1ST ANNUAL SATELLITE SYMPOSIUM ON THE CANNABINOIDS

THERAPEUTIC POTENTIAL OF THE CANNABINOIDS: PRESENT AND FUTURE

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St. Raphael Resort
Limassol, Cyprus, May 1-4, 2008

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Registration: May 1, 2008 (16.00 – 20.00)

Day 1
Friday, May 2nd

8.45 – 9.00	"Setting the Scene" - *Alex Makriyannis and Rik Musty*		
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10.30	Vincenzo Di Marzo	Endocannabinoids and peripheral control of energy homeostasis	3
11.00	Discussion – Leaders: *Vincenzo Di Marzo, Alex Makriyannis and Roger Pertwee*		

12.30	Lunch

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14.30	Joelle Chabry	Nonpsychoactive cannabidiol prevents prion accumulation and protects neurons against prion toxicity	7
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15.00	Ron Tuma	Modulation of the endocannabinoid system as a strategy to minimize ischemia/reperfusion injury.	9
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16.30	Reinaldo Takihashi	Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM 404 and cannabidiol in conditioned rats	19
16.45	Raphael Mechoulam	Cannabidiol research: 45 years on	20
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17.30	POSTER SESSION

19.00	DINNER

Notes:

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9.00	Emmanuel Onaivi	Targeting brain neuronal CB2 cannabinoid receptors in drug abuse and depression	22
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Megan J Dowie, et al.	Characterisation of the cannabinoid system prior to symptom onset in a transgenic mouse model of Huntington's disease	34

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Fabrício Alano Pamplona, et al.	Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats	38

THE ENDOCANNABINOID SYSTEM AS THERAPEUTIC TARGET

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Our current knowledge of the endocannabinoid system includes CB1 and CB2, two $G_{i/o}$ GPCRs involved in a number of signaling mechanisms. The endogenous molecules that modulate this biochemical system are represented by arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG). There is also evidence for other GPCRs may be linked to the endocannabinoid system; however, their roles in endocannabinoid modulation have not been fully characterized.

The levels of endocannabinoids are modulated by a number of metabolizing and biosynthetic enzymes as well as by a transporter system that remains to be fully characterized. Modulation of the endocannabinoid system, either direct (through CB1/CB2) or indirect (through enzymatic or transport inhibition) provides opportunities for the design and development of small ligands capable of effecting physiological changes and, thus, serve as potential drug candidates.

A number of medicinal chemistry efforts are currently underway to develop such medications. The approaches for such work involve high throughput screening approaches followed by lead optimization using classical medicinal chemistry concepts. Alternatively, the design of novel ligands is based on the intimate knowledge of the 3D-structure of the protein (receptor or enzyme) with which they interact. This target-based drug design utilizes a combination of computational and biophysical methods.

Earlier work had led to the development of the CB1/CB2 agonist nabilone as a medication for chemotherapy-induced nausea and pain. More recently a novel CB1 selective inverse agonist was developed for the treatment of obesity and metabolic disorders under the name Acomplia. Furthermore, a number of ongoing efforts aim at the development of novel compounds for other therapies. These include CB2 agonists for the treatment of neuropathic pain, CB1 agonists for the treatment of cachexia, as well as FAAH inhibitors for the treatment of pain and neurodegeneration.

The endocannabinoid system has been recognized as an important "druggable" target for medication development. There is an excellent chance that within the next few years novel cannabinergic medications will become available.

Acknowledgements: Supported by grants from the National Institute on Drug Abuse

PHARMACOLOGICAL STRATEGIES FOR TARGETING THE ENDOCANNABINOID SYSTEM IN THE CLINIC

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There are some disorders in which it is thought that the endocannabinoid system (ECS) upregulates through increased endocannabinoid release and/or cannabinoid receptor expression/signalling in a manner that suppresses unwanted signs and symptoms such as neuropathic pain or may even slow down the progression of diseases such as multiple sclerosis [1,2]. There are other disorders, however, including metabolic syndrome, in which the ECS appears to mediate unwanted signs and symptoms such as obesity [1]. Indeed, in line with this notion that the ECS has such "autoprotective" and "autoimpairing" roles, compounds that activate or block cannabinoid receptors are already in the clinic. These are two CB_1/CB_2 receptor agonists, delta-9-tetrahydrocannabinol and nabilone, and the CB_1-selective competitive antagonist/inverse agonist, rimonabant (Acomplia). Delta-9-tetrahydrocannabinol (Marinol) and nabilone (Cesamet) are prescribed to decrease chemotherapy-induced nausea and vomiting (both drugs) or to stimulate appetite (Marinol) and Acomplia is prescribed to reduce obesity. Delta-9-tetrahydrocannabinol is also a major constituent of Sativex, a licensed medicine that is used to relieve neuropathic and cancer pain.

Currently other CB_1 receptor competitive antagonists are in development as medicines and there is a lot of interest both in seeking out additional clinical applications for such ligands and in exploring the therapeutic potential of CB_1 allosteric antagonists. The possibility that CB_2 receptor inverse agonists may be potential medicines is also receiving significant attention. Thus, such ligands inhibit immune cell migration and show promise as anti-inflammatory agents [3]. Considerable effort is also being directed at exploring and validating pharmacological strategies that will reduce the incidence/intensity of unwanted central effects caused by CB_1 receptor agonists when these compounds are being used in the clinic to relieve symptoms such as pain. Possible strategies include administering a CB_1/CB_2 receptor agonist intrathecally or topically, a CB_1/CB_2 receptor agonist that does not readily cross the blood brain barrier, a cannabinoid receptor agonist that lacks maximal CB_1 receptor efficacy, or a CB_2-selective agonist. Other potential strategies exploit the ability of CB_1 receptor agonists to interact synergistically with a non-cannabinoid to relieve symptoms or rely on evidence that unwanted signs/symptoms of disorders such as multiple sclerosis could be alleviated by targeting allosteric sites on CB_1 receptors or by augmenting the concentration of endocannabinoids at their receptors with inhibitors of endocannabinoid cellular uptake or of endocannabinoid metabolizing enzymes such as fatty acid amide hydrolase or monoacylglycerol lipase. The need to develop strategies of this kind is driven not only by current accepted clinical uses for cannabinoid CB_1/CB_2 receptor agonists but also by emerging evidence for an ever-growing number of additional potential therapeutic applications for such ligands. Many of these strategies rely of course on the availability of suitable compounds and substantial progress has already been made in meeting this need, particularly through the development of novel CB_2-selective agonists and of inhibitors of fatty acid amide hydrolase.

1. Pertwee, R.G. (2005). *AAPS J* **7**: E625-E654
2. Pertwee, R.G. (2007). *Mol Neurobiol* **36**: 45-59
3. Lunn, C.A. *et al*. (2008). *Br J Pharmacol* **153**: 226-239

ENDOCANNABINOIDS AND PERIPHERAL CONTROL OF ENERGY HOMEOSTASIS

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The endocannabinoid (EC) system participates at several levels in the control of lipid and glucose metabolism, with the possible ultimate end of accumulating energy into fat. Mostly by activating cannabinoid CB_1 receptors, endocannabinoids: 1) stimulate food intake after food deprivation by enhancing appetite, increasing food palatability and suppressing satiety; 2) facilitate nutrient absorption by slowing down small intestine motility; and 3) maximize fat accumulation into the white adipose tissue, possibly also by reducing energy expenditure via reduced insulin sensitivity, fatty acid ω-oxidation and glucose uptake in the skeletal muscle. Following unbalanced energy intake, or because of genetic reasons, or both, the EC system can become dysregulated, and in most cases overactive, not only in the hypothalamus but also in several peripheral organs participating in energy homeostasis, and, particularly, in the intra-abdominal adipose tissue. This dysregulation might contribute to excessive visceral fat accumulation and reduced adiponectin release from this tissue, to ectopic fat formation in the liver, and to the development of insulin resistance that, coupled to excessive insulin release from the pancreas, and to pro-inflammatory effects, might contribute to the onset of type 2 diabetes and associated cardiovascular events. Peripheral EC dysregulation is likely at the basis of the mechanism of action of cannabinoid CB_1 receptor antagonists/inverse agonists (rimonabant, taranabant, AVE-1625, CP-945598, BMS646256, etc.) recently developed by several pharmaceutical companies as potential adjuvants to life-style modification for weight reduction, glycemic control and dyslipidemia in obese and type 2 diabetes patients. It also helps explaining why some of the beneficial actions of these new therapeutics appear to be partly independent from weight-loss.

THERAPEUTIC POTENTIAL OF CANNABINOIDS IN NEUROLOGICAL DISORDERS

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Neurological disorders are within the group of diseases for which cannabinoid-based medicines have been proposed as a novel type of pharmacotherapy, although, unhappily, the evidence described so far is still mostly preclinical. The rationale for this proposal derives from an extensive work with cellular or animal models of different neurological disorders. These studies have demonstrated **first** the existence of significant alterations in different elements (e.g. receptors, ligands, enzymes) of the cannabinoid signaling in those brain structures that are affected in these disorders. This is the case, for example, of the hypofunction of the cannabinoid signaling (mainly due to loss of CB_1 receptors) found in the basal ganglia in hyperkinetic disorders such as Huntington's disease (HD). By contrast, the cannabinoid signaling (e.g. CB_1 receptors and endocannabinoid ligands) becomes overactive in the basal ganglia in Parkinson's disease (PD). In addition, both diseases and also other neurodegenerative disorders, including amyotrophic lateral sclerosis, Alzheimer's disease (AD), multiple sclerosis (MS), brain trauma, cerebral ischemia or neuropathic pain, are associated with upregulatory responses in CB_2 receptors linked to the occurrence of local inflammatory events, and frequently with an increased generation of endocannabinoids. These effects have been considered as a part of an endogenous protective response against the brain damaging stimuli. **Second**, some of these changes may explain the potential found for certain cannabinoid-based compounds to alleviate specific neurological symptoms typical of these pathologies. In most of the cases, the treatments would be aimed at restoring the normal values for cannabinoid signaling which would originate changes in neurotransmitter activity at specific synapses. For example, CB_1 receptor antagonists have been reported to reduce motor inhibition in parkinsonian rats presumably by reducing the overactivity of this system and enhancing glutamate release in the striatum. CB_1 receptor antagonists may be also effective for certain memory defects typical of AD or other cognitive disorders, presumably by enhancing cholinergic activity. By contrast, enhancing rather than blocking the cannabinoid signaling has been associated with symptom relief in those disorders in which the cannabinoid signaling becomes hypofuntional, such as HD (antihyperkinetic effect) and AD (orexigenic effect). This can be also the case of Tourette's syndrome where certain plant-derived cannabinoids have been reported to reduce tics and other abnormalities. This strategy has resulted also effective in MS although, in this disease, the benefits provided by direct or indirect agonists of cannabinoid receptors seem to be the consequence of an enhancement in the endogenous adaptative response elicited by the cannabinoid signaling. **Lastly**, cannabinoids might be also effective to delay/arrest the progression of neurological disorders in those pathologies characterized by the degeneration of specific brain structures. This potential would be exerted through the capability of certain cannabinoid compounds to limit the influence of several cytotoxic events, including excitotoxicity, calcium influx, oxidative damage, microglial activation and vasoconstriction, on neuronal homeostasis.

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CANNABINOIDS FOR THE CONTROL OF MULTIPLE SCLEROSIS: INSIGHT FROM ANIMAL MODELS

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Multiple sclerosis (MS) is a major demyelinating disease of the central nervous system (CNS). Relapsing-remitting disease is driven by repeated neurological insults by the immune system that causes nerve damage. Nerve loss appears to the substrate for progressive disease and slow neurodegeneration, due to excitotoxicity and other factors can occurs independently of the immune responses that drive relapsing disease. This damage to neural networks within the CNS results in altered neurotransmission and the development of a variety of neurological signs including spasticity and pain. These are poorly controlled and has prompted people to self-medicate and perceive benefit from taking cannabis. The actions of cannabis are based on an unraveling biology and some clue for human uses can come from cell culture and particularly animal experiments. It has been found that cannabinoids can regulate synaptic transmission and consistent with this cannabinoid receptor agonists and modulators of endocannabinoids can control signs of disease such as spasticity in animal models of multiple sclerosis. However, in addition to symptom control potential animal models of MS can be shown to inhibit immune processes that may influence immunity that drive attacks and the processes that induce nerve damage. In addition cannabinoids can induce neuroprotective effects, which are distinct from immune inhibition, that suggest some potential to control progressive disease, which so far have avoided therapy.

CLINICAL TRIALS OF CANNABINOIDS IN MULTIPLE SCLEROSIS: SPASTICITY AND LOWER URINARY TRACT SYMPTOMS

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Introduction: Survey studies in Europe and North America reveal that multiple sclerosis patients with spasticity and lower urinary tract symptoms (LUTS) have frequently turned to cannabis as an alternative symptomatic treatment. Despite this anecdotal information, it has only been recently that formal randomized clinical trials (RCTs) have been organized to evaluate these claims of efficacy. This presentation will briefly discuss available study results for those symptoms employing Cannador, an oral cannabis extract with an approximate 2:1 THC:CBD ratio, and Sativex®, an oromucosal cannabis-based spray with an almost 1:1 ratio of THC and CBD. The latter was approved by Health Canada in April 2005 for prescription for central neuropathic pain in multiple sclerosis, and in August 2007, for treatment of cancer pain unresponsive to optimized opioid therapy.

Methods: Published and unpublished materials from RCTs of cannabinoid agents for treatment of spasticity and LUTS were reviewed.

Results: Cannador was employed in the CAMS study of 667 MS patients, with no change in Ashworth scale. Improvement in pain and subjective spasticity for both Cannador and Marinol was noted over placebo ($p=0.003$), as well as a decrease in walking time. A possible lower relapse rate was seen in treated groups. In a one year follow-up in 630 subjects, Marinol, but not Cannador, produced a small treatment effect on spasticity. A separate analysis showed a suggestion of a treatment effect on incontinence in MS. In GWMS0106, Sativex provided a greater than one box reduction in Numerical Rating Scale (NRS) spasticity scores in 189 subjects, significantly greater than placebo ($p=0.048$, or $p=0.013$ with one placebo outlier removed), and with 40% of subjects improving by 30% or more. Sativex also produced beneficial effects on sleep quality in numerous studies in MS. In Safety-Extension studies (SAFEX), improvements were durable over one or more years. In GWCL0403, 337 subjects were assessed, and Sativex trended toward a reduction of spasticity vs. placebo on the ITT population ($p=0.15$), with clearer improvement in the per protocol population ($p=0.034$), and with greater than 30% improvement in 36% of subjects. Integrated analysis of all MS spasticity subjects revealed significance at $p=0.025$. In a Phase III study of 135 MS patients with intractable LUTS, Sativex failed to influence incontinence incidence, due to a low baseline, but lowered bladder symptom severity ($p=0.001$), 24-hour frequency ($p=0.001$), nocturia ($p=0.01$), urgency ($p=0.07$), and Patient Global Impression of Change ($p=0.005$), with 55% reporting 30% or greater symptomatic improvement.

Conclusion: Available data support the clinical efficacy of cannabinoids in treatment of spasticity and lower urinary tract symptoms in multiple sclerosis.

NONPSYCHOACTIVE CANNABIDIOL PREVENTS PRION ACCUMULATION AND PROTECTS NEURONS AGAINST PRION TOXICITY

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Prion diseases are transmissible neurodegenerative disorders characterized by the accumulation in the central nervous system of the protease-resistant prion protein (PrPres), a structurally misfolded isoform of its physiological counterpart PrPsen. Both neuropathogenesis and prion infectivity are related to PrPres formation. Here, we report that the non-psychoactive cannabis constituent cannabidiol (CBD) inhibited PrPres accumulation in both mouse and sheep scrapie-infected cells while other structurally related cannabinoid analogs were either weak inhibitors or non-inhibitory. Moreover, after intraperitoneal infection with murine scrapie, peripheral injection of CBD limited cerebral accumulation of PrPres and significantly increased the survival time of infected mice. Mechanistically, CBD did not appear to inhibit PrPres accumulation *via* direct interactions with PrP, destabilization of PrPres aggregates, or alteration of the expression level or subcellular localization of PrPsen. However, CBD did inhibit the neurotoxic effects of PrPres and affected PrPres-induced microglial cell migration in a concentration dependent manner. Our results suggest that CBD may protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative process, which takes place during prion infection. When combined with its ability to target the brain and its lack of toxic side effects, CBD may represent a promising new anti-prion drug.

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THERAPEUTIC POTENTIAL OF CANNABINOIDS IN ALS

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There is increasing evidence that cannabinoids have therapeutic potential in amyotrophic lateral sclerosis (ALS). ALS is the most lethal neurodegenerative disease; typical survival after diagnosis is 3 years. ALS results in the degeneration of motor neurons in the cortex, brainstem and spinal cord. As a consequence of the damage, patients experience progressive muscle weakness that can hinder movement, speech, swallowing and even breathing. New treatments for ALS are desperately needed. Studies from in vitro and in vivo rodent models of ALS have shown neuroprotection, slowing of disease progression and increased survival in cannabinoid-treated animals. Potential mechanisms of neuroprotection include the antioxidant, anti-inflammatory and anti-excitotoxic properties exhibited by these compounds. The endocannabinoid system is dysregulated in mouse models of ALS; levels of endocannabinoids increase during disease progression, CB1 receptor levels decrease and CB2 receptor levels increase. There is also evidence of dysregulation in ALS patients, where CB2 expression is elevated in activated microglia in spinal cord. Cannabinoids have been used in patients with ALS with symptomatic benefit for insomnia, spasticity and appetite. A phase I pilot open-label crossover study of dronabinol (THC) in 19 patients with ALS showed that patients received up to 10 mg a day with no significant adverse effects. 11 of the 19 patients showed a slower decline (average 27%), however this was a very small trial and not powered to see effectiveness. Another 11 of the 19 patients reported improvement in ALS related-symptoms such as fasciculations, spasticity and insomnia, but a larger trial will be needed to assess the clinical effectiveness of THC in patients with ALS. The positive results in the animals, combined with the phase I trial results, support further evaluation of cannabinoids as potential therapies for ALS.

MODULATION OF THE ENDOCANNABINOID SYSTEM AS A STRATEGY TO MINIMIZE ISCHEMIA/REPERFUSION INJURY

Ming Zhang (Temple University School of Medicine), Billy Martin (Virginia Commonwealth University), Martin Adler (Temple University School of Medicine), Raj Razdan (Organix Inc), Doina Ganea (Temple University School of Medicine) and Ronald Tuma (Temple University School of Medicine)

We have recently reported that activation of the CB2 receptor may serve to minimize damage to the central nervous system resulting from ischemia, trauma and autoimmune disease. We have shown, using a mouse middle cerebral artery occlusion model of stroke, that activation of the CB2 receptor with a selective CB2 agonist (O-1966) improves outcome (reduced infarct size, reduced edema and improved motor function). We have also shown that blocking the CB2 receptor with a CB2 antagonist (SR144528) worsens outcome. These results are supported by preliminary investigations showing worse outcome in CB2-/- mice. In addition we have shown that administration of a CB1 antagonist (SR141716) also improves outcome, indicating that activation of the CB1 receptors during ischemia/reperfusion injury contributes to damage. The greatest degree of protection from ischemia/reperfusion injury was obtained by combined administration of a CB2 agonist and a CB1 antagonist. The administration of these agents in combination also resulted in an improvement of blood flow during the occlusion period. These results indicate that the protection obtained via antagonism of the CB1 receptor and activation of the CB2 receptor occurs through at least two different mechanisms and that modulating the balance of activation of components of the endocannabinoid system may serve as an effective means to minimize ischemia/reperfusion injury.

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TRANSMUCOSAL DELIVERY OF Δ⁹-TETRAHYDROCANNABINOL FROM A PRODRUG FORMULATION

Mahmoud A. ElSohly[1,2,3], Waseem Gul[1,3], Soumyajit Majumdar[2] and Michael A. Repka[2]

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Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) and other cannabinoids have gained much interest in recent years as possible therapeutic agents for several indications. Δ⁹-THC is currently approved for human use in the USA for two limited indications (as antiemetic and appetite stimulant) in an orally administered soft gelatin capsule. Because of the limitations associated with oral administration, attempts have been made and continue to be made to overcome these limitations. As part of our interest in this area we have developed a suppository formulation containing the hemisuccinate ester of Δ⁹-THC which was successful in delivering the drug much more effectively and consistently, without the limitation of the first pass effect. While this formulation is currently under development, we have put forth additional effort to develop a buccal transmucosal delivery system, more acceptable to the general population. In this presentation, we report on an Oral Transmucosal Matrix Patch System (TMPS) incorporating Δ⁹-THC-hemisuccinate as a prodrug. The films used in the TMPS are prepared using Hot Melt Extrusion (HME) Technology which offers several advantages over the cast film technology. Several formulations were prepared and one of these preliminary formulations was investigated *in vivo* (rabbit model) for effecting bioavailability of Δ⁹-THC from TMPS containing the hemisuccinate ester. The data showed that indeed Δ⁹-THC blood levels were achieved for several hours with very low levels of 11-OH-Δ⁹-THC, indicating true transmucosal delivery. Further studies are in progress to optimize the formulation for stability and faster onset of delivery.

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CANNABINOID SIGNALING SYSTEMS AS ANALGESIC TARGETS

Andrea G. Hohmann

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Cannabis has been used for many centuries both for its medicinal properties and for its reinforcing effects. Cannabinoids produce antinociception in animal models of acute, tissue injury and nerve injury-induced persistent pain. These antinociceptive effects are mediated through activation of CB_1 and CB_2 receptor subtypes. CB_1 receptors are found predominantly within the central nervous system (CNS) whereas CB_2 receptors are found predominantly, but not exclusively, outside the CNS where they are localized to cells of the immune system. A large body of literature indicates that cannabinoids suppress nociceptive responding in behavioral studies through actions at spinal, supraspinal and peripheral levels. These actions are consistent with the distribution of cannabinoid receptors in the CNS and periphery. Cannabinoids directly suppress sensitization in nociceptive neurons, thereby providing a cellular basis for the profound antinociceptive effects of cannabinoids. The distribution of cannabinoid receptors in brain provides an anatomical basis both for the antinociceptive effects of cannabinoids as well as the psychoactive effects of these compounds. These psychoactive effects have limited the therapeutic potential of direct agonists targeting CB_1 receptors. Pharmacological and transgenic approaches have provided considerable insight into the functional roles of endocannabinoid signaling systems in suppressing pain. Activation of endocannabinoid signaling systems would be expected to produce a more circumscribed and beneficial spectrum of biological effects compared to the psychoactive ingredient in cannabis. Under conditions of injury, cannabinoid CB_2 receptors are also upregulated in the CNS and periphery, providing a potential analgesic target for the treatment of persistent pain states. Pathways controlling endocannabinoid deactivation (e.g. inhibitors of fatty acid amide hydrolase and monoacylglycerol lipase) are also discussed as potential analgesic targets. However, the highly interactive nature of lipid signaling pathways must also be considered when pharmacological strategies aimed at manipulating levels of endocannabinoids are viewed as targets for drug development. These factors, however, also raise the exciting possibility that synergistic antinociceptive effects can be attained by adjunctive pharmacological approaches (e.g. co-administration of inhibitors of endocannabinoid hydrolysis and cyclooxygenase). Strategies aimed at exploiting the therapeutic potential of the cannabinoid signaling system for suppressing pathological pain states while limiting the unwanted psychoactive effects are emphasized. Therapeutic applications of cannabinoid-based analgesics for treatment of inflammatory, neuropathic and cancer pain are discussed.

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BENEFICIAL AND DETRIMENTAL CLINICAL IMPLICATIONS OF CANNABINOID / OPIOID INTERACTIONS ON IMMUNE RESPONSES

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Morphine, delta[9]-tetrahydrocannabinol (THC), and anandamide were all shown to give dose-related immunosuppression as assayed by a test of in vitro antibody formation (PFC assay) when added to mouse spleen cells in vitro. When each drug was tested alone, similar sigmoidal dose-response curves resulted, with suppression observed at doses between 10^{-7} and either 10^{-9}M (morphine), 10^{-10} M (THC) or 10^{-11} M (anandamide). Immunosuppression by morphine was blocked by pretreatment with naltrexone, and immunosuppression by THC or anandamide was blocked by the CB_2 antagonist, SR144528, but not by the CB_1 antagonist, SR141716. The maximal immunosuppression observed for each drug alone was 50%. The hypothesis was tested that the combination of an opioid and a cannabinoid would result in 100% suppression. A combination of morphine plus THC was tested using a paradigm in which the drugs were combined at a ratio of their individual ED_{50}s calculated in ◎g/ml. This solution was then serially diluted and tested in the PFC assay. The combination resulted in a novel dose-response curve, with minimal suppression at the high dose and suppression at lower doses than observed with either agonist alone. Thus, the combination of morphine and THC used in vitro gave a subadditive effect at a high dose and a superadditive effect at a low dose. Incubation of the cells with naltrexone, CTAP (mu antagonist), or the CB_2 antagonist, SR144528, yielded a dose-response curve similar to that of either agonist alone. The mechanism for these unanticipated drug interactive effects in the immune system is not known, but should be of considerable interest. These results also raise the question as to whether combinations of opioids and cannabinoids used for analgesia might have unwanted immunosuppressive effects. Conversely, the immunosuppressive properties of the cannabinoids might be used therapeutically, as they are inhibiting immune function via CB_2 and not CB_1 receptors.

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TBA

THE USE OF SATIVEX IN THE TREATMENT OF NEUROPATHIC PAIN: RATIONALE AND RESULTS

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Sativex is derived from extracts of selected strains of *Cannabis sativa L.* which produce high and reproducible yields of the principal cannabinoids THC and CBD. It is given as a spray for sub-lingual or oro-mucosal administration, and each 100μL spray contains 2.7 mg THC and 2.5 mg CBD. This mode of administration is designed to avoid the substantial first pass effect which makes oral administration of cannabinoids unpredictable, and to provide dosing flexibility for the patient. Both CBD and THC have a pharmacology that suggests an analgesic effect and supports their clinical investigation as analgesics in neuropathic pain. Analgesic activity for THC is most likely related to its effects at the CB1 receptor, while CBD has been shown to have several pharmacologic effects which may contribute to its analgesic efficacy.

Neuropathic pain results from nerve damage and has typically been classified as central - where the lesion lies within the CNS - or peripheral, where the lesion affects a peripheral nerve. There is a well-recognised need for better pain relief than is provided by currently available agents, which include anti-depressants, anti-convulsants, opioids and topically applied local anaesthetics.

Sativex has undergone randomised controlled clinical studies in several models of chronic neuropathic pain, both central and peripheral. These include multiple sclerosis, brachial plexus avulsion, peripheral neuropathy with allodynia, and diabetic neuropathy. It has also been studied in cancer pain. In all clinical studies, Sativex has been used as add-on therapy in patients who have failed to gain adequate relief from currently available agents.

Overall, more than 2500 patients and subjects have been included in Sativex clinical trials, and many of these have gone on to long-term open-label treatment. This has confirmed that the improvement seen in short-term use is maintained in the long-term, and that no evidence of tolerance to Sativex has developed.

Despite the fact that it has been exclusively studied in treatment-resistant populations, results have consistently shown Sativex to produce significantly more responders than placebo, with a relatively low burden of adverse events. As a result of these studies, Sativex is now approved as a prescription medicine in Canada for the relief of neuropathic pain in adults with multiple sclerosis, and for the relief of persistent background cancer-related pain.

CANNABIS IN PAINFUL HIV-ASSOCIATED SENSORY NEUROPATHY: A RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Objective This study was conducted to determine the effect of smoked cannabis on the neuropathic pain of HIV-associated sensory neuropathy and an experimental pain model.

Methods We conducted a prospective randomized placebo-controlled trial in the inpatient General Clinical Research Center at San Francisco General Hospital between May 2003 and May 2005 involving adults with painful HIV-associated sensory neuropathy. Patients were randomly assigned to smoke either cannabis (3.56% tetrahydrocannabinol) or identical placebo cigarettes with the cannabinoids extracted three times daily for 5 days. Cannabis and placebo were obtained from the U.S. National Institute of Drug Abuse. Primary outcome measures included ratings of chronic pain and the percentage of participants achieving >30% reduction in pain intensity. Acute analgesic and anti-hyperalgesic effects of smoked cannabis were assessed using a cutaneous heat stimulation procedure and the heat/capsaicin sensitization model.

Results 50 patients completed the entire trial. Smoked cannabis reduced daily pain by 34% (median reduction; IQR=-71, -16) vs 17% (IQR=-29, 8) with placebo ($p=0.03$). Greater than 30% reduction in pain was reported by 52% in the cannabis group and by 24% in the placebo group ($p=0.04$). The first cannabis cigarette reduced chronic pain by a median of 72% vs 15% with placebo ($p<0.001$). Cannabis reduced experimentally-induced hyperalgesia to both brush and von Frey hair stimuli ($p\leq0.05$) but appeared to have little effect on the painfulness of noxious heat stimulation. No serious adverse events were reported.

Conclusion Smoked cannabis was well tolerated and effectively relieved chronic neuropathic pain from HIV-associated sensory neuropathy. The findings are comparable to oral drugs used for chronic neuropathic pain. Additional studies have supported the clinical benefit of cannabis in neuropathic pain.

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CANNABINOIDS IN ACUTE PAIN: CLINICAL EVIDENCE OF INDUCED HYPERALGESIA

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Numerous animal studies have demonstrated anti-nociceptive and anti-hyperalgesic properties of plant-derived (Δ9-tetrahydrocannabinol, THC) and synthetic (e.g. nabilone) cannabinoid compounds. Their potential role in clinical acute pain, however, still has to be defined. In this abstract, the available clinical evidence of anti-nociceptive as well as recently observed pro-nociceptive effects of cannabinoids in acute pain is reviewed. Only inconsistent data exist from controlled clinical trials with cannabinoids in acute postoperative pain. In the 80ies, two small-sized studies investigated the parenterally applied synthetic cannabinoid levonantradol in postoperative pain. Two other, more recent trials were performed with oral THC. Whereas in the smaller trial no significant analgesia was produced by a single dose of 5mg THC, a well-designed multicenter dose-escalation trial using oral cannabis extract (cannador) showed a small, but significant dose-related reduction in postoperative patient-controlled analgesia requirements. In trials using human pain models of acute nociceptive or inflammatory pain and induced hyperalgesia, oral THC or cannabis extract often failed to produce significant analgesic and anti-hyperalgesic effects, although these experimental approaches were comparable to animal experiments where cannabinoids were effective. In one study, various pain thresholds were determined in normal skin of healthy volunteers after a single oral dose of 20mg THC. No analgesic effects could be found, but decreased pain thresholds (i.e. hyperalgesia) were observed (heat pain thresholds, ice water immersion test) compared to placebo and morphine. In a recent placebo-controlled, randomised, double-blind crossover study, we used a human sunburn model (acute inflammatory pain with primary and secondary hyperalgesia) and intradermal capsaicin (acute hyperalgesia) to investigate the analgesic and anti-hyperalgesic efficacy of THC-calibrated cannabis extract (cannador). A single oral dose (20mg THC) did not alter heat pain thresholds, but significantly decreased electrical pain thresholds. These results were strikingly consistent with the previous report, although different human pain models were used. In another placebo-controlled trial, the synthetic cannabinoid HU-210 - topically administered on normal skin 24 h prior to topical capsaicin application – produced significant, but mild and short-lasting analgesic effects, whereas we did not find any significant effects of prior oral THC on capsaicin-evoked immediate pain, skin flare reaction and hyperalgesia after intrademal capsaicin injection. Interestingly enough, similar results were seen in postoperative pain after 2mg nabilone. Significantly higher pain scores were reported compared to placebo, ketoprofen or only 1mg nabilone. As recently shown by Wallace et al., not only oral cannabinoids , but also smoked cannabis produced hyperalgesia. In this study, an analgesic effect of smoked cannabis on capsaicin-evoked spontaneous pain was reported, demonstrating a complex and not well understood dose dependency. Despite inconsistent weak, dose-dependent analgesic effects, oral cannabinoids proved more or less ineffective against clinical and experimentally induced acute pain and hyperalgesia. However, evidence has accumulated from various human acute pain models that cannabinoids like THC may induce acute hyperalgesia under these conditions. This effect is not restricted to the oral application route. Neither the dose-relationship, nor the underlying mechanisms of this pro-nociceptive activity are clear.

THE USE OF CANNABINOIDS IN NEUROPSYCHIATRIC CONDITIONS

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This overview presents research on Cannabinoids in neuropsychiatric conditions from our laboratory and from those of others as well. First, we review the preclinical data, in models on the following: the effect of delta-9-THC (THC) + Cannabidiol (CBD) in normal volunteers. We show that CBD reduces anxiety, marijuana effect and psychotic-like symptoms. We further demonstrate that CBD is anxiolytic, antidepressant and antipsychotic proprerties, and it reduces hyperlocomotion from stimulants in several animal models. In addition, we show that c-Fos expression is reduced like Haloperidol.

In human studies, we demonstrate that CBD has no toxicity in human studies in long term administration.

We also show that CBD has an anxiolytic effect in humans during an anxiety inducing public speaking test. CBD also reduced fear of fearful faces compared with THC administration.

We continue to show that CBD has anti-mania effect in and animal model of mania and that a small number of patients with Bipolar Affective Disorder showed some relief from CBD. We also present data on the anticonvulsant (anti-epileptic) effect of CBD.

Data on the neuroprotective effect of CBD are also presented with positive results.

Data on the effect of CBD on Parkinson's disease (PD) are also presented, with some positive results.

Although the mechanisms of CBD are still unknown, a considerable body of evidence suggests that it has biological effects, particularly anti-anxiety and antipsychotic properties.

Clinical trials are clearly needed to confirm these possible effects in the treatment of the clinical anxiety (Panic, GAD, SAD, OCD) and neurological conditions (PD and Epilepsy).

Our Power-Point presentation is available by e-mail at Richard.Musty@uvm.edu

CANNABINOIDS AND ENDOCANNABINOIDS IN THE TREATMENT OF DEPRESSION

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Several anecdotal reports and clinical studies have indicated that cannabis may serve as an effective treatment for people suffering from mood disorders, including depression.
However, the neurobiological mechanism underlying this antidepressant effect remains poorly understood. The goals of our research were to assess if cannabinoid agonists (WIN55,212-2 and delta-9-tetrahydrocannabinal/THC) as well as endocannabinoid enhancers (URB 597) possess antidepressant-like effects in animal models of depression and if, like clinically used antidepressants, they are able to increase the neurotransmission of serotonin (5-HT), the monoamine mainly involved in mood regulation.

At low doses, the CB_1 receptor (CB_1R) agonist WIN55,212-2 exerts potent antidepressant-like properties in the rat forced-swim test (FST). This effect is CB_1R-dependent since it was blocked by the CB_1R antagonist rimonabant, and 5-HT-mediated since it was abolished by pretreatment with the 5-HT-depleting agent pCPA. Then, using in vivo electrophysiology, we showed that low doses of WIN55,212-2 dose-dependently enhanced dorsal raphe nucleus 5-HT neuronal activity through a CB_1R-dependent mechanism. On the other hand, high doses of WIN55,212-2 were ineffective in the FST and decreased 5-HT neuronal activity through a CB_1R-independent mechanism. The CB_1R agonist-induced enhancement of 5-HT neuronal activity was abolished by total or medial prefrontocortical, but not by lateral prefrontocortical transection. Furthermore, 5-HT neuronal activity was enhanced by the local microinjection of WIN55,212 into the ventromedial prefrontal cortex.

The FAAH blocker URB 597 shows antidepressant-like properties at the FST and the tail-suspension test in a dose-dependent manner. URB 597 also acutely increases 5-HT firing activity, but the effect onsets 20 minutes and peaks 2 hours after injection. This effect was prevented by rimonabant. Increasing doses of URB 597 does not produce a biphasic effect, but a sigmoidal profile. Unlike direct CB_1R agonists, URB 597 does not exert rewarding effects in the conditioned place preference test or produce generalization to the discriminative effects of THC in rats. These results demonstrate that CB_1R agonists and FAAH inhibitors possess antidepressant-like properties by modulating 5-HT neuronal activity. However, since FAAH inhibitors exhibit a safer pharmacological profile, they present a more favorable option for the development of novel antidepressants.

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FACILITATION OF CONTEXTUAL FEAR MEMORY EXTINCTION AND ANTI-ANXIOGENIC EFFECTS OF AM404 AND CANNABIDIOL IN CONDITIONED RATS

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Studies suggest an important role of the endocannabinoid (eCB) system in the modulation of emotional states and extinction of aversive memories in animals. Particularly, enhancement of eCB transmission by the inhibition of uptake/metabolism seems to be an interesting pharmacological approach. The aim of this study was to investigate the central effects of the eCB uptake/metabolism inhibitor AM404 and the phytocannabinoid with different mechanisms of action cannabidiol (CBD) on the extinction of contextual fear memories in rats. Male Wistar rats were conditioned and injected i.c.v. with AM404 or CBD 24 h later. The animals were subjected to three consecutive 9-min non-reinforced exposures to the conditioning context (extinction sessions). A 3-min drug-free test of contextual memory was performed 24 h after the last extinction session to investigate long-lasting effects. Injection of AM404 (1.0 μg/μl, i.c.v.) and CBD (2.0 μg/μl, i.c.v.) facilitated the extinction of contextual fear memory, with long-lasting effects. This response was antagonized by the CB1-selective antagonist SR141716A (0.2 mg/kg, i.p.), but not by the TRPV1-selective antagonist capsazepine (5.0 μg/μl, i.c.v.), thus suggesting the involvement of CB1 cannabinoid receptors in the facilitation of extinction by these drugs. Anxiolytic-like effects might have contributed to the facilitation of extinction as suggested by an antianxiogenic effect in the fear-potentiated plus-maze test. These results complement other lines of evidence suggesting a role of the eCB system in the modulation of the emotional states and highlight that CBD, a non-psychoative phytocannabinoid could be an interesting pharmacological approach to reduce the anxiogenic effects of stress and promote the extinction of fear memories, such as PTSD.

CANNABIDIOL RESEARCH: 45 YEARS ON

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The structure and stereochemistry of Cannabidiol (CBD) were elucidated by our group in 1963. Its pharmacological effects are still being investigated:

Diabetes type 1. Administration of CBD to 11-14 week old female non-obese diabetes-prone (NOD) mice, which are either in a latent stage or with initial symptoms of diabetes, ameliorates the manifestations of the disease. Diabetes was diagnosed in only 32% of the mice in the CBD-treated group, compared to 100% in the untreated group. Histological examination of the pancreata of CBD-treated mice revealed more intact islets than in the controls.

Alzheimer's disease. Adult male mice received a single intraventricular injection of Aβ (β amyloid peptide) and then were treated with CBD (20 mg/kg ip) for three weeks. Animals injected with scrambled 25-35 peptide (SCR) served as negative controls. SCR or Aβ controls were injected vehicle ip for three weeks. Spatial navigation was assessed following the first week of treatment by means of the water maze, and the object recognition test was performed after 2 week treatment. SCR treated animals learned to find the hidden platform in the water tank over the five day training period, while Aβ-injected mice showed a significant impairment. CBD prevented Aβ-induced cognitive deficit. In summary, CBD prevented the cognitive impairment as a consequence of Aβ administration in vivo.

Hepatic encephalopathy. Hepatic encephalopathy is a neuropsychiatric complication occurring in both acute and chronic liver failure, and is a major clinical problem in treating patients with liver insufficiency. Brain levels of 2-AG were elevated almost three-fold 3 days after injection of the hepatotoxin thioacetamide (TAA) to mice, implying brain damage due to liver failure. Consistent with its role in neuroprotection, treatment with exogenous 2-AG improved behavioral indices such as cognitive, motor and neurological function. Interestingly, the effect of administration of 2-AG along with SR141716A was more pronounced than either administration of 2-AG or SR141716A alone, suggesting that combination of CB_1 blockade and CB_2 selective activation is the most effective treatment for HE. Surprisingly CBD causes the same positive effects.

Conditioned gaping. Rats received four conditioning trials in which they were injected with lithium chloride immediately before placement in a distinctive odor-laced context. This procedure led to conditioned gaping. When administered before testing, CBD (1 and 5, but not 10 mg/kg) suppressed conditioned gaping.

OVERVIEW: ADDICTIVE POTENTIAL OF CANNABINOIDS

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The reward circuitry of the brain consists of an "in series" circuit of dopaminergic (DA) neurons in the ventral tegmental area (VTA), nucleus accumbens (NAc), and that portion of the medial forebrain bundle that links the VTA and NAc. This reward circuitry also has important projections to the ventral pallidum (VP), amygdala, and the frontal and cingulate cortex. This same brain circuitry also appears to be crucial to the phenomenon or relapse to drug-seeking behavior in animals (and humans) long abstinent from drug-taking behaviors. Addictive drugs have in common that they activate this core DA reward system - producing the subjective "hit," "high," or "rush" that the drug addict seeks, and also trigger relapse to drug-seeking behavior. Addictive drugs also have in common a highly distinctive and characteristic pattern of effects on behaviors relating to the function of this core DA reward/relapse system. Thus, addictive drugs enhance electrical brain-stimulation reward within the core DA reward circuitry; enhance DA neural activity and DA neural tone within it; produce conditioned place preference (CPP), an animal model of incentive motivation; are self-administered; and trigger relapse to drug-seeking behavior in animals extinguished from drug-seeking and drug-taking behaviors. Cannabinoids were long supposed to be qualitatively different from other reward-enhancing drugs both in reward efficacy and in the underlying neurobiological substrates activated. That supposition is no longer supportable. It is now clear that cannabinoids activate these brain reward processes and reward- and relapse-related behaviors in similar fashion to other reward-enhancing drugs. This presentation will briefly review the evidence for this claim, and will conclude that while cannabinoids activate the brain's reward and relapse circuitries in a manner consistent with other reward-enhancing and relapse-producing drugs, the neural mechanisms by which this happens may distinguish the cannabinoids as a pharmacological class. In terms of potential therapeutics, the possible role for cannabinoids as antidepressant medications and for cannabinoid antagonists as anti-addiction, anti-craving, and anti-relapse medications for the treatment of addictive disease will be noted.

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TARGETING BRAIN NEURONAL CB2 CANNABINOID RECEPTORS IN DRUG ABUSE AND DEPRESSION

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Major depression and addiction are mental health problems associated with stressful events in life with high relapse and reoccurrence even after treatment. Marijuana remains one of the most widely used drugs, and much progress in cannabinoid research has revealed the existence of an elaborate endocannabinoid physiological control system (EPCS) in animals and humans (Onaivi et al, 2008), with increasing new knowledge on the scientific and therapeutic basis for the use of cannabinoids (CBs) as medicine. The expression of CB1 cannabinoid receptors (CB1-Rs) in the brain and periphery has been well studied but CB2-Rs have received much less attention than CB1-Rs. Both CB1-Rs and CB2-Rs are widely distributed in peripheral tissues with CB2-Rs particularly enriched in immune tissues (Suigiura and Waku, 2000). The functional neuronal expression of CB2-Rs in the brain had therefore been ambiguous and controversial. This is because CB2-Rs were previously thought to be expressed primarily in some peripheral tissues, like the spleen and other immune cells and has therefore been traditionally referred to as the peripheral CB-Rs (Gerard et al, 1990, Munro et al, 1993, Griffin et al, 1999). Nevertheless, many studies have now identified and characterized glial and neuronal CB2-Rs (Van Sickle et al., 2005, Gong et al 2006, Onaivi et al 2006, Benito et al, 2008) in the central nervous system. However, many features of CB2-R gene structure, regulation and variation remain poorly characterized compared to the CB1-R. This poor characterization of CB2-R gene structure and polymorphisms hampers progress in the determination of functional role of CB2-Rs particularly in neurodegenerative and neuropsychiatric disorders. In this study we tested the hypothesis that genetic variants of *CB2* gene might be associated with depression in a human population and that alteration in *CB2* gene expression may be involved in the effects of abused substances including opiates, cocaine and ethanol in rodents. We demonstrate that a high incidence of (Q63R) but not (H316Y) polymorphism in the *CB2* gene was found in Japanese depressed subjects. CB2-Rs and their gene transcripts are expressed in the brains of naïve mice and are modulated following exposure to stressors and administration of abused drugs. Mice that developed alcohol preference had reduced *CB2* gene expression and chronic treatment with JWH015 a putative CB2-R agonist, enhanced alcohol consumption in stressed but not in control mice. The direct intracerebroventricular microinjection of CB2 anti-sense oligonucleotide into the mouse brain reduced mouse aversions in the plus-maze test, indicating the functional presence of CB2-Rs in the brain that modifies behavior. Using electron microscopy we report on the sub cellular localization of CB2-Rs that are mainly on post-synaptic elements in rodent brain. Our data demonstrate the functional expression of CB2-Rs in brain that may provide novel targets for the effects of cannabinoids in depression and substance abuse disorders beyond neuro-immunocannabinoid activity.

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CANNABINOID DEPENDENCE: PREVALENCE, CHARACTERISTICS AND THE THERAPEUTIC POTENTIAL OF AGONIST MEDICATIONS IN TREATING CANNABIS USE DISORDERS

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Abstract: Cannabis is the most widely used illicit drug in the developed world and dependence rates in some regions have shown dramatic increases recently. Controlled laboratory studies have demonstrated that chronic exposure to cannabis can result in tolerance to select acute effects and that a valid withdrawal syndrome can occur following abrupt discontinuation. Clinical surveys also indicate that heavy cannabis use can be associated with a number of psychosocial problems typical of drug-use disorders (e.g. loss of controlled use, negative health consequences). The failure of behavioral treatments for cannabis use problems to achieve high rates of success suggests that adjunct pharmacotherapies may be needed for those seeking treatment for cannabis dependence. Of several candidate medications examined in laboratory experiments to date, oral administration of the CB1 agonist dronabinol (THC) appears to have the most therapeutic potential. The advantages and limitations of an agonist pharmacotherapy approach to treating cannabis dependence will be discussed. In addition, comments will be made regarding the risk for dependence developing in patients who chronically use cannabinoids in the treatment of other medical disorders.

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MANIPULATION OF FATTY ACID AMIDE HYDROLASE FUNCTIONAL ACTIVITY ALTERS SENSITIVITY AND DEPENDENCE TO ETHANOL

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The aim of this study was to examine the role of fatty acid amide hydrolase (FAAH) on the ethanol sensitivity, preference and dependence. The deletion of FAAH gene or the pharmacological inhibition of FAAH by URB597 (0.1 mg/kg) markedly increased the consumption and preference for ethanol when offered a choice between ethanol and tap water in a two-bottle choice paradigm. The study further revealed that URB597 specifically acts through FAAH and that cannabinoid-1 (CB_1) receptor is critical for N-arachidonoyl ethanolamide (AEA) mediated ethanol-reinforced behavior as revealed by lack of URB597 effect in both FAAH and CB_1 -/- mice compared to vehicle treated -/- mice. The FAAH -/- mice displayed a lower sensitivity to hypothermic and sedative effects to acute ethanol challenge. The FAAH -/- mice also exhibited a reduction in severity of handling-induced convulsions (HIC) following withdrawal from chronic ethanol exposure. The basal levels of CB_1 receptor and proenkephalin (PENK) gene expressions, and CB_1 receptor and ⊙-opioid (MO) receptor-mediated G-protein activation were found to be significantly lower in the caudate-putamen (CPu), nucleus accumbens core (AcbC) and shell (AcbS) of FAAH -/- than +/+ mice. Interestingly, the MO receptor-stimulated G-protein signaling was greater in the striatum of FAAH -/- than +/+ mice following voluntary ethanol consumption.

These findings suggest that an elevation in the AEA content and its action on the limbic CB_1 receptor and MO receptor might contribute to ethanol-reinforced behavior. Treatment with drugs that decrease AEA tone might prove useful in reducing excessive ethanol consumption.

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CANNABINOIDS AS POTENTIAL ANTICANCER AGENTS

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Δ^9-Tetrahydrocannabinol (THC) and other cannabinoids inhibit the growth of tumour cells both in vitro and in various xenograft models of cancer. Cannabinoid antitumour activity is usually dependent on the engagement of cannabinoid CB receptors and relies on the modulation of key cell signalling pathways. This reduces tumour cell growth by inducing apoptosis of tumour cells as well as by other mechanisms such as impairment of tumour angiogenesis, inhibition of tumour cell proliferation and invasion, and promotion of tumour cell differentiation. Remarkably, the antitumour effect of cannabinoids seems to be rather selective for tumour cells, supporting the notion that cannabinoid receptors regulate cell survival/death pathways differently in tumour and non-tumour cells. During the last years we have identified a new route that mediates cannabinoid-induced apoptosis of cancer cells via the endoplasmic reticulum stress response and have conducted a pilot clinical study in which patients with recurrent glioblastoma multiforme were administered THC intratumourally. Altogether, these studies, which have contributed to establish the cannabinoid and cancer issue as a very active research field, may set the basis for future clinical trials aimed at evaluating the potential antitumour activity of cannabinoids.

TOWARDS A CANNABINOID-BASED THERAPY FOR GLIOMAS

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Cannabinoid administration curbs the growth of several types of tumor xenografts in rats and mice including gliomas. This anti-tumoral action of cannabinoids relies on the ability of these agents to induce apoptosis of tumor cells as well as of inhibiting tumor angiogenesis. Recent findings have unravelled that cannabinoids activate an endoplasmic reticulum (ER) stress-evoked response [involving the up-regulation of the transcriptional co-activator p8 and its target the pseudo-kinase Tribbles homologue 3 (TRB3)] that mediates the cell death-promoting actions of these agents. Based on this preclinical evidence, a pilot clinical trial has been recently run to investigate the anti-tumoral activity of THC on recurrent gliomas.

In this talk, I will discuss some of the possible strategies that can be undertaken in order to enhance the efficacy of cannabinoid-based therapies for gliomas. These strategies may include: (i) the identification of the molecular mechanisms responsible for the resistance of glioma cells to cannabinoid treatment (development of selective therapies) and; (ii) the utilization of agents that enhance the antitumoral activity of cannabinoids (development of combinational therapies).

INHIBITION OF HUMAN GLIOMA CELL INVASIVENESS AND TUMOR ANGIOGENESIS BY CANNABIDIOL, A NON-PSYCHOACTIVE CANNABINOID COMPOUND

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Malignant gliomas are, among the common primary brain tumors, the most frequent cancer characterized by the biggest vascularization. Their high ability to invade the surrounding brain parenchyma is a leading cause of tumor recurrence and treatment failure. In this regard, we recently demonstrated that the non-psychoactive cannabinoid compound cannabidiol (CBD) can be effective, both *in vitro* and *in vivo*, in limiting human glioma cells growth (1) and impairing tumor cells migration, through a mechanism independent by the stimulation of CB1/CB2 cannabinoid and Gi/Go-coupled receptors (2). Since tumor cell motility represents a fundamental aspect in tumor invasion, in the present work we were interested in analyzing further the ability of CBD in inhibiting glioma cell migration and invasiveness. Among the various factors involved in the acquisition of increasing levels of malignancy, matrix metalloproteinases (MMPs) are a group of enzymes that play a pivotal role in promoting tissue breakdown and remodelling during angiogenesis and invasiveness through degradation of extracellular matrix components.

Therefore, since MMP-2 is one of the most important MMPs in the spreading of glioma, we investigated the influence of CBD on MMP-2 production and activity. We found that U87 glioma cells exposed *in vitro* for 24 h to different concentrations of CBD showed a significant inhibition of MMP-2 release in the supernatants of cell cultures, as evaluated by ELISA assay. Moreover, CBD was able to alter the MMP-2 gelatinolitic activity, as detected by gelatine zymography analysis. Using a scratch wound healing assay, we found also that the *in vitro* exposure to CBD for 16 and 24 h, induced a significant inhibition in the rate of glioma cells invasion into the artificial wounded areas. Finally, CBD inhibited the HUVEC endothelial cells growth and altered the formation of the capillary-like structures in matrigel assay.

In conclusion, CBD through multiple mechanisms inhibits glioma cells growth/invasiveness and endothelial cell growth, and could represent a potential compound in gliomas therapy.

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ANTITUMORAL POTENTIAL OF CANNABINOIDS IN BREAST CANCER: EFFECTS ON CELL PROLIFERATION

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Breast tumors are one of the most common human neoplasias and one of the leading causes of death among Western women. Although mortality rates have significantly decreased during recent years, mainly due to early diagnosis by mammograms, there is still an obvious need for alternative treatments because (i) the side effects associated to current therapies drastically diminish the patients' quality of life, (ii) some patients remain reluctant to conventional therapies and (iii) most recurrences are still lethal, with survival rates around 2 years. There is increasing evidence that cannabinoids possess antitumoral properties. Thus, a wide variety of cannabinoid compounds, including phytocannabinoids, endocannabinoids and synthetic cannabinoids, exert antiproliferative actions on a broad spectrum of tumour cells *in vitro*. It has been proposed that cannabinoids exert their antiproliferative effects on breast cancer cells (BCCs) by mechanisms that include, amongst others, the control of the progression through the cell cycle, the induction of apoptosis and/or the modulation of hormone and growth factor receptors. It is important to highlight that non-transformed mammary epithelial cells seem to be insensitive to cannabinoids. Some authors have also shown that, in certain circumstances, cannabinoids may stimulate BCC proliferation and even metastasis. The absence/presence of cannabinoid receptors seems to play a pivotal role in this particular proliferation induction/proliferation inhibition decision. Of interest, recent data points to a correlation between CB2 expression and breast cancer poor prognosis. In summary, data obtained during the last decade suggest that cannabinoids might be useful tools for the management of breast cancer.

BREAST CANCER: INVASION AND METASTASIS

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Many studies have described the potential anti-tumor properties of (endo)cannabinoids in breast cancer as well as in other tumor types. Recently it has been proposed that (endo)cannabinoids could also interfere with tumor invasiveness and metastatic processes. Metastasis is a multifactor process that includes the acquisition of a motile and invasive phenotype. We have shown that a metabolically stable anandamide analogue, 2-methyl-2'-F-anandamide (Met-F-AEA), arrests the growth of K-ras-dependent thyroid tumors, induced and/or already established in vivo and it inhibits the formation of metastatic nodules in the Lewis lung carcinoma model: these effects being largely mediated by cannabinoid CB1 receptors. Recently we described that Met-F-AEA treatment is able to inhibit the cell migration of the highly invasive and metastatic breast cancer cell line, MDA MB 231, as tested by in vitro migration assays with type IV collagen, the major component of the basement membrane. This effect was antagonized by pre-treatment with the CB1 antagonist.
The hypothesis that CB1 receptor stimulation could interfere with metastatic processes was also verified in a model of metastatic infiltration *in vivo*. To this end the murine breast cancer TSA-E1 cells were injected in syngenic C57BL/6N mice to induce lung metastases. Animals were divided into three groups and anandamide plus vehicle (0.5 mg/kg/ dose), anandamide plus rimonabant (0.7 mg/kg/dose), or the vehicle alone, were administered i.p. every 72 h. Anandamide significantly reduced the number and dimension of metastatic nodes, evaluated 21 days after, this effect being reduced by CB1 antagonist. Integrins, focal adhesion kinase (FAK), and CD44 have been associated with the development of a more invasive and metastatic phenotype. We hypothesized that CB1 receptor stimulation might induce a less invasive phenotype in metastatic breast cancer cells through the interference with one of these targets. FAK and Src, both located at adhesion plaques, are involved in cell motility, adhesion and invasion as well as in cell proliferation and survival. We found that total FAK and Src levels were not modified by anandamide treatment, whereas a remarkable decrease of FAK and Src phosphorylation was induced, in a way antagonized by rimonabant. Because in the cell migration regulation this pathway interconnected with Rho-Rho kinase pathway, we supposed that CB1 receptor agonist could affect the RhoA-GTPase activity and the downstream signalling and that this alteration might decrease their metastatic potential *in vitro*. Indeed the Rho family of small GTPases (RhoA, Rac1, Cdc42) may play an essential role in the dissemination of carcinomas, transducing extracellular signals into intracellular events that lead to the remodeling of the actin cytoskeleton, ultimately controlling the cell morphology and a variety of functions such as cell motility, aggregation, polarity, and contraction. Anandamide (10 μM, 1 h) inhibited RhoA activity and blocked its translocation from cytosol to cell membrane, an event needed for RhoA activation, consequently affecting actin organization. The effect is recovered by CB1 antagonist or by selective CB1 knocking down. Overexpression of a dominant negative of RhoA activity and treatment of these cells with a Rho-associated protein kinase (ROCK) inhibitor, Y 27632, mimicked Met-F-AEA effects on actin organization and cell migration. We suggest that the inhibitory effect of Met-F AEA on tumor cell migration could be related to RhoA-ROCK dependent signaling pathway.

Our data demonstrate that crucial events of the metastatic process are targeted by CB1 receptor activation, leading to inhibition of metastases formation in mice models. We suggest that CB1 receptor might be a target for therapeutic strategies aimed at retarding the breast carcinomas growth and progression.

PHARMACOLOGICAL ACTIONS OF CANNABINOID LIGANDS IN THE EYE.

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Cannabinoids reduce intraocular pressure (IOP) and may have efficacy in the treatment of glaucoma, a leading cause of irreversible blindness that is associated with the development of ocular hypertension and damage to the optic nerve. However, most of the cannabinoids tested, are known to result in undesirable neurobehavioral effects, particularly at doses that provide effective reductions in IOP. Abnormal cannabidiol (Abn-CBD), and Cannabigerol-dimethyl heptyl (CBG-DMH), are behaviourally inactive cannabinoids with hypotensive and vasorelaxative properties (Begg et al., 2005; Maor, et al., 2005). Both these compounds are thought to exert their actions, at least in part, via a pharmacological target that is unique from CB_1. The objective of our study was to compare the ocular pharmacodynamic effects of Abn-CBD and CBG-DMH to the synthetic cannabinoid, Methanadamide (MA) and the aminoalkylindole cannabinoid ligand, WIN55212-2, on IOP in Brown Norway rats.

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

Abn-CBD and CBG-DMH administration (2.5-10.0 mg/kg) resulted in a dose-dependent reduction of IOP in rat eyes ($p<0.001$). The IOP-lowering effect of Abn-CBD and CBG-DMH was significantly reduced by IP pre-administration of 2.5 mg/kg of O-1918 ($p<0.001$), a selective antagonist of non-CB_1/CB_2 novel endothelial cannabinoid receptor, and SR141716A, a CB_1 specific receptor antagonist ($p< 0.05$), but not by the CB1 antagonist, AM251 (2.5 mg/kg), or the CB_2 antagonist, AM630 (2.5 mg/kg). The observed reduction in IOP was comparable to that obtained following IP administration of 1.7 and 2.5 mg/kg of MA and 1.7 mg/kg of the WIN55212-2. However, the IOP lowering actions of MA and WIN were unaffected by O-1918 and blocked by AM251 ($p<0.01$), which did not antagonize the IOP lowering actions of Abn-CBD or CBG-DMH.

This study demonstrates that the behaviourally inactive cannabinoids, Abn-CBD and CBG-DMH, decrease IOP in the normotensive rat eye. The efficacy of Abn-CBD and CBG-DMH in lowering IOP was similar to that of MA and WIN55212-2, although the observed disparity in O-1918 antagonism implies that the pharmacodynamics of Abn-CBD and CBG-DMH may involve non-CB_1 targets. All cannabinoids tested lowered IOP independent of actions at CB_2 receptors. Both Abn-CBD and CBG-DMH have the potential to function as novel ocular hypotensive cannabinoids devoid of psychoactive side-effects.

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CANNABINOIDS AND THE GUT: POTENTIAL THERAPEUTIC APPLICATIONS

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A multitude of physiological effects and putative pathophysiological roles have been proposed for the endogenous cannabinoid system in the gastrointestinal tract. *In vivo* pharmacological studies in rodents have shown that CB_1 receptor agonists inhibit emesis, gastric acid secretion, experimental-induced gastric ulcers, relaxation of the lower oesophageal sphincter, gastric emptying, intestinal motility and secretion, exert antiproliferative actions, attenuate visceral pain and reduce inflammation. CB_2 receptor activation may affect visceral pain and inflammation. Cannabinoid receptors may also be indirectly activated by inhibitors of endocannabinoid inactivation, which increase levels of intestinal endocannabinoids and result in anti-inflammatory, anti-diarrhoeal and anticancer effects. These diverse effects lead to potential use of cannabinoid agents in a large variety of functional, inflammatory, or neoplastic diseases in the digestive tract.

THE SKELETAL ENDOCANNABINOID SYSTEM

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In mammals, including humans, bone metabolism is manifested as an ongoing modeling/remodeling process whereby the bone mineralized matrix is being continuously renewed. Recently, the main components of the endocannabinoid system were reported in the skeleton. Osteoblasts, the bone forming cells, and other cells of the osteoblastic lineage, as well as osteoclasts, the bone resorbing cells, and their precursors, synthesize the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG). These cells also express diacylglycerol lipases (DAGLs), enzymes critically involved in 2-AG biosynthesis. DAGL expression increases progressively during osteoblast differentiation. CB1 cannabinoid receptors are present in sympathetic nerve terminals in close proximity to osteoblasts. Activation of these CB1 receptors by elevated bone 2-AG levels communicates brain-to-bone signals as exemplified by traumatic brain injury-induced stimulation of bone formation. In this process, the retrograde CB1 signalling inhibits norepinephrine release and alleviates the tonic sympathetic restrain of bone formation. CB2 receptors are expressed by osteoblasts and osteoclasts. Their activation stimulates bone formation and suppresses bone resorption. CB2-deficient mice display a markedly accelerated age-related bone loss. Ovariectomy-induced bone loss can be both prevented and rescued by a CB2 specific agonist. When administered chronically, a mixture of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) stimulates fracture healing in normal rats. CB2 signalling in osteoblasts involves activation of the MAP kinases Erk1/2, but not p38. The Erk1/2 activation is prolonged, suggesting the absence of tolerance development. The stimulation of Erk1/2 phosphorylation is shared by CB2 specific and cannabinoid receptor nonspecific agonists. Hence, synthetic CB2 ligands, which are stable and orally available, provide a basis for developing novel therapies, free of psychotropic effects, to combat osteoporosis and enhance the repair of fractures and other skeletal deficiencies.

THE ENDOCANNABINOID SYSTEM IS PRESENT IN THE UNICELLULAR ORGANISM TETRAHYMENA. IDENTIFICATION OF N-ACYLETHANOLAMINES.

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The endocannabinoid system is a lipid signaling system and is characterized in a number of mammalian cells. In previous studies, we reported that major components of the endocannabinoid system such as Fatty acid amide hydrolase (FAAH) and N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD), are present in the unicellular eukaryote *Tetrahymena pyriformis*, with characteristics similar to the mammalian ones. The ciliate *Tetrahymena* is a model organism for molecular and cellular biology studies and its genome sequence is available.

In the present study we report the identification and quantification of N-acylethanolamines (NAEs) and acylglycerols (AcGs) of *Tetrahymena pyriformis* and *Tetrahymena thermophila* by LC-atmospheric pressure chemical ionization-MS. N-gamma-linolenoyl, N-eicosenoyl, N-linoleoyl, N-palmitoyl, N-steroyl and N-oleoyl ethanolamines as well as the corresponding monoacylglycerols were identified. Anandamide and 2-AG seem to be present in much lower concentration that could not be quantified. NAEs were found for the first time in *Tetrahymena.*

NAEs, such as N-arachidonoyl (anandamide), N-linoleoyl, N-alpha-linolenoyl and N-homogammalinoleoyl ethanolamine were hydrolyzed by *Tetrahymena* cell homogenate to ethanolamine and the respective fatty acid, apparently by the action of FAAH; the metabolism was almost abolished by the specific FAAH inhibitor AM374.

AcGs, such as 2-AG and 2-OG were also hydrolyzed to glycerol and the respective fatty acid by the action of FAAH and a MAGL-like enzyme since in the presence of the specific FAAH inhibitor AM374 or URB597 hydrolysis was inhibited by ~60%. MAGL activity was further characterized in the presence of URB597.

The presence of major components of the endocannabinoid system in this protist suggests the importance of the endocannabinoid system throughout the evolution and that the ciliate *Tetrahymena* can be used as a model organism for the study of drugs that target the enzymes involved on the endocannabinoid metabolism such as FAAH and MAGL.

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CHARACTERISATION OF THE CANNABINOID SYSTEM PRIOR TO SYMPTOM ONSET IN A TRANSGENIC MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurodegenerative disease, usually with mid-life onset. The disease is characterised by cognitive dysfunction, motor deficits and psychiatric symptoms, progressive deterioration and ultimate death. Neuropathological features include cell dysfunction and death in the basal ganglia and cortex. Since there are no current effective pharmacological treatments available it is vital to further our understanding of the disease processes, in particular early dysfunctions that could be targeted therapeutically.

CB1 receptor binding is lost from key brain regions early in the disease in human post mortem tissue (Glass et al., 2000). In mice transgenic for Huntington's disease (R6 lines) there are major discrepancies in cannabinoid data from different studies. We reported previously that there is 78 ± 5% CB1 receptor binding in the striatum of R6/1 mice compared to wildtype (WT) at 20 weeks (Glass et al., 2004). In contrast McCaw and colleagues evaluated CB1 mRNA and found this to be at undetectable levels at 10 weeks of age in these mice (McCaw et al., 2004). These differences may reflect differences between colonies influenced by factors such as living conditions, since environmental enrichment has been shown to alter CB1 receptor expression (Glass et al, 2004).

% WT	Striatum	Globus Pallidus	Substantia Nigra
D1	76 ± 2. *	-	92 ± 1 *
D2	68 ± 3 *	-	-
$GABA_A$	107 ± 3	108 ± 4	100 ± 5

Comparison of CB1 receptor protein, binding, mRNA, and levels of endocannabinoids have not been previously described in presymptomatic age R6/1 transgenic mice. We demonstrate that R6/1 mice have significantly decreased CB1 mRNA in the striatum to levels of 73% of WT littermates at 12 weeks of age. Total protein levels, determined by immunohistochemistry, are however not significantly different to WT in the striatum or globus pallidus. Protein levels in the substantia nigra are significantly decreased to 81% of WT. CB1 receptor binding demonstrates significant decreases in all basal ganglia regions evaluated, but this is still at levels equal to 80-90% of WT. The endocannabinoid 2-arachidonoyl glycerol is found to be at significantly increased levels in the cortex (147%) and anandamide significantly decreased in the hippocampus (67%), compared to WT. Binding to colocalised dopamine D1 and D2 receptors also demonstrate decreases (see Table) while there was no change in $GABA_A$ receptor levels.

A full characterisation of the cannabinoid system and their colocalised receptors in R6/1 mice at 12 weeks of age is valuable for understanding disease pathogenesis prior to onset of behavioural symptoms; these results suggest that 12 weeks would be a suitable timepoint to consider therapeutic intervention, in this mouse colony.

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ANTIDEPRESSANT-LIKE BEHAVIORAL EFFECTS OF CB2 CANNABINOID LIGANDS IN THE MOUSE FORCED SWIM TEST

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The forced swim test (FST) is a reliable model with predictive validity for screening anti-depressants. There are reports that marijuana use modulates mood and possesses anti-depressant properties. Furthermore, marijuana continues to be one of the most widely used drugs and much progress in cannabinoid research has revealed an elaborate endocannabinoid physiological control system (EPCS) in animals and humans, with increasing new knowledge on the scientific and therapeutic basis for the use of marijuana as medicine. The EPCS consists of genes encoding cannabinoid (CB1-Rs and CB2-Rs), their endogenous ligands, endocannabinoids (eCBs) and proteins that synthesize and degrade the eCBs. Both CB1-Rs and CB2-Rs are distributed in the brain and peripheral tissues, but brain CB2-Rs have received much less attention than CB1-Rs. Results from our previous collaborative studies indicate that a genetic variant of *CB2* gene is involved in anorexia, substance abuse and depression in a human population. In this on going studies we are testing the hypothesis that CB2-Rs are involved in the anti-depressant-like effects of cannabinoids in the mouse forced swim test. On day one, all animals were pre-exposed for 15 min- swim test prior to the 5 min- swim test on day two. We treated acutely, male mouse strains in different groups with CB2-R agonist BML-190 or the antagonist AM630 using 1-10 mg/kg dose, and measured the duration and number of immobility during the 5 min- test session. The combined administration of AM630 (5 mg/kg), 20 mins- before the administration of the agonist BML-190 (5 mg/kg) to the strains of mice were evaluated to determine the specificity and involvement of CB2-Rs in the FST. We report that our preliminary results indicate that the effects of CB2-R compounds in the FST were dose and strain dependent. There was reduced immobility with the CB2-R antagonist AM630 at a low dose (1.0 mg/kg) especially with the DBA/2 mice when compared to the responses of C57Bl/6 and BALBc mice. The effect of CB2-R ligands in the forced swim test provides further behavioral evidence for the involvement of CB2-Rs in depression. The neurobiological basis for CB2-R activity in depression and interaction with or without CB1-Rs in emotionality remains to be determined.

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DIRECT AND INDIRECT MODULATION OF CANNABINOID SYSTEM IN PSYCHOTIC-LIKE SYMPTOMS INDUCED BY PCP

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Emerging evidences point to a possible antipsychotic-like effect of anandamide, as anandamide levels in cortico-spinal fluid of antipsychotic naive schizophrenic patients are markedly elevated and inversely correlated with psychopathological symptoms. On these basis, it is of great interest to investigate the effect of pharmacological modulation of cannabinoid system in phencyclidine (PCP) model of schizophrenia. To this purpose, we used an acute PCP injection (3.5mg/kg i.p.) in order to reproduce positive schizophrenic-like symptoms and we tested the hypothesis that cannabinoid compounds would modulate the hyperlocomotion, stereotypies and ataxia induced PCP.

Male Lister Hooded rats received an acute administration of ⌀^{9}-THC (0.5mg/kg i.p.) or the anandamide uptake inhibitor AM404 (3mg/kg i.p.) respectively 30 min and 60 min prior PCP. Both these compounds were able to counteract the PCP-induced psychotic-like symptoms, inhibiting the increase of the locomotor activity and reducing stereotyped behaviours and ataxia.

To better understand the putative involvement of CB1 receptor in the protective effects of AM404 we administered the CB1 receptor antagonist AM251 (0.5mg/kg, i.p.) 30 min prior PCP in association with AM404. AM251 reversed the protective effect of AM404 on locomotion. These results suggested a partial involvement of CB1 receptor in the restorative effect of AM404 providing evidence that other systems may take part in this complex mechanism.

Since anandamide may also act on vanilloid receptor TRPV1, we assessed the effect of TRPV1 receptor antagonist, capsazepine (10mg/kg i.p.), 30 min prior PCP on the positive effect of AM404. Capsazepine reversed AM404 protective effects only on stereotipyes and ataxia.

This study demonstrated a dual role of CB1 and TRPV1 receptors in modulating positive effect of cannabinoids suggesting that CB1 receptor stimulation might be related with the regulation of locomotor activity, on the contrary TRPV1 receptor might be involved in the control of stereotyped behaviour and ataxia.

Our results put forward that pharmacological modulation of the cannabinoid and vanilloid systems can represent a new perspective in the treatment of psychotic schizophrenia symptoms.

CRUCIAL ROLE OF THE CB2 CANNABINOID RECEPTOR IN THE REGULATION OF CENTRAL IMMUNE RESPONSES DURING NEUROPATHIC PAIN

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Neuropathic pain is a clinical manifestation of nerve injury difficult to treat even with potent analgesic compounds. Here, we identify CB2 cannabinoid receptors as a new potential therapeutic target for neuropathic pain. CB2 knockout mice and wild-type littermates were exposed to sciatic nerve injury, and both genotypes developed a similar hyperalgesia and allodynia in the ipsilateral paw. Most strikingly, knockouts also developed a contralateral mirror-image pain, associated with an enhanced microglial expression in the contralateral spinal horn. In agreement, hyperalgesia, allodynia and microglial activation induced by sciatic nerve injury were attenuated in transgenic mice overexpressing CB2 receptors. Microarray analysis revealed a selectively increased interferon-response and prostaglandin synthesis in CB2 knockouts exposed to neuropathic pain. The enhanced manifestations of neuropathic pain were replicated in irradiated wild-type mice receiving bone marrow transplantion from knockouts, thus demonstrating the crucial role of CB2 receptors in the modulation of central immune responses during neuropathic pain.

SHORT- AND LONG-TERM EFFECTS OF CANNABINOIDS ON THE EXTINCTION OF CONTEXTUAL FEAR MEMORY IN RATS

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Modulation of fear memory extinction by the endocannabinoid (eCB) system has been recently in focus. While there is compelling evidence that endocannabinoids promote the extinction of cued-fear memories, less is known of their role in contextual fear memories extinction. Previous results from our group (Psychopharmacology (2006) 188: 641-9) pointed to a facilitation of contextual fear memory extinction by a low dose of a cannabinoid agonist, with unclear suggestion of within-session effects. Hence, the aim of the present study was to investigate the effects of cannabinoid drugs in the short- and long-term extinction of conditioned fear using an extended extinction protocol. Male Wistar rats were placed in a conditioning chamber and after 3 min received a footshock (1.5mA, 1s). The next day, they were injected i.p. with a cannabinoid agonist (WIN55212-2, 0.25 mg/kg), an inhibitor of eCB uptake/metabolism (AM404, 10 mg/kg), an antagonist of CB_1 cannabinoid receptors (SR141716A, 1 mg/kg) or control (10% DMSO + 0.1% Tween80) and 20 min later re-exposed to the conditioning chamber for 30 min (extinction training). No-Extinction groups received the same drug treatment, but were exposed for 3 min to the conditioning chamber. A drug-free test of contextual memory (3 min) was performed 7 days later. Time (s) of freezing behavior was used as fear memory index during extinction training and drug-free test. The cannabinoid agonist WIN55212-2 and the inhibitor of eCB uptake/metabolism AM404 facilitated short-term extinction, whereas the CB_1 antagonist SR141716A had a minimal effect during extinction training. WIN55212-2 or AM404 groups, and the SR141716 group showed less and more freezing, respectively, than the control group in the drug-free test ($p<0.05$). The long-term consequences of pharmacological treatments were in line with the effects observed during extinction training ($r=0.66$, $p<0.0001$, Pearson's correlation) and were dependent on the extinction protocol, since no alteration in freezing time was observed in No-Extinction controls. Our present findings extend previous evidence that the eCB system modulates contextual fear memories extinction and shows that short-term pharmacological effects induce long-term consequences, suggesting alterations in mnemonic processes underlying defensive freezing behavior.