INTRODUCTION
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BASIC SCIENCE

Numerous studies have shown the existence of functional links between the endogenous cannabinoid and opioid systems. However, extensive research is still needed to elucidate the biochemical mechanisms involved in this cannabinoid-opioid interaction. Mice lacking mu- (MOR), delta- (DOR) and kappa- (KOR) opioid receptors have been generated and some specific pharmacological effects induced by cannabinoids have been reported to be modified in these animals. In order to clarify further the possible mechanisms involved in this modification of cannabinoid responses we have now evaluated the expression and functional activity of cannabinoid receptors in different brain structures in these mutant animals. For this purpose, we have performed quantitative receptor autoradiography of CB1 cannabinoid receptors and activation of GTP-binding proteins by CB1 agonists in the brain of wild-type and homozygous MOR, DOR and KOR knockout mice. There were no significant differences in the levels of CB1 receptors in the brain of MOR mutant mice. In contrast, the efficacy of CB1 receptor activation by the cannabinoid agonist WIN 55 212-2 was dramatically reduced in the caudate-putamen of MOR knockout animals. The density of CB1 receptors as well as the stimulation of GTP-binding proteins by WIN 55 212-2 were significantly increased in the substantia nigra of mice deficient in DOR. Finally, there were no major changes in the levels and functional activity of CB1 cannabinoid receptors in any brain region in KOR knockout mice. Taken together, these results indicate that deletion of MOR and DOR causes alterations in cannabinoid receptor levels and functional activity in specific brain structures, which could explain some of the functional interactions observed between these two neuronal systems.


Diacylglycerol (DAG) lipase activity is required for axonal growth during development and for retrograde synaptic signaling at mature synapses. This enzyme synthesizes the endocannabinoid 2-arachidonoyl-glycerol (2-AG), and the CB1 cannabinoid receptor is also required for the above responses. We now report on the cloning and enzymatic characterization of the first specific sn-1 DAG lipases. Two closely related genes have been identified and their expression in cells correlated with 2-AG biosynthesis and release. The expression of both enzymes changes from axonal tracts in the embryo to dendritic fields in the adult, and this correlates with the developmental change in requirement for 2-AG synthesis from the pre- to the postsynaptic compartment. This switch provides a possible explanation for a fundamental change in endocannabinoid function during brain development. Identification of these enzymes may offer new therapeutic opportunities for a wide range of disorders.

Animal and human studies have suggested that cannabidiol (CBD) may possess anxiolytic properties, but how these effects are mediated centrally is unknown. The aim of the present study was to investigate this using functional neuroimaging. Regional cerebral blood flow (rCBF) was measured at rest using (99m)Tc-ECD SPECT in 10 healthy male volunteers, randomly divided into two groups of five subjects. Each subject was studied on two occasions, 1 week apart. In the first session, subjects were given an oral dose of CBD (400 mg) or placebo, in a double-blind procedure. SPECT images were acquired 90 min after drug ingestion. The Visual Analogue Mood Scale was applied to assess subjective states. In the second session, the same procedure was performed using the drug that had not been administered in the previous session. Within-subject between-condition rCBF comparisons were performed using statistical parametric mapping (SPM). CBD significantly decreased subjective anxiety and increased mental sedation, while placebo did not induce significant changes. Assessment of brain regions where anxiolytic effects of CBD were predicted a priori revealed two voxel clusters of significantly decreased ECD uptake in the CBD relative to the placebo condition (p<0.001, uncorrected for multiple comparisons). These included a medial temporal cluster encompassing the left amygdala-hippocampal complex, extending into the hypothalamus, and a second cluster in the left posterior cingulate gyrus. There was also a cluster of greater activity with CBD than placebo in the left parahippocampal gyrus (p<0.001). These results suggest that CBD has anxiolytic properties, and that these effects are mediated by an action on limbic and paralimbic brain areas.

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RATIONALE. Recently, Delta(9)-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, and synthetic cannabinoid receptor agonists reportedly reduced the head-twitches induced by the 5-HT(2A/2C) receptor agonist 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane (DOI) in mice, which is mediated via the activation of 5-HT(2A) receptor. However, the effect of endogenous cannabinoid anandamide on the head-twitch response has not been studied. OBJECTIVES. In this study, we investigated the effect of anandamide on the DOI-induced head-twitch response in mice. METHODS. Five minutes after the injection of DOI (5 mg/kg IP), the number of head-twitches was counted for a 5-min period. THC or anandamide was injected IP 60 min or 10 min before the number of head-twitches was counted, respectively. RESULTS. THC and anandamide each reduced the DOI-induced head-twitch response. The inhibition of the DOI-induced head-twitch response by THC was reversed by SR141716A (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide), a CB(1) receptor antagonist, while the effect of anandamide was not blocked by SR141716A. Cyclooxygenase (COX) inhibitors such as aspirin and indomethacin reversed the inhibition of the DOI-induced head-twitch response by anandamide. On the other hand, COX inhibitors did not affect the inhibition of the DOI-induced head-twitch response by THC. CONCLUSIONS. Taken together, these findings suggest that the endocannabinoid anandamide may inhibit 5-HT(2A) receptor-mediated function via the arachidonic acid cascade, but not via a direct interaction with the CB(1) cannabinoid receptor, and that the mechanism of its action is clearly different from that of THC.


The major psychoactive constituent of cannabis, Delta(9)-tetrahydrocannabinol, affects emotional states in humans and laboratory animals by activating brain cannabinoid receptors. A primary endogenous ligand of these receptors is anandamide, the amide of arachidonic acid with ethanolamine. Anandamide is released in selected regions of the brain and is deactivated through a two-step process consisting of transport into cells followed by intracellular hydrolysis. Pharmacological blockade of the enzyme fatty acid amide hydrolase (FAAH), which is responsible for intracellular anandamide degradation, produces anxiolytic-like effects in rats.
without causing the wide spectrum of behavioral responses typical of direct-acting cannabinoid agonists. These findings suggest that anandamide contributes to the regulation of emotion and anxiety, and that FAAH might be the target for a novel class of anxiolytic drugs.


Basal forebrain cholinergic neurons project to diverse cortical and hippocampal areas and receive reciprocal projections therefrom. Maintenance of a fine-tuned synaptic communication between pre- and postsynaptic cells in neuronal circuitries also requires feedback mechanisms to control the probability of neurotransmitter release from the presynaptic terminal. Release of endocannabinoids or glutamate from a postsynaptic neuron has been identified as a means of retrograde synaptic signalling. Presynaptic action of endocannabinoids is largely mediated by type 1 cannabinoid (CB1) receptors, while fatty-acid amide hydrolase (FAAH) is involved in inactivating some endocannabinoids postsynaptically. Alternatively, vesicular glutamate transporter 3 (VGLUT3) controls release of glutamate from postsynaptic cells. Here, we studied the distribution of CB1 receptors, FAAH and VGLUT3 in cholinergic basal forebrain nuclei of mouse and rat. Cholinergic neurons were devoid of CB1 receptor immunoreactivity. A fine CB1 receptor-immunoreactive (ir) fibre meshwork was present in medial septum, diagonal bands and nucleus basalis. In contrast, the ventral pallidum and substantia innominata received dense CB1 receptor-ir innervation and cholinergic neurons received CB1 receptor-ir presumed synaptic contacts. Consistent with CB1 receptor distribution, FAAH-ir somata were abundant in basal forebrain and appeared in contact with CB1 receptor-containing terminals. Virtually all cholinergic neurons were immunoreactive for FAAH. A significant proportion of cholinergic cells exhibited VGLUT3 immunoreactivity in medial septum, diagonal bands and nucleus basalis, and were in close apposition to VGLUT3-ir terminals. VGLUT3 immunoreactivity was largely absent in ventral pallidum and substantia innominata. We propose that specific subsets of cholinergic neurons may utilize endocannabinoids or glutamate for retrograde control of the efficacy of input synapses, and the mutually exclusive complementary distribution pattern of CB1 receptor-ir and VGLUT3-ir fibres in basal forebrain suggests segregated input-specific signalling mechanisms by cholinergic neurons.


The present studies were conducted to test the hypothesis that activation of peripheral cannabinoid CB2 receptors would suppress hyperalgesia evoked by intradermal administration of capsaicin, the pungent ingredient in hot chili peppers. The CB2 selective cannabinoid agonist AM1241 (33, 330 micro g/kg i.p.) suppressed the development of capsaicin-evoked thermal and mechanical hyperalgesia and allodynia. AM1241 also produced a dose-dependent suppression of capsaicin-evoked nocifensive behavior. The AM1241-induced suppression of each parameter of capsaicin-evoked pain behavior was completely blocked by the CB2 antagonist SR144528 but not by the CB1 antagonist SR141716A. AM1241 (33 micro g/kg i.pl.) suppressed capsaicin-evoked thermal and mechanical hyperalgesia and allodynia following local administration to the capsaicin-treated (ipsilateral) paw but was inactive following administration to the capsaicin-untreated (contralateral) paw. Our data indicate that AM1241 suppresses capsaicin-evoked hyperalgesia through a local site of action. These data provide evidence that actions at cannabinoid CB2 receptors are sufficient to normalize nociceptive thresholds and produce antinociception in persistent pain states.

The amygdala is thought to mediate memory consolidation of amphetamine-induced conditioned place preference, a behavioral paradigm that requires memory for an association between environmental cues and the affective state produced by the drug treatment. Here we show that amphetamine induces long-term synaptic depression (LTD) in the amygdala. Amphetamine LTD is not affected by dopamine, serotonin 1A, and norepinephrine alpha2 receptor antagonists but is blocked by the cannabinoid CB1 receptor antagonist AM251. It is mimicked by the CB1 agonist WIN55212-2 and facilitated and partially occluded by endocannabinoid uptake inhibitor AM404. Both amphetamine and WIN55212-2 LTDs are associated with an increase in the ratio of paired-pulse facilitation and a decrease in the frequency but not the amplitude of miniature EPSCs. They are also sensitive to block by P/Q type calcium channel blocker and occluded by each other, indicating that these two forms of synaptic plasticity share a common underlying mechanism. Loading postsynaptic neuron with calcium chelator blocked amphetamine LTD in some but not all neurons tested. However, in the presence of AM404, amphetamine LTD was present in all neurons recorded. These results suggest that amphetamine-induced endocannabinoid release depends on a rise in intracellular calcium and the incomplete block of LTD in some neurons may be attributable to the spillover of endocannabinoid from nearby cells. The finding that endocannabinoids underlie the synaptic actions of amphetamine may open a new avenue for the treatment of psychostimulants addiction.


The past decade has witnessed a rapid expansion of our understanding of the biological roles of cannabinoids and their cognate receptors. It is now certain that Delta(9)-tetrahydrocannabinol, the principle psychoactive component of the Cannabis sativa plant, binds and activates membrane receptors of the 7-transmembrane domain, G-protein-coupled superfamily. Several putative endocannabinoids have since been identified, including anandamide, 2-arachidonyl glycerol and noladin ether. Synthesis of numerous cannabinomimetics has also greatly expanded the repertoire of cannabinoid receptor ligands with the pharmacodynamic properties of agonists, antagonists and inverse agonists. Collectively, these ligands have proven to be powerful tools both for the molecular characterisation of cannabinoid receptors and the delineation of their intrinsic signalling pathways. Much of our understanding of the signalling mechanisms activated by cannabinoids is derived from studies of receptors expressed by tumour cells; hence, this review provides a succinct summary of the molecular pharmacology of cannabinoid receptors and their roles in tumour cell biology. Moreover, there is now a genuine expectation that the manipulation of cannabinoid receptor systems may have therapeutic potential for a diverse range of human diseases. Thus, this review also summarises the demonstrated antitumour actions of cannabinoids and indicates possible avenues for the future development of cannabinoids as antitumour agents.


The presence of cannabinoid1 (CB1) receptors on primary afferent fibres may provide a novel target for cannabinoid analgesics. The present study investigated the ability of peripheral CB1 receptors to modulate innocuous and noxious transmission in noninflamed rats and rats with peripheral carrageenan inflammation. Effects of peripheral injection of arachidonyl-2-choroethylamide (ACEA; 10 and 30 micro g in 50 micro L), a selective CB1 receptor agonist, on mechanically evoked responses of dorsal horn neurons were studied in noninflamed rats and rats with peripheral carrageenan inflammation. Peripheral injection of ACEA (30 micro g in 50 micro L) significantly inhibited innocuous (12 g) mechanically evoked responses of spinal neurons in noninflamed (27 +/- 4% of control; P < 0.01) and inflamed (12 +/- 8% of control; P < 0.05) rats. Similarly, noxious (80 g) mechanically evoked responses of spinal neurons were inhibited by peripheral injection of ACEA (30 micro g in 50 micro L) in noninflamed rats (51 +/- 9% of control; P < 0.01) and rats with peripheral carrageenan inflammation (21 +/- 8% of control; P < 0.01). Inhibitory effects of ACEA were significantly greater in rats with peripheral carrageenan inflammation than in noninflamed rats (P < 0.05). Inhibitory effects of ACEA were significantly
Peripheral injection of SR141716A alone did not alter mechanically evoked responses of spinal neurons in either group of rats. These data demonstrate that activation of peripheral CB1 receptors can inhibit innocuous and noxious somatosensory processing. Furthermore, following peripheral inflammation there is an enhanced inhibitory effect of a peripherally administered CB1 receptor agonist on both innocuous and noxious mechanically evoked responses of spinal neurons.


In this study we investigated the effect of cannabinoids on [3H]glutamate release from hippocampal synaptosomes of rat and CB1-null mutant mouse. In the rat, cannabinoid receptor agonists, i.e. CP55,940 (EC50, 0.84 microm), WIN55,212-2 (EC50, 3.47 microm), ACEA (EC50, 17.8 microm), and R-(-)-methanandamide (EC50, 19.8 microm) concentration-dependently inhibited the 25-mm-K+ depolarization-evoked release of [3H]glutamate and, among them, WIN55,212-2 displayed the greatest efficacy. The CB1 receptor antagonists SR141716A (1-5 microm) and AM251 (1 microm) and the VR1 vanilloid receptor antagonist capsazepine (10 microm) did not antagonize the effect of the agonists. SR141716A by itself attenuated the evoked [3H]glutamate release. WIN55,212-2 inhibited the release of [3H]glutamate in CB1 -/- mice as well. These data demonstrate that the action of cannabinoids on glutamate release in the hippocampus is pharmacologically distinct and independent from the cloned CB1 receptor.


A fully automated procedure using alkaline hydrolysis and headspace solid-phase microextraction (HS-SPME), followed by on-fiber derivatization and gas chromatographic-mass spectrometric (GC-MS) detection has been developed for determination of cannabinoids in hemp food samples. After addition of a deuterated internal standard, the sample was hydrolyzed with sodium hydroxide and submitted to direct HS-SPME. After adsorption of analytes on on-fiber derivatization, the fiber was placed directly into the headspace of a second vial containing N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA), before GC-MS analysis. Linearity was good for Delta(9)-tetrahydrocannabinol (THC), cannabidiol, and cannabiol; regression coefficients were greater than 0.99. Depending on the characteristics of the matrix the detection limits obtained ranged between 0.01 and 0.17 mg kg(-1) and the precision between 0.4 and 11.8%. In comparison with conventional liquid-liquid extraction this automated HS-SPME-GC-MS procedure is substantially faster. It is easy to perform, solvent-free, and sample quantities are minimal, yet it maintains the same sensitivity and reproducibility. The applicability was demonstrated by analysis of 30 hemp food samples. Cannabinoids were detected in all of the samples and it was possible to differentiate between drug-type and fiber-type Cannabis sativa L. In comparison with other studies relatively low THC concentrations between 0.01 and 15.53 mg kg(-1) were determined.


The therapeutic potential of cannabinoids has been described previously for several inflammatory diseases, but the molecular mechanisms underlying the anti-inflammatory properties of cannabinoids are not well understood. In this study, we investigated the mechanism of action of a novel synthetic cannabinoid, [(+)(6aS,10aS)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(1H-imidazo 1-2-ylsulfanyl)methyl]-6a,7,10,10a-tetrahydro-6H-dibenzo[b,d]pyran (PRS-211,092) that has no psychotropic effects but exhibits immunomodulatory properties. Treatment with PRS-211,092 significantly decreased Concanavalin A-induced liver injury in mice that was accompanied by: 1) promotion of early gene expression of interleukin (IL)-6 and IL-10 that play a protective role in this model; 2) induction of early gene expression of the suppressors of cytokine signaling (SOCS-1 and 3), followed by 3) inhibition of several pro-inflammatory mediators, including IL-2, monocyte chemoattractant protein-1 (MCP-1), IL-1 beta, interferon-gamma, and
tumor necrosis factor alpha. Based on these results, we propose a mechanism by which PRS-211,092 stimulates the expression of IL-6, IL-10 and the SOCS proteins that, in turn, negatively regulates the expression of pro-inflammatory cytokines. Negative regulation by PRS-211,092 was further demonstrated in cultured T cells, where it inhibited IL-2 production and nuclear factor of activated T cells activity. These findings suggest that this cannabinoid derivative is an immunomodulator that could be developed as a potential drug for hepatitis as well as for other short- or long-term inflammatory diseases.


Cannabidiol (CBD) is a new drug candidate for treatment of rheumatic diseases. However, its oral administration is associated with a number of drawbacks. The objective of this study was to design a transdermal delivery system for CBD by using ethosomal carriers. CBD ethosomes were characterized by transmission electron microscopy, confocal laser scanning microscopy and differential scanning calorimetry. Results indicated that CBD and phosphatidylcholine form an eutectic mixture. In vivo application of ethosomal CBD to CD1 nude mice produced a significant accumulation of the drug in the skin and in the underlying muscle. Upon transdermal application of the ethosomal system to the abdomen of ICR mice for 72 h, steady-state levels were reached at about 24 h and lasted at least until the end of the experiment, at 72 h. Furthermore, transdermal application of ethosomal CBD prevented the inflammation and edema induced by sub-plantar injection of carrageenan in the same animal model. In conclusion, ethosomes enable CBD’s skin permeation and its accumulation in a depot at levels that demonstrate the potential of transdermal CBD to be used as an anti-inflammatory treatment.


Recently, cannabinoids have been shown to possess antitumor properties. Because the psycho-activity of cannabinoid compounds limits their medicinal usage, we undertook the present study to evaluate the in vitro antiproliferative ability of CBD, a non-psychoactive cannabinoid compound, on U87 and U373 human glioma cell lines. The addition of CBD to the culture medium led to a dramatic drop of mitochondrial oxidative metabolism (MTT test) and viability in glioma cells, in a concentration-dependent manner, already evident 24 h after CBD exposure with an apparent IC50 of 25 micro M. The antiproliferative effect of CBD was partially prevented by the CB2 receptor antagonist SR144528 and alpha-tocopherol. By contrast, the CB1 cannabinoid receptor antagonist SR141716, capsazepine (vanilloid receptor antagonist), the inhibitors of ceramide generation or PTX did not counteract CBD effects. We also show, for the first time, that the antiproliferative effect of CBD was correlated to induction of apoptosis, as determined by cytofluorimetric analysis and ssDNA staining, which was not reverted by cannabinoid antagonists. Finally, CBD administered s.c. to nude mice at the dose of 0.5 mg/mouse, significantly inhibited the growth of subcutaneously implanted U87 human glioma cells. Concluding, the non-psychoactive CBD was able to produce a significant antitumor activity both in vitro and in vivo, thus suggesting a possible application of CBD as an antineoplastic agent.


In the present work we investigated on rat splenocytes long-term interactions between opioid and cannabinoid drugs in terms of a common regulation of cAMP intracellular pathway. Both morphine and the synthetic cannabinoid compound CP-55,940 inhibited in a concentration-dependent manner the intracellular cAMP level in splenocytes stimulated by forskolin. The in vitro combination of submaximal concentrations of the two drugs did not yield any additive effect on the inhibition induced by the two drugs. In splenocytes taken from rats chronically treated with CP-55,940 (0.2 mg/kg i.p., twice a day for 4.5 days) or morphine (5 mg/kg s.c., twice a day for 6.5 days) and in vitro exposed to either CP-55,940 or morphine, it was found a desensitisation and cross-desensitisation to the inhibitory effects on cAMP production induced by the two drugs. Binding experiments on the cannabinoid receptors level in spleen coronal sections after in vivo chronic administration of morphine, revealed that there was no changes in
the binding of \([\text{H}(3)]\)-CP-55,940. Thus, these results strengthen the hypothesis of cAMP as part of the common intracellular pathway shared by opiates and cannabinoids at immune cell level.


Traumatic brain injury (TBI) is the most common cause of mortality and morbidity in adults under 40 years of age in industrialized countries. Worldwide the incidence is increasing, about 9.5 million people are hospitalized per year due to TBI, and the death rate is estimated to be more than one million people per year. Recently BAY 38-7271 has been characterized as a structurally novel, selective and highly potent cannabinoid CB(1)/CB(2) receptor agonist in vitro and in vivo with pronounced neuroprotective efficacy in a rat traumatic brain injury model, showing a therapeutic window of at least 5 h. Furthermore, neuroprotective efficacy was also found in models of transient and permanent occlusion of the middle cerebral artery and brain edema models as well. In this article we review the in vitro and in vivo pharmacology of BAY 38-7271, the results from acute and subacute toxicity studies, pharmacokinetics and drug metabolism in animals and healthy male volunteers. In phase I studies BAY 38-7271 was safe and well tolerated when administered by i.v. infusion for either 1 or 24 h. As the doses of BAY 38-7271 in animals needed for maximal neuroprotective efficacy were significantly lower than those inducing typical cannabinoid-like side effects, it is to be expected that the compound will offer a novel therapeutic approach with a favorable therapeutic window for the treatment of TBI or cerebral ischemia.


The cannabinoid CB(1) receptor transmembrane helix (TMH) 3-4-5-6 region includes an aromatic microdomain comprised of residues F3.25, F3.36, W4.64, Y5.39, W5.43, and W6.48. In previous work, we have demonstrated that aromaticity at position 5.39 in CB(1) is crucial for proper function of CB(1). Modeling studies reported here suggest that in the inactive state of CB(1), the binding site of the CB(1) inverse agonist/antagonist SR141716A is within the TMH3-4-5-6 aromatic microdomain and involves direct aromatic stacking interactions with F3.36, Y5.39, and W5.43, as well as hydrogen bonding with K3.28. Further, modeling studies suggest that in the active state of CB(1), the CB agonist WIN55,212-2 binds in this same aromatic microdomain, with direct aromatic stacking interactions with F3.36, W5.43, and W6.48. In contrast, in the binding pocket model, the CB agonist anandamide binds in the TMH2-3-6-7 region in which hydrogen bonding and C-H-pi interactions appear to be important. Only one TMH3 aromatic residue, F3.25, was found to be part of the anandamide binding pocket. To probe the importance of the TMH3-4-5-6 aromatic microdomain to ligand binding, stable transfected cell lines were created for single-point mutations of each aromatic microdomain residue to alanine. Improper cellular expression of the W4.64A was observed and precluded further characterization of this mutation. The affinity of the cannabinoid agonist CP55,940 was unaffected by the F3.25A, F3.36A, W5.43A, or W6.48A mutations, making CP55,940 an appropriate choice as the radioligand for binding studies. The binding of SR141716A and WIN55,212-2 were found to be affected by the F3.36A, W5.43A, and W6.48A mutations, suggesting that these residues are part of the binding site for these two ligands. Only the F3.25A mutation was found to affect the binding of anandamide, suggesting a divergence in binding site regions for anandamide from WIN55,212-2, as well as SR141716A. Taken together, these results support modeling studies that identify the TMH3-4-5-6 aromatic microdomain as the binding region of SR141716A and WIN55,212-2, but not of anandamide.


This study examined whether the cannabinoid antagonist, SR 141716A, could be established as a discriminative stimulus in rhesus monkeys treated with Delta-sup-9-tetrahydrocannabinol (Delta-sup-9-THC). Stimulus control was established with SR 141716A (1.0
mg/kg) in 3 Delta-sup-9-THC-treated monkeys (1.12 mg/kg/day) in 113-124 sessions. The SR
141716A discriminative stimulus was dose related, attenuated by an acute injection of Delta-sup-
9-THC, and not mimicked by cocaine or ketamine. SR 141716A-appropriate responding
occasioned by temporary discontinuation of Delta-sup-9-THC treatment was attenuated by Delta
-sup-9-THC and not ketamine. The SR 141716A discriminative stimulus in Delta-sup-9-THC-
treated monkeys appears to be mediated by cannabinoid receptors and could be related to Delta
-sup-9-THC withdrawal. ((c) 2003 APA, all rights reserved)

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The endocannabinoids are a family of lipid messengers that engage the cell surface
receptors that are targeted by Delta(9)-tetrahydrocannabinol, the active principle in marijuana
(Cannabis). They are made on demand through cleavage of membrane precursors and are
involved in various short-range signalling processes. In the brain, they combine with CB(1)
cannabinoid receptors on axon terminals to regulate ion channel activity and neurotransmitter
release. Their ability to modulate synaptic efficacy has a wide range of functional consequences
and provides unique therapeutic possibilities.

55212-2, a cannabinoid agonist, interact to evoke synergistic hypothermia." J Pharmacol Exp
Ther.

Cannabinoids evoke profound hypothermia in rats by activating central CB1 receptors.
Nitric oxide (NO), a prominent second messenger in central and peripheral neurons, also plays a
crucial role in thermoregulation, with previous studies suggesting pyretic and antipyretic functions.
Dense nitric oxide synthase (NOS) staining and CB1 receptor immunoreactivity have been
detected in regions of the hypothalamus that regulate body temperature, suggesting that intimate
NO-cannabinoid associations may exist in the CNS. The present study investigated the effect of
L-NAME, a NO synthase inhibitor, on the hypothermic response to WIN 55212-2, a selective
cannabinoid agonist, in rats. WIN 55212-2 (1-5 mg/kg, i.m.) produced dose-dependent
hypothermia that peaked 45-90 min post-injection. L-NAME (10-100 mg/kg, i.m.) by itself did not
significantly alter body temperature. However, a non-hypothermic dose of L-NAME (50 mg/kg)
potentiated the hypothermia caused by WIN 55212-2 (0.5-5 mg/kg). The augmentation was
strongly synergistic, indicated by a 2.5-fold increase in the relative potency of WIN 55212-2. The
inactive enantiomer of WIN 55212-2, WIN 55212-3 (5 mg/kg, i.m.), did not produce hypothermia
in the absence or presence of L-NAME (50 mg/kg), confirming that cannabinoid receptors
mediated the synergy. The present data are the first evidence that drug combinations of NOS
blockers and cannabinoid agonists produce synergistic hypothermia. Thus, NO and cannabinoid
systems may interact to induce super-additive hypothermia.

signaling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1
receptors." Br J Pharmacol.
indicating that CB1 receptors are not constitutively active in these preparations. At higher concentrations (1-2.5x10^-5 M), both antagonists reduced basal G-protein activity in control and ADA-treated membranes, but had no effect when A1 receptor signaling was blocked with DPCPX. Moreover, the CB1 antagonists right-shifted A1 agonist dose-response curves without affecting maximal responses, suggesting competitive mode of antagonist action. The CB1 antagonists did not affect muscarinic acetylcholine or GABAB receptor signaling. When further optimizing G-protein activation assay for the labile endocannabinoid 2-arachidonoylglycerol (2-AG), we show, by using HPLC, that pretreatment of cerebellar membranes with methyl arachidonoyl fluorophosphonate (MAFP) fully prevented enzymatic degradation of 2-AG and concomitantly enhanced the potency of 2-AG. In contrast to previous claims, MAFP exhibited no antagonist activity at the CB1 receptor. The findings establish an optimized method with improved signal-to-noise ratio to assess endocannabinoid-dependent G-protein activity in brain membranes, under assay conditions where basal adenosinergic tone and enzymatic degradation of 2-AG are fully eliminated.


Cannabinoids have been shown to modulate central autonomic regulation and baroreflex control of blood pressure (BP). The presence of cannabinoid CB1 receptors on fibers in the NTS suggests that some presynaptic modulation of transmitter release could occur in this region which receives direct afferent projections from arterial baroreceptors and cardiac mechanoreceptors. This study, therefore, was performed to determine the mechanism(s) of effects of microinjection of an endocannabinoid, arachidonylethanolamide (anandamide, AEA), into the NTS on baroreflex sympathetic nerve responses produced by phenylephrin (PE)-induced pressure changes in anesthetized rats. AEA was found to prolong reflex inhibition of renal sympathetic nerve activity (RSNA), suggesting an increase in baroreflex sensitivity. This effect of AEA was blocked by prior microinjection of SR141716 to block cannabinoid CB1 receptors. To determine if this baroreflex enhancement by AEA involved a GABAA mechanism, the baroreflex response to AEA was tested after prior blockade of postsynaptic GABAA receptors by bicuculline (BIC), which alone prolonged the baroreflex inhibition of RSNA, AEA shortened the duration of RSNA inhibition, suggesting a possible presynaptic inhibition of glutamate release previously obscured by a more dominant GABAA effect. To support a possible physiological role for AEA, the concentration of AEA in the NTS was measured after a PE-induced increase in BP. AEA content in the NTS was increased significantly over normotensive animals. These results support the hypothesis that AEA content is increased by brief periods of hypertension and suggest that AEA can modulate the baroreflex through activation of CB1 receptors within the NTS, possibly modulating effectiveness of GABA and/or glutamate neurotransmission.


To date, N-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol are the best studied endocannabinoids and are thought to act as retrograde messengers in the central nervous system (CNS). By activating presynaptic cannabinoid CB(1) receptors, they can reduce glutamate release in dorsal and ventral striatum (nucleus accumbens) and alter synaptic plasticity, thereby modulating neurotransmission in the basal ganglia and in the mesolimbic reward system. In this review, we will focus on the role of the endocannabinoid system within these neuronal pathways and describe its effect on dopaminergic transmission and vice versa. The endocannabinoid system is unlikely to directly affect dopamine release, but can modify dopamine transmission through trans-synaptic mechanisms, involving gamma-aminobutyric acid (GABA)-ergic and glutamatergic synapses, as well as by converging signal transduction cascades of the cannabinoid and dopamine receptors. The dopamine and endocannabinoid systems exert a mutual control on each other. Cannabinergic signalling may lead to release of dopamine, which can act via dopamine D(1)-like receptors as a negative feedback mechanism to
counteract the effects of activation of the cannabinoid CB(1) receptor. On the other hand, dopaminergic signalling via dopamine D(2)-like receptors may lead to up-regulation of cannabinergic signalling, which is likely to represent a negative feedback on dopaminergic signalling. The consequences of these interactions become evident in pathological conditions in which one of the two systems is likely to be malfunctioning. We will discuss neurological and psychiatric disorders such as Parkinson’s and Huntington’s disease, drug addiction and schizophrenia. Furthermore, the possible role of the endocannabinoid system in disorders not necessarily depending on the dopaminergic system, such as eating disorders and anxiety, will be described.


Mammalian fertility absolutely depends on synchronized development of the blastocyst to the stage when it is competent to implant, and the uterus to the stage when it is receptive to implantation. However, the molecular basis for the reciprocal interaction between the embryo and the uterus remains largely unexplored. One potentially important mechanism involves signaling between an evolutionarily conserved G protein-coupled protein cannabinoid receptor, CB1, that is expressed at high levels on the surface of the trophectoderm and anandamide (N-arachidonoylethanolamine), an endocannabinoid ligand found to be produced at higher levels by the uterus before implantation and then down-regulated at the time of implantation. Using genetic, pharmacological, and physiological approaches, we show here that anandamide within a very narrow range regulates blastocyst function and implantation by differentially modulating mitogen-activated protein kinase signaling and Ca(2+) channel activity via CB1 receptors. Anandamide at a low concentration (7 nM) induces extracellular regulated kinase phosphorylation and nuclear translocation in trophectoderm cells without influencing Ca(2+) channels, and renders the blastocyst competent for implantation in the receptive uterus. In contrast, anandamide at a higher concentration (28 nM) inhibits Ca(2+) channel activity and blastocyst competency for implantation without influencing mitogen-activated protein kinase signaling. Besides uncovering a potentially important regulatory mechanism for synchronizing blastocyst and uterine competency to implantation, this observation has high clinical relevance, because elevated levels of anandamide induce spontaneous pregnancy loss in women.


Both endocannabinoids through cannabinoid receptor type I (CB1) receptors and dopamine through dopamine receptor type D1 receptors modulate postsynaptic inhibition in substantia nigra by changing GABA release from striatonigral terminals. By recording from visually identified pars compacta and pars reticulata neurons we searched for a possible corelease and interaction of endocannabinoids and dopamine. Depolarization of a neuron in pars reticulata or in pars compacta transiently suppressed evoked synaptic currents which were blocked by GABA(A) receptor antagonists (inhibitory postsynaptic currents [IPSCs]). This depolarization-induced suppression of inhibition (DSI) was abrogated by the cannabinoid CB1 receptor antagonist AM251 (1 microM). A correlation existed between the degree of DSI and the degree of reduction of evoked IPSCs by the CB1 receptor agonist WIN55,212-2 (1 microM). The cholinergic receptor agonist carbachol (0.5-5 microM) enhanced DSI, but suppression of spontaneous IPSCs was barely detectable pointing to the existence of GABA release sites without CB1 receptors. In dopamine, but not in GABAergic neurons DSI was enhanced by the dopamine D1 receptor antagonist SCH23390 (3-10 microM). Both the antagonist for CB1 receptors and the antagonist for dopamine D1 receptors enhanced or reduced, respectively, the amplitudes of evoked IPSCs. This tonic influence persisted if the receptor for the other ligand was blocked. We conclude that endocannabinoids and dopamine can be co-released. Retrograde signaling through endocannabinoids and dopamine changes inhibition independently from each other. Activation of dopamine D1 receptors emphasizes extrinsic inhibition and activation of CB1 receptors promotes intrinsic inhibition.
CLINICAL SCIENCE


Huestis and Cone reported in [J. Anal. Toxicol. 22 (1998) 445] that serial monitoring of Delta(9)-THC-COOH/creatinine ratios in paired urine specimens collected at least 24h apart could differentiate new drug use from residual Delta(9)-THC-COOH excretion following acute marijuana use in a controlled setting. The best accuracy (85.4%) for predicting new marijuana use was for a Delta(9)-THC-COOH/creatinine ratio \( \geq 0.5 \) (dividing the Delta(9)-THC-COOH/creatinine ratio of specimen no. 2 by the specimen no. 1 ratio). In previous studies in this laboratory [J. Anal. Toxicol. 23 (1999) 531 and Forensic Sci. Int. 133 (2003) 26], urine specimens were collected from chronic marijuana users \( \geq 24h \) or \( \geq 48h \) apart in an uncontrolled setting. Subjects with a history of chronic marijuana use were screened for cannabinoids with the EMIT((R)) II Plus cannabinoids assay (cut-off 50ng/ml) followed by confirmation for Delta(9)-THC-COOH by GC-MS (cut-off 15ng/ml). Creatinine was analyzed as an index of dilution. The objective of the present study was to evaluate whether creatinine corrected specimens could differentiate new marijuana or hashish use from the excretion of residual Delta(9)-THC-COOH in chronic marijuana users based on the Huestis 0.5 ratio. Urine specimens (N=376) were collected from 29 individuals \( \geq 96h \) between urine collections. The mean urinary Delta(9)-THC-COOH concentration was 464.4ng/ml, mean Delta(9)-THC-COOH/creatinine ratio (ng/(ml Delta(9)-THC-COOHmoll creatinine)) was 36.8 and the overall mean Delta(9)-THC-COOH/creatinine ratio of specimen 2/mean Delta(9)-THC-COOH/creatinine ratio of specimen 1 was 1.37. The Huestis ratio calculation indicated new drug use in 83% of all sequentially paired urine specimens. The data were sub-divided into three groups (Groups A-C) based on mean Delta(9)-THC-COOH/creatinine values. Interindividual mean Delta(9)-THC-COOH/creatinine values ranged from 4.7 to 13.4 in Group A where 80% of paired specimens indicated new drug use (N=10) and 20.4-39.6 in Group B where 83.6% of paired specimens indicated new drug use (N=7). Individual mean Delta(9)-THC-COOH/creatinine values ranged from 44.2 to 120.2 in Group C where 84.5% of paired urine specimens indicated new marijuana use (N=12). Correcting Delta(9)-THC-COOH excretion for urinary dilution and comparing Delta(9)-THC-COOH/creatinine concentration ratios of sequentially paired specimens (collected \( \geq 96h \) apart) may provide an objective indicator of ongoing marijuana or hashish use in this population.


A young Italian male was investigated for possession of illicit marijuana in Rome. In his house, police found 80 cannabis plants, the plants were different sizes and located in a room with ultraviolet light, naphthalene, as a grey-white powder, was also found in his house. The man indicated that he used it for cannabis cultivation.


The International Association for Cannabis as Medicine 2nd Conference on Cannabinoids in Medicine focused on new clinical research with cannabis and single cannabinoids (Delta(9)-tetrahydrocannabinol, CT-3) and on animal research with possible therapeutic implications. The meeting brought together basic researchers, clinicians and physicians to facilitate an exchange of knowledge and experience in this field. Even a talk by a
patient with multiple sclerosis was included in a workshop on neurology. Current clinical research with cannabinoids focuses mainly on chronic pain and neurological disorders adding to accepted indications such as anorexia in AIDS-wasting and antiemetic effects in cancer chemotherapy. First results are promising and larger studies are underway or have recently been completed and are awaiting publication. New basic research opens further areas of possible uses for modulators of the endogenous cannabinoid system, including osteoporosis, cancer and inflammation. A workshop on psychiatry focused on effects of cannabis use on onset, incidence and the course of schizophrenia. Basic and clinical research shows that adolescents might be more vulnerable than adults to possible psychiatric effects of cannabinoids. It was concluded that possible side effects of cannabinoids should be taken into account but do not preclude a legitimate medical use.


This preliminary test of a brief intervention designed to stimulate GP incorporation of cannabis enquiry was followed up after 2-3 months. Intervention comprised face-to-face discussion based on principles of motivational interviewing, with informational adjunct. Substantially more positive attitudes and greater clinical activity were observed following receipt of intervention.


BACKGROUND: Multiple sclerosis is associated with muscle stiffness, spasms, pain, and tremor. Much anecdotal evidence suggests that cannabinoids could help these symptoms. Our aim was to test the notion that cannabinoids have a beneficial effect on spasticity and other symptoms related to multiple sclerosis. METHODS: We did a randomised, placebo-controlled trial, to which we enrolled 667 patients with stable multiple sclerosis and muscle spasticity. 630 participants were treated at 33 UK centres with oral cannabis extract (n=211), Delta9-tetrahydrocannabinol (Delta9-THC; n=206), or placebo (n=213). Trial duration was 15 weeks. Our primary outcome measure was change in overall spasticity scores, using the Ashworth scale. Analysis was by intention to treat. FINDINGS: 611 of 630 patients were followed up for the primary endpoint. We noted no treatment effect of cannabinoids on the primary outcome (p=0.40). The estimated difference in mean reduction in total Ashworth score for participants taking cannabis extract compared with placebo was 0.32 (95% CI -1.04 to 1.67), and for those taking Delta9-THC versus placebo it was 0.94 (-0.44 to 2.31). There was evidence of a treatment effect on patient-reported spasticity and pain (p=0.003), with improvement in spasticity reported in 61% (n=121, 95% CI 54.6-68.2), 60% (n=108, 52.5-66.8), and 46% (n=91, 39.0-52.9) of participants on cannabis extract, Delta9-THC, and placebo, respectively. INTERPRETATION: Treatment with cannabinoids did not have a beneficial effect on spasticity when assessed with the Ashworth scale. However, though there was a degree of unmasking among the patients in the active treatment groups, objective improvement in mobility and patients' opinion of an improvement in pain suggest cannabinoids might be clinically useful.

BEHAVIOURAL SCIENCE

AIM: To examine the evidence on the association between cannabis and depression and evaluate competing explanations of the association. METHODS: A search of Medline, PsychInfo and EMBASE databases was conducted. All references in which the terms 'cannabis', 'marijuana' or 'cannabinoid', and in which the words 'depression/depressive disorder/depressed', 'mood', 'mood disorder' or 'dysthymia' were collected. Only research studies were reviewed. Case reports are not discussed. RESULTS: There was a modest association between heavy or problematic cannabis use and depression in cohort studies and well-designed cross-sectional studies in the general population. Little evidence was found for an association between depression and infrequent cannabis use. A number of studies found a modest association between early-onset, regular cannabis use and later depression, which persisted after controlling for potential confounding variables. There was little evidence of an increased risk of later cannabis use among people with depression and hence little support for the self-medication hypothesis. There have been a limited number of studies that have controlled for potential confounding variables in the association between heavy cannabis use and depression. These have found that the risk is much reduced by statistical control but a modest relationship remains. CONCLUSIONS: Heavy cannabis use and depression are associated and evidence from longitudinal studies suggests that heavy cannabis use may increase depressive symptoms among some users. It is still too early, however, to rule out the hypothesis that the association is due to common social, family and contextual factors that increase risks of both heavy cannabis use and depression. Longitudinal studies and studies of twins discordant for heavy cannabis use and depression are needed to rule out common causes. If the relationship is causal, then on current patterns of cannabis use in the most developed societies cannabis use makes, at most, a modest contribution to the population prevalence of depression.


The following Kaplan/Damphouse hypothesis was tested and cross validated: The use of marijuana either predicts to or has a greater effect on increasing the degree of violent behavior for a group that is low on delinquent behavior, than it does for a group that scores high on these behaviors. For the conventional, non-delinquent sub-group, a higher degree of significant relationship between degree of marijuana use and degree of violent behavior was found, compared to the degree of this type of relationship than was found for either cocaine/crack use, amphetamine use, or tranquilizer/sedative use. For example, for the commission of the offense of Attempted Homicide/Reckless Endangerment: for the conventional, non-delinquent group there was a highly significant relationship to the degree of marijuana use; but there was a non-significant relationship between this type of offense and the degree of use of each of the other types of drugs. Thus, this special disinhibition effect was found only for marijuana, and not for other drugs, regardless of whether they were stimulant types of drugs, or were sedative drugs.


Smoking among teens and college students is a significant public health challenge. Tobacco, marijuana, and alcohol continue to be the most commonly abused drugs by teens and young adults. Educational efforts have resulted in increased awareness of the mortality and morbidity attributed to smoking, second-hand smoke, and prenatal exposure to tobacco. Short- and long-term consequences of marijuana use are well documented in the literature, but they have received less wide spread attention. Even less well known is the relationship between these substances. Does use of one lead to use of the other? Are there synergistic and/or antagonistic effects when these substances are used together? We need answers to these questions to understand the prevalence of use and the impact of these drugs on our nations youth and young adults. The gateway theory of drug use is often used to describe the progression from using alcohol or tobacco, to marijuana, and later use of other drugs like MDMA, cocaine, and heroin. While tobacco use does commonly precede marijuana use, we propose that marijuana may be a "gateway drug" to tobacco smoking. Our research with university students is suggesting that cigarette-smoking initiation often follows or coincides with marijuana.
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