INTRODUCTION
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BASIC SCIENCE

Biochemical investigations have identified putative enzymatic pathways for the synthesis and metabolism of endogenous cannabinoids. Anandamide amidase is an enzyme that metabolizes anandamide into arachadonic acid and ethanolamine. Using in vitro methods, various inhibitors of amidase have been identified. The present studies were undertaken to determine if the amidase inhibitor AM 374 could enhance the effects of intraperitoneal (IP) injections of anandamide. Three studies were conducted to investigate the effects of various drug treatments on fixed ratio 5 operant lever pressing for food reinforcement. In the first study, the effects of different doses of anandamide were assessed, and it was demonstrated that 5.0 and 10.0 mg/kg anandamide IP significantly suppressed lever pressing, while 2.5 mg/kg produced very little effect. The second study tested the effects of intraventricular (ICV) injections of AM 374, and it was observed that doses up to 10.0, 20.0 and 40 microg AM 374 had no significant effect upon lever pressing. The third study investigated the combined effect of AM374 with a low dose of anandamide. Rats received two drug injections: one ICV and one IP. Four different drug treatments were assessed: 1) ICV vehicle + IP vehicle, 2) ICV vehicle + 2.5 mg/kg anandamide IP, 3) ICV 20.0 microg AM 374 + IP vehicle, and 4) ICV 20 microg AM 374 + 2.5 mg/kg anandamide IP. Combined administration of AM 374 plus anandamide led to a significant decrease in lever pressing compared to either AM374 or anandamide administered alone. Observations of the animals treated with the combination of AM374 plus anandamide indicated that the drug combination resulted in motor slowing, which is consistent with the notion that stimulation of cannabinoid receptors produced a motor deficit that interfered with lever pressing. Although AM374 produced no effect on its own, this amidase inhibitor did enhance the behavioral effect of a low dose of anandamide. These results are consistent with the notion that AM 374 inhibited the enzymatic breakdown of exogenously injected anandamide. This type of procedure can be used to assess a variety of different compounds for their ability to inhibit cannabinoid metabolism.


The endocannabinoid system is still poorly understood. Recently, the basic elements that constitute it, i.e., membrane receptors, endogenous ligands, and mechanisms for termination of the signaling process, have been partially characterized. There is a considerable lack of information, however, concerning the distribution, concentration, and function of those components in the human body, particularly during pathological events. We have studied the status of some of the components of the endocannabinoid system, fatty acid amide hydrolase and cannabinoid CB1 and CB2 receptors, in postmortem brains from patients with Alzheimer's
disease. Using specific polyclonal antibodies, we have performed immunohistochemical analysis in hippocampus and entorhinal cortex sections from brains of Alzheimer’s disease patients. Our results show that both fatty acid amide hydrolase and cannabinoid CB2 receptors are abundantly and selectively expressed in neuritic plaque-associated astrocytes and microglia, respectively, whereas the expression of CB1 receptors remains unchanged. In addition, the hydrolase activity seems to be elevated in the plaques and surrounding areas. Thus, some elements of the endocannabinoid system may be postulated as possible modulators of the inflammatory response associated with this neurodegenerative process and as possible targets for new therapeutic approaches.


Activation of cannabinoid CB1 receptors reduces glutamatergic synaptic transmission in the rodent striatum and is involved in the normal control of motor function by the basal ganglia. Here we investigated CB1 receptor regulation of glutamate release and uptake and synaptic transmission in the rat striatum. We show that CB1 receptor activation reduces both the release and uptake of [3H]glutamate in striatal slices. We also demonstrate that both activation of CB1 receptors and inhibition of glutamate uptake reduce corticostriatal synaptic transmission in a mutually occlusive manner and that both forms of depression are dependent on metabotropic glutamate receptor (mGluR) activation. We propose that CB1 receptor activation in the striatum decreases glutamate transporter activity and that the resulting increase in synaptic cleft glutamate concentration causes the activation of presynaptic mGluRs, which then decrease glutamate release.


This review will consider studies concerning the effects of cannabinoid receptor agonists and antagonists on memory in laboratory animals. Two subtypes of cannabinoid receptors have been identified to date: the central CB1 subtype and the peripheral CB2 subtype. The receptor which specifically binds Delta9-tetrahydrocannabinol (Delta9-THC) and related compounds in rat and human brain has been discovered and cloned by a number of researchers. This cannabinoid receptor is localized with high concentrations in different brain areas, including hippocampus and amygdala, which play an important role in the modulation of memory. In recent years evidence has been obtained that cannabinoids influence memory processes. It has been shown, for example, that Delta9-THC impairs memory in rats, mice and monkeys tested in a variety of experimental conditions (radial maze, instrumental discrimination tasks, Morris water maze, etc.). In some of these researches the effect of Delta9-THC was antagonized by the CB1 receptor antagonist SR 141716A, showing the involvement of this subtype of cannabinoid receptor in its effect. Anandamide, arachidonylethanolamide, was recently discovered as the first endogenous ligand for the cannabinoid receptor. It has been reported to stimulate CB1 receptors and to mimic the pharmacological effects of cannabinoids. Experiments carried out by our group have shown that anandamide impairs memory consolidation in random bred mice (CD1), exerts genotype-dependent influences on memory in inbred strain of mice (C57 BL/6 and DBA/2), and that opioid and dopaminergic systems might be involved in its effects.


The antinociceptive properties of cannabinoids in persistent pain are not fully elucidated. We investigated the effect of repeated treatment with the synthetic cannabinoid receptor agonist WIN 55,212-2 on the neuropathic pain induced in rats by chronic constriction of the sciatic nerve. WIN 55,212-2 administered daily throughout the development of neuropathy reversed the hyperalgesia, at a dose (0.1 mg kg(-1), s.c.) that had no effect on the nociceptive responses of either paw contralateral to the sciatic ligation or of animals subjected to sham surgery. At 14 days after injury, the levels of mediators known to be involved in neuropathic pain, such as prostaglandin E2, NO and the neuronal NOS, were increased. Repeated treatment with WIN
55,212-2 abolished these increases. In the light of the current clinical need for neuropathic pain treatments, these findings indicate that cannabinoid agonists, at doses devoid of psychoactive effects, could constitute important compounds for the development of new analgesics.


In this study, we have assessed the activation of the cannabinoid CB2 receptor (CB2-R) in a model of mouse myocardial ischemia/reperfusion (I/R). The results show that treatment of animals with WIN55212-2, a CB1/CB2-R agonist, given 30 min before induction of I/R, significantly reduced the extent of infarct size (IS) in the area at risk, as measured 2.5 h later, with almost a 51% inhibition observed at the dose tested of 3.5 mg/kg intraperitoneally (i.p.). The protective effect of WIN55212-2 was almost abolished by the selective CB2-R antagonist AM630 (1 mg/kg i.p.) and not affected by the selective CB1-R antagonist AM251 (3 mg/kg i.p.). The CB2-R antagonist administered alone produced a slight but significant (P<0.05) increase in IS compared with vehicle alone. The protection afforded by WIN55212-2 was paralleled by lower values of myeloperoxidase activity and interleukin-1beta and of the CXC chemokine ligand 8 into the injured tissue. In conclusion, we demonstrate for the first time that exogenous and endogenous CB2-R activation reduces the leukocyte-dependent myocardial damage associated with an I/R procedure.


The fatty acid amide class of compounds, which include the endocannabinoid anandamide and the sleep-inducing compound oleamide, have been shown in vitro to have a multiplicity of actions upon different neurochemical systems. In the present issue of this journal, Leggett et al present data indicating that oleamide functionally activates CB1 cannabinoid receptors in vitro. The significance of their finding is discussed in this commentary.


RATIONALE. Opioid and cannabinoid CB(1) receptor antagonists reduce the motivation to consume alcohol when taken individually but their effectiveness in combination is not yet known. OBJECTIVE. The effects of naloxone/naltrexone and SR 141716 alone and in combination were examined on beer consumption in rats. METHODS. In a progressive ratio paradigm rats were trained to lick at a tube for either beer (4.5% ethanol v/v) or near-beer (beer containing <0.5% ethanol v/v) under a progressive ratio schedule of reinforcement. They were then tested with naloxone (0.3, 0.6 or 1.2 mg/kg IP), SR 141716 (0.15, 0.3 or 0.6 mg/kg IP) and their combination. In a continuous access paradigm, other rats were given beer or near-beer in their home cages for several weeks and the effects of repeated (4 day) administration of naltrexone (0.3, 0.6 or 1.2 mg/kg), SR 141716 (0.15, 0.3 or 0.6 mg/kg) and their combination were assessed. RESULTS. In the progressive ratio paradigm SR 141716, naloxone and their combination were more effective in reducing the break points for beer rather than near-beer. The two lowest dose combinations produced a synergistic reduction in break points. The highest dose combination reduced break points for both beer and near-beer and effects were more additive than synergistic. In the continuous access paradigm, the low doses of the drugs selectively reduced beer consumption in a synergistic fashion with higher doses having a less selective and more additive effect. CONCLUSIONS. The combined, low dose treatment has possible clinical efficacy in treating alcohol craving in humans.


Huntington's disease (HD) is a late onset progressive genetic disorder characterised by motor dysfunction, personality changes, dementia and premature death. The disease is caused by an unstable expanded trinucleotide (CAG) repeat encoding a polyglutamine stretch in the IT15
gene for huntingtin, a protein of unknown function. Transgenic mice expressing exon one of the human HD gene with an expanded polyglutamine region develop many features of human HD. Exposure of these mice to an "enriched" environment delays the onset of motor disorders and slows disease progression [Nature 404 (2000) 721]. We have compared the levels of receptor binding of a range of basal ganglia neurotransmitter receptors believed to be important in HD, in normal mice and R6/1 transgenic HD mice housed in either enriched or standard laboratory environments. HD mice housed in a normal environment show a loss of cannabinoid CB1 and dopamine D1 and D2 receptors in the striatum and the corresponding output nuclei of the basal ganglia. HD mice exposed to an enriched environment show equivalent loss of D1 and D2 receptors as their "non-enriched" counterparts; in contrast, the "enriched" mice show significantly less depletion of CB1 receptors. In the brains of humans diagnosed with HD cannabinoid CB1 receptors are selectively lost from the basal ganglia output nuclei prior to the development of other identifiable neuropathology [Neuroscience 97 (2000) 505]. Our results therefore show that an enhanced environment slows the rate of loss of one of the first identifiable neurochemical deficits of HD. This suggests that delaying the loss of CB1 receptors, either by environmental stimulation or pharmacologically, may be beneficial in delaying disease progression in HD patients.


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-arachidonoyl 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.


RATIONALE. Experimental evidence from animal studies suggests reciprocal functional interactions between endogenous brain cannabinoid and opioid systems. There is recent evidence for a role of the opioid system in the modulation of the reinforcing effects of synthetic cannabinoid CB1 receptor agonists in rodents. Since Delta(9)-tetrahydrocannabinol (THC), the natural psychoactive ingredient in marijuana, is actively and persistently self-administered by squirrel monkeys, this provides an opportunity to directly study involvement of opioid systems in the reinforcing effects of THC in non-human primates. OBJECTIVES. To study the effects of naltrexone, an opioid antagonist, on THC self-administration behavior in squirrel monkeys. METHODS. Monkeys pressed a lever for intravenous injections of THC under a ten-response, fixed-ratio (FR) schedule with a 60-s time-out after each injection. Effects of pre-session treatment with naltrexone (0.03-0.3 mg/kg intramuscularly, 15 min before session) for 5 consecutive days on self-administration of different doses of THC (2-8 micro g/kg per injection) were studied. RESULTS. Self-administration responding for THC was significantly reduced by pretreatment with 0.1 mg/kg naltrexone for five consecutive daily sessions. Naltrexone pretreatment had no significant effect on cocaine self-administration responding under identical conditions. CONCLUSIONS. Self-administration behavior under a fixed-ratio schedule of
intravenous THC injection was markedly reduced by daily pre-session treatment with naltrexone, but remained above saline self-administration levels. These findings demonstrate for the first time the modulation of the reinforcing effects of THC by an opioid antagonist in a non-human primate model of marijuana abuse.


Systemic or intraventricular administration of cannabinoids causes analgesic effects, but relatively little is known for their cellular mechanism. Using brainstem slices with the mandibular nerve attached, we examined the effect of cannabinoids on glutamatergic transmission in superficial trigeminal caudal nucleus of juvenile rats. Exogenous cannabinoid receptor agonist WIN 55,212-2 (WIN), as well as the endogenous agonist anandamide, hyperpolarized trigeminal caudal neurons and depressed the amplitude of excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) monosynaptically evoked by stimulating mandibular nerves in a concentration-dependent manner. The inhibitory action of WIN was blocked or fully reversed by the CB1 receptor antagonist SR 141716A. WIN had no effect on the amplitude of miniature excitatory postsynaptic currents (mEPSCs) recorded in the presence of tetrodotoxin or cadmium. The inhibitory effect of WIN on EPSCs was greater for those with longer synaptic latency, suggesting that cannabinoids have a stronger effect on C- fibre EPSPs than on Ad-fibre EPSPs. Ba2+ (100 micro M) blocked the hyperpolarizing effect of cannabinoids, but did not affect their inhibitory effect on EPSPs. The N-type Ca2+ channel blocker omega-conotoxin GVIA (omega-CgTX) occluded the WIN-mediated presynaptic inhibition, whereas the P/Q-type Ca2+ channel blocker omega-agatoxin TK (omega-Aga) had no effect. These results suggest that cannabinoids preferentially activate CB1 receptors at the nerve terminal of small-diameter primary afferent fibres. Upon activation, CB1 receptors may selectively inhibit presynaptic N-type Ca2+ channels and exocytotic release machinery, thereby attenuating the transmitter release at the trigeminal nociceptive synapses.


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 [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.


Cannabinoids, as a result of their ability to activate cannabinoid CB(1) receptors, have been shown to possess neuroprotective properties in vivo. In vitro studies into neuroprotective effects mediated by CB(1) receptors have in general used primary neuronal cultures derived from embryonic rodents. In the present study, we have investigated whether embryonic chick telencephalon primary cultures in serum-free medium are a useful alternative for such in vitro studies. The CB agonist CP 55940 reduced the cAMP response to 5 microM forskolin by 40 and 50% at concentrations of 3 nM and 30 nM, respectively. This reduction was blocked by the CB(1) receptor antagonist AM251, indicating the presence of functional CB(1) receptors in the cultures. Incubation of the cultures with glutamate (100 microM or 1 mM) for 1 h followed by medium
change and incubation for 24 h produced a release of the cytoplasmic enzyme lactate dehydrogenase into the medium. This release was prevented by MK-801 confirming the central role of NMDA receptors in the glutamate toxicity. However, 3-30 nM CP 55940 did not produce any neuroprotection in this model regardless as to whether dibutyryl cyclic AMP was added to the culture medium. The endocannabinoid anandamide was also without effect when added either per se or together with the related N-acyl ethanolamines palmitoylethanolamide, oleoylethanolamide and stearoylethanolamide (at relative concentrations matching those seen in rat brain after excitotoxic insult). It is concluded that embryonic chick neurons in primary serum-free culture are not a useful model for the study of neuroprotective effects mediated by CB(1) receptors in vitro.


Recent evidence indicates that the basolateral amygdala (BLA) may be involved in behavioural effects induced by cannabinoids. High levels of CB1 cannabinoid receptors have been shown in this region, where they modulate excitatory and inhibitory synaptic transmission. However, the neurophysiological effects of these opposing synaptic actions have not been investigated in vivo. To this purpose, single-unit extracellular recordings were performed in urethane anaesthetized rats in order to determine whether exogenously applied cannabinoids influenced the spontaneous or evoked electrical activity of neurons in the BLA. The effects of cannabinoids were found to be dependent on the characteristics of the neurons examined and on the properties of the agents used. We tested and compared two structurally different synthetic cannabinoid receptor agonists, the highly potent HU-210 (0.125-1.0 mg/kg, i.v.) and WIN55212-2 (WIN, 0.125-1.0 mg/kg, i.v.). With a CB1 cannabinoid receptor-dependent mechanism, HU-210 potently inhibited the firing rate of BLA interneurons whereas WIN modulated the discharge rate in a biphasic manner. By contrast, BLA projection neurons, antidromically identified from the shell of the nucleus accumbens, were significantly inhibited by WIN at all doses tested, while HU-210 administration led to less consistent effects, since only 1.0 mg/kg inhibited firing rate in the majority of recorded neurons. Additionally, WIN, but not HU-210, significantly attenuated short-latency spiking activity in BLA projection neurons evoked by electrical stimulation of the medial prefrontal cortex. In these neurons, WIN-induced effects were antagonised by the non-selective cannabinoid receptor antagonist SR141716A and by the vanilloid receptor antagonist capsazepine, but not by the selective CB1 antagonist AM-251. Taken together, our findings indicate that the overall excitability of efferent neurons in the BLA is strongly reduced by WIN in a non-CB1-dependent manner. In this effect, the contribution of a novel cannabinoid-vanilloid-sensitive putative non-CB1 receptors, the existence of which was postulated in recent reports, might play a role.


Addictive drugs are thought to alter normal brain function and cause the remodeling of synaptic functions in areas important to memory and reward. Excitatory transmission to the nucleus accumbens (NAC) is involved in the actions of most drugs of abuse, including cannabis. We have explored the functions of the endocannabinoid system at the prefrontal cortex-NAC synapses. Immunocytochemistry showed cannabinoid receptor (CB1) expression on axonal terminals making contacts with NAC neurons. In NAC slices, synthetic cannabinoids inhibit spontaneous and evoked glutamate-mediated transmission through presynaptic activation of presynaptic K(+) channels and GABA-mediated transmission most likely via a direct presynaptic action on the vesicular release machinery. How does synaptic activity lead to the production of endogenous cannabinoids (eCBs) in the NAC? More generally, do eCBs participate in long-term synaptic plasticity in the brain? We found that tetanic stimulation (mimicking naturally occurring frequencies) of prelimbic glutamatergic afferents induced a presynaptic LTD dependent on eCB and CB1 receptors (eCB-LTD). Induction of eCB-LTD required postsynaptic activation of mGlu5 receptors and a rise in postsynaptic Ca(2+) from ryanodine-sensitive intracellular Ca(2+) stores. This retrograde signaling cascade involved postsynaptic eCB release and activation of
presynaptic CB1 receptors. In the NAc, eCB-LTD might be part of a negative feedback loop, reducing glutamatergic synaptic strength during sustained cortical activity. The fact that this new form of LTD was occluded by an exogenous cannabinoid suggested that cannabis derivatives, such as marijuana, may alter normal eCB-mediated synaptic plasticity. These data suggest a major role of the eCB system in long-term synaptic plasticity and give insights into how cannabis derivatives, such as marijuana, alter normal eCB functions in the brain reward system.


It has been recently shown that cannabinoids may regulate the growth of many cell types. In the present work we examined the effect of the anandamide analogue (R)-methanandamide (MET) on androgen-dependent prostate LNCaP cell growth. We found that 0.1 microM MET had a mitogenic effect measured by [(3)H]thymidine incorporation into DNA. The effect exerted by MET was blocked by the cannabinoid receptor antagonists SR141716 (SR1) and SR144528 (SR2) as well as by the phosphoinositide 3-kinase (PI3K) inhibitor LY294002, suggesting an involvement of cannabinoid receptors and the PI3K pathway in the mechanism of MET action. MET treatment of LNCaP cells also induced an up-regulation of androgen receptor expression that was blocked by the two cannabinoid receptor antagonists SR1 and SR2. These results show for the first time that cannabinoids may modify androgen receptor expression in an androgen-dependent cell line and by this mechanism could regulate prostate cell growth.


SUMMARY: Psychopathological disorders, and depression in particular, are strongly linked to eating attitude in obese patients. The identification of cannabinoid CB1 receptors (CB1Rs) in areas of the central nervous system (CNS) that have been implicated in regulation of mood and food intake suggests that these receptors may mediate such a behavioral link. The goal of this study was to evaluate CB1R modulation of antidepressant-like effects and food intake. For this purpose, 129/SVE and C57BL/6 male mice were acutely dosed intraperitoneally (i.p.) with the CB1R inverse agonist AM251 (3-30 mg/kg) and tested, respectively, in the tail-suspension test (TST) and in the forced-swim test (FST), which have been used widely as tests sensitive to antidepressant compounds. Like the antidepressant desipramine (DMI, 16 mg/kg), AM251 significantly reduced immobility at 10 mg/kg in the TST and at 1 and 10 mg/kg in the FST. Such a decrease of immobility was not accompanied by an increase in motor activity in the open field, suggesting that occupancy of CB1R by AM251 induced antidepressant-like effects. This was supported by two additional experiments. First, the co-administration of the CB1R agonist CP55940, at a dose that did not induce motor impairment or profound hypothermia (0.01 mg/kg), reversed effects of AM251 in the TST. Secondly, effects of AM251 in the FST were absent in CB1R knockout (KO) mice. In addition to an antidepressant-like effect, AM251 reduced fasting-induced hyperphagia over a comparable dose range. Taken together, these data suggest that regulation of mood and food intake might be obtained through inverse agonism of CB1R.


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 trophoderm and anandamide (N-arachidonylethanolamine), 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 Ca2+ 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 Ca2+ channels, and renders the blastocyst competent for implantation in the receptive uterus. In contrast, anandamide at a higher concentration (28 nM) inhibits Ca2+ 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.


The purpose of this study was to investigate the effects of the endogenous cannabinoid arachidonoyl-ethanolamide, anandamide (AEA), on the activity of the hypothalamo-pituitary-adrenal (HPA) axis in cannabinoid receptor (CB(1) receptor) inactivated (KO) mice. A low dose (0.01 mg/kg i.p.) of AEA significantly increased plasma corticotropin (ACTH) and corticosterone concentrations in both wild-type (+/+) and in mutant (-/-) animals. In each case, hormone levels reached their peaks at 90 min after AEA administration. In a parallel experiment, AEA administration was preceded by the injection of SR 141716A (1.0 mg/kg), a selective and potent CB(1) receptor antagonist, or of capsaicin (5.0 mg/kg), a potent vanilloid receptor of type 1 (VR1) antagonist. The latter drugs did not prevent the effects of AEA on the HPA axis. Using Fos protein immunohistochemistry, we observed that the parvo cellular part of the hypothalamic paraventricular nucleus (PVN) was activated as early as 45 min after AEA injection and reached peak levels after 60 min in both +/+ and -/- mice. Furthermore, the CB(1) and VR1 receptor antagonists did not block the effects of AEA on Fos immunoreactivity. The results strongly support the view that activation of the HPA axis produced by AEA possibly occurs via a currently unknown (CB(x)) cannabinoid receptor present in PVN.


The purpose of the present study was to evaluate the effect of a single intraperitoneal injection of a stable analogue of endogenous cannabinoid anandamide - R-(+)-methanandamide (2.5 mg/kg) and CP 55,940 (0.25 mg/kg), an exogenous CB1 receptor-agonist, on the calcitonin (CT) immunoreactivity of the thyroid parafollicular (C) cells. Four hours after injection with both cannabinoids CT immunoreactivity, evaluated with an avidin-biotin peroxidase complex method by means of rabbit antibodies against CT, was seen to be enhanced in the parafollicular cells in comparison to those of the control group. In thyroids taken from cannabinoid-treated rats the majority of follicles, particularly those located peripherally were large in size, and had low epithelium. Moreover, dilatation of the blood vessels was observed. These changes were accompanied by a significant decrease in CT plasma level, without changes in calcium concentrations. This is the first evidence that a single injection of the cannabinoids R-(+)-methanandamide and CP 55,940 significantly decreases the activity of thyroid C cells.


We have previously shown that behavioral changes induced by cannabinoid were due to an elevation of prostaglandin E2 (PGE2) via the arachidonic acid cascade in the brain. In the present study, we investigated the participation of the prostanoid EP3 receptor, the target of PGE2 in the brain, in behavioral suppression induced by Delta8-tetrahydrocannabinol (Delta8-THC), an isomer of the naturally occurring Delta9-THC, using a one-lever operant task in rats. Intraperitoneal administration of Delta8-THC inhibited the lever-pressing behavior, which was significantly antagonized by both the selective cannabinoid CB1 receptor antagonist SR141716A and the cyclooxygenase inhibitor diclofenac. Furthermore, intracerebroventricular (i.c.v.) administration of PGE2 significantly inhibited the lever-pressing performance similar to Delta8-
THC. Prostanoid EP3 receptor antisense-oligodeoxynucleotide (AS-ODN; twice a day for 3 days, i.c.v.) significantly decreased prostanoid EP3 receptor mRNA levels as determined by the RT-PCR analysis in the cerebral cortex, hippocampus and midbrain. AS-ODN also antagonized the PGE2-induced suppression of the lever pressing. In the same way, the suppression of lever-pressing behavior by Delta8-THC was significantly improved by AS-ODN. It is concluded that the suppression of lever-pressing behavior by cannabinoid is due to activation of the prostanoid EP3 receptor through an elevation of PGE2 in the brain.

**CLINICAL SCIENCE**


In most countries Cannabis is the most widely used illegal drug. Its use during pregnancy in developed nations is estimated to be approximately 10%. Recent evidence suggests that the endogenous cannabinoid system, now consisting of two receptors and multiple endocannabinoid ligands, may also play an important role in the maintenance and regulation of early pregnancy and fertility. The purpose of this review is therefore twofold, to examine the impact that cannabis use may have on fertility and reproduction, and to review the potential role of the endocannabinoid system in hormonal regulation, embryo implantation and maintenance of pregnancy.

**BEHAVIOURAL SCIENCE**


AIMS: To examine the relationship between smoking tobacco and cannabis use among smokers in their mid-to-late teens. DESIGN AND PARTICIPANTS: Two qualitative studies in Scotland. One study used semi-structured paired interviews involving 99 16-19-year-old smokers, the other comprised eight focus groups involving 46 15-16-year-old smokers. MEASUREMENT: The interviews and focus groups explored the role and meaning of smoking in the participants' lives, smoking histories and future cessation intentions and how these related to other aspects of their lives, particularly cannabis use. FINDINGS: Cannabis use was regarded as an important and enjoyable aspect of many of the participants' lives. Importantly, cannabis use and cigarette smoking were linked inextricably. Several reported how smoking joints had been a ‘gateway’ to smoking cigarettes. While most wanted to quit smoking cigarettes, cannabis use reinforced their cigarette smoking and few wanted to stop using cannabis. CONCLUSION: National studies need to be conducted to examine how widespread the problem identified is and tobacco control initiatives and smoking cessation treatment services need to consider urgently how to overcome the barrier that a desire on the part of young people to continue cannabis smoking poses to achieving a reduction in tobacco use.


AIMS: To examine the relationship between cannabis use in adolescence/young adulthood and levels of educational attainment. DESIGN: Data were gathered over the course of a 25-year longitudinal study of a birth cohort of 1265 New Zealand children. MEASUREMENTS: Measures analysed included (a) frequency of cannabis use in adolescence and young adulthood (15-25 years); (b) levels of educational achievement to age 25 years; and (c) social, family and individual characteristics assessed prior to age 16. FINDINGS: Increasing cannabis use was associated with increasing risks of leaving school without qualifications, failure to enter university and failure to obtain a university degree. The association between cannabis use and leaving school without qualifications persisted after control for confounding factors. When due allowance was made for pre-existing levels of cannabis use there was no evidence to suggest the presence
of reverse causal pathways in which lower educational achievement led to increased cannabis use. CONCLUSIONS: Findings support the view that cannabis use may act to decrease educational achievement in young people. It is likely that this reflects the effects of the social context within which cannabis is used rather than any direct effect of cannabis on cognitive ability or motivation.


Although there has been considerable research into the adverse effects of cannabis, less attention has been directed toward subjective effects that may be associated with ongoing cannabis use. Examination of self-reported cannabis effects is an important issue in understanding the widespread use of cannabis. While reviews have identified euphoria as a primary factor in maintaining cannabis use, relaxation is the effect reported most commonly in naturalistic studies of cannabis users, irrespective of the method used. Self-reported effects in 12 naturalistic and 18 laboratory studies were compared. Regardless of methodology there was considerable variation in the effects experienced. Variation has been reported in terms of opposite effects being experienced by different individuals, variation of effects by individuals within a single occasion and between occasions of use. Factors that might explain this variation are outlined. Limitations of the available literature and suggested directions for future research are discussed. [Green B, Kavanagh D, Young R. Being stoned: a review of self-reported cannabis effects. Drug Alcohol Rev 2003;22:453 - 460] 


BACKGROUND: Although cannabis is the most widely used illicit drug in the United States, few recent American studies have examined the attributes of long-term heavy cannabis users. METHOD: Using a case-control design, we obtained psychological and demographic measures on 108 individuals, age 30-55, who had smoked cannabis a mean of 18000 times and a minimum of 5000 times in their lives. We compared these heavy users to 72 age-matched control subjects who had smoked at least once, but no more than 50 times in their lives. RESULTS: We found no significant differences between the two groups on reported levels of income and education in their families of origin. However, the heavy users themselves reported significantly lower educational attainment (P < 0.001) and income (P = 0.003) than the controls, even after adjustment for a large number of potentially confounding variables. When asked to rate the subjective effects of cannabis on their cognition, memory, career, social life, physical health and mental health, large majorities of heavy users (66-90%) reported a 'negative effect'. On several measures of quality of life, heavy users also reported significantly lower levels of satisfaction than controls. CONCLUSION: Both objective and self-report measures suggest numerous negative features associated with long-term heavy cannabis use. Thus, it seems important to understand why heavy users continue to smoke regularly for years, despite acknowledging these negative effects. Such an understanding may guide the development of strategies to treat cannabis dependence.

