INTRODUCTION

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BASIC SCIENCE


RATIONALE. CB1 receptors (CB1Rs) mediate many of the psychoactive effects of cannabinoids, and marijuana intoxication can produce neurocognitive deficits with a similarity to those seen in schizophrenia, including impairments of attention. OBJECTIVES. We thus sought to characterize the effects of a CB1R-selective agonist and antagonist on attention in the rat using a lateralized reaction time task (LRT). We hypothesized that CB1R agonists would impair performance and that CB1R antagonists might improve performance. METHODS. Subjects were trained to perform the LRT, a procedure that measured their ability to attend to and detect brief visual target stimuli. After training, we tested the effects of the CB1R agonist WIN55,212-2 (WIN; 0-2.5 mg/kg) or the CB1R antagonist SR141716A (SR; 0-1.0 mg/kg), administered alone or in combination, on visual attention performance using task conditions in which target stimulus salience was varied systematically across trials. RESULTS. The highest dose of WIN reduced correct choices in well-trained rats, with impairment greatest at the shortest stimulus durations. The highest dose of WIN also increased omissions and slowed response times. By contrast, SR itself did not produce any measurable effects on performance but was able to prevent the impairment produced by WIN. CONCLUSIONS. These results suggest that CB1Rs mediate the attentional performance impairments caused by acute administration of cannabinoid agonists and begin to unravel the possible contribution of cannabinoid systems to the pathophysiological substrates of cognitive dysfunction in schizophrenia.


The name Cannabinoid applies to a large and diverse family of compounds including plant derived, synthetic and endogenously produced chemicals, some but not all of which are psychotropic. Cannabinoids of all classes have the ability to protect neurons from a variety of insults that are believed to underlie delayed neuronal death after traumatic brain injury (TBI), including excitotoxicity, calcium influx, free radical formation and neuroinflammation. The pathways and experimental models supporting a neuroprotective role for the various classes of cannabinoids are critically reviewed vis a vis their potential to support the development of a clinically viable neuroprotective agent for human TBI.


Endocannabinoid signaling has been shown to be enhanced in several cancer tissues and malignant cells, and studies in cell lines have shown that this up-regulation might serve the purpose of providing transformed cells with a further means to inhibit their proliferation. Here we investigated the effect of inhibitors of endocannabinoid degradation on the growth of rat thyroid tumor xenografts induced in athymic mice. VDM-11, a selective inhibitor of endocannabinoid...
cellular re-uptake, and arachidonoyl-serotonin (AA-5-HT), a selective blocker of endocannabinoid enzymatic hydrolysis, both inhibited the growth in vivo of tumor xenografts induced by the subcutaneous injection of rat thyroid transformed (KiMol) cells. This effect was accompanied by significantly enhanced endocannabinoid concentrations in the tumors excised at the end of the in vivo experiments. Endocannabinoids, as well as VDM-11 and AA-5-HT, inhibited the growth in vitro of the transformed rat thyroid cells used to induce the tumors in vivo, and their effect was reversed at least in part by the cannabinoid CB1 receptor antagonist SR141716A. This compound, however, when administered alone, did not enhance, but instead slightly inhibited, the growth of rat thyroid transformed cells both in vitro and in tumor xenografts induced in vivo. These findings indicate that endocannabinoids tonically control tumor growth in vivo by both CB1-mediated and non-CB1-mediated mechanisms and that, irrespective of the molecular mechanism of their anti-proliferative action, inhibitors of their inactivation might be used for the development of novel anti-cancer drugs.


RATIONALE. A growing evidentiary body indicates cannabinoid exposure is conducive to cognitive impairment and psychotic phenomena in vulnerable individuals. In this respect, recent studies have displayed controversial results on the ability of cannabinoids to elicit sensorimotor gating alterations and attentional filtering, whose disruption is a distinctive feature of psychosis. OBJECTIVES. The goal of this study was to investigate the effects of acute, subchronic, and chronic treatment with the synthetic CB receptor agonist WIN 55,212-2 (WIN) on prepulse inhibition (PPI) of the acoustic startle reflex (ASR), a powerful paradigm for evaluation of sensorimotor gating. METHODS. Different groups of adult Sprague-Dawley rats were treated with 0.5, 1, and 2 mg/kg WIN (i.p.) acutely, as well as for 7 days and 21 days. All animals underwent testing 40 min after the last treatment and their evaluation was compared with that of animals treated with vehicle. In a separate group, the effects of WIN withdrawal were also analyzed, 24 h after discontinuation of a 21-day treatment. RESULTS. No variation in PPI was detected in any of the test groups when compared with controls, whatever the dosage and the treatment. CONCLUSIONS. These findings suggest WIN does not impair sensorimotor gating in Sprague-Dawley rats and confirm clinical evidence according to which cannabis is an unlikely causative of psychosis among non-vulnerable individuals. Nonetheless, since in other studies the same compound was shown to induce PPI alterations in Wistar rats, our results are also suggestive that genetic differences might be critical for the development of cannabis-induced cognitive disorders.


N-(hydroxyphenyl)-arachidonamide (AM404) is an inhibitor of endocannabinoid transport. We examined the effects of AM404 on glutamatergic synaptic transmission using network-driven increases in intracellular Ca(2+) concentration ([Ca(2+)]i) as an assay. At a concentration of 1 microM AM404 inhibited [Ca(2+)]i spiking by 73 +/- 8%. The cannabinoid CB(1) receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-p yrazole-3-carboxamide hydrochloride (SR141716A), the vanilloid VR(1) receptor antagonist capsaicin (CPZ), and treatment with pertussis toxin failed to block AM404-mediated inhibition. AM404 (3 microM) inhibited action-potential-evoked Ca(2+) influx by 58 +/- 3% but failed to affect calcium influx evoked by depolarization with 30 mM K(+)+, suggesting that the inhibition of electrically evoked [Ca(2+)]i increases and that [Ca(2+)]i spiking was due to inhibition of Na(+)+ channels. Palmitoylethanolamide (PMEA), capsaicin (CAP) and (5Z,8Z,11Z,14Z)-N-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide (VDM11), compounds structurally similar to AM404, inhibited [Ca(2+)]i spiking by 34 +/- 10%, 42 +/- 18% and 67 +/- 12%, respectively. Thus, AM404 and related compounds inhibit depolarization-induced Ca(2+) influx independent of cannabinoid receptors, suggesting caution when using these agents as pharmacological probes to study synaptic transmission.

The asymmetric total synthesis of natural (+)-cannabisativine 1 was completed in 19 steps and 7% overall yield. The key synthetic intermediate 29 was prepared with a high degree of stereocontrol in 12 steps starting from chiral 1-acylpyridinium salt 10. Addition of zinc enolate 11 to pyridinium salt 10 furnished dihydropyridone 12 containing two contiguous stereocenters of the correct absolute configuration. Luche reduction of ketone 16 afforded diol 17 in high yield (96%) and excellent diastereoselectivity. The Mukaiyama-Michael reaction of pyridones 27a/b with O-silyl ketene acetal 32 gave phenyl selenyl ketones 33a/b with complete stereoselectivity. Elimination of cis-beta-hydroxyselenides 34 and 35 effected the regiocontrolled preparation of tetrahydropyridine derivative 29. Several approaches to the macrocyclic ring closure of the 13-membered ring were investigated, ultimately leading to the completion of an asymmetric synthesis of the target compound with a high degree of stereocontrol.


In this study, we tested the hypothesis that a CB1 TMH3-4-5-6 aromatic microdomain, which includes F3.25(190), F3.36(201), W5.43(280) and W6.48(367) is centrally involved in CB1 receptor activation, with the F3.36/W6.48 interaction key to the maintenance of the CB1 inactive state. We have previously shown that when F3.36(201), W5.43(280) and W6.48(357) are individually mutated to alanine, a significant reduction in ligand binding affinity is observed in the presence of WIN 55,212-2 and SR141716A, but not CP 55,940 and anandamide (1). In the work presented here, we report a detailed functional analysis of the F3.36A, F3.25A, W5.43A and W6.48A mutant receptors in stable cell lines created in HEK cells for agonist-stimulated GTPgammaS binding and GIRK1/4 channel current effects in Xenopus oocytes where the mutant proteins were expressed transiently. The F3.36A mutation showed statistically significant increases in ligand-independent stimulation of GTPgammaS binding vs. WT CB1, while basal levels for W6.48A mutant were not statistically different from WT CB1. F3.36A demonstrated a limited activation profile in the presence of multiple agonists. In contrast, enhanced agonist activation was produced by W6.48A. These results suggest that a F3.36/W6.48 specific contact is an important constraint for the CB1 inactive state that may need to break during activation. Modeling studies suggest that the F3.36/W6.48 contact can exist in the inactive state of CB1 and be broken in the activated state via a chi1 rotamer switch (F3.36 trans,W6.48 g+) to (F3.36 g+,W6.48 trans). The F3.36/W6.48 interaction, therefore, may represent a "toggle switch" for activation of CB1.


Although cannabinoids have been recreationally employed for thousands of years, it was not until the discovery of their specific receptors, in the early nineties, that the molecular basis of cannabinoid activity have began to be understood. Growing research in this field has demonstrated not only that the action of cannabinoids in mammals is mainly receptor-mediated, but also that endogenous cannabinoids, such as anandamide, are produced, metabolized, and taken up across the cell membrane through a facilitated uptake process. The exogenous administration of cannabinoids, as well as the manipulation of their endogenous levels have been related to a variety of effects, such as analgesia, impairment of cognition and learning, appetite enhancement and peripheral vasodilation. Hence, the endocannabinoid system, including the CB1 and CB2 receptors, the metabolizing enzyme fatty acid amide hydrolase and the anandamide transporter, is a potential target for the development of novel therapeutic drugs in the treatment of various conditions, such as pain, feeding disorders and vascular disease among others. Although most of the research in the field of cannabinoids has been focused on their effects in the central nervous system, a growing line of evidence indicates that cannabinoids can also play a major role in the control of physiopathological functions in the cardiovascular system. In this context, endocannabinoids have been proposed as novel possible hypotensive agents,
and have been involved in the hypotension observed in septic shock, acute myocardial infarction and cirrhosis. In addition, a protective role for endocannabinoids has been described in ischemia.


Effects of the CB2-selective cannabinoid agonist AM1241 on activity evoked in spinal wide dynamic range (WDR) neurons by transcutaneous electrical stimulation were evaluated in urethane-anesthetized rats. Recordings were obtained in absence and presence of carrageenan inflammation. AM1241, administered intravenously or locally in the paw, suppressed activity evoked by transcutaneous electrical stimulation during the development of inflammation. Decreases in WDR responses resulted from a suppression of C-fiber-mediated activity and windup. A-beta- and A-delta-fiber-mediated responses were not reliably altered. The AM1241-induced suppression of electrically-evoked responses was blocked by the CB2 antagonist SR144528 but not by the CB1 antagonist SR141716A. AM1241 (33 micro g/kg i.pl.), administered to the carrageenan-injected paw, suppressed activity evoked in WDR neurons relative to groups receiving vehicle in the same paw or AM1241 in the opposite (noninflamed) paw. The electrophysiological effects of AM1241 (330 micro g/kg, i.v.) were greater in rats receiving intraplantar carrageenan compared to noninflamed rats receiving an intraplantar injection of vehicle. AM1241 failed to alter the activity of purely non-nociceptive neurons recorded in the lumbar dorsal horn. Additionally, AM1241 (330 micro g/kg, i.v. and i.pl.; 33 micro g/kg i.pl.) reduced the diameter of the carrageenan-injected paw. The AM1241-induced decrease in peripheral edema was blocked by the CB2 but not by the CB1 antagonist. These data demonstrate that activation of cannabinoid CB2 receptors is sufficient to suppress neuronal activity at central levels of processing in the spinal dorsal horn. Our findings are consistent with the ability of AM1241 to normalize nociceptive thresholds and produce antinociception in inflammatory pain states.


2-Arachidonoylglycerol (2-AG) is an endogenous cannabinoid receptor ligand. To date, two types of cannabinoid receptors have been identified: the CB1 receptor, abundantly expressed in the brain, and the CB2 receptor, expressed in various lymphoid tissues such as the spleen. The CB1 receptor has been assumed to play an important role in the regulation of synaptic transmission, whereas the physiological roles of the CB2 receptor remain obscure. In this study, we examined whether the CB2 receptor is present in human eosinophils and found that the CB2 receptor is expressed in human peripheral blood eosinophils. In contrast, human neutrophils do not contain a significant amount of the CB2 receptor. We then examined the effect of 2-AG on the motility of eosinophils. We found that 2-AG induces the migration of human eosinophilic leukemia EoL-1 cells. The migration evoked by 2-AG was abolished in the presence of SR144528, a CB2 receptor antagonist, or by pretreatment of the cells with pertussis toxin, suggesting that the CB2 receptor and Gi/o are involved in the 2-AG-induced migration. The migration of EoL-1 cells induced by 2-AG was suggested to be a result of chemotaxis. In contrast to 2-AG, neither anandamide nor free arachidonic acid elicited the migration. Finally, we examined the effect of 2-AG on human peripheral blood eosinophils and neutrophils and found that 2-AG induces migration of eosinophils but not neutrophils. These results suggest that the CB2 receptor and its endogenous ligand 2-AG may be closely involved in allergic inflammation accompanied by the infiltration of eosinophils.


Anxiety and panic are the most common adverse effects of cannabis intoxication; reactions potentiated by stress. Data suggest that cannabinoid (CB(1)) receptor modulation of amygdalar activity contributes to these phenomena. Using Fos as a marker, we tested the hypothesis that environmental stress and CB(1) cannabinoid receptor activity interact in the
regulation of amygdalar activation in male mice. Both 30 min of restraint and CB(1) receptor agonist treatment (Δ9-THC) produced barely detectable increases in Fos expression within the central amygdala (CeA). However, the combination of restraint and CB(1) agonist administration produced robust Fos induction within the CeA, indicating a synergistic interaction between environmental stress and CB(1) receptor activation. An inhibitor of endocannabinoid transport, AM404 (10 mg/kg), produced an additive interaction with restraint within the CeA. In contrast, fatty acid amide hydrolase (FAAH) inhibitor-treated mice (URB597, 1 mg/kg) and FAAH(-/-) mice did not exhibit any differences in amygdalar activation in response to restraint compared to control mice. In the basolateral (BLA) and medial amygdala, restraint stress produced a low level of Fos induction, which was unaffected by cannabinoid treatment. Interestingly, the CB(1) receptor antagonist SR141716 dose-dependently increased Fos expression in the BLA and CeA. These data suggest the CeA is an important neural substrate subserving the interactions between cannabinoids and environmental stress, and could be relevant to understanding the context-dependent emotional and affective changes induced by marijuana intoxication and the role of endocannabinoid signaling in the modulation of amygdalar activity.


Arachidonylethanolamine, which is commonly known as anandamide, was the first endogenous compound to be identified which binds to the cannabinoid receptors. Anandamide mimics many of the physiological effects of Δ9-THC, including hypothermia, antinociception, immobilization, catalepsy, and immune modulation. In the present studies we show that anandamide caused a concentration-dependent inhibition of interleukin-2 in primary splenocytes. The CB1 and CB2 antagonists, SR141716A and SR144528, when used in combination, did not antagonize the inhibition of interleukin-2 by anandamide. Additionally, neither UCM0707, the inhibitor of the putative anandamide membrane transporter (AMT), nor methyl arachidonoyl fluorophosphonate (MAFP), the inhibitor of fatty acid amidohydrolase (FAAH), were able to affect the inhibitory activity of anandamide upon interleukin-2. Interestingly, arachidonic acid caused a concentration-dependent inhibition of interleukin-2 secretion (IC50=10.3 micro M), which was similar to that of structurally-related anandamide (IC50=11.4 micro M). The inhibition of interleukin-2 by anandamide and arachidonic acid was partially reversed by pretreatment with the nonspecific cyclooxygenase inhibitors, flurbiprofen and piroxicam. Moreover, NS398, a cyclooxygenase-2 specific inhibitor, also attenuated the inhibitory effects of anandamide and arachidonic acid upon interleukin-2 secretion. Finally, pretreatment with PPARgamma-specific antagonist T0070907, partially antagonized anandamide-mediated suppression of IL-2 secretion. Collectively, the aforementioned studies suggest that inhibition of interleukin-2 secretion by anandamide is independent of CB1/CB2 and the AMT/FAAH system. Additionally, these studies also suggest that inhibition of interleukin-2 is mediated by PPARgamma, which is activated by a cyclooxygenase-2 metabolite of anandamide.


Because Δ9-THC inhibited luteinizing hormone-releasing hormone (LHRH) in male rats, we hypothesized that the endocannabinoid, anandamide (AEA), would act similarly. AEA microinjected intracerebroventricularly (i.c.v.) decreased plasma luteinizing hormone (LH) at 30 min in comparison to values in controls (P < 0.001). The cannabinoid receptor 1 (CB1-r)-specific antagonist, [N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] (AM251), produced a significant elevation in plasma LH (P < 0.01). AEA (10(-9) M) decreased LHRH release from medial basal hypothalamus incubated in vitro. These results support the concept that endogenous AEA inhibits LHRH followed by decreased LH release in male rats. In ovariectomized (OVX) female rats, AEA i.c.v. also inhibited LH release, but in this case AM251 had an even greater inhibitory effect than AEA. In vitro, AEA had no effect on LHRH in OVX rats. It seems that endogenous AEA inhibits LHRH...
followed by decreased LH release in OVX rats but that AM251 has an inhibitory action in this case. In striking contrast, in OVX, estrogen-primed (OVX-E) rats, AEA i.c.v. instead of decreasing LH, increased its release. This effect was completely blocked by previous injection of AM251. When medial basal hypothalami of OVX-E rats were incubated, AEA increased LHRH release. The synthesized AEA was higher in OVX-E rats than in OVX and males, indicating that estrogen modifies endocannabinoid levels and effects. The results are interpreted to mean that sex steroids have profound effects to modify the response to AEA. It inhibits LHRH and consequently diminishes LH release in males and OVX females, but stimulates LHRH followed by increased LH release in OVX-E-primed rats.


Cannabinoid CB1 receptors in the cerebellum mediate inhibitory effects of Delta(9)-THC (THC) on motor coordination. Intracellular effects of CB1 receptors include inhibition of adenylyl cyclase via activation of G/o-proteins. There is evidence for convergence of other neuronal receptors, such as adenosine A1 and GABAB, with the cannabinoid system on this signaling pathway to influence motor function. Previous studies have shown that brain CB1 receptors are desensitized and downregulated by chronic THC treatment, but few studies have examined the effects of chronic THC on downstream effector activity in brain. Therefore, these studies examined the relationship between CB1, adenosine A1 and GABAB receptors in cerebella of chronic vehicle and THC-treated mice at the level of G-protein activation and adenylyl cyclase inhibition. In naive cerebella, CB1 receptors produced less than additive inhibition of adenylyl cyclase with GABAB and A1 receptors, indicating that these receptors are localized on overlapping populations of cells. Chronic THC treatment produced CB1 receptor downregulation and desensitization of both cannabinoid agonist-stimulated G-protein activation and inhibition of forskolin-stimulated adenylyl cyclase. However, G-protein activation by GABAB or A1 receptors was unaffected. Interestingly, heterologous attenuation of GABAB and A1 receptor-mediated inhibition adenylyl cyclase was observed, even though absolute levels of basal, forskolin- or Gs-stimulated activity were unchanged. These results indicate that chronic THC administration produces a disruption of inhibitory receptor control of cerebellar adenylyl cyclase, and suggest a potential mechanism of cross-tolerance to the motor incoordinating effects of cannabinoid, GABAB and A1 agonists.


A number of lines of evidence make the gene that encodes the G-protein-coupled CB1/Cnr1 receptor a strong candidate to harbor variants that might contribute to individual differences in human addiction vulnerability. The CB1/Cnr1 receptor is the major brain site at which cannabinoid marijuana constituents are psychoactive as well as the principal brain receptor for endogenous anandamide ligands. It is densely expressed in brain circuits likely to be important for both the reward and mnemonic processes important for addiction. Altered drug effects in CB1/Cnr1 knockout mice and initial association studies also make variants at the CB1/Cnr1 locus candidates for roles in human vulnerabilities to addictions. However, many features of this gene's structure, regulation and variation remain poorly defined. This poor definition has limited the ability of previous association studies to adequately sample variation at this locus. We now report improved definition of the human CB1/Cnr1 locus and its variants. Novel exons 1-3, splice variant and candidate promoter region sequences add to the richness of the CB1/Cnr1 locus. Candidate promoter region sequences confer reporter gene expression in cells that express CB1/Cnr1. Common polymorphisms reveal patterns of linkage disequilibrium in European- and in African-American individuals. A 5' CB1/Cnr1 'TAG' haplotype displays significant allelic frequency differences between substance abusers and controls in European-American, African-American and Japanese samples. Post-mortem brain samples of heterozygous individuals contain less mRNA transcribed from the TAG alleles than from other CB1/Cnr1
haplotypes. CB1/Cnr1 genomic variation thus appears to play roles in human addiction vulnerability.

**CLINICAL SCIENCE**


This study evaluated the efficacy of 2 brief interventions for cannabis-dependent adults. A multisite randomized controlled trial compared cannabis use outcomes across 3 study conditions: (a) 2 sessions of motivational enhancement therapy (MET); (b) 9 sessions of multicomponent therapy that included MET, cognitive-behavioral therapy, and case management; and (c) a delayed treatment control (DTC) condition. Participants were 450 adult marijuana smokers with a Diagnostic and Statistical Manual of Mental Disorders (4th ed.; American Psychiatric Association, 1994) diagnosis of cannabis dependence. Assessments were conducted at baseline, and at 4, 9, and 15 months postrandomization. The 9-session treatment reduced marijuana smoking and associated consequences significantly more than the 2-session treatment, which also reduced marijuana use relative to the DTC condition. Most differences between treatments were maintained over the follow-up period. Discussion focuses on the relative efficacy of these brief treatments and the clinical significance of the observed changes in marijuana use.

**BEHAVIOURAL SCIENCE**


In many Western jurisdictions cannabis, unlike most other psychoactive drugs, cannot be prescribed to patients even in cases where medical professionals believe that it would ease the patient's pain or anxiety. The reasons for this prohibition are mostly ideological, although medical and moral arguments have been formulated to support it. In this paper, it is argued that freedom, properly understood, provides a sound ethical reason to allow the use of cannabis in medicine. Scientific facts, appeals to harm and autonomy, and considerations of symbolic value cannot consistently justify prohibitions.


In the last decade, a large number of studies using Delta(9)-tetrahydrocannabinol (THC), the main active principle derivative of the marijuana plant, or cannabinoid synthetic derivatives have substantially contributed to advance the understanding of the pharmacology and neurobiological mechanisms produced by cannabinoid receptor activation. Cannabis has been historically used to relieve some of the symptoms associated with central nervous system disorders. Nowadays, there are anecdotal evidences for the use of cannabis in many patients suffering from multiple sclerosis or chronic pain. Following the historical reports of the use of cannabis for medicinal purposes, recent research has highlighted the potential of cannabinoids to treat a wide variety of clinical disorders. Some of these disorders that are being investigated are pain, motor dysfunctions or psychiatric illness. On the other hand, cannabis abuse has been related to several psychiatric disorders such as dependence, anxiety, depression, cognitive impairment, and psychosis. Considering that cannabis or cannabinoid pharmaceutical preparations may no longer be exclusively recreational drugs but may also present potential therapeutic uses, it has become of great interest to analyze the neurobiological and behavioral consequences of their administration. This review attempts to link current understanding of the basic neurobiology of the endocannabinoid system to novel opportunities for therapeutic intervention and its effects on the central nervous system.
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