Joint Research Conference of the Institute for Advanced Studies
and the Israel Science Foundation

WORKSHOP PROGRAM

Cannabinoids in Biology and Medicine

October 31 – November 4, 2010

Director: Itai Bab, The Hebrew University of Jerusalem
Codirectors: Ester Fride, Ariel University Center of Samaria
            Ester Shohami, The Hebrew University of Jerusalem
            Zvi Vogel, Weizmann Institute of Science

The Workshop will take place at the Institute for Advanced Studies,
The Hebrew University, the Feldman Building, Edmond J. Safra
Campus, Givat Ram, Jerusalem, Israel
Sunday, October 31

16:00-18:30   Registration
18:30   Welcoming Reception in the IAS lobby

Monday, November 1

SESSION I  PHYSIOLOGICAL ROLES OF CANNABINOID RECEPTORS
Moderator: Zvi Vogel (Weizmann Institute of Science, Rehovot, Israel)

09:00-09:30  Andreas Zimmer (University of Bonn, Germany)
Genetic analysis of the endocannabinoid system: from mice to men

09:30-10:00  George Kunos (NIH/ NIAAA, Maryland)
The endocannabinoid system and the control of energy metabolism

10:00-10:30  Coffee Break

10:30-11:00  Sophie Lotersztajn (INSERM, Groupe Hospitalier Henri Mondor, Créteil, France)
The endocannabinoid system in liver pathophysiology

11:00-11:30  Heather Bradshaw (Indiana University, Bloomington, Indiana)
Endogenous cannabinoid regulation of uterine physiology

11:30-12:00  Itai Bab (The Hebrew University of Jerusalem, Israel)
The skeletal cannabinoid system

12:00-12:15  Yossef Tam (NIH/NIAAA, Maryland)
Peripheral CB1 receptor blockade improves metabolic parameters in diet-induced obese mice independent of adiponectin

12:15-12:30  Ruth Gallily (The Hebrew University of Jerusalem, Israel)
Therapeutic role of cannabidiol in rheumatic arthritis, diabetes and heart ischemia

12:30-14:30  Lunch/Poster session I

14:30-14:40  Prof. Ester Fride Commemoration
SESSION II  CANNABINOID RECEPTOR SIGNALING
Moderator: Ken Mackie (Indiana University, Bloomington, Indiana)

14:40-15:10  Beat Lutz (Universitätsmedizin of the Johannes Gutenberg University Mainz, Mainz, Germany)
CB1 cannabinoid receptor in the interplay between nervous system and peripheral organ functions

15:10-15:40  Mauro Maccarrone (Universitá Degli Studi Di Teramo, Teramo, Italy)
Impact of membrane environment on endocannabinoid signaling

15:40-16:10  Neta Rimmerman (Weizmann Institute of Science, Rehovot, Israel)
Compartmentalization of the machinery for anandamide and 2-AG signaling

16:10-16:40  Coffee Break

16:40-17:10  Allyn Howlett (Wake Forest University, Winston-Salem, North Carolina)
CB1 receptor signaling to the cell: speculations and supporting data

17:10-17:25  Ruth Ross (University of Aberdeen, Scotland, United Kingdom)
Modulation of GPR55 signaling by phyto-cannabinoids measured using the AlphaScreen assay

17:25-17:40  Raphael Rozenfeld (Mount Sinai School of Medicine, New York, New York)
Cannabinoid-angiotensin receptor interaction contributes to hepatic stellate cell activation

17:40-18:40  Keynote lecture
Mone Zaidi (Mount Sinai School of Medicine, New York, New York)
The pituitary-bone axis

Tuesday, November 2

SESSION III  CANNABINOIDS AND BRAIN FUNCTION
Moderator: Javier Fernández Ruiz
(Universidad Complutense, Madrid, Spain)

08:45-09:15  Ken Mackie (Indiana University, Bloomington, Indiana)
Endogenous cannabinoids and synaptic plasticity
09:15-09:45  **Yosef Sarne** (Tel Aviv University, Tel Aviv, Israel)
Cannabinoids between neuroprotection and toxicity

09:45-10:15  Coffee Break

10:15-10:45  **Esther Shohami** (The Hebrew University of Jerusalem, Israel)
Endocannabinoids and traumatic brain injury

10:45-11:15  **Javier Fernández Ruiz** (Universidad Complutense Madrid, Spain)
Neuroprotection with CB2 agonists or antioxidant cannabinoids in Parkinson’s and Huntington’s disease

11:15-11:45  **Zvi Vogel** (Weizmann Institute of Science, Rehovot, Israel)
Effect of cannabinoids on microglial cells and their protective role in an animal model of multiple sclerosis

11:45-12:00  **Haleli Sharir** (Temple University, Philadelphia, Pennsylvania)
Activity of endocannabinoids at GPR55- v-interdhamine inhibits β-arrestin recruitment

12:00-13:30  Lunch

13:30-18:00  Jerusalem Tour

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**Wednesday, November 3**

**SESSION IV  ENDOCANNABINOID SYNTHESIS AND DEGRADATION**
Moderator: **Vincenzo Di Marzo** (Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy)

09:00-09:30  **Ben Cravatt** (The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California)
The enzymatic regulation of endogenous cannabinoids and its therapeutic implications

09:30-10:00  **Dale Deutsch** (Stony Brook University, Stony Brook, New York)
FABPs and FAAHs in anandamide inactivation

10:00-10:25  Coffee Break

10:25-10:55  **Daniele Piomelli** (University of California, Irvine, California)
Pathways to endocannabinoid-based therapies
10:55-11:25 Aron Lichman (Commonwealth University, Richmond, Virginia)
A tale of two endocannabinoids: physiological function and therapeutic targets of endocannabinoid catabolic enzymes

11:25-11:45 Elliot Berry (The Hebrew University of Jerusalem, Israel)
Endocannabinoids and AMPK in Liver Disease

11:45-12:00 Joanna Slusar (Dalhouse University, Halifax, Nova Scotia, Canada)
Effects of endocannabinoid enzyme inhibition in a rat model of optic nerve injury

12:00-12:15 Christoph Buettner (Mount Sinai School of Medicine, New York, New York)
Central endocannabinoid signaling regulates hepatic glucose production and systemic lipolysis

12:15-12:30 Young Investigator Award Ceremony

12:30-14:30 Lunch/Poster Session II

SESSION V CANNABINOID PHARMACOLOGY
Moderator: George Kunos (NIH/ NIAAA, Maryland)

14:30-15:00 Vincenzo Di Marzo (Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy)
Functional and chemical relationships between the endocannabinoid and the endovanilloids systems: potential for new therapeutic drugs

15:00-15:30 Roger Pertwee (University of Aberdeen, Scotland, United Kingdom)
Emerging pharmacological strategies for exploiting cannabinoids as medicines

15:30-16:00 Pál Pacher (NIH/ NIAAA, Maryland)
Role of the CB2 receptors in inflammation and tissue injury: interplay of activated endothelium and inflammatory cells

16:00-16:30 Coffee Break

16:30-17:00 Ethan Russo (GW Pharmaceuticals, Vashon, Washington)
The phytocannabinoid-terpenoid entourage effect: nature’s pharmacological dance partners

17:00-17:45 Keynote lecture
Raphael Mechoulam (The Hebrew University of Jerusalem, Israel)
Cannabinoids in biology and medicine: the last 40 years

18:30 Reception in honor of Professor Raphael Mechoulam
Thursday, November 4

SESSION VI  CANNABINOIDS IN THE TREATMENT OF NEUROLOGICAL DISEASES AND SYMPTOMS
Moderator: Mauro Maccarrone (Università Degli Studi Di Teramo, Teramo, Italy)

09:00-09:30  Andrea Hohmann (University of Indiana, Bloomington, Indiana)
Endocannabinoid signaling systems in pain modulation

09:30-10:00  Mary Lynch (Dalhouse University, Halifax, Nova Scotia, Canada)
Clinical use of cannabinoids for pain management

10:00-10:30  Coffee Break

10:30-11:00  Cecilia Hillard (Medical College of Wisconsin, Milwaukee, Wisconsin)
Endocannabinoid signaling and regulation of stress

11:00-11:30  Linda Parker (University of Guelph, Guelph, Ontario, Canada)
Role of cannabinoids in the regulation of nausea and vomiting

11:30-12:00  Daniela Parolaro (University of Insubria, Busto Arsizio, Italy)
Endocannabinoid system and neuropsychiatric diseases

12:00-12:15  Jürg Gertsch (University of Bern, Bern, Switzerland)
Discovery of an endocannabinoid binding site in GABAA receptors

12:15-12:30  Bogna Ignatowska-Jankowska (University of Gdansk, Poland)
Effects of chronic cannabidiol administration on splenic lymphocyte numbers and body weight gains in rats: role of CB2 receptors

12:30-12:45  Irit Akirav (University of Haifa, Haifa, Israel)
Cannabinoid modulation of emotional memory

12:45-13:00  Melanie Kelly (Dalhouse University, Halifax, Nova Scotia, Canada)
Examination of cannabinoid pharmacology in isolated retinal microvessels

13:00-14:00  Lunch

14:00  Meeting adjourned
POSTER SESSION I

1. **Piscitelli F**, Sirigu AR, Bisogno T, Vacca C, Berge K, Tandy S, Cohn JS, Banni S, Di Marzo V. Istituto di Chimica Biomolecolare, CNR, Pozzuoli (NA), Italy.
Lipid profiling of high-fat-fed mice after dietary krill oil supplementation.

2. **Gennequin B**, Otte DM, Miró X, Karsak M, Zimmer A, Zimmer A.
University of Bonn, Bonn, Germany.
Generation of CB2 receptor humanized mice.

3. **Ternes S**, Otte D, Miró X, Zimmer A, Schick C, Zimmer A.
University of Bonn, Germany.
Generation of conditional knockout mice for diacylglycerol lipase α and β.

The Johannes Gutenberg University Mainz, Mainz, Germany.
Endocannabinoid signaling and fear extinction: generation of a mouse line for cell type-specific rescue from CB₁ receptor deficiency.

5. Mason-Birks SJ, Marczylo T, Lam P. **Konje JC**.
University of Leicester, Leicester Royal Infirmary, Leicester, Leicestershire, UK.
Quantification of endocannabinoids in peritoneal fluid of women with endometriosis - is there a difference between active and in-active disease?

Investigation of endogenous ligands for peroxisome proliferator activated receptors.

7. **Shu K**. Kendall DA, Chapman V, Barrett D, Jeffcoate W, Bennett AJ, Scammell BE.
University of Nottingham, Nottingham, UK.
Identification of the endocannabinoid system in human bone: modulation of osteoclast function in culture.

University of Buenos Aires, Buenos Aires, Argentina.
Interaction between nitric oxide and endocannabinoids on the regulation of oxytocin release.

University Hospital Mannheim, Germany.
The endocannabinoid N-arachidonoyl dopamine (NADA) induces cell death in primary hepatic stellate cells.

10. **Marsicano G**, INSERM, NeuroCentre Magendie, Bordeaux, France.
Endocannabinoids, THC and rimonabant in the acute control of food intake: different actors for different stages.


13. Ganjiwale, A.D., Howlett, A., Cowzik, S.M. Jawaharlal Nehru University, New Delhi, India. Structural characterization of peptide mimetic of the fourth cytoplasmic loop of CB1 cannabinoid receptor: role of phosphorylation.

14. Burdet, B., De Laurentiis, A., Rettori, E., Rettori, V., Zubilete, M.Z. University of Buenos Aires, Buenos Aires, Argentina. Cannabinoid receptor subtype 1 (CB1), IL-1β and IL-6 are increased in some areas of limbic system after chronic restraint stress.


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**POSTER SESSION II**

1. Kaiser, N., Monory, K., Lutz, B. The Johannes Gutenberg University Mainz, Mainz, Germany. Loss of CB1 cannabinoid receptors in neurons expressing D1 dopamine receptors affects anxiety, motor learning and addiction behaviour.

2. Häring, M., Kaiser, N., Monory, K., Lutz, B. The Johannes Gutenberg University Mainz, Mainz, Germany. CB1 receptor function in the control of sociability behaviors.

4. **Segev A**, Akirav I.  
University of Haifa, Haifa, Israel.  
Cannabinoids and stress hormones differentially mediate the effects of acute stress on spatial memory and neural plasticity.

5. **Ramot A**, Akirav I.  
University of Haifa, Haifa, Israel.  
Cannabinoids activation in the amygdala block the stress-induced enhancement of emotionally negative learning.

6. **Abush H**, Akirav I.  
University of Haifa, Haifa, Israel.  
Does long-term administration of cannabinoids in young adult rats cause residual cognitive deficits?

7. **Ganon-Elazar E**, Akirav I.  
University of Haifa, Haifa, Israel.  
Cannabinoids prevent the effects of stress on PTSD-like symptoms in a rat model.

University of Aberdeen, Aberdeen, UK.  
Postnatal regulation of the endocannabinoid system in a mouse model for schizophrenia.

University of Bonn, Bonn, Germany.  
Targeting neuropathic pain by a natural CB2 receptor-agonist.

10. **Kelly MEM**, Dong AX, MacIntyre JN, Zhu J, Howlett SE.  
Dalhousie University, Halifax, Nova Scotia, Canada.  
Impact of modulation of the endocannabinoid system on the intestinal microcirculation in experimental sepsis.

11. **Peskuski KN**.  
Cannabis International, Mendocino, California, USA.  
Fresh cannabis: a non-psychoactive therapeutic modality.

12. **Courtney WL**.  
Cannabis International and Assoc. Luxembourgeoise des Methodes Preventives, Ettelbruck, Luxembourg.  
Conditionally essential and essential cannabinoid acids.

13. **Hergenrather JY**, Kerr SM.  
Society of Cannabis Clinicians / California Cannabis Research Medical Group, Santa Monica, California, USA.  
Clinical improvement and reduction of immunosuppressive drug therapy in cannabis treated patients with inflammatory bowel disease.

15. **Schechter M**, Pinhasov A, Weller A, Fride E. Ariel University Center, Ariel, Israel. Effect of dam’s CB1 receptor inhibition upon offspring social behavior.


Abstracts of Lectures and Poster Presentations
SELECTIVE TARGETING OF PERIPHERAL CB1 RECEPTORS AS A POTENTIAL NEW TREATMENT FOR THE METABOLIC SYNDROME

George Kunos

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

Obesity is associated with overactivity of the endocannabinoid/CB1 receptor system. Activation of CB1 receptors increases appetite, de novo lipogenesis, and promotes insulin resistance. CB1 receptor antagonists reduce body weight and improve cardiometabolic abnormalities in experimental and human obesity, but their therapeutic potential is limited by neuropsychiatric side effects due to blockade of CB1 receptors in the CNS. We have analyzed the effects of a structurally modified analog of rimonabant with greatly reduced brain penetrance. AM6545 retains the high CB1 affinity of rimonabant (Kd 3 nM), displays 200-fold selectivity for CB1 over CB2 receptors, is orally bioavailable, and it acts as a neutral CB1 antagonist. Its low brain penetrance is due partly to its reduced lipid solubility, and partly to being a substrate to P-glycoprotein-mediated extrusion through the blood-brain barrier. Tested at the dose of 10 mg/kg, AM6545 does not affect behavioral responses mediated by CB1 receptors and lacks the anxiogenic effects of rimonabant, yet it completely blocks anandamide-induced inhibition of small intestinal peristalsis, an effect mediated by peripheral CB1 receptors on cholinergic terminals innervating the gut. In mice with genetic or high fat diet-induced obesity, AM6545 is equieffective with rimonabant in improving glucose homeostasis, fatty liver, and plasma lipid profile. These effects are due to blockade of CB1R in peripheral tissues, including the liver, as verified through the use of CB1R-deficient mice with or without transgenic expression of CB1R in the liver. These results suggest that targeting peripheral CB1R has therapeutic potential for alleviating cardiometabolic risk in obese patients.
THE ENDOCANNABINOID SYSTEM IN LIVER PATHOPHYSIOLOGY

Sophie Lotersztajn

Inserm U955, Hôpital Henri Mondor,
Université Paris-Est, Faculté de Médecine, 94010, Creteil

Chronic liver disease is responsible for about 800,000 death/year due to cirrhosis and its complications. The most common causes of liver disease worldwide are viral hepatitis, chronic alcohol consumption and non alcoholic fatty liver disease associated with the metabolic syndrome. All these conditions generate liver injury and inflammation, thereby activating liver fibrogenesis and decreasing liver regeneration. Progression of fibrosis leads to cirrhosis and the life-threatening complications of liver failure and portal hypertension, as well as to incident hepatocellular carcinoma.

Accumulating evidence indicates that the endocannabinoid system plays a crucial role in the pathophysiology of liver diseases. Indeed, CB1 and CB2 receptors have emerged as mediators of non alcoholic and alcoholic fatty liver disease, and regulate hepatic inflammation, liver regeneration, liver fibrosis and complications of cirrhosis. Steatogenic and profibrogenic properties of CB1 receptors, and their harmful impact on hemodynamic complications of cirrhosis, suggest that CB1 receptors trigger several deleterious effects, that may enhance progression of chronic liver disease to cirrhosis and its complications. Peripherally-restricted CB1 antagonists are therefore expected in patients with non-alcoholic or alcoholic fatty liver disease, at multiple steps of disease progression, and preclinical studies give real hopes in the development of active CB1 molecules devoid of central activity. Beneficial antiinflammatory and hepatoprotective effects of CB2 agonists have been demonstrated in alcoholic liver disease. Moreover, CB2 agonists display antifibrogenic effects and stimulate liver regeneration. These findings may open novel perspectives for the treatment of chronic liver disease, upon clinical development of CB2 specific agonists.
CANNABINOIDS AND REPRODUCTION:
LOOK WHO’S TALKING...

Heather Bradshaw

Psychological and Brain Sciences
Kinsey Institute for Research in Sex, Gender, and Production
Indiana University

Endometriosis is a disease that is characterized by the growth of endometrial tissue outside the uterus that is associated with chronic pelvic pain and infertility. This disorder affects 10% of women of reproductive age worldwide. While the etiology of endometriosis is unclear, a leading hypothesis posits that retrograde menstruation allows viable endometrial cells to leave the uterus where they proliferate and innervate onto visceral organs such as the ovaries, bladder, and colon. The retrograde menstruation hypothesis suggests a mechanism for how endometrial cells leave the uterus; however, it does not address how endometrial cells target specific tissues resulting in the formation of the ectopic cysts that are associated with chronic pain and infertility. An alternative hypothesis is that endometrial cells are actively signaled towards these tissues through directed migration and that it is this communication system that should be therapeutically targeted. Recently, we have shown that the endogenous lipid, N-arachidonoyl glycine (NAGly), a metabolite of the endogenous cannabinoid anandamide, is a potent activator of cellular migration through the G-protein coupled receptor, GPR18. This migration is mimicked by abnormal cannabidiol (Abn-CBD) and is blocked completely by cannabidiol (CBD), providing evidence that GPR18 is the Abn-CBD receptor and NAGly is its endogenous ligand. Here, we show that NAGly-activated endometrial migration is not effected by SR144528 or Rimonabant, however, is completely blocked by CBD. Future studies will address the efficacy of CBD treatment to arrest pathophysiological endometrial migration that is associated with endometriosis.
THE SKELETAL CANNABINOID SYSTEM

Itai Bab

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

The main components of the endocannabinoid system have been reported in bone. Osteoblasts produce N-arachidonylethanolamine (Anandamide, AEA) and 2-arachidonoylglycerol (2-AG) and express diacylglycerol lipases (DAGLs) alpha and beta, critical enzymes for 2-AG biosynthesis. CB1 is present in skeletal sympathetic terminals. CB2 is expressed in osteoblasts and osteoclasts and in their precursors. Cb1 deficient mice have a low bone mass phenotype. CB1 is involved in a 2-AG – norepinephrine negative feedback circuit that regulates osteoblasts activity. Disruption of this loop mediates the stimulation of bone formation induced by traumatic brain injury. Cb2-null animals show a markedly accelerated age-related bone loss. CB2 stimulates osteoblast proliferation and activity and restrains osteoclastogenesis. In osteoblasts, CB2 signals via a Gi-protein – Erk1/2 – Mapkapk2 – CREB pathway. A CB2 specific agonist (HU-308), which is not psychoactive, attenuates and rescues ovariectomy-induced bone loss. Polymorphisms in the coding region of the human CNR2 gene, which encodes CB2, shows a highly significant association with osteoporosis. Recently, we have identified in bone a group of endocannabinoid-like N-acyl amides. The most skeletally active member of this group is oleoyl serine (OS), which activates a non-CB Gi-protein coupled receptor, thus stimulating osteoblast proliferation and inhibiting osteoclastogenesis and increasing osteoclast apoptosis. These studies offer novel targets for the development of skeletal diagnostics and therapeutics for osteoporosis and other bone deficits.
PERIPHERAL CB, RECEPTOR (CB₁R) BLOCKADE IMPROVES METABOLIC PARAMETERS IN DIET-INDUCED OBESE MICE INDEPENDENT OF ADIPONECTIN

Yossef Tam¹, Liang Zhou¹, V. Kiran Vemuri², Alexandros Makriannis², George Kunos¹

¹Laboratory of Physiologic Studies, National Institute on Alcohol Abuse & Alcoholism, Bethesda, MD 20892, USA; ²Center for Drug Discovery, Northeastern University, Boston, MA 02115, USA

The adipocyte-derived hormone adiponectin promotes fatty-acid oxidation and improves insulin sensitivity. Chronic CB₁R blockade also increases lipid oxidation and improves insulin sensitivity in obesity, resulting in reduced cardiometabolic risk. Chronic CB₁R blockade reverses the obesity-related decline in plasma adiponectin, which has been proposed to account for its antiobesity actions.

To test this hypothesis we have investigated the metabolic actions of AM6545, a peripheral CB₁R antagonist, in 6 week-old adiponectin knockout (Adipo⁻⁻⁻) mice and their wild-type controls (Adipo⁺⁺⁺). Mice were fed high-fat (HFD) or standard diet (STD) for 19 weeks before starting daily treatment with vehicle or AM6545 (10 mg/kg, ip.) for one week, followed by intraperitoneal glucose tolerance (iGTT) and insulin sensitivity tests (iIST) and metabolic measurements. HFD induced similar weight gain, hyperglycemia, insulin and leptin resistance, hyperlipidemia and hepatic steatosis in Adipo⁻⁻⁻ and Adipo⁺⁺⁺ mice. Seven-day treatment of HFD mice with AM6545 resulted in comparable reductions in body weight, adiposity, liver triglycerides, fasting plasma glucose, insulin, leptin and triglycerides levels in Adipo⁺⁺⁺ and Adipo⁻⁻⁻ mice. Similar to reported effect of rimonabant, AM6545 treatment reversed the HFD-induced reduction in plasma adiponectin levels in Adipo⁺⁺⁺ mice.

In conclusion, HFD-induced obesity and its metabolic consequences and their reversal by short term treatment with a peripheral CB₁R antagonist were similar in the absence or presence of adiponectin. Although CB₁R blockade reverses the HFD-induced reduction in plasma adiponectin, adiponectin either does not significantly contribute to the effects on weight and metabolic parameters, or if it does, compensatory mechanisms may be activated in its absence.
CANNABIDIOL: AN OLD MEDICINE IN THE NEW MILLENNIUM
A THERAPEUTIC AGENT FOR RHEUMATIC ARTHRITIS
DIABETES AND HEART ISCHEMIA

Ruth Gallily

The Lautenberg Center of Immunology and Cancer Research, The Hebrew University of Jerusalem, Faculty of Medicine, Jerusalem, Israel

The therapeutic potential of cannabidiol (CBD), the major nonpsychoactive component of cannabis, was explored in three animal models of rheumatoid arthritis, diabetes. The first indication tested was collagen induced arthritis, a model for rheumatoid arthritis in human. Application of CBD to DBA male mice, either intraperitoneally or orally, given after onset of clinical symptoms, suppressed significantly the arthritis clinical symptoms. Moreover, a protection of the joints against severe damage was observed indicating a disease modifying effect. The second animal model tested was diabetes type 1, which develops spontaneously in NOD female mice. We have shown that CBD given either before onset of diabetes symptoms (6-8 weeks) or after onset of diabetes symptoms (11-14 weeks), reduced markedly the incidence of type 1 diabetes, from 86% in non-treated mice to 30-32% in CBD-treated ones as evident by reduced glucose levels in the sera. The treatment also reduced significantly the level of pro-inflammatory cytokines TNFα and IFNγ. The most significant finding reveals a protection of Langerhaus islets from destruction. In CBD-treated NOD mice, 50% of the islets were protected from destruction compared to 1-10% in the control.

The third animal model study was heart ischemia in rats, following ligation of the coronary artery. Heart ischemia was induced in male Sprague-Dawley rats. When CBD was administered 1h before the procedure and every 24h thereafter, for 7 days, it was found that infarct size was reduced by 66% in CBD-treated animals, despite nearly identical areas at risk. The inflammatory response of the heart was also significantly reduced in CBD-treated animals.

Our data support the concept that CBD, known to be non-toxic and non-psychotropic, could be used in humans, as a therapeutic agent for treatments of RA, Diabetes Type 1 and Myocardial Ischemia.
LIPID PROFILING OF HIGH-FAT-FED MICE AFTER DIETARY KRILL OIL SUPPLEMENTATION

Fabiana Piscitelli1, Anna Rita Sirigu2, Tiziana Bisogno1, Claudia Vacca2, Kjetil Berge3, Sally Tandy4, Jeffrey S. Cohn4, Sebastiano Banni2, Vincenzo Di Marzo1

1Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, CNR, Pozzuoli (NA), Italy; 2Dipartimento di Biologia Sperimentale, Università di Cagliari, Italy; and Nutrisearch s.r.l. Pula (CA) Italy; 3Aker BioMarine, Oslo, Norway; 4Nutrition and Metabolism Group, Heart Research Institute, Sydney, Australia

Omega-3 polyunsaturated fatty acids (ω-3-PUFA) are known to ameliorate several metabolic risk factors for cardiovascular disease. We investigated the dose-dependent effects of dietary ω-3-PUFA supplementation, given as Krill oil (KO), on metabolic parameters in high fat diet (HFD)-fed mice. Since an association between elevated endocannabinoid (EC) levels and the metabolic syndrome was reported, we also measured the levels of EC and of their direct biosynthetic precursors, as well as of lipid congeners, in inguinal and epididymal adipose tissue (AT), liver, gastrocnemius muscle, kidneys and heart. Lipids were identified and quantified using high resolution ion trap-time of flight mass spectrometry coupled to liquid chromatography (LC-IT-TOF-MS). Eight-week HFD increased EC levels in all tissues except for epididymal AT, whereas KO reduced anandamide and/or 2-AG levels in all tissues but not in the liver, in a dose-dependent manner. Levels of EC precursors were generally down-regulated, suggesting that KO affects levels of endocannabinoids in part by reducing the availability of their biosynthetic precursors. Our data suggest that KO may promote therapeutic benefit by reducing EC precursor availability and hence EC biosynthesis.
Introduction: Mammalian tissues contain two types of cannabinoid receptor, CB1 and CB2, which belong to the family of G protein coupled receptors. Endocannabinoid binding to these receptors activates several cellular signalling pathways including the inhibition of the adenylyl cyclase–cyclic AMP–protein kinase A activity, activation of mitogen-activated protein kinase cascades (extracellular-signal-regulated kinase (ERK), JUN amino-terminal kinase (JNK) and p38); and activation of the phosphatidylinositol-3-kinase–AKT pathway. In general CB1 receptors are expressed at high levels in the central nervous system, whereas CB2 receptors are found predominantly in peripheral tissues e.g. on bone and immune cells. Our team has recently identified two different variants of the human CB2 receptor hCB2Gln63 and hCB2Arg63, respectively. Interestingly, the hCB2Arg63 variant has been associated with reduced bone density, osteoporosis, and psychiatric disorders in different human population samples. These findings are in concordance with the demonstration of a reduced signaling of the hCB2Arg63 variant.

Objectives: To study the functional consequences of these gene variants in vivo, we are generating humanized mice harboring both CB2 receptor variants. These mice will be a useful tool to study the in vivo effects of selective CB2 agonists and CB2 pathomechanisms in pain, osteoporosis and immune diseases. In addition these mice could be helpful in development of new drugs, which will more precisely target the CB2 receptor.
GENERATION OF CONDITIONAL KNOCKOUT MICE FOR DIACYLGLYCEROL LIPASE $\alpha$ AND $\beta$.

S. Ternes$^1$, D. Otte$^1$, X. Miró$^1$, A.M. Zimmer$^1$, C. Schick$^2$, A. Zimmer$^1$

$^1$ Institute of Molecular Psychiatry, University of Bonn, Germany
$^2$ Haus für Experimentelle Therapie, University of Bonn, Germany

During the last decade science could denote substantial progress in the cannabinoid research field. Besides an important role in neurotransmitter balancing and neuroprotection in the central nervous system, a set of different functions in peripheral organs was assigned to the endocannabinoid system (ECS). The distribution and mode of action of both cannabinoid receptors CB1 and CB2 are well characterized by now. In contrast it is still challenging to distinguish the different signaling properties of the two main endocannabinoids anandamide and 2-AG, because both messenger molecules are capable to act on CB1 as well as on CB2 receptors. To address this problem we chose to selectively inhibit the production of 2-AG in a knockout approach. We are generating conditional knockout mice for the two isoforms of the 2-AG producing enzymes diacylglycerol lipase (DAGL) $\alpha$ and $\beta$. For the construction of conditional knockout targeting vectors, the Red®/ET® cloning system developed by Genebridges™ has been used. The application of this system utilizes the advantage of homologous recombination in $E. coli$ and prevents dependence on restriction enzymes. In addition, commercially available ES cell clones that contain a targeting construct for DAGL $\alpha$ have been used to create chimeric mice for the $dagl\alpha$ gene.

Our different knockout approaches will facilitate the detailed analysis of the physiological roles of 2-AG in the endocannabinoid system and allow to differentiate between the effects of 2-AG and other endocannabinoids.
In the brain, the endocannabinoid system is involved in the regulation of a large variety of functions. Among these is the support of extinction of fear memories. The amygdala is crucial for the modulation of this emotional memory and contains cannabinoid receptors type 1 (CB₁ receptor) and endocannabinoids. We previously demonstrated that CB₁ receptor-deficient mice are strongly impaired in short-term and long-term extinction in auditory fear-conditioning tests, with unaffected memory acquisition and consolidation. To further understand the mechanism underlying this memory extinction process we generated a novel mouse line for cell type-specific rescue from CB₁ receptor deficiency. A loxP-flanked transcriptional stop cassette in the CB₁ receptor gene locus represses CB₁ receptor expression throughout the entire body, including the brain. By crossing this line with transgenic mouse lines that express Cre recombinase selectively in glutamatergic (Nex-Cre) or GABAergic (Dlx5/6-Cre) neurons, endogenous levels of CB₁ receptor will be reactivated locally. By virus-mediated delivery into distinct brain regions of Cre recombinase under the regulation of cell type-specific promoters we will also target distinct neuronal subpopulations. This will allow us to address the physiological role of specific cell populations within the amygdala and other brain areas in the control of fear extinction. In addition, this novel mouse line will also be of value to further investigate the many aspects of endocannabinoid signaling, in processes such as neuroprotection, anxiety, stress responses, feeding behavior and pain perception, under normal as well as pathological conditions.
Quantification of Endocannabinoids in Peritoneal Fluid of Women with Endometriosis - Is There a Difference Between Active and Inactive Disease?

Sara-Jane Mason-Birks, Timothy Marczylo, Patricia Lam, Justin C Konje

Endocannabinoid Research Group (ERG)
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Background: Endocannabinoids are unsaturated fatty acid derivatives that act as ligands for cannabinoid receptors, widely distributed throughout the human body. FAAH and MAGL are the prototype enzymes that degrade these ligands. Several studies have linked the endocannabinoids to inflammation and pain. Endometriosis, a condition which causes pain affects 10-15% of women of reproductive age.

Objectives: We have previously demonstrated elevated levels of the endocannabinoid anandamide (AEA) in 12 women with endometriosis. Here we measured AEA levels in 76 women with laparoscopically confirmed endometriosis, to confirm our pilot observations and to relate the levels to the type of endometriosis and therefore by proxy pain.

Methods: Peritoneal fluid (PF) was obtained from 76 women aged between 19-49 years with laparoscopically confirmed endometriosis who had given consent to partake in the study for which Ethical approval had been granted. The endometriosis was staged according the AFS classification and also into active and inactive. The PF was processed for quantification of AEA and other endocannabinoids using our developed solid-phase method of extraction and UPLC-MS/MS.

Results: AEA levels were significantly higher (P<0.05) in all those with active endometriosis, and in those with severe and active disease. Mean levels were 0.906 vs 0.7184nM in active and inactive mild disease respectively; 0.8072 vs 0.6314 in active and inactive moderate disease and 1.1823 vs 0.4874 in active and inactive severe endometriosis.

Conclusions: AEA levels were found to be significantly higher in active endometriosis, suggesting that AEA may be involved in the pathogenesis of the disease and the symptom of pain.
Activation of the peroxisome proliferator activated receptor alpha (PPARα) modulates inflammatory pain responses, however the endogenous ligand(s) which mediate the biological effects of PPARα are unknown. The aim of this study was to evaluate the potency of ligands at PPARα, and to develop and apply a novel analytical method for the measurement of endocannabinoids and identified PPARα ligands in vivo.

TR-FRET PPARα co-activator assays were used to determine the EC\textsubscript{50} of a number of potential ligands identified from the literature. A novel LC-MS/MS method that can simultaneously identify and quantify the endocannabinoids, eicosanoids and their metabolites in biological tissue was developed to investigate the relative levels of these compounds in vivo.

The following compounds were shown to bind and activate PPARα: PEA, OEA, AEA and 8-HETE. 8-HETE was a potent PPARα ligand (EC\textsubscript{50} 30nM) compared to the endocannabinoids OEA (EC\textsubscript{50} 86µM), AEA (EC\textsubscript{50} 31µM) and PEA (EC\textsubscript{50} 2.2mM) which were much weaker ligands. A number of other HETEs (5, 12, 15, 16, 19 and 20-HETE) showed no significant binding to PPARα in the in vitro assay. Levels of these PPARα ligands in the rat spinal cord were: AEA (22.0 pmol/g), OEA (0.2 nmol/g), PEA (0.5 nmol/g) and 8-HETE (9.0 pmol/g).

The affinity of 8-HETE for PPARα as compared to other endogenous ligands, and the presence of this eicosanoid in the spinal cord, suggests that the modulation of levels of 8-HETE could be used as a potential therapeutic strategy in the treatment of chronic pain states.
IDENTIFICATION OF THE ENDOCANNABINOID SYSTEM IN HUMAN BONE: MODULATION OF OSTEOCLAST FUNCTION IN CULTURE

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Introduction: Studies in rodent models suggest that the endocannabinoid system modulates bone remodelling, via actions at the cannabinoid CB1, CB2 receptors and TRPV1. The aims of the present study were to measure levels of the endocannabinoids, and components of the endocannabinoid receptor system in human trabecular bone and to investigate the effects of the endocannabinoid AEA on human osteoclast culture.

Methods and Results: 2-AG (12.7±13.6 pmol/g), PEA (6.4±3.9 pmol/g) and OEA (1.7±0.9 pmol/g) were measured in human trabecular bone from patients undergoing elective orthopaedic surgery, using Liquid Chromatography Mass Spectrometry (LC-MS-MS). Components of the endocannabinoid system (with the exception of CB2 receptor) were detected at the mRNA level in human trabecular bone with Taqman Real-Time PCR. Osteoclasts were differentiated from U-937 cells (Human leukaemic monocyte lymphoma cells) using TPA (0.1 μg/ml) followed by TNF-α (3 ng/ml). Tartrate resistant acid phosphatase (TRAP) positive, multinucleated, calcium phosphate resorbing osteoclasts were identified from day 8 of culture. mRNA expression of TRAP and components of the endocannabinoid system (with the exception of FAAH and NAPEPLD) were significantly up-regulated with osteoclast maturation. Exposure of osteoclasts to capsazepine, but not AEA, significantly attenuated the number of TRAP positive osteoclasts. Effects of capsazepine were blocked by AEA.

Conclusion: The detection of both synthetic and catabolic enzymes of the endocannabinoids in human trabecular bone and osteoclast culture indicates local skeletal production and regulation of endocannabinoids. These data suggest that activation of TRPV1 by endogenous ligands may directly regulate osteoclast differentiation and that TRPV1 blockers may be of value in preventing bone loss.
INTERACTION BETWEEN NITRIC OXIDE AND ENDOCANNABINOIDS ON THE REGULATION OF OXYTOCIN RELEASE

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Nitric oxide (NO) synthase (NOS) is expressed in the magnocellular neurons which produce oxytocin (OXT) and its inhibition increases OXT release in vivo. In a previous study in vitro we found that anandamide (AEA) inhibits OXT release from neurohypophysis (NH) and increases its release from hypothalamus (HYP). Therefore, we investigated the participation of NO and cannabinoid receptors (CB1 and CB2) in these effects.

AEA (10⁻⁹M) increased (p<0.01) NOS activity in both HYP and NH. Sodium nitroprusside (600µM), a NO donor, decreased (p<0.01) OXT release from HYP and NH. In addition, haemoglobin (40µg/ml), a scavenger of NO, augmented the increase of OXT release induced by AEA in HYP while blocked the decrease induced in NH. The presence of AM251 (10⁻⁵M), a CB1 antagonist, completely prevented the stimulatory effect of AEA on OXT release in HYP but did not modify the inhibitory effect of AEA on OXT release from NH. On the contrary, AM630 (10⁻⁵M), a CB2 antagonist, reverted the inhibitory effect in NH and was without effect in HYP. The evaluation of CB1 and CB2 mRNA by quantitative RT-PCR showed that both are present in the HYP but in NH there is a higher quantity of CB2 mRNA. Western blot studies yielded higher amount of CB2 protein in NH that was almost undetectable in HYP.

We conclude that the opposite effects of AEA on OXT release between HYP and NH may be due to different intracellular pathways activated by these subtypes of cannabinoid receptors.

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THE ENDOCANNABINOID N-ARACHIDONYL DOPAMINE (NADA) INDUCES CELL DEATH IN PRIMARY HEPATIC STELLATE CELLS

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Introduction: Liver fibrosis is the response to chronic hepatic injury and results from an increased deposition of connective scar tissue by activated hepatic stellate cells (HSCs) and cell death in these activated HSCs enhances the resolution of this disease. We have previously shown that the endogenous cannabinoid anandamide (AEA) is a lipid mediator that blocks proliferation and induces cell death in HSCs, but not in hepatocytes. The effects on hepatic cell populations of other endocannabinoids such as N-arachidonoyl dopamine (NADA) have not been investigated so far.

Results: Similarly to AEA, NADA dose-dependently induced cell death in culture-activated HSCs. Alike AEA, NADA induced hallmarks of necrotic cell death in HSCs and ROS formation in HSCs was significantly increased by NADA. Antioxidants significantly decreased cell death, demonstrating an ROS-dependent mechanism. Pharmacological blockade of the putative cannabinoid receptors CB1 and CB2 and the Vanilloid receptor TRPV1 failed to block NADA-mediated death, indicating a receptor-independent mechanism. Interestingly, membrane cholesterol depletion with methyl-β-cyclodextrin inhibited AEA-, but not NADA-induced cell death. Resistance to NADA in hepatocytes was due to high levels of GSH and high expression of the endocannabinoid-metabolizing enzyme fatty acid amide hydrolase (FAAH). Accordingly, adenoviral overexpression of FAAH in HSCs leads to resistance of NADA-induced cell death. Conclusion: NADA efficiently induced cell death in activated HSCs mediated by ROS formation, but not through engagement of cannabinoid receptors or membrane cholesterol-rich lipid rafts. GSH and FAAH determine resistance to NADA-mediated death in hepatocytes. Thus, NADA displays potential as an endogenous antifibrotic mediator.
Recent data indicate that different neuronal populations mediate diverse effects of (endo)cannabinoids in the acute regulation of food intake. In particular, we recently showed that the endocannabinoid system (ECS) displays a bimodal regulation of stimulated food intake. Type 1 cannabinoid receptors (CB1)-dependent control of glutamatergic transmission mediate at least in part the well-known hyperphagic functions of the ECS, whereas the modulation of brain GABAergic transmission underlies a novel hypophagic effect of (endo)cannabinoid signaling.

However, the locations of the circuits responsible of these differential functions are not fully clarified yet. Moreover, the neuronal types and the anatomical regions where CB1 antagonism exerts its hypophagic effect are not known.

By using conditional CB1 mutant mice and local intracerebral treatments, we systemically investigated these issues. The results clearly indicate that the locations where endogenous activation, exogenous agonism and pharmacological blockade of CB1 receptors acutely regulate food intake are very different. Hence, these data indicate that endocannabinoids, exogenous agonists and exogenous antagonists rely on different neuronal mechanisms to exert their acute control of food intake.
POSTNATAL TREATMENT WITH SR141716A IS ASSOCIATED WITH ADHD-LIKE SYMPTOMS IN ADULTHOOD

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Attention Deficit Hyperactivity Disorder (ADHD) is characterized by inattention, impulsivity and hyperactivity. The etiological and risk factors associated with ADHD are still unclear. Recently, low birth weight was found to be one of the most important predictive factors of ADHD. We hypothesised that treatment with SR141716A might lead to ADHD-like symptoms. In this study, SR141716A was given orally to mothers (0.06 mg/ml) while feeding their offspring between postnatal days 1 to 15 (model 2). In another model we treated pregnant mothers orally (0.06 mg/ml) 12 hours before and 24 hours after giving birth. At 8 weeks of age, offspring mice were tested for pre-pulse inhibition (PPI) of the acoustic startle response. At the age of 9-10 weeks the mice were tested for motor activity in the open field and for anxiety in the ‘plus-maze’ tests. Both female and male offspring mice from each model showed a significantly reduced response in the PPI test, suggesting that their sensorimotor-gating system was affected. Both males and females displayed a significant hyperactivity in rearing and a significant increase in exploration behaviour in the open field test. In the plus-maze test, both males and females spent more time in the open arms than in the closed arms, suggesting a decreased vulnerability to anxiety-provoking situations. Taken together, these results suggest that a direct inhibition of the endocannabinoid system in offspring after birth or indirectly via the mother contributes to the development of ADHD–like behaviour in adult offspring.

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THE EFFECT OF INTRA VENOUS SYNTHETIC THC ON THE ELECTROPHYSIOLOGICAL CORRELATES OF CORTICAL ACTIVATION AND DEACTIVATION

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Introduction
Oscillations at frequencies above 20 Hz, corresponding to the beta and gamma bands, are generated by the cortex during activation, such as during processing of sensory stimuli. These fast frequencies are replaced by rhythms at around 10Hz during periods of cortical deactivation in the alert state. Previous studies, in animals and man, have shown that ∆9-tetrahydrocannabinol (THC), alters neural electromagnetic oscillations. THC can also disturb sensory processing, and an acute challenge of ∆9-tetrahydrocannabinol (THC) can produce psychosis.

Method
The effect of 1.25 mg intravenous (THC), relative to placebo, on the EEG recorded during a self-paced button pressing task and during rest with 30 second periods of eyes open and eyes closed, was investigated. A randomised, double-blind, within subject, design was used with 16 healthy adults. The amplitude was quantified using a Fast Fourier Transform, and novel algorithms for the reduction of artifacts in the gamma band were applied.

Results and Discussion
THC produced significant reductions in the amplitude of both the visual high alpha (p=0.007) and sensorimotor mu (p=0.018) rhythms. The magnitude of these two effects, in different areas of cortex, were significantly correlated across subjects (p=0.007). Data will also be presented showing the effect of THC on the gamma band, before and after artefact reduction. These results are further evidence of the altered processing of sensory stimuli with THC.
Phosphorylation is an important regulatory mechanism in receptor signaling. It has been shown that phosphorylation of the CB1 receptor disrupts modulation of ion channels by the receptor. CB1 cannabinoid receptor intracellular C-terminal tail domain (amino acids 401-417) has been shown to be critical for G(i/o) protein coupling and the proximal portion of the C-terminus of the cannabinoid CB1 receptor is a primary determinant for G-protein activation. In the present study, we have used phosphorylated peptide fragment of the C-terminal juxtamembrane region (CB1 401-417) referred here as IL4 to analyze the effect of phosphorylation on the conformation of the peptide and G-protein activation using NMR Spectroscopy. Unambiguous proton NMR assignments have been carried out with the aid of correlation spectroscopy (DQF-COSY and TOCSY) experiments and nuclear Overhauser effect spectroscopy (NOESY and ROESY) experiments. The distance constraints obtained from the NMR data have been used to generate a family of structures which have been refined using restrained energy minimization and dynamics. The conformational range of the phosphorylated IL4 peptide revealed by NMR studies has been analyzed in terms of characteristic secondary structural features. The results obtained provide insight into the mechanism by which the peptide activates G-proteins, as a first step in signal transduction.
CANNABINOID RECEPTOR SUBTYPE 1 (CB1), IL-1β AND IL-6 ARE INCREASED IN SOME AREAS OF LIMBIC SYSTEM AFTER CHRONIC RESTRAINT STRESS

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Chronic stress leads to activation of hypothalamo-pituitary-adrenal axis (HPA) and changes in some parameters in limbic areas associated with anxiety. On the other hand, there is evidence that endocannabinoid system can modulate some stress responses.

The aim of this study was to find out if a chronic restraint stress could modify cannabinoid receptors expression β as IL-1β and IL-6 mRNA in amygdala and hippocampus. Sprague-Dawley adult male rats were divided in 4 groups: control (C) and restraint stress during two hours daily for 7, 14 or 21 days (n=6-8 rats per group). The results showed that there was an increase in corticosterone plasma levels as well as adrenal gland weight in all groups submitted to restraint stress. Oxytocin (OXT) plasma levels were only significantly increased at 7d (p<0.01) and 21d (p<0.05). Hippocampal CB1 mRNA by RT-PCR and protein expression by Western blot showed an increase in all stressed groups. IL-1β and IL-6 mRNA were significantly increased in amygdala only at 14d of stress. Stressed animals showed a reduced habituation in the open field test.

We conclude that in chronic stress cytokines such as IL-1β and IL-6 were increased in amygdala probably due to anxiety and that hippocampal endocannabinoid system participates in this paradigm of stress. (BID PICT 06-0258).
CONDITIONAL MUTAGENESIS REVEALS A DIRECT ENDOCANNABINOID CONTROL ON SEROTONERGIC NEURONS

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The brainstem raphe nuclei send serotonergic projections to almost all other brain regions. We have previously shown that 7-22% of these neurons express the type 1 cannabinoid receptor (CB1). Presynaptic CB1 receptors influence physiological functions by decreasing the release of various neurotransmitters. Therefore, we proposed that CB1 on serotonergic cells might play a part in physiological functions regulated by serotonin by decreasing serotonin release. To test our hypothesis, we generated an inducible conditional CB1 knock out mouse, lacking CB1 specifically in serotonergic cells, using the Cre/loxP system. Therefore, we crossed mice carrying a tamoxifen-inducible Cre-recombinase under the control of regulatory sequences of the tryptophan hydroxylase type 2 (TPH2) gene with mice having the CB1 gene flanked by loxP sites.

To avoid possible developmental consequences of early gene deletion, Cre recombinase expression was activated by injecting 10 week old CB1<sup>fl/fl;TPH2-CreERT2</sup> mice and their wild type littermates with 1 mg tamoxifen for five days. Three weeks after the last injection, mice were subjected to a battery of behavioural assays. Lack of CB1 on serotonergic neurons caused a general increase in locomotor activity. Stress and anxiety tests showed an anxiolytic phenotype: mutants spent more time in brightly lit areas and entered more to the open arms of the elevated plus maze. Furthermore CB1<sup>fl/fl;TPH2-CreERT2</sup> mice show a significantly slower increase in bodyweight compared to their wild type littermates. Taken together we could show for the first time that serotonergic neurons are under direct control of endocannabinoids and that this interaction is of great physiological importance.
Cannabinoid CB1 receptors have been shown to be importantly involved in drug addiction, in particular to marijuana, opiates, nicotine, and alcohol. However, the role of CB1 receptors in cocaine or psychostimulant addiction is controversial. One opinion is that CB1 receptors may be involved in certain aspects of cocaine addiction such as relapse to cocaine seeking, while being uninvolved in cocaine’s acute rewarding and psychomotor-stimulating effects. By using AM251, a more recently developed CB1 receptor antagonist than SR141716A, we found that CB1 receptors are importantly involved in cocaine’s actions as assessed by intravenous cocaine self-administration under progressive-ratio reinforcement, cocaine-enhanced brain-stimulation reward and cocaine-induced reinstatement of drug-seeking behavior. Further mechanistic studies demonstrated that a GABAergic mechanism in the ventral pallidum appears to underlie the antagonism of cocaine reward by AM251, while a glutamatergic mechanism in the nucleus accumbens underlies the antagonism of relapse to cocaine-seeking behavior after AM251. These findings suggest that the endocannabinoid CB1 receptor signaling system plays an important role in mediating cocaine reward and relapse to drug-seeking behavior. We suggest that the CB1 receptor antagonist approach to the development of anti-addiction medications is not flawed, but rather that AM251 is superior to SR141716A in terms of both efficacy and freedom from undesirable side-effects.
CB1 CANNABINOID RECEPTOR IN THE INTERPLAY BETWEEN THE NERVOUS SYSTEM AND PERIPHERAL ORGAN FUNCTIONS

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The endocannabinoid system (ECS) constitutes a wide-spread signaling system in the organism, acting in a paracrine, autocrine and maybe even in an endocrine manner. Insights into the “logic” of the ECS have greatly progressed in the recent years, by extensively using pharmacological and genetic tools. The functional analysis of the CB1 receptor in the context of the entire animal has been the focus of our research. While this receptor was originally annotated as the brain-type cannabinoid receptor, it has become evident during the recent few years that CB1 receptor comprises roles not only in the central nervous system, but also in a large variety of peripheral organs. Using conditional mutagenesis in mice, cell-type and organ-specific CB1 receptor deletions have been generated and analyzed in a variety of physiological and pathophysiological processes. Caused by the wide-spread expression of CB1 receptors, it is not surprising that this receptor is involved in many different processes in various organ systems. While it is relevant and interesting to evaluate the functional importance of CB1 receptors in a particular cell type, such a specific gene inactivation will consequently also influence other cell types and organ systems. Thus, it is important to understand these interactions and to integrate CB1 receptor functions into the context of an intricate network of organ interactions. This will be exemplified with CB1 receptor mutants in GABAergic and glutamatergic neurons regarding anxiety behaviours, and with restricted mutations in CNS, adipocytes, pancreas and sympathetic neurons/adrenals, regarding metabolism and energy balance.
IMPACT OF MEMBRANE ENVIRONMENT ON ENDOCANNABINOID SIGNALING

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The type-1 cannabinoid receptor (CB\textsubscript{1}) is the most abundant G-protein-coupled receptor (GPCR) in the brain. Together with its endogenous agonists, the so-called “endocannabinoids (eCBs)”, CB\textsubscript{1} belongs to an ancient neurosignaling system that plays important control functions within the brain. Not surprisingly, a wide range of neurodegenerative and neuroinflammatory disorders, such as Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis and multiple sclerosis depend on alterations of eCBs signaling. For this reason, research on the therapeutic potential of drugs modulating the endogenous tone of eCBs is very intense.

Several GPCRs reside within subdomains of the plasma membranes that contain high concentrations of cholesterol: the lipid rafts. CB\textsubscript{1} is negatively regulated by raft disruption, while type-2 (CB\textsubscript{2}) cannabinoid receptors are unaffected. This observation seems remarkable, because CB\textsubscript{2} essentially recognizes the same ligands and triggers the same signaling pathways as CB\textsubscript{1}.

Here, I shall summarize in vitro and in vivo data on the effect of raft disruption on eCBs signaling, showing that metabolism of the two major eCBs (anandamide and 2-arachidonoylglycerol) is differentially affected by membrane cholesterol depletion. In addition, I shall discuss structural differences between CB\textsubscript{1} and CB\textsubscript{2}, that might explain the different sensitivity of these two receptor subtypes to the membrane environment.
COMPARTMENTALIZATION OF ENDOCANNABINOID SIGNALING IN MICROGLIA

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Microglial cells are resident macrophages that serve as early host defense against pathogens in the central nervous system. We recently showed that the non-psychoactive plant cannabinoid, cannabidiol (CBD) inhibits LPS-activated proinflammatory pathways in the murine microglial cell line BV-2 (Kozela et al., 2010). We further investigated the compartmentalization of CBD and endocannabinoids in fractions collected from CBD-treated BV-2 microglial cells.

BV-2 cells grown in DMEM with 5% FBS were treated with CBD (10 μM) or vehicle for 3h. The cells were then homogenized and fractionated using a detergent-free OptiPrep density gradient. Whole cell media and fractions were analyzed for the presence and distribution of selected proteins and lipids. Lipids were purified from the methanolic extracts of the samples on solid phase cartridges and quantified using liquid chromatography tandem mass spectrometry (LC/MS/MS). Protein localization was determined by Western blotting.

We found that treatment of BV-2 microglial cell line with CBD resulted in increased accumulation of the endocannabinoid N-arachidonoyl ethanolamine in cell media. In the BV-2 fractions, CBD showed high affinity to non-lipid raft fractions while endocannabinoids and related proteins showed a mixed pattern. These results will be compared to those previously reported in a dorsal root ganglion cell line (Rimmerman et al., 2008).
CB1 RECEPTOR SIGNALING IN NEURONS: SPECULATION AND SUPPORTING DATA FOR INTERACTION OF MULTIPLE PATHWAYS

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The CB₁ cannabinoid receptor (CB₁R) regulates a variety of intracellular signaling pathways in neurons. Examination of CB₁R-mediated ERK1/2 tyrosine phosphorylation in neuronal N18TG2 cells reveals a complex interaction of multiple signals. Activation by 10 nM WIN55212-2 or CP55940 occurs in three phases: 1) robust ERK1/2 activation that peaks maximally at 2-5 min, 2) decline to a low level by 10 min, and 3) a secondary rise to a plateau level that is less than maximum. Each phase is blocked by the CB₁R antagonist SR141716, pertussis toxin, and MEK inhibitor PD98059, confirming mediation by the CB₁R, Gi/o proteins, and stimulation of the Raf/MEK/ERK1/2 MAPK cascade. In-Cell Western analyses revealed the vascular endothelial growth factor receptor (VEGFR/Flk-1), epidermal growth factor receptor (EGFR), and type 1 insulin-like growth factor receptor (IGF-1R) are involved in CB₁R regulation of ERK1/2. CB₁R-mediated maximal ERK1/2 activation is ligand-independent and requires phosphatidylinositol 3-kinase, Src kinase, protein tyrosine phosphatase 1B, and CB₁R internalization. ERK1/2 activation in N18TG2 cells is modulated by CB₁R-mediated inhibition of cAMP-stimulated PKA and subsequent MEK dephosphorylation via a phosphatase. Finally, CB₁R-mediated ERK1/2 phosphorylation is under tonic regulation by the cannabinoid receptor interacting protein 1a (CRIP1a). One can speculate regarding the utility of each of these processes/pathways on CB₁R-mediated neurogenesis and differentiation.

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MODULATION OF GPR55 SIGNALLING BY PHYTOCANNABINOIDS MEASURED USING THE ALPHASCREEN ASSAY

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The orphan receptor, GPR55, belongs to the GPCR superfamily, but in contrast to cannabinoid CB1 and CB2 receptors, is coupled to Gα₁₂/₁₃ and Gαq proteins. Recently, we have demonstrated that GPR55 is expressed in breast cancer cell lines and is involved in cell migration and polarization. Furthermore, GPR55 knock-out mice are resistant to inflammatory and neuropathic pain and have increased bone mass. These data suggest that GPR55 antagonists may have therapeutic potential. As part of our program to investigate the therapeutic potential of Cannabis Sativa, we examined the pharmacology of several of its constituents at GPR55. The phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2) is one of the main downstream signalling pathways that convey agonist-induced activation of GPR55. A high-throughput system was established to test the ERK1/2 phosphorylation using the AlphaScreen Surefire assay.

We first confirmed that the endogenous lipid, L-α-lyso phosphatidylinositol (LPI), stimulates ERK1/2 phosphorylation in HEK293 cells stably expressing the human GPR55 receptor. It induced a maximal stimulation of 91% ± 9.8 (Eₘₐₓ) after 20 min with an EC₅₀ of 300 nM (95% CL 160-530). LPI-induced stimulation of GPR55 was blocked by 10 μM PD98059, a non-competitive ERK1/2 inhibitor. We then compared the ability of constituents of the plant, Cannabis Sativa, to stimulate ERK1/2 phosphorylation in hGPR55-HEK293 cells. ∆⁹-tetrahydrocannabinol (∆⁹-THC) induced a 62% ± 18 stimulation at 10 µM while ∆⁹-tetrahydrocannabivarin (∆⁹-THCV) produced a 110% ± 17 stimulation of ERK1/2 phosphorylation at the same concentration. Cannabidiol (CBD) and cannabigerol (CBG) and their structural analogues, cannabidiol acid (CBDA) and cannabigerol acid (CBGA), were also investigated in this bioassay. At a concentration of 10 µM, CBD induced a 35% ± 10 stimulation while CBDA produced a 7% ± 8 inhibition of basal, and CBG induced a 25% ± 25 stimulation while CBGA produced a 42% ± 4 inhibition of basal. Each of the phytocannabinoids was investigated at a sub-inhibitory concentration for the modulation of LPI-induced stimulation of ERK1/2 phosphorylation. CBDA and CBGA were more effective than their prototype compounds, significantly modulating LPI-stimulated ERK1/2 phosphorylation at 1 µM.

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

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CANNABINOID-ANGIOTENSIN RECEPTOR INTERACTION CONTRIBUTES TO HEPATIC STELLATE CELL ACTIVATION

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A fundamental question in liver biology is how type 1 cannabinoid receptors (CB₁R) mediate liver fibrosis. CB₁Rs are upregulated in activated hepatic stellate cells (HSCs), the cells primarily responsible for the fibrogenic response in the liver, and preventing the expression or the activation of CB₁Rs attenuates the development of fibrosis. However, the mechanism underlying the role of CB₁R in liver fibrosis remains elusive. Here we show that in recombinant systems, CB₁R interacts with the angiotensin II receptor AT₁R, resulting in an hyperreactive AT₁R. The activation of AT₁R within the AT₁R-CB₁R heteromer is under the control of CB₁R activity and basal endocannabinoid tone, and leads to coupling to multiple G proteins and enhanced signaling. We demonstrate that in activated hepatic stellate cells from alcohol treated rats where the two receptors are endogenously co-expressed, AT₁R and CB₁R are colocalized and can be isolated in interacting complexes. Furthermore, in these cells, blocking CB₁R activity, prevents angiotensin II-mediated mitogenic signaling and profibrogenic gene expression. These results underscore the importance of receptor heteromerization as a mechanism by which CB₁R contributes to the profibrogenic effects of AT₁R and to HSC activation. This study also provides evidence for the generation of context-dependent G protein-coupled receptor heteromers that occur only in a pathological state, as potential disease-specific drug targets for the treatment of liver fibrosis.
Our studies have enabled the description of a connection between the pituitary and bone, in which anterior and posterior pituitary hormones bypass traditional endocrine targets to affect the skeleton directly, and with remarkable sensitivity. These studies began with our description, in 2003, of the skeletal phenotype of mice lacking the TSH receptor\(^1\). TSH receptor haploinsufficient mice showed a normal thyroid axis, but were osteopenic, suggesting that the effects of TSH signaling were independent of thyroid hormone\(^1\). In separate studies, we found that FSH\(^\beta\) haploinsufficiency in mice caused a reduction in bone resorption and a high bone mass in the face of normal estrogen\(^2\). With the demonstration \textit{in vitro} that TSH and FSH were anti- and pro-resorptive, respectively, we concluded that these hormones affect the skeleton directly. Since then, we have shown that TSH prevents bone loss in ovariectomized mice\(^3\); that over-expression of the TSH receptor reduces osteoclast formation\(^3\); that the absence of the osteoclastogenic cytokine TNF\(\alpha\) in double mutant, TSHR\(^+/+\)/TNF\(\alpha\)^{-/-}, rescues the TSHR\(^+/+\) phenotype\(^4\); and more recently, that TSH attenuates the formation of osteoclasts from embryonic stem cells \textit{in vitro}. Furthermore, we found that oxytocin, a neurohypophyseal hormone, hitherto thought solely to modulate lactation and social bonding, regulates bone mass directly. Deletion of OT or the OT receptor causes osteoporosis resulting from reduced bone formation. Consistent with this, oxytocin stimulates the differentiation of osteoblasts to a mineralizing phenotype. In contrast, oxytocin has dual effects on the osteoclast: it stimulates osteoclast formation, but inhibits bone resorption by mature cells. Finally, the hormone ACTH bypasses the adrenals to target the skeleton directly, and can be used experimentally to prevent the osteonecrosis induced by steroid hormones\(^6\). Overall, our complementary genetic and pharmacologic studies reveal a novel pituitary-bone axis of physiologic significance.

The enrichment of CB1 cannabinoid receptors on a subset of presynaptic terminals in the CNS suggested that their ligands, the endocannabinoids, might play a prominent role in synaptic plasticity. This is the case as both endocannabinoids and CB1 receptors have been implicated in diverse forms of synaptic plasticity. These include both short (tens of seconds) and long (ten of minutes, or more) forms of plasticity. These forms of plasticity are produced by a surprisingly diverse array of mechanisms.

Short forms of endocannabinoid-mediated synaptic plasticity include depolarization-induced suppression of inhibition/excitation (DSI or DSE) and metabotropic-induced suppression of inhibition/excitation (MSI or MSE). These are produced when either depolarization or activation of PLCbeta-linked G protein-coupled receptors lead to a brief production of endocannabinoid that then inhibit presynaptic CB1 receptors. Endocannabinoids also produce a persistent form of long term depression (often abbreviated as eLTD). Here, sustained excitatory synaptic input leads to the prolonged synthesis of endocannabinoids and prolonged activation of CB1 receptors. This leads to biochemical changes in the presynaptic terminal and a long-lasting decrease in neurotransmitter release, far outlasting the period of endocannabinoid production. eLTD may affect all CB1-containing terminals in the region of increased endocannabinoid production, so even though excitatory neurotransmission starts the process, inhibitory terminals may be strongly suppressed.

Despite the basic framework for endocannabinoid-mediated synaptic plasticity that has emerged over the past ten years, important gaps in our understanding of these processes remain. This talk will discuss recent results that help to fill in some those “missing pieces.”
CANNABINOIDS BETWEEN NEUROPROTECTION AND TOXICITY

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In vitro studies indicate that cannabinoid agonists may activate different signaling pathways and induce opposite effects on cell survival. One of the factors that determine the direction of the effect is the concentration of the agonist.

The in vivo neuroprotective effect of cannabinoid drugs is well documented. Based on our previous in vitro studies, we tested the effect of ultra low doses of THC on brain functioning. A single injection of 0.002 mg/kg THC, a dose that is 3 orders of magnitude lower than the doses that evoke the conventional cannabinoid effects, induced long lasting cognitive deficits in mice. These cognitive deficits were detected by various behavioral tests that measured different aspects of memory and learning, started at 48 hours and lasted up to 5 months following the treatment. The cognitive deficits were significant and reproducible but always mild.

We next tested whether this ultra low dose of THC can activate endogenous compensatory mechanism(s) that protect the brain from a more severe insult that either follows or precedes THC (pre- or post-conditioning). THC was administered 1-7 days before, or 1-3 days after the injection of the epileptogenic agent pentylenetetrazole (PTZ, 60 mg/kg) that by itself induced long lasting cognitive damage. The ultra low dose of THC abolished the deteriorating effects of PTZ as was evaluated by the various behavioral tests 3-7 weeks after the treatment.

Concomitant biochemical experiments revealed that the single injection of 0.002 mg/kg THC induced a long term modification of ERK (extracellular signal-regulated kinase) phosphorylation: a delayed activation that peaked at 24 hours followed by a sustained suppression that was detected up to 7 weeks after the injection of THC.

Our findings suggest that extremely low doses of THC may activate neuroadaptive process(es) and induce both minor toxicity and neuroprotection.
ENDOCANNABINOIDOS AND TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) represents the leading cause of death in young individuals. It triggers the accumulation of harmful mediators, leading to secondary damage, yet protective mechanisms are also set in motion. The levels of the highly abundant endocannabinoid 2-arachidonoyl-glycerol (2-AG) are significantly elevated after TBI. When administered to mice after TBI 2-AG decreases brain edema, blood-brain-barrier disruption, infarct volume and hippocampal cell death and improves clinical recovery. Moreover, the increase of NF-κB transactivation (at 24h) and of proinflammatory cytokines (2-4hrs) is abolished. The role of CB1R in mediating these effects was demonstrated using a selective antagonist or CB1R knockout mice. In models of brain ischemia and TBI agonists of CB2R were shown to reduce white blood cell rolling and adhesion, to reduce infarct size and to improve motor function.

2-AG was shown to stimulate Ca$^{2+}$ influx in endothelial cells, to induce phosphorylation of vasodilator-stimulated phosphoprotein (VASP) via the TRPV1 receptors and to counteract the ET-1-induced Ca$^{2+}$ mobilization. Taken together, the vasodilator effects of 2-AG in addition to its anti-inflammatory properties support the notion of an endogenous neuroprotective role of endocannabinoids after TBI or stroke.

N-arachidonoyl-L-serine (AraS) is a novel endocannabinoid, recently found to improve functional outcome and to decrease lesion volume after TBI. Our findings suggest that AraS affords neuroprotection via ERK and Akt phosphorylation and induction of their downstream anti-apoptotic pathways. These protective effects are probably related to signaling via CB2 receptors and TRPV1 and BK channels but not through CB1R or GPR55 receptors.
NEUROPROTECTION WITH CB$_2$ AGONISTS OR ANTIOXIDANT CANNABINOIDS IN PARKINSON’S AND HUNTINGTON’S DISEASES

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Cannabinoids have been proposed as promising medicines to arrest/delay disease progression in neurodegenerative disorders. This includes the case of Parkinson’s disease (PD) and Huntington’s disease (HD), which are within the most important neurological disorders affecting the basal ganglia structures. Two pharmacological profiles have been proposed for cannabinoids being effective in these two disorders. On the one hand, certain cannabinoids, including the phytocannabinoids ∆9-tetrahydrocannabinol (∆9-THC) and cannabidiol (CBD), have been found to protect nigral and striatal neurons from death in PD and HD, respectively, through a mechanism independent of CB$_1$ and CB$_2$ receptors and that involves the control of endogenous antioxidant defenses. This has been observed in different experimental models of both disorders, in particular those where oxidative injury is a prominent cytotoxic mechanism. On the other hand, the activation of CB$_2$ receptors has been also associated with a slower progression of neurodegenerative events in experimental models of both disorders. This effect would be exerted through limiting the toxic influence of microglial cells on neuronal homeostasis, in particular, by reducing the generation of nitric oxide, proinflammatory cytokines and reactive oxygen species. It is important to mention that CB$_2$ receptors have been recently identified in the healthy brain, mainly in glial elements and, to a lesser extent, in certain subpopulations of neurons, and that they are dramatically up-regulated in response to different types of damaging stimuli, which supports Raphi’s idea that the cannabinoid system behaves as an endogenous neuroprotective system. This CB$_2$ receptor up-regulation was found in many neurodegenerative disorders including HD and, to a lesser extent, PD, thus explaining the beneficial effects found with their selective agonists in both disorders. In conclusion, the evidence reported so far support that those cannabinoids having antioxidant properties and/or capability to activate CB$_2$ receptors may represent a promising therapy in HD and PD deserving an urgent clinical exploitation.

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EFFECT OF CANNABINOIDS ON MICROGLIAL CELLS AND THEIR PROTECTIVE ROLE IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Cannabinoids have been shown to exert anti-inflammatory activities. Using the BV-2 mouse microglial cell line and lipopolysaccharide (LPS) to induce inflammatory response, we studied the intracellular mechanisms involved in the anti-inflammatory activity of the non-psychoactive cannabinoid, cannabidiol (CBD). We observed that CBD decreases the release of interleukin (IL)-1\beta and IL-6 proinflammatory cytokines from activated microglial cells. CBD inhibits the activation of STAT1 proinflammatory transcription factor and up-regulates STAT3, an element of homeostatic mechanism(s) inducing anti-inflammatory events. Moreover, gene array analysis demonstrated that CBD downregulates the expression of many of the LPS-stimulated pro-inflammatory genes, exerting a much greater effect than ∆\textsuperscript{9}-tetrahydrocannabinol.

We evaluated the effects of CBD in myelin oligodendrocyte glycoprotein (MOG)-induced EAE murine model of multiple sclerosis (MS) and determined the mechanisms underlying these properties. We observed that CBD (administered at disease onset) ameliorates the clinical EAE symptoms as evaluated using behavioral scores. Histochemical analysis of spinal cords (SC) of MOG-injected EAE mice treated with CBD vs MOG-only treated mice revealed that CBD downregulates infiltration/proliferation of macrophages/microglia and T cells into SC white matter.

In conclusion, we observed that CBD exerts anti-inflammatory activities \textit{in vivo} in the EAE model of MS, as well as \textit{in vitro} in microglial cells \textit{via} STAT dependent pathways.
ACTIVITY OF ENDOCANNABINOIDS AT GPR55- VIRODHAMINE INHIBITS β-ARRESTIN RECRUITMENT

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Previous studies have reported that endocannabinoids can act as agonists at GPR55, a candidate cannabinoid receptor. Anandamide (N-arachidonyl ethanolamine), 2-arachidonyl glycerol and virodhamine (O-arachidonyl ethanolamine) have been reported to potently activate GPR55 in a GTPγS binding assay (Ryberg et al, Brit J Pharmacol 2007). In particular, virodhamine acted as a full agonist at GPR55, with an EC50 value of 12 nM. It thus displayed greater potency as a GPR55 agonist than at the well characterized CB1 and CB2 cannabinoid receptors; it has been shown to act as a partial agonist at CB1 and a full agonist at CB2 (EC50 values 2920 and 380nM). We have previously reported the use of beta-arrestin-green fluorescent protein biosensor as a direct readout of GPR55 activation in U2OS cells (Kapur et al, J.Biol. Chem, 2009). Examination of a panel of ligands revealed that only lysophosphatidyl inositol (LPI), SR141716A, and AM251 were equi-efficacious GPR55 agonists; and CP55940 was a GPR55 antagonist/partial agonist. In the present study we have utilized the previously characterized U2OS cells over expressing GPR55E and β-arrestin GFP to examine the possible role of virodhamine at GPR55. Interestingly, while some basal recruitment of β-arrestin GFP was evident, it was markedly increased upon application of 30 μM virodhamine. However, when the same concentration of virodhamine was applied with either LPI (3 μM) or SR141716A (30 μM), a dose-dependent inhibition of β-arrestin GFP was observed. Therefore, virodhamine acted as an antagonist when applied together with full agonists. Our data, thus suggest that Virodhamine acts as a partial agonist at GPR55.
THE ENZYMATIC REGULATION OF ENDOGENOUS CANNABINOIDS AND ITS THERAPEUTIC IMPLICATIONS

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Endogenous cannabinoids (endocannabinoids) are an important class of signaling lipids that act on both central and peripheral cannabinoid receptors, which also mediate the effects of delta9-tetrahydrocannabinol, the active component of marijuana. The magnitude and duration of endocannabinoid signaling are tightly controlled in vivo by the action of multiple biosynthetic and degradative enzymes. Here, I will discuss our lab’s efforts to develop selective genetic and pharmacological tools to perturb the function of individual endocannabinoid metabolic enzymes. These tools have not only confirmed key roles for enzymes, such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), in endocannabinoid metabolism, but have also revealed unexpected connectivities between the endocannabinoid system and other lipid signaling pathways, including prostaglandins and lysophospholipids. Such ‘systems-level’ interactions designate endocannabinoid hydrolases as regulators of larger metabolic networks that may influence diverse physiological and pathological processes, such as cancer, inflammation, and nervous system disorders.
In 1992 I attended an ICRS meeting where Dr. Mechoulam announced that he and his collaborators had discovered a new compound that binds to the cannabinoid receptor and that it was a lipid-like substance. He said they were confirming its structure and activity and that it would be published very soon. The paper came out shortly thereafter in 1992 in *Science* (Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R.). Dr. Mechoulam gave a presentation at a CPDD meeting in 1993 and skeptics in the audience demanded to know if there were enzymes to control the breakdown and synthesis of anandamide. Raphe delighted me by quoting our enzyme work that was under review. In those days, Perkin Elmer NEN Radiochemicals had research scientists who collaborated with academia and Dr. David Ahern provided my laboratory with radioactive anandamide. This allowed us to characterize an amidase that degraded anandamide to arachidonic acid and ethanolamine. We also observed a reverse reaction by adding an excess of ethanolamine (Deutsch and Chin, 1993). Having the amidase activity (now called FAAH) permitted us (with chemists at Stony Brook and Allyn Howlett in St. Louis) the first SAR study of FAAH inhibitors in a 1994 JBC paper. This field has burgeoned (using modern methods such as Cravatt’s ABPP) with the development of new classes of very specific and potent inhibitors that are in clinical trials.

The steps involved in the inactivation of anandamide will be reviewed with emphasis on the role of FAAH in driving uptake; the FABPs (fatty acid binding proteins) functioning as intracellular transporters of anandamide, and the diffusion of anandamide across the plasma membrane and the role of cholesterol in this process.
DISCOVERY OF PERIPHERALLY RESTRICTED FAAH INHIBITORS: NEW TOOLS TO STUDY THE FUNCTIONAL ROLES OF ANANDAMIDE

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Peripheral cannabinoid receptors exert a powerful inhibitory control over pain initiation, but the endocannabinoid signal that normally engages this intrinsic analgesic mechanism is unknown. To address this question, we developed a peripherally restricted inhibitor of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the endocannabinoid anandamide. The compound, called URB937, suppresses FAAH activity and increases anandamide levels outside the central nervous system (CNS). Despite its inability to access brain and spinal cord, URB937 attenuates behavioral responses indicative of persistent pain in rodent models of peripheral nerve injury and inflammation, and prevents noxious stimulus-evoked neuronal activation in spinal cord regions implicated in nociceptive processing. CB₁ cannabinoid receptor blockade prevents these effects. The results suggest that anandamide-mediated signaling at peripheral CB₁ receptors controls the access of pain-related inputs to the CNS. Brain-impenetrant FAAH inhibitors, which strengthen this gating mechanism, might offer a new approach to pain therapy.
A TALE OF TWO ENDOCANNABINOIDS: PHYSIOLOGICAL FUNCTION AND THERAPEUTIC TARGETS OF ENDOCANNABINOID CATABOLIC ENZYMES

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The endogenous cannabinoid system has been the focus of an intense amount of research to understand its role in basic physiological processes as well as for potential therapeutic targets to treat pain, psychiatric disorders (e.g., anxiety and depression), cancer, obesity, drug abuse and dependence, and a wide variety of other medical conditions. This system consists of two cannabinoid receptors (CB₁ and CB₂), endocannabinoid ligands, including anandamide (AEA) and 2-arachidonylglycerol (2-AG), and enzymes regulating the biosynthesis and catabolism of the endogenous ligands. This talk will focus on fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the primary catabolic enzymes of AEA and 2-AG, respectively. Acute inhibition of either enzyme elicits comparable antinociceptive effects in acute and neuropathic models of pain, as well as provides gastroprotective effects against ulcers induced by high doses of nonselective inhibitors of cyclooxygenase. Additionally, inhibition of either FAAH or MAGL reduces cannabinoid or opioid precipitated withdrawal signs. While blockade of either one of these enzymes elicits very few overt pharmacological effects in the tetrad assay, indicative of cannabimimetic activity, combined blockade of FAAH and MAGL produces full tetrad effects as well as THC-like subjective effects in the drug discrimination paradigm. Chronic treatment with FAAH or MAGL inhibitors reveals dramatically distinct consequences on CB₁ receptor function. Whereas the antinociceptive effects of FAAH inhibitors are sustained following chronic administration, repeated injections of a high dose of the MAGL inhibitor, JZL184, leads to tolerance in acute and chronic pain. Moreover, mice treated chronically with JZL184 also display cross-tolerance to THC as well as WIN55212-2, and is accompanied with CB₁ receptor down-regulation and desensitization. These findings indicate that FAAH and MAGL represent viable targets for the development of new therapeutic agents. However, the observations that full inhibition of MAGL activity for a prolonged period of time results in tolerance and disturbances in brain CB₁ receptor function, suggest the importance of examining whether the analgesic effects are maintained following partial blockade of this enzyme.
Hepatic encephalopathy (HE) is a neuropsychiatric disorder of complex pathogenesis caused by acute or chronic liver failure. We studied the etiology of cerebral dysfunction in a murine model of HE induced by either bile duct ligation or thioacetamide administration. We report that stimulation of cerebral AMP-activated protein kinase (AMPK), a major intracellular energy sensor, is a compensatory response to liver failure. This function of AMPK is regulated by endocannabinoids. The cannabinoid system controls systemic energy balance via the cannabinoid receptors CB-1 and CB-2. Under normal circumstances, AMPK activity is mediated by CB-1 while CB-2 is barely detected. However, CB-2 is strongly stimulated in response to liver failure. Administration of _9-tetrahydrocannabinol (THC) augmented AMPK activity and restored brain function in WT mice but not in their CB-2 KO littermates. These results suggest that HE is a disease of energy flux. CB-2 signalling is a cerebral stress response mechanism and makes AMPK a promising target for its treatment by modulating the cannabinoid system.

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THE EFFECTS OF ENDOCANNABINOID ENZYME INHIBITION IN A RAT MODEL OF OPTIC NERVE INJURY

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The most characterized endocannabinoids, anandamide (AEA) and 2-Arachidonoylglycerol (2-AG), have both been shown to have cell survival effects in the CNS, although, the mechanisms are not clear. This study examined the neuroprotective potential of URB597 and methoxy arachidonyl fluorophosphonate (MAFP), compounds that inhibit the degradation of AEA and AEA and 2-AG, respectively, in a rat optic nerve axotomy model of retinal ganglion cell (RGC) neurodegeneration.

RGCs were retrogradely labelled using fluorogold (FG) 7 days prior to transection of the optic nerve 1 mm behind the eye globe. Vehicle, URB597 (0.03 mg/kg i.p.), or URB597 (0.3mg/kg i.p.) + MAFP (1mg/kg i.p.) was administered daily until sacrifice. Enucleated eyes were then processed for histology and quantitative analysis. FG+ RGCs and phagocytotic microglia (MG) were quantified in retinal whole-mounts across four retinal quadrants.

URB597 increased RGC survival after 1 and 2 weeks of axotomy (p<0.05) compared to vehicle-treated animals. URB597-treated retinas also had a significant reduction in phagocytotic MG after 2 weeks of axotomy (p<0.05), but not after 1 week. URB597 + MAFP also showed significant increase in RGC after 1 week of axotomy (p<0.05) but as with URB597 alone, no reduction in phagocytotic MG were seen at 1 week compared to vehicle-treated retinas.

Combination URB597 and MAFP treatment showed a trend for increased RGC survival compared to URB597 alone at 1 week. Further studies are underway to investigate whether MAFP alone can increase RGC survival following 1 or 2 week axotomy and whether MG inhibition is apparent at 2 weeks with MAFP.

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CENTRAL ENDOCANNABINOID SIGNALING REGULATES HEPATIC GLUCOSE PRODUCTION AND SYSTEMIC LIPOLYSIS

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Insulin is the master regulator of both lipid and glucose homeostasis. Insulin suppresses hepatic glucose production (hGP) through direct effects mediated through hepatic insulin receptors and indirect mechanisms, which include the activation of neuronal insulin signaling and the modulation of gluconeogenic substrate flux from adipose tissue by suppressing lipolysis in adipose tissue. We have recently shown that hypothalamic insulin is an important determinant of adipose tissue insulin action (by reducing sympathetic outflow to adipose tissue) and have confirmed that it regulates hGP. Moreover, insulin resistance caused by short-term overfeeding is in part due to impaired hypothalamic insulin action, which is a failure of intracerebroventricular (ICV) infused insulin to suppress hGP and adipose tissue lipolysis. Cannabinoid receptor type 1 (CB₁) blockade with the centrally and peripherally acting Rimonabant induces weight loss and improves glucose homeostasis while also causing psychiatric adverse effects like depression. The relative contributions of peripheral versus central EC signaling on glucose homeostasis remain to be elucidated. Since hypothalamic EC tone is elevated in obesity and diabetes and ECs can act as retrograde, inhibitory messengers in neurons, we hypothesized that elevated brain EC signaling would inhibit the propagation of insulin-induced neuronal signals, resulting in decreased insulin action potentially in the absence of any hypothalamic insulin signaling defect. Here we showed that central CB₁ activation is sufficient to impair glucose homeostasis in Sprague-Dawley rats. Through hyperinsulinemic, euglycemic clamp studies, we controlled circulating insulin and glucose levels and found that central CB₁ activation by infusing WIN55,212-2 or arachidonoyl 2’-chloroethylamide ICV acutely impaired insulin action in both liver and adipose tissue. Using isotope dilution techniques we studied both glucose and lipolytic fluxes and demonstrated that central CB1 activation is sufficient to impair the ability of hyperinsulinemia to suppress hGP and lipolysis. Conversely, in a short term model of overfeeding induced insulin resistance, central CB₁ antagonism restored hepatic insulin sensitivity. Thus, central EC tone plays an important role in regulating hepatic and adipose tissue insulin action. These results indicate that peripherally restricted CB₁ antagonists, which are likely devoid of psychiatric side effects, are also likely to be less effective than brain-permeable CB₁ antagonists in ameliorating insulin resistance.
Tobacco dependence is one of the leading preventable causes of death in the world. Nicotine plays a major role in tobacco dependence by acting directly as a reinforcer of drug-seeking and drug-taking behaviour. Also environmental factors have a major influence on the reinforcing effects of nicotine, as shown by drug self-administration and conditioned place preference tests (Viveros et al. 2006, Maldonado et al. 2006). Several pieces of evidence suggest that endocannabinoids and dopamine are neurotransmitters sharing physiological roles in the control of addiction and emotional states. Additionally, the endocannabinoid system has been implicated in nicotine dependence. Pharmacological blockade of CB1 cannabinoid receptors is able to reduce nicotine-induced conditioned place preference (CPP) (Le Foll et al. 2008) in animals and probably facilitates the cessation of smoking in humans.

In order to further investigate the functional interactions between endocannabinoids and dopamine, a line of conditional knock-out mice was generated, which lacks CB1 receptors in neurons expressing D1 dopamine receptors (D1-CB1-KO mice). These animals were tested for motor learning, anxiety and addiction. Rotarod was performed in naïve state, “Tetrade”, openfield and elevated-plus-maze after treatment with vehicle and ∆9-tetrahydrocannabinol (∆9-THC), respectively, and CPP after treatment with vehicle and nicotine, respectively. This was performed in order to uncover the importance of CB1 receptors in particular brain regions and to characterize the molecular mechanisms and neuronal substrates underlying the role of CB1 receptors in nicotine dependence. Furthermore, gene array experiments are in progress to explore gene expression changes after nicotine-induced CPP in the striatum of D1-CB1-KO mice and control wild-type littermates in order to investigate the mechanisms underlying CB1 receptor-mediated nicotine dependence.

References:
CB1 RECEPTOR FUNCTION IN THE CONTROL OF
SOCIABILITY BEHAVIORS

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Several studies have shown a relation between the endocannabinoid system (ECS) and social behavior. Acute and chronic use of cannabinoids especially during adolescence leads to a decreased social interaction in rats (O’Shea et al. 2004, 2006; Trezza & Vanderschuren 2008; Schneider et al. 2008). Furthermore, Trezza & Vanderschuren (2008) also detected an increased social behavior in rats when treated with URB597, an inhibitor of anandamide degradation. This latter finding is supported by studies with CB1 receptor-deficient mutant mice, which show a decrease in social interaction depending on the behavioral context (Haller et al. 2004; Jacob et al. 2009).

Using several conditional CB1 receptor knock out mice, we aimed in the present study at investigating the impact of cell type-specific CB1 deletion on social behavior. Evaluating animate (interaction partner) and inanimate (object) exploratory behavior in several paradigms, we could show that animate exploration was increased when CB1 receptor is deleted from GABAergic neurons, while inanimate exploration seems to be decreased. On the other hand, deletion of CB1 receptor from glutamatergic forebrain neurons resulted in a decreased animate exploration. Our results suggest that social behaviors are dependent on CB1 receptor on different neuronal populations. Decreased social drive of chronic ECS activation (by CB1 receptor agonists) probably involves CB1 receptors expressed on GABAergic neurons, while the pro-social effects of anandamide degradation inhibition might be explained by the specific activation of CB1 receptors on glutamatergic neurons.

References:
THC TABLET NAMISOL®: FIRST IN HUMAN PK, PD AND TOLERABILITY

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∆⁹-tetrahydrocannabinol (THC) can be effective against pain and spasms in multiple sclerosis (MS) patients[1]. In this study, the optimal dosing route, pharmacokinetics (PK), pharmacodynamics (PD), and tolerability were investigated of a novel formulation of pure THC, Namisol®.

Part 1 included healthy males and females (n=6/6) in a double-blind, double-dummy, randomised, cross-over study using sublingual (crushed tablet) and oral THC 5mg tablet. In part 2, males and females (n=4/5) from part 1 received oral THC 6.5, 8mg or placebo in a double-blind, randomised, cross-over, rising dose study. Pharmacodynamic (PD) measurements were: body sway; visual analogue scales (VAS) mood; VAS psychedelic; heart rate. Non-compartmental PK analysis of THC and metabolites was performed. PK and PD were analysed with a mixed model analysis of variance.

Oral administration showed a lower THC t₁/₂ (-122 min; 95%CI -181/-64) compared to sublingual. Mean oral THC t₁/₂ was 72-80 min, Tₘₐₓ was 39-56 min, and Cₘₐₓ was 2.92-4.69 ng/mL. THC dose dependently affected body sway (8mg: 60.8%; 95%CI 29.5-99.8), VAS external perception (8mg: 0.078 log mm; 95%CI 0.019-0.137), and alertness (8mg: -2.7 mm; 95%CI -4.5/-0.9). VAS feeling high (8mg: 0.256 log mm; 95%CI 0.093-0.418) and heart rate (8mg: 5.6 BPM; 95%CI 2.7-6.5) also increased. Namisol® was well tolerated.

Oral Namisol® has promising PK and PD-characteristics for further development. The variability and Tmax of THC plasma concentrations were smaller for Namisol® than reported for other studies using oral THC and nabilone[2,3]. It remains to be established whether Namisol® will also show improved clinical effect initiation and dose regulation.

References:
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CANNABINIDS AND STRESS HORMONES DIFFERENTIALLY MEDIATE THE EFFECTS OF ACUTE STRESS ON SPATIAL MEMORY AND NEURAL PLASTICITY

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Recent evidence suggests endocannabinoids play a vital role in regulating the effects of stress on memory processes. The basolateral amygdala (BLA) is a crucial structure in stress- and emotion-related behaviors, and plays a vital role in the emergence of stress-associated changes in hippocampal plasticity and memory. We examined the role of the cannabinoid, glucocorticoid and adrenergic systems in altering the effects of stress on memory in the non-aversive hippocampal dependent spatial object placement (OP) task as well as on synaptic plasticity.

Stress exposure impaired memory consolidation and retrieval, but not acquisition, of the OP task. The glucocorticoid receptor (GR) antagonist RU486 (10ng/side) or the beta-adrenergic receptor antagonist propranolol (0.75 μg/side), but not the CB1/2 receptor agonist WIN 55,212-2 (WIN; 5 μg/side), into the BLA reversed the impairing effects of the stressor on consolidation, but not retrieval, of the OP task.

We also examined the involvement of BLA cannabinoids and glucocorticoids in synaptic plasticity at the ventral subiculum (vSub)-nucleus accumbens (NAc) pathway following exposure to acute stress. This pathway is important for integration of information about environmental context and goal-directed behavior. We found that WIN and RU486 micro-injected into the BLA reversed the stress-induced impairment of long-term potentiation (LTP) in this pathway.

These results point to the important role of these three receptor systems in the BLA in mediating the effects of stress on learning and plasticity. Our findings also suggest that the effects of stress on hippocampal memory consolidation and retrieval are mediated by different mechanisms.

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CANNABINOIDS ACTIVATION IN THE AMYGDALA BLOCK THE STRESS-INDUCED ENHANCEMENT OF EMOTIONALLY NEGATIVE LEARNING

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It has been suggested that emotional memory dysfunctions and exposure to stressful events may underlie several psychiatric disorders such as depression, alcohol or substance abuse and PTSD, to name just a few.

Here, we used an alley maze to examine the effects of exposure to a mild stressor on an emotionally negative learning experience and whether cannabinoids in the basolateral amygdala (BLA) could modulate these effects. The emotionally negative learning experience was achieved by decreasing the magnitude of the expected quantity of reinforcements in the alley maze task which creates a psychological state of frustration and arousal. Immediately after decreasing the reward magnitude (i.e., frustration learning) rats were microinjected with the CB1/2 receptor agonist WIN55,212-2 (5μg/side) into the BLA and exposed to a mild stressor.

Merely exposing rats to a mild stressor following a negative experience resulted in the enhancement of memory for reward reduction as indicated by increased latency to reach the reward. However, acute administration of WIN55,212-2 into the BLA before stress exposure blocked the enhancing effects of the stressor on memory for reward reduction.

Thus, cannabinoid receptors in the BLA modulate the effects of stress on the consolidation of memory for a reduction in reward magnitude. The results may give pre-clinical support to the suggestion that cannabinoids may have a potential therapeutic value in the treatment of conditions associated with the inappropriate retention of aversive memories and stress-related disorders.
P6  DOES LONG-TERM ADMINISTRATION OF CANNABINOIDS IN YOUNG ADULT RATS CAUSE RESIDUAL COGNITIVE DEFICITS?

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Cannabis and its psychoactive constituents (cannabinoids) are the most commonly used illicit drugs. This is facilitated by the fact that users generally perceive these drugs as relatively harmless. A controversial question is whether long-term cannabis use can cause irreversible deficits in higher brain function that persist after drug use stops. It has been difficult to determine whether such deficits, observed after only hours or days of abstinence, are temporary (e.g., due to a residue of cannabinoids in the brain or to acute withdrawal effects from cannabis) or long-lasting (due to a neurotoxic effect of long-term cannabis exposure).

Rats were chronically administered with the CB1/2 receptor agonist WIN 55,212-2 (WIN; 1.2mg/kg, i.p.) during post-adolescence (PND 45-60) and tested 24 hrs, 10 days or 30 days after the last drug exposure. We found that (i) WIN impaired aversive spatial learning 24 hrs after the last WIN injection, but not afterwards, (ii) WIN impaired object recognition memory 24 hrs, but not 10 or 30 days, after the last WIN injection, (iii) WIN impaired LTP in the ventral subiculum-accumbens pathway 10, but not 30, days after the last WIN injection, (iv) WIN had no effect on LTP in the dentate gyrus, and (v) impaired non-aversive short-term spatial memory 10 and 30 days after the last WIN injection.

These findings suggest that cannabinoid exposure in young adult rats may have differential effects on learning and memory that are task- and brain region-dependent and that at least some of the long-term effects of cannabinoids are reversible.
CANNABINOIDS PREVENT THE EFFECTS OF STRESS ON PTSD-LIKE SYMPTOMS IN A RAT MODEL

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Cannabinoids have recently emerged as a therapeutic target for the treatment of stress- and anxiety-related disorders such as post-traumatic stress disorder (PTSD). Here we examined whether cannabinoids could prevent the effects of trauma exposure on behavioral and neuroendocrine measures.

We used the single-prolonged stress (SPS) model for PTSD. Rats were injected with the CB1/CB2 receptor agonist WIN55,212-2 (WIN) systemically or into the basolateral amygdala (BLA) at different time points following SPS exposure and were tested 1 week later for inhibitory avoidance (IA) conditioning and extinction, acoustic startle response (ASR), hypothalamic-pituitary-adrenal (HPA) axis function and unconditioned anxiety.

Exposure to SPS enhanced avoidance conditioning and impaired extinction while enhancing ASR, negative feedback on the HPA axis and unconditioned anxiety. WIN (0.5 mg/kg) administered 2 h or 24 h (but not 48 h) after SPS prevented the trauma-induced alterations in IA conditioning and extinction, ASR potentiation and HPA axis inhibition. WIN into the BLA (5µg/side) also prevented the SPS-induced alterations in IA and ASR and this effect was reversed by CB1 receptor antagonist AM251 (0.3ng/side and 1mg/kg, respectively), suggesting mediation by CB1 receptors. However, WIN did not prevent SPS-induced elevation of anxiety levels suggesting that although cannabinoids may be beneficial in preventing PTSD-like symptoms, they are not necessarily effective in blocking all the effects of stress exposure.

These findings suggest that cannabinoids could serve as a pharmacological treatment for stress- and trauma-related disorders and that there may be an optimal time window for intervention with cannabinoids after exposure to a highly stressful event.
POSTNATAL REGULATION OF THE ENDOCANNABINOID SYSTEM IN A MOUSE MODEL FOR SCHIZOPHRENIA

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Schizophrenia affects 1% of the population and erupts mostly at late adolescence or early adulthood. This disease is associated with impairments of emotional and cognitive functions, which can be mimicked by phencyclidine (PCP), an NMDA receptor blocker. The endocannabinoid system encompasses the classified cannabinoid receptors, CB1 and CB2, that are activated by Δ9-tetrahydrocannabinol, the major psychoactive ingredient in cannabis, but also by endogenous active lipids. The CB1 receptor is primarily expressed by neurons where it regulates neuronal migration, axonal targeting and neurotransmitter release. The expression of the CB2 receptor in the adult human brain is still controversial; however, evidence suggests that both receptors regulate neurogenesis in the developing brain. We hypothesised that early postnatal treatment of mice with PCP will induce changes in the endocannabinoid system and lead to “schizophrenia-like” behaviour. The effects of administering PCP (20 mg/kg) every other day between postnatal days 3 to 15 was evaluated with a variety of animal behaviour paradigms relevant to anxiety and symptoms associated with schizophrenia when animals had reached adulthood. Alterations in cannabinoid receptor expression were observed in different brain areas. In the PCP-treated animals, we observed a significant inhibition of the pre-pulse inhibition of the startle reflex (PPI) two months after treatment. Three months after exposure, the PCP-treated animals displayed increased anxiety. We also evaluated the effect of selective cannabinoids in this model system. Our results suggest that NMDA-induced alterations in the endocannabinoid system during postnatal development of the mouse brain contribute to the development of “schizophrenic-like” behaviour in adulthood.

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TARGETING NEUROPATHIC PAIN BY A NATURAL CB2 RECEPTOR-AGONIST

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Introduction: Neuropathic pain arises as a consequence of peripheral nerve injury. Current pharmacotherapies either lead to inadequate pain relief or psychoactive side effects. Selective CB2 receptor-agonists were found to be effective antinociceptives in animal models. E-β-caryophyllene ((E)-BCP) was now identified as a natural CB2 selective agonist.

Objectives: In this study we want to investigate the efficacy of (E)-BCP to attenuate neuropathic pain symptoms in mice.

Methods: A partial ligation of the right sciatic nerve was performed in male wild type and CB2 receptor knockout mice to induce neuropathic pain. Different doses (1, 5 and 10 mg/kg) of (E)-BCP were orally administered. Thermal hyperalgesia and mechanical allodynia were assessed at day 3, 6, 8, 10 and 14 after ligation.

Results: Oral administration of (E)-BCP attenuated neuropathic pain symptoms (hyperalgesia and allodynia) in the behavioral readouts compared to vehicle treated mice. As expected, it did not influence the behavioral responses of CB2 receptor knockout mice.

Conclusion: The presented data show that (E)-BCP is a potential analgesic substance for the treatment of neuropathic pain with high efficacy at low dose. As it is found in different spice and food plants, a daily intake with vegetable food could be an efficient modulator of persistent pain states.
IMPACT OF MODULATION OF THE ENDOCANNABINOID SYSTEM ON THE INTESTINAL MICROCIRCULATION IN EXPERIMENTAL SEPSIS

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Introduction: The endocannabinoid system (ECS) is upregulated during sepsis [1]. However, the functional outcomes of modulating endocannabinoid signaling during sepsis are currently unclear. Sepsis is a disease of the microcirculation. Impairment of the intestinal microcirculation during sepsis may cause a breakdown of gut epithelial barrier function and bacterial translocation into the systemic circulation increasing the systemic inflammatory response [2]. Consequently, the protection of the intestinal microcirculation represents a pivotal therapeutic target in sepsis. The aim of the present study was to examine the effects of CB1 and CB2 receptor modulation on the intestinal microcirculation in a model of poly-bacterial sepsis (colon ascendens stent peritonitis - CASP) using intravital microscopy (IVM).

Methods: We studied six groups of animals (Lewis rats, n=10 per group): sham operated controls (SHAM), septic controls (CASP), CASP animals treated with CB1 agonist, ACEA (2.5 mg/kg IV), CASP animals treated with CB1 antagonist, AM281 (2.5 mg/kg IV), CASP animals treated with CB2 agonist, HU308 (2.5 mg/kg IV) and CASP animals treated with CB2 antagonist, AM630 (2.5 mg/kg IV). All treatments were performed immediately after sepsis induction. Intravital microscopy of the intestinal microcirculation was performed 16 hours following sepsis induction. Leukocyte adhesion and functional capillary density (FCD) were measured in a blinded fashion.

Results: Following 16 hours of CASP-induced experimental sepsis, a significant increase of leukocyte adhesion in the intestinal submucosal venules (e.g., collecting venules (V1): SHAM 35.7±6.2 n/mm², CASP 214.4±22.6 n/mm², p<0.05) was observed. Capillary perfusion of the muscular and mucosal layers of the intestinal wall was significantly reduced (e.g., longitudinalis muscular layer: SHAM 143.5±7.6 cm/cm², CASP 77.1±7.2 cm/cm²). Treatment of CASP animals with the CB1 receptor agonist, ACEA, reduced leukocyte adhesion (V1 venules: 107.4±5.1 n/mm²), whereas CB2 receptor stimulation did not affect leukocyte adhesion. However, CB2 receptor inhibition by AM630 reduced leukocyte activation significantly (V1 venules: 60.0±14.1 n/mm²) and restored capillary perfusion (longitudinal muscular layer: 114.1±7.6 cm/cm²).

Conclusion: The data suggest that ECS signaling is involved in the impairment of the intestinal microcirculation during sepsis. Blocking CB2 receptor signaling reduces leukocyte activation and improves capillary perfusion in sepsis in rats. The long-term effect of ECS modulation needs further investigation.

FRESH CANNABIS: A NON-PSYCHOACTIVE THERAPEUTIC MODALITY

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

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

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

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

This research examines whether THCa and other phytocannabinoids, such as CBD, exhibit any psychotropic effect in human patients, using self-reporting on eating fresh cannabis leaf and flowers. Between 1975 and the present, at least 110 controlled clinical studies have been published, assessing 6,100 plus patients. October 1, 2009 until October 1, 2010, roughly 2832 of patients were consulted on their use of fresh cannabis leaf, which Dr. William Courtney had prescribed the previous year.

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

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As the life span increased from 35 to 70 years, inflammation took on characteristics of an autoimmune disorder. Dietary phytocannabinoids provide safe, long-term, daily anti-inflammatory support. In the raw plant, 95% of cannabinoids are present as cannabinoid acids, which are patented for their immune-modulating ability. Patent 6,630,507, held by the US Dept. of Health and Human Services, provides an 'Effective oral human dosage schedule' for CBD in the range of 5-20 mg/kg, for the treatment or prophylaxis of a wide range of ‘Oxidative associated diseases: inflammatory disease, ischemic reperfusion injury, Crohn’s, rheumatoid arthritis, diabetes, cataracts, neoplasia…’ In this patent, there are over a dozen references to the preventive or prophylactic benefits of cannabinoids. Behind the patents, research has detailed the diverse benefits of the phytocannabinoids. FDA has approved of CBD at 600mg/day as an Investigative New Drug approximating the 5 mg/kg schedule found in the DHHS patent. Introduction of balanced profile cannabis plants, such as Cannatonic, provide 600 mg of CBDA and THCA through use of 17 gm wet weight mature flower, in divided doses q4hr.

Historically, my private practice has changed over the years: from 2006 to June 2008 the use of dried cannabis leaf was discussed with 3,263 patients, from June 2008 to October 2009 the use juiced fresh leaf was reviewed with 3,366 patients, and from Oct. 2009 to present, juiced raw bud and leaf was reviewed with 2,626 patients. This has led to improved relief, and dramatic reduction in NSAIDs and analgesic use.

Functional Foods evolved from “Foods for Specified Health Use” in the 1980’s, which referred to “Any food or ingredient that has a positive impact on an individual’s health, physical performance, or state of mind, in addition to its nutritive value” and is 1) consumed in its naturally occurring form, 2) daily, and 3) regulates a biological process to prevent or control disease. Cannabis is unparalleled as a Functional Food. Cannabis provides complete and balanced, Essential and Conditionally Essential, Amino Acids, Fatty Acids, and Cannabinoid Acids. In the whole plant, the Essential Cannabinoid Acids / ECA are complemented by terpenoid allosteric modulators and compensatory antibiotics, targeting intracellular pathogens that would thrive in an immuno-compromising, high anti-oxidant environment. Nutritional labeling uses Daily Values, also called Reference Daily Intake Values, based on Recommended Dietary Allowances / RDA’s, last updated in1968. RDA’s meet the needs of 97-98% of the population. EAR or Estimated Average Requirements are sufficient for 50% of the population. AI, Adequate Intake, is an estimate of an unknown RDA. In 1997, the Institute of Medicine developed the Dietary Reference Intake DRI, not yet in use. When Upper Limits (UL) have not been established, it is recommended that use be limited to whole food to prevent potential adverse effects from excessive intake. ECA AI: 200-500 mg/day, acute ischemia 1-2,000 mg/day.
California physicians involved in the practice of cannabis consultations regularly encounter patients with autoimmune and idiopathic inflammatory condition; a large proportion of these have inflammatory bowel disease. The authors interviewed and examined patients seeking a physician statement of recommendation as required by California law. These patients are self-referred, non-naïve cannabis using patient in nearly all cases.

The primary aim of this study was to evaluate the efficacy of the ad lib use of natural cannabis in alleviating the symptoms of active inflammatory bowel disease both with and without concomitant use of conventional medications. A secondary aim of the study was to determine if cannabis in combination with steroids and other immunomodulators leads to better response, longer periods of disease quiescence, and reduction in the use of steroids and other immunomodulator pharmaceuticals.

We performed a retrospective chart review of 38 patients with inflammatory bowel disease who were approved to use cannabis for relief of symptoms. All patients studied had an independent diagnosis of Crohn’s disease or ulcerative colitis. Inclusion criteria included the completion of a questionnaire designed to elicit details of the clinical course and use of all medications including cannabis.

Results indicate that these patients found statistically significant improvement of their clinical course and a marked reduction or discontinuation of conventional pharmaceutical therapy associated with the regular use of cannabis. Cannabis serves as an effective immunomodulator, antispasmodic and appetite stimulant with a wide margin of safety and freedom of undesirable adverse effects compared with conventional pharmacotherapy.
MICROSPHERES OF CANNABINOIDS: A TOOL FOR IMPROVING THEIR ADMINISTRATION IN PHARMACOLOGICAL STUDIES

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Objective: One of the main areas of cannabinoid research is their usefulness as antitumoural agents. However, the difficulty in administering cannabinoids in an efficient and reproducible way, due to their viscous nature, their high lipophilicity and their biopharmaceutic characteristics, is an important problem for these investigations.

The aim of this study is to develop biodegradable microspheres for the efficient and reproducible subcutaneous administration of cannabinoids and their later release in a sustained way, thus facilitating the in vivo experiments.

Methods: Microspheres of cannabidiol and ∆9-tetrahydrocannabinol were prepared by using poly-ε-caprolactone as biodegradable polymer. Size, drug disposition into the systems and in vitro drug release from microspheres were determined. Their in vivo efficacy was evaluated using a subcutaneous glioma multiforme xenograft model in nude.

Results: Microspheres with a μ of 50 μm for cannabinoid parenteral administration was obtained. The drug was dispersed at molecular state into the polymeric matrix. In vitro studies revealed a sustained release of drug for a 10-day period. Glioma’s size decreased considerably in the treated animals versus the untreated ones.

Thus, microspheres show as an efficient alternative for cannabinoids administration.
**P15**

**EFFECT OF DAM’S CB1 RECEPTOR INHIBITION UPON OFFSPRING SOCIAL BEHAVIOR**

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**Introduction:** Early postnatal development has long-lasting influences on the behavior of individuals in adulthood. Alteration in maternal behaviors such as grooming and licking, during the first 10 days of the mouse’s life, a sensitive period for establishment of attachment processes, may impact offspring social behavior. We have previously shown that blocking of maternal endocannabinoid CB1 receptors significantly affected attachment processes. The purpose of the current study was to evaluate effects of blocking these receptors in lactating dams on the offspring’s social behavior during later developmental stages.

**Methods:** Mice Offspring of dams treated on postpartum days 1-8 with SR141716 (Rimonabant) 10 mg/kg (SRO) or vehicle (VO) underwent preference and social behavior tests post-weaning and in adulthood, respectively. Preference test: At the age of 24-28 days, 17 male and 17 female offspring were tested in a Y-maze that included an entrance box, right & left arms. Different targets were placed at the edge of each arm. Three different preference tests were performed: Dam vs. Milk (DM), Dam vs. Pup (sibling) (DP) and Pup (sibling) vs. Milk (PM). In each 4 min test, the time offspring spent in each maze was measured. The three tests were performed in succession, with a 1 min interval between the tests, keeping the same order in every repeat. Social behavior test: At the age of 13-14 weeks, 48 female and 38 male SRO and VO mice underwent a social behavior test. In each test, randomly chosen SRO and VO of unfamiliar individuals were placed in separate cages for 30 min, then animals were placed together in a new cage for 5 min and their behavior was video-recorded for further analysis. The same test was repeated in 30 min intervals. Active social interactions were measured by sniffing the partner while passive social interactions were measured from the observed time that animals were ignoring each other. Rearing was also assessed.

**Results:** Preference test: 1) both SRO and VO females preferred the dam over milk, while SRO males preferred staying more with the dam compared to VO males. 2) Both SRO males and females preferred dam over pup and pup over milk in comparison to VO mice. Social behavior test: SRO males and females were more active, showing higher levels of social sniffing and rearing than VO mice.

**Conclusions:** It is commonly accepted that changes in maternal behavior are related to modification in behavioral response later in life. This study shows that blocking CB1 receptors in lactating mouse dams causes behavioral changes in the offspring’s social behavior. We speculate that the high degree of social interactions during weaning and adulthood of SRO males and females result from reduced maternal care, contact and milk during the neonatal period (Schechter et al., ICRS abstract, 2009), The present study further indicates an important role of the endocannabinoid system in attachment processes and their impact on the individual’s future social behavior.
CANNABIDIOL, NON-PSYCHOACTIVE CANNABINOID, AMELIORATES CLINICAL SYMPTOMS AND DECREASES MICROGLIAL ACTIVATION IN MOG-TREATED MICE

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Cannabis compounds, cannabinoids, have been shown to exert anti-inflammatory activities in certain experimental models of inflammatory CNS degenerative diseases. The main obstacle for clinical application of those materials are their psychoactive properties. We evaluated the effects of a non-psychoactive cannabinoid, cannabidiol (CBD), in myelin oligodendrocyte glycoprotein (MOG)-induced EAE murine model of multiple sclerosis (MS) and determined the mechanisms underlying these properties, specifically in microglial cells.

We observed that peripherally given CBD (administered at the time of disease onset) ameliorates the clinical EAE symptoms as evaluated using behavioral and pathological scores. Histochemical analysis of spinal cords of MOG-injected EAE mice treated with CBD vs MOG-only treated mice revealed that CBD down regulates infiltration/proliferation (Iba-1 staining) and activation (Mac-2 staining) of macrophages and microglia into spinal cord white matter.

Using the BV-2 mouse microglial cell line and lipopolysaccharide to induce inflammatory response, we were screening for intracellular mechanisms that might be involved in the CBD anti-inflammatory activity. We observed that CBD decreased the release of interleukin (IL)-1β and IL-6 proinflammatory cytokines from activated microglial cells. CBD inhibited the activation of STAT1 proinflammatory transcription factor and up-regulated the STAT3 factor, an element of homeostatic mechanism(s) inducing anti-inflammatory events.

In conclusion, we observed that CBD exerts anti-inflammatory activities in vivo using the in EAE model of MS) as well as in vitro (using microglial cells). These activities may be mediated via STAT dependent pathways.
IN HEALTHY SUBJECTS CANNABIDIOL INHIBITS THC-INDUCED PSYCHOSIS & TACHYCARDIA VIA A NON-PHARMACOKINETIC MECHANISM

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Recent animal work has shown that cannabidiol (CBD) can antagonise the pharmacological effects of CB₁ agonists. This has social significance, because street marijuana in the UK is now dominated by forms devoid of CBD (sinsemilla) and epidemiological work suggests that sinsemilla is more likely to be associated with psychosis than traditional marijuana. Here we investigated the effect of intravenous (IV) CBD (5mg) versus placebo on IV THC (1.25mg) responses. The study was conducted under randomised, double-blind conditions in 6 healthy controls who attended for two laboratory visits. Findings were that at 30-minutes post-THC, psychotic experiences were lower in the CBD+THC compared to the placebo+THC arm (z= -2.3, p<0.05). CBD also inhibited THC-elicited tachycardia (t=-3.2, p=0.01). Plasma concentrations of THC were numerically higher under the CBD versus the placebo arm, but differences did not reach significance. The findings indicate that CBD ‘protects’ against THC-induced psychosis. The results are consistent with recent epidemiological work. The effect of THC on heart rate is mediated directly on the vagus n. via CB₁ receptors. It is feasible that the effect of CBD on THC-psychosis might similarly be mediated via CB₁.

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FUNCTIONAL AND CHEMICAL RELATIONSHIPS BETWEEN THE ENDOCANNABINOID AND THE ENDOVANILLOIDS SYSTEMS: POTENTIAL FOR NEW THERAPEUTIC DRUGS

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Although belonging to completely different receptor classes, the cannabinoid (CB) receptors and the transient receptor potential vanilloid type-1 (TRPV1) channels (six-transmembrane-domain non-selective cation channels gated by heat, protons and several plant toxins, including capsaicin) have much in common. Firstly, they are often expressed in the same sensory and central neurons as well as in non-neuronal cells. Secondly, they participate in several pathological conditions, and their pharmacological activation, in the case of CB receptors, and rapid desensitization, in the case of TRPV1 receptors, often results in similar beneficial effects. Lastly, they share, to some extent, the same endogenous agonists. These compounds are known as endocannabinoids, in the case of CB receptors of type 1 and 2 (CB₁ and CB₂), and as endovanilloids, in the case of TRPV1 receptors. Thus, some endocannabinoids, like N-arachidonoyl-ethanolamine (anandamide) and N-arachidonoyl-dopamine (NADA), also behave as endovanilloids. Furthermore, enzymes and proteins that inactivate the endocannabinoids and that are being proposed as targets for the development of new anti-inflammatory, analgesics, anti-emetics, anxiolytic and anticancer drugs (Di Marzo, Nat. Rev Drug Discov. 7(5):438-55 2008), also have a ligand recognition pattern similar to that of TRPV1. Therefore, it is conceivable that synthetic molecules targeting at the same time proteins of the endocannabinoid system and TRPV1 channels, might be designed and be more efficacious in the indications mentioned above, and in particular in pathological conditions during which endocannabinoids/endovanilloids are produced and CB and TRPV1 receptors are activated and sensitized, respectively. The design of “hybrid” CB/TRPV1 agonists or CB antagonists/TRPV1 agonists, and of “dual” blockers of endocannabinoid degradation and TRPV1 activity will be described in my lecture, together with their potential use in the clinic against chronic pain and affective disorders. Furthermore, examples will be given of some novel pathological conditions in which CB and TRPV1 receptors, expressed in the same or in neighboring cells, cross-talk, thus opening the way to further therapeutic applications for these molecules.
EMERGING PHARMACOLOGICAL STRATEGIES FOR
EXPLOITING CANNABINOIDS AS MEDICINES

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The discovery that the endocannabinoid system adopts an “autoprotective” role in certain disorders has prompted a search for potential strategies for activating this system with maximal selectivity by directly or indirectly targeting subpopulations of cannabinoid receptors. Some of these strategies, for example allosteric enhancement of CB₁ receptor activation by endogenously released endocannabinoids, will be briefly described. New evidence that phytocannabinoids in addition to ∆⁹-tetrahydrocannabinol (Δ⁹-THC) have actions that might be exploited in the clinic will also be presented. This has come from recent in vitro experiments in Aberdeen focusing on Δ⁹-tetrahydrocannabivarin (Δ⁹-THCV), cannabigerol (CBG) and cannabidiol (CBD). Δ⁹-THCV was found to block CB₁ receptors but activate CB₂ receptors, a combination of actions prompting hypotheses that Δ⁹-THCV has potential for the management of pain, Parkinson’s disease, myocardial infarction, stroke and chronic liver diseases. Supporting evidence for some of these hypotheses has come from subsequent in vivo experiments. CBG was found to increase the activation of α₂-adrenoceptors and block 5-HT₁ₐ receptors in mouse brain with significant potency, suggesting that it might be effective against pain, depression and unwanted side effects of antipsychotic medicines. As to CBD, a possible mechanism underlying its apparent 5-HT₁ₐ receptor-mediated in vivo effects, for example ant-emesis, has recently been identified. Also described will be (1) results from recent experiments that demonstrate the relative abilities of Δ⁹-THC and its metabolite, 11-OH-Δ⁹-THC, to target cannabinoid receptors and (2) evidence obtained in a collaboration with Raphael Mechoulam that certain omega-3 fatty acid metabolites target cannabinoid receptors and display anti-cancer activity.
THE ROLE OF CB2 RECEPTORS IN INFLAMMATION AND TISSUE INJURY

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Emerging evidence using cannabinoid 2 (CB2) receptor agonists and/or knockout mice suggests that CB2 receptor activation is protective against myocardial, cerebral and hepatic I/R injuries by decreasing the endothelial cell activation/inflammatory response (for example, expression of adhesion molecules, secretion of chemokines, and so on), and by attenuating the leukocyte chemotaxis, rolling, adhesion to endothelium, activation and transendothelial migration, and interrelated oxidative/nitrosative damage. Protective effects of CB2 receptor activation was also suggested in atherosclerosis and various other inflammatory diseases, which will be summarized in this talk.
THE PHYTOCANNABINOID-TERPENOИD ENTourage EFFECT

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From 1964 when Raphael Mechoulam isolated and synthesized tetrahydrocannabinol, it has been the primary focus of cannabis research. More recently, the synergistic contributions of cannabidiol to its pharmacology and analgesic medicinal value have been demonstrated. Other phytocannabinoids including tetrahydrocannabivarin, cannabigerol, and cannabichromene harbour additional effects of therapeutic interest. Innovative conventional plant breeding has yielded cannabis chemotypes expressing high titres of each component for future study.

This presentation will introduce another echelon of phytotherapeutic agents, the cannabis terpenoids (McPartland/Russo 2001): limonene, myrcene, α-pinene, linalool, β-caryophyllene, caryophyllene oxide, nerolidol and phytol. These half-siblings of phytocannabinoids are all flavour and fragrance components common to human diets that have been designated Generally Recognized as Safe (GRAS) by the US Food and Drug Administration and other regulatory agencies. Terpenoids are quite potent, and affect animal and even human behaviour when inhaled from ambient air, at serum levels in the single digits ng/ml. They display unique therapeutic effects that may contribute meaningfully to the entourage effects of cannabis-based medicinal extracts.

Particular focus will be placed on phytocannabinoid-terpenoid interactions that could produce synergy with respect to treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, fungal and bacterial infections (including MRSA). Scientific evidence is presented for non-cannabinoid components as putative antidotes to intoxicating effects of THC that could increase its therapeutic index. Methods for investigating entourage effects in future experiments will be proposed.

Phytocannabinoid-terpenoid synergy, if proven, increases the likelihood that an extensive pipeline of new therapeutic products is possible from this venerable plant.
Monoacylglycerol lipase (MGL) and fatty-acid amide hydrolase (FAAH) degrade the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), respectively. Selective inhibition of MGL and FAAH in the periphery may elucidate the role of endocannabinoids in controlling pain initiation. We used the formalin test to compare peripheral antinociceptive effects of a novel selective MGL inhibitor, JZL184, with the MGL-prefering inhibitor URB602 and exogenous 2-AG. Intra-paw administration of JZL184 and URB602 suppressed both early and late phases of formalin pain. Each MGL inhibitor produced additive antinociceptive effects in combination with 2-AG. Antinociceptive effects of either MGL inhibitor was also blocked by either a CB$_1$ or CB$_2$ antagonist. JZL184 inhibited MGL activity in rat paw skin without altering activity of enzymes implicated in degradation (FAAH) or synthesis (NAPE-PLD) of anandamide. URB602 also produced regionally-restricted increases in rat paw skin 2-AG levels, without altering levels of anandamide. URB937, a peripherally-restricted inhibitor of FAAH, suppressed both formalin-induced pain behavior and spinal Fos protein expression in a CB$_1$-dependent manner. We also compared peripheral antinociceptive effects of inhibitors of MGL (JZL184), FAAH (URB597), and endocannabinoid uptake (VDM11), on nociception produced by capsaicin, the pungent ingredient in hot chili peppers. JZL184 suppressed capsaicin-induced nocifensive behavior and thermal hyperalgesia through CB$_1$- and CB$_2$-dependent mechanisms but did not alter capsaicin-evoked mechanical allodynia. URB597 produced a CB$_1$-mediated suppression of capsaicin-induced mechanical allodynia without altering capsaicin-evoked thermal hyperalgesia or nocifensive behavior. Finally, VDM11 suppressed capsaicin-evoked hypersensitivity for all three dependent measures (nocifensive behavior, thermal hyperalgesia, and mechanical allodynia) with a pattern of pharmacological specificity that was mimicked by that of the MGL and FAAH inhibitor in combination. Thus, peripheral inhibition of MGL and FAAH suppresses capsaicin-evoked behavioral sensitization with distinct patterns of pharmacological specificity and in a non-overlapping and modality-specific manner. Our studies also suggest that inhibition of endocannabinoid transport was more effective in suppressing capsaicin-induced sensitization compared to inhibition of either FAAH or MGL alone. More work is necessary to validate the efficacy of inhibitors of endocannabinoid deactivation and uptake as analgesics.
CLINICAL USE OF CANNABINOIDS FOR PAIN MANAGEMENT
IN CANADA

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The potent anti-nociceptive and antihyperalgesic effects of cannabinoid agonists in animal models of acute and chronic pain, the presence of cannabinoid receptors in pain-processing areas of the brain, spinal cord and periphery and evidence supporting endogenous modulation of pain systems by cannabinoids, provide support that cannabinoids exhibit significant potential as analgesics.

A systematic review was conducted and has identified 26 randomized controlled trials examining cannabinoids in the treatment of chronic pain. Cannabinoid agents tested included synthetic analogs as well as cannabis and cannabis based extracts, these agents were tested in a number of pain conditions.

Taken together, the evidence supports that cannabinoids exhibit a moderate analgesic effect in neuropathic pain and pain caused by HIV and cancer with preliminary evidence for action in other types of pain such as spinal pain and headache. In Canada there are 4 cannabinoid agents available. These include the naturally occurring agent, cannabis, available under the Medical Marihuana Access Regulations (MMAR), a cannabis buccal spray (Sativex), a synthetic THC analog nabilone (Cesamet) and dronabinol (synthetic Δ-9-THC in sesame oil sold under the trade name Marinol). Guidelines for the use of cannabinoids available in Canada in chronic pain management have been established (Clark, Lynch et al. 2005) and updated (Clark, Lynch et al. 2007). Based on current evidence supporting that cannabinoids are analgesic and safe it is reasonable to use a cannabinoid as a second or third line agent either as a single agent or in combination with other agents exhibiting a different mechanism of action. In patients exhibiting a constellation of symptoms including nausea, anorexia or spasticity one might consider introducing a cannabinoid earlier. This talk will also review clinical experience using the above guidelines.
Considerable data have accumulated over the last 10 years that support the hypothesis that endocannabinoid activation of CB1 cannabinoid receptors regulates endocrine responses to stress. I will present data supporting this hypothesis and demonstrating further that the glucocorticoid, corticosterone, activates endocannabinoid-mediated inhibition of GABA release in the prefrontal cortex. As a result, a prefrontal cortical-amygdaloid-hypothalamic circuit is activated that inhibits CRF release in the hypothalamus. These findings suggest that enhanced endocannabinoid signaling is downstream of glucocorticoid receptor activation. I will present biochemical data testing the hypothesis that glucocorticoid receptor activation alters the expression of enzymes that are involved in the regulation of endocannabinoid synthesis, degradation and/or signaling.
REGULATION OF NAUSEA BY CANNABINOIDS

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Considerable evidence demonstrates that manipulation of the endocannabinoid system regulates vomiting in humans and other animals. The anti-emetic effect of cannabinoids has been shown across a wide variety of species that are capable of vomiting in response to a toxic challenge. CB₁ agonism suppresses vomiting, which is reversed by CB₁ antagonism, and CB₁ inverse agonism promotes vomiting. Recently, animal evidence suggests that cannabinoids may be especially useful in treating the more difficult to control symptoms of nausea and anticipatory nausea which are less well controlled by the currently available conventional pharmaceutical agents (5-HT₃ antagonists). Although rats and mice are incapable of vomiting, they display a distinctive conditioned gaping response when re-exposed to cues (flavors or context) paired with a drug that produces nausea. Cannabinoid agonists (∆⁹-THC, HU-210) and the Fatty Acid Amide Hydrolase (FAAH) inhibitor, URB-597, suppress conditioned gaping reactions (nausea) in rats as they suppress vomiting in emetic species. Inverse agonists, but not neutral antagonists, of the CB₁ receptor promote nausea, and at subthreshold doses potentiate nausea produced by other toxins (LiCl). The suppression of nausea by cannabinoid agents may be mediated by their interaction with forebrain serotonin (5-HT) systems which promote nausea. The primary non-psychoactive compound in cannabis, cannabidiol (CBD), also suppresses nausea and vomiting within a limited dose range. The anti-nausea/anti-emetic effects of CBD may be mediated by indirect activation of somatodendritic 5-HT₁₅ receptors in the dorsal raphe nucleus; activation of these autoreceptors reduces the release of 5-HT in terminal forebrain regions. Preclinical research indicates that cannabinoids, including CBD, may be effective therapeutic agents for treating both nausea and vomiting produced by chemotherapy treatment or produced by other therapeutic treatments.
ENDOCANNABINOID SYSTEM AND NEUROPSYCHIATRIC DISEASES

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In the last few years several evidences have been accumulated on the role and the pharmacological modulation of the endocannabinoid system (ECs) in mood disorders and its potential implication in psychotic disorders such as schizophrenia.

Substantial evidence implicates a deficit in endocannabinoid system in the aetiology of depression, so boosting endocannabinoid tone might be a useful new therapeutic approach for depressive disorders. Similar to the action of the conventional antidepressant, the enhancers of endocannabinoid signalling can enhance serotoninergic and noradrenergic transmission, increase neurogenesis within the hippocampus and dampen the activity within the neuroendocrine stress axis.

Moreover several lines of experimental and clinical evidence point to a dysregulation of the endocannabinoid system in schizophrenia. These findings, although still unclear at the molecular level, are consistent with the neuroanatomical distribution of cannabinoid CB1 receptors, which present a high density in brain regions implicated in schizophrenia such as PFC, basal ganglia, hippocampus and anterior cingulated cortex. Hyper- or hypo-activity of CB1 receptor function in specific brain areas can markedly affect several neuronal pathways, contributing to or counteracting the pathology. The high AEA level found in schizophrenic patients negatively correlated with psychotic symptoms, pointing to a protective role, whereas the role of 2-AG is still not clear. As a whole, the EC system presents several abnormalities in receptor as in function eCBs which, depending on the cerebral areas affected, might contribute differently to the pathology. There is a potential for pharmacological manipulation of the endocannabinoid system as a novel approach for treating schizophrenia, although experimental findings are still controversial, often with different effects depending on the drug, the dose, the species and the model used for simulating positive or negative symptoms.
DISCOVERY OF AN ENDOCANNABINOID BINDING SITE IN GABAA RECEPTORS

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GABA\(_A\) receptors are the major inhibitory neurotransmitter receptors in the brain. The endocannabinoid system is a lipid signaling network that modulates different brain functions, in part by suppression of synaptic release of GABA. The major endocannabinoid 2-arachidonoyl glycerol (2-AG), but not anandamide at physiological concentrations allosterically potentiates recombinant GABA\(_A\) receptors. This modulation is limited to low concentrations of GABA and depends on the \(\beta_2\) subunit. The amino acid residues V436 and F439 in the transmembrane segment M4 of \(\beta_2\) confer 2-AG selectivity. 2-AG also modulates \(\delta\) subunit containing receptors, known to be located extrasynaptically and respond strongly to neurosteroids. We show that in animals 2-AG exerts superadditive effects with neurosteroids both in WT and CB\(_1\)/CB\(_2\) receptor double KO mice. Our finding uncover a novel GABA\(_A\) receptor binding site for drug discovery which may have far reaching consequences for the study of locomotion, sedation and sleep behavior.
EFFECTS OF CHRONIC CANNABIDIOL ADMINISTRATION ON SPLENIC LYMPHOCYTE NUMBERS AND BODY WEIGHT GAINS IN RATS: ROLE OF CB2 RECEPTORS

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Cannabidiol (CBD) is a major non-psychoactive constituent of Cannabis that has wide therapeutic potential. In our previous study, repeated CBD administration (5 mg/kg) in rats resulted in decreased numbers of lymphocyte B, T CD4+ and T CD8+ subsets in peripheral blood. CBD treatment reduced body weight gains. The present study aimed to assess distribution of the lymphocyte subsets in the spleen, following repeated CBD administration, and the involvement of CB2 receptors.

Adult male Wistar rats received intraperitoneal injections of CBD (5 mg/kg/day), or the vehicle, for 14 consecutive days. Total and relative numbers of lymphocyte T (T CD4+, and T CD8+), B, NK subsets were determined by flow cytometry. Furthermore, the selective CB2 receptor antagonist AM630 (1 mg/kg) was administered 15 min before CBD (or the vehicle) in order to block CB2 receptors.

CBD administration produced decrease in total leukocyte number resulting from decreased numbers of lymphocytes B and T - both T CD8+ and T CD4+. Pretreatment with CB2 receptor antagonist partially inhibited CBD-induced decrease in lymphocyte number – which was most pronounced in case of T CD8+ lymphocytes. AM630 itself produced insignificant decline in lymphocyte number. Observed effects were accompanied by a decrease in body weight gain, which was reversed by CB2 antagonist.

The results indicate that CBD reduces lymphocyte number in the spleen, as it does in peripheral blood and that CB2 receptor may be partially involved. The results also confirm that CBD lowers body weight gains in rats and CB2 receptor may play a role.
CANNABINOIDS MODULATION OF EMOTIONAL MEMORY

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Cannabinoids are well known modulators of mood and emotional behavior. We have data suggesting that cannabinoids can be used as potential therapeutics for the treatment of stress and anxiety-related disorders. We have recently found (Ganon-Elazar and Akirav, 2009; J Neurosci. 29:11078-11088) that cannabinoid activation in the basolateral amygdala (BLA) using the CB1/2 receptor agonist WIN 55,212-2 (WIN; 5 μg/side) can prevent the stress-induced enhancement of inhibitory avoidance conditioning as well as the stress-induced disruption of inhibitory avoidance extinction. This reversal effect was found to be associated with alterations in the hypothalamic-pituitary-adrenal (HPA) axis, as cannabinoid activation in the BLA inhibited the stress-induced increase in plasma corticosterone levels. In the hippocampus, WIN facilitated the extinction of inhibitory avoidance, with no effect on conditioning (Abush and Akirav, 2009; Hippocampus, Oct 14). These findings support a role for cannabinoid signaling in the treatment of conditions associated with the inappropriate retention of aversive memories and stress-related disorders.

Next we examined whether cannabinoid activation could prevent the effects of trauma exposure on fear-related symptoms. We found that one week after exposure to an animal model of PTSD rats show increased conditioned and unconditioned anxiety and enhanced negative feedback of the HPA axis. We found that cannabinoid activation 2-24 hrs after trauma exposure prevented the trauma-induced alterations in conditioned anxiety and HPA axis function. These effects were found to be mediated by CB1 receptors in the BLA. Taken together, the results provide critical preclinical data that may support therapeutic application for cannabinoids in the treatment of post-trauma syndromes.
EXAMINATION OF CANNABINOID PHARMACOLOGY IN ISOLATED RETINAL MICROVESSELS

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Purpose: To examine the in vitro retinal microvascular pharmacology of the CB1 receptor (CB1R) agonist, Arachidonyl-2¢-chloroethylamide (ACEA) and abnormal cannabidiol (abn-CBD), an agonist at the abn-CBD-sensitive endothelial cannabinoid receptor (CBx).

Methods: Isolated rat retinal arteriole fragments (10-35 μm) were placed in an experimental bath on the stage of an inverted microscope and superfused with Ringers solution. Drugs were applied to the isolated vessels via a 6-channel rapid switcher. Changes in arteriole diameter were measured using a video edge detector and \([\text{Ca}^{2+}]_o\) in arteriole smooth muscle cells (SMCs) was measured using the ratiometric dye Fura2-AM and a PTI imaging system. To examine the contribution of endothelial receptors to myogenic contractility, recordings were made in both endothelium-intact vessels and in vessels denuded of endothelium.

Results: Endothelin-1 (ET-1, 10 -100 nM) caused prolonged arteriole vasoconstriction increases in \([\text{Ca}^{2+}]_o\) in SMCs. ACEA produced vasorelaxation of ET-1-constricted arterioles that was blocked by the CB1R antagonist, AM251, but not by the CBx antagonist, O-1918, or the CB2R antagonist, AM630, and was not significantly affected by endothelium removal. Abn-CBD also produced vasorelaxation of ET-1 constricted arterioles, however the abn-CBD vasorelaxation was sensitive to O-1918 block, but not to AM251 or AM630, and was abrogated by endothelial removal.

Conclusions: Both the CB1 receptor agonist, ACEA and the CBx receptor agonist, abn-CBD, produced vasorelaxation in isolated retinal arterioles. However, while the actions of ACEA were mediated independent of endothelial removal, via CB1R on SMCs, those of abn-CBD were mediated in a CB1R-independent manner via an O-1918-sensitive endothelial CBx receptor.

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