation.

FAAH AND COX-2 ENZYME INHIBITION IN THE PERIPHERY IS ANTI-NOCICEPTIVE AND ALTERS LOCAL LEVELS OF ENDOCANNABINOIDS IN THE CARRAGEENAN MODEL OF PAIN

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Activation of cannabinoid receptors by the endocannabinoids (ECs) is anti-nociceptive in models of pain. ECs with a fatty acid amide group such as anandamide (AEA), oleoylethanolamide (OEA) and palmitoyl ethanolamide (PEA) are primarily metabolised by fatty acid amide hydrolase (FAAH). ECs are also substrates for cycloxygenase-2 (COX-2, Kozak *et al.*, 2004, *Curr Pharm Des,* 10, 659-67), an enzyme which primarily metabolises arachidonic acid. Systemic administration of FAAH-inhibitors is anti-nociceptive in models of inflammatory pain. The aim of this study was to compare the effects of local peripheral inhibition of FAAH and COX-2 on nociceptive behaviour and levels of ECs in a model of inflammation. The effects of URB597, a FAAH-inhibitor and nimesulide, a COX-2-inhibitor devoid of FAAH activity, were studied on weight bearing behaviour and on levels of ECs.

Male Sprague-Dawley rats (225-300 g) received an intraplantar injection of URB597 (25 or 100 µg in 50 µl), nimesulide (50 µg in 50 µl) or vehicle (3% Tween 80 in saline), 30 min prior to intraplantar injection of carrageenan (2 mg in 100 µl) or saline. Weight bearing on the hindpaws was measured before drug injection and for 3 hrs post-carrageenan. Hindpaw skin and spinal cord were dissected immediately after the last weight bearing measurement and stored at -80^{0}C for analysis of EC levels using liquid chromatography tandem mass spectrometry (Richardson *et al*., 2007, *Anal Biochem*, 360, 216-226). Weight bearing data were analysed using one-way ANOVA followed by Neuman-Keul's post-hoc test. EC levels were analysed using non-parametric Mann-Whitney test.

Intraplantar injection of carrageenan significantly reduced weight bearing on ipsilateral hindpaw 2-3 hr post-injection (hyperalgesia), compared to saline. Levels of AEA and PEA were significantly decreased in the carrageenan-inflamed hindpaw (Table 1). Intraplantar injection of URB597 (25 and 100 µg in 50 µl) significantly increased levels of AEA and 2AG in the inflamed hindpaw (Table 1). Only the lower dose of URB597 attenuated the carrageenan-induced hyperalgesia, compared to vehicle. Intraplantar injection of nimesulide (50 µg in 50 µl) significantly increased the levels of AEA and PEA in the inflamed hindpaw (Table 1) and attenuated carrageenan-induced hyperalgesia.

Table 1: Endocannabinoid levels in the ipsilateral hindpaw skin. ~ $P < 0.05$ *vs* vehicle-saline; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ *vs* vehicle-carrageenan. Values are mean ± SEM of concentration of compounds in wet tissue (n = 6-15).

Treatment	***AEA (pmol/g)***	***2AG (nmol/g)***	***PEA (nmol/g)***
Vehicle-saline	30.14 ± 2.76	1.92 ± 0.36	22.09 ± 2.4
Vehicle-carrageenan	9.38 ± 2 ~	1.98 ± 0.38	7.78 ± 1.12 ~
URB (25 µg)-carrageenan	20.66 ± 4.85*	4.25 ± 1.14*	12.85 ± 3.08
URB (100 µg)-carrageenan	24.25 ±2.09**	18.01 ± 4.64***	6.93 ± 0.83
Nimesulide-carrageenan	16.68 ± 2.6*	2.2 ± 0.27	12.11 ± 1.16*

Our data demonstrate that effects of local peripheral inhibition of FAAH on levels of AEA and 2AG are not always associated with an attenuation of carrageenan-induced hyperalgesia. The inhibitory effects of the COX-2-inhibitor nimesulide on carrageenan-induced hyperalgesia were associated with increased levels of AEA and PEA.

ATTENUATION OF ALLERGIC CONTACT DERMATITIS THROUGH THE ENDOCANNABINOID SYSTEM

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Allergic contact dermatitis affects about 5% of men and 11% of women in industrialized countries and is one of the leading causes for occupational diseases. In this study we identified the endogenous cannabinoid system as a major regulator of cutaneous contact hypersensitivity (CHS) in a mouse model.

We used 8-10 week old CB1-receptor-deficient, CB2-receptor-deficienta and their wildtype controls. For the induction of CHS, mice were sensitized by painting 50 µl of 0.2% DNFB (1-fluoro-2,4 dinitrobenzene) on the shaved abdomen on two consecutive days. For elicitation of CHS, ears of mice were painted with 10 µl of 0.3% DNFB on day 5. Ear thickness was measured 24 h, 48 h, and 72 h after challenge using an engineer's micrometer. Drug treatments were performed 30 min before challenge, as well as 24 h and 48 h after challenge. Tissue endocannabinoid levels were quantified using internal deuterated standards in LC-MS. DNFB treated and control ears from CB1/CB2-receptor-knockout and wildtype control mice were used for gene expression analysis. Total RNA was used for realtime RT-PCR and microarray experiments (MG_430 2.0 Affymetrix GeneChips).

In an animal model for cutaneous contact hypersensitivity we show that mice lacking both known cannabinoid receptors display exacerbated inflammatory skin responses to nickel-containing ear tags and in an experimental model of cutaneous contact hypersensitivity. Furthermore, contact allergic responses were accompanied by locally elevated endocannabinoid levels and were increased by the cannabinoid receptor-specific antagonists SR141716 and SR144528. Because our data indicate that activation of the endocannabinoid system may function to dampen the CHS response, we considered the possibility that administration of cannabinoids such as Δ^9-tetrahydrocannabinol (THC) might attenuate CHS in wild type animals. Indeed, THC significantly decreased ear swelling in comparison to untreated mice. In order to gain insight into the molecular mechanism that may contribute to the increased CHS in cannabinoid receptor deficient mice, we performed a series of microarray experiments with RNA isolated from DNFB-treated ears of double knockout and wildtype mice, as well as the untreated control ears. Most interestingly, one chemokine was found to be differentially upregulated in DNFB-treated knockout ears.

Activation of the endocannabinoid system in the skin upon exposure to a contact allergen down-regulates allergic responses through modulation of chemokine production. Our results demonstrate a protective role of the endocannabinoid system in contact allergy in the skin, and suggest a novel target for therapeutic intervention.

PROPERTIES OF NABILONE: ELECTROPHYSIOLOGICAL EVIDENCE OF DECREASED CENTRAL SENSITIZATION

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Aims: We investigated how the administration of different dosages of nabilone (Cesamet®), a synthetic analog of Δ^9-tetrahydrocannabinol, may affect the experience of pain. By measuring subjective pain ratings, spinal withdrawal reflexes (WR) and somatosensory evoked potentials (SEP), it was possible to investigate how nabilone affects spinal sensitization caused by repeated electrical stimulations of the sural nerve.

Methods: 17 healthy volunteers participated in this project and were seen three times, corresponding to the double-blinded administration of placebo, 0.5mg and 1mg doses of nabilone. At the beginning of each experimental session, painful electrical stimulations of the sural nerve were given every 7 seconds for a period of 10 minutes (time 1). The administration of the drug followed and, immediately after a 2 hour waiting period, stimulations of the sural nerve were performed again (time 2). Repeating the stimulation procedure after 2 hours of rest normally produces spinal sensitization.

Results: Analyses showed that nabilone 1mg blocked spinal sensitization in 14 of our 17 subjects whereas placebo blocked sensitization in only 9 subjects ($p<0.05$). Difference in SEP P220 amplitude for the 1 mg dose parallel those of the spinal withdrawal reflex ($p<0.05$). Among the 14 subjects who showed a large nabilone effect, spinal sensitization was completely blocked, and even showed a decrease of the WR amplitude by 13,79%.

Conclusion: Nabilone (1mg) was more efficacious in attenuating sensitization than placebo and this effect was visible in WR responses as well as SEP activity.

ANANDAMIDE REGULATES EDEMA AND PAIN THROUGH DISTINCT PERIPHERAL AND CENTRAL PATHWAYS

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Fatty acid amide hydrolase (FAAH) is the primary enzyme responsible for the degradation of the endogenous cannabinoid, arachidonylethanolamide (anandamide), as well as noncannabinoid fatty acid amides. Complementary approaches of genetic deletion and pharmacological inhibition of FAAH lead to increased levels of these lipid signaling molecules in both the CNS and the periphery that are accompanied with analgesic and anti-inflammatory phenotypes. The purpose of the present study was to investigate whether elevation of these endogenous ligands through the blockade of FAAH can be used treat edema and associated hyperalgesic responses elicited by an intraplantar injection of 25µg lipopolysaccaride (LPS). FAAH (-/-) mice or wild type mice treated with the FAAH inhibitor URB597 (10 mg/kg) displayed a significant reduction in both inflammation and thermal hypersensitivity in the tail immersion and hot plate tests that persisted for 48 h. Transgenic mice in which FAAH was restricted exclusively to nervous tissue continued to exhibit the anti-edema phenotype, indicating a peripheral site of action. The CB_2 receptor antagonist SR144528 (3 mg/kg) completely blocked the anti-edema phenotype, but the anti-inflammatory effects of URB597 were not attenuated in CB_1 (-/-) or by pretreatment with the CB_1 receptor antagonist rimonabant (3 mg/kg). In contrast, rimonabant completely blocked the anti-hyperalgesic responses of FAAH (-/-) mice and URB597-treated mice. These results support the hypothesis that anandamide modulates inflammation and pain through distinct anatomical and receptor systems. This dissociation between the anti-hyperalgesia and anti-edema phenotypes raises the provocative possibility that central and peripheral FAAH systems may be targeted separately for therapeutic gain.

TONIC ENDOVANILLOID FACILITATION OF GLUTAMATE RELEASE IN BRAINSTEM DESCENDING ANTINOCICEPTIVE PATHWAYS

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The heat- and capsaicin-activated vanilloid TRPV1 receptor is one of the most widely recognized alternative molecular targets for the endocannabinoids anandamide and *N*-arachidonoyldopamine. TRPV1 is expressed in the several brain areas, including the periaqueductal gray (PAG), where it seems to be involved in the descending supraspinal pathways of nociception. Supraspinal antinociception is mediated in part by excitatory neurons originating in the PAG and impinging on OFF neurons of the rostral ventromedial medulla (RVM). We recently hypothesized, based on pharmacological and electrophysiological data, that activation of glutamate release by endogenous TRPV1 agonists in the ventrolateral (VL) PAG causes activation of OFF antinociceptive neurons of the RVM (Maione et al., *J. Pharmacol. Exp. Ther*., 2006). Here, we aimed at providing conclusive support to this hypothesis by examining in rats the effect of intra-VL-PAG injections of TRPV1 agonists and antagonists, alone or in combination, on: 1) the nocifensive response to heat in the plantar test; and 2) neurotransmitter (glutamate and GABA) release in the RVM. Furthermore, we examined, by means of immunohistochemistry, the possible localization of TRPV1 in glutamatergic or GABAergic PAG and RVM neurons using vesicular glutamate transporter 1 (VGLUT1) or vesicular GABA transporter (VGAT) as markers.

Capsaicin injection into the VL-PAG increased the threshold of thermal pain sensitivity in healthy rats, whereas the selective TRPV1 antagonist 5'-iodo-resiniferatoxin (I-RTX) evoked hyperalgesia. A *per se* inactive dose of I-RTX abolished capsaicin-mediated analgesia. Intra-VL PAG injection of capsaicin also evoked robust glutamate release in RVM microdialysates, whereas I-RTX significantly decreased the release of this neurotransmitter, and, at a dose inactive *per se*, blocked the effect of capsaicin. As a secondary effect to glutamate discharge, capsaicin also caused a faint stimulation of GABA release. TRPV1-immunoreacivity (ir) in the VL-PAG and RVM localized mostly to cell bodies. In the VL-PAG, high density of VGLUT1-ir and VGAT-ir on axons terminals surrounding TRPV1 positive cells indicated glutamatergic and GABAergic input on TRPV1-ir neurons. Also in the RVM, VGAT and VGLUT1 staining was found around somas that were clearly TRPV1-expressing, but these latter were often stained also for VGLUT1. Double immunofluorescence staining conclusively identified several TRPV1/VGLUT1 positive cells in the RVM.

The present study, together with our previous electrophysiological findings, indicates that glutamatergic neurons of the VL-PAG respond to TRPV1 stimulation by releasing glutamate into the RVM, thereby activating other TRPV1-expressing glutamatergic neurons (presumably OFF cells) in this area to produce analgesia. Importantly, in view of the results obtained with the TRPV1 antagonist alone, this pathway is tonically activated by endovanilloids, presumably anandamide, as suggested by our previous finding of TRPV1-mediated antinociception following intra-VL-PAG injection of a FAAH inhibitor (Maione et al., *J. Pharmacol. Exp. Ther*., 2006).

ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL INHIBIT CHOLINERGIC CONTRACTILITY IN THE HUMAN COLON VIA A NON-CB1 PATHWAY

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The effects of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) were determined on cholinergic contractility in strips of human colonic longitudinal muscle and circular muscle *in vitro*, in the presence of nitric oxide synthase blockade with N-nitro-L-arginine (10^{-4}M). Anandamide and 2-AG inhibited longitudinal muscle and circular muscle contractile potency in response to acetylcholine (10^{-9}-10^{-4}M) in a concentration-dependent manner. This was unaltered following pre-treatment with the cannabinoid CB1 receptor-selective antagonist AM 251 (10^{-7}M). Pretreatment with an inhibitor of anandamide catabolism, arachidonoyl trifluoromethyl ketone (10^{-5}M), in isolation caused a significant decrease in the potency of acetylcholine-evoked contraction in both longitudinal and circular muscle, but had no significant additional effect on the anandamide-induced suppression of contraction. The findings of the present study indicate that the endocannabinoids anandamide and 2-arachidonoylglycerol suppress colonic cholinergic contractility via a non cannabinoid CB1 receptor-mediated pathway. Cholinergic contraction may be tonically modulated by the products of arachidonate metabolism unrelated to endocannabinoid production. The extent of anandamide metabolism is not sufficient to influence the functional effects of exogenous administration in human colonic tissue *in vitro*.

INFLUENCE OF ACUTE HYPERTENSION AND FATTY ACID AMIDE HYDROLASE ON HAEMODYNAMIC EFFECTS OF ANANDAMIDE IN CONSCIOUS RATS

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The endocannabinoid, anandamide has been shown to cause hypotension in anaesthetised rats or mice, particularly in chronic hypertensive models. Here, we have examined the cardiovascular responses to anandamide in normotensive and acutely hypertensive, conscious rats. The role of degradation of anandamide by the fatty acid amide hydrolase (FAAH) was also explored. Male Wistar rats (350-450g; Charles River, UK) were implanted with miniaturised pulsed Doppler flow probes and catheters as described previously[1]. On the day of experiment, animals received continuous i.v. infusion of either saline (0.4ml/h), or angiotensin II (AII; 500ng/kg/h) and arginine vasopressin (AVP; 50ng/kg/h). After 45min, URB597 (3mg/kg; i.v. infusion at 2ml/h for 30min) or its vehicle was administered, followed 30min later, by bolus injection (0.12ml, i.v.) of 3mg/kg anandamide. Data are shown as mean±SEM.

Infusion of AII/AVP significantly ($P<0.05$, Mann-Whitney U test) increased the mean arterial blood pressure (BP; 149±2 vs 114±1 mmHg), reduced vascular conductance (VC) in renal (RVC; 48±3 vs 81±4), mesenteric (MVC; 21±2 vs 73±4) and hindquarter beds (HVC; 17±1 vs 42±3 (kHz/mmHg)x10^3) and reduced heart rate (HR; 230±7 vs 365±6 beats/min). The selective FAAH inhibitor, URB597 alone had no consistent haemodynamic effect (data not shown). The haemodynamic responses to anandamide are shown in Table 1.

Table 1. Cardiovascular effects of 3mg/kg anandamide. * $P<0.05$ vs baseline within group (Friedman's test); #$P<0.05$ vs corresponding values in the vehicle group (Mann-Whitney U test)

		Vehicle			URB597		
		10 s	60 s	15 min	10 s	60 s	15 min
Saline (n=9)	HR	-157±34*	-42±18*	+24±14	-248±14*	-36±13*	-5±12
	BP	+11±10	-6±3	-4±3	-2±10	+1±2	+8±1*#
	RVC (%)	-17±7*	+12±4*	+14±8	-10±7	+17±3*	-6±5#
	MVC (%)	-46±7*	-4±13	+12±6	-42±5*	-7±8	-4±6#
	HVC (%)	-56±7*	+48±11*	+7±5	-64±3*	+35±8*	+29±11*
AII/AVP (n=8 or 9)	HR	-46±33	+17±5*	+6±9	-95±13*	+2±8#	+2±14
	BP	-0±6	-16±5*	+1±4	-25±6*#	-19±4*	-12±3*#
	RVC (%)	+1±4	+32±8*	+10±6	+20±8*#	+44±7*	+25±9*
	MVC (%)	-12±6	+12±13	-0±6	-3±8	+31±13*	+35±16*#
	HVC (%)	-43±14*	+140±37*	+35±33	-40±6*	+129±25*	+71±36*

These data suggest that anandamide causes depressor effects and more pronounced vasodilator responses in acutely hypertensive, conscious rats. In acute hypertension, the duration of depressor and vasodilator effects is enhanced by URB597.

[1]Gardiner SM *et al*. (2002) *Br J Pharmacol* 135: 1889-96

This study was supported by the British Heart Foundation (PG/06/064/20985) and Anne McLaren Fellowship (WSVH).

2-AG IMPROVES COGNITIVE AND NEUROLOGICAL FUNCTION IN A MODEL OF SECONDARY BILIARY CIRRHOSIS IM MICE

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Background: Hepatic encephalopathy (HE) is a major neuropsychiatric complication of both acute and chronic liver failure. However, its pathogenesis is still unknown. It has been suggested that the cognitive deficits characterizing this state result from changes in some neurotransmitter systems in the brain including the glutamatergic, cholinergic and monoaminergic systems. Endocannabinoids (EC) function as neuromodulators via specific receptors. Recently the endocannabinoid system was found to be involved in the vasodilated state associated with liver cirrhosis. We hypothesize that it might be involved also in hepatic encephalopathy.

Methods: Female Sabra mice were subjected to ligation of the bile duct (BDL). Sham operated animals were used as controls. 10 days post-surgery, animals receiving either vehicle or 1mg/kg 2-AG were evaluated for cognitive function in the Eight Arm Maze test. Neurological function was evaluated in the Neurological severity score (NSS) test, 3 weeks post-surgery. The animals were sacrificed and their livers and brains were analyzed for 2-AG levels by GC-MS analysis.

Results**:** Brain 2-AG levels were not different between Sham and BDL mice 3 weeks post-surgery, but liver 2-AG levels elevated two-fold in BDL mice in comparison to Sham mice. Cognitive and neurological functions were significantly impaired in BDL mice and 2-AG ameliorated these deficits.

Conclusion: These results indicate an involvement of the endocannabinoid system in the pathogenesis of HE. It seems that activation of the cannabinoid system might have therapeutic potential. 2-AG levels may rise in the brain at an earlier stage of the disease and that may be the reason for the lack of difference after 3 weeks. 2-AG elevation in the liver may have an antifibrotic effect mediated via the CB_2 receptor.

CP55940, A NON-SELECTIVE CB1/CB2 AGONIST, STIMULATES BONE RESORPTION BY HUMAN OSTEOCLASTS IN VITRO

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Cannabinoid receptors (CB_1 and CB_2) are G protein coupled receptors expressed in mammalian tissues and activated by endogenous cannabinoid ligands. Recent studies in mice have shown that cannabinoid receptors are present in bone, with both CB_1 and CB_2 knockout mice displaying altered bone phenotypes. Cannabinoid receptors are known to be expressed in human peripheral blood mononuclear cells but it is yet to be determined whether human osteoclasts express cannabinoid receptors. We sought to characterise the expression of CB_1 and CB_2 on human osteoclasts and to examine the effect of CP55940, a non-selective CB_1/CB_2 agonist. Human osteoclasts were generated by culturing peripheral blood-derived monocytes from healthy donors with M-CSF and RANKL. Using real-time PCR and western blotting, both monocytes and osteoclasts generated *in vitro* were found to express CB_1 and CB_2 receptor mRNA and protein. The level of CB_1 did not appear to change throughout osteoclast differentiation, whereas the level of CB_2 appeared to decrease during differentiation (as has been reported during B cell differentiation). To study the effect of CP55940, osteoclasts were generated on plastic (to study differentiation) or on dentine discs (to study resorptive activity) in the continual presence of vehicle or CP55940. In the presence of 1nM-1μM CP55940 there was no significant change in the number of VNR-positive osteoclasts; concentrations above 1μM caused a decrease in osteoclast number, attributed to cytotoxicity. However, 1nM-1μM CP55940 significantly increased the proportion of actively-resorbing osteoclasts (i.e. cells with actin rings) and increased resorption area e.g. at 1μM, cells with actin rings were 229% +/- 31 of control ($P<0.05$); resorption area was 334% +/- 50 of control ($P<0.01$), n = 7 experiments. This demonstrates for the first time that this non-selective CB_1/CB_2 agonist stimulates the activity of human osteoclasts. This finding is consistent with recent studies showing that a CB_1 antagonist prevents bone loss in OVX mice. Together, our observations confirm that CB_1 and CB_2 are expressed on human osteoclasts and suggest that endogenous ligands for these receptors may have direct effects on osteoclastic resorption. It remains to be determined whether the stimulatory effect of CP55940 is mediated via CB_1 and/or CB_2.

ENDOCANNABINOIDS ARE PRODUCED BY BONE CELLS AND STIMULATE BONE RESORPTION IN VITRO

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The recent demonstration of abnormal bone phenotypes in cannabinoid receptor (CB_1 and CB_2) knockout mice implies a role for these receptors in bone physiology. However, the exact role of these receptors in bone is far from clear and it is not known whether their endogenous ligands, the endocannabinoids, are produced in the bone microenvironment and the effects they illicit. To study the possible role of these endocannabinoids in bone physiology, we investigated the effects of arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG) on the formation and function of human osteoclasts derived from RANKL-stimulated M-CSF-dependent peripheral blood monocytes. Osteoclasts were cultured either on dentine (to study resorption) or on plastic (to study differentiation). Treatment with 10 nM 2-AG resulted in a 6.5-fold increase in bone resorption and a 2-fold increase in the number of F-actin rings in cultures of osteoclasts compared to treatment with vehicle control. Similarly, treatment with 100 nM anandamide resulted in a 3.5-fold increase in resorption and a 1.7-fold increase in the number of F-actin rings in osteoclast cultures. It remains to be determined whether this effect is mediated via CB1 or CB2. Anandamide and 2-AG did not affect osteoclast formation except at high concentrations (10uM), when osteoclast formation was inhibited by 60 %, but with no associated reduction in the number of viable cells. However, this inhibitory effect could not be overcome by co-treatment with selective antagonists for the CB1, CB2 and TRPV1 receptor (AM251, AM630 and capsaizepine respectively) suggesting that the inhibition of osteoclast formation at high concentrations of 2-AG and anandamide is a receptor-independent effect.

To address whether endocannabinoids are produced within the bone microevironment we measured the production of anandamide and 2-AG in primary bone cells and cell lines using LC-MS/MS. Endocannabinoids were extracted from cultured cells with methanol/acetonitrile and levels were normalised to total protein content. The extraction efficiency was >95% and the limit of quantification were determined as 0.01 pmol for anandamide and 25 pmol for 2-AG. 2-AG was detected in mouse calvarial osteoblasts, osteoblast (MC3T3-E1 and MG-63), osteocyte (MLOY4) and macrophage cell lines (J774) and in human and mouse primary macrophages/osteoclast precursors and osteoclast-like cells. Anandamide was detected in calvarial osteoblasts, osteoblast and osteocyte cell lines but was not detectable in human and mouse primary macrophages or in osteoclasts. Addition of the calcitropic factor parathyroid hormone (PTH) caused a significant increase in the levels of 2-AG in the osteoblast-like cell line MC3T3-E1 (1.96± 0.59nmol/mg protein to 3.66± 1.03 nmol/mg protein). PTH also caused a significant increase in anandamide levels in calvarial osteoblasts (2.25±1.65 pmol/mg protein to 3.34± 2.56 pmol/mg protein), while treatment of mouse osteoclasts with the bacterial endotoxin LPS caused a significant increase in 2-AG levels (1.37± 0.44 nmol/mg protein to 4.81± 3.35 nmol/mg protein). We conclude that 2-AG and anandamide can be produced locally by bone cells, can be regulated by osteotropic factors and are novel activators of bone resorption by human osteoclasts.

OCULAR PHARMACOLOGY OF CANNABIGEROL-DIMETHYL HEPTYL

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Cannabinoids are known to produce therapeutic hypotensive effects in the management of patients with glaucoma, a disease in which elevated intraocular pressure (IOP) is a primary risk factor. Although a number of studies have reported the IOP-lowering effect of cannabinoids, most of cannabinoid compounds tested, are known to result in undesirable psychotropic effects. Cannabigerol-dimethyl heptyl (CBG-DMH) is a synthetic non-psychoactive cannabinoid with hypotensive and vasorelaxative properties (Maor, et al., 2005), which may offer beneficial therapy for ocular hypertension.

The objective of our study was to compare the pharmacodynamic effects of CBG-DMH to other synthetic cannabinoid agonists, Methanandamide (MA) and WIN55,212-2, on IOP in Brown Norway rats.

IOP was measured with a tonometer (TonoPen ®XL). The animals were placed on a counter and the tonometer was placed gently against the anaesthetized surface of the cornea. For each time point, IOP was measured ten times and the mean value was calculated. All drugs were administered via intraperitoneal (IP) injections, and IOP readings were taken every 15 minutes for a period of 2 hrs.

CBG-DMH administration resulted in a dose-dependant reduction of IOP in rat eyes (2.5 mg/kg: 20.9 ± 0.16 to 19.8 ± 0.31 mmHg and 10 mg/kg: 20.8 ± 0.25 to 19.45 ± 0.15 mmHg; mean ± SEM). The IOP-lowering effect of CBG-DMH was significantly reduced by IP pre-administration of 2.5 mg/kg of O-1918, a selective antagonist of non-CB_1/CB_2 novel endothelial cannabinoid receptor, and SR141716A, a CB_1 specific receptor antagonist ($p < 0.05$). The observed reduction in IOP obtained with CBG-DMH was comparable to that obtained following IP administration of 1.7 and 2.5 mg/kg of MA (19.75 ± 0.39 to 17.925 ± 0.22 and 20.18 ± 0.23 to 17.95 ± 0.16, respectively) and 1.7 mg/kg of the WIN55212-2 (21.03 ± 0.11 to 19.63 ± 0.19; mean ± SEM). However, only the IOP decrease with CBG-DMH was blocked by pre-administration of O-1918.

This study demonstrates that administration of CBG-DMH effectively decreases IOP in the rat eye. The efficacy of CBG-DMH appears to be similar to that of MA and WIN55212-2, though the observed disparity in O-1918 antagonism suggests a mechanism of action for CBG-DMH that is distinct from that of the other CB1 agonists. CBG-DMH has the potential to function as a novel ocular hypotensive cannabinoid devoid of psychotropic activity.

PHARMACOLOGICAL INHIBITION OF CANNABINOID-1 RECEPTOR PROTECTS AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY.

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Background: Doxorubicin (DOX) is one of the most potent antitumor agents available; however its clinical use is limited because of the risk of severe cardiotoxicity often leading to irreversible congestive heart failure. Endocannabinoids mediate cardiodepressive effects through cannabinoid-1 (CB_1) receptors in various pathophysiological conditions, and these effects can be reversed by treatment with CB_1 antagonists. Here, we explore the effect of pharmacological inhibition of CB_1 receptor in *in vivo* and *in vitro* models of DOX-induced cardiotoxicity.

Methods and Results: Five days following the administration of a single dose of DOX (20 mg/kg IP) to mice, left ventricular systolic pressure, +dP/dt, -dP/dt, stroke work, ejection fraction, cardiac output and load independent-indexes of contractility (Emax, PRSW, d*P*/dt–EDV) were significantly depressed (measured by Millar pressure-volume system), and the tissue level of the endocannabinoid anandamide was elevated in the myocardium, as compared to vehicle-treated controls. Treatment with the CB_1 antagonists rimonabant or AM281 markedly improved cardiac dysfunction and reduced DOX-induced apoptosis in the myocardium. DOX also decreased cell viability and induced apoptosis in the H9c2 myocardial cell line measured by flow cytometry and fluorescent microscopy, which were prevented by the preincubation of the cells with either CB_1 antagonist, but not with CB_1 and CB_2 agonists and CB_2 antagonists.

Conclusions: These data suggest that CB_1 antagonists may represent a new cardioprotective strategy against DOX-induced cardiotoxicity.

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Supported by intramural funds of NIH/NIAAA

OVER-EXPRESSING CB2 RECEPTOR UNCOVERS ITS ROLE IN REGULATING LUNG EPITHELIAL CELL CHEMOTAXIS

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Marijuana smoking is associated with airway inflammation, bronchitis, impaired cellular organization and decreased cell energetics. In order to study the role of the CB2 receptor (CB2R) on lung cell function, we generated a human lung epithelial cell line that stably over-expresses CB2R. A549 cells were transfected with self-inactivating lentiviral vectors expressing either a GFP reporter gene alone or in combination with the CB2R gene. The resulting cells were FACS-sorted to produce cell lines with matching levels of GFP expression (GFP and CB2R-GFP cells, respectively). Quantitative real-time RT-PCR demonstrated a 25-fold higher expression of CB2R mRNA in CB2R-GFP cells. The chemotactic activity of both GFP and CB2R-GFP cells was suppressed when exposed to either Δ^9-tetrahydrocannabinol (THC) or marijuana smoke extract. However, the IC_{50} for CB2R-GFP cells was more than 10-fold lower than the IC_{50} for GFP cells (< 0.1 vs 1.0 μg/ml THC). Studies using the selective CB2R antagonist, SR144528, further verified functional receptor expression and receptor involvement in THC-induced inhibition of chemotaxis. While THC exposure produced a decrease in cellular ATP levels, as reported previously, this decrease did not appear to play a role in chemotaxis inhibition since cyclosporin A, which partially protected against ATP loss, did not increase cell migration. However, CB2R over-expression produced changes in cell energetics and mitochondrial membrane potential suggesting the presence of a pathway linking this plasma membrane receptor with mitochondrial activity. Since epithelial cell chemotaxis plays an important role in functional airway remodeling, potent suppression of chemotaxis by THC could contribute to lung injury in habitual marijuana smokers. In addition, pharmacologic manipulation of the CB2R pathway could have therapeutic value. This work was supported by NIH Grant R21-DA021580 and R01-DA03018.

CAPSAICIN AFFECT NEUROLOGICAL, ACTIVITY, COGNITIVE FUNCTION AND ASTROGLIOSIS IN A MODEL OF HEPATIC ENCEPHALOPATHY ASSOCIATED WITH FULMINANT HEPATIC FAILURE IN MICE

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Background: Hepatic encephalopathy (HE), is a neuropsychiatric complication of both acute and chronic liver failure. In fulminant hepatic failure (FHF), the development of serious neurological complications can be associated with cerebral herniation and death. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active component causing the hotness of hot peppers from the genus Capsicum. It is used as an anti-inflammatory, anti-carcinogenic and analgestic product. Capsaicin acts on neural cells via vanilloid receptors subtype 1 (VR1, also known as transient receptor potential 1 - TRPV1), a non-selective cation channel, with wide distribution in the central nervous system, which can be blocked by capsazepine. Di Marzo et al. raised the possibility of an interaction between the endocannabinoid system and capsaicin-like compounds due to structural similarities between these compounds and anandamide. No evidence has been found so far for the activation of cannabinoid receptors (CB1 and CB2) by capsaicin.
Aims: The present study aimed to clarify the role of capsaicin on acute liver disease and HE in a mouse model of thioacetamid-induced fulminant hepatic failure and its mechanism of action.
Methods: Female Sabra mice were administered thioacetamide or vehicle. Mice were divided into groups with equal neurological score and administered with capsaicin, SR141716A (a CB1 receptor antagonist), capsaicin+SR141716A, SR144528A (a CB2 receptor antagonist), capsaicin+SR144528, HU308 (CB2 receptor agonist), noladin (CB1 receptor agonist) and capsazepine(Vanilloid Receptor Antagonist) one day after TAA administration. Neurological, activity, cognitive function and brain and liver histopathology were evaluated.
Results: In the liver TAA administration caused apoptosis, necrosis, bridging and inflammation. Capsaicin administration caused less apoptosis ($p<0.006$), inflammation ($p<0.009$) and more regeneration ($p<0.019$). In the brain: TAA administration caused impaired neurological score, activity and cognitive function**.** Capsaicin, SR141716A or HU308 reversed these activities, while SR144528 and noladin had no effect. These activities were related to the level of astrogliosis. Less astrogliosis in the hippocampus and the cerebellum was associated with lowering impairment of neurological, activity and cognitive function. Capsaicin, SR141716A and HU308 induced significantly less astrogliosis, while SR144528 and noladin had no effect on astrogliosis. Improved neurological score and reversed activity was mediated by the action of capsaicin on the vanilloid receptors, while cognitive function was mediated by the activity of capsaicin on the CB1 receptor.
Conclusion: These results show therapeutic effect of capsaicin on both liver and brain. They indicate the involvement of both cannabinoid and vanilloid receptors in the treatment of acute HE. The effect of capsaicin on the brain could be mediated by the improvement in the liver or directly on the brain.

BACLOFEN EFFECTS IN ALCOHOL-ABSTINENT RATS ARE ASSOCIATED WITH CHANGES IN THE LEVELS OF ENDOCANNABINOIDS AND RELATED *N*-ACYLETHANOLAMINES IN SPECIFIC BRAIN REGIONS

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Baclofen, a GABA-B receptor agonist, has been proposed as a potential treatment for alcohol addiction, since it is able to reduce alcohol intake and attenuate the intensity of alcohol withdrawal. In the present study, we examined whether the reduction by baclofen of several symptoms (e.g., anxiety, hyperlocomotion) of interrupted alcohol exposure in rats are associated with changes in the levels anandamide (AEA), 2-arachidonoylglycerol (2-AG), and related *N*-acylethanolamines (NAEs). Towards this end, brain areas associated with emotion, locomotion and feeding behavior were examined. We found that baclofen elevated the levels of AEA and related NAEs in the prefrontal cortex, but only in alcohol abstinent rats, with no significant effects in naive rats. 2-AG levels were not affected by baclofen in this brain region. Baclofen also elevated the levels of 2-AG and various NAEs, excluding AEA, in the striata of alcohol-abstinent rats. However, in this case the effect was evident when compared with the previously lowered levels of 2-AG and NAEs produced by the interruption in alcohol consumption. Baclofen did not modify AEA, 2-AG or NAE levels in the amygdala or the hypothalamus. In summary, these experiments revealed a regional pattern for the effects of baclofen on the levels of endocannabinoids and related NAEs in alcohol-abstinent rats. These observations suggest that the beneficial effects of this GABA-B receptor agonist in alcohol dependence might be related to effects on cannabinoid signaling system in specific brain regions.

Acknowledgements. The authors are grateful for the support of the National Institute on Drug Abuse (DA-018224, DA-020402, F32-DA-016825), the Gill Center for Biomolecular Science, Indiana University, Bloomington, and the Lilly Foundation Inc, Indianapolis, IN.

PMSF POTENTIATES THE DISCRIMINATIVE STIMULUS PROPERTIES AND MOUSE TETRAD EFFECTS OF CANNABINOIDS

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Anandamide (AEA) and Δ^9-tetrahydrocannabinol (THC) share common pharmacological properties; however, some of their effects have been found to differ. The most often accepted explanation for these differences has been the rather fast metabolism of AEA compared to that of THC. We previously reported that pretreatment with phenylmethylsulfonyl fluoride (PMSF), an amidase inhibitor, increases AEA's levels in brain and enhances AEA cannabimimetic effects in the tetrad. The present study examined whether pretreatment with PMSF alters the THC-like discriminative stimulus properties of several CB1 agonists including THC, AEA, and CP 55,940 and the fatty acid amide, oleamide. Male Long Evans rats were trained to discriminate 3 mg/kg THC from vehicle in 2-lever drug discrimination. THC fully and dose dependently substituted for itself when administered alone. Consistent with previous findings, this study demonstrated that AEA did not substitute for THC in the absence of PMSF; however it fully and dose dependently substituted for THC when administered with PMSF. Moreover, THC also produced a several fold more potent ED50 when administered with PMSF than when administered alone. Similar results were observed for CP 55,940. In contrast, oleamide, administered alone and in combination with PMSF, did not substitute for THC. The CB1 receptor antagonist SR141716A blocked the THC-like discriminative stimulus effects of AEA co-administered with PMSF. In general, the rate suppressant effects of the cannabinoid agonists were unchanged by co-administration of PMSF suggesting the possibility of a separate mechanism of action for response rate and discriminative stimulus effects. These results, taken together, suggest that substitution of AEA for THC when administered in the presence of PMSF is dependent upon the ability of PMSF to inhibit the breakdown of anandamide, via the enzyme fatty acid amide hydrolase (FAAH). Secondly, these results suggest selectivity for CB1 mediated cannabinoids in this model, as PMSF presumably affected metabolism of both AEA and oleamide. A subsequent study examined whether PMSF administration would enhance AEA and THC effects in the mouse tetrad. As predicted, pretreatment with PMSF greatly potentiated AEA's tetrad effects. Surprisingly, THC's tetrad effects were also potentiated by pretreatment with PMSF. The potentiation of AEA's effect in drug discrimination and the tetrad is likely to be due to PMSF's effect on the metabolism of AEA (i.e. PMSF inhibits the rapid breakdown of AEA via FAAH). In contrast, PMSF is not likely to potentiate the THC-like discriminative stimulus effects of THC and CP 55,940 and the cannabimimetic effects of THC in the tetrad via interference with their metabolism as these structurally diverse cannabinoids are predominantly metabolized through the P450 system. An alternative explanation is that PMSF-induced increases in endocannabinoids may account for potentiation of THC-like discriminative stimulus effects of THC and CP 55,940 and THC's cannabimimetic effects in the tetrad.

Acknowledgements: Research supported by NIH grants DA-03672 and DA-09789.

THE MOTIVATION TO WORK FOR FOOD AFTER SUSTAINED CB_1 BLOCKADE DURING ADOLESCENCE

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Progressive-ratio schedules of reinforcement are commonly construed to be a measure of the motivation to work for a reward. These schedules require progressively larger amounts of work to receive each subsequent reinforcer (e.g., food pellet). At some point, the amount of work required exceeds the efficacy of the resulting reinforcer and the subject ceases to respond. The last ratio completed before the cessation of responding is referred to as the "breakpoint". Food-reinforced progressive-ratio breakpoints and response rates can be modulated by changing the palatability of the food reward, by drugs that affect appetite or food intake, or by varying the level of food deprivation. Repeated peripubertal exposure to WIN 55,212 has been shown to reduce breakpoints in a progressive-ratio schedule of reinforcement up to 15 days after the final treatment. Accordingly, a series of experiments were undertaken to determine the long-term impact of sustained CB_1 blockade during adolescence. Adolescence in rats is behaviorally defined as beginning around postnatal day 28 (PD28) and extending through postnatal day 42 (PD42). In these experiments, male Long-Evans rats were treated with rimonabant (3 mg/kg, i.p.) each day throughout adolescence and into early adulthood (PD28-PD68) while being allowed unrestricted access to their normal chow. To control for the effect of the drug treatment, a separate group of rats was treated with vehicle (1 ethanol: 1 emulphor: 18 saline) while also being allowed unrestricted access to their normal chow. Since it was expected that animals chronically treated with rimonabant would weigh less than vehicle treated animals, a third group of rats was treated with vehicle while being pair-fed with a designated member of the rimonabant-treated group. By the end of the treatment period, animals chronically treated with rimonabant and their pair-fed counterparts weighed significantly less than the vehicle-treated controls (approx. 9.5%). Between PD75 and PD96, the motivation to work for food was repeatedly measured while systematically manipulating the level of food deprivation (85% to 115% of pre-testing bodyweight). For vehicle-treated animals, progressive-ratio breakpoints and response rates varied as a function of the level of food deprivation. This pattern was disrupted following sustained CB_1 blockade during adolescence and in the pair-fed group. The rimonabant-treated group, but not the pair-fed rats, also showed increased consumption of novel, palatable food. In summary, sustained reduction in food consumption during adolescence, whether accomplished by pharmacologic intervention or by food restriction, resulted in a motivation to work for food that was not as clearly related to the animal's level of food deprivation. Further, these results suggest that sustained CB_1 blockade throughout adolescence may increase consumption of palatable foods in adulthood, though the duration of this effect remains unclear.

Acknowledgements: Research supported by NIH grant DA-016644 and training grant DA-07027.

Endocannabinoid Modulation of Nicotine Reward and Dependence

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Nicotine is the addictive component in tobacco that acts on the brain to produce both rewarding effects and aversive events following repeated exposure. Recent studies have reported an interaction between nicotine and cannabinoid systems. Therefore, modulation of the cannabinoid system may have direct therapeutical implications for smoking cessation. The aim was to investigate the effects of FAAH inhibition in nicotine reward and nicotine dependence using FAAH inhibitor (URB597) and the FAAH Knock-out mice. Using the condition place preference paradigm (CPP) to measure the rewarding properties of nicotine, FAAH KO's and wild type mice pretreated with various doses of nicotine. Furthermore, wild-type mice were pretreated with the FAAH inhibitor, URB597 (0.3 -10 mg/kg i.p.) in the nicotine CPP test. FAAH KO mice show an enhanced expression of nicotine CPP (0.1 mg/kg s.c.) and pretreatment with URB597 (0.3 mg/kg and 3.0 mg/kg i.p.) enhances nicotine CPP in mice. Using a mouse model (seven-day nicotine mini-pumps infusion at 24mg/kg/day), nicotine spontaneous withdrawal signs were measured in wild-type and FAAH KO mice. In addition, spontaneous nicotine withdrawal after acute challenge with URB597 was also performed in mice. Nicotine withdrawal intensity was enhanced in both FAAH KO and URB-treated mice. However, the enhancement of nicotine reward by URB597 is 3-10 times more efficacious in nicotine CPP than nicotine withdrawal. Increasing FAAH inhibition potentiates aversion in nicotine CPP, and marginally exacerbates the physical and effective signs of nicotine withdrawal. Low doses of URB597 modulate nicotine reinforcement to a much greater extent than nicotine withdrawal. Increasing levels of endocannabinoids, like anandamide, may enhance the rewarding properties of nicotine dependence and progressively increase the adverse effects of nicotine withdrawal promoting addiction and dependence.

NEUROCHEMICAL AND BEHAVIORAL EVIDENCES THAT Δ^9-THC EXPOSURE IN ADOLESCENCE PROVOKES MOOD AND COGNITIVE ALTERATIONS IN ADULTHOOD

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There are little and often contradictory studies on the long-term neurobiological consequences of cannabinoid consumption in adolescents. The endocannabinoid system plays an important role during the different development stages of the brain as cannabinoids modulate the release and the action of different neurotransmitter and promote neurogenesis.

This work studied the long-term consequences of adolescent assumption of delta-9 tetrahydrocannabinol (THC) on mood and cognitive parameters, through behavioural and neurochemical assays. Adolescent male and female rats (35-45 PND) have been treated with increasing doses of THC for 11 days and left undisturbed until their adulthood (75 PND) when the behavioural and neurochemical assays were performed.

Adolescent THC exposure produced different effects on male and female rats in adulthood. CB1 receptor level and functionality were significantly reduced in the amygdala, VTA and nucleus accumbens of female rats whereas in males significant alterations were determined in the amygdala and hippocampus. Neither female nor male rats showed alteration in anxiety responses (Elevated plus maze and open field tests) but a significant "behavioural despair" (forced swim test) paralleled by anhedonia (sucrose preference) was present in females. In contrast, male rats did not present behavioural despair but exhibited anhedonia. This different behavioral picture was supported by biochemical parameters of depression, namely CREB alteration. In fact female rats exhibited decreased CREB activity in the hippocampus and prefrontal cortex and increased CREB activity in the NAC paralleled by increased dynorphin expression.

Finally adolescent exposure to THC did not alter short term memory but reduced spatial memory (radial maze) in both genders. In contrast long term memory (passive avoidance) was impaired only in males. In line with this picture, altered synaptic markers (synaptophisin, PSD95, GAP43) were found in the prefrontal cortex and hippocampus of pretreated rats.

The present results suggest that cannabis consumption during adolescence may induce long term behavioural effects coupled with stable alteration in neurochemical markers and synaptic plasticity.

Acknowledgements: funded by "Dipartimento delle Politiche Antidroga" of Italian Governement.

SUBCHRONIC Δ9-THC ADMINISTRATION MODULATES EXPRESSION OF MOLECULAR REGULATORS OF REWARDING DRUG ACTION IN THE STRIATUM

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Subchronic Δ9-tetrahydrocannabinol (THC) administration in laboratory animals and humans produces tolerance and a mild physiological dependence syndrome. Some individuals can become addicted to marijuana upon repeated use, but literature reports on rewarding properties of THC in animal models are conflicting. Moreover, some literature reports indicate that subchronic THC administration can sensitize laboratory animals to subsequent motor-stimulating or rewarding effects of opioids or psychomotor stimulants. These effects are thought to be mediated in part by neuronal circuits in the striatum, a brain region that includes nucleus accumbens (NAC) and caudate-putamen (CPu). Two regulatory proteins whose expression is modulated by addictive drugs are the transcription factor ΔFosB and the regulator of G-protein signaling RGS9-2. Overexpression or knockout, respectively, of these proteins in the striatum can sensitize animals to conditioned rewarding effects of psychomotor stimulants or opioids. To determine the effect of THC on expression of ΔFosB or RGS9-2 in striatum, mice were treated with escalating doses of THC for two weeks. 24 hr after the last THC injection, ΔFosB was measured immunohistochemically with a FosB antibody in sections from fixed brains, or RGS9-2 was measured by immunoblotting homogenates from dissected striata. Results showed that subchronic THC increased ΔFosB immunoreactivity (ir) by ~2.5-3.5-fold in the shell and core of NAC, but not in CPu. In striatal homogenates, RGS9-ir was decreased by ~25% in THC- compared to vehicle-treated mice, with no difference observed in RGS4-ir, RGS7-ir or Gβ5-ir. Studies were then performed in genetically modified mice to determine effects of ΔFosB or RGS9-2 on CB_1 receptor function. In inducible transgenic mice overexpressing ΔFosB in the striatum, CB_1-mediated inhibition of forskolin-stimulated adenylyl cyclase (AC) in NAC was not affected by ΔFosB overexpression. In contrast, AC inhibition by mu or kappa opioid receptors was enhanced in NAC of ΔFosB overexpressing mice. In the NAC of RGS9 knockout mice, CB_1-mediated AC inhibition was not different from that of wild-type mice. However, dopamine D_2 receptor-mediated AC inhibition was enhanced. These results indicate that subchronic THC treatment can modulate expression levels of ΔFosB or RGS9-2 in brain regions associated with motivational effects of addictive drugs, but that altered expression of these proteins does not alter CB_1 receptor signaling. These findings suggest a molecular mechanism whereby subchronic THC administration might produce cross-sensitization to opioids or psychomotor stimulants.

Acknowledgements: This work was funded by NIH grants DA-05274, DA-10770, DA-03672, DA-08227 MH-66172 and DA-14277.

COGNITIVE IMPAIRMENTS ASSOCIATED WITH PRECIPITATED WITHDRAWAL FROM THC IN MICE

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The dependence liability of cannabis remains controversial, though the existence of a clinically relevant withdrawal syndrome is increasingly well documented (e.g. Haney et al., 1999; Budney et al., 2003). Symptoms are usually subtle yet multifaceted, often including sleep and gastrointestinal disturbances, anxiety, irritability, among others. Additionally, subtle impairments in cognition reported in abstaining cannabis users may also reflect withdrawal-like processes. Importantly, avoidance of these withdrawal effects has been reported to promote relapse (e.g. Copersino et al., 2006), suggesting that pharmacotherapies designed to ameliorate withdrawal may be clinically useful in treatment-seeking cannabis users. While spontaneous withdrawal effects have been difficult to model in rodents, rimonabant (RIM) – precipitated somatic withdrawal effects (e.g. paw fluttering, head shakes) have been well characterized. This emergent sensitivity to RIM-precipitated effects following repeated THC treatment is hypothesized to reflect the same underlying adaptive changes in the endocannabinoid system believed to mediate tolerance and dependence. To better understand these processes, we have begun to characterize the consequences of precipitated withdrawal in a wide range of behavioral paradigms selected based on their relevance to the human withdrawal syndrome or lingering cognitive deficits. First, mice were administered five daily treatments of 10 mg/kg THC or Veh (i.p.), a relatively mild THC regimen. 4 ½ hours after the final treatment, mice were treated with RIM and 15 min later behavior was scored for 10 min. As expected, RIM produced significant paw fluttering in THC-treated, but not Veh-treated mice. Next, a series of experiments evaluated the effects of precipitated withdrawal in a spatial recall water maze task. Mice were trained to locate a hidden platform which was moved to a new location each day. Once stable performance was achieved, mice received five daily treatments of 10 mg/kg THC or Veh (i.p.), one hour after each daily training session. On day 5, mice received their final treatment in the AM and performed an acquisition session 4 hours later. 30 min following completion of this acquisition session, mice were treated with RIM or Veh, and their ability to return to the platform location was assessed 30 min later. This process was repeated several times over consecutive weeks until all mice were tested with each dose (the order of doses was counterbalanced, and no order effects were observed). RIM produced significant dose-related impairments of recall performance in THC-treated, but not Veh-treated mice. It does not appear that this effect can be explained by sensorimotor or motivational deficits, as performance in a control experiment where the platform was explicitly visible was unaffected. Importantly, this spatial recall effect appears much more sensitive than the somatic syndrome, as near maximal effects were observed following just 1 mg/kg RIM, compared to the 10 mg/kg RIM required to produce significant paw-fluttering. These results support the hypothesis that cognitive impairments observed in abstaining cannabis users may reflect withdrawal processes. It is hypothesized that a fuller characterization of this and other behavioral consequences of precipitated withdrawal in mice may provide a useful platform for testing novel pharmacotherapies designed for relapse-prevention, and promise to further our understanding of the adaptive changes in the endocannabinoid system resulting from chronic cannabis use.

EFFECTS OF HALOGEN SUBSTITUENTS UPON CB_1 AND CB_2 RECEPTOR AFFINITIES IN THE SERIES OF *N*-ALKYL-3-(8-HALO-1-NAPTHOYL)INDOLES

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The structure of THC, as determined in 1964 by Gaoni and Mechoulam, has a dibenzopyran nucleus. This led to the development of structure-activity relationships (SAR) based upon this skeleton. Pfizer extended these SAR to their non-traditional cannabinoids, which lack the dibenzopyran nucleus. In the late 1980's it was found by a group at Sterling-Winthrop that pravadoline, an indole based non-steroidal anti-inflammatory agent, inhibited contractions of the mouse vas deferens. It was subsequently determined that this was caused by interaction of the compound with the CB_1 receptor. This compound and other structurally related aminoalkylindoles have since been shown to exhibit typical cannabinoid pharmacology *in vivo* and *in vitro.*

Many of these indole derivatives have high affinity for both the CB_1 and CB_2 receptors. 1-Pentyl-2-methyl-3-(1-naphthoyl)indole (JWH-007) has $K_i = 9.5 \pm 4.5$ at CB_1 and $K_i = 2.9 \pm 2.6$ at CB_2. The propyl analog (JWH-015) has good affinity for the CB_2 receptor with $K_i = 13.8 \pm 4.6$ while it has weak affinity for CB_1 with $K_i = 164 \pm 22$. (Huffman, J.W.; *et.al. Bioorg. Med. Chem.* 2005, *13*, 89).. Substituents on the naphthyl ring of these indoles causes variation in the affinities for both the CB_1 and CB_2 receptors. To investigate the effect of halogen substituents upon receptor affinity the synthesis of a series of indole derivatives with halogens as substituents in the C-4 and C-8 positions of the naphthyl ring was carried out

Members of a series of *N*-alkyl-3-(8-halo-1-naphthoyl)indoles have high affinity for the CB_2 receptor. The *N*-pentyl bromo compounds also have good affinity for both the CB_1 and CB_2 receptors while the *N*-pentyl iodo compounds have high selectivity for the CB_2 receptor. Specifically, *N*-pentyl-3-(8-bromo-1-naphthoyl)indole (JWH-424) has affinity for both the CB_1, $K_i = 20.9 \pm 3.4$ and CB_2, $K_i = 5.44 \pm 0.18$, while JWH-417, the 8-iodo analog, shows 22-fold selectivity for CB_2 with a K_i at CB_2 of 3.27 ± 0.09 nM. The 2-methylindole derivative of this compound is 40-fold selective for the CB_2 receptor. This trend is reversed in the *N*-propyl analogs where the 8-bromo series displays 34 to 52-fold selectivity for the CB_2 receptor. The completion of the 8-halo series is in progress and the synthesis of the 8-chloro series is currently being carried out. Syntheses as well as further SAR data will be discussed for this series of compounds and these data will be compared to SAR for other series of cannabimimetic indoles.

ATROPISOMERS AS PROBES OF THE CANNABINOID RECEPTORS

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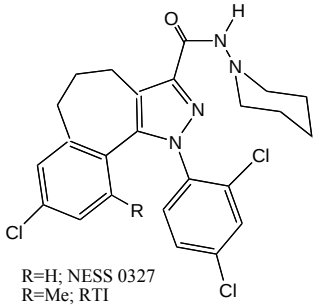
Most ligands that bind to receptors, such as CB1, have multiple conformers that can be adopted, of which only one is the active form. Towards the goals of identifying the active conformation of ligands and developing more active and potentially specific ligands, medicinal chemists have prepared constrained analogs of active leads. The concept behind a constrained analog is that structurally locking a ligand into an active conformation affords a molecule that does not require an expenditure of energy to adopt a binding conformation. Such an energy saving, results in a higher binding affinity (a measure of the energy advantage of a bound versus an unbound ligand).

A recently reported analog of SR141716 is the high affinity NESS 0357, which partially constrains the rotational conformations of the 4-chlorophenyl ring in the 5-position of SR via a three-carbon bridge between the pyrazole 4-position and the 4-chlorophenyl ring. The resulting seven-member ring favors two ring pucker conformations that are conformational isomers due to restricted rotation about the 5-position single bond. If the energy of the ring pucker were too great to surmount at ambient or physiological conditions, then two enantiomeric isomers would result. One isomer would be responsible for binding while its enantiomer would not bind. Identifying the binding enantiomer would provide physical evidence of the binding conformation to supplement and test the computational evidence of the binding conformation of the ligand.

We have obtained NMR, chromatographic and computational evidence that the energy separation between the enantiomers of NESS 0327 is too low to isolate the two conformers. However, we have synthesized a methyl-substituted analog with the same constraining bridge where the energy barrier is sufficient to isolate the enantiomeric atropisomers. Assayed in hCB1 against tritiated SR141716, the methyl-analog (as an enantiomeric mixture) exhibits a Ki = 15.9 nM versus Ki = 6.36 nM for NESS 0327. Assayed against tritiated CP 55,940, the respective Ki's were 41.0 nM versus 20.4 nM. The atropisomers of the corresponding methyl-substituted intermediate carboxylic acids were chromatographically resolved as their diasteriomeric esters and characterized by NMR spectroscopy before further transformation to the target hydrazides and assay of the separate atropisomers.



R=H; NESS 0327
R=Me; RTI

HOW SENSITIVE IS FAAH TO 'CANNABINOID RECEPTOR' LIGANDS?

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The fatty acid ethanolamine (FAE) family includes anandamide, *N*-palmitoyl–ethanolamine (PEA) and *N*-oleoylethanolamine (OEA). Members of the FAE family are agonists at multiple 7-transmembrane receptors (CB_1, CB_2, GPR55, GPR119), and show agonist activity at transmitter-gated channels (TRPV1), as well as multiple nuclear receptors, in particular, the peroxisome poliferator-activated receptors (PPAR). Given that FAEs appear to be hydrolysed principally through the action of the enzyme fatty acid amide hydrolase (FAAH), I have investigated whether ligands active at these receptors may also regulate FAAH activity in preparations from rat liver.

A microtitre plate-based spectrofluorometric assay for FAAH activity was employed, which assesses degradation of oleamide, capturing the evolved ammonia as an OPA derivative. Ligands were assessed for inhibitory activity at 100 μM, alongside the 'standard' inhibitor URB597 (10 μM), which completely abrogated FAAH activity.

Investigating structure-activity relationships for FAEs indicated a rank order of anandamide > *N*-stearoylethanolamine, OEA > *N*-elaidoylethanolamine, *N*-stearoylethanolamine, PEA. The synthetic analogue *N*-benzoylethanolamine was ineffective.

Of the CB_1/CB_2 receptor ligands, only THC (64 % control) and HU-210 (84 %) evoked significant inhibitions of FAAH activity, while THCV, WIN55212-2, rimonabant, SR144528, AM630 and CBD were ineffective.

As expected, the TRPV1 agonist NADA inhibited FAAH activity (54 %), as did olvanil (70 %), while piperine, PPAHV, isovelleral and capsaicin were without effect.
Amongst the PPARα agonists, clofibrate and WY14643 evoked modest inhibitions (70 and 79 %, respectively), while gemfibrozil and fenofibrate were inactive. Agonists at PPARβ (bezafibrate and GW0742) were ineffective, as was rosiglitazone (PPARγ agonist). Of the PPAR antagonists, GW6471 (α) was inactive, while GW9662 (γ) evoked a small inhibition (70 %).

Taken together, these results indicate relatively modest effects of representative compounds active at 'cannabinoid receptors'. Whether these agents accumulate sufficiently in intact cell preparations or *in vivo* to allow significant inhibition of FAAH activity and the consequent accumulation of endocannabinoids remains to be determined.

NOVEL CB_2 RECEPTOR LIGANDS

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Since the characterization of Δ^9-THC in the sixties, many improvements have been made in the cannabinoids pharmacology and more particularly with the discovery, in the nineties, of two G-protein coupled receptors: the CB_1 receptor, which is mainly located in the central nervous system (CNS), and the CB_2 receptor which is mainly expressed in the immune system. Thanks to the development of a great variety of synthetic ligands, the study of these receptors has revealed a large therapeutic potential for the CB_1 receptors ligands. Surprising enough the potential of modulating the CB_2 cannabinoid receptor activity has been less extensively studied. Recent published works have shown that it plays a role in the control of pain, inflammation, osteoporosis and cell proliferation. These data suggested that the CB_2 receptor constitutes an attractive target for the development of potent therapeutic compounds.

We previously described the synthesis and pharmacological evaluation of a novel series of 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives (e.g. ALICB-179, *K*i CB_1 > 2000 nM, *K*i CB_2 = 15.8 nM) exhibiting a CB_2 receptor agonists profile.

	X	**Y**	***h*CB$_1$ *K*i (nM)**	***h*CB$_2$ *K*i (nM)**
ALICB 179	O	CO-NH	> 2000	15.8 ± 1.4
ALICB 256	O	CH_2-NH	> 2000	> 2000
ALICB 270	O	NH-CO	> 2000	25.5 ± 1.3
ALICB 386	S	CO-NH	> 2000	18.2 ± 2.8

Here we present the synthesis and binding profile of novel representatives of this class of compounds. Competition binding studies were performed on hCB_1 and hCB_2-CHO cells using [^{3}H]-SR141716 and [^{3}H]-CP-55,940, respectively, as radioligands.

Our novel pharmacological data allowed us to delineate more precisely the pharmacophoric requirement in this series of compounds. In addition, our results prove the CB_2-selectivity of 4-quinoline derivatives.

Acknowledgements: Supported by the Université catholique de Louvain (ES, BB, DML) and by the Fonds National de la Recherche Scientifique (GGM).

IDENTIFICATION OF SECOND GENERATION PROSTAMIDE ANTAGONISTS (AGN 211334-6)

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The prostamides (prostaglandin – ethanolamides) and prostaglandin (PG) glyceryl esters are biosynthesized from the respective endocannabinoids anandamide and 2- arachidonyl glycerol. Early studies have suggested that the pharmacological profiles of prostamide $F_{2\alpha}$ and PGE_2 glyceryl ester are unique and unrelated to prostanoid receptors. This has recently been supported by the identification of a selective prostamide antagonist AGN 204396. Here we report the activity of second generation prostamide antagonists AGN 211334,35,and 36.

Effects on human recombinant PG receptors were studied using a FLIPR instrument: stable co-transfection of cDNAs encoding the receptors and a chimeric G protein allowed functional activity to be assessed as a Ca^{2+} signal for all receptors. The isolated feline iris was used as a key preparation where prostanoid FP receptor and prostamide activity co-exist.

Potent prostamide antagonists were designed by substituting the CH_2 at position 3 with an oxygen. The prostamide antagonists AGN 211334,35,36 did not block the prostanoid FP or other PG-sensitive receptors at concentrations up to 30μM. Similarly, in the cat iris, AGN 211334-6 did not block the effects of $PGF_{2\alpha}$. The effects of prostamide $F_{2\alpha}$ in the cat iris were antagonized by AGN 211334,35,and 36, AGN 211334 being approximately 10 fold more potent than the protypical antagonist AGN 204396. $I.C._{50}$ values were AGN 211334=236nM, AGN 211335=356nM, AGN 211336=303nM, AGN 204396=2635nM. The identification of second generation prostamide antagonists provides further support for the prostamide receptor hypothesis. AGN 211334,35, and 36 may also provide agents sufficiently potent for studies in living animals.

NOVEL BENZIMIDAZOLE DERIVATIVES AS SELECTIVE CB2 AGONISTS

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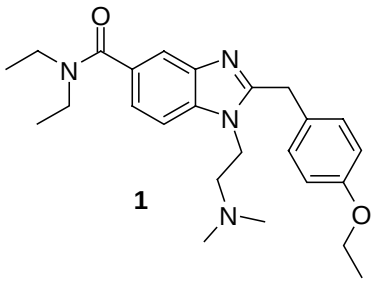
The endocannabinoid system has been known to mediate a complex array of biological effects through two distinct G-coupled protein receptors, namely CB1 and CB2. It is known that the side effects associated with cannabinoid derivatives are mostly related to the CB1 receptor located in the CNS. A compound targeting selectively the CB2 receptors located in the periphery would likely be devoid of any of these unwanted effects. Some of the reported CB2 functions included B-cell differentiation, altered antigen processing in macrophage, peripheral antinociception and antitumor properties. In a CB2 high-throughput program previously initiated at our company, compound (**1**) was obtained as an initial hit compound. This benzimidazole derivative demonstrated very good binding affinity towards the CB2 receptor with good selectivity over CB1. We report herein the preparation and evaluation of a novel class of selective CB2 benzimidazole agonists based on this scaffold.



1

Ki CB2: 36 nM
EC_{50}: 38nM (58%)
Ki CB1/CB2: >130

IDENTIFICATION OF NOVEL, SMALL MOLECULE INVERSE AGONISTS OF CB1 CANNABINOID RECEPTORS

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Inverse agonists of CB1 cannabinoid receptors have potential therapeutic utility in CNS disorders, including addictive syndromes, schizophrenia and Parkinson's disease, as well as metabolic disorders including obesity. We identified multiple novel structural classes of CB1 inverse agonists and characterized compounds from structural Class I in detail. CB1-462, a compound exemplifying structure Class I, binds to human and rat CB1 receptors with pKi values of 9.3 and 8.6, respectively. CB1-462 is an inverse agonist in cellular proliferation assays and GTPγS-binding assays, with approximately 10 nM potency in each. CB1-462 is highly selective for CB1 receptors over CB2, and over 60 other GPCRs, transporters and ion channels. CB1-462 is well absorbed and distributes readily into the CNS. CB1-462 reverses CP 55,940 induced analgesia and hypothermia in mice, and is orally active as an appetite suppressant, reducing food intake in fasted Sprague-Dawley rats. Chronic administration of CB1-462 to Zucker rats over a 14-day period resulted in a significant decrease in body weight compared to vehicle-treated animals. CB1-462 has pro-cognitive actions in radial arm maze and novel object recognition paradigms and augments apomorphine-induced rotations in the 6-hydroxy dopamine lesion model of Parkinson's disease. These results demonstrate that CB1-462 has therapeutic potential for a wide array of indications.

BENZAZOLYL ARYL UREAS: STRUCTURALLY NOVEL CLASS OF CB1 RECEPTOR LIGANDS

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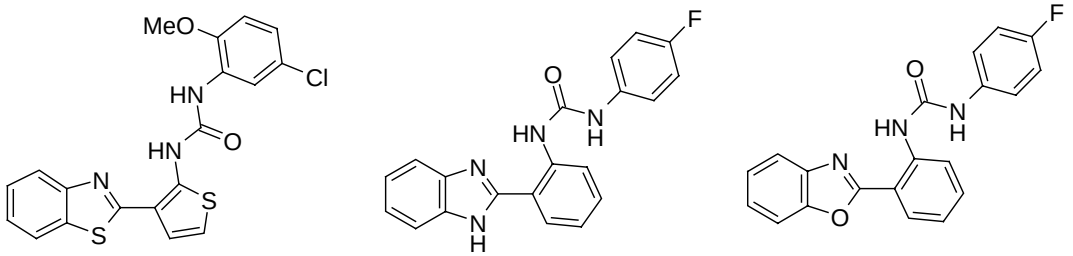
Virtual screening was applied in a search for novel cannabinergic compounds. One of the hit compounds, HTS10889, *N*-[3-(1,3-benzothiazol-2-yl)-2-thienyl]-*N*'-(5-chloro-2-methoxyphenyl) urea (Figure 1), showed antagonistic activity for the CB1 receptor in the [^{35}S]GTPγS binding assay inhibiting HU210-stimulated responses in rat cerebellar membranes. Consequently, it was used as a template for a series of novel benzazolyl aryl urea derivatives. The best compounds 1 and 2 presented in Figure 1 showed specific and moderate CB1 antagonist activity with IC_{50} values of 2.9 μM and 4.5 μM, respectively. The compounds were proven to be CB1 selective since they did not show any activity in the [^{35}S]GTPγS binding assay in the CHO cells expressing hCB2 receptors. The compounds were also tested for their affinities for the CB1 and CB2 receptors. Interestingly, the novel compounds did not have any effect on binding of [^{3}H]CP 55,940, however, with higher concentrations they significantly increased the binding of [^{3}H]WIN 55,212. Although the observed inconsistencies in the data may result from the differences in biochemical methodologies, the possibility that these compounds interact with an allosteric site at the CB1 receptor can not be excluded. Future studies will aim at improving the biological activity of these compounds and studying their potential as allosteric modulators.



HTS10889 **1** **2**

Figure 1. The hit compound HTS10889 and its synthetic analogues.

Acknowledgements: The skillful technical help of Mrs. Minna Glad and Mrs. Katja Hötti is greatly appreciated. The study was funded by the Technology Agency of Finland (TEKES), the Graduate School of Bioorganic and Medicinal Chemistry, Orion Research Foundation, Finnish Cultural Foundation, and a grant from NIDA (DA7215).

THE SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-SUBSTITUTED PHENOLIC *N*-ALKYL CARBAMATES AS NOVEL MGL-INHIBITORS

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The cannabimimetic effects of 2-arachidonoyl glycerol (2-AG), one of the main endocannabinoids, *in vivo* are weak because of its rapid degradation to arachidonic acid by the specific enzyme, monoacylglycerol lipase (MGL). The blocking of the activity of MGL by specific enzyme inhibitors leads to increased levels of 2-AG, resulting in favourable therapeutic effects such as pain reduction.

We describe here the synthesis and biological characterization of a series of 4-substituted phenolic *N*-alkyl carbamates as potent MGL-like inhibitors (Figure 1). The compounds were prepared by treatment of various 4-substituted phenols with suitable alkyl isocyanates. All the compounds synthesized in this study were tested for their potential MGL-like inhibitory activity in the assay described previously.[1]

These novel compounds inhibited MGL-like activity with IC_{50} values at low-micromolar range.

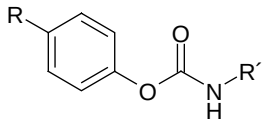

Figure 1. Chemical structure of 4-substituted phenolic *N*-alkyl carbamates, wherein R is a hydrogen bond forming heterocyclic or acyclic moiety and R´ is an alkyl chain of 6 to 12 carbon atoms.

[1] Saario et al. *Biochem. Pharmacol.* 2004, 67, 1381

A META-ANALYSIS OF RECEPTOR-LIGAND BINDING STUDIES

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Introduction: A meta-analysis, unlike a literature review, synthesizes previous studies into new results, and identifies sources of heterogeneity amongst studies. Heterogeneity in cannabinoid receptor-ligand binding studies may arise from methodological factors, such as: 1) use of PMSF to prevent the breakdown of AEA; 2) use of centrifugation versus rapid filtration to separate free and bound radioligand; 3) use of brain homogenates versus brain sections; 4) differences between Kd and Ki values, and Ki variance due to the use of dissimilar radioligands; 5) use of native tissues versus transfected cells; 6) differences between human and rodent receptors.

Methods: MEDLINE was searched for articles published in any language through 2006. Three reviewers considered studies for inclusion, and resolved disagreements by consensus. To be accepted for analysis, an article met the following inclusion criteria: 1) The study examined CB_1 or CB_2 in human (*Homo sapiens, Hs*) or rat (*Rattus norvegicus, Rn*). Data was limited to normal wildtypes. Studies of chimeric constructs were excluded (eg, F-11 and NG108-15 cells), as were studies of tissues with unidentified receptors. 2) Studies were limited to nine frequently tested cannabinoids: anandamide, (AEA), cannabidiol (CBD), cannabinol (CBN), CP55,940, HU210, SR141716A, THC, WIN55,212-2, and 2-arachidonoyl glycerol (2-AG).
3) Studies reported ligand affinity as Kd or Ki, measured in nM units. IC_{50} studies were included after conversion of IC_{50} to Ki using the Cheng-Prusoff equation.

Results: A total of 211 affinity studies met inclusion criteria. Meta-regression detected data heterogeneity arising from methodological factors: the use of sectioned tissue, the use of PMSF (in brain tissue for AEA), and choice of radioligand. Native brain tissues exerted greater affinity than transfected cells, but the trend fell short of significance. Surprisingly little heterogeneity was generated by centrifugation versus filtration (except for AEA). The mean Ki of THC and AEA differed significantly between *Hs*CB_1 and *Rn*CB_1, but not between *Hs*CB_1 and *Hs*CB_2. The mean Kd of CP55,940 and WIN55212-2 differed significantly between *Hs*CB_1 and *Rn*CB_1 and between *Hs*CB_1 and *Hs*CB_2. SR141716A differed between *Hs*CB_1 and *Rn*CB_1.

Conclusion: The meta-analysis identified several sources of heterogeneity amongst affinity studies, including phenotypic divergences between orthologs.

EFFECTS OF REPEATED ADMINISTRATION OF Δ^9-THC ON CANNABINOID CB_1 RECEPTOR DENSITY AND FUNCTION IN ADOLESCENT AND ADULT RATS

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Previous research (Sim-Selley et al, 2002) has shown that repeated administration of Δ^9-tetrahydrocannabinol (THC) produces down-regulation and desensitization of CB1 receptors in different brain regions, as well as tolerance to cannabinoid-mediated behaviors. Previous behavioral studies (Wiley et al, 2006) indicate age-dependent differences in the magnitude of tolerance produced. The aim of this study was to compare the effect of repeated administration of THC on the magnitude of desensitization and down-regulation in adolescent and adult, male and female rats. Adolescent [postnatal day 29 (PN29)] and adult (>PN60) Long- Evans rats were injected (i.p) twice daily with 10mg/kg THC or vehicle (7.8% tween 80 and 92.2% saline) for 9.5 days. On day 11, animals were sacrificed and brains were dissected. Tissues were collected from the prefrontal cortex, ventral midbrain, bilateral striatum, cerebellum, hippocampus, periaqueductal gray, anterior cingulated cortex, hypothalamus, and spinal cord, tissue was then stored at -80°C until day of assay. CB_1 receptor levels and function were assessed using [^{3}H]SR141716A and agonist-stimulated [^{35}S]GTPγS binding, respectively. The magnitudes of desensitization and down-regulation were determined by comparing the vehicle treated animals to the THC treated animals. Our results indicate a significant decrease in CP-55940 stimulated [^{35}S]GTPγS binding and [^{3}H]SR141716A binding in the prefrontal cortex of THC treated male and female adolescence rats compared to the vehicle groups. This demonstrates that there is desensitization and down regulation of CB_1 receptors following repeated administration of THC. Comparing the males and female adolescent data shows that there is no significant difference in the magnitude of down regulation between but there is a significant difference between the magnitudes of desensitization (decrease in G-protein activation). This shows that although down regulation is unaffected by gender, there is a possibility that the degree of desensitization is indeed influenced by gender. The future direction of this study is to compare the effects of repeated THC treatment between adolescent and adult male and female rats with an aim to investigate any possible differences between the degree of desensitization and down regulation.

Acknowledgements: Research supported by NIH grants DA-016644 (JLW) and DA-14277 (LJS).

References

1. Sim-Selley LJ and Martin BR (2002) Effect of chronic administration of R-(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2) or delta(9)-tetrahydrocannabinol on cannabinoid receptor adaptation in mice. *J Pharmacol Exp Ther* Vol. 303, Issue 1, pp.36-44
2. Wiley J. et al (2006) Pharmacological effects of acute and repeated administration of {Delta}9-tetrahydrocannabinol in adolescent and adult rats. *J Pharmacol Exp Ther* 106. pp.108-126

EXPRESSION, PURIFICATION AND COMPLETE MS CHARACTERIZATION OF HUMAN CB1 AND CB2 CANNABINOID RECEPTORS

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The CB1 and CB2 cannabinoid receptors belong to the GPCR superfamily and are associated with a variety of physiological and pathophysiological processes. Both receptors, with several lead compounds at different phases of development, are potentially useful targets for drug discovery. For this reason, fully elucidating the structural features of these membrane-associated proteins would be extremely valuable in designing more selective, novel therapeutic drug molecules. As a first step towards obtaining information on the structural features of the drug-receptor complex, we present the full mass spectrometric (MS) analysis of the recombinant human cannabinoid CB1 and CB2 receptors. This first complete proteomic characterization of GPCR beyond rhodopsin were accomplished by a combination MALDI TOF and several LC/MS approaches involving nanocapillary liquid chromatography, coupled to either a quadrupole-linear ion trap or linear ion trap-FTICR mass spectrometers. Both receptors with incorporated FLAG and HIS6 epitope tags were functionally expressed in baculovirus cells. The CB1 receptor purification was based on IMAC affinity chromatography, while the CB2 receptor was purified using a single step of anti-FLAG M2 affinity chromatography. To overcome the difficulties involved with in-gel digestion, due to the highly hydrophobic nature of this membrane-associated protein, we conducted in-solution trypsin and chymotrypsin digestions of purified and desalted samples in the presence of a low concentration of CYMAL5. The cannabinoid receptor peptides were fingerprinted directly by MALDI TOF MS or characterized after separation using nanoLC chromatography followed by nanospray ESI analysis. The full MS coverage of the cannabinoid CB1 and CB2 receptors can be reported confidently, based on the overlapping sequence data obtained using MALDI TOF, the highly mass accurate LTQ-FT and the 4000 Q-Trap mass spectrometers. Mass spectrometric identification of all amino acid residues in the cannabinoid receptors is a key step toward the “Ligand Based Structural Biology” approach developed in our laboratory for characterizing ligand binding sites in GPCRs using a variety of covalent cannabinergic ligands.

This work has been supported by grants from the National Institutes of Drug Abuse: DA09158, DA00493, DA03801, DA07215, and DA07312.

CANNABINOID RECEPTOR-STIMULATED NITRIC OXIDE (NO) PRODUCTION IN NEURONAL CELLS IS ASSOCIATED WITH cGMP ACCUMULATION AND S-NITROSYLATION OF PROTEINS

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Cannabinoid receptor agonists have been shown to stimulate nitric oxide (NO) production in the N18TG2 cell model. These findings suggest that the CB_1 receptor-stimulated NO production may contribute to signal transduction pathways via cGMP production and S-nitrosylation. NO binds to guanylyl cyclase (GC) and mediates the conversion of GTP to cGMP. In real-time reverse transcription polymerase chain reaction studies, we show that CB_1 but not CB_2 receptors are expressed; NO-sensitive GC α1, α2, and β1 (and minimally β2) subunits are expressed; and protein kinase G1 (PKG1) but not PKG2 are expressed in the N18TG2 cells all of which are, proteins comprising the NO-sensitive cGMP pathway. Stimulation of the cells with the CB_1 agonist CP55940 or WIN55212-2 for 5 min or 20 min resulted in an increase in cGMP levels, and this could be blocked by the NO-sensitive GC inhibitor, 1H-[1,2,4,]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). Translocation of the GC-β1 subunit from the cytosol to the membrane fraction occurs concomitantly with cGMP production. Within 5 min of exposure to CP55940 or WIN55212-2, the GC-β1 levels in the 100,000 X g membrane pellet were increased significantly. After 1h there was a significant decrease of GC-β1 from the cytosolic fraction, and upon long term cannabinoid drug treatment (48h), the cytosolic GC-β1 was replenished by expression of new mRNA. Translocation of GC-β1 was blocked by the CB_1 antagonist rimonabant and by the Gi/o inhibitor pertussin toxin, suggesting that the CB_1 and Gi/o proteins are required for translocation. The second NO-directed signal transduction pathway, S-nitrosylation, is a post-translational modification that attaches a $–NO^-$ group onto cysteine residues in target proteins. This modification has been shown to play a role in regulation of proteins such as caspases, hemoglobin, ryanodine receptor, and cytoskeletal components. S-nitrosylation of multiple proteins in N18TG2 cells peaked at 1 to 4 h of treatment with WIN55212-2 and (R)-methanandamide, as detected by a biotin switch assay. The results of this study reveal that CB_1 receptors promote the rapid production of cGMP, probably via GC-α1β1, which is the predominate heterodimer expressed in the N18TG2 cells, and this second messenger can act via PKG1 to phosphorylate target proteins. NO-sensitive GC translocation and S-nitrosylation are reversible processes important in the long-term (hours) cannabinoid-mediated signal transduction in neuronal cells. The physiological ramifications of cannabinoid-stimulated NO signal transduction pathways in neuronal cells remains to be defined.

Supported by NIDA grants R01-DA03690, U24-DA12385, K05-DA00182, NIGMS grant and S06-GM008049, and NCMHD grant P20-MD00175.

MECHANISMS OF TETRAHYDROCANNABINOL-INDUCED CYTOTOXICITY IN MOUSE J774-1 MACROPHAGES: INVOLVEMENT OF CB_2 RECEPTOR AND p38 MAPK

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The major psychoactive component of marijuana, tetrahydrocannabinol (THC) is known to exert cytotoxicity in thymocytes[1], splenocytes[1] and lymphocytes[2, 3]. These immune cells express the peripheral CB_2 receptor that is implicated in the cytotoxic process. In addition, THC also exerts the cytotoxicity in macrophages[3], but its precise mechanism is unclear. In this study, we examined the cytotoxicity of Δ^8-THC in mouse macrophage J774-1 cell line, a possible involvement of cannabinoid receptors and their cell signaling pathways. Real-time polymerase chain reaction analysis using primers specific for CB_2 and CB_1 receptors indicated that J774-1 cells mainly expressed CB_2 receptor. Δ^8-THC induced cell death of J774-1 in a concentration-dependent and exposure time-dependent manner, as assessed by the MTT assay. Fifty % of cytotoxic concentration for 1, 3 and 6 hr exposures to Δ^8-THC were > 20, 11.7 and 5.38 μM, respectively. The induction of cell death by Δ^8-THC (8 μM, 6 hr) was prevented by pretreatment of SR144528, a CB_2 receptor-selective antagonist (4 μM, $p < 0.001$), or pertussis toxin, a G_i protein inhibitor (0.8 μg/ml, $p < 0.001$). Furthermore, SB203580, a p38 MAPK inhibitor (12 μM), significantly reduced the cytotoxic effect ($p < 0.001$), whereas SP600125, a JNK inhibitor (5 μM), significantly enhanced the cytotoxicity ($p < 0.001$). These results suggest that the cytotoxicity of Δ^8-THC in J774-1 cells may be induced by the activation of G_i protein-coupled CB_2 receptor, and that p38 MAPK and JNK play some roles in the regulation of the cytotoxicity.

Acknowledgements: Mouse macrophage J774-1 cell line was provided from Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). SR144528 was gifted from Sanofi-Synthelabo Recherche (Martpellier Cedex, France). This work was funded by the Academic Frontier Project for Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2005-2009) and a Grant-in-Aid for Young Scientists from the Ministry, Education, Science, Sports, and Technology of Japan.

1. Mckallip R. J. *et al.*, *J. Pharmacol. Exp. Ther.*, 302, 451-456 (2002).
2. Mckallip R. J. *et al.*, *Blood*, 100, 627-634 (2002).
3. Zhu W. *et al.*, *J. Pharmacol. Exp. Ther.*, 286, 1103-1109 (1998).

POSITIVE ALLOSTERIC MODULATORS OF CB1 RECEPTORS

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The cannabinoid CB1 receptor is well validated as a pain target that can elicit antinociception. It has been reported that anandamide, a well-characterized endocannabinoid, produces CB1-mediated antinociception. Recently, other laboratories have reported the discovery of negative allosteric modulators of CB1 receptors**.** A CB1 positive allosteric modulator (PAM) has not yet been reported, but might be used therapeutically for pain treatment by increasing the activity of endocannabinoids. To identify PAMs on human CB1 receptors, a primary screen assay was developed using [^{35}S]GTPγS binding. We used Factorial Experimental Design to establish optimal assay conditions. We obtained a 4-fold signal to noise ratio, Z' of 0.7, and stabilized the binding of [^{35}S]GTPγS to G proteins, under basal and stimulated conditions, for up to 16 hours. From primary screening of 100,000 compounds, we identified compounds that increased the anandamide potency up to 5-fold. The effects observed were specific for CB1, since those compounds showed no PAM activity on other Gi coupled receptors including CB2. We also confirmed PAM activity on CB1 receptors in rat brain membranes, where positive effects were observed on both potency and maximal effect of anandamide. To characterize our compounds further, equilibrium binding assays and dissociation kinetic studies were performed using [^{3}H]-CP55 940. Compounds didn't displace the [3H]-CP55 940 from CB1 binding sites but decreased the rate at which [3H]-CP55 940 dissociates from these sites. These results support the allosteric nature of our compounds. In conclusion, we have identified for the first time positive allosteric modulators of the CB1 receptor. It remains to be determined whether the CB1 PAM compounds are able to potentiate the activity of anandamide in more physiological systems.

AMINO-TERMINAL PROCESSING OF THE HUMAN CANNABINOID RECEPTOR 1

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The human cannabinoid receptor 1 (CB1) translocates its long amino-terminal domain (N-domain) over the endoplasmic reticulum (ER) membrane in a C-to-N direction after translation. Translocation is inefficient but can be improved by deleting part of the N-domain (Andersson et al., Mol. Pharmacol. 64 (2003) 570-7). When studying membrane assembly of heterologously expressed CB1 using metabolic pulse-chase labeling, it was found that a large fraction of the receptor molecules became processed in the N-domain, thereby generating a novel type of receptor (Nordström and Andersson, J. Recept. Signal. Transduct., 26 (2006) 259-67). The processing took place prior to ER translocation and phosphomimetic amino acid substitutions in the N-domain affected processing, indicating that phosphorylation might regulate formation of the novel receptor. It is speculated that N-domain processing is a way to either upregulate CB1, as the processed receptor assembles more readily than full-length receptor, or to create a receptor with altered signaling properties. Cellular conditions, under which processing is altered, will be discussed.

LOSS OF G-PROTEIN Gγ7 SUBUNIT ALTERS CANNABINOID CB1 AND DOPAMINE D2 RECEPTOR SIGNALING IN BASAL GANGLIA

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Our laboratory and others have shown that cannabinoid CB_1 and dopamine D_2 receptors act on overlapping pools of G-proteins in the striatum, but the specific G-proteins involved in this response have not been determined. CB_1 and D_2 receptors normally couple to $G\alpha_{i/o}$ proteins to inhibit adenylyl cyclase, whereas co-activation of CB_1 and D_2 receptors can switch the coupling of these receptors from $G\alpha_{i/o}$ to $G\alpha_s$. The current study examined the role of Gγ7 in CB_1 and D_2 mediated inhibition of adenylyl cyclase using Gγ7 knockout mice. We examined WIN 55212-2 (CB_1 receptor agonist; WIN) and quinelorane (D2 receptor agonist; QUIN) induced inhibition of adenylyl cyclase activity in caudate-putamen, nucleus accumbens and globus pallidus membranes prepared from Gγ7 wild-type and knockout mice. Membranes were incubated with forskolin and either WIN, QUIN or WIN+QUIN. In wild-type mice, WIN and QUIN inhibited adenylyl cyclase activation by 30% and 10%, respectively. However, loss of Gγ7 in knockout mice resulted in significantly less WIN-induced inhibition (15-20%) of adenylyl cyclase in both caudate-putamen and nucleus accumbens ($p<0.05$). Similarly, QUIN did not inhibit adenylyl cyclase in the Gγ7 knockout mice. A trend for less adenylyl cyclase inhibition in globus pallidus of Gγ7 knockout mice was observed for WIN, but this effect failed to reach significance. Interestingly, in caudate-putamen, nucleus accumbens and globus pallidus from Gγ7 knockout mice, the absolute level of basal and forskolin-stimulated adenylyl cyclase activity was 53-70% ($p<0.05$) lower than wild-type. These data suggest that Gγ7 is involved in both basal and forskolin-stimulated adenylyl cyclase activity, and in CB_1 and D_2 receptor-mediated adenylyl cyclase inhibition in both caudate-putamen and nucleus accumbens. In a separate series of experiments, we examined the role of Gγ3 on CB_1 receptor-mediated G-protein activity in membranes prepared from caudate-putamen and nucleus accumbens from wild-type and Gγ3 knockout mice using WIN-stimulated [^{35}S]GTPγS binding. Interestingly, loss of Gγ3 did not alter CB_1 receptor function in either caudate-putamen or nucleus accumbens. Therefore, Gγ7 is involved in CB_1 and D_2 receptor signaling in caudate-putamen and nucleus accumbens, whereas, Gγ3 does not appear to be involved in CB_1 receptor-mediated activity. These results suggest specificity of G-protein subunits in CB_1 and D_2 receptor signaling. This work was supported by NIH grants DA05274, DA14277, DA 007027, GM39867.

MUTATION OF D2.63 IN THE HUMAN CB_1 CANNABINOID RECEPTOR REVEALS STRUCTURAL REQUIREMENTS FOR SIGNAL TRANSDUCTION

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The cannabinoid receptors belong to the Class A rhodopsin-like superfamily of G-protein coupled receptors (GPCRs). So far, two cannabinoid receptors, CB_1 and CB_2, have been conclusively identified by molecular cloning and pharmacological characterization. Mutational and computational studies indicate the existence of multiple ligand recognition sites at the CB_1 receptor for structurally diverse cannabinoid ligands. These binding sites are contributed predominantly by distinct noncontiguous regions of the hydrophobic transmembrane helixes (TMHs).

Previous studies with GPCRs have identified a highly conserved, negatively charged aspartate at position 2.50 (from TMH 2) to be crucial for ligand binding and/or receptor function. D2.50 (D163 in CB_1 and D80 in CB_2) were demonstrated to be important for G-protein coupling and signal transduction and not ligand binding.

In the present study, we investigated the role of the negatively charged residue D2.63 (D176) in CB_1 receptor function by replacing it with glutamate (D2.63E) or asparagine (D2.63N). D2.63 is unique; whereas it is highly conserved in all species of the CB_1 receptor, an asparagine residue is present at equivalent position in the CB_2 receptor. This conserved residue in CB_1 receptor is located closer to the top of TMH 2 (located upstream to D2.50) towards the extracellular region making it accessible to ligands.

Recombinant hCB_1 receptors stably expressed in HEK 293 cells, were used to investigate the consequences of mutating D2.63 (to D2.63N and D2.63E) in radioligand binding studies and GTPγS functional assays. Our results suggest this aspartate residue at position 2.63 is not obligatory for ligand recognition in CB_1 receptor; however, it plays an important role in modulating agonist-stimulated receptor activation. Not surprisingly, the charge conserved substitution, D2.63E, had no significant effect on the concentration dependence of agonist-induced receptor activation. In conclusion, the presence of a negatively charged residue at the location 2.63, rather than the residue aspartate *per se* is important for modulating the signal transduction process.

Acknowledgements: This study is supported by NIH grants DA09978, DA05274.

REGULATION OF CANNABINOID RECEPTORS DURING DIFFERENTIATION OF NT2 NEURONS AND ASTROCYTES

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Until recently CB2 (cannabinoid receptor 2) was thought to function predominantly in the peripheral immune system. However, it now appears that CB2 is expressed in the brain, in addition to CB1. The precise nature of CB2 expression or even the number of cell phenotypes capable of expression in the human brain has yet to be fully determined. Undoubtedly this will add another dimension to the complexity of the cannabinoid system in the human brain.

We have used the human derived NT2/D1 cell line to investigate the cannabinoid system. These cells have the capacity to differentiate simultaneously into neuronal and astrocytic phenotypes, providing an *in vitro* model for studying neuron-astrocyte interactions and changes in gene expression during differentiation. Neuron and astrocyte generation is achieved by culturing NT2/D1 precursors for several weeks with retinoic acid (RA) followed by incubation with various mitotic inhibitors. The temporal expression of both cannabinoid receptors was investigated using a combination of semi-quantitative PCR and immunological methods to track gene expression and receptor protein levels, respectively, as the cells differentiate.

NT2/D1 precursor cells express relatively low levels of both CB1 and CB2 mRNA. Interestingly, CB1 mRNA levels were up-regulated significantly through the differentiation process. This increase in CB1 mRNA levels commenced within one day of RA differentiation and reached its maximum by day six, with a high level of expression maintained in both fully differentiated cell types. In contrast, CB2 mRNA was essentially absent from neuronal cells and abundant in astrocytic cells. CB2 receptor protein, as detected by western blot and immunocytochemistry, confirmed the mRNA representation, thus demonstrating cell-type specific expression of CB2.

This data corresponds well with the proposed distribution of the receptors in *in vivo* published data. Correspondingly, the NT2/D1 cell line provides both a convenient and relevant model in which to investigate many aspects of the CNS cannabinoid system, an area that is rapidly growing in interest with respect to the role of the CB2 receptor.

MULTIPLE TRAFFICKING PATHWAYS INVOLEVD IN CB_1 RECEPTOR AXONAL TARGETING

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The axonal polarization of the cannabinoid receptor subtype-1 (CB_1) is essential for its function in the presynaptic modulation of neurotransmitter release. In cultured hippocampal neurons the targeting of cell-surface CB_1 receptors to the axon is achieved by domain-specific, endocytic removal of receptors from the dendritic plasma membrane (Leterrier et al., 2006; McDonald et al., 2006). However, little is known about the trafficking pathways and molecular mechanisms involved in directing CB_1 receptors to the axon itself. In this study we have investigated further the nature of the trafficking pathways involved in this process.

A chase protocol utilising treatment with the protein synthesis inhibitor anisomycin, and recombinant expression of a GFP tagged CB_1 receptor chimera (McDonald et al. 2006), shows that the CB_1 receptor is transiently expressed in the somatodendritic compartment. Furthermore, a novel fluorescent CB_1 receptor fusion protein incorporating DsRED-E5 (TIMER-CB_1), the spectra of which changes over time as the fluorescent moiety matures, and a brefeldin-A pulse/ chase protocol, utilizing GFP-CB_1, shows that the CB_1 receptor traffics directly to the axon, as well as to the somatodendritic plasma membrane. Subsequent to endocytic removal from the somatodendritic cell-surface studies with the lysosomal marker lysotracker suggests that the CB_1 receptor is not targeted for degradation in lysosomes, consistent with the existence of a parallel, transcytotic pathway delivering the CB_1 receptor to the axon.

In conclusion, the CB_1 receptor is delivered to both to the axonal cell-surface and the dendritic plasma membrane where it is transiently expressed. Subsequently, somatodendritic CB_1 receptors are not targeted for degradation and this is likely to represent an additional, transcytotic trafficking pathway mediating delivery of the receptors to the axon.

This work was supported by The Anonymous Trust (Dundee), Tenovus (Scotland), and the University of Dundee.

LETERRIER C, LAINE J, DARMON M, BOUDIN H, ROSSIER J, LENKEI Z. (2006) Constitutive activation drives compartment-selective endocytosis and axonal targeting of type 1 cannabinoid receptors. *J. Neurosci.* 26**:** 3141-53.

M^{C}DONALD N.A., HENSTRIDGE C.M., CONNOLLY C.N. & IRVING A.J. (2006) An essential role for constitutive endocytosis, but not activity, in the axonal targeting of the CB_1 cannabinoid receptor. *Mol. Pharm.* Epub ahead of print.

TYPE-1 CANNABINOID RECEPTORS COLOCALIZE WITH CAVEOLIN-1 IN NEURONAL CELLS

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Introduction: Type-1 (CB1) and type-2 (CB2) cannabinoid receptors belong to the rhodopsin family of G protein-coupled receptors (GPCR), and are activated by endogenous lipids termed "endocannabinoids". Recent reports have demonstrated that CB1R functions in the context of lipid rafts, plasma membrane microdomains which may be important in limiting signal transduction. Instead CB2R and other receptors and metabolic enzymes of endocannabinoids are not localized within lipid rafts. Here we investigated whether CB1R could reside within special lipid rafts termed "caveolae".

Materials and Methods: Fractions enriched in caveolae were prepared by carbonate extraction followed by gradient centrifugation adapted to C6 cells. Cell homogenates were immunoprecipitated with rabbit anti-caveolin-1 (CAV1) IgG1, followed by SDS-PAGE and immunoblotting with rabbit anti-CAV1, anti-CB1R, anti-TRPV1 or anti-FAAH specific antibodies. In addition, C6 cells were settled on a glass coverslip at a density of $2x10^4$ cells/cm^2. After 24 h cells were fixed for 10 min with 4% paraformaldeyde and processed for immunofluorescence, acquiring data through a C1 confocal microscope (Nikon Instruments S.p.A., Florence, Italy).

Results: We show that in C6 cells CB1R localized in the same subfractions as CAV-1, a typical marker of caveolae. These two proteins also co-immunoprecipitated, and confocal microscopy showed that CB1R and caveolin-1 co-localized on the cell surface. Unlike CB1R, TRPV1 or FAAH did not co-immunoprecipitate nor co-localize with CAV1, suggesting that this protein, widely considered as a "general brake" of GPCR signaling, could act as a specific modulator of CB1R.

Conclusion: Taken together, these findings may be relevant for the manifold CB1R-dependent activities of endocannabinoids, like the regulation of apoptosis and that of neurodegenerative diseases.

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CANNABINOID RECEPTOR INTERACTING PROTEIN ($CRIP_{1A}$); EFFECTS ON CB_1 EXPRESSION AND SIGNALLING

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A recently discovered protein, Cannabinoid Receptor Interacting Protein ($CRIP_{1A}$) interacts with the C-terminal tail of the CB_1 receptor (aa 418-473). Initial studies found that although $CRIP_{1A}$ did not affect full agonist-mediated activity, it decreased the effects of the inverse agonist SR1 on Ca^{++} currents in superior cervical ganglion neuronal expression system (Liu,Y et. al., Society for Neuroscience Abstract (2004)). In the present study, we have investigated the influence of $CRIP_{1A}$ co-transfection on CB_1 receptor levels using [^{3}H]SR-141716A saturation binding assays. The effects of heterologous transfection of CB_1 and $CRIP_{1A}$ were examined in CHO cells, which were transiently transfected with CB_1 and $CRIP_{1A}$ or empty vector. Transient $CRIP_{1A}$ transfection had no effect on CB_1 expression levels. (B_{MAX} (pmol/mg): empty vector: 1.82 ± 0.34, $CRIP_{1A}$: 1.85 ± 0.35). Immunoblot analysis was used to verify the presence of $CRIP_{1A}$ in cell membrane preparations of CHO cells with transient transfection of $CRIP_{1A}$. Stable co-transfection of $CRIP_{1A}$ had no effect on CB_1 expression levels in HEK cells stably expressing CB_1 receptor (hCB_1-HEK) or CB_1 receptor and $CRIP_{1A}$ (hCB_1-HEK $CRIP_{1A}$) (B_{MAX} (pmol/mg); hCB_1-HEK: 1.87 ± 0.26, hCB_1-HEK $CRIP_{1A}$: 2.01 ± 0.29). Studies relating cell confluency levels to CB_1 receptor expression in cell membrane preparations were performed in hCB_1-HEK cells with and without stable co-expression of $CRIP_{1A}$. $CRIP_{1A}$ did not affect CB_1 expression (approx 1.5 pmol/mg at regular (100%) and high (150%) confluency), although low confluency levels (50%) lowered CB_1 expression for both cell types equally. Studies are underway to examine the effect of $CRIP_{1A}$ on the efficacy of various full, partial and inverse agonists using [^{35}S]GTPγS binding assays. Preliminary data suggests minor changes in the relative efficacies of partial and inverse agonists due to $CRIP_{1A}$. $CRIP_{1A}$ effects on agonist-induced desensitization and receptor down-regulation will be examined by incubating hCB_1-HEK cells with and without $CRIP_{1A}$ transfection with various agonists (WIN, CP, THC and vehicle) using [^{35}S]GTPγS and [^{3}H]SR-141716A binding assays. $CRIP_{1A}$'s effects on downstream effectors, including adenyly cyclase and MAP kinase will also be examined.

Acknowledgements: Funded by National Institute of Drug Abuse (DA05274 & DA14277)

HETEROLOGOUS DESENSITIZATION OF THE CXCR4 CHEMOKINE RECEPTOR BY THE CB2 CANNABINOID RECEPTOR IN HL-60 CELLS

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Chemokines and their receptors play important roles in leukocyte trafficking as well as in cancer cell metastasis. One of the major chemokine axes is the CXCR4 receptor and its ligand SDF-1α. Both SDF-1αand endocannabinoid 2-arachidonylglycerol (2-AG) are known to induce cell migration. In this study, we tested the hypothesis that there may be a heterologous desensitization of CXCR4 by CB2 receptor in human promyeloid leukemia HL-60 cells.

Treatment with SDF-1α as well as 2-AG induced the migration of HL-60 cells in a dose-dependent manner. The effect of SDF-1α was observed at 10 ng/ml and reached a plateau at 200 ng/ml. The effect of 2-AG was observed at 3×10^{-8} M with a maximum effect at 10^{-6} M. Pretreatment of HL-60 cells with 2-AG (10^{-6} M) significantly inhibited chemotaxis induced by SDF-1α. Since both CXCR4 and CB2 receptors are expressed in HL-60 cells, our migration data demonstrated that there is a heterologous desensitization of CXCR4 receptor by the activation of CB2. One of the possible mechanisms of heterologous desensitization is through the formation of heterodimers. To test this hypothesis, co-immunoprecipitation assays were performed. Our data showed that an anti-CXCR4 antibody was able to immunoprecipitate CB2 as well as CXCR4 proteins. These results indicated the formation of heterodimers between CXCR4 and CB2 receptors in the HL-60 cells. To verify this conclusion, we performed fluorescence resonance energy transfer (FRET) assays in HEK293 cells transfected with a CXCR4-CFP plasmid and a CB2-YFP plasmid. In these experiments, strong FRET signals were observed in CXRC4-CFP and CB2-YFP expressing cells. These results confirmed that heterodimers were indeed formed between the CB2 and CXCR4 receptors.

In conclusion, our data demonstrate that in HL-60 cells there is a heterologous CXCR4 receptor by the CB2 cannabinoid receptor. Furthermore, heterdimerization occurs between these two receptors, which may play an important part in the heterologous desensitization process.

Acknowledgements: Funded by NIH grants EY13632 and DA11551

BINDING MODE PREDICTION OF CONFORMATIONALLY RIGID ANANDAMIDE ANALOGS WITHIN THE CB_1 RECEPTOR

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The conformationally restricted anandamide analogs with comparable pharmacological responses to that of anandamide (Berglund et al., Drug Des. Discov. (2000)) were docked to the published homology model of the CB_1 receptor (Shim et al., Biopolymers (2003)) after refinement to improve the quality of the extracellular loops, especially the E2 loop, which appears to be critical for ligand binding (Shire et al., J. Biol. Chem. (1996)). The docking of these analogs is of particular interest in light of assessing the binding conformation of conformationally flexible anandamide, an endogenous ligand for the cannabinoid receptor, which would otherwise be computationally challenging in characterizing its binding conformation.

To determine these conformationally simplified anandamide compounds, we explored a binding region around lysine 192, based on the observation that this residue is a key binding residue for cannabinoids as well as anandamide (Song and Bonner, Mol. Pharmacol. (1996); Chin et al., 1998 J. Neurochem. (1998)), and also considered the occupation by a hydrophobic moiety of the compounds as a prerequisite for identifying candidate docking structures in comparison with the docking structure of CP55244 (Shim et al., Biopolymers (2003)), assuming that a hydrophobic moiety would dominate the CB_1 receptor binding interaction. From the structures fitting these criteria limiting the docking space, the best was assessed using two popular docking paradigms, Affinity (Accelrys, Inc.) and Glide (Schrödinger, Inc.). After the binding free energy (ΔG_{bind}) was estimated by employing the linear interaction energy (LIE) method (Åqvist and Marelius, Comb. Chem. High Throughput Screen. (2001)), it was compared to the experimental ΔG_{bind} to obtain the LIE equation: $\Delta G_{bind} = 0.27\langle\Delta U_{vdw}\rangle + 0.03\langle\Delta U_{elec}\rangle + 1.53\Delta SASA$. A good correlation with an RMSD of 0.3 kcal/mol suggested that the identified binding modes was valid to guide the plausible binding mode(s) of anandamide.

Acknowledgements: Supported by U24DA012385-06S1, R01-DA03690, and K05-DA00182

CB1 RECEPTORS ARE SITUATED TO DIRECTLY AND INDIRECTLY CONTROL SEROTONIN RELEASE

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The endocannabinoid system (ECS) possesses neuromodulatory functions by influencing the release of various neurotransmitters, including γ-aminobutyric acid (GABA), noradrenaline, dopamine, glutamate and acetylcholine. Even though there are studies indicating similar interactions between the ECS and the serotonergic system, these studies did not show whether CB1 is directly situated at the serotonergic synapse or whether CB1 controls the firing of excitatory and/or inhibitory neurons which themselves innervate serotonergic neurons. Here we show by *in situ* hybridization that a significant fraction of serotonergic neurons in the raphe nuclei contains CB1 mRNA as illustrated by the coexpression with the serotonergic marker gene tryptophane hydroxylase 2. Furthermore, by double immunohistochemistry and confocal microscopy, demonstrate CB1 protein on serotonergic fibers and synapses expressing the serotonin reuptake transporter 5HTT. Our findings indicate that the CB1-mediated regulation of serotonin release is possible and can depend on a direct cross-talk between the two systems at single cell level. Our findings also suggest indirect ways of interaction such as the control of inhibitory and excitatory input on serotonergic neurons via CB1 activation like CB1 regulated GABAergic interneurons in the raphe and/or glutamatergic projecting neurons of cortical and subcortical regions. Together, those control possibilities of CB1 on serotonin release might lead to functional implications in the modulation of emotional states.

PKC EPSILON MODULATES ENDOCANNABINOID SIGNALING

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Endocannabinoids and Δ9-tetrahydrocannabinol, the primary psychoactive compound in marijuana, bind and activate the cannabinoid CB1 receptor. The CB1 receptor is one of the most abundant G-protein coupled receptors in brain, whose activation leads to decreased neurotransmitter release in several brain regions. Current drug abuse research shows that not only does the CB1 receptor provide a locus for marijuana's psychoactive and addictive properties, but CB1 receptor activation or inhibition strongly influences drug seeking, reward and relapse associated with other commonly abused drugs. Despite the receptor's abundance and influence in the brain, little is known about mechanisms that modulate CB1 receptor signaling.

We have previously shown that protein kinase C epsilon (PKCε), a member of the novel subclass of PKC isozymes, mediates acute behavioral responses to both endogenous and exogenous cannabinoids. Here we show using the CB1 specific antagonist SR141716A, that this enhanced cannabinoid sensitivity is mediated via the CB1 receptor. Additionally, total binding sites for [3H] WIN55,212-2 are unchanged in the brains of PKCε null mice but receptor affinity for the ligand is increased compared to wild-type littermates. In whole brain membranes from the wild type mice, the CB1 receptor and PKCε co-immunoprecipitate, suggesting that PKCε may directly associate with CB1. We further show that anandamide levels are reduced in several brain areas of PKCε null mice compared to wild-type.

Our results indicate that PKCε is a novel regulator of endocannabinoid signaling. PKCε may therefore provide a therapeutic target with which to manipulate cannabinoid signaling, altering a variety of neuronal processes, including those involved in addiction to marijuana and other drugs of abuse.

ANANDAMIDE (AEA) IS INVOLVED IN DECIDUAL NITRIC OXIDE (NO) PRODUCTION INDUCED BY LIPOPOLYSACCHARIDE (LPS) ON EARLY MURINE PREGNANCY

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Nitric oxide (NO) fulfils important functions during pregnancy and has a role in implantation, decidualization, vasodilatation and myometrial relaxation. However, at high concentrations, such as those that are produced in sepsis, NO has toxic effects as it is a free radical.

Our previous results indicate that LPS, an integral part of the outer membrane of Gram negative bacteria, is capable of producing embryonic resorption in mice due to NO increased production not only in uterus but also in decidua. Recent research has revealed that LPS induces AEA synthesis in murine macrophages.

The aim of the present work was to determine the effect of LPS and AEA on decidual NO-derived reactive species production induced by LPS on early murine pregnancy. On day 7 of pregnancy female mice were sacrificed by cervical dislocation and uterus and decidua were separated in each implantation site. Decidua was then incubated for 12 h in the presence of a) LPS (1 ug/ml), b) AEA (10^{-8}M), c) LPS + AM251 (10^{-9}M) (cannabinoid type 1 (CB1) receptor antagonist) and d) LPS + SR144528 (10^{-8}M) (cannabinoid type 2 (CB2) receptor antagonist) and NO was measured in culture supernatants as NO_3^- plus NO_2^-. Both LPS and AEA were capable of increasing NO levels and NO production induced by LPS was inhibited when decidua was incubated in the presence of AM251 as well as in the presence of SR144528. We also determine protein nitration by western blot in decidua incubated for 12 h in the presence/absence of LPS. We observed an increase in tyrosine nitration in a time dependent manner.

We determined the activity of the fatty acid amide hydrolase (FAAH–the enzyme responsible for hydrolysis of AEA- (conversion of [^{3}H]-anandamide in [^{14}C]-arachidonic acid and ethanolamine)) in the presence/absence of LPS. Treatment with the endotoxin increased FAAH activity.

These results and the inhibition of LPS-induced augmentation of NO synthesis by both cannabinoid receptor antagonists suggest that AEA could be an intermediate in LPS effect.

A MIX OF ADAPTIVE EVOLUTION AND PURIFYING SELECTION IN GENES THAT EXPRESS THE ENDOCANNABINOID SYSTEM

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Introduction: Some genes and some species evolve faster or slower than others. Genetic tempo within the human lineage may offer clues regarding the evolution of human-specific traits as well as inherited mendelian diseases. The best-known endocannabinoid ligands, anandamide and 2-AG, signal at least seven receptors and involve ten metabolic enzymes. The genes expressing endocannabinoid system proteins were examined for heterogeneities in genetic tempo (number of nucleotide substitutions per unit time, also known as the Relative Rate of Evolution, RRE) and selection pressure (the ratio of nonsynonymous substitutions to synonymous substitutions scaled to that expected under neutral divergence, designated Ka/Ks). A gene with Ka/Ks < 0.25 is undergoing strong purifying selection, whereas a gene with Ka/Ks > 1.0 may be undergoing "positive Darwinian selection" (adaptive evolution), where amino acid replacements are advantageous and nonsynonymous substitutions become stabilized at a high rate.

Methods: We used BLAST to identify orthologs of eighteen endocannabinoid genes in six organisms with sequenced genomes. Ortholog nucleotide sequences were aligned with ClustalX and MacClade. Sequence alignments were utilized to compute RRE and Ka/Ks values, using three web-accessible bioinformatics platforms: SNAP (based upon a parsimony-based NG86 model), LiKaKs (distance-based LWL85 model), and FUGE (maximum likelihood tree-based method).

Results: Pair-wise comparisons of orthologs in rodents (rat and mouse) and primates (human and macaque), determined that the mean RRE of 18 genes in rodents was 2.7-fold greater than in primates, a significant difference ($p < 0.0001$). In contrast, the mean Ka/Ks was slightly higher in primates (0.13) than rodents (0.10). Human lineage-specific Ka/Ks calculations for individual genes ranged from 1.11 to 0.00, in rank order from highest to lowest: PTPN22, NAAA, TRPV1, TRPA1, NAPE-PLD, MAGL, PPARγ, FAAH1, COX2, FAAH2, ABDH4, CB2, GPR55, DAGLβ, PPARα, TRPV4, CB1, DAGLα; differences were significant ($p < 0.0001$). Compared to the human lineage, rat and mouse presented different rank orders (for example GPR55 generated the greatest Ka/Ks ratio). Within the human lineage, the mean Ka/Ks of AEA-associated genes was three times greater than the mean Ka/Ks of 2-AG-associated genes (0.314 and 0.105, respectively, $p = 0.11$, not bad for $n = 18$). This trend was not seen in the rat or mouse.

Conclusion: The endocannabinoid system is collectively under strong purifying selection, although some genes show evidence of adaptive evolution.

IMMUNOCHEMICAL STUDIES REVEAL PREDOMINANT EXPRESSION OF LYSOSOMAL *N*-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE IN MACROPHAGES

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Bioactive *N*-acylethanolamines, including the endocannabinoid anandamide and anti-inflammatory *N*-palmitoylethanolamine, are hydrolyzed to fatty acids and ethanolamine by fatty acid amide hydrolase (FAAH). In addition, we cloned cDNA of another amidohydrolase termed "*N*-acylethanolamine-hydrolyzing acid amidase (NAAA)", which catalyzed the same reaction only at acidic pH. When examined by enzyme assay and RT-PCR, NAAA was expressed in various organs and macrophage cells of rats and mice. In the present study we developed an antibody specific for NAAA in a rabbit, and performed immunochemical studies on NAAA for the first time. The specificity of the prepared anti-NAAA antibody was confirmed by Western blotting for homogenates of HEK293 cells overexpressing recombinant NAAA. We first focused on isolated alveolar macrophages and whole lung tissue of rat, both of which were reported to express high levels of NAAA. Western blotting revealed that native NAAA of alveolar macrophages and that of the lung tissues are glycosylated and proteolytically cleaved as was observed with recombinant NAAA. Immunocytochemical observation with isolated alveolar macrophages, which were allowed to phagocytose latex beads, exhibited the presence of NAAA in lysosomes. In whole lung tissue, NAAA-positive cells were mostly alveolar macrophages. In addition, we compared expression levels of NAAA between alveolar macrophages and the lung tissue. When assayed with *N*-palmitoylethanolamine as substrate at pH 5 in the presence of the FAAH inhibitor URB597, the NAAA activity in homogenates of alveolar macrophages (5.83 nmol/min/mg of protein) was 49-fold higher than that of homogenates of lung tissue (0.12 nmol/min/mg of protein). Moreover, Western blotting and RT-PCR clarified that expression levels of NAAA protein and mRNA in alveolar macrophages were much higher than those in the lung tissue. Taken together, NAAA detected in rat lung was suggested to be principally derived from alveolar macrophages. Furthermore, rat peritoneal macrophages also revealed potent expression of NAAA. Interestingly, NAAA-positive cells in rat brain were intraventricular macrophages, while microglia appeared to be negative. Although we can not rule out a possibility that NAAA is expressed at lower levels in other types of cells, our results reveal that macrophages are major NAAA-expressing cells in different tissues of rat.

PRELIMINARY STUDIES ON THE PHARMACOLOGY AND REGULATION OF THE PUTATIVE CANNABINOID RECEPTOR GPR55

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Although the cellular actions of cannabinoids are thought to be primarily mediated by the CB_1 and CB_2 subtypes of cannabinoid receptor, an increasing number of functional studies have identified novel, non CB_1/CB_2 actions of cannabinoid ligands, which point to the involvement of additional receptors. Recently it has been suggested that several cannabinoids bind to the orphan G-protein coupled receptor GPR55. In this study we have investigated the ability of cannabinoid ligands to activate GPR55 using a [^{35}S]GTPγS binding assay and agonist-induced trafficking of tagged GPR55 fusion proteins.

Following recombinant expression of GPR55 in cell lines (HEK293 and COS7), a number of putative GPR55 ligands modulated basal [^{35}S]GTPγS binding at concentrations of 100nM and 1μM; these included CP55940, HU210, O-1602, anandamide and virodhamine. In line with this, we find that in mouse brain membranes derived from $CB_1^{-/-}$ mice, virodhamine retained the ability to modulate [^{35}S]GTPγS binding. Furthermore, treatment of GPR55 expressing recombinant cell lines with certain cannabinoid ligands resulted in a rapid redistribution of HA and GFP fusions into endosomes, which is indicative of agonist-induced endocytosis.

Thus, although the pharmacology of GPR55 is consistent with it belonging to the cannabinoid receptor family it is distinct from that of the classical CB_1 or CB_2 receptors.

Supported by Organon Laboratories Ltd., MRC and TENOVUS Scotland.

FATTY ACID AMIDE HYDROLASE (FAAH) INHIBITOR URB597 ACTIVATES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA

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The fatty acid ethanolamine (FAE) family includes endocannabinoids, such as anandamide, as well as endocannabinoid-like molecules, such as *N*-palmitoyl-ethanolamine (PEA) and *N*-oleoylethanolamine (OEA). Members of the FAE family also show agonist activity at transmitter-gated channels (TRPV1), as well as nuclear receptors. In particular, the peroxisome poliferator-activated receptors (PPAR), which are exploited clinically for treatment of dyslipidemia and diabetes, are targets for FAEs. Given that FAEs appear to be hydrolysed principally through the action of the enzyme fatty acid amide hydrolase, inhibition of FAAH should lead to accumulation of a variety of FAEs. We were, therefore, prompted to investigate whether the selective FAAH inhibitor URB597 could influence PPAR activity in intact cell systems.

Our initial investigations indicated that HeLa cells exhibited no significant FAAH expression, while SH-SY5Y cells were FAAH-positive. A PPRE-linked luciferase construct (Peroxisome Proliferator Response Element) was transiently transfected into these cells either alone or in combination with PPARα or PPARγ.

In HeLa cells expressing PPRE-luciferase alone, gene transcription was unaltered in the presence of URB597 (10 μM), WY14643 (10 μM, PPARα agonist) or rosiglitazone (10 μM, PPARγ agonist), indicating an absence of significant functional endogenous PPARα or PPARγ expression in these cells. In contrast, SH-SY5Y cells transfected with PPRE-luciferase alone showed significant elevations of luciferase activity in the presence of URB597 (1.66 ± 0.07 RLU/mg protein) and rosiglitazone (3.39 ± 0.31) compared to control (0.90 ± 0.31), while WY14643 was ineffective. These data suggest endogenous expression of functional PPARγ, but not PPARα, in SH-SY5Y cells, and that URB597 is able to elevate endocannabinoid levels in these cells sufficiently to activate PPARγ.

In HeLa cells co-transfected with PPRE-luciferase and either PPARα or PPARγ, URB597 failed to alter reporter gene activity in cells co-transfected with PPARα and PPRE-luc, while PPARγ/PPRE-luciferase co-transfectants responded to both rosiglitazone (11.70 ± 1.06) and URB597 (9.55 ± 0.48) compared to basal (5.21 ± 0.37). These data suggest that URB597 activates PPARγ, but not PPARα, in cells lacking FAAH. Further investigation is needed to ascertain whether URB597 directly activates PPARγ or a further indirect mechanism is involved.

ENDOCANNABINOID SIGNALING MODULATES DENDRITIC SPINE DENSITY IN PREFRONTAL CORTICAL PYRAMIDAL CELLS

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Disturbances in prefrontal cortical function are thought to subserve the cognitive deficits and negative symptoms of schizophrenia. Dendritic spine density is reduced in the prefrontal cortex (PFC) of patients with schizophrenia, an effect likely related to decreased PFC dopamine signaling. Alterations in endocannabinoid signaling have also been demonstrated in schizophrenia. Among such changes are elevated CSF levels of anandamide, and the observation that anandamide levels are inversely related to negative symptom severity in first episode, medication-naïve schizophrenic patients. Given these data, we tested the hypothesis that elevated brain anandamide levels are associated with alterations in neuronal morphology in the prefrontal cortex using FAAH null mutant ($^{-/-}$) mice as a "hyper-anandamidergic" model.

A Golgi-Cox method was used to label layer V pyramidal cells in the prelimbic cortex of wild-type and FAAH$^{-/-}$ mice.

FAAH$^{-/-}$ mice exhibited a significant increase in overall basilar dendritic spine density, but no significant changes in total basilar dendritic length or soma size. Spine density was significantly increased in the distal dendritic tree of pyramidal cells (>140 microns from the soma). There was also a slight but significant increase in branching of proximal dendrites in FAAH$^{-/-}$, as measured by a small increase in the number of ring intersections.

These data suggest that the increased anandamide levels reported in schizophrenic patients could reduce negative symptoms or modify cognitive function by increasing or stabilizing distal dendritic spines of layer V PFC pyramidal neurons, thus increasing or maintaining the functional integrity of prefrontal cortical circuits in the face of decreased dopamine signaling.

These studies were supported by NIH Grants PO1 NS044282, RO1 MH077298, and the National Parkinson Foundation (AYD), and R01 DA016967 (CJH). We sincerely thank Dr. BF Cravatt for donation of FAAH$^{-/-}$ breeding pairs to CJH.

PHARMACOLOGICAL EVALUATION OF ENDOCANNABINOID SYNTHESIS AND RELEASE IN RAT BRAIN SLICES

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Endocannabinoid (EC) levels are governed by the balance between synthesis, metabolism and trans-membrane transport. It is important to understand the factors regulating intracellular and extracellular EC concentrations and we have, therefore, examined the effects of different excitatory stimuli, of URB597 and MAFP (methoxy fluorophosphonate; inhibitors of fatty acid amide hydrolase (FAAH)), and VDM11 [a potent and selective inhibitor of the anandamide membrane transporter (AMT)] on intra- and extracellular ECs in a brain slice preparation.

Mini-prisms (350×350μm) were prepared from the cerebral cortices of male Lister Hooded rats (>250g). Following a 60 min incubation in Krebs Henseleit buffer (KHB, pH 7, 37^{o}C) 50μl aliquots of slices were transferred to 5ml insert vials containing KHB with 1% BSA , final volume 500 μl, and exposed to drugs for 20 min, after which the slices were separated from the medium by rapid filtration under vacuum. Medium and slices were rapidly frozen in liquid nitrogen and stored at -80^{o}C prior to assay. Following organic solvent and solid phase extraction, EC levels were measured by liquid chromatography-tandem mass spectrometry (Richardson *et al.*(2007) AnalBiochem, 360(2); 216-26. Statistical analysis (Student's t-test) compared basal levels with those following drug exposure.

Depolarizing levels of KCl (50 mM) enhanced anandamide (AEA) and oleoylethanolamide (OEA) levels in the tissue by 2.7±0.2 fold (n=4, P =0.001; mean ± s.e.m.) and 2.0 ±0.2 fold (n=3, P=0.0079) respectively whilst no changes were observed for 2-arachidonoyl glycerol (2-AG) or palmitoylethanolamide (PEA). Stimulation of excitatory amino acid receptors by glutamate (10 mM) enhanced AEA synthesis non-significantly by 1.9±0.6 fold (n=3, P=0.2309) with no effect on other ECs. The cholinergic receptor agonist carbachol (1mM) and the TRPV1 agonist capsaicin (1μM) had no effects on AEA, 2-AG, OEA or PEA. Blockade of FAAH by URB597 (1μM) significantly increased levels in the slices of AEA (3.2±0.3 fold, n=33 P<0.0001), OEA by 2.0±0.2 fold (n=30, P=0.0001) and PEA by 1.5±0.1 fold (n=25, P=0.0227) but had no effect on 2-AG. The increased EC levels due to URB597 were not altered in the presence of the CB_1 receptor antagonist (SR141617A, 1μM), the TRPV1 antagonist, capsazepine (1μM) or VDM11 (1,5 and 10 μM).

MAFP(10 μM) significantly increased AEA and OEA levels in the slices by 2.7±0.5 fold (n=6, P=0.0274) and 2.3±0.4 (n=6, P=0.0162) respectively. It had no significant effects on PEA or 2-AG. VDM11 did not affect EC tissue levels.

Basal EC levels detected in the medium were; AEA (0.5±0.1), 2-AG (2.9±0.8), OEA (39.2±8.3) and PEA (243.1±37.4) pmol/ml. These were enhanced by URB597; AEA by 4.3±0.6 fold (n=5, P=0.0190), OEA 1.9±0.2 fold (n=5, P=0.0087) but PEA 1.3±0.07 fold (n=5, P=0.39) and 2-AG 1.3±0.2 fold (n=5, P=0.4801) non-significantly. The effects of the other drugs on extracellular ECs mirrored tissue changes with the exception that no increased release was observed with K^{+} (50 mM) while VDM11 (in a dose dependent manner) increased the release of AEA, OEA and PEA by 1.5 fold, with no effect on release of 2-AG. When combined with URB597, VDM11 potentiated 2-AG release (4.1 fold compared to basal).

The data presented indicate differential effects of excitatory stimuli on EC levels in brain slices and provide no evidence for tonic regulation of synthesis by CB_1 or TRPV1 receptors. The effects of URB597 suggest a high turnover rate of AEA, PEA and OEA in this preparation and that 2-AG is not subject to catabolism by FAAH. The release of ECs from cortical slices appears, in the main, to be passively driven by the trans-membrane concentration gradient. The cortical slice preparation provides, therefore, a suitable *in vitro* model for the investigation of EC synthesis, metabolism and release in the brain.

TOWARDS CLONING OF FAAH GENE IN *TETRAHYMENA*

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Fatty acid amide hydrolase (FAAH) is a key enzyme responsible for the regulation of the neuromodulatory fatty acid amides including anandamide. The aim of the present study is to clone and further characterize FAAH from *Tetrahymena*. As it has been shown by our group FAAH is present in *Tetrahymena pyriformis*, indicating the importance of this enzyme throughout the evolution; FAAH was identified as a 66 kDa protein using anti-FAAH polyclonal antibodies. Furthermore a secreted FAAH-like activity was detected. *Tetrahymena pyriformis* genome is not available. Nevertheless, the relative species *Tetrahymena hermophila's* macronuclear genome sequence project is recently completed and a variety of tools for analysing this genome are available at Tetrahymena Genome Database (TGD) and The Institute of Genomic Research (TIGR) webpages (www.ciliate.org, www.tigr.org). *In silico* studies identified sequences with similarity to human FAAH in *T. thermophila* genome. In the present study we showed that FAAH activity is also present in *T. thermophila* and the enzyme was partially characterized. Assuming that *T. pyriformis* genome should share high similarities with *T. thermophila's* genome we performed *in silico* scanning for FAAH like homologue protein sequences using BLASTP (of TGD and TIGR). The well characterised mus musculus FAAH was used as a probe and the resulted *T. thermophila* sequences were also used for *T. pyriformis*. Based on the obtained results a number of primers, complementary to regions considered to present higher conservation, were designed. Using different sets of primers, defining overlapping regions, RT-PCR was performed on total RNA isolated from *T. pyriformis* and *T. thermophila* grown in conditions enriching FAAH activity as measured using [^{3}H]anandamide. Small regions that represent FAAH homologue sequences were identified.

COMPARTMENTALIZATION OF ENDOGENOUS 2-AG INTO LIPID RAFTS IN A DORSAL ROOT GANGLION CELL LINE

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2-Arachidonoyl glycerol (2-AG) is an endogenous cannabinoid that binds CB1 and CB2 receptors and may affect synaptic transmission in a retrograde fashion. Increases in 2-AG by inhibition of monoacylglycerol lipase in the periaqueductal gray or lumbar dorsal horn are linked to a CB1-mediated stress-induced analgesia (Hohmann et al, 2005). In addition, peripheral 2-AG application induces CB2-mediated antinociception in the formalin test (Guindon et al, 2006). 2-AG appears to be derived from three putative precursors (reviewed by Sugiura et al, 2006): 1) diacylglycerol (via diacylglycerol lipase); 2) lysophosphatidylinositol (via a phospholipase C); and, 3) 2-arachidonoyl lysophosphatidic acid (via a phosphatase).

To investigate the cellular dynamics of 2-AG biosynthesis we studied the partitioning of endogenous 2-AG, diacylglycerols (1-stearoyl-2-arachidonoyl-*sn*-glycerol), diacylglycerol lipase α, phosphatidic acid and CB2 receptors into lipid raft fractions isolated from F-11 cells. F-11 cells are a fusion of rat embryonic (day 13-14) dorsal root ganglia and mouse N18TG2 neuroblastoma cells and express both CB1 and CB2 cannabinoid receptors (Platika et al., 1985; Ross et al, 2001). *Methods:* F-11 cells were homogenized and fractionated using a detergent-free OptiPrep density gradient. All lipids were partially purified from methanolic extracts of the fractions on solid phase disc cartridges and quantified using liquid chromatography tandem mass spectrometry (LC/MS/MS). Protein localization was determined by western blotting.

Results: Under basal conditions, the endogenous cannabinoid 2-AG, but not *N*-palmitoyl ethanolamine or *N*-arachidonoyl glycine, was localized to lipid rafts. The CB1 cannabinoid receptor, if present, occurred at levels below our detection limit, but CB2 receptors were expressed in high levels in F-11 cells localized to non-lipid raft fractions. In contrast, the 2-AG precursor 1-stearoyl-2-arachidonoyl-*sn*-glycerol as well as the enzyme diacylglycerol lipase α (DAGLα), which cleaves diacylglycerol to form 2-AG, and 2-AG itself were co-localized with the lipid raft markers caveolin-1, flotillin-1, cholesterol, sphingomyelin and the glycolipid ganglioside GM3.

Conclusion: The biochemical machinery for the production of 2-AG via the putative diacylglycerol pathway is localized within lipid rafts, suggesting that 2-AG synthesis occurs within lipid rafts. Whether the 2-AG is then released into the cytoplasm from the rafts or travels laterally within the membrane to engage CB2 receptors within the non-raft regions of the same cells remains a question for future investigation.

Acknowledgements. The authors are grateful for the support of the National Institute on Drug Abuse (DA-018224, DA-020402, DA-011322, F32-DA-016825), the Gill Center for Biomolecular Science, Indiana University, Bloomington, and the Lilly Foundation Inc, Indianapolis, IN.

AN INVESTIGATION OF LIPOXYGENASE INHIBITORS AS POTENTIAL INHIBITORS OF FATTY ACID AMIDE HYDROLASE

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FAAH activity is sensitive to a number of cyclooxygenase inhibitors, including indometacin and ibuprofen. Lipoxygenases (LOX) metabolise unsaturated fatty acids to produce hydroperoxides and may be inhibited by a number of structurally diverse compounds, including coumarin derivatives, such as esculetin, as well as bisphenolic analogues, such as nordihydroguiaretic acid (NDGA), and heterocyclics, such as phenidone. In this study, we have investigated whether LOX inhibitors influence FAAH activity from rat liver. FAAH activity was measured using four separate enzyme preparations, either by a previously-described spectrofluorometric methodology, using oleamide as substrate, or a radioisotopic method, using [^{3}H]-anandamide as substrate. Inhibitors were investigated at a concentration of 100 μM, in the presence of a maximal DMSO concentration of 10 %. FAAH activity (with oleamide as substrate) in the presence of NDGA and phenidone was significantly reduced (68 ± 8 and 79 ± 5 % control). Of the coumarins, caffeic acid evoked a significant inhibition (57 ± 13 %), while coumarin and o-coumaric acid were ineffective. Since the 7-hydroxycoumarins exhibited significant fluorescence activity at the wavelengths used, they were assessed in the [^{3}H]-anandamide hydrolysis assay. However, esculetin, 4-methylesculetin and umbelliferone failed to alter FAAH activity significantly (87 ± 7; 95 ± 5 and 113 ± 28 % control). Given that many of these agents are used at 50-100 μM concentrations in order to inhibit LOX activity, it is possible that some of the effects of these inhibitors may be indirect, mediated through inhibition of FAAH activity and the consequent elevation of endocannabinoids.

Acknowledgement: we are grateful to the MRC for financial support.

CIRCADIAN ASPECTS OF CYCLOOXYGENASE-2 IMMUNOLOCALIZATION IN THE MOUSE BRAIN

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While widely described as the 'inducible cyclooxygenase' due to its increased expression following tissue injury, cyclooxygenase-2 (COX-2) is also constitutively expressed in discrete regions of the rat (*Neuron* (1993)11:371-86) and mouse CNS (*Brain Res* (1996) 713:64-69). COX-2 produces prostanoids through the oxygenation of arachidonic acid. The endocannabinoids arachidnoyol ethanolamide (AEA) and 2-achidonoyl glycerol (2-AG) are additional COX-2 substrates, (*J Biol Chem* (1997) 272: 21181-21186; *J Biol Chem* (2000) 275:33744-33749) and the resulting prostamides and prostaglandin glycerol esters are prostanoid receptor ligands. In addition to promoting prostanoid receptor signaling, endocannabinoid metabolism by COX-2 mediates cannabinoid system activity. Inhibition of COX-2, but not fatty acid amide hydrolase, potentiates cannabinoid receptor signaling in the rat hippocampus (*Nat Neurosci* (2004) 7:697-8).

Both the endocannabinoid and prostaglandin systems of the CNS exhibit circadian properties. There are circadian fluctuations in both rat brain AEA and 2-AG levels (*Cell Mol Life Sci* (2004) 61: 945-50), and prostaglandin levels in cerebrospinal fluid (*Biochem Biophys Res Com* (1995) 213:625-629). In addition, prostaglandin D and E have long been implicated in respectively mediating sleep (*PNAS* (2006) 103:17949-54) and wakefulness (*PNAS* (1989) *86:5666-9*).

Taking into account the numerous studies linking both COX-2 substrates and products to circadian events, it seems likely that COX-2 expression varies with a circadian pattern. Any circadian change in COX-2 distribution is likely important, as it may alter signaling in both the endocannabinoid and prostaglandin systems within those brain regions. In this study, the distribution of COX-2 was determined by immunocytochemistry in the brains of mice perfused at noon and midnight. Similar to previous reports (*Brain Res* (1996) 713:64-69), mouse brains perfused at noon exhibited COX-2 immunoreactivity (COX-2-IR) in CA1-3 and scattered dentate gyrus cell bodies of the hippocampus, cell bodies in the amygdala, and layers II/III of the cortex. No COX-2-IR was observed in the thalamus or cerebellum. Brains perfused at midnight exhibited COX-2-IR in all areas that were immunoreactive in noon specimens. In addition, midnight specimens had COX-2-IR in processes surrounding cell bodies in layers V and VI of the visual, somatosensory, and auditory cortex. COX-2-IR was also observed in regions completely lacking reactivity in noon specimens, such as the cerebellum, where COX-2-IR was observed in purkinje cells, and within certain thalamic nuclei, where cell bodies exhibited COX-2-IR. These data indicate COX-2 distribution is temporally regulated in discrete cell populations, further supporting observations of circadian variability of the prostanoid and endocannabinoid systems in the mouse brain.

Acknowledgement: This work was funded by NIDA grant 1 K01 DA021806-01 (S.T.G.)

ENHANCEMENT OF ANANDAMIDE LEVELS BY THROMBIN AND PROTEIN KINASES C AND A IN SENSORY NEURONS. IMPLICATIONS FOR TRPV1 RECEPTOR ACTIVITY

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The endocannabinoid, *N*-arachidonoylethanolamide (anandamide), is also a full agonist at "transient receptor potential vanilloid" type 1 (TRPV1) channels, and its synthesis is triggered by elevations of the intracellular Ca^{2+} concentration. In sensory neurons of the rat dorsal root ganglia (DRG), both depolarisation and stimulation of metabotropic receptors stimulate anandamide biosynthesis (van der Stelt et al., *EMBO J*., 2005). In DRG neurons, TRPV1 is activated by anandamide, and is sensitised following treatment with anandamide as well as upon activation of protein kinases C and A (PKC, PKA) (De Petrocellis et al., *J. Neurochem*., 2001). Stimulation of metabotropic protease-activated receptors (PARs) also sensitises TRPV1 in sensory neurons via activation of PKC and PKA (Amadesi et al., *J. Physiol*., 2006, Vellani et al, paper in preparation). It is not known whether anandamide contributes to sensitization of TRPV1 induced by PARs, PKC and PKA. Here, we investigated the possibility that stimulation of PARs with thrombin, and activation of PKC or PKA, cause enhancement of anandamide levels in DRG neurons.

In DRG neuronal cultures anandamide levels measured by isotope-dilution liquid chromatography-mass spectrometry were significantly enhanced by both phorbol miristoyl acetate (PMA), a PKC activator, and forskolin, an adenylate cyclase stimulant, as well as by thrombin (a PAR-1, PAR-3 and PAR-4 activator), at appropriate concentrations. The levels of the other endocannabinoid, and TRPV1-inactive compound, 2-arachidonoyl glycerol (2-AG), were enhanced only by thrombin and to a lesser extent than anandamide, whereas the anandamide congener, *N*-palmitoyl-ethanolamine, was not affected by any of the treatments. The mechanism of action of thrombin was investigated in human embryonic kidney (HEK-293) cells, which constitutively express PAR-1, and where the compound, as well as PMA and forskolin, also exerted potent stimulatory actions on anandamide. In this cell type the effects of both PMA and thrombin were counteracted by the PKC inhibitor RO318220.

In conclusion, we report that, in DRG neurons, of the three compounds analysed, anandamide is the most sensitive to PKC activation, either directly or after thrombin receptor stimulation. PKA also stimulates anandamide and 2-AG formation. Given the activity of anandamide at TRPV1, this finding may suggest the existence of an amplificatory cascade on this receptor in sensory neurons, involving protein kinases and anandamide. Experiments are ongoing in order to investigate the role of intracellular Ca^{2+} in the effects of PKA, PKC and thrombin.

TRIFLUOROMETHYLKETONES: BROAD SPECIFICITY INHIBITORS OF 2-ARACHIDONYLGLYCEROL DEGRADATION BUT NOT ITS SYNTHESIS FROM DIACYLGLCEROL

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2-Arachidonylglycerol (2-AG) is an endocannabinoid that targets both CB1 and CB2 cannabinoid receptors. Biochemical studies demonstrate that 2-AG is rapidly inactivated in biological tissues via hydrolysis. Several enzymes are capable of 2-AG hydrolysis, including fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase. It is likely that there are other pathways of 2-AG inactivation as well, as there are many mammalian carboxylesterases with very broad substrate specificities. Inhibition of 2-AG hydrolysis in biological tissues would provide important information regarding its signaling role, but the large number of hydrolytic pathways potentially involved make this a difficult task if one relies on specific inhibitors. The trifluoromethyl ketones (TFK) are a group of extremely potent transition state analog inhibitors of esterases. We have demonstrated that the TFKs inhibit 2-AG hydrolysis in lysates of prostate cancer cells (Nithipatikom et al., Biochem. Biophys. Res. Comm. 332: 1028, 2005). The aim of the study was to investigate whether these compounds also inhibit 2-AG hydrolysis by brain tissue and to determine whether they inhibit the hydrolysis of diacylglycerol to 2-monoacylglycerol, a critical step in the synthesis of 2-AG that is carried out by an esterase as well. Four TFKs were studied: 3-(decylthio)-; 3-(octylthio)-; 3-(hexylthio)-; and 3-(butylthio)-1,1,1-trifluropropan-2-one (DETFP; OTFP; HTFP and BTFP, respectively). The effects of the four TFKs on FAAH activity were determined using mouse brain P2 membranes and [^{3}H]AEA as substrate. All four TFKs were potent and fully efficacious inhibitors of FAAH (IC50 values for DETFP, OTFP, HTFP and BTFP: 3.45; 2.30; 4.27 and 30.62 nM, respectively). The effects of the inhibitors on cytosolic brain esterases were determined in mouse brain cytosol using [^{14}C]-2-mono-oleoyl-glycerol as substrate. All four TFKs inhibited approximately 80% of 2-OG hydrolysis with IC50 values of: 0.80; 2.77; 3.67 and 35.01 μM, for DETFP, OTFP, HTFP and BTFP respectively. We examined the effects of DETFP on the conversion of 1-stearoyl-2-[^{14}C]arachidonyl glycerol to [^{14}C]2-AG by brain P2 membranes as an assay of diacylglycerol lipase activity. DETFP had no effect on this conversion at concentrations up to 100 μM. These data demonstrate that DETFP is a broad spectrum inhibitor of 2-AG hydrolysis in brain but not an inhibitor of diacylglycerol conversion to 2-monacylglycerol.

Supported by NIH grant DA09155.

ENDOCANNABINOID RETROGRADE MODULATION OF RETINAL PHOTORECEPTORS

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Goldfish cone photoreceptors contain an extensive cannabinoid system, but a function has not been identified. Our purpose was to infer the presence of endocannabinoids by measuring retrograde suppression of the cone current ($I_{K(V)}$). Whole-cell recordings were obtained from cone inner segments, in a goldfish retinal slice under voltage clamp. $I_{K(V)}$ was elicited by a pulse to +54 mV from a holding potential of -70 mV. A 50 msec puff of saline with 70 mM KCl or Group I mGluR agonist DHPG was applied directly at a mixed rod/cone (Mb) bipolar cell. The rationale was that stimulation of postsynaptic dendrites could release a retrograde transmitter, the effect of which could be measured by modulation of presynaptic membrane currents in cones. The amplitude of cone $I_{K(V)}$ decreased 25% compared to the pre-puff control. All effects were unaffected by $GABA_A$, $GABA_C$, AMPA and kainate antagonists but were blocked by the CB1 receptor antagonist SR 141716A. A FAAH inhibitor had no effect, whereas a COX-2 inhibitor prolonged the effects 10-fold. Orlistat, a blocker of 2-AG synthesis, blocked the effect of the K^+ puff. DHPG decreased $I_{K(V)}$ of cones by 32%, an effect blocked by SR141716A. The effect of DHPG was not blocked by the mGluR5 antagonist MPEP, indicating involvement of mGluR1. Mb bipolar cells are the only cells in fish retina that contain mGluR1, suggesting that they are the source of 2-AG. The suppressive effect of the K^+ puff vanished in Ca^{2+}-free, 2mM Co^{2+} saline. TMB-8 or ryanodine, blocked the effect of DHPG, but not the K^+ puff, showing that calcium influx or release from intracellular stores could mediate retrograde 2-AG release. We suggest that retrograde suppression of cone $I_{K(V)}$ is mediated by Ca^{2+}-dependent release of 2-AG from Mb bipolar cell dendrites by separate mechanisms: 1. voltage-dependent, mimicked by the K^+ puff, activated by the depolarizing ON response to light ; 2. voltage-independent, occurring under ambient illumination, mediated by tonic mGluR1/ $G_{q/11}$ activation. The negative feedback of the latter mechanism could regulate tonic glutamate release from cones within narrow limits, regardless of ambient illumination, thus maintaining sensitivity to detect contrast.

NIH grant R01 EY001682

THE REGULATION OF DIACYLGLYCEROL LIPASE ALPHA EXPRESSION

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The sn1-diacylglycerol lipases (DAGLs) catalyse the hydrolysis of diacylglycerol (DAG) to the endocannabinoid 2-arachidonoylglycerol (2-AG). Two sn1-DAG lipases have been identified and cloned, DAGLα and DAGLβ. The activity of these enzymes is required for axonal growth during development and for retrograde signalling at mature synapses. Their expression patterns correlate with these roles, changing from expression in the axonal tracts of the embryo to dendritic fields in the adult. Evidence of a dynamic expression pattern in the subventricular zone (SVZ) of the adult brain and the loss of proliferating cells following the addition of a DAGL inhibitor to the SVZ, suggests that the DAGLs may also have a role in adult neurogenesis.

The aim of this study is to characterise the promoter region of DAGLα in order to investigate the regulation of DAGLα expression. A promoter was predicted by bioinformatic analysis and cloned, such that a luciferase assay could be optimised to study promoter activity. The predicted DAGLα promoter was found to be an active promoter in a range of cell types, including neural stem cells. Deletion analysis has given rise to the identification of core promoter elements required for maximal activity and for suppression of DAGLα transcription. Conservation between species and transcription factor binding site predictions has enabled the identification of putative regulatory regions. Deletions of these sites have revealed a potential role for transcription factor Sp1 in a neural stem cell-specific regulation of DAGLα expression.

Alongside this work, endogenous levels of DAGLα have been investigated using real-time PCR and the effects of candidate factors on DAGLα expression studied. Together the data provides a starting point for elucidation of the factors involved in regulating DAGLα expression.

DEVELOPMENT OF A SENSITIVE LC/MS-IT-ToF METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF PROSTANOID DERIVATIVES OF ENDOCANNABINOIDS

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The oxygenation of the endocannabinoids (ECs) 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide by cyclooxygenase-2 (COX-2), and the subsequent action of prostaglandin synthases, was hypothesized to produce new eicosanoid derivatives: the prostaglandin glycerol esters (PG-Gs) and prostaglandin ethanolamides (PG-EAs, also known as prostamides). However, the actual occurrence and physiological function of COX-2-derived oxidative metabolites of ECs is still far from being fully established. We describe here a sensitive and specific LC/MS-IT-ToF method for the simultaneous identification and quantification of eicosanoid derivatives from tissues.

Analytes were extracted from tissues and pre-purified by open bed chromatography using a gradient elution of $CHCl_3$/MeOH to separate different class of lipids, including ECs, from PG-AEs and PG-Gs. LC separation was performed in isocratic mode using a Discovery®C18 column (15cm x 2.1mm, 5□m) and MeOH/H_2O/acetic acid (53:47:0.05 by vol.) as the mobile phase with a flow rate of 0.15 ml/min. Prostaglandin E_2, D_2, $F_{2\alpha}$ ethanolamides (PGE_2-EA. PGD_2-EA and PG $F_{2\alpha}$-EA) and prostaglandin E_2 glycerol ester (PGE_2-G) were ionised using electrospray ionization (ESI) in positive mode and mass analysis was performed with high resolution using ion trap (IT) and time of flight (ToF) spectrometers arranged in tandem. The LC method was optimized to ensure separation among all the analytes, particularly between PGE_2-EA and PGD_2-EA, which share the same molecular weight and identical fragmentation patterns. MS analysis allowed the identification of the LC peaks at retention times 15.0, 17.5, 21.5 and 24 min as PGE_2-EA, PGD_2-EA, $PGF_{2\alpha}$-EA and PGE_2-G, respectively. The m/s spectrum of all eicosanoid derivatives exhibited a sodium adduct of the molecular ion $[M + 23]^+$ as the base peak. This ion can then be further analysed by MS/MS, yielding high resolution fragment ions that correspond to the loss of water. The measured masses for each analyte correlate with the theoretical ones calculated from the corresponding chemical formulae within <10 ppm. The limit of detection (LOD) for each compound (defined as the concentration at which a signal/noise ratio of greater than 3:1) was 25 fmol for all analytes with MS, and 500 fmol with MS-MS analyses. Recovery, including extraction recovery, ranged between 35 and 45%. Deuterated standards were used in order to quantify endogenous compounds by isotope dilution. The ratio between the $[M + 23]^+$ peak areas of non deuterated (0.05-20 pmol) *vs.* deuterated (1 pmol) compounds varied linearly with the amount of non-deuterated compounds. The reproducibility of subsequent analyses of the same amount of each non-deuterated compound ranged between 95 and 100%.

The specific and sensitive method developed here represents a further step towards the assessement of the presence and role of EC oxidation products. Its application to investigate the physiological function of PG-AEs and PG-Gs, and to other classes of bioactive fatty acid amides is currently being evaluated in our laboratory.

2-ARACHIDONOYLGLYCEROL-INDUCED ENHANCED ADHESION OF HUMAN EOSINOPHILIC LEUKEMIA EOL-1 CELLS TO ADHESION MOLECULES AND ENDOTHELIAL CELLS

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2-Arachidonoylglycerol (2-AG) is an endogenous ligand for the cannabinoid receptors (Sugiura *et al*., (1995) *Biochem. Biophys. Res. Commun*. 215, 89; Mechoulam *et al*., (1995) *Biochem. Pharmacol*. 50, 83). Recently, we found that human eosinophils contain a high level of CB_2 receptor mRNA and that the endogenous ligand 2-AG induced the migration of human eosinophils in a dose-dependent manner (Oka et al., (2004) *J. Leukoc. Biol*. 76, 1002). We also found that the level of 2-AG was markedly elevated in oxazolone-induced allergic inflammation in mouse ear and that the administration of SR144528 markedly reduced allergic responses (ear swelling and the infiltration of eosinophils) (Oka et al., (2006) *J. Immunol*. 177, 8796). These results strongly suggest that 2-AG and the CB_2 receptor play crucial roles in the pathogenesis of allergic inflammation. In the present study, we examined in detail the effects of 2-AG on the adhesion of human eosinophilic leukemia EoL-1 cells, since the adhesion of inflammatory cells to the blood vessels and the extracellular matrix is an important step in various types of inflammatory reactions. We found that 2-AG markedly augmented the adhesion of EoL-1 cells to VCAM-1 and fibronection. The response was detected with as low as 10 nM 2-AG The effect of 2-AG was abolished by treatment of the cells with SR144528 or PTX, suggesting that the CB_2 receptor and Gi/Go are involved. We next investigated the intracellular signaling molecules involved in the 2-AG-induced augmented adhesion. Treatment of the cells with wortmannin, PD98059 or BAPTA-AM abrogated the effect of 2-AG, suggesting that phosphatidylinositol 3-kinase, ERK and intracellular free Ca^{2+} are essential for the response. Notably, 2-AG enhanced the adhesion of EoL-1 cells to human endothelial cells as well. The effect of 2-AG was abolished by treatment with SR144528. Thrombin and TNF-alpha also enhanced the adhesion of EoL-1 cells to endothelial cells. Interestingly, the effects of thrombin and TNF-alpha were also abolished by treatment with SR144528, suggesting that the CB_2 receptor and 2-AG were involved in thrombin- and TNF-alpha-induced augmented adhesion of EoL-1 cells to endothelial cells. Possible pathophysiological meanings of 2-AG-induced augmented adhesion of eosinophils in allergic inflammation will be discussed.

PHARMACOLOGICAL EVIDENCE FOR THE INTERACTION OF WIN 55212-2 AND THE CHEMOKINE CXCL12/SDF-1α ON BODY TEMPERATURE IN RATS

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Chemokines are a family of small (8 to 12 kDa) proteins involved in cellular migration and intercellular communication. Receptors for chemokines, opioids, and cannabinoids are members of the G-protein-linked seven-transmembrane receptor family. These receptors are widely distributed in brain and periphery. Recently, we have demonstrated a heterologous desensitization between opioid and certain chemokine receptors. The purpose of the present study is to investigate whether this interaction extends to other drugs of abuse such as cannabinoids. In the present experiments, the chemokine Stromal cell-derived growth factor-1alpha (CXCL12/SDF-1α), a member of the CXC chemokines that acts via the G protein, seven-transmembrane CXCR4 receptor, was tested for its effect on body temperature (Tb) as well as for its possible interaction with WIN 55,212-2-induced hypothermia. Male S-D rats weighing 250-300 g were used, 8-10 rats per group. Radio transmitters (Mini-Mitter, Sunriver, OR), implanted under anesthesia 5-7 days before testing, were used to measure Tb. A sterilized stainless steel C313G cannula guide (22 gauge, Plastics One Inc., Roanoke) was placed just above the preoptic anterior hypothalamus (POAH), the main area controlling Tb. WIN 55,212-2 (5-15 ng) microinjected directly into the POAH, evoked dose-dependent hypothermia at ambient temperature of 22 ± 0.3˚C. CXCL12/SDF-1α was microinjected into the POAH, and Tb was then measured for 60 min. CXCL12/SDF-1α (25-100 ng) did not affect Tb by itself. However, CXCL12/SDF-1α (100 ng/µl), microinjected directly into the PAG 30 min before injection of WIN 55,212-2, reduced significantly the WIN 55,212-2-induced hypothermia. Our results provide evidence for interaction between chemokine and cannabinoid receptors and show that the activation of CXCL12/SDF-1α receptors in the POAH interferes with the hypothermia induced by WIN 55212-2.

Acknowledgements: Funded by Grants DA06650 and DA13429 from NIDA.

INHIBITION OF HUMAN NEUTROPHIL CHEMOTAXIS BY ENDOGENOUS CANNABINOIDS AND PHYTOCANNABINODS: EVIDENCE FOR A SITE DISTINCT FROM CB_1 AND CB_2.

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The endocannabinoid anandamide binds to cannabinoid CB_1 and CB_2 receptors and has both analgesic and anti-inflammatory actions. There is a growing body of evidence suggesting that neutrophils make a crucial contribution to a number of autoimmune, autoinflammatory and neoplastic disorders. This study was directed at investigating the modulation of human neutrophil migration by phytocannabinoids, endocannabinoids and related compounds.

Peripheral polymorphonuclear neutrophils were isolated from normal whole blood by centrifugation over Polymorphprep™. The isolated cells were resuspended at a concentration of $1x10^6$ $cellsml^{-1}$ in phosphate buffered saline containing $CaCl_2$ and $MgCl_2$. *In vitro* cell migration assays were performed using a modified 48-well Boyden Chamber. Incubation lasted 30 minutes in a 5% CO_2 atmosphere at 37°C. After incubation, the migrated adherent cells on the underside of the 3μm pore filter were stained using a Diff-Quik stain set. Each well was counted in ten non-overlapping fields (x40) using a light microscope.

Here we show a novel pharmacology for inhibition of human neutrophil migration by endocannabinoids, phytocannabinoids and related compounds. The endocannabinoids anandamide and virodhamine are highly potent inhibitors of fMLP-induced migration of human neutrophils, with IC_{50} values of < 1 nM. The phytocannabinoid, (-)-cannabidiol is a partial agonist, being ~ 40 fold more potent than (+)-cannabidiol; abnormal-cannabidiol is a full agonist. This profile of agonist efficacy and potency parallels the pharmacology of the novel 'abnormal-cannabidiol' or a related orphan GPCR, which are already known to modulate cell migration. Furthermore, the 'abnormal-cannabidiol' receptor agonist, O-1602 modulated human neutrophil migration. Whilst having no effect alone, *N*-arachidonyl serine antagonised anandamide-induced inhibition of human neutrophil migration. Our data also suggest that there is cross-talk/negative co-operativity between the cannabinoid CB_2 receptor and this novel target: CB_2 receptor antagonists significantly enhance the inhibition observed with anandamide and virodhamine.

These data reveal that certain phytocannabinoids, endocannabinoids and related compounds are highly potent inhibitors of human neutrophil migration and implicate a novel pharmacological target distinct from cannabinoid CB_1 and CB_2 receptors.

Acknowledgements: Funding from Allergan Inc. and GW Pharma

2-ARACHIDONOYLGLYCEROL TRIGGERS MEGAKARYOCYTIC DIFFERENTIATION OF HAEMATOPOIETIC PROGENITOR HEL CELLS, IN A TYPE-2 CANNABINOID RECEPTOR-DEPENDENT MANNER

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Introduction: Recent experimental findings suggest a novel physiological role for endocannabinoids, as signalling molecules responsible for the control of proliferation and differentiation. Moreover at the periphery endocannabinoids play major roles in immune response, blood cells migration, and modulation of blood flow and pressure.

Materials and Methods: Erythroid and megakaryocytic differentiation was achieved by culturing cells in medium containing 60 μM hemin or 100 nM phorbol ester (TPA), respectively. For cannabinoid and vanilloid receptor studies, the membrane fractions were used in rapid filtration assays with the synthetic cannabinoid [^{3}H]CP55,940, and [^{3}H]RTX. The uptake of [^{3}H]AEA or [^{3}H]2-AG by their purported membrane transporters was performed on proliferating and differentiated intact HEL cells. The hydrolysis of AEA by fatty acid amide hydrolase (FAAH) was assessed through reversed phase-HPLC. The hydrolysis of 2-AG by monoacylglycerol lipase (MAGL) was assayed in cell supernatants incubated with [^{3}H]2-OG. The synthesis of 2-AG through the activity of diacylglycerol lipase (DAGL) was evaluated in cell extracts with [^{14}C]DAG as substrate and the release of [^{14}C]2-AG was analyzed by thin layer chromatography (TLC) and scintillation counting. Western Blot analysis was performed with specific antibodies.

Results: Here, we show that human erythroleukemia (HEL) cells have the machinery to synthesize, hydrolyze and transport the endocannabinoid 2-arachidonoylglycerol (2-AG), along with 2-AG-binding CB2 cannabinoid receptors. HEL cells are also able to take up the other major endocannabinoid anandamide (AEA), and express functional AEA-binding vanilloid receptors, but not the AEA metabolic enzymes. We also show that differentiation of progenitor HEL cells modulates the endocannabinoid system. Commitment towards the megakaryocytic phenotype reduces the synthesis of 2-AG by diacylglycerol lipase activity down to ~20%; yet, the binding efficiency of CB2 cannabinoid receptors is unchanged, thus allowing an “outside-in” signalling cascade. In addition, we demonstrate that 2-AG induces *per se* megakaryocytic differentiation, by enhancing the expression of β3 integrin subunit (GPIIIa, antigen CD61), a megakaryocyte/platelet surface antigen, and glycoprotein VI, a late marker of megakaryocytes. 2-AG also reduces the mRNA encoding for glycophorin A, a marker of erythroid phenotype. We show that these effects of 2-AG on HEL cells were all mediated by activation of CB2 receptor, that triggered an ERK-dependent signalling cascade.

Conclusion: We report for the first time the presence of a functional endocannabinoid system in haematopoietic cells. Taken together, these data point to 2-AG and CB2 receptors as critical regulators of haematopoietic differentiation.

ENDOGENOUS CANNABINOIDS DRIVE PRODUCTION OF PROSTAGLANDINS IN A MACROPHAGE CELL LINE RAW 264.7

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There is growing evidence for a relationship between the endogenous cannabinoid system and the lipid signaling molecules that comprise the prostaglandins. Notably, they share a structural homology in that both are derived from arachidonic acid. Additionally, the primary enzyme for prostaglandin production, cyclooxygenase (COX), metabolizes both anandamide and 2-AG into what are now known as prostamides. We found that at least one of these metabolites, PGE2-Glyceryl ester, occurs *in vivo*. Evidence presented here suggests that anandamide and 2-AG may activate COX leading to increased production of prostaglandins. Here we examine the role of anandamide and 2-AG on the levels of prostaglandins (PG) in the macrophage cell line RAW 264.7.

RAW 264.7 cells were cultured in DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were used when they reached 70% confluence. Prior to incubation, cells were washed twice with dPBS; then either 10μM AEA, 2-AG, or vehicle (DMSO) was added to serum-free media and incubated for 1 hour. At the end of the incubation time, media and cells were collected and lipids were extracted on C18 solid phase extraction columns. Eluents were tested for levels of PGD/E2, PGF2alpha, PGJ2, and 15-deoxy PGJ2 using MRM tandem mass spectrometric methods.

2-AG and AEA both increased production of PGD/E2, PGF2alpha, PGJ2, and 15-deoxy PGJ2 in RAW 264.7 cells. Ongoing studies are examining the pathways by which this occurs.

Acknowledgements. The authors are grateful for the support of the National Institute on Drug Abuse (DA-018224, DA-020402, F32-DA-016825), the Gill Center for Biomolecular Science, Indiana University, Bloomington, and the Lilly Foundation Inc, Indianapolis, IN.

CANNABINOID MODULATION OF PRURITIS: POTENTIAL AGENTS AGAINST ITCH

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Many recent studies have shown evidence of potential cannabinoid modulation of multiple forms of pruritis, an unpleasant cutaneous sensation that provokes the desire to scratch the skin to obtain relief. Local dermal administration of a cannabinoid agonist (HU-210) in a small human trial showed a reduction of itch sensation upon histamine administration (Dvorak et al., *Inflammation Research* (2003) 52:238-245). Also, several studies have examined the use of cannabinoids in the treatment of pruritis associated with cholestasis. Past studies in our laboratory and those of others found that acute administration of systemic rimonabant (SR141716A) consistently produce a significant increase in spontaneous scratching behavior in mice. The pruritic effects of rimonabant have been shown to be dose-dependent and reversible by several CB_1 agonists (Janoyan et al., *Pharm. Bioc. And Beh.* (2001) 71:155-162). The two main objectives of the present study were to determine the mechanisms through which rimonabant causes spontaneous scratching, and to determine whether cannabinoids possess efficacy in a traditional pruritis model.

In our studies, we used both rimonabant (ED50 to produce scratching = 8.8 mg/kg, 95% CI 3.9 to 19.9 mg/kg, ip) and the mast cell degranulator, compound 48/80, to elicit scratching behavior in mice. Using a digital video and keystroke-based observational system, we timed the duration of scratch behavior as well as recorded several other behaviors (e.g., immobility). Additionally, we recorded several mice simultaneously under a number of treatment conditions, gathering continual behavioral data for up to sixty min following treatments. We found that wild type (mean ± SEM = 62 ± 17 s) and CB_1 (-/-) mice (39 ±16 s) did not differ in baseline scratching behavior. However, rimonabant-elicited scratching was virtually abolished in CB_1(-/-) mice [CB_1(+/+) = 206 ± 26 s versus CB_1(-/-) = 20 ± 7 s]. We have also found that the combination of moderate doses of both rimonabant (10 mg/kg) and compound 48/80 (10 μg) produced a potentiated scratching response, suggesting an interaction of their respective effects [C48/80 alone: 70.2 ± 21.2 s, rimonabant alone: 113.2 ± 29.5 s, C48/80+rimonabant: 232.4 ±2 6.8 s]. In the final set of experiments, we evaluated whether THC would reduce the scratching behavior in mice treated with compound 48/80. Indeed, systemic administration of THC led to a potent reduction in compound 48/80-induced scratching (ED50 = 0.66 mg/kg, 95% CI 0.52 to 0.82 mg/kg, ip), with a narrow dose range producing effects with minimal increases in immobility/sedation. In ongoing studies the FAAH inhibitor, URB597, is tending to decrease scratching behavior elicited by compound 48/80; however, the sample size of the groups need to be increased to make definitive conclusions. This study continues a line of evidence that points to the cannabinoid system being an efficacious target for mediating the sensation of itch and pruriceptive function.

CB2 AGONISTS AFFECT T CELL FUNCTION IN EAE

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We have shown previously that WIN55212-2 has a beneficial effect in the chronic EAE model (C57BL/6-MOG_{35-55}). The effect was enhanced in the presence of a CB1 antagonist, and reversed by a CB2 antagonist, indicating that the protective effect was mediated through CB2 receptors. Subsequently, we showed that the highly selective CB2 agonist 0-1966 is protective, and that this is associated with a significant decrease in leukocyte rolling and adhesion (intravital microscopy) and spinal cord demyelination and infiltration of inflammatory cells (immunohistochemistry). In addition, the ex vivo proliferation of MOG-specific splenic T cells isolated from EAE mice treated with 0-1966, was reduced, while nonspecific proliferation in response to T cell receptor activation (anti-CD3 Abs) was not affected. Since dendritic cells (DC) are the major inducers of T cell activation, we addressed the question whether the effect of the CB2 agonist in vivo might be mediated through DC. Using FACS analysis, we demonstrate that bone marrow derived myeloid DC (BM-DC) express CB2, and very little, if any, CB1 receptors. BM-DC treated with 0-1966 show reduced CD40 expression, and do not stimulate T cell proliferation in an allergenic system. In addition, T cells cultured with CB2 agonist-treated DC have a different cytokine/chemokine profile compared to T cells cultured with DC not treated with 0-1966. There is increased production of the Th2/Treg cytokines IL-10, IL-4 and IL-13, and decreased production of IFNg (Th1 response), and of the proinflammatory chemokines KC (a neutrophil chemoattractant), MIP-1□, and most significantly MCP-1, a major chemokine in EAE. These results suggest that, in addition to effects on leukocyte rolling and adhesion, cannabinoids could affect DC function through the CB2 receptor, inducing tolerogenic and/or Th2-promoting dendritic cells.

This project is funded, in part, under a grant with the Pennsylvania Department of Health.

THE CB_1 RECEPTOR ANTAGONIST, SR141716A IMPAIRS REVERSAL LEARNING OF AN OPERANT CONDITIONING TASK

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It is now well established that exogenously administered cannabinoids (e.g.Δ^9-THC; WIN 55212-2; CP55940) reliably produce potent and fairly specific memory deficits via CB_1 receptor activation. The role of endocannabinoids (e.g. anadamide; 2-AG) in learning and memory is less clear. However, a few recent studies suggest that the 'endocannabinoid system' may play an important role in controlling certain memory processes such as facilitating 'extinction' *and/or* 'forgetting', in which extraneous information is deleted from memory storage. This phenomenon has been supported in CB_1 $^{-/-}$ mice that show impairments in extinction and reversal learning. Similarly, deficits in extinction learning following the treatment with CB_1 receptor antagonist, SR141716A (SR) have been demonstrated in wild-type mice. Here we assess the effects of SR on reversal_learning in rats trained to perform a simple two-lever operant conditioning task to better understand the role of endocannabinoids in learning and memory.

Adult, male Long Evans rats were pre-trained to perform the Delayed Non-Match to Sample (DNMS) task (i.e. short-term memory task) to criterion (~ 90% correct at 0s delay).Half the subjects were systemically administered 5.0mg/kg/day of SR (i.p.), while the rest received comparable vehicle (Pluronic F68) injections approximately 15 minutes prior to testing the performance on the reversal task, Delayed Match to Sample (DMS) task. During this reversal task the subjects were required to match instead of non-match the sample (i.e. learn the opposite rule) for a continuous period of 12 days. Each day all subjects were given 60 minutes to run 100 DMS trials. The session was terminated depending on whichever was accomplished first.

The SR group in comparison to controls produced no deficit in reversal learning (i.e. mean rate of correct responses) from day 1 to 5 (all p's > 0.05) but, demonstrated significant impairments from day 6 to12 (all p's < 0.05). Furthermore, the SR group exhibited a significantly reduced mean percentage of correct responses on day 8, 9 and 10 (i.e. late deficit in reversal learning). No significant differences in locomotor activity between the two groups were observed across all 12 days.

These results suggest that CB1-receptor mediated activation – presumably by endocannabinoids – facilitates reversal learning of this memory task with a late onset of action. This effect was purely cognitive as no motor impairments were evident following the treatment of SR. Furthermore, these results suggest that the 'endocannabinoid system' may be actively involved in modulating the processes of consolidation/re-consolidation necessary for reversal learning.

MARIJUANA AND PROSPECTIVE MEMORY

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Marijuana use is associated with impaired performance on tests of retrospective memory, that is, the ability to remember past events and facts. Previous research suggests that chronic use of marijuana impairs retrospective memory test performance. This research also suggests that individuals who under the influence of marijuana during testing perform worse on tests of retrospective memory than individuals who not under the influence of marijuana during testing. However, to date no research has been conducted to examine the relationship between marijuana use and prospective memory performance. Prospective memory is the ability to make plans, to retain them and to carry them out at the appropriate time or in the appropriate context. In other words, it is the ability to remember to do things at a later time. Everyday examples of prospective memory tasks include remembering to buy bread on the way home from work, to go to a meeting at 3 pm or to take medication at the right time. The goal of the present study was to examine the relationship between marijuana use and everyday prospective memory performance.

Undergraduate students completed an online survey concerning their everyday prospective memory performance and marijuana use. Specifically, participants completed three questionnaires to assess their everyday prospective memory performance: the Prospective Memory Questionnaire, the Prospective and Retrospective Memory Questionnaire and the Time-Cued Prospective Memory Questionnaire. To assess marijuana use participants completed a modified version of the Marijuana Screening Inventory. The original version of the inventory contains items that assess the negative impact of marijuana use on individuals' lives. We added items to this inventory to assess the frequency with which individuals use marijuana, the quantity of marijuana that individuals typically use as well as the positive impact that using marijuana has had on individuals' lives.

Preliminary results show a positive correlation between short-term prospective memory impairments and the frequency with which individuals report using marijuana. We are continuing to collect data and we hypothesize that with the resulting increased sample size we will also find correlations between prospective memory and the quantity of marijuana typically used as well as with the influence that using marijuana has had on individuals' lives.

This study enhances our understanding of the relationship between marijuana use and memory through its examination of an under studied form of memory, namely prospective memory.

ENDOCANNABINOID SIGNALING AT STRIATAL CB_1R IS CRITICAL FOR THE CONSOLIDATION OF STIMULUS-RESPONSE MEMORIES

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The rodent striatum is a large forebrain area that receives extensive convergent excitatory inputs from the cerebral cortex, limbic system and thalamus. As the primary neuroanatomical entryway into the basal ganglia, the striatum is critical for many aspects of proper motor function, and is also necessary for particular processes of nondeclarative learning and memory. These are referred to as procedural or stimulus-response (S-R) learning, terms that describe the psychological formation of behavioral habits.

The endocannabinoid (eCB) system is implicated in various functions of learning and memory, involving mechanisms of synaptic plasticity in multiple brain networks. In the dorsolateral striatum (DLS), eCBs act as retrograde messengers mediating the induction of long-term depression (LTD) at excitatory corticostriatal synapses. Whereas mechanisms of synaptic plasticity such as LTD are generally thought to represent cellular substrates of learning and memory, there is currently little direct evidence linking these phenomena to striatal mnemonic functions, such as S-R learning.

Last year we reported that direct injection of the CB1 antagonist/inverse agonist rimonabant into the rat DLS impaired learning of S-R foraging strategies on a T-maze. We have extended these findings by employing post-trial manipulations of the eCB system in the DLS. Male Sprague-Dawley rats were bilaterally implanted with injection cannulae into the DLS. Following a recovery period, animals were maintained on a food-restricted diet and subsequently habituated to obtaining a food reward on a radial arm maze, located in a dimly lit room with no extramaze visual cues. For each learning trial, the maze was arranged in a T-formation, with the central arm as the start position (determined randomly for each trial to negate the formation of relevant spatial cues). Subjects were required to make a consistent, fixed response (right or left turn, determined randomly) in order to find the reward. A criterion level of learning was set at 9 correct responses in 10 consecutive trials. After achieving criterion performance, subjects received intra-DLS rimonabant or its vehicle. Memory recall was assessed with a probe test of 10 food-rewarded performance trials conducted 72 hours later. Rats receiving rimonabant immediately post-training were significantly less successful in the S-R behavioral strategy, compared to rats receiving either vehicle or a delayed injection of rimonabant (2 hours post-training). This suggests a mnemonic role for striatal eCB signaling within a limited post-training time window. Preliminary data further suggests that facilitating striatal eCB signaling during this time window of memory consolidation may enhance the recall of S-R foraging strategies. We propose that eCB-mediated synaptic plasticity within the DLS is an important cellular mechanism for the learning of behavioral habits, consistent with a role of the eCB system in the etiology and/or treatment of mental disorders related to habitual behaviors, such as Tourette's Syndrome, obsessive compulsive disorder and addictions.

RIMONABANT DISRUPTS EXTINCTION LEARNING IN AN AVERSIVE, BUT NOT IN AN APPETITIVE, BARNES MAZE TASK

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Advances in the field of cannabinoid research have suggested blockade of CB1 receptor signaling disrupts extinction learning, or the suppression of non-reinforced behaviors. While the full scope that the endogenous cannabinoid system plays on extinction learning is still unknown, research suggests that this phenomenon may be limited to aversive conditions. Specifically, disruption of CB_1 receptor signaling impaired extinction learning in conditioned freezing, Morris water maze, and passive avoidance paradigms, but not in operant procedures in which a food reinforcement was earned. However, it is difficult to distinguish between the hedonics (i.e., aversive vs appetitive) and required responses associated with these disparate tasks. Accordingly, there were two objectives of the present study. First, we adapted the Barnes maze, a spatial memory task requiring mice to find and enter a hidden escape hole, to assess learning in either aversive or appetitive conditions. In the aversive condition, mice were required to enter a hidden box to escape aversive stimuli (i.e., bright lights and air turbulence). Conversely, in the appetitively reinforced condition, mice were water deprived for 22 hours prior to each session and trained to enter the hidden box for access to water. The second objective of the present study was to test the hypothesis that CB_1 receptor antagonism would disrupt extinction learning in both models of appetitive and aversive reinforcement. The mice acquired the Barnes maze task to a comparable degree, based on latency to find the escape box, in both appetitive (mean ± SEM = 17 ± 2 s) and aversive (17 ± 3 s) conditions by day four. To assess extinction effects, the maze was divided into six zones of equal size, each corresponding with a possible target-hole location. The percentage of time spent in the target zone, the zone previously containing the escape box, was used as the primary dependent measure for extinction as it declines with non-reinforced trials. Administration of the CB_1 receptor antagonist, rimonabant, before each extinction trial disrupted normal extinction learning under aversive conditions, as assessed by a reduction in the percentage of time spent in the target zone. Specifically, while rimonabant-treated (41 ± 3%) and vehicle-treated (42 ± 3%) groups spent a similar percentage of their time in the target zone on day one, vehicle treated mice exhibited a significant reduction by day two and by day 10 (19± 3%) spent little of their time in the zone. Conversely, mice treated with rimonabant perseverated in the target zone area across all extinction trials and spent 37 ± 8% of their time in this zone on day 10. However, this effect was not observed under appetitive conditions. Both rimonabant and vehicle-treated subjects did not differ with respect to the percentage of time spent in the target zone on the first day of extinction, 39 ± 2% and 39 ± 2% respectively, and were found to have extinguished by day five (22 ± 3%; 23 ± 4% respectively). The differential effects of rimonabant in the same task in which only the reinforcer is changed strongly support the hypothesis that the endogenous cannabinoid system plays a role in decreasing learned behavior when reinforcement is withheld in aversive paradigms, but not in appetitive tasks. Moreover, these findings suggest that pharmacotherapies that stimulate this system may provide some utility in reducing maladaptive behaviors that arise from stress or trauma.

IMPROVEMENT IN COGNITIVE AND SOCIAL COMPETENCE IN ADOLESCENT CHRONIC CANNABIS USERS. RESULTS FROM A MANUAL BASED TREATMENT PROGRAMME AT MARIA YOUTH CENTRE, STOCKHOLM, SWEDEN

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At Maria Youth Centre in Stockholm, a system-theoretical approach involving family treatment has been used since the late 1980s to help adolescent drug addicts. This treatment approach did not help young chronic cannabis users. Therefore, the treatment method for adult chronic cannabis users (Lundqvist, T and Ericsson, D 1988) was transformed into a manual based 18 sessions programme. It is a structured six-week treatment programme including sessions three times a week. The main focus is on helping the cannabis users (17-20 year) to redirect cognitive patterns and to regain intellectual control. After completion of the six-week programme, the patients are advised to take part in supportive sessions once a week for six weeks. The programme is now a regular programme at the centre.

Fifty adolescents (75 admissions), with at least six months daily use, completed the programme between year 2000 and 2004. Average age at first cannabis use was 14.2 years. At follow-up after one year, two-thirds were cannabis free; 35 per cent had had no relapses and 33 per cent had had one brief relapse, 57 per cent were free from all problematic use, including alcohol. Patients with initial problematic alcohol use were less successful. Remaining symptoms of anxiety and depression were signs that indicate that extended support are needed. Finally, improvements could be seen in their overall life situation.

The clients were assessed at admission, after six weeks and after one year as a follow-up with a battery of questionnaires consisting of Sense of coherence (SOC), Symptomchecklist-90 (SCL-90), Beck's Depression Inventory (BDI) and scales focusing on qualitative improvements in life.

After six weeks of treatment the average SOC score increased from 118 points to 138 points (normal range for a Swedish sample is 142 – 152). At follow-up the average score was 145. These changes are statistically significant. Moreover, the average score on each of the component scales (comprehensibility, manageability and meaningfulness) also increased significantly during the programme period. After one year, a further improvement can be observed, although it is not statistically significant. The overall scores on SLC-90 (50 is normal with a range of 40 – 60), improved as follows: Global severity index (GSI) – from 68 to 54.1 to 51; Positive symptom depth index (PSDI) – from 61.2 to 50.6 to 51.9; Positive symptom total (PST) – from 65.5 to 56.4 to 51.7. Improvements were statistically significant. Clients with a GSI score below 50 increased from 8 to 29 per cent. Clients showing a PSDI score below 50 increased from 18 to 54 per cent and with a PST score below 50 increased from 10 to 30 per cent). The BDI overall score and the scores on the various component scales all improved significantly during the programme (the proportion of clients with no symptoms of depression increased from 58 to 94 per cent). At follow-up after one year, a further marginal improvement could be seen. The data and the details of the treatment programme will be discussed in comparison to other cannabis treatment programs.

UPDATED GUIDELINES FOR THE USE OF CANNABINOIDS COMPOUNDS AVAILABLE IN CANADA FOR THE TREATMENT OF CHRONIC PAIN

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Three cannabinoid compounds – nabilone, a synthetic analog of delta-9-tetrahydrocannabinol (Cesamet®), synthetic delta-9-tetrahydrocannabinol (Marinol®) and the cannabis based medicine extract containing delta-9-tetrahydrocannabinol and cannabidiol (Sativex®) - are available in Canada by prescription. In 2005 the current authors published guidelines for the use of cannabinoid compounds in the treatment of chronic pain (Clark et al., Pain Res Manage 2005;10 Suppl A:44-6). At that time published information on the off-label clinical effect of nabilone and synthetic delta-9-tetrahydrocannabinol only was available to develop clinical guidelines. Since that time a cannabis based medicine extract containing delta-9-tetrahydrocannabinol and cannabidiol has been approved which is indicated as an adjunctive treatment for neuropathic pain associated with multiple sclerosis. The original guidelines only provided recommendations about the first two preparations. This is an update to the original guidelines based on clinical experience and recent clinical trial results.

Guidelines for the use of nabilone (Cesamet®) capsules

1. Initiate nabilone at 0.25 - 0.5 mg orally at night time
2. Increase dose by 0.25 - 0.5 mg every 2-3 days to a maximum dose of 3 mg BID orally.

Guidelines for the use of synthetic delta-9-tetrahydrocannabinol (Marinol®) capsules

1. Initiate synthetic delta-9-tetrahydrocannabinol at 2.5 mg orally at night time
2. Increase dose by 2.5 mg every 2-3 days to a maximum dose of 10 mg TID orally

Guidelines for the use of delta-9-tetrahydrocannabinol and cannabidiol (Sativex®) buccal spray

1. Initiate 1 buccal spray once or twice daily
2. Increase dose by 1 – 2 buccal sprays every 2-3 days to a maximum dose of 16 sprays per day.

A management pathway for the treatment of chronic pain with cannabinoid compounds available by prescription includes (a) full assessment to establish a diagnosis, (b) assessment of psychological issues and risk of addiction, (c) treatment plan that includes an overall participatory approach by the patient and (d) traditional approaches to pain management have been tried or considered and been found to be ineffective or poorly tolerated.

A practical approach to the use of cannabinoid compounds available by prescription will be provided.

VALIDATION OF TWO MODELS ESTIMATING THE TIME OF CANNABIS USE FROM PLASMA THC AND THCCOOH CONCENTRATIONS

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Introduction: Estimating the time at which cannabis was used can be important from a forensic perspective. Two models have been developed to predict the time of cannabis exposure from plasma concentrations of THC and THCCOOH (Huestis M *et. al.*, J Anal Toxicol. 1993;17:313-6.). The models have been validated in case of exposure to joints containing up to 35 mg THC. However in recent years, cannabis has become available on the drug market with an average content of 61 mg per joint. We conducted a study to investigate whether the models perform well in case of exposure to joints with high THC concentrations.

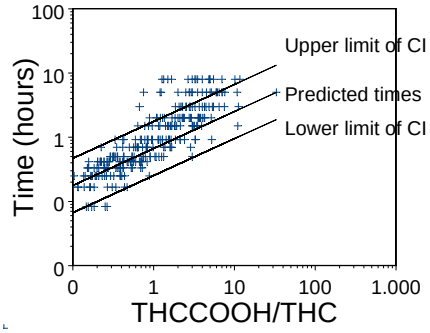
Methods: Data of a previous study -approved by the Hospital Medical Ethical Board- were used. All the participants provided informed consent. A group of 24 occasional users and a group of 6 frequent users smoked a joint containing 69.4 mg THC. Blood samples (n=336) were collected between 0-8 hours after onset of smoking, and serum THC and THCCOOH concentrations were measured by liquid chromatography/mass spectrometry with limits of quantification of 0.5, and 1.0 µg/L respectively for THC and THC-COOH. Predicted times of cannabis smoking were compared with actual smoking times. Predictions were considered correct when the actual time of exposure was within the 95% confidence interval (CI).

Results: The best results were obtained by combining both models, which allowed to predict the time of exposure within the 95% CI in 86% of the samples (Table). Model I predicted poorly. 96% of the incorrect predictions were underestimations of the time of exposure, i.e. the models estimated a shorter time than actual. Predictions were often inaccurate when actual time was ≥ 4 hours (underestimation of the time of exposure in 36 among 46 samples i.e. in 78% of the cases), even for model II (Figure).

Table. Performance of both models.

	Accuracy [a]		Underestimated time [b]	
	%	n	n	Mean (range) hours
Model I				
Occasional	51	136/266	123	1.64 (0.14-6.15)
Frequent	31	22/70	48	1.77 (0.10-7.19)
All	47	158/336	171	1.68 (0.10-7.19)
Model II				
Occasional	83	220/266	43	4.20 (0.21-7.21)
Frequent	81	57/70	10	4.61 (1.26-7.24)
All	82	277/336	53	4.27 (0.21-7.24)
Combination of CIs of both models [c]				
Occasional	86	228/266	36	3.78 (0.21-6.15)
Frequent	86	60/70	10	4.49 (1.26-6.54)
All	86	288/336	46	3.94 (0.21-6.54)

Figure. Accuracy evaluation of Model II.



a: Percentage and n samples with actual time of exposure within the 95% CI
b: Underestimated time means that the actual time was greater than the upper limit of the CI
c: Time interval defined by the lowest and highest 95% confidence limits of both models

Conclusions: Model I should be adapted for exposure to high THC concentrations and model II for blood sampling performed 4 hours or more after cannabis exposure.

RELATIONSHIP BETWEEN INITIATION OF SMOKING TOBACCO AND MARIJUANA USE IN YOUTH

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Introduction: Youth is a time characterized by risk behaviours that may be associated with harms such as smoking tobacco or marijuana use. The relationships between age of initiation of tobacco and marijuana use by youth (aged 15 to 24) will be explored.

Methods: Results are based on data from the 2005 public use file of the Canadian Tobacco Use Monitoring Survey (CTUMS), a general population telephone based survey of Canadians aged 15 years and older. Respondents were classified according to pattern and sequence of use (no use, tobacco-only, marijuana-only, tobacco first, marijuana first, same age). The associations between marijuana and tobacco use were examined through logistic and multinomial regressions. Variables controlled in the model included: gender, age, region, and household location.

Results: Youth typically used tobacco earlier than marijuana and use of one substance predicted use of the other but a greater proportion reported lifetime experience with marijuana but not tobacco. Those who had smoked marijuana-only had the youngest age of initiation of all groups (14.15 yrs). These results are the reverse of those found for respondents 25 years of age and older.

Conclusion: The results clearly highlight that the relationship between tobacco and marijuana use are changing for some of today's youth.

THE CANNABINOID SYSTEM DIFFERENTIALLY MEDIATES CEREBELLAR- AND HIPPOCAMPAL-DEPENDENT MOTOR LEARNING IN HUMANS: EVIDENCE FROM TRACE AND DELAY EYEBLINK CONDITIONING IN CANNABIS USERS

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Introduction**:** Consistent with psychomotor disturbances associated with cannabis intoxication, including memory, motor and time perception, accumulating evidence has implicated the central cannabinoid system receptor (CB1) in mediating cerebellar and hippocampal plasticity in non-human animals. Specifically, research using CB1 knockout mice has indicated a differential role for the CB1 receptor in cerebellar- versus hippocampal-dependent motor learning (Kishimoto & Kano, 2006, *J Neurosci.* 26[34]). However, the extent to which this evidence translates to humans is unclear. Thus, the present research investigated cerebellar- and hippocampal-dependent motor learning in frequent cannabis users, who may have altered CB1 function, with a classical eyeblink conditioning (EBC) procedure.

Methods**:** In the delayed version of the EBC task, a conditioned stimulus (CS; 400 ms tone) co-terminates with a corneal air puff (unconditioned stimulus; US; 50 ms), which elicits an unconditioned blink response (UR). After a number of such paired trials, a conditioned response (CR) is executed just prior to the onset of US in healthy subjects. While delay EBC is a cerebellar-dependent motor learning task, hippocampal circuitry is necessary for adaptive acquisition of the CR when a 500 ms 'trace' period of no stimulation is inserted between the CS and the US. Heavy cannabis users (abstained 12 hours prior to study; positive THC drug test) free of DSM axis I or axis II disorders, aside from cannabis abuse or dependence, completed either a delay or trace EBC task.

Results**:** Cannabis users exhibited pronounced deficits in both the acquisition and timing of the CR in cerebellar-dependent delay EBC, but acquired the CR similar to controls on the hippocampal-dependent trace EBC task.

Conclusion**:** This evidence concurs with EBC performance in CB1 knockout mice (Kishimoto & Kano, 2006) and suggests a differential role for the cannabinoid system in mediating cerebellar- and hippocampal-dependent motor learning in humans. Further, these data indicate that possible CB1 down regulation instigated by chronic exposure to the partial CB1 agonist delta-9-tetrahydrocannabinol, the primary psychoactive ingredient in cannabis, may serve as a human analogue of the animal CB1 knockout.

Acknowledgements: This work was supported in part by a grant from NIDA (NIDA 1 R03 DA019630-01) and a NARSAD Young Investigator Award; both of which were awarded to Patrick D. Skosnik. The authors wish to thank Michael Walker, Jo Anne Tracy, Richard McFall, Joseph Steinmetz, Peter Finn, and Michael Paulin for their help throughout the project.

CIRCULATING AND ADIPOSE-TISSUE ENDOCANNABINOID SYSTEM IN OBESE PATIENTS TREATED WITH SIBUTRAMINE

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Introduction: The endocannabinoid system promotes weight gain and obesity-associated metabolic disease. Weight loss interventions may influence the obesity-associated risk through modulation of peripheral endocannabinoid system activity. To address this issue, we investigated the effect of acute and chronic treatment with the serotonin and norepinephrine reuptake inhibitor sibutramine on components of the peripheral endocannabinoid system.

Methods: Twenty obese otherwise healthy subjects participated in study which started with a randomized, double blind, cross-over treatment with placebo and with 15 mg/day sibutramine for five days each, followed by 12 weeks open label sibutramine treatment. We determined anthropometric changes during treatment and sympathetic nervous system activity by microneurography in the peroneal nerve. We also quantified venous concentrations of anandamide and 2-AG and assessed the gene expression of CB1-receptors and the endocannabinoid degrading enzymes FAAH and MGL in biopsies of abdominal subcutaneous adipose tissue.

Results: Body weight did not change during five days placebo or sibutramine treatment, but decreased by 4.1±0.8 kg during the 12 weeks open label sibutramine treatment as expected ($p<0.05$). Peripheral sympathetic nervous system activity was inhibited by acute and chronic sibutramine treatment. Circulating endocannabinoid concentrations as well as expression of CB1-receptor, FAAH and MGL genes did not change with acute or chronic sibutramine treatment. We found no correlation between endocannabinoid variables and measures of sympathetic activity. However, during chronic sibutramine treatment, strong correlations were found between the changes in adipose-tissue expression of CB1-receptor, FAAH and MGL genes with genes encoding the adipokines leptin and adiponectin.

Discussion: Our study suggests that the beneficial metabolic responses to sibutramine treatment in obese subjects cannot be explained by interactions with the endocannabinoid system. Furthermore, our data suggest interactions between the endocannabinoid system in adipose tissue and adipokines such as leptin and adiponectin. These interactions await further investigation, but they may be important to further understand the role of the endocannabinoid system for the metabolic disturbances seen in human obesity.

REGULATING COMPASSION: AN OVERVIEW OF CANADA'S FEDERAL MEDICINAL CANNABIS POLICY AND PRACTICE

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Background: In response to a number of court challenges brought forth by Canadian patients who demonstrated that they benefited from the use of medicinal cannabis but remained vulnerable to arrest and persecution as a result of its status as a controlled substance, in 1999 Canada became the second nation in the world to initiate a centralized medicinal cannabis program. Over its six years of existence, this controversial program has been found unconstitutional by a number of courts, and has faced criticism from the medical establishment, law enforcement, as well as the patient/participants themselves.

Methodology: This critical policy analysis is an evidence-based review of court decisions, government records, relevant studies and recent Access to Information Act data related to the three main facets of Health Canada's medicinal cannabis policy - the Marihuana Medical Access Division; the Canadians Institute of Health Research Medical Marijuana Research Program; and the Prairie Plant Systems cannabis production facility - as well as the nation's main suppliers of therapeutic cannabis, Canada's network of unregulated compassion clubs and societies.

Results: There is a growing body of evidence that Health Canada's program is not meeting the needs of Canada's medical cannabis patient community and that the policies of the Marihuana Medical Access Division may be significantly limiting the potential individual and public health benefits achievable though the timely and effective therapeutic use of cannabis by critically and chronically ill Canadians. Over the last six years, the MMAD has only registered 1300 people, despite government studies suggesting that between 400,000 and 1,000,000 Canadians currently claim to be using cannabis for medical purposes. Contradicting their own suggestions that more research needs to be done to assess the safety and therapeutic potential of cannabis, the federal government cancelled all federal funding to the CIHR MMRP and disbanded the "Expert Advisory Committee on Marihuana for Medical Purposes, effectively eliminating government support for this much-needed research. Although Health Canada has spent over $7 million dollars on the PPS production facility since 2001, only 300 medical users are currently accessing this source of cannabis. This has resulted in over 2000 pages of complaints against this federal program, and led to a number of calls for an Auditor General investigation of the MMAD by elected officials and to ongoing legal challenges to the constitutionality of these regulations. By contrast, community-based dispensaries are currently supplying medical cannabis to over 10,000 Canadians, and are initiating or involved in a growing number of peer-reviewed research projects, all at no cost to Canadian taxpayers. It is apparent that any future success within this program will depend on the government's ability to better assess and address the concerns and needs of the nation's critically and chronically ill, to promote and fund an expanded clinical research agenda, and to work in cooperation with Canada's established network of community-based medicinal cannabis compassion clubs and societies.

TREATING NEUROPATHIC PAIN AND MUSCLE SPASTICITY IN MULTIPLE SCLEROSIS PATIENTS USING CANNABINOIDS: A SERIES OF CASE STUDIES

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Aim: To introduce practitioners to the neurobiology, neurophysiology and clinical issues involved in cannabinoid prescribing with multiple sclerosis patients. Cannabinoids are members of a family of compounds known as terpenoids. As of Sep/06 there are more than 60 cannabinoids that have been scientifically identified and labeled. All of them are ligands, to some extent. The bulk of cannabinoids are from plant source - Several synthetic cannabinoids have been formulated. Among them are: dronabinol and nabilone. These are both exogenous ligands. These two are available by prescription in North America. Nabilone, the subject of our paper, is believed to bind to the CB1 receptor site, primarily. Another cannabinoid has been synthesized by R. Mechoulam in 1991, known as anandamide. It is a mimic of an endogenous ligand. Each cannabinoid ligand, binds to one or more receptor sites. The principal cannabinoid receptor sites are: CB1, CB2, CBD and the mu Opioid. The receptor sites are distributed anatomically in the human body. Collectively, the above constructs constitutes the present-day neurobiological scientific explanation for the mechanism of action of a given cannabinoid ligand. Once the ligand has been taken up at the receptor site, the pharmacology and pharmacokinetics for that substance can be predicted, for a statistically average human subject. Clinical consultation is, however, required to determine dosing, route of administration, mode of ingestion, method of monitoring and titrating of each particular cannabinoid drug for a particular clinical patient. An attempt is given in the case histories presented, to open a practical window for the clinician, of the complexities of cannabinoid prescribing.

Methods**:** Out of a population of 900 outpatient pain patients, a number of patients who were difficult or impossible to treat with the standard medications, were selected for inclusion in this case series study.

Results: Presented in Tabular form (see Table1). Nine Patients are compared in terms of the following variables:

1. Age and Gender
2. Dose of Cesamet
    a) at date of study
    b) mode of ingestion/dosing schedule
    c) concurrent medications
    d) methodology of monitoring and titrating cannabinoid medication
3. Start/end and duration of treatment
4. Outcome measures: tests conducted/date
5. Results:
    a) phenomenological report of patients
    b) clinicians observations and notes

Conclusion**:** Although only 9 patients were retained in treatment long enough to be clinically significant to present here, each of these patients is a N=1 self selected longitudinal study. They have each failed the standard therapies, for their condition, after having tried them for varying durations, as an inclusion criterion for this case series. They have each consented to this non-standard therapy, only after having tried the standard therapies. The odds against the degree of success and duration of retention in treatment, occurring by chance, in these patients is extremely high. The medico-legal considerations in this category of patient, therefore, give a higher level of statistical significance than the size of the cohort reported on, would seem to indicate. Nonetheless, this study underlines the need for further research and randomized control trials.

CHRONIC PAIN AND CANNABINOIDS

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The political reality in various jurisdictions is that patients have access to a wide variety of modes of ingestion of cannabinoids, both legal and illegal. The pharmacokinetics of the different modes of ingestion are reviewed and compared.

The empirical efficacy of different regimes of "cannabinoid combining" is also studied by a series of case studies. In almost all cases an "opioid sparing" and "immune enhancing" effect was noted until a stable dosing platform was achieved which reduced the total "toxic load" from all ingested medications.

A sample clinical condition: "fibromyalgia" was selected because of the robust numbers of case subjects, but the same methodology can be used by extension with other conditions. Sometimes the results are what would be expected based on the pharmacokinetics of the different oral, intrabuccal, and inhaled substances. Sometimes the results are surprising, and rely on the details of the patients own clinical history and psychology. Some of the patients received a dispensation from Health Canada to buy "dried marijuana", after the study was over, and further modified and titrated their treatment regime. These results can be shared anecdotally.

ROLE OF LOX PATHWAY AND ENDOCANNABINOID SYSTEM IN ANTITUMOR ACTIVITY OF CANNABIDIOL, A NON-PSYCHOACTIVE CANNABINOID

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We recently reported that the non-psychoactive cannabinoid compound cannabidiol (CBD) is able to kill glioma cells, either in vivo or in vitro, independently of cannabinoid receptor stimulation (1,2). However, the biochemical mechanisms underlying the antitumoral effect of CBD has not been clearly clarified. On this background, in the present work we performed biochemical analysis both on glioma tumor tissues excised by nude mice exposed in vivo to CBD and on U87 glioma cell culture treated in vitro with the cannabinoid, evaluating the involvement of both COX and LOX pathways, or of the endocannabinoid system.

The in vivo exposure to CBD significantly decreased in tumor tissues the 5-LOX activity and content by about 40%, paralleled by the decrease (25%) of its product LTB4. In contrast, the COX activity and level and PGE2 amount were unaffected by the treatment. Besides, the in vivo treatment with CBD, markedly stimulated (175%) in tumor tissues the hydrolytic activity of fatty acid amide hydrolase (FAAH, the main anandamide-degrading enzyme). Concomitantly, a decrease in anandamide (AEA) level (30%) and in CB1 receptor binding (25%) was observed. The involvement of LOX enzyme in the antiproliferative effect of CBD was confirmed by data obtained in in vitro experiments. The pretreatment of U87 glioma cells with MK-886, a specific inhibitor of 5-LOX activity at doses per se not affecting cell viability, was able to significantly enhance the antimitotic effect induced by CBD (MTT test). In contrast, when the pretreatment was carried out with indomethacin (COX-1/-2 inhibitor) or celecoxib (COX-2 inhibitor), no change was observed on CBD effect. The in vitro study of the endocannabinoid system revealed that CBD was able to induce a concentration-related increase of FAAH activity in U87 cells. Moreover, when FAAH overexpressing U87 cells were used, it was found a significant reduced rate of growth compared to U87 WT (by MTT and Trypan blue exclusion tests).

In conclusion, the present investigation indicates that CBD exerts its antitumoral effects through the modulation of LOX pathway and endocannabinoid system, suggesting a possible interaction of both systems in controlling tumoral growth.

1) Massi P., Vaccani A., Ceruti S., Colombo A., Abbracchio M.P., Parolaro D. (2004) J. Pharmacol. Exp. Ther. 308:838-845.

2) Massi P., Vaccani A., Bianchessi S., Costa B., Macchi P., Parolaro D. (2006) Cell. Mol. Life Sci. 63: 2057-2066.

Funded by GW Pharmaceutical.

ANANDAMIDE CONTROLS THE MIGRATION OF MDA-MB 231 BREAST CANCER CELLS THROUGH INHIBITION OF RHO/RHO KINASE PATHWAY

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The endocannabinoid system regulates cell proliferation and migration in human breast cancer cells. The stimulation of cannabinoid CB1 receptors induced a non-invasive phenotype in MDA-MB-231 cells, a highly invasive human breast cancer cell line. In a model of metastatic spreading *in vivo*, the metabolically stable anandamide analogue, 2-methyl-2'-F-anandamide (Met-F-AEA), significantly reduced the number and dimension of metastatic nodes. This effect was antagonized by the selective CB1 antagonist SR141716. We observed that Met-F-AEA inhibited adhesion and migration of MDA-MB-231 cells on type IV collagen by modulating FAK phosphorylation. It is well known that the small GTPase Rho and its effector ROCK regulate cytoskeleton and play a crucial role in cell adhesion and motility. In this study we report a novel pathway regulating cell migration involving small GTPase RhoA. We found that Met-F-AEA inhibited of RhoA activity. Using the Rhotekin binding assay to assess RhoA activation, we observed that the treatment of MDA-MB-231 cells with Met-F-AEA for a short time period reduced the level of GTP-bound Rho. We further observed that Met-F-AEA induced a decrease in actin stress fibers, and the appearance of a dense meshwork of actin filaments around the cell periphery. Met-F-AEA markedly blocked translocation of RhoA from cytosol to membrane. Moreover, overexpression of a dominant negative mutant RhoA (N19RhoA) and treatment of these cells with Rho-associated protein kinase (ROCK) inhibitor Y-27632 induced similar effects to those of Met-F-AEA on cytoskeleton. We suggest that the inhibitory effect of Met-F-AEA on tumour cell migration is related to Rho-Rho kinase-dependent signalling pathway.

MODULATION OF THE ENDOCANNABINOID SYSTEM BY KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV)

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Kaposi's sarcoma-associated herpesvirus (KSHV) also known as human herpesvirus 8) is the etiologic agent of Kaposi's sarcoma, a endothelial neoplasm currently epidemic in AIDS patients. KSHV encodes for several unique proteins that alter target cell function, including the virion envelope-associated glycoprotein B (gB). Glycoprotein B has an RGD (Arg-Gly-Asp) motif at the extracellular amino terminus region and binds to the alpha3beta1 surface integrin, which enhances virus entry. The mechanisms of KSHV infection and latency are not fully understood but appear to involve different signaling pathways.

We now report that gB can downregulate FAAH, thus modulating the endocannabinoid tone in both HMVEC primary cells and HMEC1 cell lines. The related signaling proteins involved can trigger receptor signaling, which can modulate endothelial migration and proliferation, which are cannabinoids properties. In addition, we observed that FAAH down regulation as well as THC incubation enhanced KSHV infection and participated in KSHV-mediated transformation. The signal molecules involved may contribute to the pathogenesis of Kaposi's sarcoma and suggest that interventions in cannabinoid tone could inhibit KSHV infection/activation.

The clinical implications of these findings are of note since cannabinoids are used for their palliative effects in AIDS patients. Further studies on the subject may turn cannabinoids from palliative medicines into potential tools for modulating virus pathogenesis.

INHIBITION OF HUMAN RETINOBLASTOMA Y-79 AND WERI CELL PROLIFERATION BY THE SYNTHETIC CANNABINOID WIN-55,212-2 AND CAVEOLIN-1

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Purpose: The present study was designed to examine: i) whether cannabinoid receptor-mediated signaling plays a role in the regulation of human retinoblastoma cell growth; ii) the role of caveolin-1 as a possible tumor suppressor gene in the inhibition of human retinoblastoma cell growth.

Methods: The following methods were used; reverse transcription-polymerase chain reaction (RT-PCR), Western immunoblotting analysis, cell culture transfection and an MTS-based cell proliferation assay, in order to determine possible cannabinoid receptor-mediated signaling as well as the effects of caveolin-1 expression on human retinoblastoma Y-79 and WERI cell proliferation. Retinoblastoma cells were differentiated using serum-free conditioned media. In contrast to undifferentiated cycling tumor cells, differentiated Y-79 and WERI cells attached to the substratem, extended processes and expressed neuronal markers.

Results: RT-PCR and immunoblotting analyses revealed the expression of both CB1 and CB2 receptors in undifferentiated human retinoblastoma Y-79 and WERI cells. The expression level of CB1 receptors was higher in undifferentiated (u) Y-79 and WERI (u) cells than in differentiated (d) Y-79 and WERI (d) cells. Treatment of WERI (u) and Y-79 (u) cells with the cannabinoid receptor agonist WIN-55,212-2 resulted in a dose-dependent inhibition of cell growth. The antiproliferative effect of WIN-55,212-2 was partly blocked by AM251 and AM630, CB1 and CB2 specific antagonists, respectively.

Examination of caveolin-1 protein levels revealed that caveolin-1 was undetectable in Y-79 (u) and WERI (u) cells. However, re-expression of caveolin-1 in Y-79 (u) resulted in the inhibition of tumor cell growth.

Conclusions: These results indicate that activation of both CB1 and CB2 receptors may underlie the antiproliferative effects of WIN-55,212-2, and suggest that the cannabinoid system may be a target in the modulation of retinoblastoma cell growth. Overexpression of caveolin-1was also found to be antiproliferative, indicating that the loss of caveolin-1 may also inhibit tumor cell growth in human retinoblastoma Y-79 cells.

Supported by CIHR grant FRN 13484 to MEMK.

THE RELATIVE ABILITY OF CANNABIS AND TOBACCO SMOKE TO INDUCE CHROMOSOMAL DAMAGE IN MURINE PULMONARY CELLS

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The prevalence of cannabis smoking is increasing among Canadian youth, and often it is perceived that cannabis smoke is less harmful than that of tobacco. However, research indicates that cannabis smoke condensate contains qualitatively the same carcinogenic chemicals as tobacco smoke. Furthermore, cannabis smoke has been shown to be more cytotoxic and mutagenic than tobacco smoke, and it is associated with various adverse pulmonary effects including chronic bronchitis, edema and mucus hypersecretion. Despite these findings, epidemiological studies have failed to establish a correlation between cannabis smoking and the development of respiratory cancers. Currently, the risks of adverse effects from cannabis smoke, as compared to tobacco smoke, are not well understood. This study examined the relative ability of cannabis and Canadian flue-cured tobacco smoke condensates to induce cytogenetic damage, measured as micronuclei, in a murine lung epithelial cell line (FE1 cells). Condensates of main- and side-stream smoke from hand-rolled cannabis and tobacco cigarettes were prepared using standard (i.e., ISO) smoking conditions, as well as "extreme" conditions designed to reflect cannabis smoking habits. Pulmonary cells were exposed to the smoke condensates for a four hour period, followed by a 28 hour growth period in the presence of cytochalasin B. Two thousand binucleated cells were scored from each treatment for the presence of micronuclei. Preliminary results indicate that cannabis samples were more cytotoxic and cytostatic than tobacco samples, as demonstrated by the lower cell proliferation indices. However, at the concentrations tested, no significant increases in micronuclei were observed in cells exposed to any of the cannabis condensates. In contrast, increases in micronuclei were observed in cells exposed to mainstream tobacco condensates smoked under both the standard and extreme conditions, and both with and without the addition of exogenous a metabolic activation mixture (i.e., rat liver S9). Although the statistical analyses are still underway, the preliminary results indicate that tobacco and cannabis smoke differ substantially in their ability to induce chromosomal damage.

CANNABINOIDS SUPPRESS CHEMOTHERAPY-EVOKED NEUROPATHIC NOCICEPTION THROUGH SPINAL SITES OF ACTION

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Chemotherapeutic treatment can induce several severe side-effects including neuropathic pain. The present studies were conducted to evaluate the sites of action of cannabinoids in suppressing mechanical hypersensitivity (tactile allodynia) induced by treatment with the anti-tumor agent vincristine in rats. Tactile allodynia developed over the course of ten once-daily injections of the chemotherapeutic agent vincristine relative to groups receiving saline at the same times. Systemic administration of the cannabinoid agonist WIN55,212-2 suppressed painful peripheral neuropathy evoked by vincristine administration in rats. This suppression was mediated by both CB_1 and CB_2 receptors. Intrathecal administration of WIN55,212-2 (10 and 30 µg i.t.) suppressed vincristine-evoked tactile allodynia. By contrast, WIN55,212-3, the receptor-inactive enantiomer of WIN55,212-2, failed to alter vincristine-evoked tactile allodynia. Intrathecal co-administration of both the CB_1 antagonist SR141716 (30 µg i.t.) and the CB_2 antagonist SR144528 (30 µg i.t.) blocked the cannabinoid-induced suppression of vincristine-evoked tactile allodynia. Coadministration of both CB_1 and CB_2 antagonists with the agonist preferentially suppressed vincristine-induced tactile allodynia relative to agonist coadministration with either antagonist alone. These data suggest that the suppression of tactile allodynia induced by WIN55,212-2 was mediated by both CB_1 and CB_2 receptors. The possible presence of peripheral sites of action was assessed using site-specific injections of WIN55,212-2 in peripheral paw tissue. WIN55,212-2 (30 or 150 µg i.pl.) failed to suppress vincristine-evoked tactile allodynia following local administration into the hindpaw. By contrast, a 3 to 15-fold lower dose, administered intrathecally, normalized mechanical withdrawal thresholds to pre-vincristine levels. Our results demonstrate that cannabinoids suppress the maintenance of vincristine-induced tactile allodynia through actions mediated, at least in part, at the level of the spinal cord. Our data further suggest that cannabinoid CB_1 and CB_2 receptor subtypes may be important therapeutic targets for the treatment of chemotherapeutic neuropathy.

Acknowledgements: Supported by DA014022, DA021644 to A.G.H. and an APF/COGDOP Scholarship, APA Forest and Honaker Scholarship, and Psi Chi Graduate Research Grant to E.J.R.

DOES STRESS-INDUCED LIPOLYSIS ENHANCE THC RELEASE FROM FAT STORES?

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The main psychotropic constituent of cannabis, Δ^9-tetrahydrocannabinol (THC), is lipophilic and accumulates in fat stores at relatively high concentrations. A number of medicolegal cases indicate that former long-term cannabis users may test positive for THC even though it was confirmed that they had not recently used the drug. It was noted that such users had been exposed to an intensely stressful situation or a regime of dramatic weight loss at the time of testing. Thus, we hypothesise that long-term stored THC in fat stores may be released under conditions promoting lipolysis. First a series of experiments were conducted using an *in vitro* model. Rat epididymal adipocytes were isolated and incubated with different doses of THC (1-10 μM) and further treated with adrenaline (10 μM). A dose-dependent passive release of THC was observed with this release being enhanced by adrenaline treatment. In a second study, an *in vivo*/*in vitro* experiment was performed using epididymal adipocytes extracted from male rats exposed to THC (10 mg/kg for 10 days) and treated with adrenaline in culture. As expected an enhanced release of THC (252 ± 106 ng/ml) was demonstrated which was well above the vehicle control (39 ± 24 ng/ml). We also conducted histological studies which showed a large difference in the overall surface area and cell number of adipocytes treated with THC (S.A = 6305 ± 522 μM^2; C.N =12 ± 2 cells) compared to the vehicle control (S.A = 3211 ± 377 μM^2; C.N = 25.00 ± 2.00 cells). Therefore, it is thought that THC may increase its own storage capacity by promoting lipogenesis. A third study involved a group of male rats being dosed with THC (10 mg/kg) for 3 days before being subjected to food deprivation for 30 h. Blood sample analysis via GC-MS of THC and the inactive metabolite THC acid (THC-COOH) revealed an elevation in THC-COOH (5 ± 1 ng/mL) 20 h into food deprivation. Based on these preliminary findings, THC release from fat stores is enhanced under conditions of stress. More studies are required to confirm this phenomenon *in vivo*. In addition there needs to be an assessment on whether the THC concentrations released from fat stores would cause behavioral changes thus leading to a re-intoxicative effect.

THE CB-1 ANTAGONIST, DELTA 9 TETRAHYDROCANNABIVARIN (THCV) HAS ANTI-OBESITY ACTIVITY IN DIETARY-INDUCED OBESE (DIO) MICE

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Introduction: Cannabinoid receptor-1 antagonists have been shown to have anti-obesity activity in a number of animal models and in human clinical trials. These effects result from inhibition of the endogenous cannabinoids anandamide and 2-arachidonyl glycerol. Recently it has been shown that delta-9-tetrahydrocannabivarin (THCV), the propyl homologue of delta-9-tetrahydrocannbinol (THC) is a potent anatagonist of anandamide in the mouse vas deferens and behaves as a competitive antagonist at CB-1 and CB-2 receptors.

Methods: The current study has examined the effect of pure THCV (0.3mg/kg) and THCV extracts (Botanical Drug Substances (BDSs)) containing THCV/THC in the ratio 3/1 in comparison with rimonabant (10mg/kg) and AM251 (10mg/kg) on food intake, body weight and fatness, energy expenditure and glucose and lipid parameters in mice made obese by pre-feeding a 'Western' diet for 12 weeks.

Results: In a single dose food intake study, THCV BDS produced a dose-related decrease in 24h food intake with the dose of 30mg/kg reducing food intake by more than 50%.

In a chronic study (28-day repeat dose study) THCV BDS (30mg/kg) reduced food intake over the first few days as did rimonabant and AM251, but after 5 days food intake was similar to controls. In contrast, body weight gain was reduced throughout a 28 day study period by both pure THCV and THCV BDS and mice had a reduced body fat content, and reduced plasma leptin concentrations. Measurement of energy expenditure by indirect calorimetry showed that both pure THCV and THCV BDS produced substantial increases in both 24h energy expenditure and the thermic response to food. AM251 produced similar effects but rimonabant produced only a slight increase.

Conclusion: These studies show that pure THCV and THCV BDS produce anti-obesity effects in vivo in a DIO mouse model mainly by increasing energy expenditure rather than reducing food intake. Such effects merit further study.

ACUTE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL AND AM251 IN A MURINE FEEDING PARADIGM: MAPPING OF NEURAL SUBSTRATES

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For 20 years, Δ^9-tetrahydrocannabinol (Δ^9-THC) has been approved to treat the appetite loss seen in wasting syndromes. However, the last decade has seen dramatic advancements defining the role that cannabinoids (CBs) play in appetite control. The licensing last year of rimonabant in the UK opened the door on a new class of drugs for the management of appetite and metabolic disorders. However, the question as to how CBs modulate food intake remains to be fully elucidated. Although a number of brain regions, including hypothalamic and mesolimbic structures, have been implicated (Kirkham *et al.*, Br.J.Pharmacol. 136 (2002) 550-7; Verty *et al.*, Neuropharmacology 49 (2005) 1101-9), we have found no studies examining which brain areas are activated during a CB-mediated feeding response. Therefore, we sought to map neural activation after acute Δ^9-THC or AM251 administration in a murine feeding paradigm.

Two separate batches of 48 male C57/BL6J (18-26g) mice were allocated to 4 treatment groups. Mice were fasted for 18 hours prior to the beginning of the dark period, when one batch was injected with Δ^9-THC (vehicle, 0.01, 0.5, 3.0 mg/kg; $N = 12$) and the other with AM251 (vehicle, 1, 3, 10 mg/kg; $N = 12$). Thirty minutes after injections pre-weighed palatable pellets were placed in the test chamber. Feeding behaviour (frequency and duration), food intake and locomotor activity were monitored simultaneously. After a washout period of one week the experiment was repeated with the exception that 45 minutes after food presentation all the mice were euthanized, the brains removed and sectioned on a cryotome (20 μm) at 8 coronal levels. A ^{33}P labelled *zif268* probe was hybridised to the brain sections and the resultant autoradiograms were quantified for relative optical density (ROD) bilaterally at 39 loci. All data were analysed using one or two-way ANOVAs followed by *post hoc* Dunnett t-tests as appropriate.

No significant differences in food intake were detected between vehicle and Δ^9-THC treated groups. However, 3mg/kg Δ^9-THC treated mice spent a longer duration eating compared with control (32 ± 4% vs 22 ± 2%; $p < 0.05$). At this dose, there were also changes in *zif268* expression in 6 brain regions: reductions in visual ($p < 0.01$), somatosensory ($p < 0.05$), cingulate ($p < 0.05$) and ventral orbital ($p < 0.01$) cortices and increases in posterior ($p < 0.05$) and ventromedial ($p < 0.01$) hypothalamic areas. Interactions between treatment and laterality were also observed in a further 5 brain regions, including the nucleus accumbens and the ventral tegmental area. In contrast, AM251 caused dose ($p < 0.001$) and time-dependent ($p < 0.001$) reductions in food intake. This was associated with a dose-dependent reduction in feeding duration ($p < 0.01$).

This is the first demonstration of dissociation between effects on neural activation in hypothalamic and extrahypothalamic areas following acute CB administration.

PRENATAL EVENTS INDUCE DEPRESSION-LIKE SYMPTOMS AND AFFECT SENSORIMOTOR GATING: MEDIATION BY ENDOCANNABINOIDS?

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The offspring of women exposed to stressful events during pregnancy, are more vulnerable to anxiety-provoking events, while the etiology of schizophrenia has been ascribed in part, to prenatal stress. The endocannabinoid-CB receptor system has been shown to be intimately involved in the stress response and in emotional processing, depression and the induction of schizophrenia, while we also know that the system is functional throughout gestation and early postnatal life.

We hypothesized that prenatal stress may alter the development of the endocannabinoid-CB receptor system, thus affecting emotional processing and sensorimotor gating, which is associated with symptoms of schizophrenia.

Methods: Pregnant mice were subjected to a regimen of "ultramild" stress (prenatal stress, PS) throughout gestation. Male and female offspring, at adulthood, were assessed for 1) behavioral response to the CB_1 receptor agonist HU210 (0.1 mg/kg); 2) depression-like behavior in the Porsolt-forced swim test; 3) sensorimotor gating in the 'prepulse inhibition" (PPI) assay; 4) anandamide and 2-arachidonoyl glycerol were determined in the frontal cortex and hippocampus of PS offspring using GC-MS analyses.

Results: 1) PS attenuated the disruptive effect of HU210 on the startle response and on PPI but only in male offspring; 2) PS significantly enhanced immobility in the forced swim test, suggesting depression-like behavior in prenatally stressed male and female mice. 3) Although endocannabinoid levels did not differ between stress and nonstressed offspring, PS males displayed a selective enhancement of anandamide over 2AG in the hippocampus.

Conclusion: "Ultramild" prenatal stress causes depression-like behavior in both sexes but only attenuated the disruptive effect of HU210 on sensorimotor gating and the acoustic startle response in males. The relative increase in anandamide that was exclusively seen in male stressed offspring, may underlie the PS-induced sex-specific behavioral changes in schizophrenia-like behavior. Our data add further information to the growing evidence for a role of anandamide in the development and course of schizophrenia.

Acknowledgement: This work was supported in part by the National Institute for Psychobiology in Israel. NG was supported by the ministry of immigrant absorption in Israel

POTENTIATION OF BENZODIAZEPINE-INDUCED SLEEP BY DELTA-9-TETRAHYDROCANNABINOL

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Delta-9-Tetrahydrocannabinol (THC) exhibits a variety of pharmacological effects, such as immobility, motor incoordination, and synergism with sedative-hypnotics. Cannabinoid receptor is subdivided into CB_1 and CB_2 receptors, and the CB_1 receptor is abundant in the CNS and peripheral neurons systems, whereas the CB_2 receptor expression is restricted to lymphoid organs. The pharmacological effects of THC seemed to be mediated through CB_1 receptor, since the CNS effects of the cannabinoid are inhibited by coadministration of CB_1 receptor antagonists such as SR141716A and AM251, but not a CB_2 receptor antagonist, SR144528. We revealed the prolonging effects of THC on pentobarbital-induced sleep in mice, and the pharmacological effect was antagonized by SR141716A and AM251, but not SR144528 [1)]. It is well known that barbiturates act on the $GABA_A$ receptor complex consisting of the benzodiazepine receptor and chloride channel. We previously reported the prolongation of diazepam-induced sleep in mice by pretreatment of THC [2)]. Therefore, the potentiation mechanism of THC for diazepam-induced sleep was examined in this study. SR141716A, AM251, or SR144528 (2 mg/kg, i.v.) was pretreated 10 min before injection with THC (10 mg/kg, i.v.). Diazepam (60 mg/kg) was suspended in 1% Tween 80 saline, and then injected by i.p. 15 min after the injection of THC. The sleeping time was measured as the time between loss of the righting reflex and the recovery. The cannabinoid receptor antagonists themselves did not affect the diazepam-induced sleeping time. CB_1 antagonists, SR141716A and AM251, reversed the prolongation of diazepam-induced sleeping time by THC, while SR144528 did not. Radio receptor assay was also performed using a specific ligand, [^{3}H]CP55940, and mouse brain synaptic membrane. The membrane fraction was incubated with 0.4 nM of [^{3}H]CP55940, fatty acid free BSA, 1mM of EDTA, 3 mM of $MgCl_2$ in 50 mM of Tris-HCl (pH 7.4) in the presence of THC (100 nM), pentobarbital (1 mM) or flunitrazepam (10 uM). Nonspecific binding was determined adding 10 uM of THC in the mixture. THC inhibited specific [^{3}H]CP55940 binding to the synaptic membrane, while pentobarbital and flunitrazepam did not affect the specific binding. These results suggest that the interaction site of THC with benzodiazepine ($GABA_A$ receptor) might be a downstream from the CB_1 receptor, and that the CB_1 receptor plays some role on synergistic effect of the cannabinoid with anesthetics such as barbiturates and benzodiazepines.

Acknowledgements: SR141716A and SR144528 were gifted from Sanofi-Synthelabo Recherche, (Martpellier Cedex, France). Funded by the Academic Frontier Project for Private Universities from the Ministry of Education, Culture, Sports, and Technology of Japan 2005-2009.

Kimura T., Takaya M., Yamaori S., Funahashi T., Watanabe K., Yamamoto I. ICRS 2006 (Hungary), Abstract, p.136.

2) Yamamoto I., Kimura T., Yoshida H., Watanabe K., Yoshimura H., Res. Commun. Subst. Abuse, 13, 299-313 (1992).

CBD AND THE NEURAL CORRELATES OF ANXIETY

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Aims: The study sought to examine the neurophysiological effects of cannabidiol (CBD) on the emotional processing using functional Magnetic Resonance Imaging (fMRI). Method: Fifteen healthy male participants (age range 18-35) with a lifetime exposure to cannabis of 15 times or less were recruited in a double blind event-related fMRI design. Prior to each scanning session, participants were given an oral dose of either 600mg CBD or a placebo. The blood levels of drugs were monitored via an intravenous line, while systolic and diastolic blood pressure and heart rate (beats per minute) were recorded manually. During the scan, subjects were presented with 10 different facial identities, each identity expressing 50% or 100% intensities of fear or a neutral expression. Neuropsychological performance and symptoms ratings were recorded at baseline, immediately before scanning (1 hr), immediately after scanning (2 hr), and one hour post scanning (3 hr). Results: CBD had no significant effect on the gender discrimination task. Reaction times were significantly faster when processing 100% fearful faces than compared to 50% fearful and neutral faces. CBD had a significant effect on brain activation in response to faces with emotional expressions, decreasing activation in the right posterior cingulate gyrus and in the right cerebellum, when compared to placebo. Furthermore, a significant interaction effect was observed. In the right cingulate gyrus CBD attenuated activation during the processing of intense fearful faces but had no effect of neural response to neutral or mild fearful faces. Conclusion: CBD significantly modulates the neurophysiological response associated with anxiety.

Acknowledgements: JASC and AWZ are recipients of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) fellowships. JASC was recipient of a CNPq (#200984/01-2). Supported in part by the FAPESP fellowship (grant 02/13197-2) and Psychiatryc Research Trust..

ACTIVATION OF THE ENDOGENOUS CANNABINOID SYSTEM BY ANANDAMIDE HAS EFFECTS ON MOTIVATION AND ANXIETY THAT ARE REVEALED BY FATTY ACID AMIDE HYDROLASE (FAAH) INHIBITION

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Converging evidence suggests the endocannabinoid system is an important constituent of neuronal substrates involved in brain reward processes and emotional responses to stress. Here, we evaluated motivational effects of intravenously administered anandamide, an endogenous ligand for cannabinoid CB_1 receptors, in Sprague-Dawley rats, using a place-conditioning procedure in which drugs abused by humans generally produce conditioned place preferences (reward), depending on dose and procedural details. Anandamide (0.03 to 3 mg/kg intravenous) produced neither conditioned place preferences nor aversions. However, when rats were pre-treated with the fatty acid amide hydrolase (FAAH) inhibitor URB597 (cyclohexyl carbamic acid 3'-carbamoyl-3-yl ester; 0.3 mg/kg intraperitoneal), which blocks anandamide's metabolic degradation, anandamide produced dose-related conditioned place aversions. In contrast, URB597 alone showed no motivational effects. Like URB597 plus anandamide, the synthetic CB1-receptor ligand WIN 55,212-2 (50 to 300 µg/kg, intravenous) produced dose-related conditioned place aversions. Development of place aversions with URB597 plus anandamide and with WIN 55,212-2 were prevented by pretreatment with the CB1-receptor antagonist AM251 (3 mg/kg intraperitoneal). When anxiety-related effects of anandamide and URB597 were evaluated in a light-dark box, both anandamide (0.3 mg/kg) and URB597 (0.1 and 0.3 mg/kg) produced anxiolytic effects when given alone, but produced anxiogenic effects when combined. The high 3 mg/kg dose of anandamide produced anxiogenic effects and depressed locomotor activity when given alone and these effects were potentiated after URB597 treatment. Thus, additive interactions between the effects of endocannabinoid system activation on brain reward processes and anxiety may account for the aversive effects of high levels of endocannabinoid system activation.

Acknowledgements: The research was supported in part by the Intramural Research Program of the NIH, National Institute on Drug Abuse.

THE ANXIOLYTIC RESPONSE TO CANNABIDIOL IS MODULATED BY THE ADENOSINE A_1 RECEPTOR BUT NOT BY THE CANNABINOID CB_1 OR CB_2 RECEPTORS

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Cannabidiol (CBD) is known to have anxiolytic effects. In humans, it has been observed to reduce anxiety associated with co-administration of high doses of tetrahydrocannabinol (THC), while in rodents, treatment with CBD increases the time spent in the open arms of an elevated plus-maze, a model of anxiety. Because CBD binds poorly to known cannabinoid receptors, the mechanism by which it reduces anxiety is unknown.

Recently, we have shown that CBD blocks the uptake of adenosine through competitive binding to an equilibrative adenosine transporter (ENT1), and, in doing so, enhances signaling at adenosine receptors (Carrier et al, PNAS 103:7895, 2006). Activation of some adenosine receptors can have anti-anxiety effects, and ethanol, another inhibitor of adenosine transport, has been shown to decrease anxiety through augmented activation of adenosine A_1 receptors. Here, we tested the hypothesis that the anxiolytic effect of CBD in the elevated plus-maze is due to increased activation of A_1 receptors and not to activation of either CB_1 or CB_2 receptors.

In male ICR mice, CBD significantly increased the amount of time spent in the open arms of the elevated plus-maze, without affecting total arm entries, beginning at a dose of 1 mg/kg (i.p.). This effect was reversed by pretreatment with the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 3 mg/kg). Furthermore, the anxiolytic effect of CBD was ablated in A_1 receptor null, C57Bl/6 mice, but not in mice null for the CB_1 or CB_2 receptor. These results demonstrate that the anxiolytic effect of CBD requires activation of A_1 adenosine receptors, and support the hypothesis that CBD exerts many of its effects as an indirect agonist of adenosine receptors.

Supported by NIH grants DA016967 and HL077707

ENDOGENOUS CANNABINOIDS CONTRIBUTE TO STRESS ADAPTATION

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Mounting evidence indicates that the endocannabinoid system may act as a buffer against the effects of stress. The aim of the present experiments, therefore, was to test the role of the endocannabinoid system on the adrenal corticosterone response to repeated restraint-stress exposure. As expected, a single episode of 30 min restraint produced a significant increase in plasma corticosterone concentrations, and this response was significantly reduced following 9 daily exposures to restraint. Acute administration of the CB_1 receptor antagonist AM251 (1 mg/kg) to chronically stressed animals prior to exposure to the final stress on day 9 resulted in a significant reversal of habituation of the corticosterone secretion. This effect was specific to the stress state, as administration of AM251 to chronically stressed animals under non-stress conditions did not significantly alter corticosterone secretion. Further, AM251 administration prior to acute exposure to a single episode of restraint stress did not potentiate stress-induced corticosterone secretion, suggesting that changes in the endocannabinoid system over the course of repeated restraint exposure contribute to the development of stress adaptation. Based on these findings, we then tested whether blocking endocannabinoid metabolism could enhance the process of adrenal habituation. Administration of the FAAH inhibitor, URB597 (0.3 mg/kg) during repeated restraint or immediately prior to the first or last episode of restraint had no significant effects on the magnitude of the corticosterone response. Taken together, the findings suggest a strong influence of the endocannabinoid system on stress adaptation. The extent to which repeated stress exposure involves other modes of altered endocannabinoid metabolism, including changes in 2-AG activity, remains to be seen.

ROLE OF CB1 RECEPTOR SYSTEM IN A PHARMACOLOGICAL MODELS OF SCHIZOPHRENIA

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Clinical and laboratory findings suggest that cannabinoid signalling is implicated in schizophrenia, however the interaction remains poorly understood, as data are often contradictory. On the basis of the intriguing but still confusing results present in literature, the aim of the present study is to investigate the role of the CB1 receptor system and signalling in a pharmacological model of schizophrenia.

The pharmacological model is based on repeated injections of the non-competitive NMDA antagonist, phencyclidine (PCP, 2,5 mg/kg, chronic intermittent treatment for 1 month). This is a validated model that mirrors the metabolic hypofunction and some of the neurochemical abnormalities observed in schizophrenia, such as a decrease of parvalbumin mRNA expression, that reflect a down-regulation of expression due to decreased activity of GABAergic interneurons. 72 hours after the last PCP injection, brains have been analyzed for CB1 receptor binding and CP-55,940-stimulated [^{35}S]GTPγS binding. Our results show that CB1 receptor levels are not modified by the pharmacological treatment but significant alterations in the coupling to G proteins are found in specific cerebral areas such as prefrontal cortex (-23%), globus pallidus (+71%), hippocampus (-34%) and substantia nigra (-28%), areas involved in the control of motor, emotional and cognitive states.

On the animals exposed to sub-chronic PCP, we then performed behavioural tests such as locomotor activity, object recognition and sucrose preference. PCP treated rats showed increased stereotyped behaviour, whereas any alterations in locomotor activity and sucrose preference were observed. Moreover, PCP treated animals showed an impairment in the object recognition test, a popular protocol to study learning and memory, suggesting the presence of altered cognitive function, a typical sign of schizophrenia. When PCP rats were co-treated with a low dose of THC (0.5 mg/kg, daily for three weeks) that per se was not able to induce any significant alterations in memory parameters, a worsening in cognitive parameters was found. Co-treated animals showed an CB1 receptor functionality that only partially overlapped the picture produced by PCP alone.

Taken together, our findings suggest that altered cannabinoid signalling is present in animal models of schizophrenia. Whether this alteration is produced by schizophrenia or able per se to favour schizophrenic-like symptoms is still a matter of speculation.

CYTOTOXIC EFFECT OF TETRAHYDROCANNABINOL ON MOUSE NEUROBLASTOMA CELLS

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Tetrahydrocannabinol (THC), a major psychoactive constituent of marijuana, exerts cytotoxicity in cultured hippocampal and cortical neurons. These neurotoxicities have been suggested to be attributed to generation of free radicals by cyclooxygenase in hippocampal neurons[1] and the activation of c-Jun N-terminal kinase (JNK) in cortical neurons[2]. However, a mechanism underlying THC cytotoxicity in neuroblastoma has not been fully understood. In this study, we examined cytotoxic potential of Δ^8-THC in mouse neuroblastoma C-1300N18 (N18) cell line. Real-time polymerase chain reaction analysis using primers specific for CB_1 or CB_2 receptors indicated that N18 cells expressed the mRNA for CB_1 receptor (0.464 copies/copy of β-actin mRNA) but not CB_2 receptor (< 0.001 copies/copy of β-actin mRNA). Δ^8-THC induced the cell death in a concentration-dependent and exposure time-dependent manner, as assessed by the MTT assay. Fifty % cytotoxic concentrations for 1, 3 and 6 hr exposures to Δ^8-THC were 9.64, 5.12 and 4.25 μM, respectively. Fluorescence microscopic analysis by staining with Hoechst 33258 indicated that Δ^8-THC (5 μM, 3 hr) caused the nuclear fragmentation in N18 cells. Furthermore, z-DEVD-fmk (100 μM), a caspase-3 inhibitor, significantly prevented Δ^8-THC-induced cell death ($p < 0.01$), as assessed by the MTT assay. In addition, the cytotoxicity induced by Δ^8-THC (5 μM, 3 hr) was significantly suppressed by the addition of AM251, a CB_1 receptor antagonist (2 μM, $p < 0.001$), and forskolin, an adenylate cyclase activator (20 μM, $p < 0.001$). By contrast, SP600125, a JNK inhibitor (5 μM), did not affect the cytotoxic effect of Δ^8-THC. These results suggest that the cytotoxicity of Δ^8-THC in neuroblastoma N18 cells may be induced by the activation of CB_1 receptor, but its underlying mechanism appears to be different at least in part from that in cortical neurons.

Acknowledgements: Mouse neuroblastoma C-1300N18 cell line was provided from Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). This work was funded by the Academic Frontier Project for Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2005-2009) and a Grant-in-Aid for Young Scientists from the Ministry, Education, Science, Sports, and Technology of Japan.

1. Chan G. *et al.*, *J. Neurosci.*, 18, 5322-5332 (1998).
2. Downer E. J. *et al.*, *Br. J. Pharmacol.*, 140, 547-557 (2003).

BEHAVIOURAL EFFECTS OF CHRONIC TREATMENT WITH CANNABINOID RECEPTOR AGONISTS HU210 AND Δ^9-TETRAHYDROCANNABINOL IN A TRANSGENIC MOUSE MODEL OF HUNTINGTON'S DISEASE

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The endogenous cannabinoid neurotransmitter system has broad ranging neurological and peripheral activity, and has been implicated in the pathology of several diseases, one of which is Huntington's disease (HD). Loss of cannabinoid CB1 receptors from the basal ganglia is one of the earliest neurochemical alterations observed in HD. In cultured cells expressing the mutated huntingtin protein, cannabinoids have shown neuroprotective effects and cannabinoids have been evaluated for therapeutic potential in lesion models of HD, but never in transgenic HD mice. In the present study, R6/1 transgenic HD mice were treated daily from 12 weeks of age, for a period of 8 weeks with either the full agonist HU210 (0.01 mg/kg, i.p.), or the partial agonist Δ^9-tetrahydrocannabinol (10 mg/kg, i.p.). Age-matched wild-type littermates also received treatment, while control groups received vehicle alone. Mice were evaluated for motor and cognitive behaviour changes throughout the course of treatment, with behavioural tests including the accelerating rotarod, Y-maze, rear paw clasping and locomotor cells. While both drugs were administered at doses with therapeutic effectiveness in other murine CNS disease models, neither cannabinoid significantly altered HD progression. Both compounds resulted in acute behavioural responses, demonstrating that they targeted the brain at appropriate concentrations. Mice treated with HU210 experienced a significant increase in the number of seizures upon handling, suggesting the dose to be sufficient for receptor activation but potentially in excess of a therapeutic level. While this study suggests HU210 and THC at these doses are ineffective for HD treatment in these transgenic mice, further studies are required to determine the efficacy of other drugs or dosage regimes that target cannabinoid signalling.

LACK OF CHRONIC EFFECT OF LEVODOPA AND BROMOCRIPTINE N CEREBRAL CANNABINOID TYPE 1 RECEPTORS: AN IN VIVO MICROPET STUDY

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Introduction**:** Several evidences suggest a tight association between the cannabinoid system and brain dopaminergic circuits involved in movement disorders. The aim of this study was to investigate and quantify the in vivo chronic effect of the commonly used anti-parkinsonian drugs, levodopa and bromocriptine on cannabinoid-type 1 receptor (CB1R) binding using a novel high-affinity and subtype-selective PET radioligand [^{18}F]-MK9470 (Merck Inc, USA).

Method: Six adult healthy Wistar rats (female, body weight 205-266g) were investigated in baseline condition and after chronic (during 1 week daily) exposure to levodopa (6mg/kg IP; 1.5mg/kg carbidopa IP) and bromocriptine (4mg/kg IP). Acquisitions were conducted on a FOCUS 220 system after 50 mg/kg pentobarbital IP anesthesia, and using 18 MBq of ^{18}F-MK9470 (60 min dynamic). Images were reconstructed using filtered back projection, anatomically standardized to Paxinos space and analyzed by voxel-based statistical parametric mapping (SPM2) using paired t-tests.

Results: No absolute changes in ^{18}F-MK9470 binding were found upon chronic exposure to levodopa and bromocriptine (all $p_{height}>0.001$ uncorrected). However, regional relative ^{18}F-MK9470 binding was significantly increased in the hippocampus (peak average value 6.4%, $p_{height}<0.001$ uncorrected, $p_{cluster}<0.0001$) and the cerebellum (peak average value 9.7%, $p_{height}<0.001$ uncorrected, $p_{cluster}<0.0001$) after bromocriptine administration, while levodopa induced no changes in relative ^{18}F-MK9470 binding.

Conclusion: Chronic exposure to the anti-parkinsonian drugs levodopa and bromocriptine induces no major changes in the cerebral CB1R availability. These in vivo findings suggest that chronic use of levodopa and bromocriptine will not interfere with human PET imaging using [^{18}F]-MK9470.

Research Support: This work was supported by the Research Council of K.U.Leuven (OT/05/58). KVL is Senior Clinical Investigator of the Flemish Fund of Scientific Research.

CB1 CANNABINOID RECEPTORS IN AN IN VITRO MODEL OF HUNTINGTON'S DISEASE

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In human HD, loss of the CB1 cannabinoid receptor from medium spiny projection neurons of the striatum occurs prior to other receptor disturbances (Glass et. al 2000). However, environmental enrichment, which delays the loss of CB1 receptors, also slows motor deterioration in an R6/2 mouse model of HD (Glass et. al 2004). Thus, loss of the CB1 receptor may be critical for Huntington's Disease pathology. We have developed an *in vitro* model of Huntington's Disease by transfection of either 25 (control) or 97 (HD) glutamine-repeat N-terminal huntingtin under inducible control. Using the Alamar Blue assay, we find that neither transfection of these cells with human CB1 receptor, nor the manipulation of CB1 signal transduction with HU210, WIN55212-2, or SR141716A, significantly affects the cytotoxicity profile associated with mutant huntingtin expression. We demonstrate, however, using ELISA, that CB1 cannabinoid receptors are lost rapidly from the cell surface following expression of mutant huntingtin. Confocal analysis suggests that CB1 receptors also colocalize with huntingtin, which may function as a trafficking adapter for the receptor. Thus while this model system may not be appropriate for investigating the efficacy of cannabinoids in treating HD cell death, it may provide a useful tool for determining the mechanism by which receptors are lost in human HD.

THE ENDOCANNABINOID SYSTEM IS ACTIVATED IN RESPONSE TO SPINAL CORD INJURY IN RATS

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Most human spinal cord injuries result from traumas that fracture or dislocate the spinal column. Some of them produce penetrating wounds but the majority result in transient compression or contusion of the spine. Endocannabinoids have been shown to act as neuroprotective and immunomodulatory mediators after lesions of the nervous system. Moreover, the production of endocannabinoids is activated "on demand" following to many types of damage, including chronic constriction injury of the sciatic nerve in rats (Petrosino et al., *Neuropharmacology*, 2007). In the present study, we have explored the response of the endocannabinoid system following spinal cord injury. With this purpose, we submitted male Wistar rats to laminectomy of the 8th thoracic vertebra (sham-operated animals) or to moderate spinal cord contusion (lesioned animals) and sacrificed them at different time points after lesion. Using isotope dilution-liquid chromatography-mass spectrometry we found in lesioned animals a several-fold and time-dependent elevation of the spinal cord concentrations of the endocannabinoids, anandamide and 2-arachidonoylglycerol. The spinal cord concentrations of the anti-inflammatory anandamide congener, and cannabinoid receptor-inactive compound, palmitoylethanolamide were also dramatically elevated. In addition, we have studied the modulation of cannabinoid receptors by Western Blotting and quantitative PCR. Our results show that a spinal cord contusion activates the endocannabinoid system in rats and points to this endogenous signalling system as a target for future studies related to the pathology and therapeutic treatment of this problem.

Acknowledgements: [1] Funded by grants from the Fondo de Investigaciones Sanitarias, Spain (04/2120) and from the Consejería de Sanidad de la Junta de Comunidades de Castilla-La Mancha (06054-00). [2,3]Partly supported by Epitech, S.r.l.

PRO-APOPTOTIC ACTIONS OF THE CB1 RECEPTOR ANTAGONIST AM251 IN CULTURED CEREBELLAR GRANULE CELLS

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Endocannabinoids are though to play an important role in neuronal function, including modulation of synaptic transmission, neurite outgrowth and cell fate. In this study we have examined the effects of altered cannabinoid receptor signaling on neuronal morphology and survival in cerebellar granule cells at different stages in culture (CGCs).

CGCs were cultured from animals at P7 and maintained in culture medium supplemented with 20mM KCl in a humidified atmosphere at 37ºC. Young (1DIV) and mature (7DIV) CGCs were treated with cannabinoid ligands and then stained for axonal (GAP43) and dendritic (MAP2) markers. Cell viability was also assessed using a propidium iodide stain that binds fragmented DNA and a TUNNEL assay to highlight dead and/or dying cells. Finally, Caspase-3 labeling was used as a marker for the induction of apoptotic pathways. Overnight treatment of cells with the CB1 receptor antagonist AM251 (10uM) at 1DIV resulted in axonal and dendritic retardation, and an increased incidence of propidium iodide and TUNNEL positive cells, together with elevated Caspase-3 staining. However, treatment with a CB2 receptor antagonist AM630 (10uM) or vehicle (1:1000 DMSO) did not alter neurite outgrowth or influence cell viability. Interestingly, in mature cultures (following synapse formation), treatment with AM251 had no clear effect on CGC morphology and viability.

These results suggest that the endocannabinoid system is important in the early development of rat cerebellar granule neurons and by interfering with the CB1 receptor activation we have induced an apoptotic event in the young, but not older cells. This has implications in the therapeutic use of CB1 antagonists where they may interfere with normal neuronal development.

Supported by the MRC

REDISTRIBUTION OF THE CB1 RECEPTOR FROM GABAERGIC TO GLUTAMATERGIC SYNAPSES DURING THE EPILEPTOGENIC PHASE FOLLOWING PILOCARPINE-INDUCED STATUS EPILEPTICUS

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It has been well established that cannabinoids exhibit anticonvulsant properties through their interaction with the CB1 receptor (Wallace et al, 2001, *Eur J Pharmacol*; Marsciano et al, 2003, *Science*). There is growing evidence that the endocannabinoid system regulates excitatory and inhibitory synaptic transmission during epilepsy. Our lab has recently shown that a long term and permanent redistribution of hippocampal CB1 receptors occur in the epileptic rat (Wallace, et al, 2003, *JPET*; Falenski et al, 2007, *Neuroscience*). Prior to the establishment of epileptic seizures in the pilocarpine model of acquired epilepsy, a 1 to 4 week quiescent (seizure-free) period follows status epilepticus (SE) when epileptogenesis takes place. During epileptogenesis, a number of plasticity changes take place which cause the transformation of a normal brain to epileptic following an injury. Using immunofluorescent staining, this study examines the expression of the CB1 receptor at inhibitory (GABAergic) and excitatory (glutamatergic) synapses during the epileptogenic phase following pilocarpine-induced SE.

Rats were perfused with 4% paraformaldehyde at various timepoints following pilocarpine-induced SE. Timepoints of interest included 1 week, 1 month, and 4 months post-SE. Following perfusion, brains were removed and processed for cryosectioning. Immunofluorescent co-localization analysis was performed on naïve and post-SE brains with a specific antibody to the CB1 receptor in combination with antibodies to the vesicular glutamate transporter 1 (VGLUT1) or the vesicular GABA transporter (VGAT). These transporters were used as markers for CB1 expression at excitatory and inhibitory synapses respectively. In naïve tissue, CB1 receptor expression is prominent on inhibitory synaptic terminals in the stratum pyramidale of the CA1 and CA3 regions of the hippocampus as shown by co-localization with VGAT. At 1 week post-SE, CB1 receptor expression is decreased throughout the hippocampus compared to naïve tissue. At 1 month post-SE, CB1 receptor co-localization with VGLUT1 begins to increase, and by 4 months post-SE, there is a marked increase of CB1 receptor expression on excitatory synaptic terminals. Therefore, this redistribution of CB1 receptor expression from inhibitory synapses to excitatory synapses during epileptogenesis allows for a better understanding of the role of the endocannabinoid system during the development of epilepsy.

ALTERATION OF THE LYMPHOCYTE ENDOCANNABINOID SYSTEM AS A POTENTIAL BIOMARKER OF HUNTINGTON'S DISEASE

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Introduction: The search for peripheral markers of neurodegenerative diseases aims at identifying molecules that could help in monitoring the effects of future therapeutics in easily accessible cells. Here we focused on the involvement of the endocannabinoid system in Huntington's disease (HD).

Materials and Methods: We isolated peripheral lymphocytes from HD patients and healthy controls. Then, we analyzed the activity of the fatty acid amide hydrolase (FAAH), the enzyme that degrades the endocannabinoid anandamide (AEA), through RP-HPLC and FAAH protein content through ELISA and Western Blot. For cannabinoid receptor studies, the membrane fractions were used in rapid filtration assays with the synthetic cannabinoid [^{3}H]CP55,940. Moreover, endogenous AEA levels were quantified through FL-HPLC.

Results: We found that the FAAH activity was dramatically decreased (down to less than 10%) in HD compared to healthy subjects. Concomitantly, the endogenous levels of AEA were ~6-fold higher in HD *versus* healthy lymphocytes, while the other elements of the endocannabinoid system were not affected by HD. Low FAAH activity in HD lymphocytes was not due to down-regulation of protein expression, but rather to blockage of enzyme activity by a cytosolic and irreversible inhibitor. Finally, pre-HD patients showed defective FAAH activity, as did the brain of HD patients compared with healthy controls.

Conclusion: Taken together, our data indicate that FAAH activity in HD lymphocytes mirrors metabolic changes which take place in the brain, and is a robust, non-genetic peripheral marker of HD.

LEVELS OF ENDOCANNABINOIDS AND RELATED SIGNALING LIPIDS IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's Disease is (HD) is an inherited, autosomal dominant condition caused by an expanded trinucleotide (CAG) repeat resulting in degeneration of the striatum and corticostriatal pathway. Growing evidence suggests a role for the endogenous cannabinoid system in the progression of HD. There is consistent evidence that CB1 receptors are down-regulated in the striatum in HD animal models as well as in humans. Here, using a recently developed mouse line with 140 CAG repeats knocked-in to the huntingtin mouse gene (KI) we measured the production of endocannabinoids and related signaling lipids in WT and KI mice at the time of symptom onset (26-27 weeks of age).

Striatum and cortex were dissected from 8 WT and 8 KI mice and flash frozen until lipid extractions, which were performed with 20 volumes of methanol and sonication. Lipids were further purified on C8 solid phase extraction columns. Rapid separation of analytes was obtained using 10 µl injections of the 100% methanol elution onto a C8 reversed phase column. Mass spectrometric analysis was performed with a triple quadrupole mass spectrometer using electrospray ionization. Levels of each compound were analyzed by multiple reactions monitoring (MRM) on the LC/MS/MS system. The levels of production of each lipid were quantified as moles per gram tissue.

Levels of anandamide (AEA), *N*-arachidonoyl glycine (NAGly), 2-arachidonoyl glycerol (2-AG), and 1-stearoyl-2-arachidonoyl-sn-glycerol (the 2-AG precursor; DAG) were significantly increased in KI striatum (see table) perhaps driving the down-regulation of CB1 recpetors. Whereas the levels of DAG in the cortex also increased in KI mice, levels of 2-AG and *N*-palmitoyl glycine significantly decreased. The levels of AEA and NAGly in cortex were unchanged in KI mice (see table). These data provide further evidence that the endogenous cannabinoid system plays a role in the pathophysiology of HD.

	Striatum WT	Striatum KI	Cortex WT	Cortex KI
DAG	5.1E-10±4.6E-11	**8.2E-10±1.4E-10***	3.4E-10±4.4E-11	**6.3E-10±1.1E-10***
2-AG	9.3E-09±6.4E-10	**1.3E-08±6.1E-10***	4.8E-09±2.1E-10	**4.1E-09±1.6E-10***
AEA	3.1E-11±5.3E-12	**8.0E-11±3.3E-12***	3.62E-10±1.03E-11	3.611E-10±1.269E-11
PEA	8.9E-10±1.0E-10	1.1E-09±3.7E-11	9.9E-10±3.7E-11	9.9E-10±1.0E-10
NAGly	7.2E-11±6.3E-12	**1.2E-10±5.3E-12***	4.87E-11±4.7E-12	4.98E-11±4.62E-12
PALGly	4.2E-10±3.5E-11	3.4E-10 ±2.9E-11	5.8E-11±1.1E-11	**2.94E-11±3.3E-12***

Acknowledgements. The authors are grateful for the support of the National Institute on Drug Abuse (DA-018224, DA-020402, F32-DA-016825), NIH (NS 35663), NSF (2004016218), the Gill Center for Biomolecular Science, Indiana University, Bloomington, and the Lilly Foundation Inc, Indianapolis, IN.

CYTOKINE REGULATION OF CANNABINOID RECEPTORS 1 & 2: A MULTIPLE SCLEROSIS PERSPECTIVE

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Introduction: Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) white matter which is characterized by focal T cell and macrophage infiltrates, demyelination, axonal injury and loss of neurological function. Various cytokines which drive inflammatory responses are secreted by T cells and macrophages and are thought to be key mediators of the autoimmune attack against CNS myelin. Elevated mRNA and protein levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), have been detected in MS lesions, cerebrospinal fluid (CSF) and peripheral blood monocytes. Interestingly, the activation of cannabinoid receptors 1 and 2 has profound effects, mostly inhibitory, on cytokine production and strongly appears to reduce symptoms associated with MS. Although these inhibitory actions are thought to be mediated through neuro- and immunoregulatory mechanisms, few have investigated the regulation of these receptors in MS. Hence, this study first examined the expression level of these two cannabinoid receptors and compared it to that of pro-inflammatory cytokines in MS blood. Secondly, hypothesizing that cytokine increase in MS enhances CB1 and CB2 mRNA, we investigated the effects of cytokines on CB1 and CB2 expression in blood and peripheral blood mononuclear cells (PBMCs).

Method: 1) RNA was extracted from whole blood donated by normal (n=38: 26 females; 12 males) and MS (n=44: 29 females; 15 males; which included 6 secondary progressive MS; 38 relapsing-remitting MS) subjects. CB1, CB2, IL-6, IL-1β, and TNF-α mRNA from each sample was then measured and compared by quantitative reverse transcriptase PCR (RT-PCR). 2) PBMCs that were isolated from heparinised whole blood and untreated whole blood from normal subjects (n=10) were stimulated with the three pro-inflammatory cytokines and incubated for 18hrs at 37° C (5% CO2). RNA extraction, RT-PCR and protein analysis effectuated through flow cytometry using indirect staining methods for CB1 and CB2 expression were also performed.

Results: The cannabinoid receptors and cytokines mRNA were found to be significantly up-regulated in MS blood when compared to that of normal subjects. Moreover, the cytokine-stimulated normal PBMCs and whole blood expressed distinctive increases in CB1 and CB2 mRNA and protein levels as opposed to non-stimulated media. However, cytokine-induced CB2 mRNA was more elevated than CB1 in PBMCs and whole blood.

Conclusion: The parallel up-regulation of pro-inflammatory cytokines and cannabinoid receptors in MS blood suggests a role for cytokines not only in the pathogenesis of MS but also in the regulation of cannabinoid receptors. Such regulation may therefore be induced as a protective mechanism against pro-inflammatory cytokine actions that may have detrimental effects as observed in MS.

MICE LACKING α-SYNUCLEIN OR BEARING A MUTATED FORM OF HIS PROTEIN HAVE LOW LEVELS OF CB_1 RECEPTORS IN THE BASAL GANGLIA

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Recent observations have suggested that losses and/or malfunctioning of the cannabinoid signaling system, mainly at the level of the central CB_1 receptor, may be an early event in the pathogenesis of different chronic neurodegenerative disorders, including Huntington's chorea [Lastres-Becker et al., Brain Res. 929, 236-242 (2002)] and Parkinson's disease [González et al., Brain Res. 1046, 195-206 (2005)]. These losses might trigger excitotoxicity, inflammation or other cytotoxic events that are normally under the control of CB_1 receptors. In the present study, we wanted to examine the status of CB_1 receptors in the basal ganglia in two models affecting the *PARK-1* (α-synuclein) gene, that have been associated with early parkinsonism: (i) mice deficient in the α-synuclein gene [Cabin et al., J. Neurosci. 22, 8797-8807 (2002)], and (ii) mice overexpressing a mutated form of α-synuclein [Gispert et al., Mol. Cell. Neurosci. 24, 419-429 (2003)]. These mice exhibited progressive changes of dopamine levels in the nigrostriatal pathway and of spontaneous motor activity, while no evidence for protein aggregation or neurodegeneration was observed. They also exhibited a significant reduction of mRNA levels for the CB_1 receptor in the striatum, in parallel to marked reductions in the number of binding sites in the globus pallidus and, in particular, the substantia nigra. Interestingly, the down-regulatory responses found for CB_1 receptors in both models were more marked in the case of mice lacking α-synuclein than in the mice overexpressing a mutated form of this protein. It is important to remark that these receptor losses occurred in a situation where dopaminergic dysfunction rather than neuronal death is the major event that takes place. Therefore, one may consider that CB_1 receptor losses would not require a massive destruction of dopamine neurons in the substantia nigra to be expressed, thus stressing the notion that this might be an early event presumably involved in the pathogenic process.

Acknowledgements: Supported by a grant from MCYT SAF2006-11333

AGONIST-INDUCED DOWNREGULATION OF THE CB_1 RECEPTOR RESULTS IN THE EXPRESSION OF STATUS EPILEPTICUS-LIKE ACTIVITY IN THE HIPPOCAMPAL NEURONAL CULTURE MODEL OF ACQUIRED EPILEPSY

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Previous studies from our laboratory have shown that exogenous cannabinoids infer CB_1-receptor dependent anticonvulsant activity in both in vivo and in vitro models of status epilepticus (SE) and acquired epilepsy (AE) (Blair et al., 2006, *JPET*, 317(3),1072-78; Wallace et al., 2003, *JPET*, 307(1), 129-37), and that the endocannabinoid system acts to tonically regulate epileptic seizure discharge and termination through CB_1 receptor activation (Deshpande et al., 2007, *Neur. Lett*, 411, 11-16). Although acute cannabinoid treatment has been shown to infer CB_1-receptor dependent anticonvulsant activity, little is known concerning the effects of prolonged exposure to CB_1 agonists on the epileptic phenotype. Tolerance to cannabinoids has been shown to be associated with a number of changes in neuronal synaptic transmission, some of which include loss of LTP, hyperexcitability and decreased seizure threshold. This study was carried out to evaluate the effects of prolonged exposure to CB_1 agonists on seizures in an in vitro model of AE.

The hippocampal neuronal culture model of low-Mg^{++} induced spontaneous recurrent epileptiform discharges (SREDs) was utilized. Hippocampal cultures were exposed to Mg^{++}-free media for 3 hours resulting in the permanent expression of SREDs. Following exposure to Mg^{++}-free media, cultures were returned to maintenance media containing 1 μM of either WIN 55,212-2 (+WIN) or WIN 55,212-3 (-WIN) for 24 hours. Parallel control groups were carried which included non-epileptic cultures treated with either +WIN or –WIN. Cultures were evaluated immunocytochemically for membrane CB_1 receptor expression and electrophysiologically using whole-cell current-clamp analysis.

Exposure to +WIN (1 μM) for 24 hours resulted in a dramatic decrease in immunocytochemical staining of surface CB_1 receptor expression in both control and epileptic (SREDs) cultures, while no change in CB_1 receptor expression was observed in cultures exposed to 24 hours of –WIN. In epileptic cultures, the downregulation of CB_1 receptor expression by +WIN resulted in the induction of continuous high-frequency (SE-like) seizure discharges, while –WIN treated epileptic cultures showed no change from the SRED activity observed in this model. Downregulation of CB_1 receptor expression by +WIN treatment in control, non-epileptic, cultures did not produce SE-like activity or hyperexcitability. The results from this study further substantiate a role for a tonic CB_1 receptor-dependent endocannabinoid regulation of seizure discharge and suggest that prolonged exposure to cannabinoids may result in exacerbation of seizure activity in the epileptic phenotype.

POTENTIAL ANTI-INFLAMMATORY ACTIONS OF THE ELMIRIC (LIPOAMINO) ACIDS

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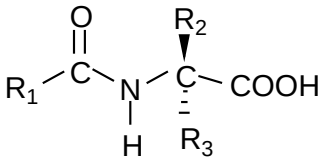
A library of amino acid-fatty acid conjugates (elmiric acids) was synthesized and evaluated for activity as potential anti-inflammatory agents. The compounds were tested *in vitro* for their effects on cell proliferation and prostaglandin production and compared with their effects on *in vivo* models of inflammation. LPS stimulated RAW 267.4 mouse macrophage cells was the *in vitro* model and phorbol ester-induced mouse ear edema served as the principal *in vivo* model. The prostaglandin responses were found to be strongly dependent on the nature of the fatty acid part of the molecule. Polyunsaturated acid conjugates produced a marked increase in media levels of immunoreactive 15-deoxy-PGJ_2 with minimal effects on PGE production. It is reported in the literature that prostaglandin ratios in which the J series predominates over the E series promote the resolution of inflammatory conditions. Several of the elmiric acids tested here produced such favorable ratios suggesting that their potential anti-inflammatory activity occurs *via* a novel mechanism of action. The ear edema assay results were generally in agreement with the prostaglandin assay findings indicating a connection between them.

Note: We have defined elmiric acids as compounds that conform to the general structure shown for which a short hand nomenclature system is proposed. Using this system N-arachidonylglycine would be written as: EMA-1 (20:4). EMA stands for elmiric acid; each amino acid constituent is assigned a number, e.g. 1=glycine; 2=alanine, etc. The identity of the acyl substituent is indicated in parentheses; e.g. (20:4)=arachidonoyl; (16:0)=palmitoyl, etc. We are proposing this nomenclature to simplify the naming of these compounds; it has not been approved or adopted by any official body.



Acknowledgments.This publication was made possible by grants DA17969 (SHB) and DA13691 (RBZ) from National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD. Its contents are solely the responsibility of the author and do not necessarily represent the official views of the National Institute on Drug Abuse. We thank Dr. Akbar Ali for obtaining the NMR spectra of several of the elmiric acids used in this report. Thanks are also due to James and Barbara Evans for the precise mass spec data.

FAAH INHIBITION IS NOT THE MAJOR MECHANISM OF ANTIHYPERALGESIA OF URB597

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In recent years there has been an effort to develop drugs that act on the endocannabinoid system, with the goal of avoiding the psychotropic effects of exogenous cannabinoids. A key component of this system is fatty acid amide hydrolase (FAAH), which catalyzes the hydrolysis of the endocannabinoid anandamide to arachidonic acid and ethanolamine (Deutsch, DG and Chin, SA, *Biochem Pharmacol,* (1993) 46:791-796). The objective of the present study was to evaluate the antihyperalgesic activity of URB597, a selective inhibitor of FAAH that has been reported to have analgesic and anti-inflammatory effects (Jayamanne, A, et al., *Br J Pharmacol*, (2006) 147:281-288). URB597 at 30 mg/kg, i.p., significantly attenuated carrageenan-induced thermal hyperalgesia in rats with a response of 175% of baseline. URB597 completely reversed the carrageenan-induced deficit in hindpaw weight bearing. Additionally, URB597 at 30 mg/kg, i.p. reversed mechanical hyperalgesia by 35% at 24 hours after FCA treatment. URB597 also significantly decreased locomotor activity at 30 mg/kg, i.p. URB597 did not produce antinociception in the formalin, writhing, L-5 ligation model of neuropathic pain, incisional pain or hot plate assays at doses as high as 30 mg/kg, i.p. Importantly, URB597 completely inhibited brain FAAH activity at doses as low as 1 mg/kg, i.p., yet significant antihyperalgesia was observed only at a 30-fold higher dose. This suggests a dissociation between inhibition of brain FAAH activity and efficacy in these hyperalgesia models. In conclusion, URB597 displayed NSAID-like activity in several pain models, but only at doses that caused decreases in locomotor activity. Furthermore, inhibition of central FAAH activity does not appear to be the predominant mechanism by which URB597 produced its antihyperalgesic activity.

ACTIVATION OF PERPHERAL CB1 RECEPTORS PRODUCES ROBUST ANALGESIA IN RODENT INFLAMMATORY AND NEUROPATHIC PAIN MODELS

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Cannabinoids show analgesia in man but use is limited by their psychoactive properties. One way to avoid CB1R-mediated central side effects is to develop CB1R agonists with limited CNS penetration. Activation of peripheral CB1Rs is suggested to be analgesic, but what is the relative contribution of peripheral CB1Rs to analgesic effects in chronic pain? We have addressed this by exploring the analgesic properties of two peripherally restricted CB1R agonists: AZ001 and AZ002 in animal pain models. AZ001 and AZ002 are full CB1R agonists with potency to cloned rat CB1R receptor (AZ001 EC50=34 nM, Emax=111% and AZ002 EC50=6.1 nM, Emax=103%). AZ001 and AZ002 exhibit a very low brain:plasma ratio (0.04 ± 0.04 and 0.04 ± 0.02, respectively) as compared with the centrally acting CB1R agonist Win 55-212,2 (brain:plasma ratio of 2.3 ± 1.5). The analgesic properties of AZ001 and AZ002 were assessed in inflammatory and neuropathic pain in rats (carrageenan-induced inflammation and spinal nerve ligation or SNL respectively). Systemic administration of AZ001 (0.3-2.5 μmol/kg s.c.) and AZ002 (1-7 μmol/kg) completely reversed heat hyperalgesia (plantar method) in carrageenan model and mechanical allodynia (MVF, up and down) in SNL model. AZ001 and AZ002 produced comparable in vivo efficacy to Win 55-212, 2 with less observed CNS side effects in both pain models. We tested the specificity of these in vivo effects using the CB1R selective antagonist, rimonabant, and CB1R KO mice. Rimonabant (10 umol/kg i.p., 15 min before AZ001) completely reversed the anti-hyperalgesic effects of AZ001 in the carrageenan model. AZ002 produced robust anti-heat hyperalgesia effects in mice with a Freund's complete adjuvant (FCA)-induced inflammation of the tail that were completely absent in CB1R KO mice. We confirmed that a peripheral action of AZ001 and AZ002 was sufficient for analgesia by local injection into the hind paw. Intraplantar administration of AZ001 or AZ002 (40nmol/rat) significantly attenuated heat-hyperalgesia in rat carrageenan or FCA induced inflammatory pain whereas systemic administration of the same dose had no effect. Our results clearly show that peripherally restricted CB1R agonists are able to produce robust analgesia with much less CNS side effects in animal pain models via peripheral CB1R mechanisms. Peripherally restricted CB1R agonists provide an interesting approach to improve analgesic therapy in chronic pain patients.

LACK OF CHRONIC EFFECT OF PARACETAMOL ON CEREBRAL CANNABINOID-TYPE 1 RECEPTORS: AN IN VIVO MICROPET STUDY

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Introduction: Several lines of evidence suggest that the endocannabininoid system may be involved in analgesia. Recently, Ottani and coworkers (Eur J Pharmacol, 2006) indicated that the analgesic effect of paracetamol may result from activation of the cannabinoid system. We evaluated changes in cannabinoid-type 1 receptor (CB1R) binding using a novel high-affinity and subtype-selective PET radioligand [^{18}F]-MK9470 (Merck Inc, USA) in vivo after chronic administration of paracetamol.

Methods: Nine female Wistar rats (age 3 months) were anesthetized with 50 mg/kg pentobarbital and injected with 18 MBq [^{18}F]-MK9470 in two conditions: at baseline and after chronic treatment with paracetamol (2 weeks daily intraperitoneal injection, 60 mg/kg). Images were acquired dynamically for 60 minutes on a Concorde Focus 220 microPET and were reconstructed using filtered back projection. Parametric standard uptake values (evaluated 40-60 min. postinjection) were anatomically standardized to Paxinos space and analyzed using voxel-based statistical parametric mapping (SPM2) using paired t-tests.

Results: On a group basis, no absolute changes in [^{18}F]-MK9470 binding were found. After chronic paracetamol treatment, regional relative [^{18}F]-MK9470 binding was significantly increased in the cerebellum (peak average value 7.1%, $p_{height}<0.001$ uncorrected, $p_{cluster}<0.005$ corrected).

Conclusions: Chronic i.p administration of paracetamol does not produce major changes in CB1R availability in the rat brain. Although Bmax and Kd cannot be separated from the current experiment and the status of the endocannabinoids were not evaluated, the results of this study suggest that the in vivo mechanism of action of paracetamol does not involve blockade of the CB1R directly. However, the sensitivity of this tracer to changes in endogenous CB1R agonists levels is unknown.

Acknowledgements: KG is Research Assistant and KVL is Senior Clinical Investigator of the Flemish Fund of Scientific Research.

References:
Ottani A., Leone S., Sandrini M., Ferrari A., Bertolini A., 2006. The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors. Eur J Pharm 531, 280-281.

PROTECTIVE ROLE OF ALIAMIDES AND ENDOCANNABINOID SYSYEM DURING λ-CARRAGEENIN-INDUCED GRANULOMA FORMATION

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Granuloma formation is one of the most representative features of chronic inflammation. The inflammatory process is characterized by the release of several pro-inflammatory and pro-angiogenic mediators including IL-1, VEGF, TNF-α and nitric oxide (NO). Cannabinoids (CB) from *Cannabis sativa,* in addition to the well known psychotropic effects, affect the immune system by the activation of CB_1 and CB_2 receptors. Subsequent to CB receptor cloning, endogenous ligands (endocannabinoids), represented by arachidonoylethanolamine or anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG), were also identified. Moreover, a second generation of endogenous substances that interact with the endocannabinoid system, such as palmitoylethanolamine (PEA), have been recognized. Endocannabinoids and PEA exert a wide range of pharmacological activities including anti-inflamatory and analgesic actions. The aim of this study was to investigate the role of the endocannabinoid system in a model of chronic inflammation, granuloma in rats. Granuloma was induced by subcutaneous implantation of two λ-carrageenin-soaked sponges on the back of male Wistar rats. Animals were sacrificed 4 days after implant and tissues around the sponges were weighed. In homogenates from the granulomatous tissues we evaluated: (i) the amounts of the principal endocannabinoid ligands, AEA, 2-AG and PEA by liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry (LC-APCI-MS); (ii) CB_1, CB_2, TNF-α and iNOS protein expression by Western blot analysis; (iii) the infiltration of polymorphonucleated leukocytes (PMN) by myeloperossidase assay, and (iv) the extent of neoangiogenesis by measuring haemoglobin (Hb) content. Arachidonoylserotonin (AA-5-HT), a specific inhibitor of endocannabinoid enzymatic hydrolysis, and PEA were administered locally at time of implantation (t0). Our results show a down-regulation of AEA, 2-AG and PEA levels and an up-regulation of the expression of both CB receptor types in granulomatous tissues compared to tissues from saline-soaked sponges. Treatment with AA-5-HT (25 μM) and PEA (20 μM) significantly inhibited granuloma formation when given at t0. Moreover AA-5HT and PEA reduced iNOS and TNF-α expression, PMN infiltration and Hb content in λ-carrageenin-induced granuloma. The results of the present study underline that in a model of chronic inflammation, i.e. λ-carrageenin-induced granuloma formation, the down-regulation of endocannabinoid ligands might account for the progression of the inflammatory scenario, and that increase of endocannabinoid levels produced by an inhibitor of endocannabinoid degradation, or the treatment with PEA, are able to prevent the inflammatory process and the angiogenesis therewith associated.

Work partly supported by Epitech S.r.l.

INHIBITORY EFFECTS OF SPINAL UCM707 ON EVOKED RESPONSES OF DORSAL HORN NEURONES IN NEUROPATHIC AND SHAM-OPERATED RATS

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Levels of endocannabinoids are increased in the spinal cords of rats following peripheral nerve injury (Petrosino *et al*., (2007) *Neuropharmacology*; 52(2): 415-422). Recent work by our group demonstrated inhibitory effects of the FAAH-inhibitor URB597 in neuropathic rats (Jhaveri *et al.,* (2006) *J.Neurosci.*;26(51):13318-27). An alternative way to modulate levels of endocannabinoids is by inhibiting their uptake with compounds such as UCM707 (López-Rodríguez, M.L. *et al.,* (2001) *J.Med Chem.,* 44: 4505-4508). In Here, effects of a spinal administration of UCM707 on mechanically (10-100g)-evoked responses of dorsal horn (DH) neurones in neuropathic (SNL) and sham-operated rats were studied.

Tight ligation of spinal nerves L5 and L6 was performed in Male Sprague Dawley rats. A control group of rats received sham surgery. In isoflurane anaesthetised rats extracellular single unit recordings of deep convergent DH neurones (laminae VI and VI) were made 14-17 days post-surgery. Peripheral mechanically-evoked responses (von Frey monofilaments of bending forces 10-100g) of spinal neurones were measured. Effects of a spinal administration of UCM707 (10, 50 and 100µg/50µl) or vehicle (3% Tween80 in saline) on mechanically-evoked responses of spinal neurones were studied at 10 min intervals for 60 min. The contribution of the CB_1 receptor to the effects of UCM707 was studied with the CB_1 receptor antagonist AM251 (1µg/50µl).

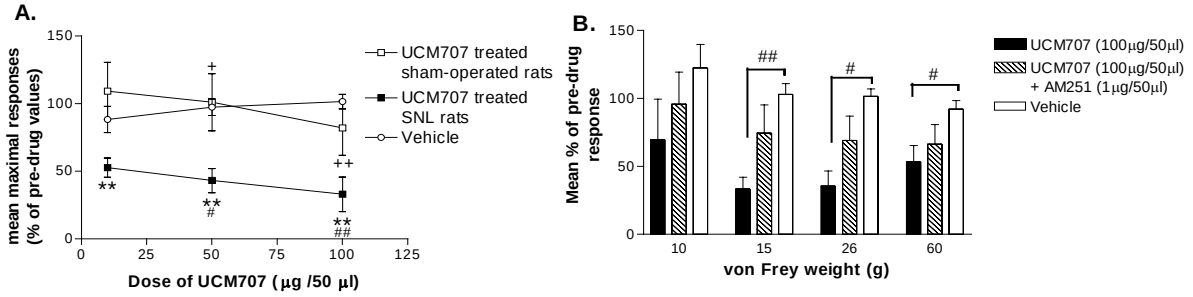

Figure A. Effects of UCM707 (10-100µg/50µl) or vehicle on 26g-evoked responses of dorsal horn neurones in SNL and sham-operated rats. Statistical comparisons between effects of UCM707 and vehicle was performed using a 2-way ANOVA with a Bonferroni post hoc test. #p<0.05, ##p<0.01. Statistical analysis comparing effects of UCM707 or vehicle to pre-drug values was performed using a one way ANOVA with dunnet's post hoc test. **p<0.01 (SNL), +p<0.05, ++p<0.01 (sham). Figure B. Effects of vehicle or UCM707 (100g/50µl) alone or following pre-treatment with AM251 (1µg/50µl) on mechanically-evoked responses of dorsal horn neurones in SNL rats. Data are expressed as mean maximal effect ± SEM.

Spinal administration of UCM707 (10-100µg/50µl) significantly inhibited mechanically-evoked responses of DH neurones in SNL, but not sham-operated rats compared to pre-drug controls. Effects of the highest dose of UCM707 (100µg/50µl) on high-weight (26-100g) evoked responses in SNL rats were significant, compared to vehicle (Figures A and B). Mean maximal inhibitory effects of UCM707 were observed at 30-40 min post drug administration. Effects of UCM707 (100µg/50µl) on mechanically (10-100g)-evoked responses were not significantly blocked following pre-treatment with the CB_1 antagonist AM251 (Figure B).

These data suggest there is a greater endogenous cannabinoid tone in neuropathic rats compared to sham-operated rats. These differences in effects of UCM707 between sham-operated rats and neuropathic rats suggest there is a functional plasticity of the endocannabinoid tone following peripheral nerve injury.

EFFECTS OF INHIBITION OF MICROGLIA ON NOCICEPTION AND LEVELS OF ENDOCANNABINOIDS IN THE SPINAL CORD OF NEUROPATHIC RATS

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Microglia provide support and protection to neurons in the central nervous system, and are rapidly activated in response to nerve injury. This process involves morphological, immunophenotypic and functional responses, including the release of pro-inflammatory cytokines, endocannabinoids (ECs) and the upregulation of CB_2 receptors. Inhibition of microglia using the tetracycline derivative, minocycline, attenuates the development of hyperalgesia and allodynia in animal models of neuropathic pain (Raghavendra, V. et al. (2003) J Pharmacol Exp Ther. 306(2):624-30; Zanjani, T.M. et al. (2006) Eur J Pharmacol. 538(1-3):66-72). The aim of this study was to determine the effects of minocycline on mechanical allodynia (touch-evoked pain behaviour) and levels of ECs in the spinal cord of spinal nerve ligated (SNL) rats.

Male Sprague-Dawley rats (80-90 g) received intra-peritoneal injection of minocycline (30 mg/kg) or vehicle (sterile water) 1 hour prior to SNL (tight ligation of L5 and L6 spinal nerves) or sham-surgery (day 0). Rats were then dosed with minocycline daily until day 14. Development of mechanical allodynia was assessed using von Frey hairs to determine withdrawal threshold on days 1, 3, 5, 7, 10 and 14. On day 15, rats were killed by stunning and decapitation and the hindpaw tissue was dissected rapidly onto dry ice. Samples were stored at -80^{o}C until analysis. The ECs AEA, 2-arachidonyl glycerol (2-AG) and palmitoyl ethanolamide (PEA) were extracted and levels were quantified using a targeted liquid chromatography- tandem mass-spectrometry approach, based on a published method Richardson, D. et al., (2007) Anal Biochem 360(2): 216-26).

		AEA (pmol/g)	2-AG (nmol/g)	PEA (nmol/g)
Veh-SNL	Ipsi	65.86±6.9 **	52.21±4.5	4.20±0.39 **
	Contra	28.42±0.9	54.40±4.0	8.80±1.0
Mino-SNL	Ipsi	116.9±22.8 ** $^{\phi}$	4.53±0.9 **$^{\phi\phi}$	31.59±5.6 ** $^{\phi\phi}$
	Contra	53.72±2.3	47.66±4.4	5.01±0.5

Figure 1. Effects of minocycline (mino, 30 mg/kg) or vehicle (veh) on levels of AEA, 2-AG and PEA in the ipsilateral (ipsi) and contralateral (contra) spinal cord of SNL rats. Data are expressed as mean ± SEM and were analysed using non-parametric Mann-Whitney test,** $P < 0.01$ vs contralateral values $^{\phi}$ $P < 0.05$, $^{\phi\phi}$ $P < 0.001$ ipsilateral Mino-SNL *vs* ipsilateral veh-SNL.

Levels of AEA were elevated in the ipsilateral spinal cord of SNL rats, compared to contralateral spinal cord, whereas levels of PEA were decreased compared to contralateral spinal cord. Minocycline increased levels of AEA and PEA in the ipsilateral spinal cord, compared to the contralateral spinal cord, and compared to vehicle treated SNL rats. Although levels of 2-AG which were not altered by SNL surgery, they were significantly reduced in the ipsilateral spinal cord of minocycline treated SNL rats.

CHANGES IN ENDOCANNABINOID SIGNALING IN DORSAL ROOT GANGLION NEURONS IN A MODEL OF CANCER PAIN

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Anandamide (AEA) signaling is terminated by its hydrolysis by the intracellular enzyme fatty acid amide hydrolase (FAAH). Here we determined the contribution of FAAH in modulating inhibitory effects of AEA in control and cancer related pathology. In a novel in vitro model, isolated adult murine dorsal root ganglion (DRG) neurons were cultured alone or co-cultured with fibrosarcoma cells for 48 hr. Responses of DRG neurons to AEA were assayed by measuring inhibition of Ca^{2+} transients evoked by depolarization with 50 mM KCl. AEA (10 nM) inhibited evoked Ca^{2+} transients in the majority of small DRG neurons in control conditions. Higher concentrations of AEA were ineffective unless neurons were treated with URB 597, an inhibitor of FAAH. Similarly, methanandamide, which is resistant to hydrolysis by FAAH, mimicked the effect of AEA when used at a concentration of 10 nM but had a greater inhibitory effect at a higher concentration. Interestingly, when DRG neurons were co-cultured with fibrosarcoma cells, AEA had a greater inhibitory effect and the change was accompanied by decreased intensity and distribution of FAAH-immunoreactivity. Importantly, results in vitro paralleled data generated in vivo. Whereas peripheral administration of AEA into the hind paw had no analgesic effect in an assay for mechanical nociception, intraplantar administration of AEA ipsilateral to the tumor in a model of cancer pain was anti-hyperalgesic. Importantly, the hyperalgesia was accompanied by decreased FAAH-immunoreactivity in DRG neurons ipsilateral to the tumor. Together these data demonstrate that fibrosarcoma cells modify the endocannabinoid metabolism of DRG neurons and suggest that supplementing the production of peripheral endocannabinoids may be beneficial in the treatment of cancer-related pain. Supported by DA11471, CA091007, and a grant from the Academic Health Center of the University of Minnesota.

GROUP I AND GROUP III METABOTROPIC GLUTAMATE RECEPTORS REGULATE ENDOCANNABINOID-MEDIATED STRESS-INDUCED ANALGESIA

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In previous work we showed that a nonopioid form of stress-induced analgesia (SIA) is mediated by endogenous cannabinoids such as 2-arachidonylglycerol (2-AG) in the midbrain periaqueductal gray (Hohmann et al. (2005) *Nature* 435: 1108-1112). However, the mechanisms underlying mobilization of 2-AG *in vivo* remain unknown. The present studies were therefore conducted to further examine the role of 2-AG in SIA. 2-AG is synthesized *in vitro* through the consecutive activation of two distinct enzymes–phospholipase C (PLC) and diacylglycerol lipase (DGL). First, diacylglycerol (DAG), the 2-AG precursor, is formed from PLC-mediated hydrolysis of membrane phospholipid precursors. DAG is subsequently hydrolyzed by DGL to form 2-AG. We examined whether activation of group I metabotropic glutamate receptors (mGluRs), which are positively coupled to PLC, would enhance SIA. Microinjection of the group I mGluR agonist DHPG into the dorsolateral PAG (dPAG) enhanced SIA through a CB_1-dependent mechanism. Blockade of group I mGluR5 with MPEP, but not group I mGluR1 with CPCCOEt, suppressed SIA following microinjection into the same site. The group I mGluR antagonists failed to suppress SIA following microinjection of the same doses deliberately off-site. We also evaluated whether pharmacological inhibitors of DGL would suppress SIA. Microinjection of either the selective DGL inhibitor tetrahydrolipstatin (THL) or the nonselective DGL inhibitor RHC80267 into the dPAG suppressed SIA. By contrast, off-site microinjection of either DGL inhibitor failed to suppress SIA. The enhancement of SIA induced by activation of group I mGluRs was also dependent upon DGL. The DGL inhibitor THL blocked the enhancement of SIA induced by DHPG. We also examined whether SIA would be suppressed by blockade of presynaptic group III mGluRs in the PAG. Blockade of group III mGluRs with UBP1112 into the dPAG induced a dose-dependent suppression of SIA. Our results are consistent with the hypothesis that exposure to an environmental stressor stimulates biosynthesis of 2-AG through the PLC/DGL pathway to induce SIA. Our data further suggest that this process may be initiated by activation of group I mGluRs and prevented by blockade of group III mGluRs. Our findings collectively suggest that independent presynaptic and postsynaptic mGluR mechanisms regulate endocannabinoid signaling in the PAG to induce SIA. (Supported by DA014022, DA014265, DA021644 to A.G.H.).

N-PALMITOYL GLYCINE INDUCES CALCIUM MOBILIZATION IN NATIVE DORSAL ROOT GANGLION CELLS AND NITRIC OXIDE PRODUCTION IN F-11 CELLS EXPRESSING NEURONAL NITRIC OXIDE SYNTHASE

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The *N*-acyl glycines *N*-arachidonoyl glycine (NAGly) and *N*-palmitoyl glycine (PalGly) are important modulators of pain and inflammation. NAGly is a putative ligand of GPR18 inducing calcium mobilization in GPR18-transfected CHO cells and suppressing thermal and chemical-induced pain in a rat model (Kohno et al, 2006; Huang et al 2001, Burstein, 2002). PalGly induces calcium mobilization in a dorsal root ganglion (DRG) cell line (F-11) and inhibits firing of nociceptive wide dynamic range neurons following thermal stimulation in a rat model. Here we tested whether PalGly produced calcium mobilization in capsaicin-sensitive/insensitive native rat DRG cells. Since the activity of neuronal nitric oxide synthase (nNOS) is regulated by changes in intracellular calcium (Alderton et al, 2001) we examined whether PalGly-induced calcium increases in a DRG cell model (F-11) correlated with nitric oxide (NO) production.

Method: Single cell calcium mobilization was measured in Fura-2/AM loaded native DRG cells alternately excited at 340 and 380nm. Nitric oxide production following application of *N*-acyl glycines or endocannabinoids was measured using the cell-permeant fluorescence dye DAF-2/DA in F-11 cells imaged in a Flexstation II fluorescence imaging plate handler. The expression of nitric oxide synthase (nNOS) in F-11 cells was determined by western blotting.

Results: Single cell calcium imaging revealed intracellular calcium mobilization following PalGly treatment in capsaicin-sensitive/insensitive native DRG cells. Both phosphorylated and non-phosphorylated forms of nNOS were highly expressed in F-11 cells that were capsaicin-insensitive. PalGly, N-arachidonoyl dopamine, and 2-arachidonoyl glycerol but not NAGly or N-arachidonoyl ethanolamine (AEA) induced significant increases in nitric oxide production. PalGly-induced increases in nitric oxide production were blocked by the receptor operated or voltage-gated calcium channel inhibitor SK&F 96365 that also inhibits PalGly induced calcium mobilization in F-11 cells.

Conclusion: PalGly but not NAGly induces increases in intracellular calcium and NO production in F-11 cells. The inhibitory effect of PalGly on wide dynamic range neuronal firing following thermal stimulation may be linked to increases in nitric oxide production by DRG cell subtypes.

Acknowledgements. The authors are grateful for the support of the National Institute on Drug Abuse (DA-018224, DA-020402, F32-DA-016825), the Gill Center for Biomolecular Science, Indiana University, Bloomington, and the Lilly Foundation Inc, Indianapolis, IN.

SYNERGISTIC ANTINOCICEPTIVE EFFECTS OF URB597 AND DICLOFENAC IN A MOUSE VISCERAL PAIN MODEL

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Combinations of drugs are frequently used to achieve an enhanced therapeutic effect and limited side effects associated with each agent. For example, combinations of nonsteroidal antiinflammatory drugs (NSAIDs) and opioids are widely used in the management of pain, allowing enhanced analgesia with reduced side effects. Direct acting cannabinoid agonists are promising therapeutic drugs that have analgesic properties similar to that of opioids, but do not produce respiratory depression. However, the beneficial effects of cannabinoids for pain treatment are counterbalanced by their psychotomimetic side effects. An alternative strategy is to augment levels of endogenous cannabinoids, such as anandamide. Indeed, genetic deletion or pharmacological blockade of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of anandamide, has been demonstrated to produce analgesic and antiinflammatory phenotypes in the absence of motor deficits. Importantly, NSAIDs as well as cannabinoids are known to elicit analgesic actions in visceral pain models. However, there are presently no available reports examining FAAH inhibitors in these models or the interaction between FAAH inhibitors and NSAIDs in pain models. Accordingly, there were two goals of the present study. First, we evaluated FAAH (-/-) mice and mice treated with FAAH inhibitors (URB597 and OL-135) in the acetic acid writhing test. Second, we evaluated the analgesic effects of URB597 given in combination with diclofenac, an NSAID. FAAH (-/-) mice or mice treated with URB597 or OL-135 displayed a significant antinociceptive phenotype in this writhing test, suggesting the potential role of FAAH/endocannabinoid system in visceral pain processing. FAAH (-/-) mice showed a 40% reduction in writhing compared to wild type mice, whereas URB597 (10 mg/kg) and Ol-135 (30 mg/kg) showed 90% and 75% inhibition of writhing, respectively. URB597 elicited a similar magnitude of antinociceptive effects in CB_1 (-/-) and wild type mice, indicating a non CB_1 receptor mechanism of action. Interactions between FAAH inhibitors and NSAIDs were analyzed using an isobolographic analysis. URB597 (1-10 mg/kg; ED_{50} (95% CI) = 2.1 (1.5-2.8) mg/kg), a commonly employed FAAH inhibitor, and diclofenac sodium (3-30 mg/kg; ED_{50} (95% CI)= 9.8 (8.2-11.7) mg/kg), a commonly used NSAID, either alone or in combination, produced dose-dependent antinociception in the writhing test. Combination administration of URB59597 and diclofenac given in ratios of 1:1, yielded a synergistic interaction, with respective ED_{50} (95% CI) values of 0.24 (0.20-0.28) mg/kg and 1.18 (0. 88-1. 58) mg/kg. Taken together, the results of the present study suggest that FAAH represents a promising target for the treatment of pain and combination of FAAH inhibitors and NSAIDs may have great utility to treat visceral pain.

EFFECT OF THC AND TETRAHYDROCANNABIVARIAN (THCV) ON ANTINOCICEPTION IN THE TAIL FLICK TEST

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Some of the most compelling evidence in favor of using marijuana (and or THC) for medicinal purposes has been in the area of pain management (Lynch, Young & Clark, 2006). Usually, comparisons are made with opiates. However, the CB1 receptor has been shown to inhibit the release of glutamate (Godino, Torres, & Sanchez-Prieto, 2007) suggesting that comparisons with anti-nociceptive compounds that block glutamatergic neurotransmission may be more meaningful. Therefore, in the present study we compared the anti-nociceptive effectiveness of eTHC to the glutamate antagonist ketamine (e- is the abbreviation used to indicate that these are extracts, see below). In addition, the anti-nociceptive properties of marijuana are usually attributed to the actions of THC or CBD. In this study we extended testing to include the cannabis extract eTHCV.

Subjects. Thirty-four male C57bl6yj mice were randomly selected from our colony and then randomly assigned to one of four drug conditions: vehicle, ketamine 10 mg/kg, eTHC 8 mg/kg or eTHCV 20 mg/kg (i.p.). The eTHC and eTHCV were cannabis extracts obtained from GW Pharma.

Methods: Tail-flick test for Anti-nociception. During each test session animals were placed in a Plexiglas restraining tube (Braintree Scientific, USA) and allowed to habituate for five minutes. Three trials were then undertaken. During each trial, the tail was carefully placed 1 cm into a water bath maintained at 55 degrees C. The time taken for the animal to remove its tail up to a maximum of 10 s was measured. The inter-trial interval was 10 s. Animals received a total of two test sessions. A drug-free baseline test and then seven days later a second test that occurred 30 minutes after an i.p. injection of the appropriate drug.

Results: The maximum percent effect (MPE) was calculated for each subject. The MPE is a standard measure of anti-nociception for the tail-flick test (Ozdogan, Lahdesmaki, and Scheinin 2006). The greater the MPE, the greater anti-nociceptive effect.
Statistical analyses indicated as significant drug effect on the MPE, $F :(3.30)=5.233$, $p < 0.005$. Post-hoc analyses were completed using Tukey's Honestly Significant Difference (HSD) test. Analyses indicated that mice that received either THC or THCV showed a significantly larger MPE relative to controls, as did ketamine (positive control). There were no significant differences between eTHC, eTHCV and ketamine.

Discussion: These data demonstrate that both eTHC and eTHCV produce anti-nociception in the tail-flick test at a level that was comparable to a 10 mg/kg does of ketamine. At the dosages selected here, both eTHC and ketamine slow motor responses in about 50% of the subjects, but eTHCV does not effect motor responses until the dose exceeds 40 mg/kg (unpublished observations). Since eTHCV has been shown to be a competitive antagonist of CB_1 receptors and the synthetic form of THCV (O4394) has been shown to produce anti-nociception in the tail flick test, (Pertwee, Thomas, Stevenson, Ross, Varvel, Lichtman, Martin & Razdan 2007) these data extend these findings to eTHCV. Combinations of eTHC and eTHCV should be a subject of further investigation.

Acknowledgement: The authors thank GW Pharma Ltd for the extracts used in this study.

ATRAUMATIC AUTOIMMUNE DISORDERS TREATED WITH CANNABIS

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California law allows physicians to recommend and/or approve cannabis use by patients. In a practice dedicated to cannabis-using patients (Mikuriya's) a medical questionnaire is completed by the applicant and reviewed with the physician. Diagnoses and supporting documentation are entered into the patient's file. Codification with ICD-9 diagnoses affords standardized reporting usually required for billing.

The Oakland Cannabis Buyers' Cooperative (OCBC) issues identification cards and maintains a database of patients. A registered nurse (Galli) verifies the ICD-9 diagnoses with the recommending physician.

To determine the frequency with which cannabis is being used to treat atraumatic autoimmune disorders —conditions involving inflammatory response, excluding psychological conditions and those resulting directly from trauma— 35, 232 OCBC records were reviewed and 7,776 cases were found to meet the criteria. AIDS related illness was the most numerous (1,884 cases) followed by hepatitis C (723 cases), degenerative arthritis (577), asthma (470), fibromyalgia (447), cancers (444), and premenstrual syndrome (404).

The surprisingly diverse set of conditions for which cannabis provides relief confirms that the cannabinoids play a role in immunologic processes but raises questions about mechanisms of action.

EFFECTS OF Δ-9 THC ON SYSTEMIC CANDIDIASIS IN MICE

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The immunomodulatory activity of Cannabinoids has been well established in various *in vivo* infectious models. Δ-9 THC has been shown to facilitate an immune shift from Th_1 to Th_2 in response to antigenic challenge with bacteria and viruses, demonstrated by lowered serum levels of interferon-gamma (IFN-γ), interleukin-2 (IL-2) and increased in IL-4. To date, no study has been reported to investigate these effects in response to antigenic challenge with a fungal pathogen. The aim of the present study is to investigate the effects of pre-treatment with Δ-9 THC on systemic candidiasis in mice

Eight-week old Female Swiss-Webster mice were given various doses ($1\text{-}12x10^6$ cells/mouse) of *Candida albicans* cells I.V. and monitored for weight, activity, grooming and morbidity for 10 days. Mice were euthanized by cardiac puncture under anesthesia; blood and kidneys were collected and assayed for colony forming units and cytokine levels (IFN-γ, IL-2 and IL-4) by Sandwich ELISA. To determine if Δ-9 THC worsens systemic candidiasis, two I.P injections of Δ-9 THC will be given, one 48 hours and one 24 hours prior to I.V. injection with *C. albicans*.

Preliminary studies showed that mice injected with $3\text{-}12x10^6$ yeast cells died within 24 hours. However, 80% of the mice that received $2x10^6$ yeast cells survived but showed significant weight loss 10 days post I.V. injection of yeast cells. Mice that received $1x10^6$ yeast cells did not exhibit weight reduction, lethargy, reduced grooming behavior or increased morbidity.

Since Δ-9 THC has been shown to bias towards a Th_2 response, we expect that in our systemic candidiasis model, Δ-9 THC pre-treatment will worsen the progression of infection. More specifically, we expect Δ-9 THC to increase IL-4 level in whole blood while decreasing IFN-γ and IL-2. We also expect increased weight loss, lethargy and morbidity in addition to an increase in CFU in kidney tissue of mice pre-treated with Δ-9 THC. These outcomes will be reported at the meeting.

Acknowledgements: Funded by NIH grant SO6 GM53933

ANTIDEPRESSANT POTENTIAL OF THE NEUTRAL CANNABINOID CB1 RECEPTOR ANTAGONIST VCHSR1 AND PIMSR1

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Over ten years ago, SR141716 (rimonabant), the first antagonist for the cannabinoid CB1 receptor was developed**.** This molecule however, also has inverse agonist and at high doses, agonist properties. Further, psychiatric drugs including anti-depressant medication, frequently cause undesirable weight gain, which however, may be prevented using rimonabant (Gobshtis et al., Eur J Pharmacol 2007). The antagonist/inverse agonist/agonist properties of rimonabant complicate the interpretation of its role as an *in vivo* antagonist and as a compound involved in emotionality-related functions. Recently, several neutral CB1 receptor antagonists have been developed. Their simpler mechanism of action may clarify the role of CB1 receptor antagonism in mediating behavioral effects.

In the present study we characterized the neutral CB1 receptor antagonists VCHSR1 (5-(4-Chlorophenyl)-3-[(E)-2-cyclohexylethenyl]-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole, 5, 12.5 and 25 mg/kg) and PIMSR1 (5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-[(E)-piperidinoiminomethyl]-1H-pyrazole, 5.5 mg/kg) using *in vivo* assays. Thus we investigated their effects in the 'forced swim' test for 'antidepressant' effects and the 'plus maze' for antianxiety effects. We also monitored potential side effects on motor activity and body temperature. Finally we studied the antagonist effects toward Δ^9-tetrahydrocannabinol (THC, 20 mg/kg)-induced effects in the 'tetrad' of cannabinoid-activity (for VCHSR1) .

Methods: Female Sabra strain mice were used and all drugs were administered *i.p.*

Results: Neither antagonist affected body temperature or motor activity in an open field. No 'anti-anxiety' potential was recorded. However, significant 'antidepressant' activity could be demonstrated for both antagonists. Surprisingly, in two separate experiments spaced 6 months apart, VCHSR1 did not antagonize THC-induced activity in the 'tetrad'.

Conclusions: 1) The existence of an unidentified CB1 receptor subtype may explain the lack of antagonism by VCHSR1 and PIMSR1. 2) An endogenous cannabinoid tone seems to be important for the neurochemical regulation of depressive-like behavior but not for anxiety-like behavior. Finally, the absence of spontaneous effects on body temperature and motor activity suggests that these compounds should be further developed as antidepressants which are hopefully, devoid of side effects.

Acknowledgements: This work was supported in part, by an intramural grant from CJS; NG was supported in part by the ministry for immigrant absorption in Israel.

THC INHIBITION OF CYSTEINE CATHEPSIN UPREGULATION DURING AN INFLAMMATORY RESPONSE

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Cannabinoids, including the major psychoactive component of marijuana delta^9-tetrahydrocannabinol (THC), modulate a variety of immune responses. While cannabinoids can have adverse effects on the immune system, they have been proposed as possible therapeutics to control inflammation. The drugs' ability to suppress inflammatory mediators, including chemokines and cytokines, indicate the usefulness of cannabinoids for disease treatment. Cannabinoids may mediate their effects through receptor dependent and independent mechanisms. Cannabinoid receptor subtype 1 (CB1) and receptor subtype 2 (CB2) have differential expression in leukocytes. Cysteine cathepsins B, L and S are endosomal/lysosomal proteases that participate in numerous physiological systems. Cathepsin expression and activity are altered during various inflammatory diseases, including rheumatoid arthritis, atherosclerosis, neurodegenerative diseases and cancers. We developed assays to measure proteolytic activity of cathepsins B, L and S inside live cells by flow cytometry and spectrofluorophotometry. Using bacterial lippopolysaccharide (LPS) to induce inflammation, we investigated the ability of THC to suppress cysteine cathepsins during an inflammatory response. Proteolytic activity of cathepsins B, L and S significantly increased in P388D1 macrophages stimulated with LPS, and THC interfered with this upregulation. Dose response studies showed that 1 nM THC was sufficient to inhibit cathepsin enhancement in LPS-stimulated cells. Furthermore, P388D1 macrophages expressed CB2 mRNA, but had no detectable CB1 mRNA in their resting state indicating a role for the CB2 receptor. Utilizing a CB2-/- macrophage cell line, we explored CB2 receptor participation in THC inhibition of cysteine cathepsin upregulation. THC did not affect cathepsin activity in LPS-stimulated cells lacking CB2 expression. These results indicate cannabinoids via the CB2 receptor interfere with increased cysteine cathepsin activity during an inflammatory response. Our findings support the possibility of receptor selective agonists as therapeutic treatment during inflammatory diseases to prevent cathepsin involvement in pathological tissue destruction.

Acknowledgements: Funded by National Institutes of Health (Grant no. P50DA05275 and RO1ES07199), Department of Health and Human Services (Grant no. T32AI07407) and A.D. Williams Trust Funds.

INVESTIGATION OF THE ROLE OF CB1 RECEPTORS ON PITUITARY PROLACTIN SECRETION IN CB1 RECEPTOR KNOCK-OUT MICE USING REVERSE HEMOLYTIC PLAQUE ASSAY

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It has been previously suggested that the endocannabinoids might have a role in the regulation of prolactin and LH secretion. The most of the research groups have came to the conclusion that these influences can be exerted at both hypothalamic and pituitary levels. As far as the pituitary site of action is concerned, it is important that CB1 receptor immonoreactivity can be detected in the anterior lobe (AL), namely on gonadotropes and lactortopes of the pituitary gland. At the same time *in vitro* and *in vivo* studies have demonstrated that the main effect of endocannabinoids on the AL is inhibitory. Studies have also revealed that in CB1 receptor gene deficient (CB1 KO) mice the basal secretion of LH is lower, while PRL is higher compared to wild type (WT) controls. The aim of our present studies was to investigate the lack of CB1 receptors on basal as well as on the dopamine (DA) and thyroid hormone releasing hormone (TRH) induced changes of PRL secretion. Dispersed AL cell obtained from WT and homozygous CB1 KO male and female mice were used. PRL release was detected by the reverse hemolytic plaque assay (RHPA). The percentage of plaque forming cells (number of lactotropes), the mean plaque area (the average amount of PRL released) and the total plaque area (total amount of hormone released) were measured and calculated. No difference have been detected in the number of PRL secreting cells and in the average amount of PRL release of lactotropes obtained from female WT and CB1 KO mice. In contrast, CB1 KO males have exhibited two-fold higher number of PRL cells compared to the number of lactotropes of WT mice. DA was equally able to inhibit the release of PRL in both CB1 KO and WT mice and there was no difference between females and males in this respect. Following DA treatment, the percentage of PRL secreting cells has significantly decreased on cells obtained from male mice, but no effect of DA was detected on cells obtained from females. Stimulatory effect of TRH was diminished in CB1 KO females but it has no influence in males obtained either from WT or CB1 KO groups. These results indicate that CB1 receptors have a role on PRL secretion only in male mice, while there are no consequences in females. Endogenous cannabinoids most likely responsible to the lower number of lactotropes found in male mice, and their possible role in a tonic inhibition on the proliferation of PRL cells needs to be further investigated. This work was supported by the Hungarian National Research Grant (OTKA 68170 and 68176 to GMN)

CANNABIDIOL (CBD) FOR THE TREATMENT OF BIPOLAR AFFECTIVE DISORDER

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It is well-known that cannabis can cause adverse effects, including psychosis, anxiety and mania. However, anecdotal reports suggest that some patients use marijuana to relieve both depression and mania symptoms. Cannabidiol (CBD), a major compound from c*annabis sativa*, was formerly proposed as a cannabinoid devoid of psychopharmacological activity. Potential antipsychotic, anticonvulsive, antidepressant, hypnotic and anxiolytic effects of CBD have been suggested based on preclinical and clinical data. These observations led to the hypothesis that CBD may have mood stabilizing properties in bipolar affective disorder (BAD). Therefore, the aim of the present study was to directly investigate for the first time the efficacy and safety of CBD in two patients with BAD. This was an in-patient study and the efficacy, tolerability and side effects were assessed by means of the YMRS, BPRS and UKU scales and weekly plasma measurements of CBD were obtained. Both patients were assessed with the Portuguese version of the Structured Clinical Interview for DSM-IV and met criteria for bipolar I disorder experiencing a manic episode without comorbid conditions and were previously responsive to mood stabilizing treatment. The first patient (Ms A., a 34-year-old woman) was given placebo for the initial 5 days, and from the 6th to 30th day (inclusive) she received CBD (initial oral dose of 600 mg reaching 1200 mg/day). From the 6^{th} to the 20^{th} she received adjunctive olanzapine (oral dose of 10 to 15 mg). On day 31st, CBD treatment was discontinued and replaced by placebo for 5 days. The second patient (Ms. B., 36-year-old man) received the same treatment, but adjunctive olanzapine was not given. Ms. A. showed symptoms improvement while on olanzapine *plus* CBD, but showed no additional improvement during CBD monotherapy. Ms. B. had no symptoms improvement with any dose of CBD during the trial. Both patients tolerated CBD very well and no side-effects were reported. These preliminary data suggest that CBD may not be effective for the treatment of BAD with manic features. However, double-blind, controlled clinical trials would be necessary to further confirm these observations.

Acknowledgements: JASC and AWZ are recipients of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) fellowships. Supported in part by the FAPESP fellowship (grant 02/13197-2). Cannabidiol was kindly provided by THC-Pharm (Frankfurt, Germany).

N-ARACHIDONYLETHANOLAMIDE-INDUCED INCREASE IN AQUEOUS HUMOR OUTFLOW FACILITY

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This study was conducted to study the effects of *N*-arachidonylethanolamide (anandamide, AEA) on aqueous humor outflow facility and to investigate the possible existence and activity of Fatty Acid Amide Hydrolase (FAAH), an AEA metabolic enzyme in the trabecular meshwork (TM) tissues.

The effects of AEA on aqueous humor outflow facility were measured using a porcine anterior segment perfused organ culture model. Anterior segment explants were mounted on a perfusion chamber and perfused with culture medium at a constant pressure of 7.35 mmHg maintained at 37ºC and 5% CO_2. After explants had stabilized, different doses of AEA was administered to the perfusion medium and observed for 5 hrs. To investigate whether FAAH is involved in regulating the effects of AEA on aqueous humor outflow, the anterior segments were pre-treated with the FAAH inhibitor URB597 for 30 minutes prior to AEA application. SR141716A, an antagonist selective for CB1, was administered to determine the involvement of CB1 cannabinoid receptors on the outflow effects of AEA. Western blot analysis was used to study the expression of FAAH and a Thin-Layer Chromatography (TLC) approach was used to measure the enzymatic activity of FAAH on porcine TM tissues.

Administration of 10 nM of AEA caused a transient enhancement [163 ± 18 % of basal (mean ± SE)] of aqueous humor outflow facility. In the presence of 100 nM of URB597, a FAAH inhibitor, the effect of 10 nM of AEA on outflow facility was prolonged by at least 4 hours. The AEA-induced enhancement of outflow facility was blocked by 1 μM of SR141716A, a CB1 antagonist. In Western blot studies, positive signals were detected on porcine TM tissues with an anti-FAAH antibody. In the enzyme activity studies using TM tissues, the rate of hydrolysis of [^{3}H] AEA was protein concentration-dependent. The enzyme activity of AEA hydrolysis was 0.15 ± 0.04 nmol min^{-1} mg^{-1} protein (mean ± SE), and this activity was reduced by 86.6 ± 5.3% with the addition of 100 nM of URB597.

The results from this study demonstrate that administration of AEA increases aqueous humor outflow facility and this effect of AEA involves CB1 cannabinoid receptors. In addition, this study demonstrates the existence and the activity of FAAH in the trabecular meshwork tissues, and indicates that this enzyme is involved in regulating the effects of AEA on aqueous humor outflow.

Acknowledgements: Funded by NIH grants EY13632 and DA11551

CANNABINOIDS ELICIT ANTIDEPRESSANT-LIKE BEHAVIOUR AND ACTIVATE SEROTONERGIC NEURONS THROUGH THE MEDIAL PREFRONTAL CORTEX

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.Preclinical and clinical studies demonstrate that marijuana modulate mood and possess antidepressant-like properties, mediated by the agonistic binding of its cannabinoid constituents on brain CB1 receptors. The action of CB1 receptor agonists on the serotonergic system, the major modulatory component involved in mood control, which is implicated in the pathophysiology of depression and in the action of antidepressants, remains however poorly understood. In this study, we demonstrated that a low dose of the direct CB1 receptor agonist WIN55,212-2 exerts potent CB1-dependent antidepressant-like properties in the rat forced-swim test (FST). Then, using in vivo electrophysiology, we showed that low doses of WIN55,212-2 dose-dependently enhanced dorsal raphe nucleus 5-HT neuronal firing activity via a CB1-dependent mechanism. On the other hand, high doses of WIN55,212-2 were ineffective in the FST, and decreased 5-HT neuronal firing activity through a vanilloid-sensitive mechanism. The CB1 agonist-induced enhancement of 5-HT neuronal firing activity was abolished by medial prefrontocortical transection but not by lateral prefrontocortical transection. Furthermore, 5-HT neuronal firing activity was significantly enhanced following local microinjection of WIN55,212 into the ventromedial prefrontal cortex but not after microinjection into the lateral prefrontal cortex. Similarly, intra-ventromedial prefrontal cortex microinjection of WIN55,212-2 produced an antidepressant-like effect in the FST. These results demonstrate that CB1 receptor agonists possess antidepressant-like properties and modulate 5-HT neuronal firing activity via the ventromedial prefrontal cortex.

Acknowledgements: Funded by the Canadian Psychiatric Research Foundation and the McGill University Faculty of Medicine and MUHC scholarships.

NORADRENERGIC MEDIATION OF THE ANTIDEPRESSANT EFFECT OF LONG-TERM CANNABINOID ADMINISTRATION

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Previous research from our laboratory and others, has shown that enhanced activity at the CB_1 receptor elicits an anti-depressant effect in the rat forced swim test. The present study aimed to assess the effects of chronic treatment with a CB_1 receptor agonist in the forced swim test, and further determine the mechanism of this effect. In experiment one, 0.1 mg/kg of HU-210, a potent CB_1 receptor agonist, or vehicle was administered for 10 days. On day 11 a 15 min pre-test swim session was performed, and 24h later a 5 min test session was given during which behavioral parameters were assessed. During the 24h period between swim sessions animals were administered either 10 mg/kg of desipramine, a norepinephrine reuptake inhibitor, or vehicle given at 23.5, 5, and 1 hour prior to the 5 minute test session. Administration of HU-210 and desipramine alone both had an anti-depressant effect, in that they significantly reduced the time spent in immobility during the test session. This reduction in immobility for both treatments was due to a selective increase in struggling behavior, suggesting that the antidepressant effect of HU-210 was noradrenergic in nature. In support of this hypothesis, the combined treatment of HU-210 and desipramine produced a maximal reduction in immobility. In Experiment two, to determine if the antidepressant effect of chronic cannabinoid treatment was mediated by increased noradrenergic transmission, we examined if this effect was reversed by treatment with antagonists to either the α_1 or β noradrenergic receptors. Again, 0.1 mg/kg of HU-210 or vehicle was administered for 10 days, with the 15 minute pre-test swim on day 11. At 23.5, 5 and 1 hour prior to the 5 minute test session either 1 mg/kg of prazosin (a α_1-NE receptor antagonist), 2.5 mg/kg of propanolol (a β-NE receptor antagonist) or vehicle was administered. As before, chronic HU-210 alone significantly reduced immobility and increased struggling. The reduction in immobility was reversed by both the α_1 and β NE antagonists. The concurrent increase in struggling following HU-210 treatment was only partially attenuated by the β-NE antagonist, and was unaffected by the α_1-NE antagonist. These results show that chronic treatment with a CB_1 receptor agonist produces an anti-depressant response in the rat forced swim test, and that this effect is likely mediated by enhanced noradrenergic neurotransmission.

ANTIDEPRESSANT PROPERTIES OF THE HIPPOCAMPAL CANNABINOID CB1 RECEPTOR

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In recent years, converging evidence has outlined a potential role for the endocannabinoid system in the etiology and treatment of depression. Specifically, global pharmacological enhancement of cannabinoid CB1 receptor activity has been shown to elicit an antidepressant effect in the rat forced swim test. Further, exposing rats to chronic unpredictable stress is associated with robust reductions in both 2-arachidonylglycerol (2-AG) content and CB1 receptor density within the hippocampus, whereas chronic antidepressant treatment increases CB1 receptor density in the hippocampus. With this in mind, the purpose of the present study was to assess whether local infusions of HU-210 (a CB1 receptor agonist) or URB-597 (a fatty acid amide hydrolysis [FAAH] inhibitor) into the hippocampus would produce antidepressant-like effects in the rat forced swim test. Rats were implanted with bilateral cannulae aimed at the dentate gyrus of the hippocampus and were randomly assigned to five separate conditions: HU-210 (1.0 μg or 2.5 μg), URB597 (0.5 μg or 1.0 μg), or dimethyl sulfoxide (vehicle control). Infusions occurred three times prior to a 5 min test session, in which the duration of immobility, swimming and struggling was scored. Results demonstrated that administration of both the high and low dose of HU-210 elicited an antidepressant effect, as seen by a significant reduction in time spent in immobility and an increase in the amount of time spent swimming. In contrast, administration of the FAAH inhibitor URB597 had no effect on immobility at either dose. These results demonstrate the importance of hippocampal CB1 receptors in regulating emotional behavior, but demonstrate a dissociation between the neuroanatomical sites of action of direct CB1 receptor agonists and FAAH inhibitors.

PERIPHERAL ANANDAMIDE EFFECTS LINKED TO TRPV1 VANILLOID RECEPTOR PROTEIN AND CGRP CONTENT

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In previous studies we have shown that the *in vitro* exposure to 10 μM anandamide caused greater relaxations in mesenteric beds isolated from endotoxemic compared to untreated rats(Orliac *et al.*,2003), as well as in untreated mesenteries obtained from female compared to male rats(Peroni *et al.*,2004). The aim of the present experiments was to study the role, on peripheral anandamide effects, played by the transient receptor potential vanilloid receptor (TRPV1) expression as well as by the calcitonin gene-related peptide (CGRP) content.

Six hours after the intraperitoneal injection of 5 mg.kg-[1] lipopolysaccharide (LPS) to male Sprague-Dawley rats there was a significant increase in the abundance of TRPV1 receptor protein assayed by Western blot in tongue tissue , that was employed as a representative model of systemic TRPV1 expression . In the mesenteric arteries, the density of the CGRP-positive nerves determined by immunohistochemistry, as well as the anandamide-induced release of CGRP measured by radioimmunoassay, were also significantly increased 6 h after LPS administration.

On the other hand, the content of CGRP in untreated mesenteric beds was higher in female compared to male rats, reduced by ovariectomy and restored to control values 21 days after a 3 weekly i.m. administration of 450 μg.kg-[1] 17β-oestradiol. When applied to male rats, this latter treatment also increased the mesenteric content of CGRP, as well as the anandamide-induced relaxations, up to the same levels to those observed in female rats. Moreover, although the basal release of CGRP in the isolated mesenteric beds was equivalent in either sex, the *in vitro* exposure to anandamide increased the release of CGRP solely in female mesenteries.

Is is concluded that the augmented vascular relaxations caused by anandamide in the septic shock, as well as the higher vascular effects in untreated female compared to male rats, is linked to the enhancements in TRPV1 receptor protein and/or CGRP content, that were either constitutive, as in the case of females compared to males, or inducible, as in the case of septic shock.

Orliac,M.L. *et al*.(2003) *J.Pharmacol.Exp.Ther*., 304,179-184
Peroni,R.N. *et al*.(2004) *Eur.J.Pharmacol*.,493,151-160

Acnowledgements:Research supported by grants FONCTY,PICT 5-14107 and CONICET,PIP 5695.

INHIBITION MECHANISM OF CANNABINOIDS FOR RAT TESTIS 3β-HYDROXYSTEROID DEHYDROGENASE / Δ^5-Δ^4 ISOMERASE ACTIVITY

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Three major cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabinol (CBN) exert a variety of pharmacological and toxicological effects. Furthermore these cannabinoids have some effects on the endocrine system, since the chemical structures of the cannabinoids are similar to those of some steroid hormones. 3β-Hydroxysteroid dehydrogenase / Δ^5-Δ^4 isomerase (3β-HSD), which catalyzes the conversion of Δ^5-3β-hydroxysteroids to Δ^4-3β-hydroxysteroids, is a key enzyme in biosynthesis of many steroid hormones. In this study, we examined inhibitory effects of the cannabinoids on rat testicular microsomal 3β-HSD activity. Testicular microsomal fraction was prepared from male Sprague-Dawley rat (8 weeks old). Dehydroepiandrosterone (DHEA) as a substrate was incubated with the microsomal fraction in the presence of NAD^+, and then androstenedione formed was determined by GC-MS after extraction with ethyl ether (IS, 5α-cholestane). These cannabinoids significantly inhibited 3β-HSD activities at a concentrations greater than 10 μM (Δ^9-THC), 100 μM (CBD) and 50 μM (CBN). Fifty % inhibitory concentrations of Δ^9-THC, CBD and CBN were 42.0, 51.3 and 87.0 μM, respectively. Kinetic analysis using double reciprocal plots indicated that the type of inhibition by three cannabinoids was uncompetitive showing decrease in K_m and V_{max} values (control: K_m 2.0 μM, V_{max} 0.39 nmol/min/mg protein). The present results suggest that these cannabinoids may have inhibitory effects on the reproductive system through the inhibition of 3β-HSD, a key enzyme in biosynthesis of most of steroid hormones.

Acknowledgiment: Funded by the Academic Frontier Project for private Universities from the Ministry of Education, culture, Sports, Science, and Technology of Japan (2005-2009)

C-FOS EXPRESSION RELATED TO VOMITING IS BLOCKED FOLLOWING DELTA-9-TETRAHYDROCANNABINOL INJECTION IN THE LEAST SHREW

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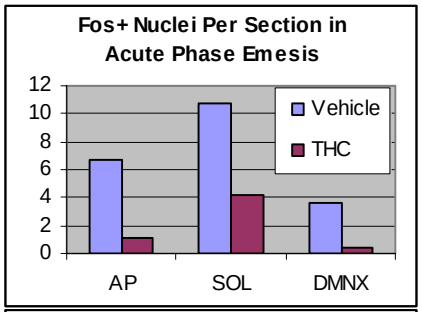
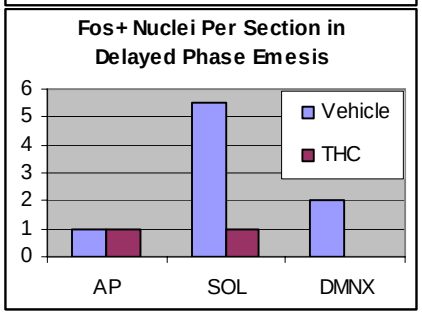
INTRODUCTION: Vomiting is a frequent side effect of cisplatin (CIS) chemotherapy and can result in its premature discontinuation. CIS induces vomiting in an acute phase, shortly post-administration, and a delayed phase, a separate bout occurring days later. Cannabinoids (CB's) are known to be potent inhibitors of vomiting. To help elucidate the substrates of CB-mediated inhibition of acute and delayed-phase vomiting, we used immunohistochemical labeling for c-fos (a neural activity marker), potential emetogens serotonin (5HT) and Substance P (SP), and CB receptors 1 and 2 (CB1R, CB2R), in the least shrew emesis model.

METHODS: Thirty-two least shrews (*Cryptotis parva*, 3.8–5.1g) were used. For acute studies, shrews were given mealworms and a Δ^9–THC (2.5 mg/kg ip) or vehicle injection. Twenty minutes later CIS (10 mg/kg ip) was injected, and the shrew monitored for vomiting for another 30-40 min. It was then anesthetized and fixed via transcardial perfusion 1 h post-vomiting (usually 20-30 min post-injection), or 90-100 min post-injection when no vomiting occurred. For delayed phase studies, shrews were injected with CIS as above, and 26-29 h post-CIS shrews were given food and THC and monitored/perfused as described. Series of brain sections were then processed for c-fos (fos), 5HT, SP, CB1R, and CB2R immunohistochemistry. Fos+ nuclei were counted, and the distribution of fos+ nuclei relative to the other antigens determined.

RESULTS: Emesis-related fos-immunoreactivity (FIR) was present in the nucleus of the solitary tract (Sol), area postrema (AP), and dorsal motor nucleus of the vagus (DMNX), following acute emesis, and in the Sol and DMNX following delayed emesis. The Sol was also strongly CB1R-positive. The dorsal raphe (DR) had increased FIR following delayed-phase emesis. Fos+ nuclei were generally fewer in delayed phase compared to acute phase emesis. All areas correlated with dense SP innervation. Many DR fos+ nuclei were also serotonergic. Δ^9–THC injection blocked emesis and reduced or eliminated FIR in the brainstem, as shown in the tables to the right.





CONCLUSIONS: Acute and delayed-phase emesis activated brainstem nuclei correlated with gastrointestinal motility. Δ^9–THC inhibits this activation in both phases, suggesting that endocannabinoids have excellent anti-emetic potential. The dense SP innervation prevalent in FIR areas, and possible involvement of the DR in the delayed phase, suggest that both SP and 5HT are significant neurotransmitters in the emetic reflex arc.

PARTICIPATION OF ENDOCANNABINOIDS IN THE CONTROL AND PERIPHERAL INHIBITION OF SALIVARY SECRETION BY ALCOHOL

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It is known that alcohol consumption decreases salivary secretion. Recently, we demonstrated that both cannabinoid receptors (CB_1 and CB_2) are present in the submandibulary gland of the rat (SMG). These receptors are coupled to Gi proteins that respond by inhibiting adenylyl cyclase activity. We hypothesized that ethanol (EtOH) might act through the cannabinoid system to inhibit salivary secretion in adult male Wistar rats. The gastric administration by gavage of EtOH (3g/Kg) inhibited at 1h the metacholin-stimulated saliva secretion that could be partially restored by intraglandular injection of the CB_1 antagonist, AM251 (6.10^{-4}M). Incubation *in vitro* of SMGs slices in the presence of AEA (10^{-9}M) or EtOH (0.1M) for 30 min, significantly reduced the forskolin-increase of cAMP content ($p<0.001$ and $p<0.05$, respectively). The inhibitory effect of AEA was blocked significantly by AM251 (10^{-5}M) and CB_2 antagonist, AM630 (10^{-5}M). However, the decrease of cAMP content by EtOH, was significantly blocked by AM251 but not AM630. Indicating that EtOH activated only CB_1 receptor in the SMG. Furthermore, anandamide synthase activity measured by the radioconversion of ^{14}C-arachidonic acid and ethanolamine to ^{14}C-AEA was increased significantly ($p<0.001$) in SMG 1h after gastric administration of EtOH.

In summary, the present results demonstrated that the inhibitory effect produced by alcohol on salivary secretion is mediated, at least in part, by the endocannabinoid system. (Supported by Grant BID 1201 OC-AR, PICT 03/14264 and UBA-O-025)

THE EFFECT OF CENTRAL AND PERIPHERAL AM251 ON THE ESTABLISHMENT OF LITHIUM-INDUCED CONDITIONED GAPING IN RATS

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Conditioned gaping in rats serves as a rat model of nausea. In the first experiment, rats were administered 1.25 mg/kg ip of AM251, 30 min prior to receiving a 2-min intraoral infusion of 0.1% saccharin solution which was followed by LiCl (130 mg/kg of 0.15 M) or saline. Seventy-two hr later, they were intraorally infused with saccharin solution and the mean frequency of gapes was measured. Replicating previous findings using SR141716, the CB_1 antagonist, AM251, potentiated the establishment of lithium-induced conditioned gaping reactions. In a second experiment, rats were surgically implanted with bilateral intracerebroventricular (ICV) cannulae into the lateral ventricles. The design of conditioning and testing was the same as the first experiment, except that the AM251 was delivered by ICV injection at doses of 0.0, 0.125 and 0.250 μg. When administered centrally, AM251 did not potentiate lithium-induced conditioned gaping reactions, but it did suppress hedonic reactions elicited by saccharin solution.

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ENDOCANNABINOIDS ACTING VIA HEPATIC CB_1 RECEPTORS MEDIATE ALCOHOL-INDUCED FATTY LIVER IN MICE

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Chronic alcohol abuse can lead to the development of fatty liver, which has been atrtributed to enhanced hepatic lipogenesis and decreased fatty acid β-oxidation. Obesity is also frequently associated with fatty liver, and high fat diets in rodents induce steatosis as well as increased hepatic lipogenesis. Endogenous cannabinoids and their receptors are present in the liver, and activation of hepatic CB_1 cannabinoid receptors increases *de novo* lipogenesis and decreases fatty acid β-oxidation. Furthermore, the hepatic endocannabinoid/CB_1 receptor system becomes tonically active in response to a high fat diet and is required for the development of steatosis and obesity (Osei-Hyiaman et al., *J. Clin. Invest.* 115:1298, 2005). Chronic ethanol exposure increases the synthesis of endocannabinoids in neuronal cells. Together, these findings may suggest endocannabinoid involvement in alcohol-induced fatty liver. In the present study, mice exposed to a liquid alcohol diet for 3 weeks developed steatosis, as verified by histology and elevated hepatic triglyceride levels, and hepatocellular damage, as indicated by elevated serum alanine aminotransferase. These changes were significantly attenuated by simultaneous daily treatment of the mice with 3 mg/kg rimonabant. Alcohol exposure also increased the hepatic levels of 2-arachidonoylglycerol (2-AG) and CB_1 receptor mRNA. In order to dissect the specific function of CB_1 receptors in hepatic cells, we used conditional mutagenesis to obtain a liver-targeted deletion of the CB_1 gene. "Floxed"-CB_1 mice were crossed with mice expressing the Cre recombinase in hepatocytes (C57BL/6-TgN[AlbCre]21Mgn). In double mutant mice, the deletion of the receptor was restricted to hepatocytes. When exposed to the same alcohol diet, mice with either global or liver-specific genetic ablation of CB_1 receptors did not develop steatosis or hepatocellular damage. Additionally, compared to wild-type mice on the same alcohol diet, both types of CB_1 knockout mice had elevated hepatic levels of activated AMP kinase, increased expression and activity of carnitine palmitoyl transferase-1 (the rate limiting enzyme in fatty acid β-oxidation), and decreased expression of sterol response element binding protein-1c and its target fatty acid synthase. These findings indicate that endocannabinoids, most likely 2-AG, acting via hepatic CB_1 receptors mediate ethanol-induced steatosis by increasing *de novo* lipogenesis and decreasing fatty acid β-oxidation. Targeting the endocannabinoid system may offer a novel therapeutic strategy for alcoholic liver disease.

RIMONABANT AND AM251 SHOW PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARγ)-LIKE PROPERTIES

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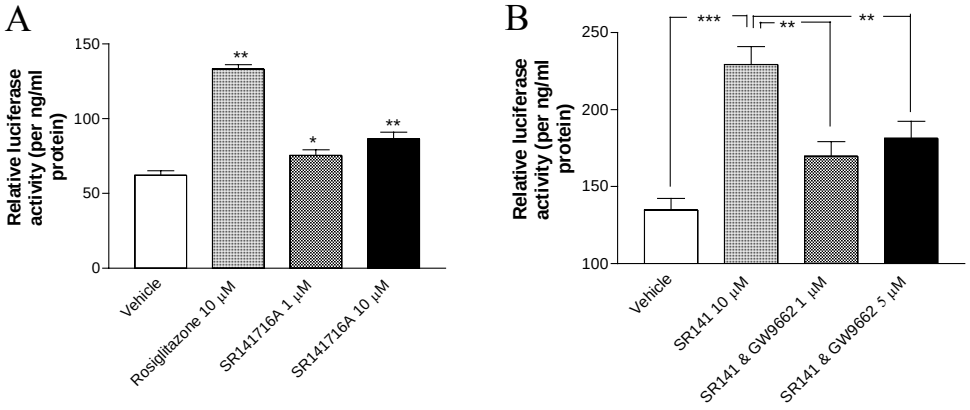
The CB_1 receptor antagonist, rimonabant (SR141716A) reduces weight and improves lipid profiles and glucose control in obese patients (Scheen *et al*., 2006), although it is unclear whether all the metabolic effects of rimonabant can be attributed to CB_1 receptor antagonism. We have shown that Δ^9-tetrahydrocannabinol (THC) (O'Sullivan *et al*., 2005) and other cannabinoids (O'Sullivan *et al*., 2006) exhibit peroxisome proliferator-activated receptor gamma (PPARγ) properties, and postulated that some of the metabolic effects of rimonabant might be through activation of PPARγ. The aims of the present study were therefore to examine the effects of rimonabant, and the structurally similar compound, AM251, on PPARγ activity.

In transactivation assays in cultured HEK293 cells, rimonabant and AM251 (at concentrations from 100 nM to 10 μM) activated PPARγ, transiently expressed in combination with retinoid X receptor gamma and a luciferase reporter gene, in a concentration-dependent manner (Fig 1A). This effect was antagonised by the PPARγ antagonist GW9962 (Fig 1B). Similar increases in the transcriptional activity of PPARγ were stimulated by rimonabant and AM251 in Chinese hamster ovary (CHO) cells.



In the isolated rat aorta, THC causes time-dependent, PPARγ-mediated vasorelaxation (O'Sullivan *et al*., 2005). Both rimonabant and AM251 similarly caused time-dependent vasorelaxation, however, these responses were not antagonised by the PPARγ antagonist GW9962 or reduced by protein synthesis inhibition, and are therefore unlikely to be due to PPARγ activation. Rimonabant and AM251 both stimulated adipocyte differentiation in cultured 3T3 L1 cells at concentrations from 100 nM to 5 μM, a charateristic property of PPARγ ligands (Mueller *et al*., 2002). In binding studies, it was found that rimonabant and AM251 did not directly bind to PPARγ, suggesting that PPARγ-like activation by these compounds may be through indirect mechanisms. The significance of these findings will be discussed.

ENDOCANNABINOID SYSTEM AND THE CARDIOVASCULAR CONSEQUENCES OF CIRRHOSIS

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Advanced liver cirrhosis is associated with hyperdynamic circulation i.e. systemic hypotension, decreased peripheral resistance and cardiac dysfunction termed as cirrhotic cardiomyopathy. We have previously demonstrated the role of endocannabinoids and vascular CB_1 receptors in the development of generalized hypotension and mesenteric vasodilation in cirrhosis (Batkai et al., *Nat. Med*. 7:827, 2001). In this study we explore the pathogenic role of the endocannabinoid system in cardiac dysfunction associated with cirrhosis. Rats with CCl_4-induced cirrhosis developed tachycardia, decreased cardiac contractility as documented through the use of the Millar pressure-volume microcatheter system, low blood pressure, decreased peripheral resistance and elevated mesenteric blood flow. Bolus i.v. injection of the CB_1 antagonist AM-251 (3 mg/kg) acutely increased mean blood pressure as well as cardiac contractility and reduced mesenteric blood flow, whereas no such changes were elicited by AM-251 in control rats. Furthermore, the myocardial content of anandamide increased 2.7-fold in cirrhotic vs. control rats without any change in 2-arachidonoylglycerol (2-AG) levels, whereas in the cirrhotic liver both 2-AG (6-fold) and anandamide (3.5-fold) were markedly increased compared to control. CB_1 receptor expression in the heart of cirrhotic animals was not different from the controls, as verified by Western blotting. We conclude that activation of cardiac CB_1 receptors by endogenous anandamide contributes to the reduced cardiac contractility in cirrhosis, and CB_1 receptor antagonists may be used to improve contractile function in cirrhotic cardiomyopathy and, possibly, in other forms of heart failure. Furthermore, the pro-fibrotic function of hepatic CB_1 receptors has been recently documented (Teixeira-Clerc et al., *Nat. Med*. 12:671, 2006), and the observed marked elevations in hepatic endocannabinoid levels may contribute to the development of cirrhosis in these animals. CB_1 receptor blockade may therefore represent a novel strategy in the medical management of cirrhosis, as it could not only slow down the progression of fibrosis, but would also correct the associated cardiac dysfunction.

Supported by intramural funds of NIAAA

IS THE CB1 RECEPTOR EXPRESSED IN SKELETAL AND CARDIAC MUSCLE?

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The Cannabinoid-1 Receptor (CB1) is a G protein-coupled receptor that is highly expressed in the brain and which mediates the orexigenic effects of endogenous cannabinoids, as demonstrated by studies showing that CB-1 antagonists are efficacious anorexigenic agents. Additional studies have provided support for a peripheral metabolic effect of CB1 antagonists by demonstrating increased beta-oxidation of fatty acids. Since skeletal muscle is a major locus of fatty acid oxidation, we performed a study to determine CB1 expression in muscle tissue. Using qPCR and western blotting, it was determined that expression of CB1 was very low or undetectable in skeletal and cardiac muscle from rat and dog , as well as in CB1-null (-/-) mouse brain. In contrast, high levels of CB1 expression were detected in rat and dog cerebellum and wild-type mouse brain, tissues in which CB1-mediated pharmacological activity has been reported. Radioligand binding studies were also performed in membranes from skeletal and cardiac muscle tissue from dog. There was no detectable specific binding of cannabinoid radioligands to membrane preparations of skeletal muscle tissues (specifically semitendinosus, soleus, and diaphragm) or cardiac muscle tissues (atria and ventricle) from dog, while there were significant levels of radioligand binding observed in membranes from cerebellar tissue. Evaluation of rat and dog skeletal muscle and heart tissues by double-antibody immunofluorescence confirmed that CB1 was not expressed in these tissues, while there was considerable CB1 staining in rat and dog cerebellar Purkinje cells. Based on these analyses, we conclude that the CB1 receptor is not expressed at detectable levels in skeletal or cardiac muscle in rat or dog.

N-ARACHIDONOYL GLYCINE INCREASES UTERINE TONE AND INDUCES CALCIUM MOBILIZATION IN MYOMETRIAL CELLS

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Huang and colleagues demonstrated that N-arachidonoyl glycine (NAGly) is an endogenous compound found throughout the body in amounts equivalent to anandamide and confirmed its antinociceptive properties. Last year at this meeting we presented evidence that NAGly is synthesized from anandamide. Also, in previous work present here, we showed that production of NAGly and anandamide increase in the uterus on the morning of estrus, which is a time of a significant increase in uterine contractions. Additionally, we showed that NAGly induces an increase in uterine contractions *in vivo* on the morning of estrus. Here, using an organ bath preparation and a calcium-mobilization assay, we examine the role of NAGly activation on uterine tissue.

Uterine strips were isolated from Sprague-Dawley rats on the morning of estrus and mounted in a 20-ml tissue bath containing De Jalon's solution (consisting of [mM] NaCl, 154; KCl, 5.6; NaHCO3, 5.9; glucose, 2.5; CaCl2, 0.27), gassed with 95% CO2 and 5% O2, and maintained at 32ºC. 10uM or DMSO vehicle were added to the buffer and the tension on the line was measured before, during, and after a 15 minute application of the drug.

Primary non-pregnant human smooth myocytes (Cambrex, Walkersville, MD) were cultured in SmGM-2 modifed MCDB 131 medium as recommended by the supplier and used at passages 5-7. Cells were plated on 96-well, Corning CellBIND-coated, black with clear bottom plates 24-48 hours prior to assay. Prior to imaging, cells were loaded with 3μM Fura-2 AM for 60 minutes in the following buffer: 145mM NaCl, 5mM KCl, 1mM Na_2HPO_4, 0.5mM $MgCl_2$, 1mM $CaCl_2$, 10mM HEPES, and 5mM Glucose titrated to 7.4pH. Cells were then washed three times with the same buffer then imaged using the FlexStation II (Molecular Devises; Sunnyvale, CA).

In isolated uterine strips, NAGly was shown to significantly increase uterine tone. Additionally, NAGly was shown to significantly increase calcium mobilization in human myometrial cell in a dose-dependent manner. These data demonstrate that NAGly is acting as a direct signaling molecule on myometrial cells and initiating calcium mobilization, which is likely playing a role in the facilitation of uterine tone and contractions. Future studies are aimed at the mechanism of action of NAGly's effects on myometrial cells.

Acknowledgements. The authors are grateful for the support of the National Institute on Drug Abuse (DA-018224, DA-020402, F32-DA-016825), the Gill Center for Biomolecular Science, Indiana University, Bloomington, and the Lilly Foundation Inc, Indianapolis, IN.

CB2 RECEPTORS MODULATE ENTERIC NEURON AND GLIAL ACTIVATION IN A MODEL OF LIPOPOLYSACCARIDE INFLAMMATION

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An in vivo lipopolysaccharide (LPS) challenge results in enhanced gastrointestinal motility (Mathison et al., BJP;142:1247-54). In this inflammatory model, the inhibitory actions of CB1 receptor agonists observed in saline-treated animals were abolished whereas CB2 receptor agonists were found to exert a novel inhibitory action on motility. The aim of the present study is to investigate the mechanism by which CB2 receptor agonists can normalize LPS-enhanced gastrointestinal transit.

Rats were either treated with vehicle or LPS (65 μgkg-1, i.p.); after 80 minutes animals were injected with drug or vehicle, and at 120 minutes ileum tissues were removed for Fos immunohistochemistry. Ileal segments from other vehicle or LPS treated rats were mounted in organ baths and electrical field stimulation was applied in the presence or absence of CB1 and CB2 receptor agonists.

Immunohistochemical studies demonstrate that in vivo LPS treatment significantly induces Fos in both enteric glia and neurons compared to control tissues. Treatment with the CB2 receptor agonist JWH133 significantly attenuates the Fos activation in both enteric glia and neurons, and this action is reversed by the CB2 receptor antagonist AM630. In the in vitro functional studies, JWH133 had no effect on the electrically-evoked (cholinergically-mediated) twitch in the vehicle treated tissues compared to control responses, whereas the CB1/CB2 receptor agonist WIN55, 212 reduced the amplitude of the twitch responses in a concentration-dependant manner. In the LPS treated tissues, JWH133 was able to reduce the twitch response in a concentration dependent manner compared with vehicle control. WIN55, 212 reduced the amplitude of the twitch responses in a concentration-dependant manner and there was no significant difference in the actions of WIN55,212 between the vehicle and LPS-treated tissues.

These data indicate that in a LPS model of inflammation, both enteric glia and neurons are activated, indicating a possible novel role of glia in LPS-enhanced gastrointestinal motility. Furthermore, activation of CB2 receptors under these conditions attenuates neuronal and glial activation as well as cholinergic neurotransmission in LPS-treated tissues. It would appear that LPS stimulates the enteric nervous system resulting in enhanced motility, which is normalized by CB2 receptor activation.

Acknowledgments: Canadian Association of Gastroenterology, Canadian Institute of Health Research, Janssen Ortho & Alberta Heritage Foundation for Medical Research.

CO-EXPRESSION OF THE CB1 CANNABINOID RECEPTOR mRNA AND THE ALPHA7 NICOTINIC RECEPTOR mRNA IN RAT HIPPOCAMPUS

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Both alpha7 nicotinic and CB1 cannabinoid receptors are highly concentrated in the hippocampus, a structure known to be involved in learning and memory. Moreover, it has been claimed that the hippocampal alpha7 receptors play an important role in schizophrenia and that schizophrenics are prone to consume marijuana. We, therefore, studied the relation between the expressions of these receptors at the cellular level. Using double *in-situ* hybridization technique to detect the alpha7 (riboprobe radioactively labeled) and the CB1 (riboprobe labeled with digoxigenin) receptor mRNAs, we found high cellular co-expression of the mRNAs of these receptors in the hippocampus. We determined that 80-87% of the alpha7 receptor expressing cells co-expresses the CB1 receptor and that 70-80% of the CB1 receptor expressing cells expresses high amounts of alpha7 mRNA. Most of these cells were found in the CA1, CA3 and the dentate gyrus regions of the hippocampus. Moreover, we found that all alpha7/CB1 cells were GABAergic interneurons as they were immunostained with an anti GABA antibody. The presence of alpha7 and CB1 mRNAs in the same cell population suggests that acetylcholine and endocannabinoids (as well as nicotine and delta-9-THC) could modulate GABAergic neurotransmission (probably in opposite direction) in the same hippocampal cells and that these GABAergic neurons are likely to participate in the biological effects of at least two drugs of abuse. **On sabbatical leave from the Weizmann Institute of Science, Rehovot, Israel.*

ALTERATIONS IN PLASMA ENDOCANNABINOIDS (eCB) IN 4-WEEK ABSTINENT ALCOHOLICS: A BIOLOGICAL MARKER FOR HEDONIC TONE?

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Chronic alcohol abuse significantly alters the CRF and noradrenergic responses to stress. Previous preclinical research has suggested that endocannabinoids (eCBs) like anandamide (AEA) play an important role in the regulation of stress-coping behaviors. Some basic research also indicates that eCBs may be involved in alcohol seeking behavior. However, there is no data on eCB levels and responses to psychological stress in humans. Thus, the aim of the present study was to assess endocannabinoid responses to individualized emotional stress and to alcohol cues in 4-week abstinent alcoholics compared to healthy social drinkers. Eight 4-week abstinent alcoholics (AD patients) engaged in inpatient treatment and 10 healthy social drinkers participated in three laboratory sessions in which they were exposed to individualized stress, alcohol cue and neutral relaxing situations using guided imagery procedures, one imagery per day with randomized order of presentation. Alcohol craving, subjective anxiety, cardiovascular and plasma anandamide levels were assessed at baseline and immediately following the imagery over the course of 75 minutes. Findings indicated that baseline anandamide levels were significantly lower in AD patients compared to social drinkers ($p<.0001$). Furthermore, anandamide response to neutral relaxing situations and alcohol cue exposure were higher than the stress condition (p's $<.001$) in social drinkers but no such response differences were evident in AD patients. These findings indicate that alcoholics show suppressed endocannbinoid levels with a lack of responsivity to hedonic cues while healthy social drinkers showed increased peripheral endocannabinoid levels in response to positive emotional and hedonic stimuli. The findings suggest that chronic alcohol abuse is associated with a dysregulated hedonic state and that pharmacological manipulation of the endocannabinoid system may provide important therapeutic targets in the treatment of alcoholism.

Acknowledgements: This research was supported by NIH grants R01-AA113892 and K02-DA17232.

DIFFERENCES IN OPIOID PEPTIDE AND DOPAMINE RECEPTOR GENE EXPRESSION IN THE STRIATUM OF CB_1(-/-) VERSUS CB_1(+/+) C57BL/6 MICE ARE AGE- AND SEX-DEPENDENT

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The endocannabinoid system, including CB_1 cannabinoid receptors and endocannabinoid agonists, interacts with opioid and dopaminergic systems in the striatum. Using reverse transcription and real-time polymerase chain reaction analyses, we found differences in gene expression in CB_1 (-/-) compared with CB_1 (+/+) mice (C57BL/6 background) that were sex- and age-specific (2-3 month juveniles versus 6 month adults). We previously reported significantly greater gene expression of the opioid peptides proenkephalin and prodynorphin in the striatum from transgenic CB_1 (-/-) mice compared with CB_1 (+/+) littermates (Gerald et al., 2006, Brain Research). We now report that gene expression of both prodynorphin and proenkephalin were greater in adult female CB_1 (-/-) compared with CB_1 (+/+) mice, but only prodynorphin was greater in juvenile female CB_1 (-/-) compared with CB_1 (+/+) mice. There were no significant genotypic differences in opioid peptide gene expression found in male mice. Although opioid peptide gene expression was different between genotypes in females, the expression of δ-opioid or μ-opioid receptors was not influenced by CB_1 receptor expression, age or sex.

Upon investigation of gene expression for striatal dopamine receptors, we found that within the D_1-like family, D_1 receptor gene expression was approximately 100-fold greater than D_5 receptor expression. D_1 receptor expression not differ between genotype, age or sex. D_5 receptor gene expression was greater in CB_1 (-/-) compared with CB_1 (+/+) in juvenile females only, but not in males at any age or in adult females. Among the D_2-like dopamine receptors, D_2 receptors were expressed approximately 100-fold greater than D_3 and 1000-fold greater than D_4 receptors in the striatum. There was no genotype-, age- or sex-related difference in expression of D_2 receptors. Expression of D_3 receptors was greater in CB_1 (-/-) compared with CB_1 (+/+) juvenile females with no difference in juvenile males. Expression of D_4 receptors was approximately two-fold greater in CB_1 (-/-) compared with CB_1 (+/+) juvenile or adult females, but there was no genotype difference in males. These results suggest neuromodulatory differences in the striatum that are not only dependent on CB_1 receptor regulation, but are also sex- and/or age-specific.

Supported by NIDA grants U24DA12385 and K05-DA00182, NIGMS grant S06-GM08049, and NCMHD Export grant P20-MD00175.

BIDIRECTIONAL MODULATION OF CORTICOSTRIATAL SYNAPSES AFTER REPEATED Δ9-THC EXPOSURE

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The dorsal striatum regulates motor output and appears to contribute to the development of drug dependence and addiction. It has been proposed that bidirectional changes at corticostriatal synapses may be involved in memory and drug habits. High-frequency stimulation of excitatory cortical inputs to the striatum induces either a long-lasting increase (HFS-LTP) or decrease (HFS-LTD) of synaptic transmission. Both forms of long-term synaptic plasticity are dependent on glutamate and dopamine release in the striatum.

In the brain, the cannabinoid receptor CB1 (CB1R) is the primary molecular target of Δ9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana. Agonists of CB1R reduce glutamate release in the dorsal striatum and retrograde endocannabinoid signalling is required for HFS-LTD. Additionally, endocannabinoid and dopaminergic systems functionally interact to modulate striatal signalling. These acute effects of CB1R activation are likely to contribute to the motor depressant properties of marijuana.

Chronic cannabinoid exposure results in tolerance to cannabinoid-induced locomotor effects. We therefore investigated whether repeated THC administration differentially modulates LTD and LTP at corticostriatal synapses. We monitored long-term synaptic plasticity in the striatum of mice exposed to a pharmacological protocols inducing THC tolerance (10 mg/Kg, 2 daily s.c. injections, 4.5 d treatment). 1d after the final injection, we performed extracellular recordings of glutamate-driven population spikes in coronal brain slices containing the dorsolateral striatum, where CB1R are more prominently expressed. Plasticity was induced by a high-frequency stimulation protocol (4 x 1s-long 100-Hz trains, repeated every 10 s), delivered by a bipolar stimulating electrodes placed in proximity of the white matter overlaying the striatum.

In vehicle-treated mice, this stimulation protocol triggered a known form of endocannabinoid mediated LTD that, conversely, was not observed after chronic exposure to THC. This effect was prevented by *in-vivo* treatment with the cannabinoid antagonist SR141716A (3 mg/Kg), administered before each THC injection. Consistent with these data, the same pharmacological treatment induced CB1R down regulation and desensitization in the dorsolateral striatum.

Glutamatergic synapses in the dorsolateral striatum are also capable of expressing an NMDA- and dopamine- dependent form of LTP, after removal of Mg^{2+} from the bathing medium. In these experimental conditions, the HFS protocol induced an LTP of comparable magnitude in both vehicle and THC-treated mice. However, after the induction of LTP by HFS, low-frequency stimulation (2Hz, 10 min) depotentiated the synaptic strength to pre-LTP levels in control mice, but it was unable to restore baseline synaptic responsiveness in tolerant mice.

These results provide the first evidence that chronic THC exposure blocks LTD and the reversal of LTP in the dorsolateral striatum. This suggests that a bidirectional modulation of corticostriatal synaptic plasticity may be relevant for the development and maintenance of cannabinoid addictive processes.

CHRONIC ADMINISTRATION OF NICOTINE DOES NOT INFLUENCE CEREBRAL CANNABINOID-TYPE 1 RECEPTOR AVAILABILITY : AN IN VIVO MICROPET STUDY

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Introduction : Several lines of evidence suggest that there may be a functional interaction between central nicotinic and endocannabinoid systems. We evaluated changes in cannabinoid-type 1 receptor (CB1R) binding using a novel high-affinity and subtype-selective PET radioligand [^{18}F]-MK9470 (Merck Inc, USA) in vivo after chronic administration of nicotine.

Methods : Six female Wistar rats (age 3 months) were anesthetized with 50 mg/kg pentobarbital and injected with 18 MBq [^{18}F]-MK9470 in two conditions : baseline and post chronic (2 weeks daily IP injection 1 mg/kg). Images were acquired on a Concorde Focus 220 microPET dynamically in 60 minutes. Parametric static standard uptake (SUV) between 40-60 min p.i. images were reconstructed using filtered back projection, anatomically standardized to Paxinos space and analyzed by voxel-based statistical parametric mapping (SPM2) using paired t-tests.

Results : On a group basis, no absolute or relative changes in [^{18}F]-MK9470 binding were found at the $p < 0.001$ level (uncorrected). Only at a more liberal threshold of $p_{height} < 0.005$ uncorrected, a very modest increase in tracer binding in the cerebellum was observed (peak average value 1.8%, $p_{cluster}=0.002$ corrected).

Conclusions : Chronic ip administration of nicotine does not produce significant changes in CB1R availability in the rat brain. These results suggest that chronic nicotine usage is not likely to interfere with human PET imaging using [^{18}F]-MK9470.

Research Support : This work was supported by the Research Council of K.U.Leuven (OT/05/58). KVL is Senior Clinical Investigator of the Flemish Fund of Scientific Research.

ABUSE LIABILITY EVALUATION OF CANNABINOIDS IN CLINICAL STUDIES

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The clinical evaluation of abuse liability (AL) is an important component of the safety evaluation of drugs that act on the cannabinoid system. Because of the historical association of cannabinoids with abuse and the relatively recent emergence of these compounds as a legitimate therapeutic class, the evaluation of AL for cannabinoids requires a specialized approach. A typical AL design is a randomized, double-blind, placebo- and active-controlled crossover study in healthy volunteers with non-therapeutic experience with the class of interest. However, cannabinoids present unique challenges that need to be addressed using innovative design strategies. For an AL study, the selected study population and positive comparator are typically based on the pharmacology of the investigational drug. For cannabinoid-1 (CB-1) receptor full agonists, which have been extensively studied, the choice of comparator and population is fairly clear. However, many investigational drugs act on the cannabinoid system in a way that has not yet been characterized with respect to AL (e.g., inverse agonists, antagonists, partial agonists) and hence there is a lack of precedent on which to base design decisions. In addition, many compounds have varying degrees of affinity for other receptor systems. In these cases, the selection of appropriate study population and comparator(s) requires a different approach, which includes a careful assessment of available non-clinical and clinical data. Another issue is the slow release of cannabinoids from adipose tissue, which in crossover studies, can increase the risk of carryover effects from the investigational drug, the comparator(s), or from cannabis misuse pre-study or during washout intervals. Although in most cases subjective and cognitive effects of cannabinoids do not persist beyond 24-48 hours, sustained receptor occupancy may decrease or alter the effects of subsequent treatments. Strategies to address carryover issues will be presented including modified crossover designs, in-clinic 'washout', urine drug screening, and statistical analysis. The selection of outcome measures will also be detailed; these should assess not only subjective 'euphoria', drug liking and value but also cannabinoid-specific effects such as perceptual, cardiovascular, appetite, and cognitive effects. These measures can help to pharmacologically distinguish the investigational drug from cannabis, which can impact on scheduling decisions. Concurrent cognitive measures are used to assess the relative potential for impairment but also provide objective confirmation that the subject's ability to respond to subjective questionnaires has not been compromised by the perceptual or cognitive changes associated with cannabinoid drugs. Next day measures are standard in AL studies, but should be interpreted with caution due to recall/memory effects. In conclusion, due to the high AL of some drugs in this class, the clinical AL evaluation is an important aspect of cannabinoid drug development that often requires specialized approaches.

CANNABINOIDS ENHANCE SLOW OSCILLATION IN VENTRAL TEGMENTAL AREA DOPAMINE NEURONS

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The dopaminergic (DA) system is part of a rich functional and anatomical neural network crucial for many cognitive processes and motor functions.
Among the well recognized single spiking and bursting pattern, ventral tegmental area (VTA) DA cells exhibit robust oscillatory activities characterized by repetition of clusters of action potentials at low frequencies (slow oscillation, SO 0.5-1.5 Hz).
This range of frequency is in the low delta band, which is most apparent in slow wave sleep and in general anaesthesia. However, SO can also be detected in the awake state and may play a role in information processing by linking single-neuron activity to behaviour. The potential significance of SO is also highlighted by the fact that it depends on prefrontal cortex (PFC) inputs and noradrenaline receptor activation, and it can be differentially affected by different drugs. Since cannabinoids modulate PFC and LC neuronal activity, enhance firing rate and burst firing of VTA DA neurons, we sought to investigate the role of CB1 receptors in the expression of SO. To this aim, we performed single cell electrophysiological recording from VTA DA neurons in chloral hydrate anaesthetized rats.
Using spectral analysis the present study revealed a pronounced SO in the firing activity of the majority (62.5%) of DA neurons recorded in the VTA.
WIN22512-2 (WIN, 0.5 mg/kg i.v.), significantly enhanced the expression of SO activity (mean power between 0.5-1.5 Hz, $P_{0.5\text{-}1.5}$, from 0.28±0.08 to 4.38±2.10, n=7, $p<0.01$). Administration of the CB1 antagonist SR141716A (0.5 mg/kg i.v.), ineffective *per se*, prevented the effect of WIN. Moreover, since studies highlighted the role of $\alpha 1$ adrenergic receptors in the expression of SO, we administered the selective $\alpha 1$ antagonist prazosin (0.1 mg/kg i.v.) before WIN. Prazosin did not affect SO *per se*, but reduced the effects of WIN ($P_{0.5\text{-}1.5}$ 1.99±0.74 post prazosin+WIN, n=5 $P>0.05$ *vs.* baseline), indicating that the promotion of SO in DA neurons by CB1 agonists is, at least partially, dependent on noradrenergic inputs. On the other hand, prazosin did not antagonized WIN-induced changes in firing rate and bursting activity of DA neurons.
Functions of neuronal SO include input selection, facilitation of synaptic plasticity, and promotion of synchrony between functionally related neurons, all of which are modulated by cannabinoids. Thus, the enhancement of SO evoked by cannabinoids may have consequences on DA information processing related with ability of DA neurons to fire in distinct temporal domains.

CB1 RECEPTOR KNOCKOUT MICE WITH C57BL/6 BACKGROUNDS DISPLAY REDUCED COCAINE-INDUCED INCREASES IN LOCOMOTION AND EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS

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Previous studies suggest that cannabinoid CB1 receptors are not critically involved in cocaine's rewarding effects, largely as a result of findings with CB1-KO mice demonstrating that deletion of CB1 receptors failed to alter cocaine self-administration, cocaine-induced increases in extracellular dopamine (DA) in the nucleus accumbens (NAc), cocaine-induced conditioned place preference or cocaine-induced behavioral sensitization. These results were observed in CB1-KO mice generated from CD1 genetic backgrounds (Ledent et al., Science, 283:401-4, 1999). In the present study, we used CB1-KO mice generated from C57BL/6 backgrounds (Zimmer et al., PNAS, 96:5780-5, 1999), and found that CB1-KO mice displayed a very robust decrease in basal locomotor activity (~80%) and extracellular DA in the NAc (~40%), when compared with their wild-type littermates. We further found that cocaine-induced increases in locomotion and NAc DA (~50%) were decreased significantly in male CB1-KO mice, when compared with their wild-type littermates. To further determine whether such decreases are mediated by inactivation of CB1 receptors, we observed the effects of the CB1 receptor antagonists SR141716 and AM251 on locomotor activities. We found that either SR141716 or AM251 significantly inhibited locomotor activities only in the wild-type, but not in CB1-KO mice. These data suggest that deletion or pharmacological blockade of CB1 receptors inhibits basal and cocaine-induced increases in locomotion and NAc DA, therefore supporting an important role for CB1 receptors in cocaine's rewarding effects. The ineffectiveness of CB1 receptor deletion on cocaine's actions in previous studies may well be related to the use of CB1-KO mice with different genetic backgrounds.

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

ENDOCANNABINOID-MEDIATED RETROGRADE SIGNALING IN ALCOHOL-INDUCED DEPRESSION OF EXCITATORY POSTSYNAPTIC CURRENTS IN CULTURED HIPPOCAMPAL NEURONS

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Alcohol increases endocannabinoids levels and inhibits transport in neuronal cells including hippocampal neurons, although the molecular mechanisms by which this occurs and physiological significance are unknown. In the current investigation, we show the participation of endocannabinoids and its receptors in the effect of acute alcohol on the spontaneous synaptic transmission (mEPSCs) using whole-cell voltage clamp in cultured hippocampal neurons. Immunocytochemical studies suggested that these neurons have highest levels of cannabinoid type-1 receptor (CB1) and are colocalized with synaptophysin, a presynaptic marker but not with postsynaptic protein, PSD-95. These observations suggest that CB1 receptors are localized exclusively on presynaptic neurons. Exposure of alcohol (50mM) for 20 min decreased both amplitude and frequency of mEPSCs (6% and 78%, respectively compared to control), suggesting that alcohol modulate both pre- and post-synaptic mechanisms. To understand the contribution of endocannabinoid system in alcohol action, we used CB1 receptor antagonist rimonabant (SR141716A). Rimonabant antagonized the effects of alcohol on both amplitude and frequency of mEPSCs. Further, AEA transport inhibitors (AM404 and UCM707) (1μM) and fatty acid amidohydrolase (FAAH) inhibitor (URB597) (1μM) enhanced alcohol-induced inhibition of mEPSC frequency. Rimonabant antagonized the effects of AM404, UCM707 and URB597 on alcohol-induced inhibition of mEPSC frequency. These results suggest alcohol-induced release of endocannabinoids that diffuse retrogradely and inhibit presynaptic neurotransmitter release. The current finding highlights the endocannabinoid system as a potential target in the treatment or prevention of alcoholism.

Acknowledgements: Funded by NIAAA grant (BSB, AA14411).

EXTINCTION AND REINSTATEMENT OF DRUG-SEEKING BEHAVIOR UNDER A SECOND-ORDER SCHEDULE OF INTRAVENOUS THC INJECTION IN SQUIRREL MONKEYS

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THC self-administration behavior by squirrel monkeys can be consistently maintained using a fixed-ratio schedule in which every 10th lever-pressing response during daily sessions produces an i.v. injections of THC (1-16 µg/kg) and each injection is followed by a one-min timeout (Tanda *et al*., 2000; Justinova *et al.* 2003). We have now studied maintenance, extinction and reinstatement of drug-seeking behavior under a more complex, second-order, fixed-interval schedule of i.v. THC injection with fixed-ratio units of brief-light presentation. Under this second-order schedule, each fixed-ratio 10 unit completed by the monkey during a 30-min fixed-interval of time (FI 30 min) produces only a brief 2-sec flash of an amber light; the first fixed-ratio component completed after the 30-min interval elapses produces 10 consecutive pairings of the light and i.v. injections of THC (1-8 µg/kg/injection) and ends the daily session. With this schedule, drug-seeking behavior during the session occurs in the absence of the direct pharmacological effects of THC, since THC self-administration occurs only at the end of each session. Under this second-order schedule, responding was markedly reduced either by substitution of vehicle for THC or by pre-session treatment with the cannabinoid CB_1 receptor antagonist rimonabant (SR141716). Responding was also markedly reduced by removal of the brief-stimulus presentations during the session. During vehicle-extinction sessions, intravenous administration of the cannabinoid CB_1 agonists THC (10-80 µg/kg), anandamide (30-560 µg/kg), and methanandamide (10-100 µg/kg), and the opioid agonist morphine (0.19-1.50 mg/kg), before the start of the session induced a marked reinstatement of drug-seeking behavior. In contrast, pre-session intravenous administration of cocaine (0.03-1 mg/kg) or rimonabant (0.03-1 mg/kg) did not reinstate drug-seeking behavior. The reinstatement of drug-seeking behavior produced by THC was blocked by pretreatment with the cannabinoid antagonist rimonabant, but not by pretreatment with the opioid antagonist naltrexone. Similarly, the reinstatement of drug-seeking behavior produced by morphine was blocked by pretreatment with the opioid antagonist naltrexone, but not by pretreatment with the cannabinoid antagonist rimonabant. This study shows that cannabinoid-seeking behavior in primates is reinstated by priming injections of both cannabinoid and opioid agonists. When cannabinoid and opioid antagonists were administered to prevent the reinstatement, the bi-directionality of cannabinoid-opioid interactions was not observed.

Acknowledgements: The research was supported in part by the Intramural Research Program of the NIH, National Institute on Drug Abuse.

CYTOCHROME P450 1 ISOFORM-SELECTIVE INHIBITION OF MAJOR CANNABINOIDS, Δ^9-TETRAHYDROCANNABINOL, CANNABIDIOL AND CANNABINOL

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Δ^9-Tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabinol (CBN), major constituents of marijuana, are mainly metabolized by human cytochrome P450 (CYP) 2C9 and CYP3A4. On the other hand, some of the major cannabinoids inhibit these CYP-mediated drug oxidations. In addition, it has been recently reported that Δ^9-THC inhibits the human CYP1A1 activity. The human CYP1 family consists of CYP1A1, CYP1A2 and CYP1B1, and plays important roles in the bioactivation of procarcinogens and the drug metabolism. However, the inhibitory profile of cannabinoids against human CYP1 isoforms has not been fully understood. In this study, we investigated the effects of three major cannabinoids on 7-ethoxyresorufin *O*-deethylase (EROD) activity of recombinant human CYP1 enzymes and human liver microsomes. All cannabinoids used (0.025-25 μM) inhibited the EROD activity (150 nM 7-ethoxyresorufin) of these CYP1 enzymes, with IC_{50} values ranging from 0.188 to 9.83 μM. Δ^9-THC most potently inhibited CYP1B1, whose IC_{50} (3.85 μM) was approximately one-half of the other isoforms' value. CBD was the most potent CYP1A1 inhibitor, whose IC_{50} (0.537 μM) was approximately one-tenth of the other isoforms' value. CBN markedly inhibited CYP1A2 and CYP1B1, whose IC_{50}s (0.188 and 0.278 μM, respectively) were approximately one-tenth of the CYP1A1's value. In the case of human liver microsomes, the inhibition patterns were similar to those of recombinant CYP1A2, indicating that CYP1A2 predominantly attributed to the EROD activity of human liver microsomes. The type of inhibition by major cannabinoids against recombinant CYP1 enzymes was competitive, except that the inhibition of CYP1A1 by Δ^9-THC shifted competitive to a mixed type with an increase in cannabinoid concentrations. These results suggest that the inhibitory potential and mechanism of the major cannabinoids depend on human CYP1 isoforms, and that CBN could lead to possible drug-drug interactions with CYP1A2 substrates in human livers.

Acknowledgement: Funded by the Academic Frontier Project for Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2005-2009).

CANNABIGEROL BEHAVES AS A PARTIAL AGONIST AT BOTH CB_1 AND CB_2 RECEPTORS

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There is evidence that the plant cannabinoid, cannabigerol (CBG), is anti-inflammatory (Formukong *et al.*, 1988) and also that inflammation can be alleviated by compounds that target CB_2 receptors as either agonists or inverse agonists (Klein, 2005; Lunn *et al.*, 2006). Accordingly, this investigation was directed at establishing whether CBG binds to CB_2 receptors at clinically-relevant (sub-micromolar) concentrations and whether it behaves as a CB_2 receptor agonist or inverse agonist as measured by inhibition or enhancement of forskolin-induced stimulation of cyclic AMP production by Chinese Hamster Ovary (CHO) cells transfected with the human CB_2 receptor (hCB_2). CBG was compared in this assay with the established CB_1/CB_2 receptor agonist, CP55940, and also with cannabidiol (CBD), Δ^9-tetrahydrocannabivarin (THCV) and Δ^9-tetrahydrocannabinol (THC). The ability of CBG to interact with CB_1 receptors on mouse brain membranes was also investigated.

Our experiments were performed using methods described elsewhere (Ross *et al*., 1999; Thomas *et al.*, 2007). CBG, CBD, THCV and THC were obtained from GW Pharmaceuticals and all compounds were dissolved in DMSO.

CBG displaced [^{3}H]CP55940 from specific binding sites on membranes prepared from hCB_2-CHO cells (K_i = 337 nM) and, at submicromolar concentrations, inhibited the ability of 5 μM forskolin to stimulate cyclic AMP production by these cells, albeit with an efficacy less than that of CP55940. CBD, THCV and THC also behaved as CB_2 receptor partial agonists in this bioassay. Further experiments showed that CBG can displace [^{3}H]CP55940 from specific binding sites on mouse brain membranes (K_i = 439 nM) and stimulate binding of [^{35}S]GTPγS to these membranes, again with an efficacy less than that of CP55940.

In conclusion, CBG shares the ability of THC to behave as a partial agonist at both CB_1 and CB_2 receptors. CBD and THCV were also found to behave as CB_2 receptor partial agonists. Additional experiments are required to establish first, whether CBG, THCV and/or CBD can activate CB_2 receptors in a cell line or tissue in which these receptors are less over-expressed than in our hCB_2-CHO cells, and second, whether the reported anti-inflammatory properties of CBG and CBD result at least in part from CB_2 receptor activation by either or both of these cannabinoids.

Formukong, EA *et al.* (1988). *Inflammation* **12**: 361-371.
Klein, TW (2005). *Nat Rev Immunol* **5**: 400-411.
Lunn, C.A. *et al.* (2006). *J Pharmacol Exp Ther* **316**: 780-788.
Ross, RA *et al.* (1999). *Br J Pharmacol* **126**: 665-672.
Thomas, A *et al.* (2007). *Br J Pharmacol* advance online.publication.

Funded by GW Pharmaceuticals, BBSRC and NIDA.

AGONIST-SPECIFIC PLEIOTROPY IN CB_1 RECEPTOR SIGNALLING IN HUMAN TRABECULAR MESHWORK CELLS

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In humans, intraocular pressure is determined by the secretion of aqueous humor by the ciliary epithelium and outflow resistance via the trabecular and uveoscleral routes. Cannabinoid receptors are present on tissues of both the inflow and outflow pathways and cannabinoid ligands have been shown to decrease intraocular pressure in humans, though the cellular mechanisms currently remain unclear. To investigate the pharmacology of CB_1 receptor (CB_1) activation in cells of the trabecular meshwork (TM), we used ratiometric calcium imaging, western, and infrared In-Cell Western™ (ICW) analysis in HTM5 cells, a transformed cell line derived from human trabecular meshwork.

The aminoalkylindole WIN55212-2 (WIN), a cannabinoid receptor agonist, evoked a gradual increase in $[Ca^{2+}]_i$ in HTM5 cells (1-100 μM). These responses were mediated by CB_1 as they were inhibited in the presence of AM251 (1-10 μM), a CB_1-specific antagonist, and were unaffected by the CB_2 receptor antagonist AM630. The increase in $[Ca^{2+}]_i$ was dependent upon activation of phospholipase-C (PLC) and mobilization of intracellular Ca^{2+} stores. The CB_1-induced $[Ca^{2+}]_i$ increase was pertussis toxin (PTX)-insensitive and, therefore, independent of $G_{i/o}$ coupling, but was attenuated in cells expressing a dominant negative $G_{q/11}$ α subunit, implicating a CB_1-$G_{q/11}$ signalling pathway. The increase in $[Ca^{2+}]_i$ was agonist-specific, as the non-classical cannabinoid CP55940 and the eicosanoid anandamide were unable to initiate a significant increase (0.1-10 μM).

Western and ICW analysis demonstrated that WIN also activates the mitogen activated protein kinase (MAPK) pathway in HTM5 cells. We observed a dose-dependent increase in phosphorylation of extracellular signal-regulated kinase (ERK) in response to WIN (5-10 μM). In contrast to the WIN-mediated increase in $[Ca^{2+}]_i$, ERK phosphorylation was sensitive to both AM251 and pre-treatment with PTX implicating CB_1-$G_{i/o}$ coupling in the signalling mechanism. This MAPK signalling was not agonist-specific as both CP55940 and methanandamide increased the level of ERK phosphorylation in manner that was sensitive to PTX and AM251.

This study suggests that endogenously expressed CB_1 receptors are capable of both pleiotropy and agonist-specific signal transduction in HTM5 cells. WIN activation of CB_1 initiates both $G_{q/11}$ and $G_{i/o}$ signal transduction pathways, resulting in elevated $[Ca^{2+}]_i$ and ERK phosphorylation, respectively. The coupling of CB_1 to $G_{q/11}$ and PLC-dependent increases in Ca^{2+} were specific to WIN and were not observed with other CB_1 agonists, including anandamide and CP55940, although these compounds were able to activate $G_{i/o}$-dependent increases in ERK phosphorylation.

AGONIST-SPECIFIC INTRACELLULAR TRAFFICKING OF CANNABINOID RECEPTOR 1

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As is the case for a number of G-protein coupled receptors (GPCRs), Cannabinoid Receptor 1 (CB1) internalises via a well characterised clathrin-mediated pathway following agonist-induced or constitutive activation. Subsequent to internalisation, the fate of GPCRs tends to be divergent between receptor types. While some receptors rapidly recycle to the cell surface following removal of agonist, others remain sequestered in the cytoplasm or are degraded. To date, very limited information is available regarding the intracellular trafficking of CB1.

We have applied an immunofluorescence-based high-throughput quantitative assay and western blotting to investigate the fate of CB1 following acute and chronic stimulation with cannabinoid agonists in HEK-293 cells stably expressing HA-tagged rat CB1.

In accordance with the results of a previous study (Hsieh et al., 1999), following the induction of receptor internalisation with 100nM WIN55212-2 (WIN), receptor recycling can be detected 60 mins after agonist washout, and near-complete repopulation of the cell surface results within 3 hours. In stark contrast to this, no receptor recycling is detected for at least 3.5 hours following internalisation with 100nM HU-210 (HU). We have verified that these agonists produce equivalent rates and extents of CB1 internalisation.

Somewhat paradoxically, we observe that chronic treatment with 100nM WIN for 2-3 hours induces a significant reduction in total CB1, whereas only a subtle decrease in expressed CB1 is observed following stimulation with 100nM HU over the same time course.

The present results appear to indicate that the binding of these two agonists induces CB1 to enter divergent sorting pathways, perhaps through differential interaction with adaptor proteins or induction of signal transduction pathways. Current experiments aim to investigate this hypothesis further by co-localising internalised receptors with various endosomal markers.

This novel phenomenon indicates that cannabinoid drugs with similar receptor affinities and efficacies can produce profoundly different receptor fates. The importance of receptor intracellular trafficking in short- and long-term drug efficacy and the development of drug tolerance denotes that these findings may have profound implications for the design of cannabinoid therapeutics.

Hsieh, C., Brown, S., Derleth, C., et al. (1999). J Neurochem. 73:493-501.

PHARMACOLOGICAL CHARACTERIZATION OF NOVEL CB_2 RECEPTOR LIGANDS

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The CB_2 cannabinoid receptor is extensively expressed by immune cells and was recently described in the SNC under both pathological and physiological conditions. The interest for the CB_2 cannabinoid receptors has been growing recently with suggested applications in alleviating pain and inflammation, cough, dermatitis, and treating cancers. In light of these applications, the development of potent and selective CB_2 ligands will result in powerful therapeutic tools.

We previously described the synthesis, binding, and molecular modeling studies of a novel series of 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives acting as selective and potent CB_2 receptor agonists. The prototypic compound, ALICB-179, shows nanomolar affinity for the human CB_2 receptor (Ki = 15.8 nM) coupled to a good selectivity for the CB_2 over the CB_1 cannabinoid receptor.

Here we present the pharmacological characterization of novel representatives of this class of compounds. Competition binding studies were performed on hCB_2-CHO cells and on murine spleen tissues using [^{3}H]-CP55,940. The functionality of the ligands was determined by [^{35}S]-GTPγS binding studies and by investigating the adenylyl cyclase activity.

Our data demonstrate the presence of both agonists and inverse agonists within the family 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives. In addition, our results prove the versatility of the selected scaffold for developing CB_2 ligands.

Acknowledgements : Supported by the Fonds National de la Recherche Scientifique (GGM), and by the Université catholique de Louvain (ES, BB, DML).

A TOXICOLOGY SCREEN OF THCV AND THCV BDS IN ZEBRAFISH

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Background: Zebrafish (*Danio rerio*) are ideally suited for predictive toxicology screening primarily because their physiology and development closely parallel mammalian systems and they develop extremely rapidly, ex utero. They are transparent through embryo- and organogenesis which allows every event in early development to be observed visually. Three days after fertilization, the embryo is essentially complete, with a functioning heart, circulatory and nervous system. This rapid development is comparable to three months of human development. A number of laboratories have developed toxicity assays based on the use of the zebrafish embryo to assess compounds for developmental toxicity, neurotoxicity, cardiotoxicity, hepatotoxicity and acute toxicity.

Objective: The aim of this study was to assess the potential of the delta-9-tetrahydrocannabivarin (THCV) and THCV Botanical Drug Substance (THCV BDS), to produce acute toxicity, embryotoxicity and cardiotoxicity in the zebrafish.

Methods: Doses of 0.01, 0.1, 1, 10 and 20μM of both THCV and THCV BDS were assessed for acute toxicity and embryotoxicity. Doses of 0.1, 1, 5, 10, 15 and 20μM of both THCV and THCV BDS were assessed for cardiotoxicity.

Results: With regard to acute toxicity, both THCV and THCV-BDS shows acute lethality from 10μM (2850 ng/ml). With regard to embryotoxicity, both THCV and THCV-BDS showed toxicity from 10μM. With regard to cardiotoxicity, both THCV and THCV-BDS THCV-BDS caused a reduction in heart rate followed by bradycardia from 5μM and an atrio-ventricular arrhythmia from 10μM with ventricular asystole evident in some larvae. The No Observed Effect Level (NOEC) and Lowest Observed Effect Level (LOEC) data are presented below.

Assay	Test Item	NOEC (μM)	LOEC (μM)
Acute Toxicity	THCV BDS	1	10
	Pure THCV	1	10
Embryotoxicity	THCV BDS	1	10
	Pure THCV	1	10
Cardiotoxicity	THCV BDS	1	5
	Pure THCV	1	5

Conclusion: THCV and THCV BDS has low potential to cause in vivo toxicity in the zebrafish. Doses up to 1μM (285 ng/ml) produced no toxic effects in any of the assays. The toxicity of pure THCV and THCV BDS appears similar. Given that the bioavailability of THCV is likely to be similar to THC at similar doses, THCV is likely to be well tolerated, with low potential for toxicity at clinical doses.

LACK OF HUMAN CYTOCHROME P450 INDUCTION BY SATIVEX®

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Objective: The aim of this study was to assess the potential of the delta-9-tetrahydrocannabinol Botanical Drug Substance (THC BDS), Cannabidiol (CBD) BDS and a 1:1 % (v/v) mixture of THC BDS:CBD BDS, (the active constituents in Sativex®) to induce the major human cytochrome P450 enzymes involved in drug metabolism.

Methods: Cultured human hepatocytes were maintained in culture for 5 days during which time they were exposed to multiple doses of test item or positive control chemicals for 72 hours. The test items were: THC BDS, CBD BDS and 1:1 % (v/v) THC BDS:CBD BDS at 3 concentrations: 0.01, 0.1 and 1 μM (3.14, 31.4 and 314 ng/ml) tested as the principal active cannabinoid. Prototypical CYP450 enzyme inducing agents were used as positive controls (Rifampicin – CYP2C and CYP3A and omeprazole – CYP1A). Incubation media containing the cannabinoid components, positive controls and solvents was replaced every 24 hours. The appropriate wells were then incubated at 37°C for up to 2 hours with fresh incubation media containing the appropriate CYP450 substrates: ^{14}C-Testosterone (100 μM) to assess CYP3A4 activity; ^{14}C-Phenacetin (50 μM) to assess CYP1A2 activity; ^{14}C-Diclofenac (10 μM) to assess CYP2C9 activity and ^{14}C-S-Mephenytoin (100 μM) to assess CYP2C19 activity. CYP2D6 is a non-inducible human CYP450 enzyme and was therefore not assayed for. Potential inductive effects were assessed by comparing the extent of metabolite formation by human hepatocytes following multiple exposure to the test items positive controls and solvents.

Results: Results at the highest concentration of cannabinoids (1 μM, 314 ng principal cannabinoid/ml) are summarised below:

Cannabinoid	CYP450 Enzyme			
	1A2	2C9	2C19	3A4
THC BDS	< 5 %	0%	0	< 5 %
CBD BDS	< 10 %	0%	< 3%	< 10%
THC BDS : CBD BDS	< 10 %	0%	0	0%

Data expressed as: [% of inductive effect observed by relevant prototypical enzyme inducer]

Conclusion: A test article was considered an enzyme inducing agent if the inductive response was ≥40% of the prototypical inducing agent for the corresponding enzyme. As all the test items all produced an inductive response of less than 10% of the corresponding positive control agents, none were considered to be inducing agents of human CYP450 at concentrations up to 314ng/ml. Given that typical plasma concentrations of THC and CBD are of the order of 5-30ng/ml when Sativex® is administered in therapeutic doses, the potential for Sativex® to produce CYP P450 induction in the clinical setting is low.

EXPLORATION OF NATURAL AND SYNTHETIC *N*-ALKYL AMIDES AS SOURCE FOR NEW CANNABINOID RECEPTOR TYPE-2 (CB_2) SELECTIVE LIGANDS

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We recently reported that certain anti-inflammatory *N*-alkyl amides from purple coneflower (*Echinacea* spp.) constitute a new class of cannabinomimetics, which selectively bind to and activate the cannabinoid type-2 (CB_2) receptor (Raduner et al., 2006). In the present study, we have investigated whether chain length and substitution of the headgroup of this class of natural products can result in new compounds with nM affinities to the CB2 receptor. More than 30 *N*-alkyl amide derivatives were synthesized. A comparison of the preliminary structure-activity relationship of *N*-alkyl amides with the endogenous cannabinoid *N*-arachidonoyl ethanolamine (anandamide) clearly indicates that these compound classes bind distinctively to the CB2 receptor. Moreover, unlike anandamide, *N*-alkyl amides and 2-arachidonoyl glycerol (2-AG) trigger CB2-receptor dependent intracellular calcium transients in myelo-monocytic cells. We have therefore correlated the binding affinity to intracellular calcium transient responses. In dodecanoic acid derivatives, the 2*E*,4*E* double bonds were found to be crucial for optimal binding to CB2 while only the 2*E* double bond appears to be required for the moderate CB1 affinity. The most active compounds were isobutylamides (Ki ~ 60 nM). We show that certain derivatives segregate and form micelles, which are no longer able to interact with the receptor. Thus, self-assembling of these compounds directly influences CB2 affinity. Aggregation was not observed for the endogenous cannabinoids anandamide and 2-AG. *N*-alkyl amides from other plant sources, such as *Lepidium meyenii* and *Artemisia dracunculus* showed weak affinity to the CB2 receptor (Ki = 2 - 5 μM).

Based on the dodeca-2*E*,4*E*-diene chain a fluorescent nitrobenzooxadiazole ligand was synthesized which moderately but selectively binds to CB2 (Ki ~ 1.5 μM) over CB1 (Ki > 30 μM) . Biological characterization suggests that this compound could be a valuable tool to study CB2 receptor localization and as fluorescent ligand for displacement studies. Since analogs of palmitoylethanolamide have been shown to inhibit the uptake and degradation of [^{3}H]-anandamide (Jonsson et al., 2001) we tested the *N*-alkyl amide derivatives on [^{3}H]-anandamide uptake into HL60 cells and metabolism by fatty acid amide hydrolase (FAAH). Overall, *N*-isobutyl amides represent interesting lead structures for the development of anti-inflammatory drugs and tool compounds for cannabinoid research.

Raduner, S., Majewska, A., Chen, J.Z., Xie, X.Q., Hamon, J., Faller, B., Altmann, K.H., Gertsch, J. (2006) *J. Biol. Chem.* **281**, 14192-206.
Jonsson, K.O., Vandevoorde, S., Lambert, D.M., Tiger, G., Fowler, C.J. (2001) *Br. J. Pharmacol.* **133**, 1263-75.

SCREENING OF PLANT EXTRACTS FOR NEW CB2-SELECTIVE AGONISTS REVEALS NEW PLAYERS IN CANNABIS SATIVA

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CB2 receptor agonists have been reported to be effective in the control of different disease states, such as inflammation and pain. CB2 agonists are therefore promising candidates for the development of drugs. Our ongoing search for new CB2-selective agonists has led us to screen diverse plant extract libraries. Examination of the hits revealed that highly lipophilic isoprenoids competitively bind to the THC binding site in CB2 as shown by displacement of the radioligand [^{3}H]-CP55940. At a concentration of 10 μg/mL (ppm), *Cannabis sativa* oil devoid of cannabinoids displaced the radioligand [^{3}H]-CP55940 by more than 90%. Similar hits were also obtained with *n*-hexane extracts of *Piper nigrum*, *Smyrnium rotundifolium*, and *Juniperus* spp. Bioactivity-guided fractionation of the complex mixtures revealed that certain ubiquitously occurring sesquiterpenes of the humulan and caryophyllan types show selective affinity to CB2 over CB1. Since these classes of compounds are poorly soluble in water the most promising sesquiterpene was co-incubated at a fixed concentration of 200 nM with varying concentrations of cannabinol, and [^{3}H]-CP55940 displacement experiments on both CB1 and CB2 receptors were performed. The sesquiterpene showed a significant additive effect at the CB2 receptor but not at CB1 receptor. The apparent Ki value for CB2 was 700 nM under bovine serum albumin (BSA)-free conditions. Our data show that the Ki value is determined by solubility rather than receptor interaction. In order to characterize the effect of ligand binding we assessed intracellular calcium transient responses in CB2 expressing HL60 cells. In HEPES buffer containing bovine serum albumin the effect size of the sesquiterpene was approximately the same as obtained with the endogenous cannabinoid 2-arachidonoyl glycerol (EC_{50} = 5.8 μM), thus indicating an agonistic mechanism. The CB2-selective antagonist SR144528 (10 μM), which did not show an effect on intracellular calcium, fully blocked the effects triggered by both 2-AG and the sequiterpene. Like 2-AG, this compound was effective in CD3-induced T-lymphocyte proliferation assays where it showed a concentration and CB2-dependent inhibition. Moreover, LPS-induced tumor necrosis alpha (TNF-α) expression from human primary monocytes/macrophages was dose-dependently inhibited by both 2-AG and the CB2 receptor-binding sesquiterpene.

Our data provide the first proof of CB2-selective functional agonists in *Cannabis sativa* other than cannabinoids. This finding may therefore have implications for the therapeutic applications of *Cannabis sativa* extracts containing sesquiterpenes.

MAPPING CANNABINOID-MEDIATED G-PROTEIN ACTIVITY IN THE 3D RECONSTRUCTED MOUSE BRAIN

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Receptor-mediated G-protein activity has been examined in specific regions thought to mediate cannabinergic effects. However, the relationship between ligand type and localization of receptor-mediated G-protein activity has not been systematically addressed using a novel 3D unbiased image analysis approach.

Coronal sections from naïve mice were cut in a cryostat with an interslice spacing of 200 μm throughout the entire neuroaxis and processed for autoradiography. Maximally effective concentrations (10 μM) of cannabinoid agonists, methanandamide (m-AEA) and WIN 55,212-2 (WIN), were compared in their ability to activate G-proteins, and the neuroanatomical localization of this activity was determined using agonist-stimulated [^{35}S]GTPγS autoradiography. We used a voxel-based analysis technique, Statistical Parametric Mapping (SPM), to localize differences in magnitude of [^{35}S]GTPγS binding in the 3D reconstructed mouse brain. Each autoradiographic section was digitized, quantitated, realigned to its neighbor using an intensity-based registration algorithm, and all aligned sections were then imported as a volumetric image array. Reconstructed brain volumes were spatially normalized to a common coordinate space defined by a study-specific brain template, which is derived from the average of all reconstructed brain volumes in the study. SPM facilitates a semi-automated procedure for creating statistical images and localizing changes on a whole-brain basis by applying a general linear model to each voxel. SPM allows efficient exploration of large data sets, while circumventing the limited sampling and subjective nature of a regions-of-interest analysis. In addition, SPM provides 3D visualization of images, which can be rotated or resliced in any plane to reveal the internal anatomical detail and spatial extent of agonist-stimulated [^{35}S]GTPγS binding.

Regions that showed the highest levels of receptor-mediated G-protein activity included: caudate-putamen, globus pallidus, hippocampus, substantia nigra, and cerebellum. Overall, receptor-mediated G-protein activity was lower using m-AEA as compared to WIN, which is in agreement with m-AEA acting as a partial agonist. [^{35}S]GTPγS autoradiography demonstrated a unique global profile of receptor-mediated G-protein activity, varying both in magnitude and neuroanatomical localization, when using a maximally effective dose of either m-AEA or WIN. These results show ligand dependent regulation of cannabinoid receptor function that can be spatially localized and mapped throughout the 3D reconstructed mouse brain.

Acknowledgements: This work was supported by NIH Grants DA14277 and DA05274

Nguyen PT, Holschneider DP, Maarek JM, Yang J, Mandelkern MA (2004) Statistical parametric mapping applied to an autoradiographic study of cerebral activation during treadmill walking in rats. *NeuroImage* 23: 252-9

ASSESSMENT OF CANNABINOID CB1 RECEPTOR AGONISTS IN VITRO

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The development of CB1 agonists as effective analgesics involves multi-parameter optimization of compounds. The first requirements are *in vitro* assays of appropriate physiological relevance, to provide SAR around the compounds' interaction with CB1 receptors and to guide synthetic chemistry. CB1 agonists, acting via alpha or beta/gamma subunits of G-proteins, induce several cellular changes that may be used as functional assay endpoints *in vitro*. In this study, we assessed a simple panel of assays to report affinity and agonist activity of novel compounds at CB1 receptors.

The potency and efficacy of test compounds were determined in GTPγ[^{35}S] binding assays using membranes prepared from HEK-293S cells expressing cloned human CB1 and rat CB1 receptors. Competition experiments were used to measure binding of [^{3}H]-CP55940, in the presence of various concentrations of unlabeled test compounds, to the same membranes. A subset of CB1 agonists was assessed further at a CRO (CEREP) for ability to inhibit electrically induced contraction of *mouse vas deferens*, a more traditional assay of CB1 agonism using isolated tissue in an organ bath.

We confirmed that, for over 600 agonists, their potency in the human CB1 GTPγ[^{35}S] assay correlated well with binding affinity at the receptor, and similar activity was observed between human and rat CB1. Compounds that acted as agonists in the human CB1 GTPγ[^{35}S] also showed agonism in the *mouse vas deferens* assay. The assays described herein were validated with reference compounds and used to identify CB1 agonists for further characterization *in vivo*.

We conclude that the CB1 assays using [^{3}H]-CP55,940 and GTPγ[^{35}S] binding are relevant SAR-driving assays, and despite the complexity of CB1 signaling pathways, simple G-protein activation predicts agonism in a more physiological system (mouse *vas deferens*) and identifies new chemical entities for further testing in rodent pain models.

THE PET RADIOLIGAND [^{11}C]MEPPEP BINDS REVERSIBLY AND WITH HIGH SPECIFIC SIGNAL TO CANNABINOID CB1 RECEPTORS IN NON-HUMAN PRIMATE BRAIN

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Phytocannabinoids are arguably the world's oldest pharmaceuticals, but have limited therapeutic potential due to their sub-optimal drug-like properties. Recent convergence of advances in biology, molecular pharmacology, medicinal chemistry, and medical imaging provides the opportunity to improve the design of compounds that target the cannabinoid system for CNS indications. Key to the success of neuroscience therapeutic development is the availability of biomarkers to provide information regarding CNS penetration and target occupancy. Through a cross functional partnership between the NIH and industry, a selective CB1 receptor PET ligand, ^{11}C-MePPEP, was created and evaluated in non-human primates to determine spatial and temporal measures of receptor occupancy in brain. Following intravenous injection, ^{11}C-MePPEP reached high levels in central compartments with regional uptake consistent with the distribution of CB1 receptors. High tracer emission levels were observed in cerebellum and striatum and much lower levels observed in thalamus and pons. Pre-administration of two CB1 selective antagonists at pharmacological doses displaced ^{11}C-MePPEP binding indicating greater than 89% specific binding in CB1 receptor rich brain regions. These preclinical studies suggest that ^{11}C-MePPEP may be useful for measuring occupancy of experimental CB1 antagonists in human subjects.

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