INTRODUCTION
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BASIC SCIENCE

Arachidonylethanolamide (AEA or anandamide) is believed to be the endogenous ligand of the cannabinoid CB1 and CB2 receptors. CB1 receptors have been found localized on fibers in the spinal trigeminal tract and spinal trigeminal nucleus caudalis. Known behavioural effects of anandamide are anti-nociception, catalepsy, hypothermia and depression of motor activity, similar to Delta(9)-tetrahydocannabinol, the psychoactive constituent of cannabis. It may be a possible therapeutic target for migraine. In this study we looked at the possible role of the CB1 receptor in the trigeminovascular system, using intravital microscopy to study the effects of anandamide against various vasodilator agents. Anandamide was able to inhibit dural blood vessel dilation brought about by electrical stimulation by 50%, CGRP by 30%, capsaicin by 45% and nitric oxide by 40%. CGRP8-37 was also able to attenuate NO-induced dilation by 50%. The anandamide inhibition was reversed by the CB1 receptor antagonist, AM251. Anandamide also reduced the blood pressure changes caused by CGRP injection, this effect was not reversed by AM251. It would seem that anandamide acts both pre-synaptically, to prevent CGRP release from trigeminal sensory fibers, and post-synaptically to inhibit the CGRP-induced NO release in the smooth muscle of dural arteries. CB1 receptors appear to be involved in the NO/CGRP relationship that exists in causing headache and dural blood vessel dilation. It also seems that some of the blood pressure changes caused by anandamide are mediated by a non-cannabinoid receptor, as AM251 was unable to reverse these effects. It can be suggested that anandamide is tonically released to play some form of modulatory role in the trigeminovascular system.


Mice devoid of CB1 cannabinoid receptors (CB1-/- mice) provide a unique opportunity to further investigate the role of CB1 receptors in exocannabinoid and endocannabinoid effects. CB1-/- mice (N=18) and their wild type littermates (CB1+/+ mice; N=12) were placed in standard mouse operant chambers and trained to lever press under a fixed-ratio 10 schedule of reinforcement. When stable lever press responding under the fixed ratio10 schedule had been established, cannabinoids and non-cannabinoids were administered to both groups. CB1+/+ mice acquired the lever press response more readily than CB1-/- mice. Delta(9)-THC decreased lever press responding in CB1+/+ mice only, whereas methanandamide, a metabolically stable endocannabinoid analog, produced similar response rate decreases in both genotypic groups. Similar to Delta(9)-THC, another endocannabinoid analog O-1812 decreased responding in CB1+/+ mice, but not in CB1-/- mice. The CB1 receptor antagonist SR141716A, blocked the effects of Delta(9)-THC, but not those of methanandamide. As methanandamide binds poorly to CB2 receptors, these results suggest possible non-CB1, non-CB2 mechanisms of action for methanandamide-induced behavioral disruption of lever press responding. Ethanol and morphine
elicited greater response decreases in CB1-/- mice than in CB1+/+ mice, suggesting a possible role of CB1 receptors in the rate disruptive effects of these drugs. In contrast, diazepam did not produce between group differences, suggesting that CB1 receptors are not involved in diazepam-induced disruption of lever press responding.


Recent reports indicate a higher frequency of brain infections with opportunistic amebae of the genus Acanthamoeba among immune compromised individuals, including AIDS patients. We have demonstrated, using a murine model of Granulomatous Amebic Encephalitis (GAE), that the major psychoactive and immune suppressive component in marijuana delta-9-tetrahydrocannabinol (THC) exacerbates infection by these amebae. Mice administered THC and infected with Acanthamoeba exhibited dose-related higher mortalities than infected vehicle controls. The greater severity of disease for THC-treated mice was accompanied by decreased accumulation of macrophage-like cells at focal sites of infection in the brain. Furthermore, THC administration resulted in decreased levels of mRNA for the pro-inflammatory cytokines interleukin-1alpha, interleukin-1beta, and tumor necrosis factor alpha for neonatal rat microglia co-cultured with Acanthamoeba. These results indicate a potential for marijuana to alter the capacity of brain macrophage-like cells to mount a full complement of immune responsiveness to brain infection by opportunistic amebae.


Cannabinoids and opioids both produce analgesia through a G-protein-coupled mechanism that blocks the release of pain-propagating neurotransmitters in the brain and spinal cord. However, high doses of these drugs, which may be required to treat chronic, severe pain, are accompanied by undesirable side effects. Thus, a search for a better analgesic strategy led to the discovery that delta 9-tetrahydrocannabinol (THC), the major psychoactive constituent of marijuana, enhances the potency of opioids such as morphine in animal models. In addition, studies have determined that the analgesic effect of THC is, at least in part, mediated through delta and kappa opioid receptors, indicating an intimate connection between cannabinoid and opioid signaling pathways in the modulation of pain perception. A host of behavioral and molecular experiments have been performed to elucidate the role of opioid receptors in cannabinoid-induced analgesia, and some of these findings are presented below. The aim of such studies is to develop a novel analgesic regimen using low dose combinations of cannabinoids and opioids to effectively treat acute and chronic pain, especially pain that may be resistant to opioids alone.


Two inhibitors of the cellular uptake of the endocannabinoid anandamide, (R)-N-oleoyl-(1’-hydroxybenzyl)-2’-ethanolamine and (S)-N-oleoyl-(1’-hydroxybenzyl)-2’-ethanolamine (OMDM-1 and OMDM-2, respectively), were recently synthesized, and their in vitro pharmacological activity described. Here we have assessed their activity in two typical pharmacological responses of cannabimimetic compounds. We first examined whether these compounds exert any effect per se on locomotion and pain perception in rats, and/or enhance the effects of anandamide on these two processes. We compared the effects of the novel compounds with those produced by a previously developed selective inhibitor, N-arachidonoyl-(2-methyl-4-hydroxyphenyl)amine (VDM-11). When assayed alone, OMDM-1 and OMDM-2 (1-10 mg/kg, i.p.) did not affect any of the five motor parameters under investigation, although the former compound exhibited a trend for the inhibition of ambulation, fast movements, and speed in rats. OMDM-2 and, to a lesser extent, VDM-11 (5 mg/kg, i.p.) enhanced the motor-inhibitory effects of a noneffective dose (2 mg/kg, i.p.) of anandamide, while OMDM-1 did not. In a typical test of acute analgesia, OMDM-2 and VDM-11 (1-10 mg/kg, i.p.), but not OMDM-1, significantly enhanced the time spent by rats on a "hot plate." However, the same compounds (5 mg/kg, i.p.) did not enhance the analgesic effect of a subeffective dose (2 mg/kg, i.p.) of anandamide, whereas OMDM-1 exerted a strong trend
towards potentiation (P=0.06). We next explored the possible use of the two novel compounds in a pathological condition. Thus, we determined if, like other previously developed anandamide reuptake inhibitors, OMDM-1 and OMDM-2 inhibit spasticity in an animal model of multiple sclerosis—the chronic relapsing experimental allergic encephalomyelitis in mice. As previously shown with a higher dose of VDM-11, both novel compounds (5 mg/kg, i.v.) significantly reduced spasticity of the hindlimb in mice with chronic relapsing experimental allergic encephalomyelitis. We suggest that OMDM-1 and, particularly, OMDM-2 are useful pharmacological tools for the study of the (patho)physiological role of the anandamide cellular uptake process, and represent unique templates for the development of new antispastic drugs.


This study investigated the possible behavioral mechanisms underlying the anorectic effect of the cannabinoid CB(1) receptor antagonist/inverse agonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR141716A). Male or female rats were food-restricted and trained to emit stable responding in daily 10-min, fixed ratio 10 food-reinforced operant sessions. Under these conditions, as well as under free-feeding conditions, SR141716A inhibited food-maintained responding (ED(50) values ranging from 0.92 to 2.52 mg/kg, i.p.). In the same operant procedure, SR141716A suppressed intracranial self-stimulation with a potency which was slightly lower than the anorectic potency (ED(50): 4.50 mg/kg). As assessed during a 10-min test period SR141716A (1-10 mg/kg) did not affect activity counts; suggesting that the observed inhibition of operant behavior is not a direct consequence of impairment of locomotor activity. SR141716A, however, attenuated saccharin-preference in a conditioned taste aversion paradigm (ED(50): 6.45 mg/kg). Although the data support the suggestion that the anorectic effect of SR141716A results from an attenuating effect on the rewarding effect of food, the contribution of drug-induced aversion/malaise cannot be excluded.

Duarte, C., R. Alonso, et al. (2003). "Blockade by the Cannabinoid CB1 Receptor Antagonist, Rimonabant (SR141716), of the Potentiation by Quinelorane of Food-Primed Reinstatement of Food-Seeking Behavior." Neuropsychopharmacology.

It has been shown previously that the selective cannabinoid CB1 receptor antagonist, rimonabant (SR141716), reduced the intake of palatable food as well as the self-administration of several drugs of abuse, suggesting that endocannabinoid systems play a role in brain reward function. The present study investigated whether a cannabinoid step was involved in food-seeking behavior induced by explicit stimuli, using an operant reinstatement procedure in rats. Experimental sessions consisted of a 15-min food rewarded period, followed by a 45-min extinction period. Rimonabant did not affect the response reinstatement induced by noncontingent delivery of food pellets, but prevented (0.03-0.3 mg/kg) the potentiation by quinelorane, a dopamine D3 receptor-prefering agonist, of food-seeking behavior. A possible link between cannabinoid processes and D3- and/or D2-mediated dopaminergic transmission was further investigated by studying Fos protein expression in cortico-limbic structures in D3 (D3-/-) and D2 (D2-/-) knockout mice. Rimonabant (10 mg/kg) increased Fos immunoreactivity in the prefrontal cortex (pFCortex) and in the shell but not the core of the nucleus accumbens (NAcc). Fos induction by this dose of rimonabant was not seen in mice lacking CB1 receptors, providing clear evidence for the involvement of CB1 receptors. In the NAcc shell, the effect of rimonabant was suppressed in D3-/-, but remained unchanged in D2-/- mice. In contrast, Fos expression by rimonabant in the pFCortex was impervious to D2 or D3 receptor deletion. In conclusion, these data indicate first that rimonabant prevented the enhancement by quinelorane of the appetitive value of food pellets unexpectedly delivered during extinction and second that rimonabant effects might involve D3 receptor-mediated processes. Overall, these results are consistent with the notion that endocannabinoid functions control brain reward processes and in particular the capacity of explicit stimuli to precipitate food-seeking behavior. Neuropsychopharmacology advance online publication, 17 December 2003; doi:10.1038/sj.npp.1300370

Two non-psychotropic cannabinoids, cannabidiol (CBD) and cannabidiol-dimethylheptyl (CBD-DMH), induced apoptosis in a human acute myeloid leukemia (AML) HL-60 cell line. Apoptosis was determined by staining with bisBenzimide and propidium iodide. A dose dependent increase of apoptosis was noted, reaching 61 and 43% with 8 microg/ml CBD and 15 microg/ml CBD-DMH, respectively, after a 24 h treatment. Prior exposure of the cells to gamma-irradiation (800 cGy) markedly enhanced apoptosis, reaching values of 93 and 95%, respectively. Human monocytes from normal individuals were resistant to either cannabinoids or gamma-irradiation. Caspase-3 activation was observed after the cannabinoid treatment, and may represent a mechanism for the apoptosis. Our data suggest a possible new approach to treatment of AML.


Wasabi, horseradish and mustard owe their pungency to isothiocyanate compounds. Topical application of mustard oil (allyl isothiocyanate) to the skin activates underlying sensory nerve endings, thereby producing pain, inflammation and robust hypersensitivity to thermal and mechanical stimuli. Despite their widespread use in both the kitchen and the laboratory, the molecular mechanism through which isothiocyanates mediate their effects remains unknown. Here we show that mustard oil depolarizes a subpopulation of primary sensory neurons that are also activated by capsaicin, the pungent ingredient in chilli peppers, and by Delta(9)-tetrahydrocannabinol (THC), the psychoactive component of marijuana. Both allyl isothiocyanate and THC mediate their excitatory effects by activating ANKTM1, a member of the TRP ion channel family recently implicated in the detection of noxious cold. These findings identify a cellular and molecular target for the pungent action of mustard oils and support an emerging role for TRP channels as ionotropic cannabinoid receptors.


We have reported that injection of marijuana cannabinoids, such as Delta(9)-tetrahydrocannabinol (THC), into mice, followed by infection with Legionella pneumophila (Lp), suppresses the development of cell-mediated immunity T helper 1 (Th1) activity. These effects are accompanied by suppression of interleukin (IL)-12 and interferon (IFN) gamma production and enhancement of IL-4 production suggesting THC-induced T helper cell biasing. In the current report, other T helper cell biasing mechanisms were studied. Mice were injected with THC followed 18 h later by a challenge infection with Lp. Two-hour post-infection, spleens were removed and analyzed for mRNA to either IL-12Rbeta2 or GATA3 gene products. The results showed that THC suppressed IL-12Rbeta2 but increased GATA3. Receptor antagonists for CB1 (SR141716A, SR1) and CB2 (SR144528, SR2) were also injected to analyze the involvement of cannabinoid receptors. It was determined that SR1 attenuated the THC suppression of IL-12Rbeta2, while SR2 attenuated the increase in GATA3 mRNA. These results suggest that THC suppresses Th1 biasing activity such as IL-12Rbeta2 by a CB1 mediated mechanism and enhances the Th2 biasing activity, GATA3, by a CB2 mechanism. This dichotomy of receptor involvement might result from differential expression and/or signaling function of CB1 and CB2 on Th1 and Th2 cells.


Cannabinoids are known to attenuate learning and memory in both humans and animals. In rodents, disruptive effect of cannabinoids on memory, reversed by SR 141716, a specific CB(1) receptor antagonist, was shown in behavioral tests based on conditioning. There are no data concerning the influence of cannabinoids on recognition memory. Recently, the improvement of recognition memory in cannabinoid CB(1) receptor knock-out mice was reported. Therefore, the purpose of the present study was to determine whether a stable analogue of...
endogenous cannabinoid anandamide, R-(+)-methanandamide (0.25 and 2.5 mg/kg, ip) and a potent CB(1) receptor agonist, CP 55,940 (0.025 and 0.25 mg/kg ip) affect recognition memory in rats evaluated in an object recognition test, based on discrimination between the familiar and a new object presented at 1h interval. Because cannabinoids at the higher doses can produce motor inhibition, the influence of both compounds on psychomotor activity was evaluated in an open field test. CP 55,940 and R-(+)-methanandamide, at both doses given once, 15 min before the learning trial, significantly attenuated recognition memory, measured by the difference in exploration of a new object and a duplicate of the familiar object. Moreover, CP 55,940 at the higher dose significantly attenuated ambulation, and bar approaches, and at both doses also rearings, evaluated in an open field, performed immediately after an object recognition test, while R-(+)-methanandamide at both doses did not alter locomotor and exploratory activity of rats. This is the first evidence that cannabinoids impair recognition memory in rats.


**RATIONALE.** The 5-HT(3) antagonist, ondansetron (OND), and the cannabinoid, Delta(9)-tetrahydrocannabinol (Delta(9)-THC), have been shown to interfere with emesis; however, their relative and/or combined effectiveness in suppressing vomiting produced by the chemotherapeutic agent, cisplatin, is unknown. **OBJECTIVES.** To evaluate the potential of: 1) a broad range of doses of Delta(9)-THC and OND to prevent cisplatin-induced vomiting and retching in the Suncus murinus (house musk shrew), 2) combined treatment with ineffective individual doses of Delta(9)-THC and OND to prevent cisplatin-induced vomiting and retching, 3) the CB(1) receptor antagonist, SR141716, to reverse the antiemetic effects of OND, and 4) cannabidiol (CBD), the principal non-psychoactive component of marijuana, to reverse cisplatin-induced vomiting in the shrew. **METHODS.** Shrews were injected with various doses of OND (0.02-6.0 mg/kg), Delta(9)-THC (1.25-10 mg/kg) and a combination of ineffective doses of each (0.02 mg/kg OND+1.25 mg/kg Delta(9)-THC) prior to being injected with cisplatin (20 mg/kg) which induces vomiting. Shrews were also injected with CBD (5 mg/kg and 40 mg/kg) prior to an injection of cisplatin. **RESULTS.** OND and Delta(9)-THC both dose-dependently suppressed cisplatin-induced vomiting and retching. Furthermore, a combined pretreatment of doses of the two drugs that were ineffective alone completely suppressed vomiting and retching. CBD produced a biphasic effect, suppressing vomiting at 5 mg/kg and potentiating it at 40 mg/kg. **CONCLUSIONS.** A low dose of the non-intoxicating cannabinoid CBD may be an effective antiemetic treatment and combined doses of OND and Delta(9)-THC that are ineffective alone suppresses cisplatin-induced emetic reactions in shrews.


A series of novel 3,4-diarylpyrazolines was synthesized and evaluated in cannabinoid (hCB(1) and hCB(2)) receptor assays. The 3,4-diarylpyrazolines elicited potent in vitro (hCB(1)) antagonistic activities and in general exhibited high CB(1) vs CB(2) receptor subtype selectivities. Some key representatives showed potent pharmacological in vivo activities after oral dosing in both a CB agonist-induced blood pressure model and a CB agonist-induced hypothermia model. Chiral separation of racemic 67, followed by crystallization and an X-ray diffraction study, elucidated the absolute configuration of the eutomer 80 (SLV319) at its C(4) position as 4S. Bioanalytical studies revealed a high CNS-plasma ratio for the development candidate 80. Molecular modeling studies showed a relatively close three-dimensional structural overlap between 80 and the known CB(1) receptor antagonist rimonabant (SR141716A). Further analysis of the X-ray diffraction data of 80 revealed the presence of an intramolecular hydrogen bond that was confirmed by computational methods. Computational models and X-ray diffraction data indicated a different intramolecular hydrogen bonding pattern in the in vivo inactive compound 6. In addition, X-ray diffraction studies of 6 revealed a tighter intermolecular packing than 80, which also may contribute to its poorer absorption in vivo. Replacement of the amidine -NH(2) moiety...
with a -NHCH(3) group proved to be the key change for gaining oral bioavailability in this series of compounds leading to the identification of 80.


Cannabinoids have been shown to impair cognition in vivo and block long-term potentiation (LTP), a candidate experimental model of learning and memory in vitro, via cannabinoid receptor (CB1) activation. cis-Oleamide (cOA) is an endogenous sleep-inducing lipid with putative cannabinomimetic properties. We hypothesise that cOA is cannabinomimetic and perform a comparative study with synthetic and endogenous cannabinoids on their effects on synaptic conditioning via two different patterns of stimulation in the hippocampal slice. CB1 agonists, R(+)-WIN55212-2 and anandamide, but not cOA blocked high frequency stimulation (HFS-LTP). R(+)-WIN55212-2 and cOA (stereospecifically) attenuated responses to theta-burst-LTP, while anandamide did not. The anandamide transport inhibitor, AM404, attenuated HFS-LTP, an effect reversed by the CB1 receptor antagonist SR141716A but not mimicked by the vanilloid receptor agonist capsaicin. TFNO, an inhibitor of fatty acid amide hydrolase (FAAH), the enzyme responsible for degrading anandamide, failed to block HFS-LTP alone or in combination with cOA. On the contrary, this combination was as effective as cOA on its own in attenuating theta-burst-LTP. cOA effects on theta-burst-LTP were prevented in the presence of the GABA(A) receptor blocker picrotoxin, but not by pretreatment with SR141716A. These findings suggest that cOA neither directly activates CB1 receptors nor acts via the proposed "entourage" effect [Nature 389 (1997) 25] to increase titres of anandamide through FAAH inhibition. The selective effects of cOA on theta-burst-conditioning may reflect modulation of GABAergic transmission. Anandamide uptake inhibition, but not blockade of FAAH, effectively increases synaptic concentrations of endocannabinoids.


The ability of the endogenous fatty acid amide, cis-oleamide (ODA), to bind to and activate cannabinoid CB1 and CB2 receptors was investigated. ODA competitively inhibited binding of the nonselective cannabinoid agonist [(3)H]CP55,940 and the selective CB1 antagonist [(3)H]SR141716A to rat whole-brain membranes with Kᵢ values of 1.14 micro M (0.52-2.53 micro M, Hill slope=0.80, n=6) and 2.63 micro M (0.62-11.20 micro M, Hill slope=0.92, n=4), respectively. AEA inhibited [(3)H]CP55,940 binding in rat whole-brain membranes with a Kᵢ of 428 nM (346-510 nM, Hill slope=-1.33, n=3). ODA competitively inhibited [(3)H]CP55,940 binding in human CB1 (hCB1) cell membranes with a Kᵢ value of 8.13 micro M (4.97-13.32 micro M, n=2). In human CB2 transfected (hCB2) HEK-293T cell membranes, 100 micro M ODA produced only a partial (42.5 +/- 7%) inhibition of [(3)H]CP55,940 binding. ODA stimulated [(35)S]GTPgammaS binding in a concentration-dependent manner (EC50=1.64 micro M (0.29-9.32 micro M, R(2)=0.99, n=4-9), with maximal stimulation of 188 +/- 9% of basal at 100 micro M. AEA stimulated [(35)S]GTPgammaS binding with an EC50 of 4.45-24.42 micro M, R(2)=1.00, n=3, 195 +/- 4% of basal at 300 micro M). In human CB2 transfected (hCB2) HEK-293T cell membranes, 100 micro M ODA produced only a partial (42.5 +/- 7%) inhibition of [(3)H]CP55,940 binding. ODA stimulated [(35)S]GTPgammaS binding with an EC50 of 10.43 micro M (4.45-24.42 micro M, R(2)=1.00, n=3, 195 +/- 4% of basal at 300 micro M). Trans-oleamide (trans-ODA) failed to significantly stimulate [(35)S]GTPgammaS binding at concentrations up to 100 micro M. ODA (10 micro M)-stimulated [(35)S]GTPgammaS binding was reversed by the selective CB1 antagonist SR141716A (IC50=2.11 nM (0.32-13.77 nM), R(2)=1.00, n=6). The anatomical distribution of ODA-stimulated [(35)S]GTPgammaS binding in rat brain sections was indistinguishable from that of HU210. Increases of similar magnitude were observed due to both agonists in the striatum, cortex, hippocampus and cerebellum. ODA (10 micro M) significantly inhibited forskolin-stimulated cyclic AMP (cAMP) accumulation in mouse neuroblastoma N1E 115 cells (P<0.02, n=11). ODA-mediated inhibition was completely reversed by 1 micro M SR141716A (P<0.001, n=11) and was also reversed by pretreatment with 300 ng ml(-1) pertussis toxin (P<0.001, n=6). These data demonstrate that ODA is a full cannabinoid CB1 receptor agonist. Therefore, in addition to allosteric modulation of other receptors and possible entourage effects due to fatty acid amide hydrolase inhibition, the effects of ODA may be mediated directly via the CB1 receptor.

Cortical information processing requires an orchestrated interaction between a large number of pyramidal cells and albeit fewer, but highly diverse GABAergic interneurons (INs). The diversity of INs is thought to reflect functional and structural specializations evolved to control distinct network operations. Consequently, specific cortical functions may be selectively modified by altering the input-output relationship of unique IN populations. Here, we report that persistently active cannabinoid receptors, the site of action of endocannabinoids, and the psychostimulants marijuana and hashish, switch off the output (mute) of a unique class of hippocampal INs. In paired recordings between cholecystokinin-immunopositive, mossy fiber-associated INs, and their target CA3 pyramidal cells, no postsynaptic currents could be evoked with single presynaptic action potentials or with repetitive stimulations at frequencies <25 Hz. Cannabinoid receptor antagonists converted these "mute" synapses into high-fidelity ones. The selective muting of specific GABAergic INs, achieved by persistent presynaptic cannabinoid receptor activation, provides a state-dependent switch in cortical networks.


We investigated the distribution and function of cannabinoid (CB)1 receptors in the submucosal plexus of the guinea pig ileum. CB1 receptors were found on both types of submucosal secretomotor neurons, colocalizing with VIP and NPY, the non-cholinergic and cholinergic secretomotor neurons, respectively. CB1 receptors colocalized with TRPV1 receptors on paravascular nerves and fibers in the submucosal plexus. In the submucosal ganglia, these nerves were preferentially localized at the periphery of the ganglia. In denervated ileal segments, CB1 receptor immunoreactivity in submucosal neurons was not modified, but paravascular and intraganglionic fiber staining was absent. Short circuit current (Isc) was measured as an indicator of net electrogenic ion transport in Ussing chambers. In the ion transport studies, Isc responses to capsaicin, which activates extrinsic primary afferents, and to electrical field stimulation (EFS), were reduced by pretreatment with the muscarinic antagonist, atropine; abolished by tetrodotoxin, but were unaffected by VIP receptor desensitization, hexamethonium, AMPA or NMDA glutamate receptor antagonists. The responses to capsaicin and EFS were reduced by 47+/-12% and 30+/-14%, respectively, by the CB1 receptor agonist, WIN 55,212-2. This inhibitory effect was blocked by the CB1 receptor antagonist, SR141716A. Isc responses to forskolin or carbachol, which act directly on the epithelium, were not affected by WIN 55,212-2. The inhibitory effect of WIN 55,212-2 on EFS-evoked secretion was not observed in extrinsically denervated segments of ileum. Taken together, these data show cannabinoids act at CB1 receptors on extrinsic primary afferent nerves, inhibiting the release of transmitters that act on cholinergic secretomotor pathways.


The endogenous cannabinoid system has been shown to play a crucial role in controlling neuronal excitability and synaptic transmission. In this study we investigated the effects of a cannabinoid receptor (CB-R) agonist WIN 55,212-2 (WIN) on excitatory synaptic transmission in the rat ventral tegmental area (VTA). Whole-cell patch clamp recordings were performed from VTA dopamine (DA) neurons in an in vitro slice preparation. WIN reduced both NMDA and AMPA EPSCs, as well as miniature EPSCs (mEPSCs), and increased the paired-pulse ratio, indicating a presynaptic locus of its action. We also found that WIN-induced effects were dose-dependent and mimicked by the CB1-R agonist HU210. Furthermore, two CB1-R antagonists, AM281 and SR141716A, blocked WIN-induced effects, suggesting that WIN modulates excitatory synaptic transmission via activation of CB1-Rs. Our additional finding that both AM281 and SR141716A per se increased NMDA EPSCs suggests that endogenous cannabinoids, released from depolarized postsynaptic neurons, might act retrogradely on presynaptic CB1-Rs to suppress
glutamate release. Hence, we report that a type of synaptic modulation, previously termed depolarization-induced suppression of excitation (DSE), is present also in the VTA as a calcium-dependent phenomenon, blocked by both AM281 and SR141716A, and occluded by WIN. Importantly, DSE was partially blocked by the D2DA antagonist eticlopride and enhanced by the D2DA agonist quinpirole without changing the presynaptic cannabinoid sensitivity. These results indicate that the two pathways work in a cooperative manner to release endocannabinoids in the VTA, where they play a role as retrograde messengers for DSE via CB1-Rs.


Cannabinoid receptor agonists produce analgesia for pains of non-cranial origin. However, their effectiveness for craniofacial pains is currently unclear. In the present study, the cannabinoid CB1/CB2 receptor agonist, WIN 55,212-2 (WIN), was bath applied to the brainstem while activity of spinal trigeminal nucleus caudalis (Vc) neurons evoked by transcutaneous electrical stimulation was recorded in isoflurane anesthetized rats. Neurons were characterized using mechanical and electrical stimulation of the face, and were classified as either low-threshold mechanoreceptive (LTM) or wide dynamic range (WDR). LTM neurons responded to light brushing of the receptive field and received only Aβ primary afferent fiber input. WDR neurons showed a graded response to mechanical stimulation, responding maximally to noxious stimuli, and demonstrated both A- and C-fiber evoked activity. In addition, WDR neurons displayed longer latency, C-fiber mediated post-discharge (PDC) activity after repetitive stimulation. Local bath application of 2.0 mg/ml WIN significantly reduced PDC activity (3+/−1% control, P<0.01), C-fiber evoked activity (58+/−9% control, P<0.01), and Aβ evoked activity (57+/−10% control, P<0.01) in WDR neurons. In contrast, LTM Aβ-fiber evoked activity increased after local administration of WIN (204+/−52% control, P<0.01). SR141716A, a CB1 receptor antagonist, prevented the effects of WIN on WDR PDC and LTM Aβ evoked activity. These results indicate that cannabinoid receptor agonists may be effective agents for craniofacial pain. Furthermore, the particular sensitivity of PDC activity, a measure of neuronal hyperexcitability, to cannabinoid receptor agonists may be relevant to the treatment of persistent craniofacial pain.


The influence of substances of abuse on the progression of HIV-1 infection is controversial, and pharmacologic factors have been postulated as a potential explanation for conflicting data arising from epidemiological studies and animal models. In the present study, cell culture models of HIV-1 infection were used to test this hypothesis. The synthetic cannabinoid WIN 55,212-2 was found to potently inhibit HIV-1 expression in a concentration- and time-dependent manner in CD4(+) lymphocyte and microglial cell cultures. In sharp contrast, morphine either inhibited or stimulated viral expression, depending upon the time of drug exposure, and marked differences were observed between CD4(+) and microglial cells. Also, WIN 55,212-2 inhibited the stimulatory effect of morphine in HIV-1 infected CD4(+) cells. These in vitro findings support the notion that pharmacologic factors need to be considered in epidemiological studies and animal models that pertain to HIV-1 infection.

were being proposed. As the scenario becomes subsequently more complicated, and the experimental tasks to be accomplished correspondingly more numerous, we briefly review in this article the latest 'additions' to the endocannabinoid system together with earlier breakthroughs that have contributed to our present knowledge of the biochemistry and pharmacology of the endocannabinoids.


Lung macrophages provide a first line of host defense against inhaled pathogens and their function is impaired in the lungs of inhaled substance abusers. In order to investigate the mechanism for this impairment, alveolar macrophages (AM) were recovered from nonsmokers (NS), regular tobacco smokers (TS), marijuana smokers (MS), or crack cocaine smokers (CS), and evaluated for their production of nitric oxide (NO) and the role of NO as an antimicrobial effector molecule. AM from NS and TS efficiently killed Staphylococcus aureus and their antibacterial activity correlated closely with the production of nitrite and the expression of mRNA encoding for inducible nitric oxide synthase (iNOS). In contrast, AM collected from MS and CS exhibited limited antimicrobial activity that was not affected by an inhibitor of iNOS, or associated with expression of iNOS. Treatment with either granulocyte/macrophage colony-stimulating factor (GM-CSF) or interferon-gamma restored the ability of these cells to produce NO and to kill bacteria. These findings confirm a significant role for NO as an antibacterial effector molecule used by normal human AM and suggest that this host defense mechanism is suppressed by habitual exposure to inhaled marijuana or crack cocaine in vivo.


In addition to their inhibitory effects, cannabinoids also exert stimulatory activity which can be detected at the cellular level. In a previous study, we demonstrated a stimulatory effect of the synthetic cannabinoid receptor agonist desacetylevonantradol (DALN) on Ca(2+) flux into N18TG2 neuroblastoma cells, and suggested a dual mechanism: one pathway mediated by PKA and the other one by protein kinase C (PKC). Here we studied the PKC-mediated effect of DALN on Ca(2+) influx. The stimulatory effect of DALN on Ca(2+) influx was partially blocked by the PKC inhibitor chelerythrine, by the metalloproteinase inhibitor o-phenanthroline and by the MEK (mitogen-activated protein-kinase kinase, MAPK kinase) inhibitor PD98059. Immunoblotting of ERK1/2 MAPK demonstrated phosphorylation by DALN, and indicated the involvement of vascular endothelial growth factor (VEGF) receptor tyrosin kinases (RTKs) in MAPK activation as it was blocked by oxindole-1. Transactivation of the VEGFR-MAPK cascade by DALN involved CB1 cannabinoid receptors coupled to Gi/Go GTP-binding proteins as it was blocked by SR141716A and by pertussis toxin (PTX). The pharmacological implications of this novel mechanism of cannabinoid activity are discussed.

structural requirements of the putative carrier, we synthesized a series of structurally different compounds 1-8 and evaluated their capacity as uptake inhibitors. They showed different inhibitory capacity in PC-3 cells, with (9Z,12Z)-N-(fur-3-ylmethyl)octadeca-9,12-dienamide (4, UCM119) being the most efficacious, with maximal inhibition and IC50 values of 49% and 11.3±0.5 micro M, respectively. In conclusion, PC-3 cells possess a complete inactivation system for anandamide formed by an uptake process and the enzyme FAAH. These results suggest a possible physiological function of anandamide in the prostate, reinforcing the role of endocannabinoid system as a neuroendocrine modulator.


Retrograde synaptic signaling has long been recognized as a fundamental feature of neural systems. However, the cellular specificity and functional consequences of fast retrograde communication are not well understood. We have focused our efforts on understanding the role that endocannabinoids play in regulating synaptic inhibition in sensory neocortex. Recent studies have implicated endocannabinoids as the retrograde signaling molecules that underlie depolarization-induced suppression of inhibition, or DSI. This short-term form of presynaptic depression is triggered by postsynaptic depolarization and likely plays an important role in information processing. In the present study we investigated the cellular and synaptic specificity of endocannabinoid signaling in sensory cortex using whole-cell recordings from layer 2/3 pyramidal neurons (PNs) in acute brain slices. We report that GABAergic interneurons that are depolarized by muscarinic receptor stimulation provided the majority of DSI-susceptible inputs to neocortical PNs. This subclass of interneurons generated large, fast postsynaptic currents in PNs which were transiently suppressed by either postsynaptic depolarization or a brief train of action potentials. Neocortical DSI required activation of the type 1 cannabinoid receptor (CB1R) but not metabotropic glutamate or GABA receptors. Using focal drug application, we found that the DSI-susceptible afferents preferentially synapse on the perisomatic membrane of PNs, and not on the apical dendrites. Together, these results suggest that endocannabinoid-mediated DSI in the cortex can transiently and selectively depress a subclass of PN inputs. Although the physiological implications remain to be explored, this suppression of somatic inhibition may alter the excitability of principal neurons and thereby modulate cortical output.


The residual neuropsychological effects of marijuana abuse in man indicate a dysfunction of the attentional/executive systems. Moreover, experimental investigations suggest that repeated, intermittent (subchronic) Delta(9)-tetrahydrocannabinol (THC), the main psychoactive ingredient of marijuana, alters neurotransmission in the frontal cortex of rats and humans, a key neural site mediating attention and executive functions. In the present studies, the acquisition and performance of a test of visuospatial attention (the lateralized reaction time task) after subchronic THC administration (10.0 mg/kg twice daily for 14 days) was examined. Rats previously administered THC showed impairments in this self-paced version of the classic multiple-choice serial reaction time task, which persisted 14 days after the final drug administration. Longer time points were not examined. These attentional impairments were transiently reversible with an acute amphetamine (0.5 mg/kg) challenge. These behavioral data demonstrate that chronic THC administration to rats induces an attentional deficit, similar to that observed in humans who abuse marijuana. Finally, amphetamine’s ability to reverse the attentional impairments provides indirect evidence that monoaminergic deficits may be linked to the cognitive dysfunction. Neuropsychopharmacology advance online publication, 17 December 2003; doi:10.1038/sj.npp.1300316

Administration of the CB(1) receptor antagonist SR 141716 [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] suppresses intake of highly palatable (high carbohydrate) food. However, the effects of SR 141716 on intake of foods with varying macronutrient compositions, and in non-deprived animals have not been well studied. In the present study, non-deprived rats were injected intraperitoneally with SR 141716 (0.03-3.0 mg/kg) and presented with a high carbohydrate, high fat, or normal chow diet. Food intake and locomotor activity were recorded for 120 min. Results showed that SR 141716 significantly suppressed food intake irrespective of the composition of the test food without affecting locomotor activity. These data suggest that food deprivation or high palatability of the ingesta are not necessary to observe the suppressive effects of SR 141716 on food intake.


Cannabis is under clinical investigation to assess its potential for medicinal use, but the question arises as to whether there is any advantage in using cannabis extracts compared with isolated Delta9-trans-tetrahydrocannabinol (Delta9THC), the major psychoactive component. We have compared the effect of a standardized cannabis extract (SCE) with pure Delta9THC, at matched concentrations of Delta9THC, and also with a Delta9THC-free extract (Delta9THC-free SCE), using two cannabinoid-sensitive models, a mouse model of multiple sclerosis (MS), and an in-vitro rat brain slice model of epilepsy. Whilst SCE inhibit spasticity in the mouse model of MS to a comparable level, it caused a more rapid onset of muscle relaxation, and a reduction in the time to maximum effect compared with Delta9THC alone. The Delta9THC-free extract or cannabidiol (CBD) caused no inhibition of spasticity. However, in the in-vitro epilepsy model, in which sustained epileptiform seizures were induced by the muscarinic receptor agonist oxotremorine-M in immature rat piriform cortical brain slices, SCE was a more potent and again more rapidly-acting anticonvulsant than isolated Delta9THC, but in this model, the Delta9THC-free extract also exhibited anticonvulsant activity. Cannabidiol did not inhibit seizures, nor did it modulate the activity of Delta9THC in this model. Therefore, as far as some actions of cannabis were concerned (e.g. antispasticity), Delta9THC was the active constituent, which might be modified by the presence of other components. However, for other effects (e.g. anticonvulsant properties) Delta9THC, although active, might not be necessary for the observed effect. Above all, these results demonstrated that not all of the therapeutic actions of cannabis herb might be due to the Delta9THC content.

CLINICAL SCIENCE


OBJECTIVES: To examine prevalence and patterns of smoked marijuana and perceived benefit and to assess demographic and clinical factors associated with marijuana use among HIV patients in a public health care setting. METHODS: Participants (n = 252) were recruited via consecutive sampling in public health care clinics. Structured interviews assessed patterns of recent marijuana use, including its perceived benefit for symptom relief. Associations between marijuana use and demographic and clinical variables were examined using univariate and multivariate regression analyses. RESULTS: Overall prevalence of smoked marijuana in the previous month was 23%. Reported benefits included relief of anxiety and/or depression (57%), improved appetite (53%), increased pleasure (33%), and relief of pain (28%). Recent use of marijuana was positively associated with severe nausea (odds ratio [OR] = 4.0, P = 0.004) and recent use of alcohol (OR = 7.5, P < 0.001) and negatively associated with being Latino (OR = 0.07, P < 0.001). No associations between marijuana use and pain symptoms were observed. CONCLUSIONS: The findings suggest that providers be advised to assess routinely and better understand patients' "indications" for self-administration of cannabis. Given the estimated prevalence, more formal characterization of the patterns and impact of cannabis use to alleviate HIV-associated symptoms is warranted. Clinical trials of smoked and noncombustible marijuana are needed to determine the role of cannabinoids as a class of agents with potential to improve quality of life and health care outcomes among patients with HIV/AIDS.

The majority of North American pregnant women experience some degree of nausea and vomiting, usually in the first few months of pregnancy. Women utilize many coping strategies, including self-treatment with herbal medicine and other alternative therapies. In a qualitative study of self-care in pregnancy, birth and lactation within a non-random sample of 27 women in British Columbia, Canada, 20 women (74%) experienced pregnancy-induced nausea. Ten of these women used anti-emetic herbal remedies, which included ginger, peppermint, and Cannabis. The safety and efficacy of each of these herbal remedies is discussed here. Only ginger has been subjected to clinical trials among pregnant women, though all three herbs were clinically effective against nausea and vomiting in other contexts, such as chemotherapy-induced nausea and post-operative nausea. While safety concerns exist in the literature for all three herbs with regards to their use by pregnant women, clinical evidence of harm is lacking.

BEHAVIOURAL SCIENCE


OBJECTIVE: To compare associations of alcohol, cannabis, and cocaine abuse and traffic crash risk for "at fault" crashes and all crashes. DESIGN: A historical cohort study. SETTING: Toronto, Ontario. Patients or subjects: Subjects beginning treatment at the Centre for Addictions and Mental Health (CAMH) in 1994 for abuse of alcohol, cannabis, cocaine, and all combinations of these substances (n = 590, with 411 drivers). A control group consisted of 518 records from the Ontario registry of registered drivers, frequency matched for age and sex and residence. INTERVENTIONS: CAMH subjects took part in therapeutic programs. Pre-intervention (11 115 driver-years) and post-intervention intervals (8550 driver-years) were defined and compared. MAIN OUTCOME MEASURES: Crash and collision rates, adjusted relative risks (ARRs) of crash involvement and of "at fault" crashes were computed using Poisson regression to control for variations in time at risk, age, and sex of participants. RESULTS: Pre-treatment, significant ARRs of 1.49 to 1.79 for all crashes were found for abusers of cannabis, cocaine, or a combination. ARRs increased by 10%-15% for "at fault" crashes. Post-treatment, all associations were very modest for all abuse types. Only younger and male drivers had a significantly increased risk, which was stronger for "at fault" than for all crashes. CONCLUSIONS: Abuse of cannabis and cocaine pre-treatment was more strongly related to "at fault" crashes than to all crashes. Interaction between these substances means that the effects of combined abuse cannot be predicted from simple main effects.


OBJECTIVES: We examined the prevalence of substance use among American adults aged 35 years, and we considered adulthood predictors and the impact of adolescent substance use. METHODS: National panel data were drawn from the Monitoring the Future study. Logistic regressions were conducted to assess the impact of demographics, life experiences, and adolescent substance use on smoking, heavy drinking, prescription drug misuse, marijuana use, and cocaine use at 35 years of age. RESULTS: Factors related to increased likelihood of substance use include high school use, unemployment, and noncustodial parenthood. Lower use was associated with being female, a college graduate, a professional, married, or a custodial parent. CONCLUSIONS: Among those aged 35 years, substance use was still rather prevalent and was a function of adulthood roles, experiences, and previous use.

The authors examined changes in college students' illicit drug use, patterns of polydrug use, and the relationship between students' ages of initiation of substance use and later use of marijuana and other illicit drugs between 1993 and 2001. Data from 119 US colleges and universities in the Harvard School of Public Health College Alcohol Study were used in the study. They found significant increases in percentages of students' use of marijuana in the past 30 days (from 13% to 17%), past year (from 23% to 30%), and lifetime (from 41% to 47%) between 1993 and 2001, with most of the increase occurring between 1993 and 1997. Past 30-day use of other illicit drugs increased from 4% to 7% and past year use increased from 11% to 14%. More than 98% of marijuana and other illicit drug users used another substance. They also either smoked, were binge drinkers, and/or were users of another illicit drug. Drug prevention programs should emphasize heavy alcohol use and smoking and should start when students are in high school or earlier.


OBJECTIVE: Neurotransmitter release of GABAergic and glutamatergic neurons may be significantly influenced by cannabinoid CB1 receptors located at presynaptic nerve terminals. GABA and glutamate have been reported to be involved in the pathogenesis of severe alcohol withdrawal-induced seizures and delirium tremens. The aim of this study is to test the potential influence of a bi-allelic cannabinoid receptor gene (CNR1) polymorphism (G1359A) on severe alcohol withdrawal syndromes. METHODS: Based upon a sample size estimation, 196 subjects meeting DSM IV and ICD10 criteria for alcohol dependence and 210 non-alcoholic controls were recruited for study. CB1 polymorphisms were determined using polymerase chain reaction (PCR). History of alcohol withdrawal-induced delirium tremens, seizures and other alcohol withdrawal-related phenotypes were obtained using the SSAGA (Semi-Structured Assessment of Genetics in Alcoholism). Data were corroborated with information from the inpatients' clinical files. RESULTS: Allele frequencies of the CNR1 G1359A polymorphism were within the range reported by previous studies. After correcting for multiple testing, no association of the A- or G-allele of CNR1 polymorphism with a history of alcohol withdrawal-induced seizures was detected. In addition, no significant relationships with other alcoholism-related phenotypes were found. CONCLUSION: This study failed to confirm an earlier report of a potential role of a CNR1 polymorphism in the pathogenesis of delirium tremens.


OBJECTIVE: Past research has not fully explained why black youth are less likely than white youth to use alcohol and other substances. One plausible yet underexamined explanation is the "religion hypothesis," which posits that black youth are more likely than white youth to abstain because they are more religious than white youth. The present study tested this hypothesis empirically. METHOD: The study examined data from large, nationally representative samples of white and black 10th graders from the Monitoring the Future project. RESULTS: Relative to white students, black students are more likely to abstain from alcohol, cigarettes and marijuana and are more highly religious. Consistent with the "religiosity hypothesis," race differences in abstinence are substantially reduced when race differences in religiosity are controlled. Unexpectedly, however, highly religious white youth are more likely than highly religious black youth to abstain from alcohol and marijuana use. CONCLUSIONS: Although religion is an important protective factor against alcohol and other substance use for both white and black adolescents, it appears to impact white youth at an individual level, whereas for black youth the influence of religion seems greatest at the group level. Future research should seek to better understand the mechanisms through which religion promotes adolescents' abstinence from the use of drugs and should seek to explain why the magnitude of its effect varies for black and white adolescents.

This research examines the responsiveness of the demand for marijuana to changes in its money price and criminal status using data on individuals from the Australian National Drug Strategy's Household Surveys (NDSHS). The results suggest that both the prevalence of marijuana use and the conditional demand for marijuana in the general population are responsive to changes in its money price. Significant differences are found in the effect of price on participation in marijuana use across age-groups, with participation by youth more price sensitive than participation by older age-groups. Similarly, the effect of the legal status of marijuana use on the participation decision is found to differ across age-groups and gender. Specifically, decriminalisation is associated with an increase in the prevalence of use by males over the age of 25. There is no evidence that decriminalisation significantly increases participation in marijuana use by either young males or females, or that decriminalisation increases the frequency of use among marijuana users.