INTRODUCTION
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BASIC SCIENCE

Endocannabinoids are crucial for the extinction of aversive memories, a process that considerably involves the amygdala. Here, we show that low-frequency stimulation of afferents in the lateral amygdala with 100 pulses at 1 Hz releases endocannabinoids postsynaptically from neurons of the basolateral amygdala of mice in vitro and thereby induces a long-term depression of inhibitory GABAergic synaptic transmission (LTDi) via a presynaptic mechanism. Lowering inhibitory synaptic transmission significantly increases the amplitude of excitatory synaptic currents in principal neurons of the central nucleus, which is the main output site of the amygdala. LTDi involves a selective mGluR1 (metabotropic glutamate receptor 1)-mediated calcium-independent mechanism and the activation of the adenyl cyclase-protein kinase A pathway. LTDi is abolished by the cannabinoid type 1 (CB1) receptor antagonist SR141716A and cannot be evoked in CB1 receptor-deficient animals. LTDi is significantly enhanced in mice lacking the anandamide-degrading enzyme fatty acid amidylase hydrolase. The present findings show for the first time that mGluR activation induces a retrograde endocannabinoid signaling via activation of the adenyl cyclase-protein kinase A pathway and the release of anandamide. Furthermore, the results indicate that anandamide decreases the activity of inhibitory interneurons in the amygdala. This disinhibition increases the activity of common output neurons and could provide a prerequisite for extinction by formation of new memory.


Abstract The possible interactions between Delta9-tetrahydrocannabinol (Delta9-THC) and nicotine remain unclear in spite of the current association of cannabis and tobacco in humans. The aim of the present study was to explore the interactions between these two drugs of abuse by evaluating the consequences of Delta9-THC administration on the somatic manifestations and the aversive motivational state associated with nicotine withdrawal in mice. Acute Delta9-THC administration significantly decreased the incidence of several nicotine withdrawal signs precipitated by mecamylamine or naloxone, such as wet-dog-shakes, paw tremor and scratches. In both experimental conditions, the global withdrawal score was also significantly attenuated by acute Delta9-THC administration. This effect of Delta9-THC was not due to possible adaptive changes induced by chronic nicotine on CB(1) cannabinoid receptors, as the density and functional activity of these receptors were not modified by chronic nicotine administration in the different brain structures investigated. We also evaluated the consequences of Delta9-THC administration on c-Fos expression in several brain structures after chronic nicotine administration and withdrawal. c-Fos was decreased in the caudate putamen and the dentate gyrus after mecamylamine precipitated nicotine withdrawal. However, acute Delta9-THC administration did not modify c-Fos expression under these experimental conditions. Finally, Delta9-THC also reversed conditioned place aversion associated to naloxone precipitated
nicotine withdrawal. Taken together, these results indicate that Delta9-THC administration attenuated somatic signs of nicotine withdrawal and this effect was not associated with compensatory changes on CB(1) cannabinoid receptors during chronic nicotine administration. In addition, Delta9-THC also ameliorated the aversive motivational consequences of nicotine withdrawal.


The present review evaluates the evidence that the endocannabinoid system plays in the development of tolerance to alcohol. The identification of a G-protein-coupled receptor, namely, the cannabinoid receptor (CB1 receptor), which was activated by Delta(9)-tetrahydrocannabinol (Delta(9)-THC), the major psychoactive component of marijuana, led to the discovery of endogenous cannabinoid agonists. Until now, four fatty acid derivatives identified to be arachidonylethanolamide (AEA), 2-arachidonylglycerol (2-AG), 2-arachidonylglycerol ether (noladin ether) and virodhamine have been isolated from both nervous and peripheral tissues. Both AEA and 2-AG have been shown to mimic the pharmacological and behavioural effects of Delta(9)-THC. The role of the endocannabinoid system in the development of tolerance to alcohol was not known until recently. Recent studies from our laboratory have implicated for the first time a role for the endocannabinoid system in development of tolerance to alcohol. Chronic alcohol treatment has been shown to down-regulate CB1 receptors and its signal transduction. The observed downregulation of CB1 receptor function results from the persistent stimulation of the receptors by AEA and 2-AG, the synthesis of which has been shown to be increased by chronic alcohol treatment. The enhanced formation of endocannabinoids may subsequently influence the release of neurotransmitters. It was found that the DBA/2 mice, known to avoid alcohol intake, have significantly reduced CB1 receptor function in the brain, consistent with other studies in which the CB1 receptor antagonist SR 141716A has been shown to block voluntary alcohol intake in rodents. Similarly, activation of the CB1 receptor system promoted alcohol craving, suggesting a role for the CB1 receptor gene in excessive alcohol drinking behaviour and development of alcoholism. Ongoing investigations may lead to a better understanding of the mechanisms underlying the development of tolerance to alcohol and to develop therapeutic strategies to treat alcoholism.


This study examined the safety and efficacy of gamma vinyl-GABA (GVG, vigabatrin) for the treatment of methamphetamine and/or cocaine addiction. A total of 30 subjects, who met DSM-IV criteria for methamphetamine and/or cocaine dependence, were enrolled in an open label 9-week safety study. The protocol was specifically designed to include extensive visual field monitoring as well as outcome measures of therapeutic efficacy. Patients were screened twice weekly for the presence of urinary cocaine, methamphetamine, heroin, alcohol, and marijuana. In total, 18/30 subjects completed the study and 16/18 tested negative for methamphetamine and cocaine during the last 6 weeks of the trial. GVG did not produce any visual field defects or alterations in visual acuity. Furthermore, it did not produce changes in vital signs even with continued use of methamphetamine and cocaine. Thus, under conditions that appear to be appropriate for the successful treatment of methamphetamine and/or cocaine addiction, GVG is safe. Synapse 55:122-125, 2005. (c) 2004 Wiley-Liss, Inc.


Previous studies in the hippocampus and cerebellum demonstrate that depolarisation of postsynaptic neurones stimulates the rapid synthesis and release of an endocannabinoid that retrogradely interacts with pre-synaptic CB(1) to modulate neurotransmitter release. This study evaluated whether depolarisation of second order neurones in the dorsal horn of the spinal cord by the AMPA receptor agonist, (S)-AMPA, would modulate sensory neurotransmission via release of endocannabinoids. Using an isolated rat dorsal horn with dorsal root attached in vitro
preparation the release of calcitonin gene-related peptide (CGRP) after electrical stimulation of the dorsal roots was measured. Superfusion of either WIN55,212-2 (1μM) or (S)-AMPA (1μM) significantly attenuated CGRP release in a CB(1)-dependent manner (SR141716A, 5μM). This provides indirect pharmacological evidence for an AMPA-evoked release of endogenous cannabinoids inhibiting peptide release from primary afferent neurons. This study confirms that CGRP release from the dorsal horn is modulated via CB(1) activation. Furthermore a depolarising stimulus also modulates CGRP release, potentially via the release of endogenous cannabinoids.


The CB1 cannabinoid receptor antagonist, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (rimonabant; SR141716A), and oleoylethanolamide (OEA) are known to reduce food consumption, by, at least partially, a peripheral regulation of feeding. The effects of systemic SR141716A or OEA (5 mg/kg) administrations on food consumption in 24 h food-deprived and fed rats were investigated. In fasted rats, SR141716A and OEA produced an inhibition in food intake measurable the first 20 min following injection. The increase in ghrelin levels observed in the vehicle-injected rats was abolished in animals receiving OEA and significantly reduced with SR141716A. Neither OEA nor SR141716A modified glucagon-like peptide-1 (7-36) amide portal levels 20 min after the administration. In fed rats, plasma ghrelin levels of SR141716A- and OEA-treated rats were 35% lower as compared with those of the vehicle-injected rats. These results show an influence of cannabinoid agents on circulating ghrelin levels and suggest that their short-term action on appetite seems to be in accordance with the control of secretion of gastrointestinal orexigenic peptides, mainly expressed in the upper part of the gastrointestinal tract.


Recent work in the field of gastrointestinal pharmacology of cannabinoids has focused on enteric endocannabinoid and endovanilloid systems and their modulation in pathophysiological conditions. CB(1) receptor immunoreactivity was detected on enteric cholinergic neurones and vasoactive intestinal peptide-containing submucosal ganglion cells, on discrete nuclei of the dorsovagal complex (involved in emesis) and on central and peripheral vagal terminals, thus controlling gastroesophageal reflux and gastrointestinal motility. CB(1) receptor activation by endocannabinoids inhibited induced fluid secretion and inflammation in animal models and reduced proliferation of cultured colorectal cancer cells. Endocannabinoids also activate cannabinoid CB(2) and vanilloid VR1 receptors in certain inflammatory states. Thus endocannabinoid metabolism could provide a useful therapeutic target for many gastrointestinal disorders.


BAY 59-3074 (3-[2-cyano-3-(trifluoromethyl)phenoxyl]phenyl-4,4,4-trifluoro-1-butane-sulfonate) is a structurally novel cannabinoid CB(1)/CB(2) receptor partial agonist with analgesic properties. The present study was performed to confirm its receptor binding profile in a highly sensitive in vivo assay. Rats (n=10) learned to discriminate BAY 59-3074 (0.5 mg/kg, p.o., t-1 h) from vehicle in a fixed-ratio: 10, food-reinforced two-lever procedure after a median number of 28 training sessions. BAY 59-3074 generalized dose-dependently (ED(50): 0.081 mg/kg, p.o.) and the cue was detectable between 0.25 and 4 h after administration. The selective cannabinoid
CB(1) receptor antagonist SR 141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride] blocked the discriminative effects of BAY 59-3074 (ID(50): 1.79 mg/kg, i.p.). Complete generalization was also obtained after i.p. administration of BAY 59-3074 (ED(50) value: 0.41 mg/kg), and the reference cannabinoids BAY 38-7271 [(−)-R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-butanesulfonate, 0.011 mg/kg], CP 55,940 [(−)-cis-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxy-propyl)cyclohexanol, 0.013 mg/kg], HU-210 [(−)-11-OH-Delta(8)-tetrahydrocannabinol dimethylheptyl, 0.022 mg/kg], WIN 55,212-2 [(−)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6H-pyrrolo[3,2,1-ij]quinolin-6-one, 0.41 mg/kg], and (−)-Delta(9)-tetrahydrocannabinol (0.41 mg/kg). Non-cannabinoids with analgesic properties, such as morphine, amitriptyline, carbamazepine, gabapentin, and baclofen, did not generalize to the cue. It is concluded that the discriminative stimulus effects of BAY 59-3074 are specifically mediated by cannabinoid CB(1) receptor activation.


Peripheral cannabinoid 2 receptors (CB2 receptors) modulate immune responses and attenuate nociceptive behaviour in models of acute and persistent pain. The aim of the present study was to investigate whether peripheral CB2 receptors modulate spinal processing of innocuous and noxious responses and to determine whether there are altered roles of CB2 receptors in models of persistent pain. Effects of local administration of the CB2 receptor agonist JWH-133 (5 and 15 microg/50 microL) on mechanically evoked responses of spinal wide dynamic range (WDR) neurons in noninflamed rats, rats with carrageenan-induced hindpaw inflammation, sham-operated rats and spinal nerve-ligated (SNL) rats were determined in anaesthetized rats in vivo. Mechanical stimulation (von Frey filaments, 6-80 g) of the peripheral receptive field evoked firing of WDR neurons. Mechanically evoked responses of WDR neurons were similar in noninflamed, carrageenan-inflamed, sham-operated and SNL rats. Intraplantar injection of JWH-133 (15 microg), but not vehicle, significantly (P < 0.05) inhibited innocuous and noxious mechanically evoked responses of WDR neurons in all four groups of rats. In many cases the selective CB2 receptor antagonist, SR144528 (10 microg/50 microL), attenuated the inhibitory effects of JWH-133 (15 microg) on mechanically evoked WDR neuronal responses. The CB1 receptor antagonist, SR141716A, did not attenuate the inhibitory effects of JWH-133 on these responses. Intraplantar preadministration of JWH-133 also inhibited (P < 0.05) carrageenan-induced expansion of peripheral receptive fields of WDR dorsal horn neurons. This study demonstrates that activation of peripheral CB2 receptors attenuates both innocuous- and noxious-evoked responses of WDR neurons in models of acute, inflammatory and neuropathic pain.


Cannabis is the most commonly used illicit drug in western societies, in particular among young people. It is consumed even by women during pregnancy and lactation, which result in a variety of disturbances in the development of their offspring, because, like other habit-forming drugs, cannabinoids, the psychoactive ingredients of marijuana, can cross the placental barrier and be secreted in the maternal milk. Through this way, cannabinoids affect the ontogeny of various neurotransmitter systems leading to changes in different behavioral patterns. Dopamine and endogenous opioids are among the neurotransmitters that result more affected by perinatal cannabinoid exposure, which, when animals mature, produce changes in motor activity, drug-seeking behavior, nociception and other processes. These disturbances are likely originated by the capability of cannabinoids to influence the expression of key genes for both neurotransmitters, in particular, the enzyme tyrosine hydroxylase and the opioid precursor proenkephalin. In addition, cannabinoids seem to be also able to influence the expression of genes encoding for neuron-glia cell adhesion molecules, which supports a potential influence of cannabinoids on the processes of cell proliferation, neuronal migration or axonal elongation in which these proteins are involved. In support of this possibility, CB(1) receptors, which represent
the major targets for the action of cannabinoids, are abundantly expressed in certain brain regions, such as the subventricular areas, which have been involved in these processes during brain development. Finally, cannabinoids might also be involved in the apoptotic death that occurs during brain development, possibly by influencing the expression of Bcl-2/Bax system. Also in support of this option, CB(1) receptors are transiently expressed during brain development in different group of neurons which do not contain these receptors in the adult brain. This paper will review all evidence relating cannabinoids to the expression of key genes for neural development, trying to establish the future research addressed to elucidate the mechanisms involved in the epigenetic action of cannabinoids during brain development.


The endogenous cannabinoid system is an ubiquitous lipid signalling system that appeared early in evolution and which has important regulatory functions throughout the body in all vertebrates. The main endocannabinoids (endogenous cannabis-like substances) are small molecules derived from arachidonic acid, anandamide (arachidonylethanolamide) and 2-arachidonoylglycerol. They bind to a family of G-protein-coupled receptors, of which the cannabinoid CB1 receptor is densely distributed in areas of the brain related to motor control, cognition, emotional responses, motivated behaviour and homeostasis. Outside the brain, the endocannabinoid system is one of the crucial modulators of the autonomic nervous system, the immune system and microcirculation. Endocannabinoids are released upon demand from lipid precursors in a receptor-dependent manner and serve as retrograde signalling messengers in GABAergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine. Endocannabinoids are transported into cells by a specific uptake system and degraded by two well-characterized enzymes, the fatty acid amide hydrolase and the monoacylglycerol lipase. Recent pharmacological advances have led to the synthesis of cannabinoid receptor agonists and antagonists, anandamide uptake blockers and potent, selective inhibitors of endocannabinoid degradation. These new tools have enabled the study of the physiological roles played by the endocannabinoids and have opened up new strategies in the treatment of pain, obesity, neurological diseases including multiple sclerosis, emotional disturbances such as anxiety and other psychiatric disorders including drug addiction. Recent advances have specifically linked the endogenous cannabinoid system to alcoholism, and cannabinoid receptor antagonism now emerges as a promising therapeutic alternative for alcohol dependence and relapse.


Anatomical studies have shown that the G-protein-coupled cannabinoid receptor-1 (CB1) is selectively expressed in a subset of GABAergic interneurons. It has been proposed that these cells regulate rhythmic activity and play a key role mediating the cognitive actions of marijuana and endogenous cannabinoids. However, the physiology, anatomy, and synaptic connectivity of neocortical CB1-expressing interneurons remain poorly studied. We identified a population of CB1-expressing interneurons in layer II/III in mouse neocortical slices. These cells were multipolar or bitufted, had a widely extending axon, and exhibited a characteristic pattern of irregular spiking (IS) in response to current injection. CB1-expressing-IS (CB1-IS) cells were inhibitory, establishing GABAergic receptor-mediated synapses onto pyramidal cells and other CB1-IS cells. Recently, electrical coupling among other classes of cortical interneurons has been shown to contribute to the generation of rhythmic synchronous activity in the neocortex. We therefore tested whether CB1-IS interneurons are interconnected via electrical synapses using paired recordings. We found that 90% (19 of 21 pairs) of simultaneously recorded pairs of CB1-IS cells were electrically coupled. The average coupling coefficient was 6%. Signaling through electrical synapses promoted coordinated firing among CB1-IS cells. Together, our results identify a population of electrically coupled CB1-IS GABAergic interneurons in the neocortex that share a unique morphology and a characteristic pattern of irregular spiking in response to current injection. The synaptic interactions of these cells may play an important role mediating the cognitive actions of cannabinoids and regulating coherent neocortical activity.

Acetylcholine stimulates the release of endothelium-derived arachidonic acid (AA) metabolites including prostacyclin and epoxyeicosatrienoic acids, which relax coronary arteries. However, mechanisms of endothelial cell (EC) AA activation remain undefined. We propose that 2-arachidonylglycerol (2-AG) plays an important role in this pathway. An AA metabolite isolated from bovine coronary ECs was identified as 2-AG by mass spectrometry. In ECs pretreated with the fatty acid amidohydrolase inhibitor, diazomethylarachidonyl ketone (DAK, 20 micromol/L), methacholine (10 micromol/L) stimulated 2-AG release was blocked by the phospholipase C inhibitor, U73122 (10 micromol/L) or the diacylglycerol lipase inhibitor, RHC80267 (40 micromol/L). In U46619-preconstricted bovine coronary arterial rings, 2-AG relaxations averaging 100% at 10 micromol/L were inhibited by endothelium removal, DAK, the hydrolase inhibitor, methyl arachidonoylfluorophosphate (10 micromol/L), the cyclooxygenase inhibitor, indomethacin (10 micromol/L) but not the CB1 cannabinoid receptor antagonist, SR141716 (1 micromol/L). The cytochrome P450 inhibitor, SKF525a (10 micromol/L) or the EET antagonist, 14,15-EEZE (10 micromol/L) further attenuated the indomethacin-resistant relaxations. The non-hydrolyzable 2-AG analogs, noladin ether, 2-AG amide and 14,15-EET glycerol amide did not induce relaxation. Nitro-L-arginine-resistance relaxations to methacholine were also inhibited by U73122, RHC80267 and DAK. 14,15-EET glycerol ester increased opening of large-conductance K(+) channels 12-fold in cell-attached patches of isolated smooth muscle cells and induced relaxations averaging 95%. These results suggest that methacholine stimulates EC 2-AG production through phospholipase C and diacylglycerol lipase activation. 2-AG is further hydrolyzed to AA, which is metabolized to vasoactive eicosanoids. These studies reveal a role for 2-AG in EC AA release and the regulation of coronary tone.


Echinacea plant preparations are widely used in the prevention and treatment of common cold. However, so far no molecular mechanism of action has been proposed. We analyzed the standardized tincture Echinaforce(TM) and found that it induced de novo synthesis of tumor necrosis factor alpha (TNF-alpha) mRNA in primary human monocytes/macrophages, but not TNF-alpha protein. Moreover, LPS-stimulated TNF-alpha protein was potently inhibited in the early phase but prolonged in the late phase. A study of the main constituents of the extract showed that the alkylamides dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides (1/2), trienoic (3) and dienoic acid (4) derivatives are responsible for this effect. The upregulation of TNF-alpha mRNA was found to be mediated by CB2 receptors, increased cAMP, p38/MAPK and JNK signaling, as well as NF-kappaB and ATF-2/CREB-1 activation. This study is the first to report a possible molecular mechanism of action of Echinacea, highlighting the role of alkylamides as potent immunomodulators and potential ligands for CB2 receptors.


The arachidonic acid derivative, 2-arachidonoyl-glycerol (2-AG), was initially isolated from gut and brain; it is also produced and released from blood and vascular cells. Many of the 2-AG-induced cellular responses (i.e., neuromodulation, cytoprotection and vasodilation) are mediated by cannabinoid receptors CB(1) and CB(2). The findings presented here demonstrate the expression of CB(1), CB(2) and TRPV1 receptors on cerebrovascular endothelial cells (HBEC). The expression of TRPV1, CB(1) and CB(2) receptor mRNA and proteins were demonstrated by RT-PCR and polyclonal antibodies, respectively. The endocannabinoid 2-AG, and other related compounds [anandamide (ANA), methanandamide (m-ANA), N-(4-hydroxyphenyl-arachidonyl-ethanolamide) (AM404) and capsaicin] dose-dependently stimulated Ca(2+) influx in HBEC. The selective TRPV1 receptor antagonist (capsazepine), CB(1) receptor antagonist (SR141716A) and CB(2) receptor antagonist (SR144528) inhibited these responses.
The effects of capsaicin, a specific agonist for TRPV1 receptors, were inhibited by capsazepine, but only weakly by CB(1) or CB(2) receptor antagonists. 2-AG also induced phosphorylation of vasodilator-stimulated phosphoprotein (VASP); this response was mediated by VR1 receptors. These studies clearly indicate that 2-AG and other related compounds may function as agonists on VR1 receptors, as well as CB(1) and CB(2) receptors, and implicated these factors in various HBEC functions.


Deficits in cognitive functioning and flexibility are seen following both chronic stress and modulation of endogenous cannabinoid (eCB) signaling. Here, we investigated whether alterations in eCB signaling might contribute to the cognitive impairments induced by chronic stress. Chronic stress impaired reversal learning and induced perseveratory behavior in the Morris water maze without significant effect on task acquisition. These cognitive impairments were reversed by exogenous cannabinoid administration, suggesting deficient eCB signaling underlies these phenomena. In line with this hypothesis, chronic stress downregulated CB(1) receptor expression and significantly reduced the content of the endocannabinoid 2-arachidonylglycerol within the hippocampus. CB(1) receptor density and 2-arachidonylglycerol content were unaffected in the limbic forebrain. These data suggest that stress-induced downregulation of hippocampal eCB signaling contributes to problems in behavioral flexibility and could play a role in the development of perseveratory and ruminatory behaviors in stress-related neuropsychiatric disorders. Neuropsychopharmacology advance online publication, 3 November 2004; doi:10.1038/sj.npp.1300601.


This review presents the remarkable research during the past several years indicating that some of the pharmacological and behavioral effects of alcohol, including alcohol drinking and alcohol-preferring behavior, are mediated through one of the most abundant neurochemical systems in the central nervous system, the endocannabinoid signaling system. The advances, with the discovery of specific receptors and the existence of naturally occurring cannabis-like substances in the mammalian system and brain, have helped in understanding the neurobiological basis for drugs of abuse, including alcoholism. The cDNA and genomic sequences encoding G-protein-coupled cannabinoid receptors (CB1 and CB2) from several species have now been cloned. This has facilitated discoveries of endogenous ligands (endocannabinoids). To date, two fatty acid derivatives characterized to be arachidonylethanolamide and 2-arachidonylglycerol have been isolated from both nervous and peripheral tissues. Both these compounds have been shown to mimic the pharmacological and behavioral effects of Delta(9)-tetrahydrocannabinol, the psychoactive component of marijuana. The involvement of the endocannabinoid signaling system in tolerance development to drugs of abuse, including alcohol, were unknown until recently. Studies from our laboratory demonstrated for the first time the downregulation of CB1 receptor function and its signal transduction by chronic alcohol. The observed downregulation of CB1 receptor binding and its signal transduction results from the persistent stimulation of receptors by the endogenous CB1 receptor agonists arachidonylethanolamide and 2-arachidonylglycerol, the synthesis of which is increased by chronic alcohol treatment. The deletion of CB1 receptor has recently been shown to block voluntary alcohol intake in mice, which is consistent with our previous findings where the DBA/2 mice known to avoid alcohol intake had significantly reduced brain CB1 receptor function. These findings suggest a role for the CB1 receptor gene in excessive alcohol drinking behavior and development of alcoholism. Ongoing investigations may lead to the development of potential therapeutic agents to modulate the endocannabinoid signaling system, which will be helpful for the treatment of alcoholism.

Multiple sclerosis is increasingly recognized as a neurodegenerative disease which is triggered by inflammation in the central nervous system (CNS). Demyelination-associated axonal or neuronal damage is a primary cause of disability and has thus far not been successfully targeted by available drug therapies. The neuroprotective properties of both endogenous and administered cannabinoids have been shown in in vivo and in vitro models of CNS damage following excitotoxic, oxidative, traumatic and ischaemic insults, with a predominantly apoptotic effector mechanism. In this study a foetal mouse telencephalon aggregate cell culture system was developed to compare tissue from cannabinoid receptor 1 knockout mice with wildtype counterparts. Aggregate formation and neurofilament/myelin basic protein accumulation were dependent on the age of foetal dissection and species used. Following treatment with interferon-gamma, levels of myelin basic protein, neurofilament, neuronal dephosphorylation and caspase 3 activation were assessed in telencephalon tissue in vitro. Cytokine treatment resulted in significant loss of the neuronal marker neurofilament-H in cannabinoid receptor 1 knockout cultures but not in wildtypes, indicating that presence of the cannabinoid receptor 1 gene can be neuroprotective. Caspase 3 activation was higher in cultures from knockout animals, indicating an apoptotic mechanism of cell death. Dephosphorylated neurofilament levels were significantly elevated in knockout mice, lending support to the premise that neurofilament dephosphorylation is a marker for neuronal damage. Taken together, these results indicate that neuroprotection could be elicited through the cannabinoid receptor 1, and point towards a potential therapeutic role for cannabinoid compounds in demyelinating conditions such as multiple sclerosis.


AIMS: Previous studies have shown that CB1 cannabinoid receptors are involved in the behavioural effects induced by chronic ethanol administration in Wistar rats by using SR 141716, a CB1 cannabinoid receptor antagonist. These studies have now been extended to investigate the effect of acute and chronic alcoholization on blood ethanol concentration (BEC) and ethanol preference in CB1 knockout (-/-) mice. METHODS: BEC was monitored for a period of 8 h in both CB1(-/-) male mice and CB1 male wild-type (+/+) mice, which had received an acute i.p. injection of ethanol in 1, 3 or 5 g/kg doses. Ethanol preference was assayed in both groups of male mice in non-forced ethanol administration and forced chronic pulmonary alcohol administration for 14 and 39 days, respectively. RESULTS: After an acute intraperitoneal ethanol injection of 5 g/kg, CB1(-/-) mice showed a significant higher BEC during the ethanol elimination stage than the CB1(+/+) mice. However, those in the 1 and 3 g/kg groups showed no significant difference. A 2-3 fold increase in BEC was observed in CB1(-/-) mice on days 10 and 11 after commencement of forced chronic pulmonary alcoholization in comparison with CB1(+/+) mice, although comparable BEC values were assayed in both groups on day 12. In addition, these CB1(-/-) mice showed a significantly lower preference for ethanol than CB1(+/+) mice. CONCLUSIONS: The studies on CB1(-/-) and CB1(+/+) mice have clearly confirmed the involvement of CB1 receptor on ethanol induced behavioural effects and also revealed that CB1 receptors may be implicated in ethanol absorption/distribution, particularly after administration of high ethanol doses.


It is well known that United States paper currency in general circulation is contaminated with trace amounts of illicit substances such as cocaine, heroin and marijuana. As is the case with cocaine, differentiating "background levels" of the various cannabinoid constituents of Cannabis sativa L., namely, Delta(9)-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD) contaminating currency found in the general circulation from currency associated with illegal drug activity is imperative if a legal nexus is to be established with the latter. We analyzed 165 randomly collected paper currency notes from 12 U.S. cities (N = 125) and 4 foreign countries (N = 40) for THC, CBD, CBN, 11-nor-9-carboxy-Delta(9)-tetrahydrocannabinol, and 11-hydroxy-Delta(9)-tetrahydrocannabinol. Uncirculated U.S. $1 notes were added as negative controls. Drug residues were washed from individual bills, extracted using a liquid-liquid extraction protocol, derivatized, and quantitated by gas chromatography-mass spectrometry by selected ion monitoring. For the U.S. $1 currency, THC was present in
1.6% (2 notes), CBN 10.31% (13 notes), CBD 1.6% (2 notes). The following concentrations were determined: 0.085 microg/bill and 0.146 microg/bill for THC; 0.014-0.774 microg/bill (mean 0.166 microg/bill) for CBN; and 0.032 microg/bill and 0.086 microg/bill for CBD. For the foreign currency (Colombia, Qatar, India, and New Zealand), THC and CBN were present in 22.5% (9 notes). The following concentration ranges were determined: THC 0.026-0.065 microg/bill (mean 0.049 microg/bill), CBN 0.061-0.197 microg/bill (mean 0.115 microg/bill). All of the positive THC and CBN were found in the New Zealand polypropylene notes. This study demonstrated that marijuana (cannabinoids) may contaminate both paper and plastic currency.


OBJECTIVE:: To investigate the effect of SR141716, a selective CB1 receptor antagonist, on energy expenditure and on glucose uptake in isolated soleus muscle of Lep(ob)/Lep(ob) mice. DESIGN:: Female Lep(ob)/Lep(ob) mice (8-10 weeks old) were treated with SR141716 (10 mg/kg, i.p. once daily) or vehicle for 7 days. MEASUREMENTS:: Oxygen consumption, daily food and water intake, body weight and glucose uptake in isolated soleus muscle. RESULTS:: SR141716 (10 mg/kg, i.p. once daily) resulted in a significant reduction of daily food intake (P<0.01) and body weight (P<0.05) 5 days after daily treatment. Body weight continued to be lower for the rest of the treatment period (P<0.05). There was no significant difference in body weight between the pair-fed and vehicle-treated animals. A 7-day treatment with SR141716 (10 mg/kg, i.p. once daily) caused 37% increase in basal oxygen consumption compared to that of vehicle-treated (90 min mean; P<0.01), and a significant 68% increase in glucose uptake in isolated soleus muscle preparations. CONCLUSION:: It is concluded that SR141716 has a direct effect on energy expenditure suggesting that the antiobesity effect of SR141716 is due to activation of thermogenesis in addition to the initial hypophagia. The increase in soleus muscle glucose uptake with SR141716 treatment may contribute to the improved glycaemia seen in the previous studies.International Journal of Obesity advance online publication, 23 November 2004; doi:10.1038/sj.ijo.0802847.


Among the studies that investigate the vasorelaxation induced by anandamide, one of the most frequent differences is the use of distinct solvents that could modify vascular function and explain the controversial results described. The aims of this study were: to evaluate the influence of different cannabinoid vehicles in vascular function of rat aorta, and to compare the vasorelaxation induced by anandamide dissolved in different vehicles. Vehicles were: ethanol (70%), Tween 80/ethanol (2:1 and 1:1), 1:1:18 (Tween 80/ethanol/saline) and dimethylsulphoxide (DMSO) 0.5%. All the vehicles tested, except DMSO 0.5%, modified the vascular and/or the endothelial function in rat aorta rings. Anandamide caused a time- and concentration-dependent vasorelaxation in all the experimental groups except in ethanol group, but the mechanisms involved in its vasorelaxation appear to be different depending on the vehicle used. The results obtained with vehicles containing Tween 80 suggest a non-endothelial component in the vasorelaxation caused by anandamide, while those obtained with DMSO at 0.5% suggest an endothelial component in this vasorelaxation.


Over the past few years, advances in the investigation of the neurochemical circuits involved in the development and treatment of alcohol dependence have identified peptides and receptors as potential key targets in the treatment of problems related to alcohol consumption. The endogenous opioid system is modified by alcohol intake in areas of the brain related to reward systems, and differential basal levels of opioid gene expression are found in rodents with a high preference for ethanol. This suggests a greater vulnerability to alcohol consumption in relation to differences in genetic background. Further evidence of the involvement of opioid peptides in alcohol dependence is the ability of the opioid antagonist naltrexone to reduce alcohol
intake in animal models of dependence and in alcohol-dependent patients. Abundant evidence indicates that the activation of cannabinoid receptors stimulates the release of opioid peptides, therefore the cannabinoid receptor antagonists may presumably alter opioid peptide release, thus facilitating the reduction of ethanol consumption. However, little is known about the effects of ethanol on the endogenous cannabinoid system, the vulnerability of cannabinoid receptors to alcohol intake or their neurochemical implications in reducing consumption of alcohol. In this paper, we review the role of opioid and cannabinoid receptor systems, their vulnerability to alcohol intake and the development of dependence, and the targeting of these systems in the treatment of alcoholism.


N-Arachidonylethanolamine (anandamide) is an endogenous agonist of the cannabinoid CB1 and CB2 receptors and displays many of the same receptor-mediated physiological effects as Delta(9)-tetrahydrocannabinol (Delta(9)-THC), the active component of marijuana. As with any neurotransmitter, there must be tight control of anandamide receptor-mediated signaling and a means of rapid removal of the molecule from the system. Thus, the process by which anandamide is transported into the cell for metabolism has been a topic of much interest and has been implicated as a potential drug target in the treatment of several disease states that are reported to have an association with the endocannabinoid system. In this review, we will discuss the current models proposed for the mechanism of anandamide transport, the progress that has been made in the development of compounds that specifically inhibit anandamide transport, the observed effects of anandamide transport inhibition in vivo, and finally, potential therapeutic applications of compounds that inhibit anandamide transport.


AIMS: Recent studies suggest that cannabinoid receptor agonists may promote relapse to drug-seeking behaviour after a period of abstinence. In this study, the ability of Delta(9)-tetrahydrocannabinol (THC) to reinstate previously reinforced responding for alcoholic and non-alcoholic beverages was assessed in rats using a novel lick-based paradigm. METHODS: Rats were initially given free access to beer (containing 4.5% ethanol v/v), near-beer (a beverage that looks and tastes like beer but contains <0.5% ethanol v/v) or isocaloric sucrose in their home cages for 3 weeks. They were then trained to lick at a tube to self-administer the pre-exposed beverage in operant chambers under a VR10 schedule in 30-min sessions daily. After approximately 3 weeks of such access, the rats underwent an extinction procedure, so that licking at the tube produced no reward. Once responding had ceased, the rats were subjected to various reinstatement tests. RESULTS: In Experiment 1, the cannabinoid receptor agonist Delta(9)-THC (1 mg/kg) significantly reinstated responding, previously reinforced with beer or near-beer. The effect was unlikely to be caused by increased appetite because 24 h food-deprivation had no such effect. Exposure to cat odour in the test chamber failed to reinstate responding for beer or near-beer and caused a complete inhibition of responding. In Experiment 2, Delta(9)-THC (0.3 and 1 but not 3 mg/kg) again reinstated beer-seeking behaviour while the 1 mg/kg dose also reinstated responding in sucrose trained animals. Midazolam (0.15 mg/kg but not 0.5 or 1.5 mg/kg) produced a modest reinstatement of beer-seeking but had no effect on sucrose-seeking behaviour. CONCLUSIONS: The finding that Delta(9)-THC can reinstate alcohol-seeking provides the impetus for further research into the involvement of the cannabinoid system in alcohol craving. However, the reinstatement of near-beer and sucrose-seeking behaviour caused by Delta(9)-THC suggests a relatively non-specific effect. This may perhaps be related to the stressor-like effects of cannabinoids, and their ability to activate key neural circuitry in the amygdala and bed nucleus of the stria terminals.

Pharmacological effects of cannabinoid ligands are thought to be mediated through cannabinoid CB(1) and CB(2) receptor subtypes. Sequence analysis revealed that rat and human cannabinoid CB(2) receptors are divergent and share 81% amino acid homology. Pharmacological analysis of the possible species differences between rat and human cannabinoid CB(2) receptors was performed using radioligand binding and functional assays. Pronounced species selectivity at the rat cannabinoid CB(2) receptor (50- to 140-fold) was observed with AM-1710 (3-(1,1-Dimethyl-heptyl)-1-hydroxy-9-methoxy-benzo[c]chromen-6-one) and AM-1714 (3-(1,1-Dimethyl-heptyl)-1-9-dihydroxy-benzo[c]chromen-6-one). In contrast, JWH-015 ((2-Methyl-1-propyl-1H-indol-3-yl)-naphthalen-1-yl-methanone) was 3- to 10-fold selective at the human cannabinoid CB(2) receptor. Endocannabinoid ligands were more human receptor selective. Cannabinoid CB(2) receptor antagonist, AM-630 ((6-Iodo-2-methyl-1-(2-morpholin-4-yl-ethyl)-1H-indol-3-yl)-(4-methoxy-phenyl)-methanone) was more potent at the rat receptor in radioligand and functional assays than that of the human receptor. The findings of the pharmacological differences between the human and rat cannabinoid CB(2) receptors in this study provide critical information for characterizing cannabinoid ligands in in vivo rodent models for drug discovery purpose.


The role of endocannabinoid signaling in the response of the brain to injury is tantalizing but not clear. In this study, transient middle cerebral artery occlusion (MCAo) was used to produce ischemia/reperfusion injury. Brain content of N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol were determined during MCAo. Whole brain AEA content was significantly increased after 30, 60 and 120 min MCAo compared with sham-operated brain. The increase in AEA was localized to the ischemic hemisphere after 30 min MCAo, but at 60 and 120 min, was also increased in the contralateral hemisphere. 2-Arachidonoylglycerol content was unaffected by MCAo. In a second set of studies, injury was assessed 24 h after 2 h MCAo. Rats administered a single dose (3 mg/kg) of the cannabinoid receptor type 1 (CB(1)) receptor antagonist SR141716 prior to MCAo exhibited a 50% reduction in infarct volume and a 40% improvement in neurological function compared with vehicle control. A second CB(1) receptor antagonist, LY320135 (6 mg/kg), also significantly improved neurological function. The CB(1) receptor agonist, WIN 55212-2 (0.1-1 mg/kg) did not affect either infarct volume or neurological score.


There are several similarities between the behavioral and neurochemical effects of lead (Pb(2+)) and the cannabinoids. Both Pb(2+) exposure and cannabinoid treatment decrease exploratory behavior. Pb(2+)-induced hyperactivity has been observed in rats and fish. By comparison, cannabinoids increase locomotor activity at higher doses in rats. Moreover, Pb(2+) exposure produces learning and memory impairments as do the cannabinoids. Many of the behavioral effects of Pb(2+) are thought to be due, in part, to the ability of Pb(2+) to either inhibit or mimic the actions of calcium (Ca(2+)). At low concentrations, Pb(2+) enhances basal release of neurotransmitter from presynaptic terminals by increasing intracellular free Ca(2+) concentrations. Pb(2+) also decreases evoked neurotransmitter release due to blockade of voltage-gated Ca(2+) channels. Interestingly, the endocannabinoids (eCBs) including N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) are synthesized in response to increases in intracellular Ca(2+) and activate the CB(1) receptor that inhibits voltage-gated Ca(2+) channels. We tested the hypothesis that waterborne Pb(2+) exposure significantly affects whole-brain eCB content in adult male and female fathead minnows (Pimephales promelas). Waterborne Pb(2+) exposure (1.0ppm) resulted in a time-dependent accumulation of Pb(2+) in bone in both males and females. Brain AEA and 2-AG content were significantly greater in females compared to males. Pb(2+) exposure increased brain AEA content in males at 7 and 14 days of exposure and increased brain 2-AG content at 14 days. Pb(2+) exposure had no effect on either brain AEA or 2-AG content in females at any of the time points examined. As eCBs serve
as activity-dependent retrograde inhibitors of neurotransmitter release, the increase in brain eCB content would accentuate Pb(2+)-induced decreases in evoked neurotransmitter release in male but not female fathead minnows.


The therapeutic use of cannabinoids, the components of cannabis sativa L., was investigated in numerous researches in detail. Animal studies revealed that cannabinoid receptor agonists alter pain-associated behaviour, have immune-suppressive properties, suppress tumor growth, modulate sensitisation processes and influence memory and learning. Those effects are mediated by two membrane-bound cannabinoid receptors and as mechanisms of signal transduction blockade of ion channels, inhibition of adenylate cyclase and retrograde inhibition of neurotransmitter release are currently being discussed. In clinical studies oral administration of cannabinoids indicated beneficial results during the therapy of multiple sclerosis, weight loss, nausea and vomiting due to chemotherapy, and intractable pruritus. However, therapy of chronic pain conditions revealed conflicting results and unequivocal success could not have been delivered due to unwanted side effects. Further multicentre studies are required to estimate cannabinoids as novel therapeutic tools for the treatment of chronic pain.


Zebra finch song is a form of vocal communication learned during at least two distinct stages of late postnatal development. During the first of these stages, termed auditory learning, nestlings memorize the song pattern of an adult male tutor, usually the father. During the second stage, sensory-motor learning, these song patterns are practiced and refined until a good copy is produced by adulthood. Vocal learning has made zebra finches a useful model for studying drug effects during vocal development. Prior work has shown that daily exposure to a modest dosage of the cannabinoid agonist WIN55212-2 (WIN) alters sensory-motor learning by reducing stereotypy scores and numbers of note types learned. Here we report that these two effects are produced independently during subperiods of the sensory-motor learning stage. Additional temporally distinct WIN effects during sensory-motor learning include differential incorporation of tutor-derived and improvised note types. We have also evaluated acute and chronic effects of WIN exposure on ability to encode a tutor’s song during auditory learning, finding significant effects on stereotypy and distinct effects on note duration and internote intervals. Taken together, these results demonstrate the presence of distinct subperiods of cannabinoid sensitivity during zebra finch auditory and sensory-motor vocal development.


Singing by adult male zebra finches is a learned behavior important for courtship, kin recognition, and nest defense (Zann, 1996) and is inhibited by both brief periods of limited food availability and systemic injection of cannabinoids. These similar effects on singing, combined with increasing evidence for endocannabinoid involvement in feeding behavior, led us to evaluate a possible shared mechanism. We found that limited food availability both reduces singing in a cannabinoid antagonist-reversible manner and increases levels of the endocannabinoid 2-arachidonyl glycerol in various brain regions including the caudal telencephalon, an area that contains auditory telencephalon including the L2 subfield of L (L2) and caudal medial nidopallium (NCM). Development and use of an anti-zebra finch cannabinoid receptor type 1 (CB1) antibody demonstrates distinct, dense cannabinoid receptor expression within song regions including Area X, IMAN (lateral magnocellular nucleus of anterior nidopallium), HVC, RA (robust nucleus of arcopallium), and L2. NCM receives L2 projections and is implicated in integration of auditory information. Activity in this area, determined through expression of the transcription factor ZENK, is increased after exposure to unfamiliar song. Because previous work has shown that these novel song-stimulated increases in NCM activity are mitigated by cannabinoid exposure, we tested and found that similar effects on ZENK expression are produced by limiting food. Limited food-related reductions in the activity of NCM neurons were reversed by the cannabinoid...
antagonist SR141716A (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide), implicating CB1 cannabinoid receptor involvement. Taken together, these experiments indicate a link between feeding state and gene expression related to auditory perception that is mediated by endocannabinoid signaling.


Efficient reuptake of synaptically released glutamate is essential for preventing glutamate receptor overstimulation and neuronal death. Glutamate transporters play a vital role in removing extracellular glutamate from the synaptic cleft. This study analyzed the expression of the glial (GLAST) and neuronal (EAAC1) subtypes of glutamate transporter in the cerebellum of male and female offspring exposed pre- and postnatally to Delta9-tetrahydrocannabinol (THC, the main component of marijuana). Pregnant rats were administered saline or THC from gestational day 5 to postnatal day 20 (PD20). The expression of glutamate transporters was examined at PD20, PD30 and PD70 (10 and 50 days after THC withdrawal) to analyze the short- and long-term effects of prenatal THC exposure. The expression of the glutamate transporter GLAST in astroglial cells and EAAC1 in Purkinje neurons decreased in THC-exposed offspring compared to controls. This reduction was observed at all ages but mainly in males. Moreover, the glial glutamate transporter level in THC-exposed rats (quantified by Western blot) was lower than in control rats. These results suggest that THC exposure during cerebellar development may alter the glutamatergic system not only during the period of drug exposure but in the postnatal stage following withdrawal. The down-regulation reported here might reflect an abnormal maturation of the glutamatergic neuron-glia circuitry.


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.


The antinociceptive action of cannabinoids in acute and inflammatory pain states have been well-documented. There is also accumulating evidence suggesting that cannabinoids are effective analgesics in chronic pain conditions. WIN 55,212-2, a mixed CB1 and CB2 cannabinoid receptor agonist, has been shown to be effective against hyperalgesia and allodynia in painful peripheral mononeuropathy. Recently, in addition to their spinal and supraspinal antinociceptive
action, cannabinoids have also reported to exert local analgesic effects. The aim of this study is to observe the effect of a high affinity cannabinoid, WIN 55,212-2, on tactile allodynia and thermal hyperalgesia in diabetic rats. Diabetes was produced with the injection of a single dose of streptozocin (50mg/kg, i.p.) and this procedure resulted in neuropathic pain behaviors in the hindlimbs. Mechanical allodynia was detected by application of von Frey filaments to the plantar surface of the foot, and thermal hyperalgesia was studied using the Hargreaves’ method; however, thermal hyperalgesia did not develop in diabetic rats. With its higher doses, both systemic (3 and 10mg/kg, i.p.) and peripheral (30mug, i.p.,l.) injections of WIN 55,212-2 reduced mechanical allodynia. These results suggest that WIN 55,212-2 has an antiallodynic effect in streptozocin-induced diabetic rats and may be a promising approach in the treatment of diabetic neuropathy.


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 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.


The fourth cytoplasmic domain, the so-called C-terminal juxtamembrane segment or Helix VIII, has been identified in numerous G-protein coupled receptors and exhibits unique functional characteristics. Efforts have been devoted to study the juxtamembrane segment in order to understand the biological importance of the segment in G-protein activation of the cannabinoid CB1 and CB2 receptors. Recent biochemical data revealed that the CB1 C-terminal juxtamembrane peptide fragment (CB1401-417) can directly activate the G-protein, and also showed that the specificity of the signal transduction activation by the C-terminal juxtamembrane region is unique to the CB1 receptor but not to the CB2 receptor. However, there is not yet reported experimental work on the conformational analyses and structural comparison between the respective Helix VIII segments of the two receptors. In the present study, we have examined the conformational specificities of the cytoplasmic helical domains for both cannabinoid receptors. 3D structural features of two synthetic CB1 and CB2 peptides, CB1I397-G418 and CB2I298-K319, respectively in membrane-mimetic DPC micelles, were studied using a combined high-resolution NMR and computer modeling approach. Comparisons of the NMR determined structures of the two peptides as well as their correspondent mutant peptides revealed their conformational properties and salt bridge dissimilarity, which might help to understand the
different structural roles of the fourth cytoplasmic helices in the function and regulation of CB1 and CB2 receptors.


Opioids and cannabinoids produce antinociception through activity at spinal, supraspinal and peripheral sites. Tolerance to the antinociceptive effects of both the opioids and the cannabinoids develop when these agents are administered chronically. Although mutual potentiation of antinociceptive effects have been reported between opioids and cannabinoids, the development of antinociceptive cross-tolerance between these systems has not been demonstrated consistently. In the present investigation, we explored the possibility of antinociceptive cross-tolerance between systemic or topical morphine and systemic or topical cannabinoids in mice. Mice were made tolerant to morphine either by the subcutaneous (s.c.) implantation of a morphine pellet or repeated topical administration and then challenged with the mixed CB(1) and CB(2) receptor agonist WIN55,212-2 given s.c. or topically. Antinociception was indicated by increased tail-flick latencies to noxious radiant heat. Implantation with morphine pellets did not attenuate the antinociceptive potency of systemic or topical WIN 55,212-2. Moreover, twice-daily topical administration of morphine did not attenuate the antinociceptive potency of WIN 55,212-2 applied topically. These observations suggest that opioids and cannabinoids produce antinociception through mechanisms that are independent of each other at either the systemic or peripheral levels.


The vanilloid receptor type 1 (TRPV1/VR1) is a non-specific calcium-permeable ionotropic cation channel expressed in the peripheral sensory system as well as in the central nervous system. An endogenous ligand for TRPV1 is arachidonoyl ethanolamide (anandamide), which also activates the metabotropic cannabinoid receptor 1 (CB1). Previous studies in this laboratory reported CB1 receptors and CB1-mediated effects on voltage-gated currents in goldfish cones and bipolar cells. In this study, we show TRPV1-like-immunoreactivity (TRPV1-L-IR) by immunoblot analysis of goldfish retina and rat brain homogenates with a guinea pig polyclonal antibody against the C-terminus of the rat TRPV1. Light-level immunocytochemistry showed restriction of the guinea pig-TRPV1 antibody to a very narrow band in the outer plexiform layer in goldfish and zebrafish retinas. However, no immunoreactivity was detected using rabbit-polyclonal antibodies against the C or N-termini of the rat TRPV1. Pre and post-embedding electron microscopy (EM) immunocytochemistry revealed that TRPV1-L-IR (using the guinea pig antibody) was restricted to synaptic ribbons of all cones and many rods, but never was observed at the synaptic ribbons of bipolar cells. While pre-embedded tissue showed diffuse label associated only with photoreceptor-synaptic ribbons, analysis of post-embedded tissue showed label tightly restricted to synaptic ribbons of all cones and many rods. Oblique sections showed that immunogold particles were confined to the outer electron dense region of the ribbons, with few or no gold particles in the ribbon core or associated with tethers or vesicles. Although TRPV1-L-IR described here, does not necessarily represent TRPV1 antigen associated with synaptic ribbons, these data provide an unequivocal label with which to study the functional dynamics of ribbon formation and degradation in teleost photoreceptors.

**CLINICAL SCIENCE**


The etiology of the pruritus of cholestasis is unknown. It is inferred that the pruritogen(s) is produced in the liver, excreted in bile, and as a result of cholestasis it accumulates in plasma. It may follow, logically, that the removal of the substance(s) that mediate pruritus leads to its resolution. The problem with this approach, however, is that the substance(s) is unknown; thus, it is not possible to reduce its serum levels specifically. Oral cholestyramine, a resin that is not
absorbed, is associated with increased fecal excretion of certain substances, including cholesterol and bile acids. Many patients respond to treatment with cholestyramine with a relief of pruritus, which unfortunately may be temporary, but is well tolerated in general and it seems reasonable to prescribe it as an initial therapy. When pruritus is not relieved by resins, the use of opiate antagonists (e.g., naloxone and naltrexone) is supported by data from controlled clinical trials. Butorphanol is an agonist at the kappa opioid receptor and an antagonist at the mu opioid receptor with minimal or absent abuse potential. The use of butorphanol spray in selective patients may be a therapeutic alternative. In uncontrolled observations dronabinol, an agonist at the cannabinoid B1 receptor, and sertraline, a serotonin reuptake inhibitor, have been reported to be associated with the relief of pruritus. The cannabinoidergic and serotonergic systems participate in the mediation of nociception; therefore, there appears to be a rationale for the use of these drugs to treat pruritus. Data from controlled clinical trials on the use of dronabinol and sertraline, however, are not available at present.


Multiple sclerosis is a common human demyelinating disease of the central nervous system (CNS), and it is thought to involve autoimmune responses to CNS myelin antigens. Current symptomatic therapies for multiple sclerosis are in some cases ineffective and may have a high risk of serious side effects. This has led some multiple sclerosis patients to self-medicate with cannabis, which anecdotal evidence suggests may be beneficial in controlling symptoms such as spasticity, pain, tremor and bladder dysfunction. In support of these claims, results from experimental studies have suggested that cannabinoid-based treatments may be beneficial in a wide number of diseases. Furthermore, recent research in animal models of multiple sclerosis has demonstrated the efficacy of cannabinoids in controlling disease-induced symptoms such as spasticity and tremor, as well as in ameliorating the severity of clinical disease. However, these initially promising results have not yet been fully translated into the clinic. Although cannabinoid treatment of multiple sclerosis symptoms has been shown to be both well tolerated and effective in a number of subjective tests in several small-scale clinical trials, objective measures demonstrating the efficacy of cannabinoids are still lacking. Currently, a number of large-scale phase III clinical trials are under way to further elucidate the use of cannabinoids in the symptomatic treatment of multiple sclerosis. This review highlights the recent advances in our understanding of the endocannabinoid system, discusses both the experimental and clinical evidence for the use of cannabinoids to treat multiple sclerosis and explores possible future strategies of cannabinoid therapy in multiple sclerosis. (c) 2004 Prous Science. All rights reserved.


Chromatographic separation of highly polar basic drugs with ideal ionspray mass spectrometry volatile mobile phases is a difficult challenge. A new quantification procedure was developed using hydrophilic interaction chromatography-mass spectrometry with turbo-ionspray ionization in the positive mode. After addition of deuterated internal standards and simple clean-up liquid extraction, the dried extracts were reconstituted in 500 microL pure acetonitrile and 5 microL was directly injected onto a Waters Atlantis HILIC 150- x 2.1-mm, 3-microm column. Chromatographic separations of cocaine, seven metabolites, and anhydroecgonine were obtained by linear gradient-elution with decreasing high concentrations of acetonitrile (80-56% in 18 min). This high proportion of organic solvent makes it easier to be coupled with MS. The eluent was buffered with 2 mM ammonium acetate at pH 4.5. Except for m-hydroxy-benzoylecgonine, the within-day and between-day precisions at 20, 100, and 500 ng/mL were below 7 and 19.1%, respectively. Accuracy was also below +/- 13.5% at all tested concentrations. The limit of quantification was 5 ng/mL (%Diff < 16.1, %RSD = 4.3) and the limit of detection below 0.5 ng/mL. This method was successfully applied to a fatal overdose. In Switzerland, cocaine abuse has dramatically increased in the last few years. A 45-year-old man, a known HIV-
positive drug user, was found dead at home. According to relatives, cocaine was self-injected about 10 times during the evening before death. A low amount of cocaine (0.45 mg) was detected in the bloody fluid taken from a syringe discovered near the corpse. Besides injection marks, no significant lesions were detected during the forensic autopsy. Toxicological investigations showed high cocaine concentrations in all body fluids and tissues. The peripheral blood concentrations of cocaine, benzoylecgonine, and methylecgonine were 5.0, 10.4, and 4.1 mg/L, respectively. The brain concentrations of cocaine, benzoylecgonine, and methylecgonine were 21.2, 3.8, and 3.3 mg/kg, respectively. The highest concentrations of norcocaine (about 1 mg/L) were measured in bile and urine. Very high levels of cocaine were determined in hair (160 ng/mg), indicating chronic cocaine use. A low concentration of anhydroecgonine methylester was also found in urine (0.65 mg/L) suggesting recent cocaine inhalation. Therapeutic blood concentrations of fluoxetine (0.15 mg/L) and buprenorphine (0.1 microg/L) were also discovered. A relatively high concentration of Delta(9)-THC was measured both in peripheral blood (8.2 microg/L) and brain cortex (13.5 microg/kg), suggesting that the victim was under the influence of cannabis at the time of death. In addition, fluoxetine might have enhanced the toxic effects of cocaine because of its weak pro-arrhythmogenic properties. Likewise, combination of cannabinoids and cocaine might have increase detrimental cardiovascular effects. Altogether, these results indicate a lethal cocaine overdose with a minor contribution of fluoxetine and cannabinoids.


Although exposure to exocannabinoids (e.g. marijuana) is associated with adverse pregnancy outcome, little is known about the biochemistry, physiology, and consequences of endocannabinoids in human pregnancy. In these studies, we measured the levels of the endocannabinoid anandamide (N-arachidonoyylethanolamine, AEA) by HPLC-mass spectrometry in 77 pregnant and 25 nonpregnant women. The mean +/- sem plasma AEA levels in the first, second, and third trimesters were 0.89 +/- 0.14, 0.44 +/- 0.12, and 0.42 +/- 0.11 nm, respectively. The levels in the first trimester were significantly higher than those in either the second or third trimester. During labor, AEA levels were 3.7 times nonlaboring term levels (2.5 +/- 0.22 vs. 0.68 +/- 0.09 nm, P < 0.0001). During the menstrual cycle, levels in the follicular phase were significantly higher than those in the luteal phase (1.68 +/- 0.16 vs. 0.87 +/- 0.09 nm, P < 0.005). Postmenopausal and luteal-phase levels were similar to those in the first trimester. These findings suggest that successful pregnancy implantation and progression requires low levels of AEA. At term, AEA levels dramatically increase during labor and are affected by the duration of labor, suggesting a role for AEA in normal labor.


Understanding the relationship of (9)-tetrahydrocannabinol (THC) concentrations in oral fluid and plasma is important in interpretation of oral fluid test results. Current evidence suggests that THC is deposited in the oral cavity during cannabis smoking. This "depot" represents the primary or sole source of THC found when oral fluid is collected and analyzed. In this research, oral fluid and plasma specimens were collected from six subjects following smoking of cannabis cigarettes containing 1.75% and 3.55% THC. There was at least one week between each cannabis administration. Plasma specimens were analyzed by gas chromatography-mass spectrometry (GC-MS) and paired oral fluid specimens were analyzed by radioimmunoassay (RIA). In addition, one individual's oral fluid specimens were also analyzed by GC-MS. These data are unique in that they represent simultaneous or near simultaneous collection of oral fluid and plasma specimens in subjects following controlled cannabis dosing. The first oral fluid specimen, collected from one subject at 0.2 h following initiation of smoking, contained a THC concentration of 5800 ng/mL (GC-MS). By 0.33 h, the THC concentration in oral fluid had fallen to 81 ng/mL. From approximately 0.3 h through 4.0 h, the mean (+/- SD) THC ratio of oral fluid to plasma THC concentrations was 1.18 (0.62) with a range of 0.5 to 2.2. Within 12 h, both oral fluid and plasma THC concentrations generally declined below 1 ng/mL. RIA analyses of oral fluid
specimens for six subjects demonstrated the same pattern of initial high levels of contamination immediately after smoking, followed by rapid clearing, and a slower decline over 12 h. Mean THC oral fluid concentrations by RIA at 0.2 h were 864 ng/mL and 4167 ng/mL compared to plasma concentrations of 52 ng/mL and 230 ng/mL at 0.27 h following the low- and high-dose cannabis cigarettes, respectively. The similarity in oral fluid and plasma THC concentrations following the dissipation of the initial "contamination" indicates the likelihood of a physiological link between these specimens. Recent studies have shown that sublingual or transmucosal administration of pure THC results in direct absorption of intact THC into the bloodstream, thereby bypassing the gastrointestinal tract. The current study demonstrates that THC is deposited in the oral cavity and remains for up to 24 h following cannabis smoking. The decline in THC oral fluid concentration over this time suggests that there may be absorption of THC into blood as previously shown with pure THC. Passive cannabis exposure studies appear to indicate that positive oral fluid tests for THC can occur shortly after cannabis smoke exposure, but results were negative within 1 h. Consequently, when very recent passive exposure to cannabis smoke can be ruled out, it is concluded that a positive oral fluid test provides credible evidence of active cannabis use.


Obesity has been described as a global epidemic. Its increasing prevalence is matched by growing costs, not only to the health of the individual, but also to the medical services required to treat a range of obesity-related diseases. In most instances, obesity is a product of progressively less energetic lifestyles and the over-consumption of readily available, palatable, and highly caloric foods. Past decades have seen massive investment in the search for effective anti-obesity therapies, so far with limited success. An important part of the process of developing new pharmacologic treatments for obesity lies in improving our understanding of the psychologic and physiologic processes that govern appetite and bodyweight regulation. Recent discoveries concerning the endogenous cannabinoids are beginning to give greater insight into these processes. Current research indicates that endocannabinoids may be key to the appetitive and consummatory aspects of eating motivation, possibly mediating the craving for and enjoyment of the most desired, most fattening foods. Additionally, endocannabinoids appear to modulate central and peripheral processes associated with fat and glucose metabolism. Selective cannabinoid receptor antagonists have been shown to suppress the motivation to eat, and preferentially reduce the consumption of palatable, energy-dense foods. Additionally, these agents act to reduce adiposity through metabolic mechanisms that are independent of changes in food intake. Given the current state of evidence, we conclude that the endocannabinoids represent an exciting target for new anti-obesity therapies.


This review examines the development of cannabinoid CB1 receptor antagonists as a new class of therapeutic agents for drug addiction. Abused drugs (alcohol, opiates, Delta(9)-tetrahydrocannabinol (Delta(9)-THC) and psychostimulants, including nicotine) elicit a variety of chronically relapsing disorders by interacting with endogenous neural pathways in the brain. In particular, they share the common property of activating mesolimbic dopamine brain reward systems and virtually all abused drugs elevate dopamine levels in the nucleus accumbens. Cannabinoid CB1 receptors are expressed in this brain reward circuit and modulate the dopamine-releasing effects of Delta(9)-THC and nicotine. Rimonabant (SR141716), a CB1 receptor antagonist, blocks both the dopamine-releasing and the discriminative and rewarding effects of Delta(9)-THC in animals. Blockade of CB1 receptor activity by genetic invalidation also decreases rewarding effects of opiates and alcohol in animals. Although CB1 receptor blockade is generally ineffective in reducing the self-administration of cocaine in rodents and primates, it reduces the reinstatement of extinguished cocaine-seeking behavior produced by cocaine-associated conditioned stimuli and cocaine priming injections. Similarly, CB1 receptor blockade is effective in reducing nicotine-seeking behavior induced by re-exposure to nicotine-associated stimuli. Some of these findings have been recently validated in humans. In clinical trials, Rimonabant blocks the subjective effects of Delta(9)-THC in humans and prevents relapse to
smoking in ex-smokers. Findings from both clinical and preclinical studies suggest that ligands blocking CB1 receptors offer a novel approach for patients suffering from drug dependence that may be efficacious across different classes of abused drugs.


Oral fluid testing for Delta(9)-tetrahydrocannabinol (THC) provides a convenient means of detection of recent cannabis usage. In this study, the risk of positive oral fluid tests from passive cannabis smoke exposure was investigated by housing four cannabis-free volunteers in a small, unventilated, and sealed room with an approximate volume of 36 m³. Five active cannabis smokers were also present in the room, and each smoked a single cannabis cigarette (1.75% THC). Cannabis smoking occurred over the first 20 min of the study session. All subjects remained in the room for approximately 4 h. Oral fluid specimens were collected with the Intercept DOA Oral Specimen Collection Device. Three urine specimens were collected (0, 20, and 245 min). In addition, three air samples were collected for measurement of THC content. All oral fluid specimens were screened by enzyme immunoassay (EIA) for cannabinoids (cutoff concentration = 3 ng/mL) and tested by gas chromatography-tandem mass spectrometry (GC-MS-MS) for THC (LOQ/LOD = 0.75 ng/mL). All urine specimens were screened by EIA for cannabinoids (cutoff concentration = 50 ng/mL) and tested by GC-MS-MS for THCCOOH (LOQ/LOD = 1 ng/mL). Air samples were measured for THC by GC-MS (LOD = 1 ng/L). A total of eight oral fluid specimens (collected 20 to 50 min following initiation of smoking) from the four passive subjects screened and confirmed positive for THC at concentrations ranging from 3.6 to 26.4 ng/mL. Two additional specimens from one passive subject, collected at 50 and 65 min, screened negative but contained THC in concentrations of 4.2 and 1.1 ng/mL, respectively. All subsequent specimens for passive participants tested negative by EIA and GC-MS-MS for the remainder of the 4-h session. In contrast, oral fluid specimens collected from the five cannabis smokers generally screened and confirmed positive for THC throughout the session at concentrations substantially higher than observed for passive subjects. Urine specimens from active cannabis smokers also screened and confirmed positive at conventional cutoff concentrations. A biphasic pattern of decline for THC was observed in oral fluid specimens collected from cannabis smokers, whereas a linear decline was seen for passive subjects suggesting that initial oral fluid contamination is cleared rapidly and is followed by THC sequestration in the oral mucosa. It is concluded that the risk of positive oral fluid tests from passive cannabis smoke inhalation is limited to a period of approximately 30 min following exposure.


Only a few studies have been carried out in children on the prevention of chemotherapy-induced acute emesis. 5-HT3 antagonists have been shown to be more efficacious and less toxic than metoclopramide, phenothiazines and cannabinoids. The optimal dose and scheduling of the 5-HT3 antagonists has not been identified. Combinations of a 5-HT3 antagonist and dexamethasone show increased efficacy with respect to 5-HT3 antagonists alone. All pediatric patients receiving chemotherapy of high or moderate emetogenic potential should receive a combination of a 5-HT3 antagonist and dexamethasone to prevent acute emesis. No studies have specifically evaluated antiemetic drugs in the prevention of chemotherapy-induced delayed and anticipatory emesis in children.


Health Canada may be poised to emulate the Netherlands’ system of distributing marijuana to HIV/AIDS and other patients through pharmacies. Meanwhile, revisions to the much-criticized medical marijuana regulatory system are under development.
BEHAVIOURAL SCIENCE


The authors review the literature examining the validity and significance of cannabis withdrawal syndrome. Findings from animal laboratory research are briefly reviewed, and human laboratory and clinical studies are surveyed in more detail. Converging evidence from basic laboratory and clinical studies indicates that a withdrawal syndrome reliably follows discontinuation of chronic heavy use of cannabis or tetrahydrocannabinol. Common symptoms are primarily emotional and behavioral, although appetite change, weight loss, and physical discomfort are also frequently reported. The onset and time course of these symptoms appear similar to those of other substance withdrawal syndromes. The magnitude and severity of these symptoms appear substantial, and these findings suggest that the syndrome has clinical importance. Diagnostic criteria for cannabis withdrawal syndrome are proposed.


We used PET (15)O and a modified version of the Stroop task to determine if 25-day abstinent heavy marijuana (MJ) users have persistent deficits in executive cognitive functioning (ECF) and brain activity. Performance on a modified version of the Stroop task and brain activity was compared between 25-day abstinent, heavy marijuana users (n = 11), and a matched comparison group (n = 11). The 25-day abstinent marijuana users showed no deficits in performance on the modified version of the Stroop task when compared to the comparison group. Despite the lack of performance differences, the marijuana users showed hypoactivity in the left perigenual anterior cingulate cortex (ACC) and the left lateral prefrontal cortex (LPFC) and hyperactivity in the hippocampus bilaterally, when compared to the comparison group. These results suggest that marijuana users display persistent metabolic alterations in brain regions responsible for ECF. It may be that marijuana users recruit an alternative neural network as a compensatory mechanism during performance on a modified version of the Stroop task. These differences in brain activity may be a common denominator in the evolution of maladaptive behaviors such as substance abuse and other neuropsychiatric disorders.


Cannabis has been used for recreational, medicinal and religious purposes in different cultures since ancient times. There have been various reports of adverse effects due to or associated with cannabis consumption, including psychotic episodes. Historically, our understanding of these clinical observations has been significantly hindered by a lack of knowledge regarding their underlying neurobiological and pharmacological processes. However, the discovery of the endogenous cannabinoid system has allowed a greater understanding of these adverse effects to develop. From a clinical perspective, toxic or transient psychotic reactions to the administration of herbal cannabis preparations or specific cannabinoid compounds have to be differentiated from longer-lasting, persistent schizophrenia-like disorders associated with the use of cannabis/cannabinoids. The latter are most likely to be associated with a predisposition or vulnerability to schizophrenia. Interestingly, the recently suggested role of the endogenous cannabinoid system in schizophrenia not related to previous cannabinoid consumption introduces an additional perspective on the mechanism underlying cannabis-associated schizophrenia-like disorders, as well as on the effects of cannabis consumption in schizophrenia. At present, acute psychopharmacological treatment options for cannabis-associated transient and persistent schizophrenia-like psychotic episodes are similar and are based on the use of benzodiazepines and antipsychotics. However, new pharmacological strategies using the endogenous cannabinoid system as a primary target are under development. Long-term psychotherapeutic treatment
options involve case management strategies and are mainly based on specialised psychotherapeutic programmes to encourage cannabis users to stop their use of the drug.

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