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BASIC SCIENCE

Addiction

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Endocrinology

Presynaptic actions of endocannabinoids mediate (alpha)-MSH-induced inhibition of oxytocin cells


We recently showed that central injections of alpha-melanocyte-stimulating hormone (alpha-MSH) inhibits oxytocin cells and reduces peripheral release of oxytocin, but induces oxytocin release from dendrites. Dendritic oxytocin release can be triggered by agents that mobilize intracellular calcium. Oxytocin, like alpha-MSH, mobilises intracellular calcium stores in oxytocin cells, and triggers presynaptic inhibition of afferent inputs that is mediated by cannabinoids, and we hypothesized that this mechanism might underlie the inhibitory effects of alpha-MSH. To test this, we recorded extracellularly from identified oxytocin and vasopressin...
cells in the anesthetised rat SON. Retrodialysis of a CB1 receptor antagonist to the SON blocked the inhibitory effects of i.c.v. injections of alpha-MSH on the spontaneous activity of oxytocin cells. We then monitored synaptically-mediated responses of SON cells to stimulation of the organum vasculosum of the lamina terminalis (OVLT); this evoked a mixed response comprising an inhibitory component mediated by GABA and an excitatory component mediated by glutamate, as identified by the effects of bicuculline and CNQX applied to the SON by retrodialysis. Application of CB1 receptor agonists to the SON attenuated the excitatory effects of OVLT stimulation in both oxytocin and vasopressin cells; while alpha-MSH attenuated the responses of oxytocin cells only. Thus alpha-MSH can act as a 'switch', it triggers oxytocin release centrally but at the same time, through initiating endocannabinoid production in oxytocin cells, inhibits their electrical activity and hence peripheral secretion.

Gastrointestinal

Genetics

Infectious Diseases

Marijuana for cholera therapy

Cannabinoid antagonist AM 281 reduces mortality rate and neurologic dysfunction after cecal ligation and puncture in rats

OBJECTIVES:: The purpose of this study was to examine whether anandamide, an endogenous cannabinoid receptor ligand, is involved in the pathogenesis of septic encephalopathy. DESIGN:: Prospective, controlled study. SUBJECTS:: Male Wistar rats (7 wks old) were randomly divided into four groups as follows: group 1, control (0.5 mL of saline injected subcutaneously); group 2, sham (surgical abdominal incision and suturing were performed, but ligation and puncture of the cecum were omitted); group 3, cecal ligation and puncture (CLP); group 4, CLP + AM 281 ([N-morpholin-4-yl]-5-[2,4-yl]-5-[2,4-dichlorophenyl]-4-methyl-1H-pyrazole-3-carboxamide) as the cannabinoid receptor antagonist (1 mg/kg intraperitoneally). INTERVENTIONS:: Sepsis was induced by CLP under pentobarbital anesthesia (10 mg/kg intraperitoneally) with 1% isoflurane. A 2-Fr high-fidelity micromanometer catheter was inserted into the left ventricle via the right carotid artery to assess hemodynamics. Each of the rats was neurologically assessed at 30 mins and 12, 24, and 48 hrs after the treatment. The cytoplasmic levels of caspase-3 in the hippocampi were assayed before surgery and at 30 mins and 24 and 48 hrs after surgery using Western blotting techniques. To examine the effects of AM 281 on neurologic function and mortality rate, we set another control group treated solely with AM 281. Selective inducible nitric oxide synthase inhibitor, L-N6-(1-iminoethyl)-lysine (4 mg/kg), was injected intraperitoneally immediately after CLP to produce the CLP + L-N6-(1-iminoethyl)-lysine group to exclude the influence of depressed hemodynamics on neurologic impairment. MEASUREMENTS AND MAIN RESULTS:: It was found that administration of AM 281 could prevent the hemodynamic changes induced by sepsis. Reflex responses, including the pinna, corneal, paw or tail flexion, and righting reflexes, and the escape response significantly decreased in the CLP and CLP + L-N6-(1-iminoethyl)-lysine groups at 48 hrs after the surgery. In contrast, no changes in these reflex responses were found between the CLP + AM 281 and control and sham groups. Administration of AM 281 on neurologic function and mortality rate in the control group were found. Tissue caspase-3 levels were elevated at 48 hrs after CLP in the CLP alone group (means +/- sd: control, 3.9 +/- 0.4; sham, 4.2 +/- 0.4; CLP, 7.1 +/- 1.0 [p < .01]; CLP + AM 281, 4.0 +/- 0.5 densitometric units). In addition, administration of AM 281 also decreased the mortality rate (p < .05). CONCLUSIONS:: Administration of AM 281 prevented the hemodynamic changes and development of neurologic dysfunction occurring in association with septic shock, and could decrease the mortality rate in experimentally induced septic shock in rats. Although further studies are necessary to determine
whether endogenous cannabinoids cause septic encephalopathy in rats directly or via their effects on systemic hemodynamics, the beneficial effects of AM 281 on these rats might have significant therapeutic implications in cases of septic encephalopathy.

**Immunology**

2-Arachidonoylglycerol, an endogenous cannabinoid receptor ligand, enhances the adhesion of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes


2-Arachidonoylglycerol (2-AG), an endogenous cannabinoid receptor (CB1 and CB2) ligand, enhanced the adhesion of HL-60 cells differentiated into macrophage-like cells to fibronectin and the vascular cell adhesion molecule-1. The CB2 receptor, Gi/Go, intracellular free Ca(2+) and phosphatidylinositol 3-kinase were shown to be involved in 2-AG-induced augmented cell adhesion. 2-AG also enhanced the adhesion of human monocytic leukemia U937 cells and peripheral blood monocytes. These results strongly suggest that 2-AG plays some essential role in inflammatory reactions and immune responses by inducing robust adhesion to extracellular matrix proteins and adhesion molecules in several types of inflammatory cells and immune-competent cells.

A novel cannabinoid CB2 receptor-selective inverse agonist blocks leukocyte recruitment in vivo.


The expression of the cannabinoid CB2 receptor on peripheral immune cells suggests that compounds specific for CB2 might be effective anti-inflammatory agents. In this report we present the initial biological profiling of a novel triaryl bis-sulfone, Sch.336 which is selective for the human cannabinoid CB2 receptor (hCB2). Sch.336 is an inverse agonist at hCB2, as shown by its ability to decrease GTPgammaS binding to membranes containing hCB2, by the ability of GTPgammaS to left-shift Sch.336 binding to hCB2 in these membranes, and by the compound's ability to increase forskolin-stimulated cAMP levels in CHO cells expressing hCB2. In these systems, Sch.336 displays a greater potency than that reported for the CB2-selective dihydropyrazole SR144528. In vitro, Sch.336 impairs the migration of CB2-expressing recombinant cell lines to the cannabinoid agonist 2-arachidonoylglycerol. In vivo, the compound impairs migration of cells to cannabinoid agonist HU-210. Oral administration of the Sch.336 significantly inhibited leukocyte trafficking in several rodent in vivo models, induced either by specific chemokines or by antigen challenge. Finally, oral administration of Sch.336 blocked ovalbumin-induced lung eosinophilia in mice, a disease model for allergic asthma. We conclude that selective cannabinoid CB2 inverse agonists may serve as novel immunomodulatory agents in the treatment of a broad range of acute and chronic inflammatory disorders in which leukocyte recruitment is a hallmark of disease pathology.

**Molecular biology**

Plasma membrane and lysosomal localization of CB1 cannabinoid receptor are dependent on lipid rafts and regulated by anandamide in human breast cancer cells


In this report we show, by confocal analysis of indirect immunofluorescence, that the type-1 cannabinoid receptor (CB1R), which belongs to the family of G-protein-coupled receptors, is expressed on the plasma membrane in human breast cancer MDA-MB-231 cells. However, a substantial proportion of the receptor is present in lysosomes. We found that CB1R is associated with cholesterol- and sphingolipid-enriched membrane domains (rafts). Cholesterol depletion by methyl-beta-cyclohextrin (MCD) treatment strongly reduces the flotation of the protein on the raft-fractions (DRM) of sucrose density gradients suggesting that CB1 raft-association is cholesterol dependent. Interestingly binding of the agonist, anandamide (AEA) also impairs DRM-association of the receptor suggesting that the membrane distribution of the receptor is dependent on rafts and is possibly regulated by the agonist binding. Indeed MCD completely blocked the clustering of CB1R at the plasma membrane. On the contrary the lysosomal localization of CB1R was impaired by this treatment only after AEA binding.
**Neuroscience**

*Endocannabinoid identification in the brain: studies of breakdown lead to breakthrough, and there may be NO hope*

Endocannabinoids are a class of fatty acid derivatives defined by their ability to interact with the specific cannabinoid receptors that were originally identified as the targets of Delta9-tetrahydrocannabinol (Delta9-THC), the psychoactive component of cannabis. Endocannabinoids have been implicated in a growing number of important physiological and behavioral events. A full understanding of the functions of endocannabinoids will involve knowing which ones are active, and how they are produced, during any given physical event. However, studying these small lipids in the brain presents many technical challenges. New selective pharmacological tools promise to be very useful in unraveling the complexities of endocannabinoid signaling, but parallel developments from the investigation of the cellular neurophysiology of the endocannabinoid systems highlight the difficulties remaining.

*AM 251 produces sustained reductions in food intake and body weight that are resistant to tolerance and conditioned taste aversion*

The cannabinoid 1 (CB(1)) receptor has been implicated in the regulation of food intake. Here, we examine the effect of the CB(1) receptor antagonist AM 251 on food intake and body weight over a prolonged period. Further, we examine whether AM 251 produces conditioned taste aversion (CTA) and if sustained antagonism at central receptors contributes to its anorectic effect. The effect of AM 251 of food intake and body weight was examined in daily (1 mg kg(-1)) and 5-day (5 mg kg(-1)) dosing schedules. Matching reductions in food intake and body weight were observed in both paradigms. A single administration of AM 251 (5 mg kg(-1)) significantly reduced food intake for 4 days. Tolerance to the anorectic effects of AM 251 did not develop in either dosing strategy. Active avoidance of AM 251 (3; 5 mg kg(-1), i.p.) was examined using a CTA assay. Rats showed no evidence of CTA associated with AM 251. We investigated the sustained effect of AM 251 (5 mg kg(-1), i.p.) on CB(1) receptors in the hypothalamus using Delta(9)-tetrahydrocannabinol (6 mg kg(-1), i.p.) induced hypothermia. AM 251 initially blocked hypothermia, but this effect was not seen 2 or 4 days later. The results demonstrate that smaller, or infrequent, administrations of AM 251 can produce sustained reductions in food intake and body weight in rat. Reductions in food intake were sustained longer than AM 251 antagonized the effects of a CB(1) receptor agonist in the hypothalamus, and occurred independently of CTA. *British Journal of Pharmacology* advance online publication, 31 October 2005; doi:10.1038/sj.bjp.0706439.

*Activation of CB(1) and CB(2) receptors attenuates the induction and maintenance of inflammatory pain in the rat*

The aim of the present study was to investigate the effects of cannabinoid agonists on established inflammatory hyperalgesia. We have compared the effects of pre-administration versus post-administration of a potent non-selective cannabinoid agonist HU210 and a selective CB(2) receptor agonist JWH-133 on hindpaw weight bearing and paw oedema in the carrageenan model of inflammatory hyperalgesia. For comparative purposes we also determined the effects of the mu-opioid receptor agonist morphine and the COX2 inhibitor rofecoxib in this model. At 3h following intraplantar injection of carrageenan (2%, 100μl) there was a significant (P<0.001) reduction in weight bearing on the ipsilateral hindpaw, compared to vehicle treated rats and a concomitant increase in ipsilateral hindpaw volume (P<0.001), compared to vehicle treated rats. Systemic administration of HU210 (10μg/kg) and JWH-133 (10μg/kg) at 3h following injection of carrageenan, significantly attenuated decreases in ipsilateral hindpaw weight bearing (P<0.05 for both) and paw volume (P<0.001 for both). Pre-administration of HU210 and JWH-133 had similar effects on weight bearing in this model. Pre-administered HU210 also significantly decreased carrageenan-induced changes in paw volume (P<0.001), this was not the case for JWH-133. Effects of post-administered HU210 and JWH-133 on ipsilateral hindpaw weight
bearing and paw volume were comparable to the effect of systemic post-administration of morphine and rofecoxib (3mg/kg for both). In summary, both HU210 and JWH-133 attenuated established inflammatory hypersensitivity and swelling, suggesting that cannabinoid-based drugs have clinical potential for the treatment of established inflammatory pain responses.

Ex-vivo imaging of fatty acid amide hydrolase (FAAH) activity and its inhibition in the mouse brain

There is recent behavioral evidence that FAAH inhibitors produce a sub-set of cannabinoid receptor agonist effects, suggesting both anandamide-specific behavioral functions, and possible regional differences in FAAH inhibitory effects. Here we introduce a novel imaging method to quantify regional differences in brain FAAH activity. Upon intravenous [(3)H]anandamide administration, brain FAAH activity generates [(3)H]arachidonic acid, which is promptly trapped in membrane phospholipids. As a result, WT brains accumulate tritium in a regionally specific manner that is dependent upon regional FAAH activity, while brains from FAAH KO mice show a uniform [(3)H]anandamide distribution. Increasing doses of anandamide+[(3)H]anandamide fail to alter regional tritium accumulation, suggesting insensitivity towards this process by anandamide-induced changes in regional cerebral blood flow. Regional tritiated metabolite levels in WT brains were highest in the somatosensory and visual cortex, and thalamus. Treatment with methylarachidonyl fluorophosphonate (MAFP) (1 mg/kg, i.p.), reduced regional tritium accumulation in the somatosensory and visual cortex (p<0.01), and at higher doses, the thalamus (p<0.05). The selective FAAH inhibitor CAY10435, while having similar efficacy as MAFP in reducing tritium in the thalamus, somatosensory and visual cortex, also reduces caudate putamen and cerebellum (p<0.01) activity. These data indicate FAAH activity generates heterogeneous regional accumulation of [(3)H]anandamide and metabolites, and suggest the modulation of endocannabinoid tone by FAAH inhibitors depends upon not only the dose and compound used, but also the degree of FAAH expression in the brain regions examined. This imaging method determines regionally-specific FAAH inhibition and can elucidate the in vivo effects of pharmacological agents targeting anandamide inactivation.

Species and strain differences in the expression of a novel glutamate-modulating cannabinoid receptor in the rodent hippocampus

A novel, non-CB1 cannabinoid receptor has been defined by the persistence of inhibition of glutamatergic EPSPs by the cannabinoid receptor agonist WIN55,212-2 in mice lacking the cloned CB1 receptor (CB1(-/-)) (Hajos et al., 2001). This novel receptor was also distinguished from CB1 by its sensitivity to the antagonist SR141716A and its insensitivity to the antagonist AM251 (Hajos & Freund, 2002). We have chosen to refer to this putative receptor as CBsc due to its identification on Schaffer collateral axon terminals in the hippocampus. We examined properties of CBsc receptors in Sprague Dawley (SD) rats and two strains of wild-type (WT) mice (C57BL/6J and CD1) used as backgrounds for two independent lines of CB1(-/-) mice (Ledent et al., 1999; Zimmer et al., 1999). The inhibition of synaptic glutamate release by WIN55,212-2 was observed in hippocampal slices from WT CD1 mice and SD rats but was absent in WT C57 mice. We also found that AM251 and SR141716A antagonized the effect of WIN55,212-2 in hippocampal slices from CD1 mice and SD rats demonstrating a lack of selectivity of these ligands for CB1 and CBsc receptors in these animals. The results indicate that the glutamate-modulating CBsc cannabinoid receptor is present in the hippocampi of CD1 mice and SD rats but not in C57BL/6J mice. Thus, we have identified animal models that may permit the study of cannabinoids independently of the novel CBsc receptor (C57(CB1+/-)), the CBsc receptor independently of the cloned CB1 receptor (CD1(CB1-/-)), or in the absence of both receptors (C57(CB1--)).

Enhancement of Spontaneous and Heat-Evoked Activity in spinal Nociceptive Neurons by the Endovanilloid/Endocannabinoid N-Arachidonoyldopamine (NADA)

N-arachidonoyldopamine (NADA) is an endogenous molecule found in the nervous system that is capable of acting as a vanilloid agonist via the TRPV1 receptor and as a
cannabinoid agonist via the CB1 receptor. Using anesthetized rats, we investigated the neural correlates of behavioral thermal hyperalgesia produced by NADA. Extracellular single cell electrophysiology was conducted to assess the effects of peripheral administration of NADA (i.pl.) on nociceptive neurons in the dorsal horn of the spinal cord. Injection of NADA in the hindpaw caused increased spontaneous discharge of spinal nociceptive neurons compared to injection of vehicle. The neurons also displayed magnified responses to application of thermal stimuli ranging from 34 to 52 degrees C. NADA-induced neural hypersensitivity was dose-dependent (EC50= 1.55 microg) and TRPV1 dependent, as the effect was abolished by co-administration of the TRPV1 antagonist 5'-iodoresiniferatoxin (I-RTX). In contrast, co-administration of the CB1 antagonist SR 141716A did not attenuate this effect. These results suggest that the enhanced responses of spinal nociceptive neurons likely underlie the behavioral thermal hyperalgesia, and implicate a possible pain-sensitizing role of endogenous NADA mediated by TRPV1 in the periphery.

CB1 receptor knockout mice show similar behavioral modifications to wild-type mice when enkephalin catabolism is inhibited


Behavioral and biochemical studies have suggested a functional link between the endogenous cannabinoid and opioid systems. Different hypotheses have been proposed to explain the interactions between opioid and cannabinoid systems such as a common pathway stimulating the dopaminergic system, a facilitation of signal-transduction- and/or a cannabinoid-induced enhancement of opioid peptide release. However, at this time, all the studies have been performed with exogenous agonists (delta-9-tetrahydrocannabinol or morphine), leading to a generally excessive stimulation of receptors normally stimulated by endogenous effectors (anandamide or opioid peptides) in various brain structures. To overcome this problem, we have measured various behavioral responses induced by the stimulation of the endogenous opioid system using the dual inhibitor of enkephalin-degrading enzymes, RB101, in CB1 receptor knockout mice. Thus, analgesia, locomotor activity, anxiety and antidepressant-like effects were measured after RB101 administration (80 and 120 mg/kg i.p. or 10 mg/kg, i.v.) in CB1 receptor knockout mice and their wild-type littermates. In all the experiments, inhibition of enkephalin catabolism produced similar modifications in behavior observed in CB1 knockout and wild-type mice. These results suggest limited physiological interaction between cannabinoid and opioid systems.

Molecular mechanisms of cannabinoid protection from neuronal excitotoxicity


 Cannabinoids protect neurons from excitotoxic injury. We investigated the mechanisms involved by studying N-methyl-D-aspartate (NMDA) toxicity in cultured murine cerebrocortical neurons in vitro and mouse cerebral cortex in vivo. The cannabinoid agonist R(+)-[2,3-dihydro-5-methyl-3-[[morpholinyl]methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (R(+)-Win 55212) reduced neuronal death in murine cortical cultures treated with 20 mM NMDA, and its protective effect was attenuated by the CB1R antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR141716A). Cultures from CB1R-knockout mice were more sensitive to NMDA toxicity than cultures from wild-type mice. The in vitro protective effect of R(+)-Win 55212 was reduced by pertussis toxin, consistent with signaling through CB1R-coupled G-proteins. The nitric oxide synthase (NOS) inhibitors 7-nitroindazole (7-NI) and N-nitro-L-arginine methyl ester (L-NAME) also reduced NMDA toxicity. In addition, CB1R and neuronal NOS were coexpressed in cultured cortical neurons, suggesting that cannabinoids might reduce NMDA toxicity by interfering with the generation of NO. NOS activity in cerebral cortex was higher in CB1R-knockouts than in wild-type mice, and 7-NI reduced NMDA lesion size. R(+)-Win 55212 inhibited NO production after NMDA treatment of wild-type cortical neuron cultures, measured with 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM) diacetate, and this effect was reversed by SR141716A. In contrast, R(+)-Win 55212 failed to inhibit NO production in cultures from CB1R knockouts. Dibutyryl-cyclic adenosine monophosphate (dbcAMP) blocked the protective effect of R(+)-Win 55212, and this
was reversed by the protein kinase A inhibitor N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (H89). Cannabinoids appear to protect neurons against NMDA toxicity at least partly by activation of CB1R and downstream inhibition of PKA signaling and NO generation.

**Anandamide suppression of Na(+) currents in rat dorsal root ganglion neurons**

Anandamide, the ethanolamide of arachidonic acid, is an endogenous cannabinoid. It is an agonist at CB(1) and CB(2) cannabinoid receptors as well as the vanilloid receptor, VR1. It is analgesic in inflammatory and neuropathic pain. Both central and peripheral mechanisms are considered to participate in its analgesia. Primary sensory neurons express Na(+) currents that are involved in the pathogenesis of pain. We examined the effect of anandamide on tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na(+) currents in rat dorsal root ganglion neurons. Anandamide inhibited both Na(+) currents in a concentration-dependent manner. At a membrane potential of -80 mV, the current inhibition was greater in TTX-S than TTX-R currents (K(d); 5.4 μM vs. 38.4 μM). The activation and inactivation became faster in TTX-R current but not in TTX-S current. Anandamide did not alter the activation voltage in either type of current. It, however, produced a hyperpolarizing shift of the steady-state inactivation voltage in both types of currents. The maximum availability at a large negative potential was not reduced by anandamide. Thus, anandamide seems to affect inactivated Na(+) channels rather than resting channels. The inhibition of Na(+) currents was not reversed by AM 251 (a CB(1) antagonist), AM 630 (a CB(2) antagonist) or capsazepine (a VR1 antagonist), suggestive of a direct action of anandamide on Na(+) channels. The inhibition of Na(+) currents in sensory neurons may contribute to the anandamide analgesia.

**Elevation of endocannabinoid levels in the ventrolateral periaqueductal grey through inhibition of fatty acid amide hydrolase affects descending nociceptive pathways via both CB1 and TRPV1 receptors**

In the ventrolateral periaqueductal grey (PAG), activation of excitatory output neurons projecting monosynaptically to OFF cells in the rostral ventromedial medulla (RVM) causes antinociceptive responses, and is under the control of cannabinoid CB1 and vanilloid TRPV1 receptors. We studied in healthy rats the effect of elevation of PAG endocannabinoid (anandamide and 2-arachidonoylglycerol [2-AG]) levels produced by intra-PAG injections of the inhibitor of fatty acid amide hydrolase, URB597, on: 1) nociception in the "plantar test"; and 2) spontaneous and tail-flick related activities of RVM neurons. Depending on the dose or time elapsed since administration, URB597 (0.5-2.5 nmol/rat) either suppressed or increased thermal nociception via TRPV1 or CB1 receptors, respectively. TRPV1 or cannabinoid receptor agonists, capsaicin (6 nmol) and WIN55,212-2 (4 nmol), also suppressed or enhanced nociception, respectively. URB597 dose-dependently enhanced PAG anandamide and 2-AG levels, with likely subsequent activation of TRPV1/CB1 receptors and only CB1 receptors, respectively. The TRPV1-mediated antinociception and CB1-mediated nociception caused by URB597 correlated with enhanced or reduced activity of RVM OFF cells, suggesting that these effects occur via stimulation or inhibition of excitatory PAG output neurons respectively. Accordingly, several ventrolateral PAG neurons were found by immunohistochemistry to co-express TRPV1 and CB1 receptors. Finally, at the highest doses tested, URB597 (4 nmol/rat) and, as previously reported, WIN55,212-2 (25-100 nmol) also caused CB1-mediated analgesia, correlating with stimulation (possibly dis-inhibition) of RVM OFF cells. Thus, endocannabinoids affect the descending pathways of pain control by acting at either CB1 or TRPV1 receptors in healthy rats.

**Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia**

Endocannabinoids, acting via type 1 cannabinoid receptors (CB(1)), are known to be involved in short-term synaptic plasticity via retrograde signaling. Strong depolarization of the postsynaptic neurons is followed by the endocannabinoid-mediated activation of presynaptic CB(1) receptors, which suppresses GABA and/or glutamate release. This phenomenon is termed
depolarization-induced suppression of inhibition (DSI) or excitation (DSE), respectively. Although both phenomena have been reported to be present in the basal ganglia, the anatomical substrate for these actions has not been clearly identified. Here we investigate the high-resolution subcellular localization of CB(1) receptors in the nucleus accumbens, striatum, globus pallidus and substantia nigra, as well as in the internal capsule, where the striato-nigral and pallido-nigral pathways are located. In all examined nuclei of the basal ganglia, we found that CB(1) receptors were located on the membrane of axon terminals and preterminal axons. Electron microscopic examination revealed that the majority of these axon terminals were GABAergic, giving rise to mostly symmetrical synapses. Interestingly, preterminal axons showed far more intense staining for CB(1), especially in the globus pallidus and substantia nigra, whereas their terminals were only faintly stained. Non-varicose, thin unmyelinated fibers in the internal capsule also showed strong CB(1)-labeling, and were embedded in bundles of myelinated CB(1)-negative axons. The majority of CB(1) receptors labeled by immunogold particles were located in the axonal plasma membrane (92.3%), apparently capable of signaling cannabinoid actions. CB(1) receptors in this location cannot directly modulate transmitter release, because the release sites are several hundred micrometers away. Interestingly, both the CB(1) agonist, WIN55,212-2, as well as its antagonist, AM251, were able to block action potential generation, but via a CB(1) independent mechanism, since the effects remained intact in CB(1) knockout animals. Thus, our electrophysiological data suggest that these receptors are unable to influence action potential propagation, thus they may not be functional at these sites, but are likely being transported to the terminal fields. The present data are consistent with a role of endocannabinoids in the control of GABA, but not glutamate, release in the basal ganglia via presynaptic CB(1) receptors, but also call the attention to possible non-CB(1)-mediated effects of widely used cannabinoid ligands on action potential generation.

The cannabinoid receptor antagonist SR-141716A induces penile erection in male rats: Involvement of paraventricular glutamic acid and nitric oxide

The cannabinoid CB1 receptor antagonist SR141716A (0.5, 1 and 2 mug) induces penile erection when injected into the paraventricular nucleus of male rats. The pro-erectile effect of SR 141716A occurs concomitantly with an increase in the concentration of NO(2)(-) and NO(3)(-) in the paraventricular dialysate obtained by means of intracerebral microdialysis. Both penile erection and NO(2)(-) increase induced by SR 141716A were reduced by the prior injection into the PVN of the cannabinoid CB1 agonists WIN 55,212-2 (5 mug) or HU 210 (5 mug), given into the paraventricular nucleus at doses unable to induce penile erection or to modify NO(2)(-) concentration. SR 141716A responses were also reduced by nitro-l-arginine methylester (20 mug), a non-selective NO synthase inhibitor, S-methyl-l-thiocitrulline (20 mug), a selective neuronal NO synthase inhibitor, the excitatory amino acid NMDA receptor antagonist dizocilpine ((+)MK 801) (1 mug), or the GABA(A) receptor agonist muscimol (0.2 mug) injected into the PVN 15 min before SR 141716A. In contrast, the inducible NO synthase inhibitor L-N(6)-(1-iminoethyl)lysine (20 mug), the GABA(B) receptor agonist baclofen (0.2 mug), the mixed dopamine receptor antagonist cis-flupenthixol (10 mug), and the oxytocin receptor antagonist d(CH(2))(5)Tyr(Me)-Orn(8)-vasotocin (1 mug), were ineffective. Despite its inability to reduce penile erection and NO(2)(-) increase induced by SR 141716A when injected into the PVN, d(CH(2))(5)Tyr(Me)-Orn(8)-vasotocin (1 mug) reduced almost completely penile erection without reducing paraventricular NO(2)(-) increase when injected into the lateral ventricles 15 min before SR 141716A. The present results show that SR 141716 induces penile erection by a mechanism (possibly activation of excitatory amino acid neurotransmission), which causes the activation of neuronal NO synthase in paraventricular oxytocinergic neurons mediating penile erection.

Ultra-low dose naltrexone enhances cannabinoid-induced antinociception

Both opioids and cannabinoids have inhibitory effects at micromolar doses, which are mediated by activated receptors coupling to Gi/o-proteins. Surprisingly, the analgesic effects of opioids are enhanced by ultra-low doses (nanomolar to picomolar) of the opioid antagonist, naltrexone. As opioid and cannabinoid systems interact, this study investigated whether ultra-low
dose naltrexone also influences cannabinoid-induced antinociception. Separate groups of Long-Evans rats were tested for antinociception following an injection of vehicle, a sub-maximal dose of the cannabinoid agonist WIN 55 212-2, naltrexone (an ultra-low or a high dose) or a combination of WIN 55 212-2 and naltrexone doses. Tail-flick latencies were recorded for 3 h, at 10-min intervals for the first hour, and at 15-min intervals thereafter. Ultra-low dose naltrexone elevated WIN 55 212-2-induced tail flick thresholds without extending its duration of action. This enhancement was replicated in animals receiving intraperitoneal or intravenous injections. A high dose of naltrexone had no effect on WIN 55 212-2-induced tail flick latencies, but a high dose of the cannabinoid 1 receptor antagonist SR 141716 blocked the elevated tail-flick thresholds produced by WIN 55 212-2+ultra-low dose naltrexone. These data suggest a mechanism of cannabinoid-opioid interaction whereby activated opioid receptors that couple to Gs-proteins may attenuate cannabinoid-induced antinociception and/or motor functioning.

Agonistic Properties of Cannabidiol at 5-HT1a Receptors

Cannabidiol (CBD) is a major, biologically active, but psycho-inactive component of cannabis. In this cell culture-based report, CBD is shown to displace the agonist, [3H]8-OH-DPAT from the cloned human 5-HT1a receptor in a concentration-dependent manner. In contrast, the major psychoactive component of cannabis, tetrahydrocannabinol (THC) does not displace agonist from the receptor in the same micromolar concentration range. In signal transduction studies, CBD acts as an agonist at the human 5-HT1a receptor as demonstrated in two related approaches. First, CBD increases [35S]GTPgammaS binding in this G protein coupled receptor system, as does the known agonist serotonin. Second, in this GPCR system, that is negatively coupled to cAMP production, both CBD and 5-HT decrease cAMP concentration at similar apparent levels of receptor occupancy, based upon displacement data. Preliminary comparative data is also presented from the cloned rat 5-HT2a receptor suggesting that CBD is active, but less so, relative to the human 5-HT1a receptor, in binding analyses. Overall, these studies demonstrate that CBD is a modest affinity agonist at the human 5-HT1a receptor. Additional work is required to compare CBD’s potential at other serotonin receptors and in other species. Finally, the results indicate that cannabidiol may have interesting and useful potential beyond the realm of cannabinoid receptors.

Endocannabinoids Control the Induction of Cerebellar LTD

The long-term depression (LTD) of parallel fiber (PF) synapses onto Purkinje cells plays a central role in motor learning. Endocannabinoid release and LTD induction both depend upon activation of the metabotropic glutamate receptor mGluR1, require postsynaptic calcium increases, are synapse specific, and have a similar dependence on the associative activation of PF and climbing fiber synapses. These similarities suggest that endocannabinoid release could account for many features of cerebellar LTD. Here we show that LTD induction is blocked by a cannabinoid receptor (CB1R) antagonist, by inhibiting the synthesis of the endocannabinoid 2-arachidonyl glycerol (2-AG), and is absent in mice lacking the CB1R. Although CB1Rs are prominently expressed presynaptically at PF synapses, LTD is expressed postsynaptically. In contrast, a previously described transient form of inhibition mediated by endocannabinoids is expressed presynaptically. This indicates that Purkinje cells release 2-AG that activates CB1Rs to both transiently inhibit release and induce a postsynaptic form of LTD.

Effects of intrathecal anandamide on somatosensory evoked responses in rats
were injected intrathecally to the first (control), second, third and fourth groups, respectively. Five minutes later, second SEP traces were started. In every SEP trace, two negative waves (N1, N2) following positive deflections were obtained. The latency and amplitudes of these waves assessed were compared in each group. In control and second groups, the parameters of these waves before and after the injections were not significantly different. However, in the third and fourth groups, latencies of N1 and N2 after injections were found significantly longer. This effect was dose dependent. In any of the groups, no significant changes were detected in the amplitudes after injections. In conclusion, anandamide, when injected intrathecally in pharmacological doses caused an induction of moderate conduction delay in SEP systems.

Kappa- and delta-opioid receptor functional activities are increased in the caudate putamen of cannabinoid CB receptor knockout mice


The purpose of this study was to examine the functional interaction between endogenous opioid and cannabinoid receptor systems in the caudate putamen and nucleus accumbens. We therefore examined by autoradiography the functional activity and density of micro-, kappa- and delta-opioid receptors in both brain regions of cannabinoid CB(1) receptor knockout mice. Functional activity was estimated by measuring agonist-stimulated [(35)S]GTPgammaS binding. Results showed that deletion of the CB(1) cannabinoid receptor markedly increased kappa-opioid (50%) and delta-opioid (42%) receptor activities whereas no differences were found in micro-opioid receptor in the caudate putamen. In contrast, binding autoradiography showed a similar density of micro-, kappa- and delta-opioid receptors between mutant and wild-type mice. No differences were found in densities or activities of micro-, kappa- and delta-opioid receptors between mutant and wild-type mice in the nucleus accumbens. Taken together, our results revealed that deletion of CB(1) cannabinoid receptors produced a pronounced increase in the activity of kappa- and delta-opioid receptors in the caudate putamen. This endogenous interaction between opioid and cannabinoid receptors may be relevant to further understand a variety of neuroadaptative processes involving the participation of opioid receptors, such as motor behaviour, emotional responses and drug dependence.

Oncology

*R(+)-Methanandamide Elicits a Cyclooxygenase-2-Dependent Mitochondrial Apoptosis Signaling Pathway in Human Neuroglioma Cells*


PURPOSE: Cannabinoids have been associated with tumor regression and apoptosis of cancer cells. Recently, we have shown that R(+)-methanandamide (R(+)-MA) induces apoptosis of H4 human neuroglioma cells via a mechanism involving de novo expression of the cyclooxygenase-2 (COX-2) enzyme. The present study investigated a possible involvement of a mitochondrial-driven pathway in this process. METHODS: Cell death was determined by the WST-1 cell viability test, and changes in apoptotic parameters [i.e., release of mitochondrial cytochrome c, activation of caspases, cleavage of poly(ADP-ribose) polymerase (PARP)] were detected by Western blotting. RESULTS: H4 cells treated with R(+)-MA showed typical signs of mitochondrial apoptosis, i.e., release of mitochondrial cytochrome c into the cytosol and activation of initiator caspase-9. Moreover, cleavage of the executor caspase-3 was observed following cannabinoid treatment. Cells were fully protected from apoptotic cell death by the caspase-3 inhibitor Ac-DEVD-CHO, indicating a crucial role for caspase-3 activation in R(+)-MA-elicited apoptosis. Furthermore, cleavage of the caspase-3 target protein PARP was registered. All of the aforementioned effects were substantially reduced by the selective COX-2 inhibitor celecoxib (1 muM) at a pharmacologically relevant, nonapoptotic concentration. CONCLUSION: R(+)-MA-induced apoptosis is mediated via a mitochondrial-dependent pathway that becomes activated, at least in part, through up-regulation of the COX-2 enzyme.

Cannabinoids and cancer

Marijuana has been used in medicine for millennia, but it was not until 1964 that delta9-tetrahydrocannabinol (delta9-THC), its major psychoactive component, was isolated in pure form and its structure was elucidated. Shortly thereafter it was synthesized and became readily available. However, it took another decade until the first report on its antineoplastic activity appeared. In 1975, Munson discovered that cannabinoids suppress Lewis lung carcinoma cell growth. The mechanism of this action was shown to be inhibition of DNA synthesis. Antiproliferative action on some other cancer cells was also found. In spite of the promising results from these early studies, further investigations in this area were not reported until a few years ago, when almost simultaneously two groups initiated research on the antiproliferative effects of cannabinoids on cancer cells: Di Marzo's group found that cannabinoids inhibit breast cancer cell proliferation, and Guzman's group found that cannabinoids inhibit the growth of C6 glioma cell. Other groups also started work in this field, and today, a wide array of cancer cell lines that are affected is known, and some mechanisms involved have been elucidated.

**Ophthalmology**

**Pharmacology**

*Design, Synthesis, and Binding Studies of New Potent Ligands of Cannabinoid Receptors*


Despite their different chemical structures, Delta(9)-tetrahydrocannabinol (THC) and anandamide (AEA) have common pharmacological properties. This study was aimed at finding new cannabinoid receptor ligands that overcome the instability of AEA and its analogues. To this end we planned the synthesis of a series of compounds which retained both a rigid structure, like that of plant cannabinoids, and a flexible portion similar to that of anandamide. Binding studies on CB(1) and CB(2) receptors, anandamide membrane transporter (AMT), and fatty acid amide hydrolase (FAAH) showed that some of the newly developed compounds have high affinity and specificity for cannabinoid CB(1) and CB(2) receptors. Compound 25 is a potent CB(1) and CB(2) ligand, with affinity constants significantly lower than AEA and similar to WIN 55-212, compound 52 is a potent CB(2) ligand, although not very selective over CB(1) receptors, and compound 43 is CB(2) ligand, with at least a 26-fold selectivity over CB(1) receptors. Compound 25 behaved as an inverse agonist at CB(1) receptors as assessed in the cyclic AMP functional assay.

*New bicyclic cannabinoid receptor-1 (CB(1)-R) antagonists*


A series of conformationally constrained bicyclic derivatives derived from SR141716 was prepared and evaluated as hCB(1)-R antagonists and inverse agonists. Optimization of the structure-activity relationships around the 2,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one derivative 2a led to the identification of two compounds with oral activity in rodent feeding models (2h and 4a). Replacement of the PP group in 2h with other bicyclic groups resulted in a loss of binding affinity.

*The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits cell proliferation and increases markers of adipocyte maturation in cultured mouse 3T3 F442A preadipocytes*


Adipocyte cell proliferation is an important process in body fat mass development in obesity. Adiponectin or Acrp30 is an adipokine exclusively expressed and secreted by adipose tissue, which regulates lipid and glucose metabolism and plays a key role in body weight regulation and homeostasis. Adiponectin mRNA expression in adipose tissue and plasma level of adiponectin are decreased in obesity and type 2 diabetes. In obese rodents, the selective CB1 receptor antagonist rimonabant reduces food intake, body weight and improves lipid and glucose parameters. We have previously reported that rimonabant stimulated adiponectin mRNA expression in adipose tissue of obese fa/fa rats, by a direct effect on adipocytes. Here we report that rimonabant (10 to 400 nM) inhibits cell proliferation of cultured mouse 3T3 F442A preadipocytes in a concentration-dependent manner. In parallel to this inhibitory effect on
preadipocyte cell proliferation, rimonabant (25 to 100 nM) stimulates mRNA expression and protein levels of two late markers of adipocyte differentiation (adiponectin and GAPDH) with a maximal effect at 100 nM, without inducing the accumulation of lipid droplets. Furthermore, treatment of mouse 3T3 F442A preadipocytes with rimonabant (100 nM) inhibits basal and serum-induced p42/44 MAPkinase activity. These results suggest that rimonabant-inhibition of MAPkinase activity may be one of mechanisms involved in the inhibition of 3T3 F442A preadipocyte cell proliferation and stimulation of adiponectin and GAPDH expression. The inhibition of preadipocyte cell proliferation and the induction of adipocyte late "maturation" may participate in rimonabant-induced anti-obesity effects and in particular the reduction of body fat mass.

Structural-activity relationship study on C-4 carbon atom of the CB(1) antagonist SR141716: synthesis and pharmacological evaluation of 1,2,4-triazole-3-carboxamides

A series of 1,2,4-triazole-3-carboxamides has been prepared from alkyl-1,2,4-triazole-3-carboxylates under mild conditions. The ability of these triazoles to displace [(3)H]-CP55940 from CB(1) cannabinoid receptor was measured. However, they showed only poor to moderate binding affinities, indicating that substitution of the C-4 pyrazole atom of the CB(1) reference compound SR141716 by a nitrogen atom results in loss of affinity. Further investigations for functionality indicated that the compound 6a exhibited significant cannabinoid antagonistic properties in the mouse vas deferens functional assay. This leads us to the conclusion that 6a binds at a different CB(1) binding site or at a new cannabinoid receptor subtype.

1-Benzhydryl-3-phenylurea and 1-Benzhydryl-3-phenylthiourea Derivatives: New Templates among the CB(1) Cannabinoid Receptor Inverse Agonists

New 1-benzhydryl-3-phenylurea derivatives and their 1-benzhydryl-3-phenylthiourea isosteres were synthesized and evaluated for their human CB(1) and CB(2) cannabinoid receptor affinity. These compounds proved to be selective CB(1) cannabinoid receptor ligands, acting as inverse agonists in a [(35)S]-GTPgammaS assay. The affinity of 3,5,5'-triphenylimidazolidine-2,4-dione and 3,5,5'-triphenyl-2-thioximidazolidin-4-one derivatives, possessing the 1-benzhydryl-3-phenylurea and 1-benzhydryl-3-phenylthiourea moiety, respectively, was also evaluated. In conclusion, the 1-benzhydryl-3-phenylurea scaffold seems to be a new interesting template of CB(1) cannabinoid receptor inverse agonists.

Tricyclic Pyrazoles. 3. Synthesis, Biological Evaluation, and Molecular Modeling of Analogues of the Cannabinoid Antagonist 8-Chloro-1-(2',4'-dichlorophenyl)-N-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide

A series of analogues of 8-chloro-1-(2',4'-dichlorophenyl)-N-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide 4a (NESS 0327) (Ruiu, S.; Pinna, G. A.; Marchese, G.; Mussinu, J. M.; Saba, P.; Tambaro, S.; Casti, P.; Vargiu, R.; Pani, L. Synthesis and Characterization of NESS 0327: A Novel Putative Antagonist of CB(1) Cannabinoid Receptor. J. Pharmacol. Exp. Ther. 2003, 306, 363-370) was synthesized and evaluated for their affinity to cannabinoid receptors. Depending on the chemical modification of the lead structure that was chosen, compounds 4b, 4c, 4i, 4l, and 4m still proved to be potent binders of the CB(1) receptor. Moreover, several analogues (4c, 4d, 4e, and 4m) demonstrated superior CB(2) receptor binding affinities compared to the parent ligand. Compounds 4b, 4c, 4i, and 4l displayed the most promising pharmacological profiles, having the highest selectivity for CB(1) receptors with K(i)(CB(2)) to K(i)(CB(1)) ratios of 11 250, 2000, 3330 and 4625, respectively. Compound 4c increased the intestinal propulsion in mice and antagonized the effect induced by the CB(1) receptor agonist WIN 55,212-2. Finally, molecular modeling studies were carried out on a set of tricyclic pyrazoles (2a-4a) and on rimonabant 1 (SR141716A), indicating that high CB(1) receptors affinities were consistent for the tricyclic derivatives, both with a nonplanar geometry of the tricyclic cores and with a precise orientation of the substituent (chlorine) on this ring system.
**Delta(9)-tetrahydrocannabinol immunochemical studies: haptens, monoclonal antibodies, and a convenient synthesis of radiolabeled delta(9)-tetrahydrocannabinol**


Immunopharmacotherapy as an approach to combat drugs of abuse has become an active area of investigation. Marijuana is the most commonly used illicit drug in the U.S. The main active chemical in marijuana is Delta(9)-tetrahydrocannabinol (Delta(9)-THC); hence, monoclonal antibodies with high affinity and specificity for Delta(9)-tetrahydrocannabinol could be valuable immunopharmacotherapeutic intervention and diagnostic tools. We have synthesized immunoconjugates that induce an effective immune response to Delta(9)-THC and describe a convenient synthesis of radiolabeled Delta(9)-THC. We demonstrate the value and use of this probe to select anti-Delta(9)-THC antibodies that bind Delta(9)-THC with good affinity. The synthetic route to radiolabeled Delta(9)-THC has enabled the correct assessment of the affinity of these antibodies to their ligand and may facilitate future binding studies between Delta(9)-THC and its analogues and the cannabinoid receptors.

**Virtual Screening of Novel CB2 Ligands Using a Comparative Model of the Human Cannabinoid CB2 Receptor**


To identify novel selective CB2 lead compounds, a comparative model of the CB2 receptor was constructed using the high-resolution bovine rhodopsin X-ray structure as a template. The CB2 model was utilized both in building the database queries and in filtering the hit compounds by a docking and scoring method. In G-protein activation assays, 1-isoquinolyl[3-(trifluoromethyl)phenyl]methanone (40, NRB 04079) was found to act as a selective agonist at the human CB2 receptor.

**Physiology**

**Plant Sciences**

**Psychiatry**

**Participation of the cannabinoid system in the regulation of emotional-like behaviour**


The endocannabinoid system has been involved in the control of several neurophysiological and behavioural responses. Indeed, recent studies have suggested that the cannabinoid system could represent an important substrate for the control of emotional behaviour, and further research would probably help to identify new promising therapeutic targets. This paper reviews the results obtained in different animal models used to investigate emotional states after the manipulation of the endocannabinoid system. Cannabinoid compounds can induce anxiogenic- and anxiolytic-like responses in rodents depending on the experimental conditions. Studies using knockout mice lacking the CB1 cannabinoid receptors have shown the participation of this receptor in several behavioural responses including anxiety- and depressive-like states. Furthermore, the endocannabinoid system regulates the hypothalamic-pituitary adrenal axis, which is involved in providing an appropriate response to stressful situations. Recent studies have also demonstrated that the endocannabinoids can function as retrograde messengers, modulating the release of different neurotransmitter, including opioids, GABA and cholecystokinin that have been classically involved in the control of anxiety-like responses. All this recent information has further clarified the role played by the endogenous cannabinoid system in the control of emotional behaviour and provides data to support a new possible therapeutic use of cannabinoid compounds.
Reproductive Biology

Modulation of ATP-mediated contractions of the rat vas deferens through presynaptic cannabinoid receptors

The effect of R-(+)-[2,3-dihydro-5-methyl-3-[(morpholiny)methyl]pyrrol[1,2,3-de]-1,4-benzoxazin-yl]-1(naphthalenyl)methanone mesylate (WIN 55,212-2; a cannabinoid receptor agonist) was investigated on contractions of the bisected (epididymal and prostatic portions) rat vas deferens to assess the role of cannabinoid receptors in sympathetic ATP neurotransmission. WIN 55,212-2 inhibited the electrically induced contractions in both portions of the rat vas deferens. In the presence of the alpha(1)-adrenoreceptor antagonist prazosin, electrical stimulation produces a contraction mediated exclusively by ATP. In this condition, WIN 55,212-2 in the prostatic portion elicited a concentration-dependent inhibition that was antagonized by N-piperidinyl-[8-chloro-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide] (NESS 0327), a selective cannabinoid CB(1) receptor antagonist. NESS 0327 caused a parallel dextral displacement of the WIN 55,212-2 concentration-response curve. It is suggested that activation of pre-junctional cannabinoid receptors on sympathetic nerves of the vas deferens modulates ATP neurotransmission.

Respiratory

Toxicology

CLINICAL SCIENCE

Adverse events

Small intestine volvulus in a hashish body-packer

Case reports

Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up

OBJECTIVE: To test the effectiveness and long term safety of cannabinoids in multiple sclerosis (MS), in a follow up to the main Cannabinoids in Multiple Sclerosis (CAMS) study. METHODS: In total, 630 patients with stable MS with muscle spasticity from 33 UK centres were randomised to receive oral Delta(9)-tetrahydrocannabinol (Delta(9)-THC), cannabis extract, or placebo in the main 15 week CAMS study. The primary outcome was change in the Ashworth spasticity scale. Secondary outcomes were the Rivermead Mobility Index, timed 10 metre walk, UK Neurological Disability Score, postal Barthel Index, General Health Questionnaire-30, and a series of nine category rating scales. Following the main study, patients were invited to continue medication, double blinded, for up to 12 months in the follow up study reported here. RESULTS: Intention to treat analysis of data from the 80% of patients followed up for 12 months showed evidence of a small treatment effect on muscle spasticity as measured by change in Ashworth score from baseline to 12 months (Delta(9)-THC mean reduction 1.82 (n = 154, 95% confidence interval (CI) 0.53 to 3.12), cannabis extract 0.10 (n = 172, 95% CI -0.99 to 1.19), placebo -0.23 (n = 176, 95% CI -1.41 to 0.94); p = 0.04 unadjusted for ambulatory status and centre, p = 0.01 adjusted). There was suggestive evidence for treatment effects of Delta(9)-THC on some aspects of disability. There were no major safety concerns. Overall, patients felt that these drugs were helpful in treating their disease. CONCLUSIONS: These data provide limited evidence for a longer term treatment effect of cannabinoids. A long term placebo controlled study is now needed to establish whether cannabinoids may have a role beyond symptom amelioration in MS.
**Clinical trials**

**Cannabinoid-1-receptor blockade to treat obesity and cardiovascular risk factors**


**Cannabinoid 1 receptor blockade to treat obesity and cardiovascular risk factors**


**Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis**


Objectives. To assess the efficacy of a cannabis-based medicine (CBM) in the treatment of pain due to rheumatoid arthritis (RA). Methods. We compared a CBM (Sativex) with placebo in a randomized, double-blind, parallel group study in 56 patients over 5 weeks of treatment. The CBM was administered by oromucosal spray in the evening and assessments were made the following morning. Efficacy outcomes assessed were pain on movement, pain at rest, morning stiffness and sleep quality measured by a numerical rating scale, the Short-Form McGill Pain Questionnaire (SF-MPQ) and the DAS28 measure of disease activity. Results. Seventy-five patients were screened and 58 met the eligibility criteria. Thirty-one were randomized to the CBM and 27 to placebo. Mean (s.d.) daily dose achieved in the final treatment week was 5.4 (0.84) actuations for the CBM and 5.3 (1.18) for placebo. In comparison with placebo, the CBM produced statistically significant improvements in pain on movement, pain at rest, quality of sleep, DAS28 and the SF-MPQ pain at present component. There was no effect on morning stiffness but baseline scores were low. The large majority of adverse effects were mild or moderate, and there were no adverse effect-related withdrawals or serious adverse effects in the active treatment group. Conclusions. In the first ever controlled trial of a CBM in RA, a significant analgesic effect was observed and disease activity was significantly suppressed following Sativex treatment. Whilst the differences are small and variable across the population, they represent benefits of clinical relevance and show the need for more detailed investigation in this indication.

**Recent cannabis abuse decreased stress-induced BOLD signals in the frontal and cingulate cortices of cocaine dependent individuals**


Previous neuroimaging studies showed that use of marijuana can alter patterns of cortical activation during rest or a task challenge. We used functional magnetic resonance imaging to examine whether recent cannabis abuse contributed to stress-induced blood-oxygen-level-dependent (BOLD) contrast in a group of cocaine-dependent individuals. Emotional stress was induced using the script-guided imagery paradigm, in which subjects imagined being in a real-life stressful situation and, as a control, in a neutral situation, while BOLD signals of their brain were acquired with a 1.5 T scanner. Abstinent cocaine-dependent subjects with recent marijuana abuse (n=8) were compared with abstinent cocaine-dependent subjects who had not abused marijuana recently (n=18). The two groups were otherwise matched in their demographic characteristics and drug use history. All subjects were abstinent for at least 15 days and drug free as confirmed by urine drug screening before the imaging session. Recent cannabis abusers demonstrated hypo-activation in frontal cortical areas including the perigenual anterior cingulate during increased emotional stress. In contrast, at the same statistical threshold, no brain regions showed increased activation in recent cannabis abusers compared with non-abusers. The group difference in the perigenual anterior cingulate remained even when lifetime cocaine and alcohol consumption was accounted for in covariance analysis. These results provide evidence that recent cannabis abuse is associated with decreased activation in the frontal cortex during an emotional stress task. The results suggest an abnormal cognitive control mechanism during affective processing in association with heavy cannabis use.
Forensic Science

Genetic Studies

Policy

Reviews

Drug Insight: appetite suppressants

The term 'appetite suppressant' is used to denote drugs that act primarily on the neurochemical transmitters of the central nervous system to reduce food intake. In addition to drugs that release or mimic the effect of norepinephrine (noradrenaline), this could include drugs that inhibit: reuptake of norepinephrine or 5-hydroxytryptamine (also known as serotonin); bind to the gamma-aminobutyric acid receptors or the cannabinoid receptors; and some peptides that reduce food intake. The sympathomimetic drugs phentermine, diethylpropion, benzphetamine, and phendimetrazine--so named because they mimic many effects of norepinephrine--are only approved in a few countries, and then only for short-term use. Sibutramine, a norepinephrine-5-hydroxytryptamine reuptake inhibitor, is approved for long-term use. Several new mechanisms for drug action are under investigation. Appetite suppressants should be viewed as useful adjuncts to diet and physical activity and might help selected patients to achieve and maintain weight loss.

Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia

BACKGROUND: Rimonabant, a selective cannabinoid-1 receptor (CB1) blocker, has been shown to reduce body weight and improve cardiovascular risk factors in obese patients. The Rimonabant in Obesity-Lipids (RIO-Lipids) study examined the effects of rimonabant on metabolic risk factors, including adiponectin levels, in high-risk patients who are overweight or obese and have dyslipidemia. METHODS: We randomly assigned 1036 overweight or obese patients (body-mass index [the weight in kilograms divided by the square of the height in meters], 27 to 40) with untreated dyslipidemia (triglyceride levels >1.69 to 7.90 mmol per liter, or a ratio of cholesterol to high-density lipoprotein [HDL] cholesterol of >4.5 among women and >5 among men) to double-blind therapy with either placebo or rimonabant at a dose of 5 mg or 20 mg daily for 12 months in addition to a hypocaloric diet. RESULTS: The rates of completion of the study were 62.6 percent, 60.3 percent, and 63.9 percent in the placebo group, the group receiving 5 mg of rimonabant, and the group receiving 20 mg of rimonabant, respectively. The most frequent adverse events resulting in discontinuation of the drug were depression, anxiety, and nausea. As compared with placebo, rimonabant at a dose of 20 mg was associated with a significant (P<0.001) mean weight loss (repeated-measures method, -6.7+/-.5 kg, and last-observation-carried-forward analyses, -5.4+/-0.4 kg), reduction in waist circumference (repeated-measures method, -5.8+/-0.5 cm, and last-observation-carried-forward analyses, -4.7+/-0.5 cm), increase in HDL cholesterol (repeated-measures method, +10.0+/-.1.6 percent, and last-observation-carried-forward analyses, +8.1+/-1.5 percent), and reduction in triglycerides (repeated-measures method, -13.0+/-.3.5 percent, and last-observation-carried-forward analyses, -12.4+/-3.2 percent). Rimonabant at a dose of 20 mg also resulted in an increase in plasma adiponectin levels (repeated-measures method, 57.7 percent, and last-observation-carried-forward analyses, 46.2 percent; P<0.001), for a change that was partly independent of weight loss alone. CONCLUSIONS: Selective CB1-receptor blockade with rimonabant significantly reduces body weight and waist circumference and improves the profile of several metabolic risk factors in high-risk patients who are overweight or obese and have an atherogenic dyslipidemia.

Rimonabant (Acomplia), specific inhibitor of the endocannabinoid system
The endocannabinoid system plays a major role in the regulation of body energy by stimulation of the appetite in the hypothalamus and increase of fat accumulation in adipocytes. The blockade of the cannabinoid system (CB1) by the specific inhibitor (rimonabant) decreases food intake and adiposity in animals and in humans. Moreover rimonabant lowers tobacco addiction. Clinical studies (RIO-LIPIDS and RIO-EUROPE) have recently confirmed that rimonabant combined with a hypocaloric diet over 1 year, promoted significant decrease of body weight, waist circumference and improvement of dyslipidemia. Rimonabant was well tolerated with mild and transient side effects. The future place of rimonabant in the strategy of obesity is still to be clarified.

Cannabinoids

Since the discovery of an endogenous cannabinoid system, research into the pharmacology and therapeutic potential of cannabinoids has steadily increased. Two subtypes of G-protein coupled cannabinoid receptors, CB(1) and CB(1), have been cloned and several putative endogenous ligands (endocannabinoids) have been detected during the past 15 years. The main endocannabinoids are arachidonoyl ethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), derivatives of arachidonic acid, that are produced "on demand" by cleavage of membrane lipid precursors. Besides phytocannabinoids of the cannabis plant, modulators of the cannabinoid system comprise synthetic agonists and antagonists at the CB receptors and inhibitors of endocannabinoid degradation. Cannabinoid receptors are distributed in the central nervous system and many peripheral tissues, including immune system, reproductive and gastrointestinal tracts, sympathetic ganglia, endocrine glands, arteries, lung and heart. There is evidence for some non-receptor dependent mechanisms of cannabinoids and for endocannabinoid effects mediated by vanilloid receptors. Properties of CB receptor agonists that are of therapeutic interest include analgesia, muscle relaxation, immunosuppression, anti-inflammation, antiallergic effects, improvement of mood, stimulation of appetite, antiemesis, lowering of intraocular pressure, bronchodilation, neuroprotection and antineoplastic effects. The current main focus of clinical research is their efficacy in chronic pain and neurological disorders. CB receptor antagonists are under investigation for medical use in obesity and nicotine addiction. Additional potential was proposed for the treatment of alcohol and heroine dependency, schizophrenia, conditions with lowered blood pressure, Parkinson's disease and memory impairment in Alzheimer's disease.

Cannabinoids in multiple sclerosis: urgent need for long term trials

The cannabinoid system and its importance in the perinatal period

The cannabinoid system has been recently described, including the endogenous ligands, mainly arachidonic acid derivatives, and their specific receptors. Endocannabinoids are involved in the modulation of synaptic transmission, through which they exert their psychoactive, motor and antinociceptive effects, among others; they also exert extraneural effects, mainly immunomodulation and vasodilation. Recent data suggest that the cannabinoid system might play an important role in human ontogeny and could participate in the implantation and early development of the embryo, in fetal brain development, and in the beginning of breast feeding after birth. In addition, the vasodilatory effect of cannabinoids, together with inhibition of the release of excitotoxic amino acids and cytokines, as well as modulation of oxidative stress and the toxic production of nitric oxide, justify the growing evidence pointing to a possible neuroprotective effect of cannabinoids in perinatal asphyxia.

A therapeutic role for cannabinoid CB(1) receptor antagonists in major depressive disorders

Cannabinoid receptors in the CNS have been implicated in the control of appetite, cognition, mood and drug dependence. Recent findings support the hypothesis that cannabinoid CB(1) receptor blockade might be associated with antidepressant and anti-stress effects. A novel
potential antidepressant drug class based on this mechanism is supported by the neuroanatomical localization of CB(1) receptors and signal transduction pathways that are involved in emotional responses, together with the antidepressant-like neurochemical and behavioral effects induced by CB(1) receptor antagonists. Selective CB(1) receptor antagonists are in development for the treatment of obesity and tobacco smoking, and could be tested for antidepressant efficacy because recent results of clinical studies suggest that they would also treat comorbid symptoms of depression such as cognitive deficiencies, weight gain, impulsivity and dependence disorders. Thus, CB(1) receptor antagonism might constitute an integrated pharmacotherapeutic approach that impacts the affective, cognitive, appetitive and motivational neuronal networks involved in mood disorders.

Surveys

BEHAVIOURAL SCIENCE

Addiction

The role of corticotropin-releasing factor-like peptides in cannabis, nicotine, and alcohol dependence


The corticotropin-releasing factor (CRF)-like peptides, which include the mammalian peptides CRF, urocortin 1, urocortin 2, and urocortin 3, play an important role in orchestrating behavioral and physiological responses that may increase an organism's chance of survival when confronted with internal or external stressors. There is, however, evidence that a chronic overactivity of brain CRF systems under basal conditions may play a role in the etiology and maintenance of psychiatric disorders such as depression and anxiety disorders. In addition, there is evidence of a role for CRF-like peptides in acute and protracted drug abstinence syndromes and relapse to drug-taking behavior. This review focuses on the role of CRF-like peptides in the negative affective state associated with acute and protracted withdrawal from three widely abused drugs, cannabis, nicotine, and alcohol. In addition, we discuss the high comorbidity between stress-associated psychiatric disorders and drug dependence. A better understanding of the brain stress systems that may underlie psychiatric disorders, acute and protracted drug withdrawal, and relapse to drug-taking behavior may help in the development of new and improved pharmacotherapies for these widespread psychiatric disorders.

Effect of the cannabinoid CB(1) receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats


RATIONALE: It has been suggested that endocannabinoid mechanisms are involved in the control of ethanol consumption. OBJECTIVES: The aims of the present study were (1) to evaluate the role of the endocannabinoid system in the control of operant ethanol self-administration and in the reinstatement of ethanol seeking, when induced by stress or conditioned stimuli and (2) to offer new insights on the specificity of such a role. METHODS: Rats were administered intraperitoneally with the selective cannabinoid CB(1) receptor antagonist, SR-141716A, 30 min before operant self-administration or reinstatement sessions. Two schedules of reinforcement, the fixed-ratio 1 (FR1) and the progressive ratio (PR), were used to study 10% (w/v) alcohol and 5.0% sucrose self-administration. NaCl (2% w/v) intake in sodium-depleted rats was studied only under the FR1 program. RESULTS: Treatment with SR-141716A (0.3-3.0 mg/kg) significantly attenuated FR1 alcohol self-administration and lowered the break point for ethanol under PR. SR-141716A also markedly inhibited the reinstatement of alcohol seeking elicited by presentation of cues predictive of drug availability. Conversely, the cannabinoid antagonist did not prevent the reinstatement of alcohol seeking induced by foot-shock stress. Lever pressing for sucrose under FR1 and PR schedules was also significantly decreased by SR-141716A treatment, whereas the drug modestly and only at the highest dose decreased 2% NaCl self-administration. CONCLUSIONS: Results emphasize that endocannabinoid mechanisms play
a major role in the control of ethanol self-administration and in the reinstatement of conditioned ethanol seeking. However, these effects extend to the control of operant behaviours motivated by natural rewards (i.e. sucrose). On the other hand, SR-141716A only weakly reduces NaCl self-administration in sodium-depleted rats, in which salt intake is largely controlled by homeostatic mechanisms. Overall, these observations demonstrate that the inhibition of operant behaviour following blockade of CB(1) receptors by SR-141716A is linked to a reduction of reward-related responding and is not related to drug-induced motor deficits.

The Marijuana Ladder: Measuring motivation to change marijuana use in incarcerated adolescents

The purpose of this study was to determine if a modified version of the Contemplation Ladder, a measure of motivation to change marijuana use among incarcerated adolescents (Marijuana Ladder; ML), was related to marijuana use and treatment engagement. Participants (N=122) in this study were all incarcerated at a state juvenile correctional facility in the Northeast. Adolescents were assessed at the beginning of their incarceration, 2 months into their incarceration, and 3 months after their release. There was a significant negative relationship between ML scores and marijuana use and a significant positive relationship between ML scores and treatment engagement. When controlling for prior marijuana use and age, ML scores at baseline significantly added to the prediction of marijuana use and treatment engagement among incarcerated adolescents. Results support the concurrent validity and the predictive validity of the ML. This measure has the potential to provide important information for Juvenile Justice Facilities that might aid in treatment planning and discharge planning for incarcerated adolescents. In addition, researchers may find a quick visual analog measure of motivation to change marijuana use with good psychometric properties useful.

Driving studies

Policy
Gonzales v Raich: The US supreme court’s consideration captured the public policy debate about the medical use of marijuana

Response to the American Academy of Pediatrics report on legalization of marijuana

Population studies
Beyond Treatment Effects: Predicting Emerging Adult Alcohol and Marijuana Use among Substance-Abusing Delinquents

Secondary analyses of a randomized clinical trial examined the effects of 4 putative risk factors and 2 protective factors in predicting drug use among 80 emerging adults treated 5 years earlier for delinquency and alcohol and/or marijuana use disorders. Frequency of marijuana use and the number of comorbid psychiatric disorders in adolescence predicted cannabis use in emerging adulthood. Increasing academic competence at high levels of social competence predicted less marijuana use. At emerging adulthood, greater use of alcohol and marijuana were associated with both criminality and psychopathology. ((c) 2005 APA, all rights reserved).

Substance Use among School-Based Youths in Northern Mexico

A small base of research suggests that adolescent substance use is a growing public health concern in Mexico. Employing confidential methods, the International Longitudinal Survey of Adolescent Health was administered to assess substance use among 1,238 students in northern Mexico. A large proportion of students indicated lifetime use of tobacco and alcohol.
Gender differences in tobacco, alcohol, and marijuana were also evident. The current findings are congruent with the sparse extant data on youths’ substance use in Mexico and highlight the need for early prevention interventions.

**Assessing cannabis use in adolescents and young adults: what do urine screen and parental report tell you?**

Objectives: Our analysis compares three approaches to detect the most common drug abused in early adulthood, cannabis: (1) report on direct structured interview; (2) indirect parental report; and (3) urine toxicology screen. Methods: We examined data on 207 subjects (36% also met criteria for alcohol abuse; 9% for alcohol dependence) derived from two prospective and ongoing family studies of boys and girls with or without attention-deficit/hyperactivity disorder (ADHD). Assessments relied on the Schedule for Affective Disorders and Schizophrenia (K-SADS-E; under 18 years of age) and on the Structured Clinical Interview for DSM-IV (SCID-IV; over 18 years of age). Urine samples were analyzed with Auccusign DOA5 (on-site screening assay). Results: Ninety-seven percent (97%) of individuals, who reported no use of cannabis within the past month, had a negative urine screening and 79% of individuals, who endorsed cannabis abuse/dependence, had a positive urine screening. The sensitivity of the direct structured interview report was 91%, the specificity 87%, the positive predicting value 67%, and the negative predictive value 97%. Indirect parental reports were found to be less informative on cannabis use than direct report. Conclusion: Direct report of cannabis use, abuse, or dependence during the structured interview is both sensitive and specific when compared to urine toxicology screens and indirect parental reports.

**God forbid! Substance use among religious and nonreligious youth**

Among a predominately Mexican and Mexican American sample of preadolescents, religiosity protected against lifetime alcohol, cigarette, and marijuana use and recent alcohol and cigarette use when religious affiliation was controlled. When religiosity was controlled, however, adolescents with no religious affiliation and adolescents who were religiously affiliated reported similar substance use outcomes. Interaction effects demonstrated that the protective effect of greater religiosity operated more strongly in some religions than in others for selected outcomes. Overall, the impact of religiosity on reported drug use did not differ significantly for more and less acculturated Latino youth. ((c) 2005 APA, all rights reserved).

**Lifetime associations between cannabis, use, abuse, and dependence and panic attacks in a representative sample**

**BACKGROUND:** The present investigation evaluated lifetime associations between cannabis use, abuse, and dependence and panic attacks after controlling for alcohol abuse, polysubstance use, and demographic variables. **METHODS:** Data for this study were obtained as part of a large statewide survey, the Colorado Social Health Survey (CSHS). Participants were contacted using randomly sampled household addresses (72% response rate) and interviews took place in participants’ homes. Participants consisted of a representative sample from the Colorado general adult population (n=4745; 52% female). The Diagnostic Interview Schedule was administered to obtain diagnoses. **RESULTS:** After controlling for polysubstance use, alcohol abuse, and demographic variables, lifetime history of cannabis dependence, but not use or abuse, was significantly related to an increased risk of panic attacks. Additionally, among participants reporting a lifetime history of both panic attacks and cannabis use, the age of onset of panic attacks (M=19.0 years of age) was significantly earlier than for individuals with a lifetime panic attack history but no cannabis use (M=27.6 years of age). **CONCLUSIONS:** Structured interview data suggest lifetime cannabis dependence is significantly associated with an increased risk of panic attacks.
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