INTRODUCTION
Here is the latest summary of research abstracts. Please note that the International Cannabinoid Research Society is meeting in Clearwater, Florida June 24-27th 2005. The conference schedule is now available at www.cannabinoidsociety.org.

BASIC SCIENCE

Anandamide (arachidonyl-ethanolamide, AEA) is an important endogenous cannabinoid ligand isolated from porcine brain. AEA has a flexible molecular structure with a series of four non-conjugated double bonds, a hydrophobic alkyl chain, and a carboxamide head group. It is known that AEA binds to cannabinoid receptor and induces cannabimimetic activity. However, questions still remain about the three-dimensional arrangement of the pharmacophoric groups of AEA that facilitate its interaction with cannabinoid receptor, a member of transmembrane G-protein coupled receptors (GPCRs). Such information is of critical importance for the design of novel analogs of potential therapeutic values. In the present studies, we developed a combined approach of 2D high-resolution NMR and computer modeling to investigate conformational features of AEA in solution. The developed method and experimental data is then applied to study the structural properties of AEA in a membrane-like environment that will be reported elsewhere. In addition to the measured NOEs, the dihedral angle constraints were for the first time being used as experimentally-determined structural constraints for performing molecular dynamics simulations to refine the NMR-determined AEA conformations. Our results showed that AEA prefers an extended pseudo-helical conformation in solution with two oxygen atoms pointing towards the same side and a straight pentyl chain, which was an averaged conformation observed on the basis of NMR time scale. The results were correlated to the computer predicted AEA models reported by others. The established NMR-based computational approach provides an alternative way to explore further the detailed conformational properties of AEA that encodes important pharmacophoric and conformational information regarding the activation of cannabinoid receptors.


Cannabinoid CB(1) receptors are involved in ocular physiology and may regulate intraocular pressure (IOP). However, endocannabinoid levels in human ocular tissues of cornea, iris, ciliary body, retina, and choroid from normal and glaucomatous donors have not been investigated. Anandamide (N-arachidonoyl ethanolamine; AEA), 2-arachidonoylglycerol (2-AG), and the anandamide congener, palmitoylethanolamide (PEA), were detected in all the human tissues examined. In eyes from patients with glaucoma, significantly decreased 2-AG and PEA levels were detected in the ciliary body, an important tissue in the regulation of IOP. The findings suggest that these endogenous compounds may have a role in this disease, particularly with respect to regulation of IOP.

The neuroprotective effects of Delta(9)-tetrahydrocannabinol (THC) were examined using an in vitro model in which the AF5 CNS cell line was exposed to toxic levels of N-methyl-d-aspartate (NMDA), an agonist of the NMDA glutamate receptor. NMDA toxicity was reduced by THC, but not by the more specific cannabinoid receptor agonist, WIN55,212-2. Addition of dibutyryl cAMP (dbcAMP) to the culture medium did not alter the neuroprotective effect of THC and did not unmask a neuroprotective effect of WIN55,212-2. The cannabinoid antagonist SR141716A did not inhibit the neuroprotection induced by THC or alter the response to WIN55,212-2, even in the presence of dbcAMP, indicating that the neuroprotective effect of THC was cannabinoid receptor-independent. On the other hand, both THC and WIN55,212-2 produced cellular toxicity at higher dosages, an effect which was blocked in part by SR141716A. Capsaicin, an antioxidant and vanilloid receptor agonist, also produced a protective effect against NMDA toxicity. The protective effect of capsaicin was blocked by co-application of ruthenium red, but was not blocked by the specific vanilloid receptor antagonist capsazepine, and the transient receptor potential vanilloid type 1 (TRPV1) and ANKTM1 transcripts were not detected in AF5 cells. Thus, the neuroprotective effects of THC and capsaicin did not appear to be mediated by TRP ion channel family receptors. The antioxidant alpha-tocopherol prevented neurotoxicity in a dose-dependent manner. Therefore, THC may function as an antioxidant to increase cell survival in NMDA-induced neurotoxicity in the AF5 cell model, while higher dosages produce toxicity mediated by CB1 receptor stimulation.


Cannabinoid (CB) receptor agonists have potential utility as anti-inflammatory drugs for the treatment of many disease conditions. In the present study, we investigated the effects of the synthetic CB(2) ligand, JWH-133 on the production of interleukins (ILs), IL-12 and IL-10 by lipopolysaccharide (LPS) or Theiler's virus (TMEV)-activated macrophages. JWH-133 evoked a concentration-related inhibition (10 nM-5 muM) of LPS/IFN-gamma induced IL-12p40 release. The effect of JWH-133 (100 nM) was significantly blocked by the CB(2) antagonist SR-144528 (1 muM). Macrophages infected with TMEV increased IL-12p40 production and activation of CB(2) receptors by JWH-133 (100 nM) inhibited it. The inhibitory effect of JWH-133 (100 nM) on IL-12p40 production may involve extracellular-regulated kinase (ERK1/2) signaling: (i) JWH-133 induced a greater and sustained activation of ERK1/2 kinase in comparison with the level of activation observed following LPS; (ii) the inhibition of ERK1/2 by the specific inhibitor PD98059 increased LPS-induced IL-12p40 production in the presence or absence of JWH-133 suggesting a negative regulation of ERK pathway on IL-12p40 biosynthesis. Activation of CB(2) receptors by JWH-133 (10 nM-5 muM) enhanced IL-10 release by LPS/IFN-gamma-activated macrophages and addition of SR144558 (1 muM) totally blocked the effect of JWH (100 nM). Inhibition of ERK by PD98059 significantly suppressed IL-10 production by LPS-activated macrophages. Endogenous IL-10 plays a modulatory role in IL-12 production. Blocking IL-10 with neutralizing antibody resulted in increased IL-12p40 secretion by LPS-activated macrophages in the absence or presence of JWH-133. In contrast, the addition of exogenous mIL-10 reduced the secretion of IL-12p40 in response to LPS. British Journal of Pharmacology advance online publication, 11 April 2005; doi:10.1038/sj.bjp.0706215.


Marijuana and its major psychotropic component, Delta(9)-tetrahydrocannabinol, stimulate appetite and increase body weight in wasting syndromes, suggesting that the CB(1) cannabinoid receptor and its endogenous ligands, the endocannabinoids, are involved in controlling energy balance. The endocannabinoid system controls food intake via both central and peripheral mechanisms, and it may also stimulate lipogenesis and fat accumulation. Here we discuss the multifaceted regulation of energy homeostasis by endocannabinoids, together with its applications to the treatment of eating disorders and metabolic syndromes.

Cannabinoid CB1 receptor (via Gs) and dopamine D2 receptor (via Gi/o) antagonistically modulate goldfish cone membrane currents. As ON bipolar cells have CB1 and D1 receptors, but not D2 receptors, we focused on whether CB1 receptor agonist and dopamine interact to modulate voltage-dependent outward membrane K+ currents I\(K(V)\) of the ON mixed rod/cone (Mb) bipolar cells. Whole-cell currents were recorded from Mb bipolar cells in goldfish retinal slices. Mb bipolar cells were identified by intracellular filling with Lucifer yellow. The bath solution was calcium-free and contained 1 mM cobalt to block indirect calcium-dependent effects. Dopamine (10 μM) consistently increased I\(K(V)\) by a factor of 1.57 +/- 0.12 (S.E.M., n = 15). A CB receptor agonist, WIN 55212-2 (0.25-1 μM), had no effect, but 4 μM WIN 55212-2 suppressed I\(K(V)\) by 60%. If I\(K(V)\) was first increased by 10 μM dopamine, application of WIN 55212-2 (0.25-1 μM) reversibly blocked the effect of dopamine even though these concentrations of WIN 55212-2 had no effect of their own. If WIN 55212-2 was applied first and dopamine (10 μM) was added to the WIN-containing solution, 0.1 μM WIN 55212-2 blocked the effect of dopamine. All effects of WIN 55212-2 were blocked by coapplication of SR 141716A (CB1 antagonist) and pretreatment with pertussis toxin (blocker of Gi/o) indicating action via CB1 receptor activation of G protein Gi/o. Coactivation of CB1 and D1 receptors on Mb bipolar cells produces reciprocal effects on I\(K(V)\). The CB1-evoked suppression of I\(K(V)\) is mediated by G protein Gi/o, whereas the D1-evoked enhancement is mediated by G protein Gs. As dopamine is a retinal "light" signal, these data support our notion that endocannabinoids function as a "dark" signal, interacting with dopamine to set retinal sensitivity.


OBJECTIVE: While endogenous cannabinoids regulate various physiologic functions, their role in the intestinal tract is unclear. We continuously recorded colonic motility in conscious guinea pigs. Mechanisms of action then were investigated using guinea pig taenia caecum in vitro. DESIGN: Prospective experimental observations using the cannabinoid agonists 2-arachidonoylglycerol (2-AG) and WIN55212-2; a cannabinoid antagonist, AM281; and ion-channel antagonist. SETTING: University research laboratory. SUBJECTS: Thirty guinea pigs (20 for in vivo study, 10 for in vitro). MEASUREMENTS AND MAIN RESULTS: Colonic motility was monitored in vivo using telemetry via a force transducer attached to the guinea pig taenia caecum. Taenias isolated from other guinea pigs were studied in vitro to assess cannabinoid effects on muscle contractions evoked pharmacologically or electrically. Immediately after cannabinoid injection in conscious guinea pigs, taenial relaxation began peaking at 30 to 40 min. In animals pretreated with AM281, a CB1 cannabinoid receptor antagonist, cannabinoid evoked relaxation was less evident. In vitro, cannabinoids suppressed KCl-induced taenial contractions; this suppression was opposed by charybdotoxin, a Ca(2+)-activated K(+) channel inhibitor, but not AM281. Cannabinoids decreased amplitude of repeated contractions evoked by electrical stimulation (an effect inhibited by AM281) but not muscle tension. CONCLUSIONS: Cannabinoids decreased intestinal tract tension in vivo, apparently via central CB1 receptors. This differs from peristaltic suppression.


We wanted to search for the mechanism(s) responsible for the brief pressor response induced by anandamide in urethane-anaesthetized rats. The anandamide-induced pressor effect was not modified by the antagonists of cannabinoid CB(1) and vanilloid TRPV(1) receptors, SR 141716A (3 mumol kg(-1)) and capsazepine (1 mumol kg(-1)), respectively, by bilateral vagotomy and by pithing. Replacement of urethane by pentobarbitone virtually abolished the pressor effect of anandamide, both in pithed and vagotomized and in 'intact' rats (i.e. not treated in this manner). The pressor effect of anandamide was reduced by the nonselective TRPV family inhibitor ruthenium red (3 mumol kg(-1)) and by the blocker of L-type calcium channels nifedipine (1 mumol kg(-1)), both in pithed urethane-anaesthetized rats and in 'intact' urethane-
anaesthetized rats. The nonselective beta-adrenoceptor antagonist propranolol (0.1 or 0.3 mumol kg\(^{-1}\)) and the nonselective NMDA receptor antagonist MK-801 (1 mumol kg\(^{-1}\)) diminished the anandamide-induced vasopressor response in 'intact' but not in pithed rats. The inhibitory effect of propranolol in 'intact' rats was mimicked by the beta(2)-adrenoceptor antagonist ICI 118551 (1 mumol kg\(^{-1}\)), but not by the beta(1)-adrenocceptor antagonist CGP 20712 (1 mumol kg\(^{-1}\)). The present study revealed that two mechanisms may be responsible for the anandamide-induced pressor response in urethane-anaesthetized rats. The first involves the central nervous system (probably the medulla oblongata) and is sensitive to propranolol and MK-801. The second, which is located peripherally (most probably in blood vessels), is sensitive to nifedipine, ruthenium red and pentobarbitone and, hence, probably represents a Ca(2+)-dependent mode of action. 

British Journal of Pharmacology advance online publication, 18 April 2005; doi:10.1038/sj.bjp.0706195.


Cannabinoids have been reported to provide neuroprotection in acute and chronic neurodegeneration. In this study, we examined whether they are also effective against the toxicity caused by 6-hydroxydopamine, both in vivo and in vitro, which may be relevant to Parkinson's disease (PD). First, we evaluated whether the administration of cannabinoids in vivo reduces the neurodegeneration produced by a unilateral injection of 6-hydroxydopamine into the medial forebrain bundle. As expected, 2 weeks after the application of this toxin, a significant depletion of dopamine contents and a reduction of tyrosine hydroxylase activity in the lesioned striatum were noted, and were accompanied by a reduction in tyrosine hydroxylase-mRNA levels in the substantia nigra. None of these events occurred in the contralateral structures. Daily administration of Delta(9)-tetrahydrocannabinol (Delta(9)-THC) during these 2 weeks produced a significant waning in the magnitude of these reductions, whereas it failed to affect dopaminergic parameters in the contralateral structures. This effect of Delta(9)-THC appeared to be irreversible since interruption of the daily administration of this cannabinoid after the 2-week period did not lead to the re-initiation of the 6-hydroxydopamine-induced neurodegeneration. In addition, the fact that the same neuroprotective effect was also produced by cannabidiol (CBD), another plant-derived cannabinoid with negligible affinity for cannabinoid CB(1) receptors, suggests that the antioxidant properties of both compounds, which are cannabinoid receptor-independent, might be involved in these in vivo effects, although an alternative might be that the neuroprotection exerted by both compounds might be due to their anti-inflammatory potential. As a second objective, we examined whether cannabinoids also provide neuroprotection against the in vitro toxicity of 6-hydroxydopamine. We found that the non-selective cannabinoid agonist HU-210 increased cell survival in cultures of mouse cerebellar granule cells exposed to this toxin. However, this effect was significantly lesser when the cannabinoid was directly added to neuronal cultures than when these cultures were exposed to conditioned medium obtained from mixed glial cell cultures treated with HU-210, suggesting that the cannabinoid exerted its major protective effect by regulating glial influence to neurons. In summary, our results support the view of a potential neuroprotective action of cannabinoids against the in vivo and in vitro toxicity of 6-hydroxydopamine, which might be relevant for PD. Our data indicated that these neuroprotective effects might be due, among others, to the antioxidant properties of certain plant-derived cannabinoids, or exerted through the capability of cannabinoid agonists to modulate glial function, or produced by a combination of both mechanisms.


BACKGROUND AND PURPOSE: Cannabidiol has been reported to be a neuroprotectant, but the neuroprotective mechanism of cannabidiol remains unclear. We studied the neuroprotective mechanism of cannabidiol in 4-hour middle cerebral artery (MCA) occlusion mice. METHODS: Male MCA occluded mice were treated with cannabidiol, abnormal cannabidiol, anandamide, methanandamide, cannabidiol plus capsazepine, and cannabidiol plus WAY100135 before and 3 hours after MCA occlusion. The infarct size was determined after 24 hours (2,3,5-triphenyltetrazolium chloride staining). Cerebral blood flow (CBF) was measured at, before and 1,
2, 3, and 4 hours after MCA occlusion. RESULTS: Cannabidiol significantly reduced the infarct volume induced by MCA occlusion in a bell-shaped curve. Similarly, abnormal cannabidiol but not anandamide or methanandamide reduced the infarct volume. Moreover, the neuroprotective effect of cannabidiol was inhibited by WAY100135, a serotonin 5-hydroxytriptamine(1A) (5-HT(1A)) receptor antagonist but not capsazepine a vanilloid receptor antagonist. Cannabidiol increased CBF to the cortex, and the CBF was partly inhibited by WAY100135 in mice subjected to MCA occlusion. CONCLUSIONS: Cannabidiol and abnormal cannabidiol reduced the infarct volume. Furthermore, the neuroprotective effect of cannabidiol was inhibited by WAY100135 but not capsazepine, and the CBF increased by cannabidiol was partially reversed by WAY100135. These results suggested that the neuroprotective effect of cannabidiol may be related to the increase in CBF through the serotonergic 5-HT(1A) receptor.


Cannabidiol is a non-psychotomimetic compound from Cannabis sativa. It is proposed as a possible antipsychotic drug, since it can prevent some psychotomimetic-like effects of Delta(9)-tetrahydrocannabinol or apomorphine. Therefore, the aim of this work was to test the hypothesis that cannabidiol would inhibit the hyperlocomotion induced by two psychotomimetic drugs, d-amphetamine or ketamine. Male Swiss mice received i.p. injections of haloperidol (0.15-0.6 mg/kg), clozapine (1.25-5 mg/kg) or cannabidiol (15-60 mg/kg) followed by d-amphetamine (5 mg/kg) or ketamine (60 mg/kg). Thirty minutes after the first injection, the distance moved in circular arena was measured during 10 min. In another group of experiments, catalepsy was measured 30 min after haloperidol, clozapine or cannabidiol injections. Cannabidiol, like clozapine but unlike haloperidol, inhibited hyperlocomotion without inducing catalepsy. Moreover, cannabidiol itself, unlike haloperidol and clozapine, did not decrease locomotion. In conclusion, cannabidiol exhibits an antipsychotic-like profile without inducing extrapyramidal-like effects.


A series of 4,5-dihydro-1H-benzo[g]indazole-3-carboxamides (2a-k) as analogues of the previously reported CB(2) ligands 6-chloro- and 6-methyl-1-(2',4'-dichlorophenyl)-N-piperidin-1-yl-1,4-dihydroinden[1,2-c]pyrazole-3-carboxamides (1a,b) was synthesized and their affinity and selectivity towards CB(1) and CB(2) receptors were evaluated. Several of the new compounds (2a,b,c,d and i) exhibited CB(1) affinity in the nanomolar range with moderate or negligible affinity towards CB(2) receptors. Compounds 2a and c increased intestinal propulsion in mouse. Their pro-kinetic effects were reversed by the reference CB agonist CP-55,940. Consequently, in vivo CB(1) antagonistic activity was highlighted for these compounds.


Interest in cannabinoid pharmacology developed rapidly since the discovery of cannabinoids receptors and endocannabinoids. Modulation of this system is becoming a hot topic in cardiovascular pharmacology mainly at the light of recent findings. Among them, cardiac effects of cannabinoids were described with respect to their probable participation to the well-studied preconditioning phenomenon. Beneficial effects of post-infarction cannabinoids administration against ischemia-reperfusion injury were also reported. Concerning their vascular effects the situation is more complex some studies reporting pressor effects while others depressor ones. It was also proposed that the endothelium-derived hyperpolarizing factor released by various vasodilators may be an endocannabinoid an hypothesis still discussed. Finally, pathological situations concerning the cardiovascular system and including brain ischemia, hemorrhagic and endotoxic shocks were reported to be linked with endocannabinoids. However, the clinical use of cannabinoid receptors agonists or antagonists will depend on the development of non psychoactive compounds.

Several G protein-coupled receptors (GPCRs), including cannabinoid CB(1) and CB(2) receptors, show constitutive activity under heterologous expression. Such a tonic response is generated in the absence of an activating ligand, and can be inhibited by inverse agonists. Neutral antagonists, however, are silent at such receptors, but can reverse both agonist and inverse agonist responses. To date, no neutral antagonist for the CB(2) receptor has been reported. Here, by monitoring receptor-dependent G protein activation, we demonstrate that WIN55212-3 acts as a neutral antagonist at the human CB(2) (hCB(2)) receptor. WIN55212-3 alone, at concentrations \(\leq 10^{-4}\) M, behaved as a silent ligand exhibiting no agonist or inverse agonist activity. However, WIN55212-3 competitively antagonized cannabinoid agonist CP-55,940-stimulated responses (\(pA(2) = 6.1\)). Importantly, the inverse agonism evoked by SR144528 in hCB(2) was dose-dependently reversed by WIN55212-3 (\(pEC(50) = 5.3\pm 0.2\)), indicating true neutral antagonist behavior. Furthermore, WIN55212-3 also antagonized CB(1) receptor signaling in a competitive manner (\(pA(2) = 5.6\)), but behaved as a partial inverse agonist (\(pIC(50) = 5.5\pm 0.1\)) at the constitutively active human CB(1). Additionally, WIN55212-3 antagonized signaling of the human melatonin MT(1) receptor, with modest activity at the human muscarinic M4 receptor, but it was inactive towards several other GPCRs. These data identify WIN55212-3 as a true neutral hCB(2) receptor antagonist. WIN55212-3 offers a valuable tool for further characterization of ligand activities at the CB(2) receptor and may serve as a lead compound in further efforts to develop more potent and selective neutral CB(2) receptor antagonists. British Journal of Pharmacology advance online publication, 25 April 2005; doi:10.1038/sj.bjp.0706230.


The endogenous cannabinoid anandamide (AEA) is a lipid mediator that blocks proliferation and induces apoptosis in many cell types. Although AEA levels are elevated in liver fibrosis, its role in fibrogenesis remains unclear. This study investigated effects of AEA in primary hepatic stellate cells (HSCs). Anandamide blocked HSC proliferation at concentrations of 1 to 10 mumol/L but did not affect HSC proliferation or activation at nanomolar concentrations. At higher concentrations (25-100 mumol/L), AEA rapidly and dose-dependently induced cell death in primary culture-activated and in vivo-activated HSCs, with over 70% cell death after 4 hours at 25 mumol/L. In contrast to treatment with Fas ligand or gliotoxin, AEA-mediated death was caspase independent and showed typical features of necrosis such as rapid adenosine triphosphate depletion and propidium iodide uptake. Anandamide-induced reactive oxygen species (ROS) formation, and an increase in intracellular Ca(2+)i. Pretreatment with the antioxidant glutathione or Ca(2+)i-chelation attenuated AEA-induced cell death. Although the putative endocannabinoid receptors CB1, CB2, and VR1 were expressed in HSCs, specific receptor blockade failed to block cell death. Depletion of membrane cholesterol by methyl-beta-cyclodextrin inhibited AEA binding, blocked ROS formation and intracellular Ca(2+)i-increase, and prevented cell death. In primary hepatocytes, AEA showed significantly lower binding and failed to induce cell death even after prolonged treatment. In conclusion, AEA efficiently induces necrosis in activated HSCs, an effect that depends on membrane cholesterol and a subsequent increase in intracellular Ca(2+)i and ROS. The anti-proliferative effects and the selective killing of HSCs, but not hepatocytes, indicate that AEA may be used as a potential anti-fibrogenic tool. Supplementary material for this article can be found on the HEPATOLOGY website (http://www.interscience.wiley.com/jpages/0270-9139/suppmat/index.html). (HEPATOLOGY 2005;41:1085-1095.).


Immune system responsiveness results from numerous factors, including endogenous cannabinoid signaling in immunocytes termed the "immunocannabinoid" system. This system can be an important signaling pathway for immune modulation. To assess the immunomodulating role of the cannabinoid 2 (CB2) receptor, we sought polymorphisms in the human gene, identified a common dinucleotide polymorphism, and investigated its effect on endocannabinoid-induced
inhibition of T lymphocyte proliferation. The CB2 cDNA 188-189 GG/GG polymorphism predicts the substitution of glutamine at amino acid position 63 by arginine. T lymphocytes from CB2 188-189 GG/GG homozygotes had approximately twofold reduction of endocannabinoid-induced inhibition of proliferation compared with cells from CB2 188-189 AA/AA homozygotes. In GG/GG subjects, the reduced endocannabinoid inhibitory response was highly significant for N-arachidonylglycine and nearly significant for 2-arachidonyleglycerol, and a specific CB2 receptor antagonist partially blocked these effects. Also, patients with autoimmune diseases had an increased prevalence of the homozygous GG/GG genotype. Collectively, these results demonstrate reduced endogenous fatty acid amide immunomodulatory responses in individuals with the CB2 188-189 GG/GG genotype and suggest that this CB2 gene variation may be a risk factor for autoimmunity. The results also support the proposition that the CB2 receptor may represent a novel pharmacological target for selective agonists designed to suppress autoreactive immune responses while avoiding CB1 receptor-mediated cannabinoid adverse effects.


RATIONALE: The CB1 receptor antagonist SR141716A reduces food intake in rats. This effect is likely to depend on modulation of reward related processes.OBJECTIVE: To investigate the effects of SR141716A on responding for food under a second order instrumental task in which responding and consumption of food can be separated, and on Pavlovian responding for a stimulus predictive of food reward.METHODS: Instrumental responding and pellet consumption following administration of SR141716A (0-3 mg/kg) were recorded under an FI5 min FR5(5:S) operant schedule that incorporates both a 5 min initial appetitive phase and a 25 min consummatory phase. We compared the drug-induced change in responding to that recorded following a reduction in motivational state induced by pre-feeding. In a second experiment we assessed the effects of SR141716A (0-3 mg/kg) on Pavlovian approach behaviour for a stimulus (lever) associated with food reward (CS+) and a neutral stimulus (lever) not associated with reward (CS-).RESULTS: SR141716A reduced pellet consumption and instrumental responding during both the appetitive and consummatory phases of the second order schedule. Pre-feeding had a similar effect on responding during the appetitive phase, suggesting an effect on incentive motivation. SR141716A also blocked an enhancement of responding that occurred during the consummatory phase in pre-fed animals. SR141716A and pre-feeding had no effect on responding in the Pavlovian autoshaping paradigm.CONCLUSIONS: SR141716A impacts on motivational processes in both the appetitive and consummatory phases of feeding behaviour.


While it is widely accepted that Delta(9)-THC is the primary psychoactive constituent of marijuana, questions persist as to whether other components contribute to marijuana's pharmacological activity. The present experiments assessed the cannabinoid activity of marijuana smoke exposure in mice, and tested the hypothesis that Delta(9)-THC mediates these effects through a CB1 receptor mechanism of action. First, the effects of Delta(9)-THC on analgesia, hypothermia, and catalepsy were compared with those of a marijuana extract with equated Delta(9)-THC content after either i.v. administration or inhalation exposure. Second, mice were exposed to smoke of an ethanol-extracted placebo plant material or ditchweed (marijuana with minimal Delta(9)-THC but similar levels of other cannabinoids) that were impregnated with varying quantities of Delta(9)-THC. In order to assess doses, Delta(9)-THC levels in the blood and brains of drug-exposed mice were determined following both i.v. and inhalation routes of administration. Both marijuana and Delta(9)-THC produced comparable levels of antinociception, hypothermia, and catalepsy regardless of the route of administration, and these effects were blocked by pretreatment with the CB1 antagonist SR141716. Importantly, the blood and brain levels of Delta(9)-THC were similar in mice exhibiting similar behavioral effects, regardless of the presence of non-Delta(9)-THC marijuana constituents. The present experiments provide evidence that the acute cannabinoid effects of marijuana smoke exposure on analgesia, hypothermia, and
catalepsy in mice result from Delta(9)-THC content acting at CB1 receptors, and that the non-
Delta(9)-THC constituents of marijuana (at concentrations relevant to those typically found in
nature) influence these effects only minimally, if at all.

Vlachou, S., G. G. Nomikos, et al. (2005). "CB(1) cannabinoid receptor agonists increase

RATIONALE: Addictive drugs have a number of commonalities in animal behavioral
models. They lower intracranial self-stimulation (ICSS) thresholds, support self-administration,
and produce conditioned place preference (CPP). However, cannabinoids appear atypical as
drugs of abuse, since there are controversial data in the literature concerning their reinforcing
properties. OBJECTIVES: The aim of the present study was to examine the effects of
cannabinoids on brain reward using the rate-frequency curve shift paradigm of ICSS. METHODS:
Male Sprague-Dawley rats were implanted with electrodes into the medial forebrain bundle
(MFB). Rate-frequency functions were determined by logarithmically decreasing the number of
cathodal pulses in a stimulation train from a value that sustained maximal responding to one that
did not sustain responding. After brain stimulation reward thresholds stabilized rats received
intraperitoneal (IP) injections of the potent CB(1) receptor agonists WIN 55,212-2 (graded doses
0.1, 0.3, 1 and 3 mg/kg), CP 55,940 (graded doses 10, 30, 56 and 100 mug/kg), or HU-210
(graded doses 10, 30, 100 mug/kg). RESULTS: With the exception of the highest dose of all
cannabinoid agonists tested, which significantly increased the threshold frequency required for
MFB ICSS, all other doses of the tested drugs did not affect ICSS thresholds. The CB(1) receptor
agonist antagonist SR141716A reversed the actions of WIN 55,212-2 and CP 55,940, but not HU-210.
However, the selective CB(1) cannabinoid receptor antagonist AM 251 counteracted the effect of
HU-210. Both CB(1) receptor antagonists, at the doses used in the present study, did not affect
reward thresholds by themselves. CONCLUSIONS: The present results indicate that cannabinoid
agonists do not exhibit reinforcing properties in the ICSS paradigm, but rather have an inhibitory
influence on reward mechanisms. The results suggest that the anhedonic effects of cannabinoids
are probably mediated by cannabinoid CB(1) receptors.

Zhang, R., D. P. Hurst, et al. (2005). "Cysteine 2.59(89) in the Second Transmembrane Domain
of Human CB2 Receptor Is Accessible within the Ligand Binding Crevise: Evidence for Possible
CB2 Deviation from a Rhodopsin Template." Mol Pharmacol.

In this study, the sensitivity of the CB2 receptor to methanethiosulfonate (MTS)
derivatives was tested and a native cysteine residue conferring the sensitivity was identified. By
incubating HEK293 cells stably transfected with CB2 receptors and MTS derivatives such as MTS
ethylammonium (MTSEA), [(3)H]-HU-243 binding was inhibited. Pretreatment of the CB2 receptor
with cannabinoid ligands prevented this inhibition, suggesting that MTSEA modification occurred
within the binding crevice. In order to identify the cysteine(s) responsible for the MTSEA
sensitivity, ten CB2 mutants were prepared, in which the eight cysteines in transmembrane
domains or extracellular loop 2 were mutated to serine or alanine, one at a time or in
combination. Five mutants exhibited specific [(3)H]-HU-243 binding, with Kd and Bmax values
similar to wild-type CB2. However, five other mutants had no detectable ligand binding and were
not detected on cell membranes by Western blot analysis. Among the five mutants with normal
binding, only the C2.59(89)S mutant's sensitivity to MTSEA was reduced significantly. These data
demonstrate that C2.59(89) is the residue mainly conferring the inhibitory effect of MTSEA on
ligand binding. Further, the magnitude of the second-order rate constant (1.14+/-.028 M(-1)s(-1))for the MTSEA reaction with wild-type CB2 suggests that C2.59(89) resides at the margin of
the CB2 binding site crevice. The accessibility of C2.59(89) to MTSEA provides experimental
evidence for a possible conformational difference between TMH2 of CB2 versus Rho. Modeling
studies undertaken to explore the origin of such differences suggest it is possibly due to the
conformational influence of S2.54(84).
CLINICAL SCIENCE


According to the WHO, over one billion adults worldwide are currently overweight. Unless appetites are tamed by medication, or people finally learn to resist temptation, the future looks bleak.


RATIONALE: Oral Delta-9-tetrahydrocannabinol (Delta(9)-THC; Marinol) is medically available for the treatment of nausea associated with cancer chemotherapy and for wasting syndromes related to HIV/AIDS. Little is known about its reinforcing effects. OBJECTIVE: This study was conducted to characterize the reinforcing effects of oral Delta(9)-THC in experienced marijuana smokers under controlled laboratory conditions. METHODS: Ten healthy male marijuana users completed this 17-day residential study. On days 2, 6, 10, and 14, at 0900 h, participants received a "sample" oral dose of Delta(9)-THC (0, 10, 20 mg) and an alternative reinforcer, a $2 voucher (redeemable for cash at study's end). Over the next 3 days, they had 11 opportunities to self-administer either the sampled dose of Delta(9)-THC or to receive a $2 voucher. RESULTS: Participants chose active Delta(9)-THC (10 and 20 mg) more often than placebo (<two selections vs approximately four selections, respectively). However, they chose active Delta(9)-THC on less than 50% of choice opportunities. Both active Delta(9)-THC doses produced significant increases in "positive" subjective effects, impaired psychomotor performance, and increased heart rate, relative to the placebo conditions. CONCLUSION: These data indicate that oral Delta(9)-THC may have modest abuse liability in experienced marijuana smokers.


The primary goal of this prospective extended case series was to obtain the first data about the potential influence of nabilone intake on driving ability related neuropsychological functions. Six patients were investigated within a placebo controlled, double-blind crossover study of this synthetic cannabinoid (2 mg/day) in patients with multiple sclerosis and spasticity associated pain. Five neuropsychological functions (reaction time, working memory, divided attention, psychomotor speed and mental flexibility) were assessed. No indication was found of a deterioration of any of the five investigated neuropsychological functions during the 4-week treatment period with nabilone.


The endocannabinoid system, consisting of two cannabinoid receptors (CB(1) and CB(2)) and the endogenous ligands anandamide (arachidonylethanolamide (AEA)) and 2-arachidonoylglycerol (2-AG), has been shown to control food intake in both animals and humans, modulating either rewarding or quantitative aspects of the eating behavior. Moreover, hypothalamic endocannabinoids seem to be part of neural circuitry involved in the modulating effects of leptin on energy homeostasis. Therefore, alterations of the endocannabinoid system could be involved in the pathophysiology of eating disorders, where a deranged leptin signalling has been also reported. In order to verify this hypothesis, we measured plasma levels of AEA, 2-AG, and leptin in 15 women with anorexia nervosa (AN), 12 women with bulimia nervosa (BN), 11
women with binge-eating disorder (BED), and 15 healthy women. Plasma levels of AEA resulted significantly enhanced in both anorexic and BED women, but not in bulimic patients. No significant change occurred in the plasma levels of 2-AG in all the patients' groups. Moreover, circulating AEA levels were significantly and inversely correlated with plasma leptin concentrations in both healthy controls and anorexic women. These findings show for the first time a derangement in the production of the endogenous cannabinoid AEA in drug-free symptomatic women with AN or with BED. Although the pathophysiological significance of this alteration awaits further studies to be clarified, it suggests a possible involvement of AEA in the mediation of the rewarding aspects of the aberrant eating behaviors occurring in AN and BED.


No abstract.


BACKGROUND: The brain endogenous cannabinoid system modulates reward and craving pathways and consequently may affect body weight. A naturally occurring missense polymorphism in the gene encoding fatty acid amide hydrolase (FAAH), the primary enzyme for inactivation of endocannabinoids, is associated with problem drug use. AIMS: To investigate the relationship between the FAAH cDNA 385 A/A (P129T) polymorphism and overweight disorders in subjects of multiple ethnic backgrounds attending a medical screening clinic. SUBJECTS: A total of 2667 subjects of white, black and Asian ancestry were genotyped and stratified by a standardized clinic-based assessment of body mass index (BMI, weight in kilograms/(height in meters)(2) or kg/m(2)). METHODS: Subjects were genotyped for the FAAH cDNA 385 C --> A polymorphism using allele-specific oligonucleotide hybridization methods by investigators blinded to all clinical information. BMI was calculated based on exact clinical measurements and World Health Organization ranges were used to stratify subjects. Statistical methods included the Fisher exact test, Mann-Whitney U-test and multivariable logistic regression analysis. RESULTS: The homozygous FAAH 385 A/A genotype was significantly associated with overweight and obesity in white subjects (P=0.005) and in black subjects (P=0.05) but not in a small group of Asians. The median BMI for all subjects was significantly greater in the FAAH 385 A/A genotype group compared to heterozygote and wild-type groups (P=0.0001). In white subjects, there was an increasing frequency of the FAAH 385 A/A genotype with increasing BMI categories of overweight (P=0.02) and obese (P=0.006) with the same trend in black subjects. CONCLUSIONS: These results suggest a role for the FAAH 385 A/A missense polymorphism as an endocannabinoid risk factor in overweight/obesity and may provide indirect evidence to support cannabinoid antagonist treatment strategies in overweight disorders.


BACKGROUND: In animal models, cannabinoid-1 receptor (CB1) blockade produces a lean phenotype, with resistance to diet-induced obesity and associated dyslipidaemia. We assessed the effect of rimonabant, a selective CB1 blocker, on bodyweight and cardiovascular risk factors in overweight or obese patients. METHODS: patients with body-mass index 30 kg/m2 or greater, or body-mass index greater than 27 kg/m2 with treated or untreated dyslipidaemia, hypertension, or both, were randomised to receive double-blind treatment with placebo, 5 mg rimonabant, or 20 mg rimonabant once daily in addition to a mild hypocaloric diet (600 kcal/day deficit). The primary efficacy endpoint was weight change from baseline after 1 year of treatment
in the intention-to-treat population. FINDINGS: Weight loss at 1 year was significantly greater in patients treated with rimonabant 5 mg (mean -3.4 kg [SD 5.7]; p=0.002 vs placebo) and 20 mg (-6.6 kg [7.2]; p<0.001 vs placebo) compared with placebo (-1.8 kg [6.4]). Significantly more patients treated with rimonabant 20 mg than placebo achieved weight loss of 5% or greater (p<0.001) and 10% or greater (p<0.001). Rimonabant 20 mg produced significantly greater improvements than placebo in waist circumference, HDL-cholesterol, triglycerides, and insulin resistance, and prevalence of the metabolic syndrome. The effects of rimonabant 5 mg were of less clinical significance. Rimonabant was generally well tolerated with mild and transient side effects. INTERPRETATION: CB1 blockade with rimonabant 20 mg, combined with a hypocaloric diet over 1 year, promoted significant decrease of bodyweight and waist circumference, and improvement in cardiovascular risk factors.


BEHAVIOURAL SCIENCE


Aims To investigate the relationship between marijuana use prior to driving, habitual marijuana use and car crash injury Design and setting Population based case-control study in Auckland, New Zealand. Participants Case vehicles were all cars involved in crashes in which at least one occupant was hospitalized or killed anywhere in the Auckland region, and control vehicles were a random sample of cars driving on Auckland roads. The drivers of 571 case and 588 control vehicles completed a structured interview. Measurements Self reported marijuana use in the 3 hours prior to the crash/survey and habitual marijuana use over the previous 12 months were recorded, along with a range of other variables potentially related to crash risk. The main outcome measure was hospitalization or death of a vehicle occupant due to car crash injury. Findings Acute marijuana use was significantly associated with car crash injury, after controlling for the confounders age, gender, ethnicity, education level, passenger carriage, driving exposure and time of day (OR 3.9, 95% CI 1.2-12.9). However, after adjustment for these confounders plus other risky driving at the time of the crash (blood alcohol concentration, seat-belt use, travelling speed and sleepiness score), the effect of acute marijuana intake was no longer significant (OR 0.8, 95% CI 0.2-3.3). There was a strong significant association between habitual use and car crash injury after adjustment for all the above confounders plus acute use prior to driving (OR 9.5, 95% CI 2.8-32.3). Conclusions This population-based case-control study indicates that habitual use of marijuana is strongly associated with car crash injury. The nature of the relationship between marijuana use and risk-taking is unclear and needs further research. The prevalence of marijuana use in this driving population was low, and acute use was associated with habitual marijuana use, suggesting that intervention strategies may be more effective if they are targeted towards high use groups.


This study investigated the relations between anxious, depressive and borderline symptomatology, motivations for cannabis use, and cannabis use and dependence among 212 adolescents and young adults, 114 of whom were cannabis users. Motives for cannabis use were assessed using the Marijuana Motives Measure (Simons, J., Correia, C. J., Carey, K. B., & Borsari, B. E. (1998). Validating a Five-Factor Motives Measure: Relations with use, problems and alcohol motives. Journal of Counseling Psychology, 45, 265-273.). In three sets of regression analyses, motives, cannabis use frequency, and cannabis dependence served as criterion variables. First, when motives were regressed on psychopathological measures, borderline symptomatology predicted expansion motives in both boys and girls. Second, when frequency of use was regressed on motives and psychopathological measures, enhancement motives were the only significant predictor among boys and expansion motives were the only significant
predictor among girls. Finally, when cannabis dependence was regressed on motives and psychopathological measures, borderline symptomatology was the only significant predictor in boys and expansion motives were the only significant predictor in girls. This study suggests the importance of motives and borderline symptomatology in the understanding of cannabis use and dependence among adolescents and young adults.


Aims To assess if cannabis use is a risk factor for future psychotic symptoms, and vice versa, in adolescents and young adults from the general population. Design Cohort study. Setting/participants Zuid Holland study, a 14-year follow-up study of 1580 initially 4-16-year-olds who were drawn randomly from the Dutch general population. Because cannabis use is generally condoned in the Netherlands, false-negative reports of cannabis use may occur less frequently than in countries with stricter drug policies, which supports the value of the present study. Measurements Life-time cannabis use and psychotic symptoms, assessed with the Composite International Diagnostic Interview (CIDI). Findings Cannabis use, in individuals who did not have psychotic symptoms before they began using cannabis, predicted future psychotic symptoms (hazard ratio = 2.81; 95% confidence interval = 1.79-4.43). However, psychotic symptoms in those who had never used cannabis before the onset of psychotic symptoms also predicted future cannabis use (hazard ratio = 1.70; 95% confidence interval = 1.13-2.57). Conclusions The results imply either a common vulnerability with varying order of onset or a bi-directional causal relationship between cannabis use and psychosis. More research on patterns and timings of these relationships is needed to narrow down the possibilities.


Marijuana smoking produces effects that may persist for hours or days beyond the period of acute intoxication. Despite evidence that adolescence represents a period of heightened exposure to marijuana, little research exists regarding possible impairment in adolescents who smoke marijuana regularly, and none exists regarding basic behavioral processes. In the present study, adolescents who smoked marijuana on a regular basis (near daily) were compared to a control group of adolescents on a two-option experimental task designed to measure motivation. The contingencies were arranged such that one option (work), which required systematically increasing response output, initially produced greater rates of monetary reinforcement than an alternative option (non-work) that required no response output to earn money. Switching to the non-work option was interpreted as a measure of reduced motivation. Significant differences were found between the groups: the marijuana-smoking participants switched earlier to the non-work option, and derived a greater percentage of their earnings from the non-work option. These differences existed when controlling for differences in cognitive aptitude, gender, and the presence of conduct disorder. A significant correlation between cannabinoid levels and percent of earnings derived from the non-work option suggests that these effects could be associated with the presence of cannabionoids in the marijuana-smoking group.


11-Nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) distributions in postmortem specimens are rarely reported. Fifty New Jersey State Medical Examiner's cases in which automobile accident deaths suspected of involving marijuana intake were studied for the distributions of THC-COOH in postmortem urine, blood, vitreous humor, and bile specimens.
Cases were selected based on immunoassay (TDx) urine test results. If the preliminary urine test indicated the presence of THC-COOH (apparent THC-COOH concentration ≥ 20 ng/mL), urine, heart blood, vitreous humor, and bile specimens from the case were analyzed for THC-COOH concentrations by gas chromatography-mass spectrometry. The mean, standard deviation, and range of THC-COOH in heart blood, urine, and bile found in these 50 cases were 0.081, 0.082, and 0.016-0.33 microg/mL; 0.314, 0.415, and 0.044-2.33 microg/mL; and 12.9, 11.4, and 1.03-43.7 microg/mL, respectively. THC-COOH was absent (detection limit, 1 ng/mL) or at low concentration (< 10 ng/mL) in vitreous humor specimens. The mean, standard deviation, and range of the bile-to-blood and urine-to-blood ratios were 242, 196, and 17.2-888 and 4.70, 4.05, and 1.14-19.2, respectively. The highest concentrations of THC-COOH were found in bile and the lowest in vitreous humor. These findings are consistent with the high hydrophobicity nature of THC-COOH and further suggest that bile is the specimen of choice for detecting low level of THC-COOH in postmortem cases.


Recent drug-use monitoring among Houston adolescents has detected a concoction of cigarettes or marijuana sticks laced with embalming fluid and PCP ("fry"). To shed light on this mixture, the current pilot study used a qualitative approach to investigate relevant beliefs and norms associated with fry initiation and perceived addiction among 38 youth who were attending outpatient and inpatient drug-user treatment programs in the spring of 2003. Respondents perceived that addiction to fry could occur as early as initial consumption, and the majority of participants indicated that their second fry event occurred either the same day as their initial use or the next day. In addition, fry use was perceived to have extremely dangerous consequences. Youth stated that users have impaired motor skills, hallucinations, long-term mental health problems, incoherent behavior, paranoia, and aggressive behaviors. Implications for these results are discussed.


The current investigation uses a large non-clinical sample of undergraduate college students (N=189) to investigate schizotypal traits among cannabis and non-cannabis users, as well as the temporal order of the onset of these traits and cannabis use. Findings suggest that regular cannabis users are significantly more prone to cognitive and perceptual distortions as well as disorganization, but not interpersonal deficits, than non-regular users and those who have never used. Additionally, the onset of schizotypal symptoms generally precedes the onset of cannabis use. The findings do not support a causal link between cannabis use and schizotypal traits.


A valid cannabis withdrawal syndrome has been demonstrated in controlled studies with adult marijuana abusers, yet few published reports have examined cannabis withdrawal among adolescents. Adolescents presenting for outpatient substance abuse treatment, whose primary substance of abuse was cannabis, completed a questionnaire reporting the presence and severity of withdrawal symptoms during past periods of cannabis abstinence. Nearly two-thirds of the sample indicated that they had experienced four or more symptoms, and over one-third reported four or more symptoms that occurred at a moderate or greater severity. The magnitude of withdrawal severity was positively correlated with current emotional and behavioral symptoms and self-reported problems with cannabis use. These findings are consistent with previous studies, though the prevalence and magnitude of withdrawal symptoms were lower than that observed in a similar study with adult treatment seekers [Budney, A.J., Novy, P., Hughes, J.R.,
1999. Marijuana withdrawal among adults seeking treatment for marijuana dependence. *Addiction* 94, 1311-1322. Further research is needed to elucidate associations between cannabis withdrawal effects, the initiation of cessation attempts, and relapse.


Multilevel modeling techniques have become a useful tool that enables substance abuse researchers to more accurately identify the contribution of multiple levels of influence on drug-related attitudes and behaviors. However, it is difficult to determine the relative importance of the different hierarchical levels because, in the case of dichotomous outcomes, the variance components estimation involves calculations using a log-odds metric at the lowest level of estimation. We present methods introduced by Goldstein and Rasbash [Goldstein, H., Rasbash, J., 1996. Improved approximations for multilevel models with binary responses. J. Roy. Stat. Soc. A 159, 505-513.] to convert the variance components from the log-odds to the probability metric. This method provides a more logical and interpretable way to examine variation for nonlinear outcomes, which tend to be heavily utilized in substance use research. Using data from the National Household Survey on Drug Abuse [Substance Abuse and Mental Health Services Administration (SAMHSA), 2001. 1999 National Household Survey on Drug Abuse. Data Collection Final Report. Office of Applied Studies (OAS), Rockville, MD. Available at . Accessed on July 1, 2003.], we partition variation among individual, household, and neighborhood levels for the binary outcome of past year marijuana use to illustrate this approach. We also conduct a stability analysis to examine the robustness across different estimation procedures commonly available in commercial multilevel software packages. Finally, we partition the variance components using a conventional continuously distributed outcome and compare the relative magnitudes across binary and continuous outcomes.

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