INTRODUCTION

Here is the latest summary of research abstracts. A reminder also to interested investigators that the deadline for abstract submission to the 2003 ICRS meeting in Cornwall, Ontario is March 14th (www.cannabinoidsociety.org).

BASIC SCIENCE


The effects of chronic Delta(9)-tetrahydrocannabinol on cannabinoid receptor levels and receptor-G-protein coupling were investigated. Male Sprague-Dawley rats were infused continuously with low or high dose regimens of Delta(9)-tetrahydrocannabinol or vehicle for 4 days. Following treatment, rats were sacrificed for cannabinoid CB(1) receptor binding analysis or challenged with the cannabinoid CB(1) receptor antagonist, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl (SR141716A). The rats receiving Delta(9)-tetrahydrocannabinol exhibited antagonist-precipitated withdrawal signs. Each brain region (cerebellum, cortex, hippocampus and basal ganglia) from high-dose rats showed 30-70% decreases in [3H] (-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxyphenyl) cyclohexanol (WIN55212-2) B(max) values, indicating receptor down-regulation. Most regions showed decreased WIN55212-2-stimulated [35S]guanosine-5'-O-3-thiotriphosphate (GTPgammaS) binding, indicating desensitization of cannabinoid CB(1) receptors. Additional receptor binding assays in cerebellar membranes showed a significantly greater decrease in agonist than in antagonist B(max) values, indicating a lower fraction of coupled receptors after treatment. Concentration-effect analysis of five agonists revealed that the treatment resulted in greater decreases in the efficacy of low-efficacy agonists.


Several studies have shown a functional relationship between the endogenous cannabinoid and opioid systems. However, acute effects of Delta9-tetrahydrocannabinol (THC) and physical dependence were not modified in knockout mice with single deletion of mu (MOR), delta (DOR) or kappa (KOR) opioid receptors. To further investigate the neurobiological basis of cannabinoid dependence, we have evaluated acute pharmacological responses, rewarding effects, tolerance and dependence to THC in double MOR/DOR knockout mice. Antinociception and hypolocomotion induced by acute THC administration remained unaffected, whereas the hypothermic effect was slightly attenuated in these double knockout mice. During chronic THC treatment, knockout mice developed slower tolerance to the hypothermic effect, but the development of tolerance to antinociceptive and hypolocomotor effects was unchanged. The rewarding properties of THC, measured in the conditioned place preference paradigm, were reduced in knockout mice. Interestingly, the somatic manifestations of THC withdrawal were also significantly attenuated in mutant mice, suggesting that a cooperative action of MOR and DOR is required for the entire expression of THC dependence.
OBJECTIVE: To evaluate the effects of WIN 55212-2, a cannabinoid receptor agonist, on intraocular pressure and aqueous humor dynamics in normal monkeys and monkeys with glaucoma. METHODS: Intraocular pressure was measured prior to and up to 6 hours after the topical administration of WIN 55212-2 to 1 eye of 5 normal monkeys and to the glaucomatous eye of 8 monkeys with unilateral laser-induced glaucoma. Tonographic outflow facility and fluorophotometric flow rates of aqueous humor were measured in 6 normal monkeys before and after treatment. RESULTS: In normal monkeys, a single dose of WIN 55212-2 reduced intraocular pressure for 4, 5, or 6 hours, with a maximum reduction of 1.4 +/- 0.4 (mean +/- SEM) mm Hg, 2.9 +/- 0.4 mm Hg, and 3.4 +/- 0.6 mm Hg following the 0.07%, 0.2%, and 0.5% concentrations, respectively (P =.08). In 8 glaucomatous monkey eyes, the ocular hypotensive effect was maintained for 5 days with twice-daily administration of 0.5% WIN 55212-2. Outflow facility was unchanged (P =.34) and aqueous humor flow was decreased by 18% (P =.04) in the treated eyes compared with vehicle-treated contralateral control eyes in normal monkeys. CONCLUSIONS: WIN 55212-2, a cannabinoid agonist at the CB(1) receptor, reduces intraocular pressure in both normal and glaucomatous monkey eyes. A decrease of aqueous flow appears to account for the intraocular pressure reduction in normal monkey eyes. CLINICAL RELEVANCE: Cannabinoid agonists at the CB(1) receptor, a new class of antiglaucoma agents that is different from currently used clinical drugs, may have clinical potential.

The G-protein coupled cannabinoid receptors CB(1) and CB(2) are activated by Delta(9)-tetrahydrocannabinol, the psychoactive ingredient of cannabis, and mediate physiological effects of endogenous cannabinoids ("endocannabinoids"). CB(1) genes have been identified in mammals, birds, amphibians and fish, whilst CB(2) genes have been identified in mammals and in the puffer fish Fugu rubripes. Therefore, both CB(1) and CB(2) receptors probably occur throughout the vertebrates. However, cannabinoid receptor genes have yet to be identified in any invertebrate species and the evolutionary origin of cannabinoid receptors is unknown. Here we report the identification of CiCBR, a G-protein coupled receptor in a deuterostomian invertebrate - the urochordate Ciona intestinalis - that is orthologous to vertebrate cannabinoid receptors. The CiCBR cDNA encodes a protein with a predicted length (423 amino-acids) that is the intermediate of human CB(1) (472 amino-acids) and human CB(2) (360-amino-acid) receptors. Interestingly, the protein-coding region of the CiCBR gene is interrupted by seven introns, unlike in vertebrate cannabinoid receptor genes where the protein-coding region is typically intronless. Phylogenetic analysis revealed that CiCBR forms a clade with vertebrate cannabinoid receptors but is positioned outside the CB(1) and CB(2) clades of a phylogenetic tree, indicating that the common ancestor of CiCBR and vertebrate cannabinoid receptors predates a gene (genome) duplication event that gave rise to CB(1)- and CB(2)-type receptors in vertebrates. Importantly, the discovery of CiCBR and the absence of orthologues of CiCBR in protostomian invertebrates such as Drosophila melanogaster and Caenorhabditis elegans indicate that the ancestor of vertebrate CB(1) and CB(2) cannabinoid receptors originated in a deuterostomian invertebrate.


A series of 1-pentyl-1H-indol-3-yl-(1-naphthyl)methanes (9-11) and 2-methyl-1-pentyl-1H-indol-3-yl-(1-naphthyl)methanes (12-14) have been synthesized to investigate the hypothesis that cannabimimetic 3-(1-naphthoyl)indoles interact with the CB(1) receptor by hydrogen bonding to the carbonyl group. Indoles 9-11 have significant (K(i)=17-23nM) receptor affinity, somewhat less than that of the corresponding naphthoylindoles (5, 15, 16). 2-Methyl-1-indoles 12-14 have little affinity for the CB(1) receptor, in contrast to 2-methyl-3-(1-naphthoyl)indoles 17-19, which have affinities comparable to those of 5, 15, 16. A cannabimimetic indene hydrocarbon (26) was synthesized and found to have K(i)=26 +/- 4nM. Molecular modeling and receptor docking studies...
of naphthoylindole 16, its 2-methyl congener (19) and indolyl-1-naphthylmethanes 11 and 14, combined with the receptor affinities of these cannabinimetic indoles, strongly suggest that these cannabinoid receptor ligands bind primarily by aromatic stacking interactions in the transmembrane helix 3-4-5-6 region of the CB(1) receptor.


We have investigated the effects of cannabinoid agonists and antagonists on tumour necrosis factor-alpha (TNF-alpha)-induced secretion of interleukin-8 from the colonic epithelial cell line, HT-29. The cannabinoid receptor agonists ((-)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)-phenyl]4-[3-hydroxypropyl]cyclo- hexan-1-ol) (CP55,940); Delta-9-tetrahydrocannabinol; [R(+)-2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-de]1,4-benzoxazin-6-yl](1-naphthyl) methanone mesylate} (WIN55,212-2) and 1-propyl-2-methyl-3-naphthoyl-indole (JWH 015) inhibited TNF-alpha induced release of interleukin-8 in a concentration-dependent manner. The less active enantiomer of WIN55212-2, [S(-)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]1,4-be nzoxazin-6-yl](1-naphthyl) methanone mesylate (WIN55212-3), and the cannabinoid CB(1) receptor agonist arachidonoyl-2-chloroethylamide (ACEA) had no significant effect on TNF-alpha-induced release of interleukin-8. The cannabinoid CB(1) receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1,4- pyrazole-3-carboxamide hydrochloride (SR141716A; 10(-6) M) antagonised the inhibitory effect of CP55,940 (pA(2)=8.3+/-.2, n=6) but did not antagonise the inhibitory effects of WIN55212-2 and JWH 015. The cannabinoid CB(2) receptor antagonist N-(1,S)-endo1,3,3-trimethylbicyclo(2,2,1)heptan-2-yl)-5(4-chloro-3-methyl- phenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528; 10(-6) M) antagonised the inhibitory effects of CP55,940 (pA(2)=8.2+/-.8, n=6), WIN55212-2 (pA(2)=7.1+/-.3, n=6) and JWH 015 (pA(2)=7.6+/-.3, n=6), respectively. Western immunoblotting of HT-29 cell lysates revealed a protein with a size that is consistent with the presence of cannabinoid CB(2) receptors. We conclude that in HT-29 cells, TNF-alpha-induced interleukin-8 release is inhibited by cannabinoids through activation of cannabinoid CB(2) receptors.


Nonmyelinated (C-) fibers represent the majority of vagal afferents innervating the airways and lung, and play an important role in regulating the respiratory and cardiovascular functions under both normal and abnormal physiologic conditions. Studies of the relationship between the conduction velocities of the vagal afferents and their sensitivities to capsaicin and other chemical irritants reveal that C-fibers are the primary type of chemosensitive afferents in the rat lung. Furthermore, a distinct sensitivity to capsaicin and a weak response to lung inflation are the defining characteristics of these afferents. In cultured rat nodose and jugular ganglion neurons, capsaicin-sensitive cells were identified by measurement of the capsaicin-evoked calcium transients using the Fura-2-based ratiometric imaging technique. The percentage of capsaicin-sensitive neurons gradually decreases as the cell diameter increases. However, the capsaicin-sensitive neurons cannot be precisely identified solely on the basis of the cell size. Anandamide, an endogenous cannabinoid released from leukocytes and epithelial cells, consistently evokes a stimulatory effect on pulmonary C-fiber endings by activating vanilloid receptor type 1 (VR1). The discharge pattern of pulmonary C-fibers evoked by anandamide closely resembles that produced by a much lower (approximately 1/600) dose of capsaicin in the same fibers. Whether anandamide acts as a potential endogenous ligand to VR1 at the C-fiber terminals is unclear, and the physiological role of VR1 in modulating the transduction properties of these afferents also remains to be determined. Anat Rec Part A 270A:17-24, 2003.


Intraluminal administration of the endocannabinoids N-arachidonoyl-ethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG) causes inflammation similar to that caused by Clostridium difficile toxin A in the rat ileum. The effects of anandamide and 2-AG were
significantly inhibited by pretreatment with the specific capsaicin receptor (vanilloid receptor subtype 1; VR1) antagonist capsazepine. Pretreatment with the CB1 and CB2 cannabinoid receptor antagonists N-piperidino-5-(4-chlorophenyl)-1-[(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716) and N-[1S]-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methyl phenyl)-1-[(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) did not affect the responses to anandamide. It has previously been shown that intraluminal toxin A stimulates substance P (SP) release from primary sensory neurons and that pretreatment with SP receptor [neurokinin (NK)-1 receptor] antagonists inhibits the inflammatory effects of toxin A. Anandamide stimulated SP release and this was blocked by capsazepine pretreatment. Also, pretreatment with the specific NK-1 receptor antagonist (2S,3S)-3-[(3,5-bis[trifluoromethyl)phenyl]methoxy)-2-phenylpiperidine (L-733,060) significantly inhibited the inflammatory effects of both toxin A and anandamide. Toxin A increased tissue concentrations of anandamide and 2-AG in the ileum, and these effects were enhanced after pretreatment with inhibitors of fatty acid amide hydrolase, a major endocannabinoid-degrading enzyme. The toxin A-stimulated release of anandamide but not 2-AG was selective over their congeners. These results demonstrate that the endocannabinoids anandamide and 2-AG stimulate intestinal primary sensory neurons via the capsaicin VR1 receptor to release SP, resulting in enteritis, and that endocannabinoids may mediate the inflammatory effects of toxin A. 


Most actions of anandamide (AEA) are mediated by the cannabinoid 1 (CB(1)) receptor activation, but on sensory neurones it is also an agonist on the vanilloid subtype 1 receptor (VR(1)). The aim of the present study was to analyse the effect of AEA (10(-6)-10(-4) M) on inhibitory CB(1) and excitatory VR(1) receptors by measuring sensory neuropeptide release such as somatostatin, substance P and calcitonin gene-related peptide, from isolated rat tracheae. AEA (10(-6) M) was without significant effect, 10(-5) M inhibited neuropeptide release, which was abolished by the G protein-coupled receptor blocker pertussis toxin (100 ng/ml) and the CB(1) receptor antagonist SR141716A (5x10(-7) M). High concentrations of AEA (5x10(-5) M, 10(-4) M) increased the release of the peptides and this inhibition was prevented by the competitive VR(1) antagonist capsazepine (10(-5) M). These results indicate a dual, concentration-dependent action of AEA on CB(1) receptors and VR(1) on peripheral sensory nerve terminals.


RATIONALE. Marijuana has been reported to suppress nausea produced by chemotherapy treatment in human cancer patients. Although there is abundant evidence that cannabinoid agonists attenuate vomiting in emetic species, there has been little experimental evidence of their anti-nausea potential. Considerable evidence suggests that conditioned rejection reactions in rats reflect nausea. The present experiments evaluated the potential of low doses of the cannabinoid agonists, Delta-9-tetrahydrocannabinol (THC; 0.5 mg/kg, i.p.), and HU-210 (0.001 mg/kg and 0.01 mg/kg, i.p.), and the CB(1) antagonist SR-141716A in modulating the establishment and the expression of lithium-induced conditioned rejection reactions in rats. OBJECTIVESTo evaluate the effect of cannabinoids on conditioned rejection reactions, a rat model of nausea. METHODSIn experiments 1 and 2, respectively, rats were injected with cannabinoid agonists, THC (0.5 mg/kg, i.p.) and HU-210 (0.001, 0.005 or 0.01 mg/kg), 30 min prior to exposure to 0.1% saccharin solution by intraoral infusion. Immediately following saccharin exposure, they were injected with 20 ml/kg 0.15 M lithium chloride or saline. On each of two test trials, the rats were injected with the cannabinoid or vehicle 30 min prior to exposure to saccharin. In experiment 3, rats were injected with the CB(1) antagonist, SR-141716A (2.5 mg/kg) or a combination of SR-141716A and HU-210 (0.01 mg/kg) 30 min prior to an infusion of saccharin followed by injection of lithium or saline. They were given a single drug-free test trial. Experiment 4 replicated and extended the findings of experiment 3. RESULTSDelta-9-THC and HU-210 interfered with the establishment and the expression of lithium-induced conditioned rejection reactions. The suppressive effect of HU-210 on rejection reactions was reversed by pretreatment with SR-141716A. Administration of SR-141716A prior to conditioning potentiated lithium-induced
conditioned rejection reactions. CONCLUSIONSThese results indicate that the establishment and the expression of lithium-induced conditioned rejection reactions are suppressed by pretreatment with cannabinoid agents. These effects appear to be mediated by their action on the CB(1) receptor, because they are reversed by pretreatment with SR-141716A. Finally, our results suggest that endogenous cannabinoids play a role in modulation of nausea, because the antagonist potentiated lithium-induced nausea.


Baroreceptor afferent fibers synapse in the nucleus tractus solitarius (NTS) of the medulla. Neuronal cannabinoid receptors (CB1) are expressed in the NTS and central administration of CB1 receptor agonists affect blood pressure (BP) and heart rate. In addition, there is evidence that endocannabinoids are produced in the brainstem. This study examined whether changes in CB1 receptor activity in the NTS modulated the baroreceptor reflex, contributing to the changes seen in BP and heart rate. Baroreflexes were evoked in anesthetized dogs by pressure ramp stimulations of the isolated carotid sinus before and after microinjection of a CB1 receptor agonist WIN 55,212-2 (1.25-1.50 pmol) or antagonist SR141716 (2.5-3.0 pmol) into cardiovascular regions of the NTS. Microinjection of the SR141716 did not affect baseline blood pressure or baroreflex sensitivity. However, SR141716 significantly prolonged the time needed to return to the baseline level of blood pressure following the pressure ramp. Microinjection of WIN 55,212-2 had no effect on the baroreflex. These data suggest that endocannabinoids can modulate the excitability of NTS neurons involved in the baroreceptor reflex, leading to modulation of baroreflex regulation.


**BACKGROUND:** Cannabinoids exert a wide spectrum of effects in men including alterations in the reproductive system. To date, two types of cannabinoid receptors have been cloned in humans, namely CB(1) and CB(2) belonging to the G protein-coupled receptor superfamily. Although cannabinoids have functional and morphologic effects in the prostate gland, the expression of cannabinoid receptors in this tissue has never been investigated. The aim of this study was to analyze the expression of cannabinoid receptors in the human prostate gland and their regulatory effects on adenylyl cyclase activity. **METHODS:** To investigate the existence of cannabinoid receptors in prostate, we used various methods, including reverse transcriptase-polymerase chain reaction, Western blotting, and immunohistochemistry. Adenylyl cyclase activity was analyzed by measuring the cAMP produced by means of a competitive assay by using PKA. **RESULTS:** Both mRNA for CB(1) and the corresponding protein are expressed in the human prostate gland at a level comparable with the receptor expressed in cerebellum. The molecular mass of the protein estimated from Western blot analysis was 58 kDa, which is in concordance with previous data for CB(1) in other tissues. Immunohistochemical studies show that CB(1) is preferentially expressed in the epithelia of the prostate. The cannabinoid receptor expressed in the prostate negatively regulates adenylyl cyclase activity through a pertussis toxin-sensitive protein. *Prostate* 54: 95-102, 2003.


Anandamide (arachidonoylethanolamine, AEA), an endogenous agonist for both the cannabinoid CB(1) receptor and the vanilloid VR1 receptor, elicits neurobehavioral, anti-inflammatory, immunomodulatory, and proapoptotic effects. Because of the central role of nuclear factor-kappaB (NF-kappaB) in the inflammatory process and the immune response, we postulated that AEA might owe some of its effects to the suppression of NF-kappaB. This study shows that AEA inhibits tumor necrosis factor-alpha (TNFalpha)-induced NF-kappaB activation by direct inhibition of the IkappaB kinase (IkK)beta and, to a lesser extent, the IkKalpha subunits of kappaB inhibitor (IkappaB) kinase complex, and that IKKs inhibition by AEA correlates with inhibition of IkappaBalpha degradation, NF-kappaB binding to DNA, and NF-kappaB-dependent transcription in TNFalpha-stimulated cells. AEA also prevents NF-kappaB-dependent reporter
gene expression induced by mitogen-activated protein kinase kinase kinase and NF-kappaB-inducing kinase. The NF-kappaB inhibitory activity of AEA was independent of CB(1) and CB(2) activation in TNFalpha-stimulated 5.1 and A549 cell lines, which do not express vanilloid receptor 1, and was not mediated by hydrolytic products formed through the activity of the enzyme fatty acid amidase. Chemical modification markedly affected AEA inhibitory activity on NF-kappaB, suggesting rather narrow structure-activity relationships and the specific interaction with a molecular target. Substitution of the alkyl moiety with less saturated fatty acids generally reduced or abolished activity. However, replacement of the ethanolamine "head" with a vanillyl group led to potent inhibition of TNFalpha-induced NF-kappaB-dependent transcription. These findings provide new mechanistic insights into the anti-inflammatory and proapoptotic activities of AEA, and should foster the synthesis of improved analogs amenable to pharmaceutical development as anti-inflammatory agents.


We have observed rapid and extensive depletion of cellular energy stores by Delta(9)-tetrahydrocannabinol (THC) in the pulmonary transformed cell line A549. ATP levels declined dose dependently with an IC(50) of 7.5 &mgr;g/ml of THC after 24-h exposure. Cell death was observed only at concentrations >10 &mgr;g/ml. Studies using JC-1, a fluorescent probe for mitochondrial membrane potential, revealed diminished mitochondrial function at THC concentrations as low as 0.5 &mgr;g/ml. At concentrations of 2.5 or 10 &mgr;g/ml of THC, a decrease in mitochondrial membrane potential was observed as early as 1 h after THC exposure. Mitochondrial function remained diminished for at least 30 h after THC exposure. Flow cytometry studies on cells exposed to particulate smoke extracts indicate that JC-1 red fluorescence was fivefold lower in cells exposed to marijuana smoke extract relative to cells exposed to tobacco smoke extract. Comparison with a variety of mitochondrial inhibitors demonstrates that THC produced effects similar to that of carbonyl cyanide p-trifluoromethoxyphenylhydrazone, suggesting uncoupling of electron transport. Loss of red JC-1 fluorescence by THC was suppressed by cyclosporin A, suggesting mediation by the mitochondrial permeability transition pore. This disruption of mitochondrial function was sustained for at least 24 h after removal of THC by extensive washing. These results suggest that exposure of the bronchopulmonary epithelium to THC may have important health and physiological consequences.


The present study was designed to determine the potential of CB1 cannabinoid receptor modulating compounds in the treatment of L-3,4-dihydroxyphenylalanine (L-dopa)-induced dyskinesia in Parkinson's disease. In the reserpine-treated rat model of parkinsonism, administration of a high dose of L-dopa (150 mg/kg) but not of CI-APB (0.5 mg/kg) or quinpirole (0.5 mg/kg) produced a hyperkinetic state characterised by an increase in horizontal and vertical activity, which likely represent correlates of antiparkinsonian and dyskinetic activity, respectively. Injection of the CB1 cannabinoid receptor antagonist SR141716 (0.1-3 mg/kg) reduced the increase in vertical activity elicited by L-dopa without affecting the increase in horizontal activity. Injection of the CB1 cannabinoid receptor agonist WIN55,212-2 (0.1-3 mg/kg) reduced the L-dopa-induced increase in vertical activity and, at the highest dose only (3 mg/kg), also reduced horizontal activity elicited by L-dopa. WIN55,212-2 (1 mg/kg) reduced motor activity induced by both the D1 receptor agonist CI-APB (0.5 mg/kg) and the D2 receptor agonist quinpirole (0.5 mg/kg) in the reserpine-treated rat. SR141716 (1 mg/kg) had no effects on motor activity induced by CI-APB (0.5 mg/kg) or quinpirole (0.5 mg/kg) in the reserpine-treated rat. Injection of the inhibitor of endocannabinoid transport AM404 (0.1-1 mg/kg) did not affect the increase in horizontal or vertical activity elicited by L-dopa (150 mg/kg) in the reserpine-treated rat. The data suggest that both CB1 cannabinoid receptor antagonists and agonists can modulate the behavioural effects of L-dopa and may be useful for the treatment of the dyskinesia associated with long-term L-dopa treatment of Parkinson's disease.
Many tumor promoters suppress the immune system; however, the direct effect of immunosuppressants on the tumorigenic pathways of nonimmune cells in solid tissue has not been well documented. Cannabinoids were chosen to explore this question further. Cannabinoids are immune modulators that affect specific intracellular signaling pathways in leukocytes. Since these compounds are nongenotoxic, any tumorigenic effect that might be associated with these compounds would need to occur through an epigenetic mechanism. Therefore, we determined the effect of Delta(9)-THC and CBN, 2 plant-derived cannabinoids, on 2 key epigenetic markers of tumor promotion: inhibition of GJIC, which is essential in removing a cell from growth suppression, and activation of the ERK-MAPK pathway, which is crucial in activating the appropriate genes for mitogenesis. Both Delta(9)-THC and CBN reversibly inhibited GJIC at noncytotoxic doses (15 &mgr;M) in a normal diploid WB rat liver epithelial oval cell line within 20 min and activated ERK1 and ERK2 within 5 min. Inhibition of MEK with PD98059 prevented the inhibition of GJIC by either cannabinoid, suggesting that inhibition of GJIC was MEK-dependent. Based on RT-PCR analysis and employment of an antagonist of CB1 and CB2, the effects on GJIC and MAPK were independent of both cannabinoid receptors. Cannabinoids affected crucial epigenetic pathways associated with cell proliferation in a rodent liver epithelial cell model system. Copyright 2002 Wiley-Liss, Inc.


Cannabinoids and ethanol can activate the same reward pathways, which could suggest endocannabinoid involvement in the rewarding effects of ethanol. The high ethanol preference of young (6-10 weeks) C57BL/6J mice is reduced by the cannabinoid receptor 1 (CB1) antagonist SR141716A to levels observed in their CB1 knockout littermates or in old (26-48 weeks) wild-type mice, in both of which ethanol preference is unaffected by SR141716A. Similarly, SR141716A inhibits food intake in food-restricted young, but not old, wild-type mice. There are no age-dependent differences in the tissue levels of the endocannabinoids anandamide and 2-arachidonoylglycerol or the density of CB1 in the hypothalamus, limbic forebrain, amygdala, and cerebellum. CB1-stimulated guanosine 5’-[gamma-thio]triphosphate (GTP[gammaS]) binding is selectively reduced in the limbic forebrain of old compared with young wild-type mice. There is no age-dependent difference in Gi or Go subunit protein expression in the limbic forebrain, and the selective reduction in GTP[gammaS] labeling in tissue from old mice is maintained in a receptor/G protein reconstitution assay by using functional bovine brain G protein. These findings suggest that endocannabinoids acting at CB1 contribute to ethanol preference, and decreased coupling of CB1 to G proteins in the limbic forebrain by mechanisms other than altered receptor or G protein levels may be involved in the age-dependent decline in the appetite for both ethanol and food.


The heritability of nociception and antinociception has been well established in the mouse. The pharmacogenetics of morphine analgesia are fairly well characterized, but far less is known about other analgesics. The purpose of this work was to begin the systematic genetic study of non-opioid analgesics. We tested mice of 12 inbred mouse strains for baseline nociceptive sensitivity (49 degrees C tail-withdrawal assay) and subsequent antinociceptive sensitivity to systemic administration of (trans)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate hydrate (U50,488; 10-150 mg/kg), a kappa-opioid receptor agonist; (R)-(+)-(2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55,212-2; 0.5-480 mg/kg), a synthetic cannabinoid receptor agonist; epibatidine (7.5-150 mg/kg), a nicotinic receptor agonist; clonidine (0.1-5 mg/kg), an alpha(2)-adrenergic receptor agonist; and, for purposes of comparison, the prototypic mu-opioid receptor agonist, morphine (5-200 mg/kg). Robust interstrain variability was
observed in nociceptive sensitivity and in the antinociceptive effects of each of the drugs, with extreme-responding strains exhibiting antinociceptive potencies differing up to 37-fold. Unexpectedly, we observed moderate-to-high genetic correlations of strain sensitivities to the five drugs (r = 0.39-0.77). We also found moderate-to-high correlations between baseline nociceptive sensitivity and subsequent analgesic response to each drug (r = 0.33-0.68). The generalizability of these findings was established in follow-up experiments investigating morphine and clonidine inhibition of formalin test nociception. Despite the fact that each drug activates a unique receptor, our results suggest that the potency of each drug is affected by a common set of genes. However, the genes in question may affect antinociception indirectly, via a primary action on baseline nociceptive sensitivity.


Dexanabinol (HU-211) is a synthetic non-psychotropic cannabinoid and a non-competitive NMDA-receptor antagonist. The beneficial effect of dexanabinol on prevention of degeneration and promotion of regeneration was studied on the crush-injured rat optic nerve model. Sprague-Dawley rats were subjected to a calibrated crush injury of the optic nerve and treated with a single intraperitoneal injection of dexanabinol (7 mg/kg), its vehicle only or were untreated. Transmission electron microscopic analysis of the excised optic nerves was performed after 30 days. In the dexanabinol treated rats, the site of injury was traversed by unmyelinated and thinly myelinated axons, possibly indicative of regenerative growth. No such growth was detectable in the controls. Viable axons were found 0.5 mm distal to the site of injury in 6 of 8 dexanabinol treated rats, but in only 1 of 10 rats in the control groups. These results have clinical implications for the prevention of secondary degeneration and promotion of regeneration after injuries to the central nervous system.

CLINICAL SCIENCE


The use of complementary and alternative medicine (CAM) appears to be high in the general population and in patients with multiple sclerosis (MS). There are no diets or dietary supplements that are definitely effective in altering the disease course in MS. However, diets and dietary supplements that increase the intake of polyunsaturated fatty acids may produce mildly beneficial effects. Because these approaches are not definitely effective, they may be of limited interest to physicians and other conventional health providers. In contrast, for patients with MS, these interventions may be of considerable interest, because they may be mildly effective and are inexpensive and relatively safe. Vitamin D, ginkgo biloba, cannabinoids, and Padma 28 produce immunomodulatory actions and therapeutic effects in experimental autoimmune encephalomyelitis. However, for these compounds, there are not enough clinical trial data or safety information to support their use as disease-modifying therapies. The role of antioxidant compounds in MS is unclear. There is no evidence that vitamin B(12) supplementation or gluten-free diets are effective MS therapies. Conventional health providers can play an important role in the care of MS patients by being open to discuss CAM therapies and by providing objective MS-relevant CAM information.


The marijuana plant (Cannabis sativa) and preparations derived from it have been used for medicinal purposes for thousands of years. It is likely that the therapeutic benefits of smoked marijuana are due to some combination of its more than 60 cannabinoids and 200-250 non-cannabinoid constituents. Several marijuana constituents, the carboxylic acid metabolites of tetrahydrocannabinol, and synthetic analogs are free of cannabimimetic central nervous system activity, do not produce behavioral changes in humans, and are effective antiinflammatory and analgesic agents. One cannabinoid acid in particular, ajulemic acid, has been studied extensively in in vitro systems and animal models of inflammation and immune responses. This commentary
reviews a portion of the work done by investigators interested in separating the medicinal properties of marijuana from its psychoactive effects. Understanding the mechanisms of the therapeutic effects of nonpsychoactive cannabinoids should lead to development of safe effective treatment for several diseases, and may render moot the debate about "medical marijuana".

**BEHAVIOURAL SCIENCE**


**AIMS:** To probe recent evidence on apparent excess occurrence of marijuana dependence when marijuana smoking starts in adolescence. **DESIGN AND PARTICIPANTS:** A national sample of recent-onset marijuana users was identified within public data files of the National Household Survey on Drug Abuse (NHSDA), 1995-98 (1866 adolescents and 762 adults). **MEASUREMENTS:** Marijuana dependence was assessed via seven standardized questions about its clinical features, such as being unable to cut down. Multivariate response models (GLM/GEE and MIMIC) were used to evaluate adolescent excess risk and possible item biases. **FINDINGS:** Among people who had just started to use marijuana, clinical features of marijuana dependence occurred twice as often among adolescents compared to adults, even with statistical adjustment for other covariates (P < 0.01 from GLM/GEE). MIMIC analyses suggest that adolescent-onset users have somewhat higher levels of marijuana dependence, and they also provide evidence of age-associated response bias for some but not all clinical features of marijuana dependence. That is, even with level of marijuana dependence held constant, adolescent recent-onset users were more likely than adults to report being unable to cut down (P = 0.01) and tolerance (P = 0.029). **CONCLUSION:** Nosologic, methodological and substantive reasons for observed age-related excess in occurrence of marijuana dependence problems among early onset users deserve more attention in future research.


**BACKGROUND:** The aims of this research were to use data gathered over the course of a 21 year longitudinal study to examine the linkages between cannabis dependence at ages 18 and 21 and rates of psychotic symptoms taking into account previous symptom levels and other confounding factors. **METHOD:** Data were gathered during the course of the Christchurch Health and Development Study (CHDS). The CHDS is a longitudinal study of a birth cohort of 1,265 children who have been studied from birth to age 21. As part of this study, data were gathered on cannabis dependence and psychotic symptoms at ages 18 and 21. **RESULTS:** Young people meeting DSM-IV criteria for cannabis dependence had elevated rates of psychotic symptoms at ages 18 (rate ratio = 3.7; 95% CI 2.8-5.0; P < 0.0001) and 21 (rate ratio = 2.3; 95% CI 1.7-3.2; P < 0.0001). These associations were adjusted for previous psychotic symptoms and a range of other confounding factors using a generalized estimating equation model. This analysis showed that after adjustment for confounding factors, those meeting criteria for cannabis dependence still had an increased rate of psychotic symptoms (rate ratio = 1.8; 95% CI 1.2-2.6; P < 0.005). **CONCLUSIONS:** The results show that the development of cannabis dependence is associated with increased rates of psychotic symptoms in young people even when pre-existing symptoms and other background factors are taken into account.


**AIM:** To apply a new paradigm using transient changes to visual scenes to explore information processing biases relating to 'social' levels of alcohol and cannabis use. **PARTICIPANTS:** Male and female student volunteers (n = 200) not self-reporting substance-related problems. **SETTING:** Quiet testing areas throughout the university campus. **DESIGN:** A
flicker paradigm, for inducing change blindness with lighter and heavier social users of alcohol (experiment 1, n= 100) and social users and non-users of cannabis (experiment 2, n= 100), explored the associations between habitual level of use and the latency to detection of a single substance-related or neutral change made to a scene of grouped substance-related and neutral objects. MEASUREMENTS: Alcohol use was measured as the number of units of the heaviest drinking day from the previous week; cannabis use as the number of months of use in previous 12. Change-detection latency comparisons were used to evaluate processing biases. FINDINGS: In both experiments, (i) heavier social users detected substance-related changes quicker than lighter and non-users; (ii) lighter and non-users detected substance-neutral changes quicker than heavier users; (iii) heavier social users detected substance-related quicker than substance-neutral changes; and (iv) lighter and non-users detected substance-neutral changes quicker than substance-related changes. CONCLUSIONS: Alcohol and cannabis processing biases are found at levels of social use, have the potential to influence future consumption and for this reason merit further research.


AIMS: To find out how cannabis came to be subject to international narcotics legislation. METHOD: Examination of the records of the 1925 League of Nations' Second Opium Conference, of the 1894 Report of the Indian Hemp Drugs Commission and other contemporary documents. FINDINGS: Although cannabis (Indian hemp) was not on the agenda of the Second Opium Conference, a claim by the Egyptian delegation that it was as dangerous as opium, and should therefore be subject to the same international controls, was supported by several other countries. No formal evidence was produced and conference delegates had not been briefed about cannabis. The only objections came from Britain and other colonial powers. They did not dispute the claim that cannabis was comparable to opium, but they did want to avoid a commitment to eliminating its use in their Asian and African territories.


CONTEXT: Previous studies have reported that early initiation of cannabis (marijuana) use is a significant risk factor for other drug use and drug-related problems. OBJECTIVE: To examine whether the association between early cannabis use and subsequent progression to use of other drugs and drug abuse/dependence persists after controlling for genetic and shared environmental influences. DESIGN: Cross-sectional survey conducted in 1996-2000 among an Australian national volunteer sample of 311 young adult (median age, 30 years) monozygotic and dizygotic same-sex twin pairs discordant for early cannabis use (before age 17 years). MAIN OUTCOME MEASURES: Self-reported subsequent nonmedical use of prescription sedatives, hallucinogens, cocaine/other stimulants, and opioids; abuse or dependence on these drugs (including cannabis abuse/dependence); and alcohol dependence. RESULTS: Individuals who used cannabis by age 17 years had odds of other drug use, alcohol dependence, and drug abuse/dependence that were 2.1 to 5.2 times higher than those of their co-twin, who did not use cannabis before age 17 years. Controlling for known risk factors (early-onset alcohol or tobacco use, parental conflict/separation, childhood sexual abuse, conduct disorder, major depression, and social anxiety) had only negligible effects on these results. These associations did not differ significantly between monozygotic and dizygotic twins. CONCLUSIONS: Associations between early cannabis use and later drug use and abuse/dependence cannot solely be explained by common predisposing genetic or shared environmental factors. The association may arise from the effects of the peer and social context within which cannabis is used and obtained. In particular, early access to and use of cannabis may reduce perceived barriers against the use of other illegal drugs and provide access to these drugs.

BACKGROUND: Epidemiological findings suggest that cannabis use is a risk factor for the emergence of psychosis, and that the induction of psychotic symptoms in the context of cannabis use may be associated with a pre-existing vulnerability for psychosis. This study investigated in a non-clinical population the interaction between cannabis use and psychosis vulnerability in their effects on psychotic experiences in daily life. METHOD: Subjects (N = 79) with high or low levels of cannabis use were selected among a sample of 685 undergraduate university students. Experience sampling method (ESM) was used to collect information on substance use and psychotic experiences in daily life. Vulnerability to develop psychosis was measured using a clinical interview assessing the level of psychotic symptoms. Statistical analyses were performed using multilevel linear random regression models. RESULTS: The acute effects of cannabis are modified by the subject’s level of vulnerability for psychosis. Subjects with high vulnerability for psychosis are more likely to report unusual perceptions as well as feelings of thought influence than subjects with low vulnerability for psychosis, and they are less likely to experience enhanced feelings of pleasure associated with cannabis. There is no evidence that use of cannabis is increased following occurrence of psychotic experiences as would be expected by the self-medication model. CONCLUSION: Cannabis use interacts with psychosis vulnerability in their effects on experience of psychosis in daily life. The public health impact of the widespread use of cannabis may be considerable.

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