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BASIC SCIENCE

Addiction

Since the discovery of the cannabinoid CB1 receptor (CB1R) in 1988, and subsequently of the CB2 receptor (CB2R) in 1993, there has been an exponential growth of research investigating the functions of the endocannabinoid system. The roles of CB1Rs have been of particular interest to behavioral pharmacologists because of their selective presence within the
central nervous system (CNS) and because of their association with brain-reward circuits involving mesocorticolimbic dopamine systems. One potential role that has become of considerable recent focus is the ability of CB1Rs to modulate the effects of drugs of abuse. Many drugs of abuse elevate dopamine levels, and the ability of CB1R antagonists or inverse agonists to attenuate these elevations has suggested their potential application as pharmacotherapies for treating drug abuse disorders. With the identification of the selective CB1R antagonist, SR141716, in 1994, and its subsequent widespread availability, there has been a rapid expansion of research investigating its ability to modulate the effects of drugs of abuse. The preliminary clinical reports of its success in retarding relapse in tobacco users have accelerated this expansion. This report critically reviews preclinical and clinical studies involving the ability of CB1R antagonists to attenuate the effects of drugs of abuse, while providing an overview of the neuroanatomical and neurochemical points of contact between the endocannabinoid system and systems mediating abuse-related effects.


The endocannabinoid system and the cannabinoid CB(1) receptors are involved in the development of ethanol tolerance and dependence. This study aimed to investigate the in vivo sensitivity of a CB(1) receptor agonist (WIN 55,212-2) modulating the synthesis of 3,4-dihydroxyphenylalanine/dopamine/noradrenaline (DA/NA) and that of 5-hydroxytryptophan/serotonin (5-HTP/5-HT) in rat brain after ethanol treatment and withdrawal. In control rats, WIN 55,212-2 (4mg/kg, i.p., for 1h), through a mechanism sensible to the CB(1) antagonist SR 141716A, increased the synthesis of DA/NA in a slice of brainstem containing the locus ceruleus (250%) and in the hippocampus (64%), and it reduced DA/NA synthesis in the striatum (47%). WIN 55,212-2 also decreased the synthesis of 5-HTP/5-HT in the locus ceruleus (43%), hippocampus (35%) and striatum (35%). In the locus ceruleus of ethanol-treated rats, the stimulatory effect of WIN 55,212-2 on DA/NA synthesis was abolished (acute treatment) or markedly attenuated (53-55%, chronic treatment and withdrawal), whereas in the hippocampus this effect was reduced only in chronic ethanol-withdrawn rats (33%). In the striatum of ethanol-treated rats (acute, chronic and withdrawal), the inhibitory effect of WIN 55,212-2 on DA/NA synthesis was completely blunted or markedly reduced. Similarly, the inhibitory effect of WIN 55,212-2 on 5-HTP/5-HT synthesis was reduced or abolished in the three brain regions after chronic ethanol and during withdrawal. These results indicate that treatment with ethanol in rats induces a functional desensitization of CB(1) receptors modulating the synthesis of brain monoamines.


The present study examined the effect of chronic exposure to Delta(9)-tetrahydrocannabinol (THC) on heroin-induced locomotor sensitisation and Fos-immunoreactivity (Fos-IR). Adult male albino Wistar rats (n=60) were injected intraperitoneally (i.p.) 21 times with vehicle, 0.05, 0.5, or 5.0mg/kg THC (once every 48h for 41 days). Locomotor activity was assessed for 180min on pre-exposure days 1, 21, and 41. Following a 2-week washout period, rats were divided into five equal groups (n=12) and injected subcutaneously (s.c.) with vehicle or heroin (0.5mg/kg). Locomotor activity was recorded for 240min. In drug-naive rats, heroin significantly increased locomotor activity. THC pre-exposure further increased heroin-induced locomotion. After an interval of 2 weeks, rats pre-exposed to vehicle and 5.0mg/kg THC in the first part of the experiment were randomly assigned to one of four treatment groups (n=6) and injected s.c. with vehicle or 0.5mg/kg heroin and perfused 2h later. Fos-IR was examined in several brain regions. Acute heroin increased Fos-IR in drug-naive rats in the caudate-putamen (CPu; central, medial and dorsomedial regions), nucleus accumbens (NAC; core and shell regions), bed nucleus of the stria terminalis (BNST), lateral septum, central nucleus of the amygdala (CEA), periaqueductal grey (PAG; dorsolateral, dorsomedial, and lateral), and the Edinger-Westphal nucleus. Pre-exposure to THC significantly increased heroin-induced Fos-IR in the dorsomedial CPu and the NAC (core). Conversely, THC pre-exposure reduced heroin-induced Fos-IR in the BNST, CEA, and the PAG (dorsolateral and laterall). The present study
demonstrates that THC pre-exposure increases the locomotor stimulating effects of heroin and provides new evidence for the neural correlates that may underlie cannabinoid and opioid cross-sensitisation.

Thanos, P. K., E. S. Dimitrakakis, et al. (2005). "Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors." Behav Brain Res.

Cannabinoids are postulated to play a role in modulating the reinforcing effects of abused drugs, including alcohol. Experiment 1 examined alcohol self-administration in cannabinoid CB1 receptor knockout (KO), heterozygous (HT) and wild type (WT) mice in a two-bottle choice paradigm. Mice were trained in a limited 8haccess/day to 10% (v/v) ETOH (ETOH) versus water. After baseline drinking levels (% ETOH preference and total ETOH intake (g/kg)), results indicated that the CB1 knockout mice displayed significantly lower baseline ETOH consumption compared to wild type mice. Subsequently, treatment with SR141716A (5mg/kg) significantly attenuated ETOH intake in the WT and HT mice but had little effect on the knockout mice. Experiment 2 examined the CB1 WT and CB1 KO strains in a conditioned place preference (CPP) procedure between saline and 2g/kg ETOH. The CB1 WT mice spent significantly more time in the ETOH-paired versus saline-paired chambers, whereas no significant preference was observed in the CB1 KO mice. Finally, we observed that CB1 KO mice were significantly lighter than WT and HT and that SR141716A did not significantly alter body weight. These results demonstrate that the cannabinoid CB1 receptor is an essential component of the molecular pathways underlying the reinforcing effects of alcohol. Thus, medications targeting the CB1 receptors may be beneficial for the treatment of alcoholism.


The present article focuses on psychoneuroendocrine effects of cannabinoids in developing animals, with special emphasis on the perinatal, periweanling and periadolescent periods. We describe and discuss published data dealing with acute and long-term effects of exposure to cannabinoid agonists in such critical periods. Human studies have demonstrated that the consumption of marijuana by women during pregnancy affects the neurobehavioural development of their children. Investigations using animal models provide useful information for a better understanding of the long-lasting deleterious consequences of cannabis exposure during pregnancy and lactation. The increasing use of cannabis among adolescents and its associated public health problems have led to a parallel increase in basic research on appropriate animal models. Chronic administration of cannabinoid agonists during the periadolescent period causes persistent behavioural alterations in adult animals. Some of these alterations may be related to a possible increased risk of psychosis and other neuropsychiatric disorders in early onset cannabis users.

**Cardiovascular**


The rate and strength of heart contractions decreased after 10-min perfusion of rat myocardium with Krebs-Henseleit solution containing a selective cannabinoid receptor agonist HU-210 in a final concentration of 10 nM. HU-210 completely blocked the positive inotropic and chronotropic effect of beta-adrenoceptor agonist isoproterenol, decreased the basal level of cAMP, and abolished the isoproterenol-induced increase in myocardial cAMP concentration. cGMP concentration remained unchanged under these conditions. The decrease in myocardial cAMP concentration after activation of cannabinoid receptors did not correlate with changes in the strength and rate of heart contractions. Our results suggest that the negative inotropic and chronotropic effects of HU-210 are not associated with decreased cAMP concentration in the myocardium.

Although the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide share a similar pharmacology, 2-AG reportedly limits myocardial ischaemia-reperfusion injury whereas anandamide does not. We therefore investigated whether or not anandamide reduces infarct size and which, if any, of the known cannabinoid-signalling pathways are involved. Rat isolated perfused hearts were subjected to global, no-flow ischaemia (30 min) and reperfusion (1 h). Agonists were present from 5 min before ischaemia until the end of reperfusion. Antagonists, where used, were present throughout the protocol. Recovery of left ventricular developed pressure and coronary flow was incomplete in control hearts and not significantly affected by any drug treatment. In vehicle-treated hearts, 26±3% (n=13) of the left ventricle was infarcted at the end of reperfusion. Infarction of the left ventricle was significantly reduced after 1 μM anandamide (10±3%, n=7) or 1 μM methanandamide (12±4%, n=6) but not 1 μM HU210. Neither ACPA (1 μM; CB(1) receptor agonist) nor JWH133 (1 μM; CB(2) receptor agonist), individually or combined significantly affected infarct size. Anandamide (1 μM) did not reduce infarct size in the presence of the CB(1) receptor antagonist rimonabant (SR141716A, 1 μM) or the CB(2) receptor antagonist, SR144528 (1 μM). Despite sensitivity to CB(1) and CB(2) receptor antagonists, the infarct-limiting action of anandamide was not mimicked by agonists selective for CB(1) or CB(2) receptors suggesting the involvement of a novel cannabinoid site of action. British Journal of Pharmacology advance online publication, 12 September 2005; doi:10.1038/sj.bjp.0706391.


This study was designed to test the hypothesis that increased sensitivity of blood pressure to anandamide (AEA), an endocannabinoid compound, occurs during high-salt intake, which can be blocked by a selective vanilloid receptor 1 (VR1) antagonist, capsazepine (CAPZ). Intravenous administration of a metabolically stable analog, methanandamide (MethA), dose-dependently decreased mean arterial pressure (MAP) in conscious rats fed a high-sodium diet (HS) for 3 weeks but it had a minimal effect in normal sodium (NS)-treated rats. The MethA-induced decrease in MAP was significantly attenuated but not abolished by CAPZ, or a selective cannabinoid receptor 1 (CB1) antagonist, SR141716A, administered separately in HS-treated rats. The MethA-induced depressor effect was prevented by the combined administration of CAPZ and SR141716A in HS-treated rats. Likewise, administration of capsaicin, a selective VR1 receptor agonist, dose-dependently decreased MAP in both HS- and NS-treated rats. The depressor effect of capsaicin was more profound in HS-treated rats, which was prevented by CAPZ. Western blot showed that expression of VR1 but not CB1 in mesenteric arteries was increased in HS-treated compared with NS-treated rats. Therefore, these data show that: (1) HS upregulates mesenteric VR1 expression; (2) HS increases sensitivity of blood pressure to AEA; and (3) HS-induced enhancement of the depressor effect of AEA can be prevented only when both VR1 and CB1 receptors are blocked. These results indicate that AEA contributes to the prevention of salt induced increases in blood pressure via, at least in part, activating the VR1 receptor.

Endocrinology


Obesity is the main risk factor for the development of type 2 diabetes. Activation of the central endocannabinoid system increases food intake and promotes weight gain. Blockade of the cannabinoid type 1 (CB-1) receptor reduces body weight in animals by central and peripheral actions; the role of the peripheral endocannabinoid system in human obesity is now being extensively investigated. We measured circulating endocannabinoid concentrations and studied the expression of CB-1 and the main degrading enzyme, fatty acid amid hydrolase (FAAH), in adipose tissue of lean (n = 20) and obese (n = 20) women and after a 5% weight loss in a second group of women (n = 17). Circulating levels of anandamide and 1/2-arachidonoylglycerol were
increased by 35 and 52% in obese compared with lean women (P < 0.05). Adipose tissue mRNA levels were reduced by -34% for CB-1 and -59% for FAAH in obese subjects (P < 0.05). A strong negative correlation was found between FAAH expression in adipose tissue and circulating endocannabinoids. Circulating endocannabinoids and CB-1 or FAAH expression were not affected by 5% weight loss. The expression of CB-1 and FAAH was increased in mature human adipocytes compared with in preadipocytes and was found in several human tissues. Our findings support the presence of a peripheral endocannabinoid system that is upregulated in human obesity.


The discovery of cannabinoid receptors, together with the development of selective cannabinoid receptor antagonists, has encouraged a resurgence of cannabinoid pharmacology. With the identification of endogenous agonists, such as anandamide, scientists have sought to uncover the biological role of endocannabinoid systems; initially guided by the long-established actions of cannabis and exogenous cannabinoids such as Delta-tetrahydrocannabinol (THC). In particular, considerable research has examined endocannabinoid involvement in appetite, eating behaviour and body weight regulation. It is now confirmed that endocannabinoids, acting at brain CB1 cannabinoid receptors, stimulate appetite and ingestive behaviours, partly through interactions with more established orexigenic and anorexigenic signals. Key structures such as the nucleus accumbens and hypothalamic nuclei are sensitive sites for the hyperphagic actions of these substances, and endocannabinoid activity in these regions varies in relation to nutritional status and feeding expression. Behavioural studies indicate that endocannabinoids increase eating motivation by enhancing the incentive salience and hedonic evaluation of ingesta. Moreover, there is strong evidence of an endocannabinoid role in energy metabolism and fuel storage. Recent developments point to potential clinical benefits of cannabinoid receptor antagonists in the management of obesity, and of agonists in the treatment of other disorders of eating and body weight regulation.


The present work studied the long-term effects of chronic perinatal manipulation of cannabinoid CB1 receptors in male and female rats. Perinatal activation of cannabinoid CB1 receptors by chronic administration of Delta-tetrahydrocannabinol at different doses (0.1, 0.5, 2 mg/kg, p.o.) induced sexually dimorphic behavioural changes in adulthood, altering habituation of locomotion, immobility and exploratory activity. These behavioural effects were also accompanied by alterations in corticosterone levels in the adult period. Prenatal blockade of CB1 receptors by chronic administration of 3 mg/kg (s.c.) of SR141716A decreased immobility behaviour in male and female animals, without any significant changes in corticosterone plasma levels. Cannabinoid CB1 receptors appear to play an important role in the ontogeny of psychomotor behaviours, and activation or blockade of these receptors during stages of plasticity, such as the prenatal or perinatal periods, can induce long-term effects, as shown by sexually dimorphic changes in behavioural patterns in adulthood.

Gastrointestinal


Aims: Endocannabinoids are endogenous compounds that bind to the same receptors as tetrahydrocannabinol, the active component in marijuana and hashish. They have been found to have many physiological and patho-physiological functions, including mood alteration, control of feeding and appetite, motor and co-ordination activities, analgesia, immune modulation and gut motility. In this review we aim to elucidate current knowledge as to their role in liver physiology and disease. Methods: The major findings published to date concerning endocannabinoids and liver disease are described, and their implications with regard to understanding disease
mechanisms, and the development of new treatments is considered. Results: Recently, endocannabinoids have been implicated in the hemodynamic alterations occurring in cirrhosis. These changes appear to be mediated via specific cannabinoid receptors (CB1) on splanchnic and hepatic vascular endothelium. Plasma levels of endocannabinoids also seem to be elevated in hepatitis, and are involved in apoptosis of hepatocytes by a membrane mechanism not related to a specific receptor. Other studies suggest a beneficial role for cannabinoids in reducing the inflammation of experimental hepatitis. In an animal model of acute hepatic failure, both endocannabinoids and the antagonist to the CB1 receptor have been found to have a beneficial effect on neurological and cognitive function. Conclusions: Endocannabinoids appear to be involved in several aspects of acute and chronic liver disease, including vascular changes, modulation of inflammatory process and neurological function. Further research may provide new insights into the pathophysiology of liver disease, as well as a basis for novel treatment modalities.


The cannabinoid receptor agonists Delta-tetrahydrocannabinol (Delta-THC) and HU-210 were compared in terms of their effects on: (1) progressive ratio (PR) responding for food, and (2) free food intake. In the first experiment, food-deprived Wistar rats were trained on a time-constrained (60 min) PR-5 schedule for food reinforcement, in which the response requirement incremented by five lever presses for each successive reinforcer. One group of rats received vehicle, 0.5, 1 or 3 mg/kg Delta-THC (i.p.), and three other groups received HU-210 (i.p.) at three different dose ranges, spanning 0.001-0.1 mg/kg. In the second experiment, the effects of the two drugs on free food intake were tested in a separate group of non-deprived rats. For PR responding, Delta-THC significantly increased the break point (final ratio completed) and the total number of lever presses emitted. The same drug also significantly increased free food intake. However, the effects of HU-210 were quite different: it did not alter PR responding at any dose; instead, its only significant effect was to reduce free food intake at 0.06 mg/kg. These data suggest that increased motivation to obtain food might underlie the hyperphagic effects of Delta-THC. However, the synthetic agonist HU-210 has different effects: it only acts to reduce feeding behaviour, an outcome that probably reflects non-specific behavioural disruption. These findings suggest important differences between the two CB1 receptor agonists in terms of their pharmacological effects.


Numerous investigations have recently demonstrated the important roles of the endocannabinoid system in the gastrointestinal (GI) tract under physiological and pathophysiological conditions. In the GI tract, cannabinoid type 1 (CB1) receptors are present in neurons of the enteric nervous system and in sensory terminals of vagal and spinal neurons, while cannabinoid type 2 receptors are located in immune cells. Activation of CB1 receptors was shown to modulate several functions in the GI tract, including gastric secretion, gastric emptying and intestinal motility. Under pathophysiological conditions induced experimentally in rodents, the endocannabinoid system conveys protection to the GI tract (e.g. from inflammation and abnormally high gastric and enteric secretions). Such protective activities are largely in agreement with anecdotal reports from folk medicine on the use of Cannabis sativa extracts by subjects suffering from various GI disorders. Thus, the endocannabinoid system may serve as a potentially promising therapeutic target against different GI disorders, including frankly inflammatory bowel diseases (e.g. Crohn's disease), functional bowel diseases (e.g. irritable bowel syndrome) and secretion- and motility-related disorders. As stimulation of this modulatory system by CB1 receptor agonists can lead to unwanted psychotropic side effects, an alternative and promising avenue for therapeutic applications resides in the treatment with CB1 receptor agonists that are unable to cross the blood-brain barrier, or with compounds that inhibit the degradation of endogenous ligands (endocannabinoids) of CB1 receptors, hence prolonging the activity of the endocannabinoid system.

It has recently been recognized that anandamide (arachidonylethanolamide), which is an endogenous-cannabinoid (endocannabinoid), mediates septic shock. Cannabinoid means a mind-active material in cannabis (marijuana). Anandamide is mainly produced by macrophages. Cannabinoid 1 (CB1) receptor, which is one of the cannabinoid receptors, is also known to mediate hypotensive shock. The role of endocannabinoids in the progression of acute pancreatitis is unclear. The aims of this study are to clarify their relationship and to find a new therapeutic strategy by regulating the endocannabinoid signaling in acute pancreatitis. Male Wistar rats were injected with caerulein intravenously to induce mild edematous pancreatitis or injected with 5% sodium taurocholate to the bilio-pancreatic duct to induce severe necrotizing pancreatitis. The animals in the latter group were also injected with a CB1 receptor antagonist, AM251, or vehicle solution to see if the inhibition of endocannabinoids improves their survival. Plasma anandamide level was measured by the liquid chromatography/tandem mass spectrometry method. In both models of acute pancreatitis, the plasma anandamide levels were increased, and the levels were significantly higher in rats with severe necrotizing pancreatitis than those in rats with mild edematous pancreatitis. The mean arterial pressure and survival rate were significantly improved by the treatment with AM251, despite that the local inflammatory changes in the pancreas and various parameters (white blood cells, hematocrit, serum amylase, and serum interleukin-6) were similar. This is the first report to show that endocannabinoids are involved in the deterioration of acute pancreatitis and that the down-regulation of endocannabinoid signaling may be a new therapeutic strategy for severe acute pancreatitis.


Endogenous cannabinoids acting at CB1 receptors stimulate appetite, and CB1 antagonists show promise in the treatment of obesity. CB1−/− mice are resistant to diet-induced obesity even though their caloric intake is similar to that of wild-type mice, suggesting that endocannabinoids also regulate fat metabolism. Here, we investigated the possible role of endocannabinoids in the regulation of hepatic lipogenesis. Activation of CB1 in mice increases the hepatic gene expression of the lipogenic transcription factor SREBP-1c and its targets acetyl-CoA carboxylase-1 and fatty acid synthase (FAS). Treatment with a CB1 agonist also increases de novo fatty acid synthesis in the liver or in isolated hepatocytes, which express CB1. High-fat diet increases hepatic levels of the endocannabinoid anandamide (arachidonoyl ethanolamide), CB1 density, and basal rates of fatty acid synthesis, and the latter is reduced by CB1 blockade. In the hypothalamus, where FAS inhibitors elicit anorexia, SREBP-1c and FAS expression are similarly affected by CB1 ligands. We conclude that anandamide acting at hepatic CB1 contributes to diet-induced obesity and that the FAS pathway may be a common molecular target for central appetitive and peripheral metabolic regulation.


Abstract Cannabinoid receptors and the endocannabinoids anandamide and 2-arachidonoylglycerol have been suggested to regulate food intake in several animal phyla. Orthologs of the mammalian cannabinoid CB(1) and CB(2) receptors have been identified in fish. We investigated the presence of this endocannabinoid system in the brain of the goldfish Carassius auratus and its role in food consumption. CB(1)-like immunoreactivity was distributed throughout the goldfish brain. The prosencephalon showed strong CB(1)-like immunoreactivity in the telencephalon and the inferior lobes of the posterior hypothalamus. Endocannabinoids were detected in all brain regions of C. auratus and an anandamide-hydrolysing enzymatic activity with features similar to those of mammalian fatty acid amide hydrolase was found. Food deprivation for 24 h was accompanied by a significant increase of anandamide, but not 2-arachidonoylglycerol, levels only in the telencephalon. Anandamide caused a dose-dependent effect on food intake within 2 h of intraperitoneal administration to satiated fish and significantly enhanced or reduced food intake at low (1 pg/g body weight) or intermediate (10 pg/g) doses,
respectively, the highest dose tested (100 pg/g) being inactive. We suggest that endocannabinoids might variously contribute to adaptive responses to food shortage in fish.

Ward, S. J. and L. A. Dykstra (2005). "The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940)." Behav Pharmacol 16(5-6): 381-8.

It is well established that Cannabis sativa can increase appetite, particularly for sweet and palatable foods. In laboratory animals, cannabinoid CB1 receptor antagonism decreases motivation for palatable foods, and most recently, the CB1 receptor antagonist SR141716A, or rimonabant (Acomplia), was reported to produce weight loss in obese human subjects. Indeed, the endocannabinoid system plays a select role in the rewarding properties of palatable foods, and this is well characterized in laboratory animals with sweet sucrose solutions. In the present study, CB1 knockout mice (CB1 KO) and wild-type littermate mice (WT) were trained to respond for a complex sweet as well as a pure fat reinforcer under a progressive ratio (PR) schedule, to determine whether motivation to consume different palatable foods is tonically regulated by CB1 receptors. To assess sweet reinforcement, several concentrations of the liquid nutritional drink, Ensure, were presented under the PR schedule. For fat reinforcement, several concentrations of corn oil (emulsified in 3% xanthan gum) were made available. Additionally, to compare the result of genetic invalidation of the CB1 receptor to antagonism of the CB1 receptor system, the effect of SR141716A (3.0 mg/kg) on responding for Ensure and corn oil were also assessed using the PR schedule. We also assessed the effect of the CB1 agonist CP-55940 (30 μg/kg) on responding for Ensure and corn oil. CB1 KOs took significantly longer to acquire operant responding maintained by Ensure, and responding for Ensure under the PR schedule was significantly reduced in CB1 KOs as well as in WTs pretreated with SR141716A, as compared to WT controls. Additionally, pretreatment with the CB1 agonist CP-55940 increased responding for Ensure. In contrast, responding for corn oil during acquisition and under the PR schedule was not significantly different in CB1 KOs versus wild-type mice. However, SR141716A did reduce responding for corn oil in WTs, and CP-55940 significantly increased responding for corn oil. Taken together, these results suggest that CB1 receptors are preferentially involved in the reinforcing effects of a complex sweet, as compared to a pure fat, reinforcer. These data also suggest, however, that antagonism of CB1 receptors with SR141716A is sufficient to attenuate the reinforcing effect of Ensure and corn oil, while activation of the central CB1 system is sufficient to enhance Ensure and corn oil reinforcement.

**Immunology**


Several linear fatty acid dopamides (N-acyldopamines) have been identified recently in the brain. Among them, N-arachidonoyldopamine (NADA) is an endogenous lipid mediator sharing endocannabinoid and endovanilloid biological activities. We have reported previously that NADA exerts some of its biological activities through inhibition of the NF-kappaB pathway and, because this transcription factor plays a key role in HIV-1-long terminal repeat (LTR) trans activation, we have evaluated the anti-HIV-1 activity of NADA. In this study, we show that NADA inhibits vesicular stomatitis virus-pseudotyped HIV-1 infection in the human leukemia T cell line Jurkat, in primary T cells, and in the human astrocytic cell line U373-MG. Other endocannabinoids such as anandamide, 2-arachidonoylglycerol, and noladin ether did not show inhibitory activity in the HIV-1 replication assays. The anti-HIV-1 activity of NADA was independent of known cannabinoid and vanilloid receptor activation. In addition, NADA did not affect reverse transcription and integration steps of the viral cycle, and its inhibitory effect was additive with that of the reverse transcriptase inhibitor azidothymidine. NADA inhibited both TNF-alpha and HIV-1 trans activator protein-induced HIV-1-LTR activation. We also show that NADA counteracts the TNF-alpha-mediated trans activation capacity of the p65 NF-kappaB subunit without affecting its physical association to the HIV-1-LTR promoter. Moreover, NADA inhibited the p65 transcriptional activity by specifically targeting the phosphorylation of this NF-kappaB
These findings provide new mechanistic insights into the biological activities of NADA, and highlight the potential of lipid mediators for the management of AIDS.


Involvement of cannabinoid CB(2) receptor and effect of cannabinoid CB(2) receptor antagonist/inverse agonists on cutaneous inflammation were investigated. Mice ears topically exposed to an ether-linked analogue of 2-arachidonoylglycerol (2-AG-E) or selective cannabinoid CB(2) receptor agonist, {4-[4-(1,1-dimethylheptyl)-2,6-dimethoxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}-methanol (HU-308), had early and late ear swelling (0-24 h and 1-8 days after exposure, respectively). Both types of responses induced by 2-AG-E were significantly suppressed by oral administration of cannabinoid CB(2) receptor antagonist/inverse agonists, [N-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide] (JTE-907) and {N-[(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide}} (SR144528). In contrast, JTE-907 did not affect arachidonic acid-induced swelling. Orally administered JTE-907 (0.1-10 mg/kg) and SR144528 (1 mg/kg) also produced significant inhibition of dinitrofluorobenzene-induced ear swelling, with increased cannabinoid CB(2) receptor mRNA expression observed in the inflamed ear. These results suggest that cannabinoid CB(2) receptor is partially involved in local inflammatory responses and cannabinoid CB(2) receptor antagonist/inverse agonist has beneficial effects on ear swelling.

Molecular biology


Herbal cannabis, smoked in the form of marihuana or hashish, is the most common illicit drug consumed in the Western world. In the brain, cannabinoids interact with neuronal CB1 receptors, thereby producing a marked reduction of motor activity. Here, we report that the motor depressant effect produced by the cannabinoid receptor agonist (-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]trans-4-(3-hydroxypropyl)cyclohexanol (CP55,940) is attenuated by genetic inactivation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), which is abundantly expressed in the medium spiny neurons of the striatum. Point mutation of Thr34, the protein kinase A (PKA) phosphorylation site of DARPP-32, produces a similar reduction in the effect of the CB1 agonist. In contrast, point mutation of Thr75, a site on DARPP-32 specifically phosphorylated by cyclin-dependent kinase 5, does not affect the behavioral response to CP55,940. Activation of CB1 receptors, either by an agonist or by inhibition of reuptake of endogenous cannabinoids, stimulates phosphorylation at Thr34, thereby converting DARPP-32 into an inhibitor of protein phosphatase-1. Genetic inactivation either of dopamine D2 receptors or of adenosine A2A receptors reduces the phosphorylation of DARPP-32 at Thr34 and the motor depression produced by CP55,940. Our data indicate that a considerable proportion of the psychomotor effect of cannabinoids can be accounted for by a signaling cascade in striatal projection neurons involving PKA-dependent phosphorylation of DARPP-32, achieved via modulation of dopamine D2 and adenosine A2A transmission.

Neuroscience


The present study was designed to test whether chronic neuroleptic treatment, which is known to alter both expression and density of dopamine D(2) receptors in striatal regions, has effects upon function and binding level of the cannabinoid CB(1) receptor in the basal ganglia by using receptor autoradiography. As predicted, subchronic haloperidol treatment resulted in
increased binding of (3)H-raclopride and quinpirole-induced guanosine 5'-O-(gamma-[(35)S]thio)triphosphate ([(35)S]GTPgammaS) in the striatum when compared to that measured in control animals. This increased D(2) receptor binding and function after 3 days washout was normalized after a 2-week washout period. Effect of haloperidol treatment was studied for CB(1) receptor binding and CP55,940-stimulated [(35)S]GTPgammaS in the striatum, globus pallidus, and substantia nigra. (3)[H]CP55,940 binding levels were found in rank order from highest to lowest in substantia nigra > globus pallidus > striatum. Furthermore, subchronic haloperidol treatment resulted in elevated binding levels of (3)[H]CP55,940 in the striatum and the substantia nigra and CB(1) receptor-stimulated [(35)S]GTPgammaS bindings in the substantia nigra after 3 days washout. These increased binding levels were normalized at 1-4 weeks after termination of haloperidol treatment. Haloperidol treatment had no significant effect on CB(1) receptor or [(35)S]GTPgammaS binding levels in globus pallidus. The results help to elucidate the underlying biochemical mechanism of CB(1) receptor supersensitivity after haloperidol treatment. (c) 2005 Wiley-Liss, Inc.


Endocannabinoids are a new class of lipids, which include amides, esters and ethers of long chain polyunsaturated fatty acids. Anandamide (N-arachidonylethanolamine; AEA) and 2-arachidonoylglycerol are the main endogenous agonists of cannabinoid receptors, able to mimic several pharmacological effects of Delta(9)-tetrahydrocannabinol, the active principle of Cannabis sativa preparations like hashish and marijuana. It is known that the activity of AEA is limited by cellular uptake through a specific membrane transporter, followed by intracellular degradation by a fatty acid amide hydrolase. Together with AEA and congeners these proteins form the "endocannabinoid system". The endogenous cannabinoids were identified in brain, and also in neuronal and endothelial cells, suggesting a potential role as modulators in the central nervous system and in the periphery. This review summarises the metabolic routes for the synthesis and degradation of AEA, and the latest advances in the involvement of this lipid in neurovascular biology. In addition, the therapeutic potential of the modulation of endocannabinoid metabolism for neuronal and vascular system will be also reviewed.


Previous studies supporting a possible physiological role for an endogenous cannabinoid, arachidonylethanolamine (AEA, anandamide), showed a significant increase in AEA content in the nucleus tractus solitarius (NTS) after an increase in blood pressure (BP) and prolonged baroreflex inhibition of renal sympathetic nerve activity (RSNA) after exogenous AEA microinjections into the NTS. These results, along with other studies, support the hypothesis that endogenous AEA can modulate the baroreflex through cannabinoid CB(1) receptor activation within the NTS. This study was performed to characterize the physiological role of endogenously released cannabinoids (endocannabinoids) in regulating baroreflex control of RSNA through actions in the NTS. Endocannabinoid effects were assessed by measuring the RSNA baroreflex response to increased pressure after bilateral microinjections of AM404, an endocannabinoid transport inhibitor, into the NTS of adult male Sprague Dawley rats. AM404 blocks uptake of endocannabinoids and enhances the effects of any endocannabinoids released [M. Beltramo, et al., Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science, 277 (5329) (1997) 1094-1097.] into the NTS. Therefore, it was hypothesized that microinjections of AM404 should exhibit effects similar to microinjections of exogenous AEA. In this study, AM404 microinjections into the NTS were found to significantly prolong baroreflex inhibition of RSNA compared to control, similar to effects of exogenous AEA. This effect is thought to result from an increased endocannabinoid presence in the NTS, leading to prolonged CB(1) receptor activation. These results indicate that endocannabinoids released in the NTS have the potential to regulate baroreflex control at this site in the central baroreflex pathway.

Neuropathic pain is a clinical manifestation characterized by the presence of spontaneous pain, allodynia and hyperalgesia. Here, we have evaluated the involvement of CB1 cannabinoid receptors in the development and expression of neuropathic pain. For this purpose, partial ligation of the sciatic nerve was performed in CB1 cannabinoid receptor knockout mice and their wild-type littermates. The development of mechanical and thermal allodynia, and thermal hyperalgesia was evaluated by using the von Frey filaments, cold-plate and plantar tests, respectively. Pre-surgical tactile and thermal withdrawal thresholds were similar in both genotypes. In wild-type mice, sciatic nerve injury led to a neuropathic pain syndrome characterized by a marked and long-lasting reduction of the paw withdrawal thresholds to mechanical and thermal stimuli. These manifestations developed similarly in mice lacking CB1 cannabinoid receptors. We have also investigated the consequences of gabapentin administration in these animals. Gabapentin (50mg/kg/day, i.p.) induced a similar suppression of mechanical and thermal allodynia in both wild-type and CB1 knockout mice. Mild differences between genotypes were observed concerning the effect of gabapentin in the expression of thermal hyperalgesia. Taken together, our results indicate that CB1 cannabinoid receptors are not critically implicated in the development of neuropathic pain nor in the anti-allodynic and anti-hyperalgesic effects of gabapentin in this model.


RATIONALE: Delta(9)-Tetrahydrocannabinol (Delta(9)-THC) disrupts working memory. The prefrontal cortex (PFC) is involved in the processing of working memory, and its medial portion (mPFC) is part of a brain reward circuit as constituted by the mesocorticolimbic dopaminergic system. OBJECTIVE: This study examined the involvement of the mPFC in the effects of Delta(9)-THC on spatial working memory. METHODS: Ten male Wistar rats well-trained in a radial arm maze and with bilateral cannula implanted in the mPFC received Delta(9)-THC intracortically (Delta(9)-THC IC) at doses of 0 (VEH), 32, 100 or 180 mug, 5 min before a 5-s or a 1-h delayed task in order to measure a short- or long-term spatial working memory, respectively. By contrast, 11 other animals received Delta(9)-THC intraperitoneally (Delta(9)-THC IP) at doses of 0 (VEH), 0.32, 1 or 1.8 mg/kg, 30 min before a 5-s or a 1-h delayed task. Additionally, after a 15-day washout, the effect of an IP or IC pre-exposure of Delta(9)-THC was examined by repeating both dose-effect curves in a crossover order for the routes of administration. RESULTS: Delta(9)-THC IP produced significantly larger number of errors at doses of 0.32 or 1 mg/kg as compared to VEH in the 1-h post-delay performance. Delta(9)-THC 100 mug IC also produced significantly larger number of errors as compared to VEH and also to the other doses (32 or 180 mug) IC in the 1-h post-delay performance. Previous exposure to Delta(9)-THC IP or IC did not significantly affect the disruptive effect of this cannabinoid. CONCLUSIONS: Delta(9)-THC administered directly in the mPFC impaired 1-h delayed task in the radial arm maze in a manner similar to that observed for its systemic administration, suggesting that the mPFC is involved in the disruptive effects of Delta(9)-THC on spatial working memory.


Previous studies have suggested that the endocannabinoid CB1 receptor (ECBR) system is involved in stress. However, the nature of this association is complex. Here, we investigated the role of CB1 receptors in the response to stress by comparing the effects of various stress modalities in CB1 receptor deficient and wild-type mice, at adulthood and during early development. Response to acute stress was assayed by plasma corticosterone (CS) and adrenocorticotrophic hormone (ACTH), USVs and motor inhibition. The response to repeated stress was assessed by USVs and motor inhibition. Since repeated bell stress seemed to cause a cumulative fear in CB1 receptor knockout mice, these behavioral responses were also compared to those observed after a single severe stress (forced swimming). In wild-type, but not in CB1 receptor knockout mice, bell stress-induced elevations of ACTH and CS were significant. The first exposure to bell stress had no significant effect on USVs or mobility. Upon repeated exposures, significant suppression of USVs, together with behavioral inhibition, were observed in CB1
knockout but not in wild-type mice. Swim stress inhibited USVs in the knockout animals, and the profound motor inhibition displayed by all animals was greater and more prolonged in the CB1 mice. Since the knockout mice lack the CB1 receptor throughout pre- and postnatal life, the stress response in pups was also assayed (by separation-induced USVs). Wild-type pups displayed the characteristic developmental peak in USV emissions; it was completely lacking in knockout pups. We conclude that acutely, the absence of CB1 receptors reduces the neuroendocrine response and does not affect the behavioral response to moderate stress. However, upon repeated stress or acute severe stress, CB1 receptor deficiency causes persistent behavioral inhibition. Finally, the CB1 receptor plays a role in modulating the stress response from an early age. These observations suggest that CB1 receptors participate in the mediation of the stress response and that the absence of these receptors results in a greater vulnerability to stress. We suggest that the stress-induced endocrine and behavioral suppression in CB1 receptor deficient mice may serve as a model for some forms of post-traumatic stress disorder (PTSD). Further, the role of CB1 receptors in coping with stress is a lifelong function. Finally, although equivalent research has not been performed in human infants, the postnatal suppression of the stress response in CB1 receptor knockout pups may have implications when cannabinoid-based therapy is considered for children.


The current experiments were designed to study the antinociceptive effects of intrathecal (i.t.) administration of cannabinoid CB1 receptor and alpha2-adrenoceptor drugs in the nociceptive processing and also their receptor interactions. Different doses of a cannabinoid receptor agonist, CP 55,940, and an alpha2-adrenoceptor agonist, clonidine induced a dose-dependent antinociception in both phases of the formalin test. CP 55,940-induced antinociception was reduced by pretreatment of a selective cannabinoid CB1 receptor antagonist, SR 141716A, but not by pretreatment with an alpha2-adrenoceptor antagonist, yohimbine in both phases of the test. However, yohimbine and SR 141716A attenuated the antinociception induced by clonidine in the early phase but not in the late phase of the test. While SR 141716A by itself did not influence pain behaviour, the reversal effect of clonidine by SR 141716A indicate that clonidine stimulate the release of endocannabinoid(s). In conclusion, our findings may suggest that: (1) spinal cannabinoid and alpha2-adrenoceptor systems are able to induce antinociception in both phases of formalin test, and (2) the cannabinoid system may be involved in the antinociception induced by adrenoceptors in the early phase.

Lim, G., S. Wang, et al. (2005). "Central glucocorticoid receptors modulate the expression of spinal cannabinoid receptors induced by chronic morphine exposure." Brain Res.

Central cannabinoid receptors (CBRs) have been implicated in the opioid analgesic effects. However, it remains unclear as to whether the expression of central CBRs would be altered after repeated morphine exposure. Here, we show that chronic intrathecal treatment with morphine (10 mug, twice daily for 6 days) induced a time-dependent upregulation of both CB-1 and CB-2 receptors within the spinal cord dorsal horn. This morphine-induced CB-1 and CB-2 upregulation was dose-dependently attenuated by the intrathecal co-administration of morphine with the glucocorticoid receptor (GR) antagonist RU38486 (0.25, 0.5, or 2 mug). The intrathecal RU38486 treatment regimen also attenuated the development of morphine tolerance. These results indicate that the expression of spinal CBRs was altered following repeated morphine exposure and regulated by the activation of central GRs.


Prolonged exposure to cannabinoids results in desensitization of cannabinoid receptors. Here, we compared the desensitization produced by the partial agonist, Delta(9)-tetrahydrocannabinol (THC) to that produced by the full agonist Win55,212-2 on cannabinoid-mediated inhibition of glutamatergic synaptic transmission. Synaptic activity between rat
hippocampal neurons was determined from network-driven increases in the intracellular Ca(2+) concentration ([Ca(2+)](i) spikes). To assess the effects of prolonged treatment, cultures were incubated with cannabinoids, washed in 0.5% fatty-acid-free bovine serum albumin to ensure the removal of the lipophilic drug and then tested for inhibition of [Ca(2+)](i) spiking by Win55,212-2. In control experiments, 0.1µM Win55,212-2 inhibited [Ca(2+)](i) spiking by 93 +/- 5%. Win55,212-2 produced significantly less inhibition of [Ca(2+)](i) spiking following 18-24h treatment with 1µM THC (48 +/- 5%) or treatment with 1µM Win55,212-2 (29 +/- 6%). Thus, THC produced significantly less functional desensitization than Win55,212-2. The desensitization produced by THC was maximal at 0.3µM, remained stable between 1 and 7days of preincubation and shifted the EC(50) of acute inhibition by Win55,212-2 from 27 to 251nM. Differences in the long-term effects of cannabinoid receptor agonists on synaptic transmission may prove important for evaluating their therapeutic and abuse potential.


Depolarisation-induced suppression of excitation and inhibition (DSE/DSI) appear to be important forms of short-term retrograde neuronal plasticity involving endocannabinoids and the activation of presynaptic cannabinoid CB1 receptors. We report here that CB1-dependent DSE can be elicited from autaptic cultures of excitatory mouse hippocampal neurons. DSE in autaptic cultures is both more robust and elicited with a more physiologically relevant stimulus than has been thus far reported for conventional hippocampal cultures. Autaptic DSE additionally requires filled internal calcium stores. Pharmacological experiments favour a role for 2-arachidonyl glycerol (2-AG) rather than arachidonyl ethanolamide (AEA) or noladin ether as the relevant endocannabinoid (eCB) to elicit DSE. In particular, the latter two fail to reversibly inhibit EPSCs, a quality inconsistent with the role of bona fide eCB mediating DSE. Delta9-tetrahydrocannabinol (Delta9-THC) fails to inhibit EPSCs, yet readily occludes both DSE and EPSC inhibition by a synthetic CB1 agonist, WIN 55212-2. With long-term exposure (~18 hrs), Delta9-THC additionally desensitizes CB1 receptors. Lastly, a functional endocannabinoid transporter is necessary for the expression of DSE.


Therapeutic options for amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disorder, remain limited. Emerging evidence from clinical studies and transgenic mouse models of ALS suggests that cannabinoids, the bioactive ingredients of marijuana (Cannabis sativa) might have some therapeutic benefit in this disease. However, Delta(9)-tetrahydrocannabinol (Delta(9)-THC), the predominant cannabinoid in marijuana, induces mind-altering effects and is partially addictive, compromising its clinical usefulness. We therefore tested whether cannabinol (CBN), a non-psychotropic cannabinoid, influences disease progression and survival in the SOD1 (G93A) mouse model of ALS. CBN was delivered via subcutaneously implanted osmotic mini-pumps (5 mg/kg/day) over a period of up to 12 weeks. We found that this treatment significantly delays disease onset by more than two weeks while survival was not affected. Further research is necessary to determine whether non-psychotropic cannabinoids might be useful in ameliorating symptoms in ALS.

Oncology


Cannabinoids have been shown to inhibit the growth of a broad spectrum of tumour cells. However, the molecular mechanisms involved in that effect have not been completely elucidated. Here, we investigated the possible involvement of mitogen-activated protein kinases (MAPKs) in CB(2) receptor-induced apoptosis of human leukaemia cells. Results show that stimulation of the CB(2) receptor leads to p38 MAPK activation and that inhibition of this kinase attenuates CB(2) receptor-induced caspase activation and apoptosis. These findings support a role for p38 MAPK in CB(2) receptor-induced apoptosis of human leukaemia cells.
**Ophthalmology**


Cannabinoid agonists have been shown to produce dose-related impairments in several measures of cognitive performance. However, it is unclear if low doses of cannabinoid CB1 agonists, or CB1 antagonists, can facilitate aspects of stimulus detection. The present study employed an operant procedure involving visual stimulus detection in rats. The task was found to be sensitive to the muscarinic acetylcholine antagonist scopolamine. The CB1 antagonist AM 251 did not affect stimulus detection processes across a broad range of doses. However, the novel CB1 agonist AM 411 produced a biphasic effect, with the two lowest doses (0.25 and 0.5 mg/kg) enhancing accuracy. AM 411 changed patterns of responding toward runs of consecutive errors on only one of the two levers. It produced a biphasic effect on consecutive errors on the lever associated with a higher level of errors, with decreases in errors following the lower doses (0.25 and 0.5 mg/kg) and increases following the highest dose (2.0 mg/kg). These effects were not accompanied by changes in measures of bias commonly used to uncover such patterns in rodent operant models of cognitive performance. In contrast to the cognitive impairment seen after administration of moderate to high doses of CB1 agonists, it appears that low doses of some CB1 agonists may be capable of enhancing stimulus detection processes.

**Pharmacology**


Newly developed cannabinoids may hold the promise of the development of useful and safe drugs. This study aimed to investigate the behavioral effects of the novel 1',1'-dithiolane Delta-THC analogue AMG-3, a cannabinomimetic molecule with high affinity for CB1/CB2 receptors. This analog was chosen for its binding affinity to these receptors, which is higher than that reported for Delta-tetrahydrocannabinol (Delta-THC). Behavioral responses were assessed after the administration of AMG-3 (1, 2, 4, 8 mg/kg, i.p.) in the open field, on the bar test, on the hot plate and in the intracranial self-stimulation procedure. AMG-3 increased the reactivity time on the hot plate in a dose- and time-dependent manner, indicating a long-lasting analgesic effect (at least 24 h). The substance was found dose-dependently to decrease spontaneous motor activity and to induce catalepsy, particularly at the highest dose (8 mg/kg). AMG-3 did not affect the rewarding value of intracranial self-stimulation, except to increase the reward threshold at the highest dose (8 mg/kg). The effects of the highest dose of AMG-3 on spontaneous activity and on the self-stimulation paradigm were completely reversed by pre-treatment with the CB1 receptor antagonist AM-251. These findings indicate that the administration of AMG-3 to rats elicits a specific behavioral profile, most probably associated with the activation of CB1 receptors and without effects indicating abuse potential.


Two new series of cannabinoids were prepared and their affinities for the CB(1) and CB(2) receptors were determined. These series are the (2'R)- and (2'S)-1-methoxy- and 1-deoxy-3-(2'-methylalkyl)-Delta(8)-tetrahydrocannabinols, with alkyl side chains of three to seven carbon atoms. These compounds were prepared by a route that employed the enantioselective synthesis of the resorcinol precursors to the cannabinoid ring system. All of these compounds have greater affinity for the CB(2) receptor than the CB(1) receptor and four of them, (2'R)-1-methoxy-3-(2'-methylbutyl)-Delta(8)-THC (JWH-359), (2'S)-1-deoxy-3-(2'-methylbutyl)-Delta(8)-THC (JWH-352), (2'S)-1-deoxy-3-(2'-methylpentyl)-Delta(8)-THC (JWH-255), and (2'R)-1-deoxy-3-(2'-
methylpentyl)-Delta(8)-THC (JWH-255), have good affinity (K(i)=13-47nM) for the CB(2) receptor and little affinity (K(i)=1493 to >10,000nM) for the CB(1) receptor. In the 1-deoxy-3-(2'-methylalkyl)-Delta(8)-THC series, the 2'S-methyl compounds in general have greater affinity for the CB(2) receptor than the corresponding 2'R isomers.


A stereocontrolled synthesis of (-)-CP55,940, a potent cannabinoid receptor agonist, has been attained using a novel aldolization/retro-aldolization interconversion strategy, in which a temporarily generated chiral aldol motif plays essential roles.


Novel 3,4-diarylpyrazolines 1 as potent CB(1) receptor antagonists with lipophilicity lower than that of SLV319 are described. The key change is the replacement of the arylsulfonyl group in the original series by a dialkylaminosulfonyl moiety. The absolute configuration (4S) of eutomer 24 was established by X-ray diffraction analysis and 24 showed a close molecular fit with rimonabant in a CB(1) receptor-based model. Compound 17 exhibited the highest CB(1) receptor affinity (K(i)=24nM) in this series, as well as very potent CB(1) antagonistic activity (pA(2)=8.8) and a high CB(1)/CB(2) subtype selectivity (approximately 147-fold).


The voltammetric behaviour of 2,6-dichloro-p-aminophenol (PAP) in aqueous solution at an edge plane pyrolytic graphite electrode was explored and its sensitivity to additions of substituted phenols examined. Proof of concept is shown for the electrochemical adaptation of the Gibbs reaction, where reaction of the oxidised form of PAP with substituted phenols provides an indirect methodology for the analytical detection of these compounds. This indirect protocol provides an attractive alternative to the direct electrochemical oxidation of phenolic compounds, since the latter is plagued by electrode passivation, leading to low sensitivity. It is observed that phenol, 4-phenoxyphenol, methylphenol (para and meta), nitrophenol and most importantly, tetrahydrocannabinol, can be detected voltammetrically. Such a protocol is particularly attractive for roadside testing for cannabis in drug drivers.


The prototypic cannabinoid CB1 antagonist SR 141716A is one important pharmacologic tool for examining CB1 receptors that mediate the behavioral and physiologic effects of Delta-tetrahydrocannabinol (Delta-THC). This study examined the effects of SR 141716A on the rate-decreasing, hypothermic and discriminative stimulus effects of Delta-THC in rhesus monkeys. In monkeys (n=4) responding under a multiple fixed ratio (FR-10:FR-10) schedule of food presentation and stimulus-shock termination, the potency of i.m. Delta-THC to decrease responding in the food component (ED50=0.64 mg/kg) was threefold greater than its potency in the stimulus-shock termination component (ED50=2.14 mg/kg). In the same monkeys, hypothermia was induced by Delta-THC at a dose (e.g. 0.32 mg/kg) that did not alter responding in either schedule component; the maximum decrease was 2.1 degrees C at a dose of 3.2 mg/kg. A dose of 0.32 mg/kg of SR 141716A, significantly attenuated Delta-THC-induced hypothermia without attenuating the rate-decreasing effects of Delta-THC in either component of the multiple schedule. The largest dose of i.m. SR 141716A that was studied, 1.0 mg/kg, significantly decreased rectal temperature and responding in the food component but did not significantly decrease responding in the stimulus-shock termination component of the multiple schedule. In a separate group of monkeys (n=3) that discriminated i.v. Delta-THC (0.1 mg/kg) while responding under an FR-5 schedule of stimulus-shock termination, SR 141716A (0.32 and 1 mg/kg) significantly increased the ED50 of the Delta-THC by 2.3- and 3.7-fold, respectively. Collectively, these results demonstrate that the behavioral effects of Delta-THC are not equally attenuated by SR 141716A.

The CB1 cannabinoid receptor has been shown to play important physiological roles in the central nervous system as well as peripherally and is a target for development of therapeutic medications. To gain insight on the ligand binding site(s) and structural features of activation, we designed and synthesized AM841, a classical cannabinoid affinity label which incorporates an isothiocyanate substituent as an electrophilic reactive group capable of interacting irreversibly with a suitably located and properly oriented nucleophilic amino acid residue at or near the binding site. To obtain evidence for the site of covalent attachment of AM841, C6.47, identified in part by interactive ligand docking, was mutated to serine, alanine and leucine to reduce or eliminate the nucleophilic character. WT and mutant CB1 receptors were evaluated for their abilities to recognize a series of cannabimimetic ligands. Each bound comparably to WT, excluding C6.47L, which displayed a reduced affinity for [(3)H]-CP55940, AM841, AM4043 and AM4056 and an improvement in affinity for Delta9-THC. The affinity of [(3)H]-WIN55212-2 was unchanged across all mutants. Importantly, AM841 was shown to bind irreversibly to WT CB1 but exhibited no covalent attachment with the mutants and behaved as an agonist suggesting irreversible attachment to C6.47 maintains CB1 in its active state. The evidence presented identifies C6.47 as the site of covalent bond formation with AM841 and combined with the binding data fully supports the molecular modeling. These studies present the first report of tandem applications of affinity labeling, site-directed mutagenesis and interactive ligand docking for CB1.


The properties of ES46.5K, an esterase from mouse hepatic microsomes, were compared with those of carboxylesterases from rabbit and porcine liver. The inhibitory profile with a serine hydrolase inhibitor (bis-p-nitrophenylphosphate) and detergents (sodium dodecylsulfate, Emulgen 911) was different between ES46.5K and the carboxylesterases. Bis-p-nitrophenylphosphate (0.1 mM) markedly inhibited the catalytic activity of the carboxylesterases but not that of ES46.5K. Emulgen 911 (0.05-0.25%) inhibited the catalytic activity of the carboxylesterases, whereas the detergent conversely stimulated that of ES46.5K by 150%. The two carboxylesterases catalyzed the hydrolysis of acetate esters of tetrahydrocannabinol (THC) analogues with different side chain lengths (C(1)-C(5)), although ES46.5K showed marginal activity only against the acetate of Delta(8)-tetrahydrocannabinol, a methyl side chain derivative of Delta(8)-THC. ES46.5K hydrolyzed cannabinoid esters stereospecifically and regioselectively. The esterase hydrolyzed 8alpha-acetoxy-Delta(9)-tetrahydrocannabinol (8alpha-acetoxy-Delta(9)-THC, 5.62 nmol/min/mg protein), while the enzyme did not hydrolyze 8beta-acetoxy-Delta(9)-THC, 7alpha-acetoxy-, and 7beta-acetoxy-Delta(8)-THC at all. In contrast, the carboxylesterases from rabbit and porcine liver hydrolyzed 8beta-acetoxy-Delta(9)-THC efficiently but not 8alpha-acetoxy-Delta(9)-THC. ES46.5K hydrolyzed side chain acetoxy derivatives of Delta(8)-THC at the 3'- and 4'-positions, and a methyl ester of 5'-nor-Delta(8)-THC-4'-oic acid. The enzyme, however, could not hydrolyze methyl esters of Delta(8)- and Delta(9)-THC-11-oic acid, while both carboxylesterases hydrolyzed side chain acetoxy derivatives of Delta(8)-THC and three methyl esters of THC-oic acids. These differences in stereospecificity and regioselectivity between ES46.5K and carboxylesterases suggest that the configurations of important amino acids for the catalytic activities of these enzymes are different from each other.


A new series of CB(1) ligands with high binding affinity (K(i) = 0.7-100 nM) and moderate lipophilicity (cLogD(7.4)) in the range of 2.1-4.5 has been synthesized. A structure-activity relationship study demonstrated that for the studied set of aminoalkylindoles, the molecular dipole of the ground state conformation within the series was inversely related to the affinity. The
racemic ligand with highest affinity (0.7 nM), 3-(4-fluoronaphthoyl)-1-(N-methylpiperidin-2-ylmethyl)indole, was radiolabeled with (18)F. This radioligand specifically labeled CB(1) receptors in mouse brain and accumulated in regions of high versus low CB(1) receptor density in a ratio of 1.6. The displaceable radioactivity of one enantiomer in the brains of mice determined in a pretreatment study using the CB(1) antagonist N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyra zole-3-carboxamide (SR141716) was nearly double that of the racemate for the same determination; therefore, the active enantiomer is a candidate for PET studies in animals. A pretreatment study for the other enantiomer found no displaceable radioactivity in the same group of mice; this result suggested the enantiomer was inactive.


Prostaglandin F2a 1-ethanolamide (Prostamide F2a) is a potent ocular hypotensive agent in animals and represents a new class of fatty acid amide compounds. Accumulated evidence indicated anandamide, an endogenous bioactive ligand for cannabinoid receptors, may serve as a common substrate to produce all prostamides including prostamide F2a. Following incubation of anandamide with cylooxygenase 2 (COX-2), the reaction mixture was profiled by HPLC and an intermediate metabolite was discovered and characterized as a cyclic endoperoxide ethanolamide using HPLC tandem mass spectrometry (HPLC-MS/MS). Formation of prostamide F2a was also demonstrated when the intermediate metabolite was isolated and incubated with prostaglandin F synthase. These results suggest that the biosynthesis of prostamide F2a proceeds in two consecutive steps, oxidation of anandamide to form an endoperoxide intermediate by COX-2, and reduction of the endoperoxide intermediate to form prostamide F2a by PGF synthase. This endoperoxide ethanolamide intermediate has been proposed as prostamide H2.

Plant science


The cannabinoid content of 13 different strains of cannabis plant (Cannabis sativa L.) was analyzed. Six strains fell into the "drug-type" class, with high Delta-9-tetrahydrocannabinolic acid (THCA) content, and seven strains into the "fiber-type" class, with low THCA using HPLC analysis. Genomic DNA sequence polymorphisms in the THCA synthase gene from each strain were studied. A single PCR fragment of the THCA synthase gene was detected from six strains of "drug-type" plants. We could also detect the fragment from seven strains of "fiber-type" plants, although no or very low content of THCA were detected in these samples. These were 1638bp from all 13 strains and no intron among the sequences obtained. There were two variants of the THCA synthase gene in the "drug-type" and "fiber-type" cannabis plants, respectively. Thirty-seven major substitutions were detected in the alignment of the deduced amino acid sequences from these variants. Furthermore, we identified a specific PCR marker for the THCA synthase gene for the "drug-type" strains. This PCR marker was not detected in the "fiber-type" strains.


The stable carbon and nitrogen isotopic ratios were measured in marijuana samples (Cannabis sativa L.) seized by the law enforcement officers in the three Brazilian production sites: Pernambuco and Bahia (the country's Northeast known as Marijuana Polygon), Para (North or Amazon region) and Mato Grosso do Sul (Midwest). These regions are regarded as different with respect to climate and water availability, factors which impact upon the isotope fractionations of these elements within plants. It was possible to differentiate samples from the dry regions (Marijuana Polygon) from those from Mato Grosso do Sul and Para, that present heavier rainfall. The results were in agreement with the climatic conditions of the suspected regions of origin and this demonstrates that seized samples can be used to identify the isotopic signatures of marijuana from the main producing regions in Brazil.

Alkamides are the major lipophilic constituents of Echinacea angustifolia roots. Due to their structural similarity with anandamide, we have evaluated their ability to bind to rodent cannabinoid receptors CB1 and CB2 by a standard receptor binding assay using [(3)H]CP-55,940 as a radioligand. The alkamides exhibited selective affinity especially to CB2 receptors and can therefore be considered as CB ligands. Most of the alkamides showed good metabolic stability as indicated by the similarity between affinity to CB1 determined in the presence/absence of the protease inhibitor PMSF. It is suggested that CB2 interactions may be the molecular mode of action of Echinacea alkamides as immunomodulators.


OBJECTIVE: To select the optimum extracting procedure for cannabinoids from hemp seed oil. METHODS: The optimum extracting procedure was selected with the content of cannabinoi and delta9-tetrahydrocannabinol from hemp seed oil by orthogonal test design. We have examined three factors that may influence the extraction rate: the time of extraction, the times of extraction and the amount of methanol. RESULTS: The optimum extraction condition was adding 5 ml, two times amount of methanol into hemp seed oil for 15 min. CONCLUSION: The above extraction process gave the most rational, stable, feasible and satisfactory results. The method is convenient.

Psychiatry


Clinical and laboratory findings suggest that cannabinoid signalling is implicated in schizophrenia. However, the interaction remains poorly understood, as data are often contradictory. Here we investigated wild-type (WT) and cannabinoid CB1 receptor-knockout (CB1-KO) mice in the phencyclidine-induced social withdrawal model of schizophrenia. N-methyl-D-aspartate (NMDA) antagonists (including phencyclidine) induce psychotic symptoms in humans, and are used to model schizophrenia in a variety of experimental conditions. In WT, 5 mg/kg phencyclidine increased locomotion and stereotyped behaviours, and decreased social interactions. These changes are consistent with a schizophrenia-like effect. In CB1-KOs, phencyclidine decreased locomotion, enhanced ataxia and stereotypy more markedly than in WTs, but did not affect social interactions. Locomotion showed a significant negative correlation with both ataxia and stereotypy, suggesting that in CB1-KOs, the locomotor suppressive effect of phencyclidine was secondary to changes in these variables. Our findings demonstrate that CB1 gene disruption dramatically alters the behavioural effects of the NMDA antagonist phencyclidine, suggesting that the CB1 receptor is involved in schizophrenia. As social disruption and stereotypy respectively are believed to model negative and positive symptoms of schizophrenia, our findings tentatively suggest that cannabinoids are differentially involved in these two symptom categories. These findings require verification by experiments involving CB1 receptor blockers, as the genetic and pharmacological blockade of receptors may not always provide similar results.


As persistent behavioural changes, such as increased anxiety-related behaviours, can be predicted based on the phenomenon of psychostimulant-induced neuronal plasticity, the time course (3-, 5- and 10-day time points) of the effects of both a single and repeated (daily for 7 days) i.p. administrations of cocaine (COC) and methamphetamine (MA) on anxiety-related behavioural symptoms in the elevated plus-maze test were examined in mice. Furthermore, based on the reported interactions between brain dopamine versus cannabinoid (CB) receptors and the contribution of CB receptors to the occurrence of persistent anxiety-related behavioural
symptoms, the interactions of the agonist CP 55940 (CP) and the endogenous ligands anandamide (arachidonylethanolamide: AEA), 2-arachidonylglycerol (ARA), N-arachidonyldopamine (NADA), noladin ether (NL), and virodhamine (VA) with the COC- or MA-induced anxiety-related behaviours were also studied. In both an acute experiment using a single COC (30 mg/kg) or MA (4 mg/kg) dose and a chronic experiment using repeated COC (15 mg/kg) or MA (2 mg/kg) doses, anxiety-related behavioural symptoms were observed similarly at 3- and 5-day time points, but disappeared at the 10-day time point. Among the CB ligands, the agonists CP, AEA, ARA, NADA, and NL provided strong protective effects against each parameter at 3- and 5-day time points. Therefore, it was concluded that both COC and MA caused persistent anxiety-related behavioural symptoms following both a single and repeated treatments. Since these anxiogenic effects were attenuated by the endogenous CB agonists, the involvement of brain CB receptors was suspected.


With advances in basic and clinical neuroscience, many gaps have appeared in the traditional monoamine theory of depression that have led to reformulation of the hypotheses concerning the neurobiology of depression. The more recent hypotheses suggest that melancholic depression is characterized by central glucocorticoid resistance that results in hypercortisololemia, which in turn leads to down-regulation of neurotrophins and subsequent neurodegeneration. Examining the neurobiology of depression from this perspective suggests that the endocannabinoid system may play a role in the etiology of melancholic depression. Specifically, pharmacological and genetic blockade of the cannabinoid CB1 receptor induces a phenotypic state that is analogous to melancholic depression, including symptoms such as reduced food intake, heightened anxiety, increased arousal and wakefulness, deficits in extinction of aversive memories and supersensitivity to stress. These similarities between melancholic depression and an endocannabinoid deficiency become more interesting in light of recent findings that endocannabinoid activity is down-regulated by chronic stress and possibly increased by some antidepressant regimens. We propose that an endocannabinoid deficiency may underlie some of the symptoms of melancholic depression, and that enhancement of this system may ultimately be a novel form of pharmacotherapy for treatment-resistant depression.


The present study investigated spontaneous and quinpirole-induced motor responses of in rats, following withdrawal from chronic treatment with the potent cannabinoid agonist HU-210. Withdrawal from chronic HU-210 (20 mg/kg daily, 14 days) produced a decrease in spontaneous activity at 1 and 2 days and enhanced the hyperactivity induced by acute administration of the dopamine D2 agonist quinpirole (0.5 mg/kg) at 4 days after the end of HU-210 treatment. Administration of quinpirole on day 4 of withdrawal from chronic HU-210 enhanced stereotyped responses and induced jumping behaviour. These results suggest that withdrawal from chronic exposure to cannabinoid agonists could induce a time-dependent alteration in dopamine D2 psychomotor function, leading to a behavioural disorganization, comparable to acute psychotic episodes after continuous cannabinoids.


Cannabis is one of the most commonly used illicit drugs during pregnancy, but little is known about the lasting effects of early-life exposure to this drug. In this study, male Wistar rat pups were treated daily with (-)-Delta-tetrahydrocannabinol (THC; 5 mg/kg, s.c.) or its vehicle between postnatal days (PND) 4 and 14. Drug administration during this early postnatal period in rats is analogous to the third trimester of gestation in humans, which is a major period of synaptogenesis. Rats were subsequently tested drug-free during young adulthood (PND 56) using a two-component food-motivated double Y-maze test. Each trial included distinct spatial discrimination and delayed alternation components, which permitted the simultaneous assessment of reference memory and working memory. Rats were tested for 30 trials/day, 5 days/week for 5 weeks. Results revealed no significant differences between THC- and vehicle-treated rats in the spatial discrimination task. However, compared to vehicle-treated rats, THC-treated rats committed significantly more errors, and required significantly longer to obtain 80% correct over two consecutive days in the delayed alternation task. Results suggest that neonatal THC exposure leads to a specific and lasting deficit in learning in adulthood, which is likely due to impaired working memory function.


The notoriously inconsistent effects of cannabinoids on anxiety-like behaviour may be explained by recent research on CB1 receptor knockout (CB1-KO) mice suggesting that cannabinoids exert bidirectional effects via the CB1 receptor (anxiolysis) and a novel rimonabant-sensitive neuronal cannabinoid receptor (anxiogenesis). This hypothesis is supported by the anxiogenic-like profile of AM-251, an analogue of rimonabant that is a potent and selective CB1 receptor antagonist but which, unlike rimonabant, has no activity at the novel receptor. As we have previously shown that rimonabant reduces anxiety-like behaviour in test-experienced animals only, the current study assessed the effects of AM-251 (1.5-3.0 mg/kg) in male Swiss-Webster mice that were either plus-maze-naive or had been exposed undrugged to the apparatus 24 h prior to testing. Results confirmed that prior maze experience per se significantly increases behavioural indices of anxiety without altering measures of general activity. In maze-naive mice, the lower dose of AM-251 (1.5 mg/kg) significantly reduced % open-arm time and increased grooming while the higher dose (3.0 mg/kg) additionally reduced open-arm entries and total head-dipping, and increased closed-arm returns. These anxiogenic-like effects were observed in the absence of significant changes in general activity levels. Although AM-251 had a very similar profile in maze-experienced animals, significant drug effects on open-arm avoidance measures were precluded by experiementally-induced changes in behavioural baselines (i.e. 'ceiling' effects). Nevertheless, AM-251 again significantly reduced total head-dipping and increased grooming (3.0 mg/kg) and, unlike effects in naive animals, both doses markedly reduced time spent on the centre platform and increased time spent in the enclosed arms. Against a baseline of almost total open-arm avoidance, the pattern of behavioural change in maze-experienced mice would also be consistent with an anxiogenic-like action of AM-251. Data are discussed in relation to previous
findings with rimonabant, the putative existence of a novel non-CB1 neuronal cannabinoid receptor and, more generally, the behavioural pharmacology of plus-maze ‘trial 2’.


Human and animal studies provide evidence for vulnerable periods of brain development for deleterious effects of cannabinoids. We have recently shown that pubertal chronic cannabinoid treatment leads to long-lasting behavioral deficits, whereas a comparable treatment in adult rats did not affect the animals' behavior. In the present study we examined the effects of an identical chronic cannabinoid treatment in juvenile rats, just before the onset of puberty. Treatment with the synthetic cannabinoid agonist WIN 55,212-2 (WIN) (1.2 mg/kg) or vehicle was extended over 25 days throughout the prepubertal period (postnatal days 15-40) in juvenile rats. The rats received a total of 20 injections intraperitoneally. Adult rats were tested for object recognition memory, performance in a progressive ratio (PR) operant behavior task, locomotor activity and prepulse inhibition (PPI) of the acoustic startle response. Juvenile chronic WIN administration had no effect on object recognition memory, PR performance and locomotor activity in adulthood. However, a PPI deficit was observed in WIN-treated rats when tested as adults that could be reversed by the acute administration of the dopamine receptor antagonist haloperidol (0.1 mg/kg). Additionally, juvenile cannabinoid treatment reduced the number of rearings, as well as the time spent in the center of the open field in adult rats, suggesting increased anxiety. Juvenile chronic cannabinoid treatment induced behavioral disturbances in adult rats that are less severe than those observed after pubertal cannabinoid administration. However, based on the observations of sensorimotor gating deficits and increased anxiety, we conclude that the prepubertal developmental phase, in addition to puberty, also represents a vulnerable time period for persistent adverse effects of cannabinoids.


Mood and anxiety disorders, the most prevalent of the psychiatric disorders, cause immeasurable suffering worldwide. Despite impressive advances in pharmacological therapies, improvements in efficacy and side-effect profiles are needed. The present literature review examines the role that the endocannabinoid system may play in these disorders and the potential value of targeting this system in the search for novel and improved medications. Cannabis and its major psychoactive component (-)-trans-Δ9-tetrahydrocannabinol, have profound effects on mood and can modulate anxiety and mood states. Cannabinoid receptors and other protein targets in the central nervous system (CNS) that modulate endocannabinoid function have been described. The discovery of selective modulators of some of these sites that increase or decrease endocannabinoid neurotransmission, primarily through the most prominent of the cannabinoid receptors in the CNS, the CB1 receptors, combined with transgenic mouse technology, has enabled detailed investigations into the role of these CNS sites in the regulation of mood and anxiety states. Although data point to the involvement of the endocannabinoid system in anxiety states, the pharmacological evidence seems contradictory: both anxiolytic- and anxiogenic-like effects have been reported with both endocannabinoid neurotransmission enhancers and blockers. Due to advances in the development of selective compounds directed at the CB1 receptors, significant progress has been made on this target. Recent biochemical and behavioural findings have demonstrated that blockade of CB1 receptors engenders antidepressant-like neurochemical changes (increases in extracellular levels of monoamines in cortical but not subcortical brain regions) and behavioural effects consistent with antidepressant/antisstress activity in rodents.


3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') and cannabis are two of the most commonly used illicit drugs in the western world, and are often used in combination. Very little research has examined their effect on cognitive function or behavior when combined, The
present study used a double Y-maze task to examine the acute effect of MDMA and delta9-tetrahydrocannabinol (THC, the principal psychoactive ingredient of cannabis) on mnemonic function in rats, at a range of doses representative of common human use. Experiment I (low doses) examined the effect of 0.25 mg/kg THC and 1.25 mg/kg MDMA alone and together. At these doses MDMA or THC given alone had no effect on working memory, but the co-administered drugs significantly disrupted working memory. Experiment 2 (medium doses) examined the effect of 0.5 mg/kg THC and 2.5 mg/kg MDMA given alone or together. At these doses THC, but not MDMA, impaired working memory. Although MDMA alone had no effect, it exacerbated the impairment due to THC when the drugs were co-administered. Experiment 3 (high doses) examined the effects of 1 mg/kg THC and 5 mg/kg MDMA alone and together. Both drugs significantly impaired memory when given alone, although the impairment due to MDMA was less than that caused by THC. When co-administered at these doses, the drugs caused a major disruption of behavior and this precluded ascribing a mnemonic cause to poor performance on the double Y-maze task. Taken together, these experiments demonstrate a synergistic disruption of working memory by acute co-administration of THC and MDMA.

Reproductive biology


1. In the present study, the effects of anandamide and WIN 55,212-2, cannabinoid receptor agonists, were investigated on electrical field stimulation (EFS)-induced biphasic twitch responses obtained from the epididymal and prostatic portions of rabbit vas deferens strips. 2. Anandamide and WIN 55,212-2 dose-dependently inhibited both the first and second phases of the EFS-induced twitch responses recorded from epididymal and prostatic portions of the vas deferens over the concentration range 10(-9) to 3 x 10(-6) mol/L. 3. The cannabinoid CB1 receptor antagonist AM 251 (10(-6) mol/L) and the cannabinoid CB2 receptor antagonist AM 630 (10(-6) mol/L) had no effect on the inhibitory action of anandamide on the biphasic twitch responses in the prostatic and epididymal portions of the rabbit vas deferens. 4. In both the prostatic and epididymal portions of the rabbit vas deferens, AM 251 significantly, but not completely, reversed the inhibitory effect of WIN 55,212-2 on the first phase of the twitch response. In contrast, AM 251 did not have any effect on the inhibitory action of WIN 55,212-2 in the rabbit vas deferens strips. 5. The inhibitory effects of anandamide or WIN 55,212-2 on EFS-induced twitch responses of both the prostatic and epididymal portions of the rabbit vas deferens were not altered in the presence of 10(-5) mol/L nalamoxene. 6. These results suggest that cannabinoid receptors may have a modulatory role in the regulation of sympathetic transmission in the rabbit vas deferens. However, further investigation is required to characterize the receptors involved.


Anandamide (AEA) is the endogenous ligand of cannabinoid (CB) receptors, and as such it plays several central and peripheral activities. Regulation of female fertility by AEA has attracted growing interest, yet a role for this endocannabinoid in controlling sperm function and male fertility in mammals has been scarcely investigated. In this study we report unprecedented evidence that boar sperm cells have the biochemical machinery to bind and degrade AEA, i.e. type-1 cannabinoid receptors (CB1R), vanilloid receptors (TRPV1), AEA-synthesizing phospholipase D (NAPE-PLD), AEA transporter (AMT) and AEA hydrolase (FAAH). We also show that the non-hydrolyzable AEA analogue methanandamide reduces sperm capacitation and, as a consequence, inhibits the process of acrosome reaction (AR) triggered by the zona pellucida, according to a cyclic AMP-dependent pathway triggered by CB1R activation. Furthermore, activation of TRPV1 receptors seems to play a role of stabilization of the plasma membranes in capacitated sperm, as demonstrated by the high incidence of spontaneous AR occurring during the cultural period when TRPV1 activity was antagonized by capsazepine. We show that sperm cells have a complete and efficient endocannabinoid system, and that activation of cannabinoid or vanilloid receptors controls, at different time-points, sperm functions required.
for fertilization. These observations open new perspectives on the understanding and treatment of male fertility problems.

**Toxicology**


Neonates that are exposed to cannabinoids in utero may have characteristic physical and mental developmental problems throughout their lives. The early identification of exposed neonates allows early intervention and anticipation of potential problems. Testing meconium detects maternal marijuana use over the last four months of gestation, providing a better drug exposure marker than urine. However, the distribution of metabolites in meconium is not identical to urine and analytical methods must be adapted. Both the major urine metabolite, 11-nor-9-carboxy-Delta9-tetrahydrocannabinol (9-carboxy-THC), and a minor urine metabolite, 11-hydroxy-Delta9-tetrahydrocannabinol (11-hydroxy-THC), are common in meconium. Currently published methods to extract these two metabolites for instrumental analysis are time-consuming and laborious, often involving the preparation of two fractions. This study describes a simple solid-phase extraction method and an optimized hydrolysis method that allow the preparation and analysis of both metabolites in a single extract. The limit of detection by this extraction method was 5 ng/g for both metabolites with an analytical measurement range from 10 to 500 ng/g. The recovery at 100 ng/g was greater than 62% for both analytes. The analysis of 246 cannabinoid screen positive specimens illustrated the importance of including the 11-hydroxy-THC in a meconium marijuana confirmation: 16 specimens confirmed positive for 11-hydroxy-THC only, resulting in a 6.5% increase in the positivity rate compared to 9-carboxy-THC alone.


Anandamide (N-arachidonylethanolamine) is an endogenous cannabinoid receptor ligand that has been implicated in various physiological and pathophysiological functions. In the present study, a liquid-liquid extraction-based reversed-phase HPLC method with fluorometric detection was validated and applied for the analysis of anandamide in human plasma. Following derivatization with the fluorogenic reagent 4-(N,N-dimethylaminosulfonyl)-7-(N-chloroformylmethyl-N-methyl-amino)-2,1,3-benzoxadiazole (DBD-COCl), the analyte was separated using an acetonitrile-water gradient at a flow rate of 0.8 mL/min, and spectrophotometric detection at 560 nm with an excitation wavelength of 450 nm. The retention times for anandamide and R(+)-methanandamide (internal standard) were 27.1 and 30.7 min, respectively. The validated quantification range was 1-15 ng/mL. The developed procedure was applied to determine anandamide levels in human plasma following a 24 h incubation of human whole blood at 37 degrees C in the presence or absence of phenylmethylsulfonyl fluoride, an inhibitor of the anandamide-degrading enzyme fatty acid amide hydrolase. Anandamide levels determined under both conditions were within the validated concentration range with anandamide levels being 2.3-fold higher in plasma from PMSF-treated blood. Copyright (c) 2005 John Wiley & Sons, Ltd.

**CLINICAL SCIENCE**

**Adverse events**


Marijuana is one of the most widely used recreational substances in the world, considered by many consumers as a relatively safe drug with few significant side-effects. We report the case of a 21-year-old man who suffered an acute myocardial infarction following the use of marijuana, despite having no other identifiable risk factors for an acute cardiovascular event. We review the published medical literature regarding acute cardiovascular events following
marijuana use and postulate a possible mechanism for this unusual pathological consequence of marijuana use.


AIMS: To investigate if associations between cannabis use and psychotic symptoms occur independently, or occur as a consequence of previous-other types of psychopathology.

METHODS: A 14-year follow-up study of 1580 initially 4- to 16-year-olds who were drawn randomly from the Dutch general population was conducted. At initial assessment, psychopathology was assessed with the Child Behavior Checklist. Across the 14-year follow-up period, cannabis use and psychotic symptoms were assessed with the Composite International Diagnostic Interview (CIDI). Because cannabis use is generally condoned in The Netherlands, false-negative reports of cannabis use may occur less frequently than in countries with stricter drug policies, which supports the value of the present study.

RESULTS: Survival analyses indicated that the association between cannabis use and psychotic symptoms occurred independently of initial CBCL scores.

CONCLUSIONS: The link between cannabis use and psychotic symptoms is specific, and does not depend on the earlier presence of other types of psychopathology. This indicates that research aimed at unraveling mechanisms that are responsible for this specific association is useful. Further, given the fact that cannabis use seemed to be a specific risk factor for future psychotic symptoms, prevention aimed against cannabis use may prohibit the onset of psychotic symptoms in vulnerable individuals.


Cannabis smoking is on the increase both in the United Kingdom and in the United States. For over three decades it has been known that cannabis has pathophysiological effects on the cardiovascular system, and previously an association with an increased risk of myocardial infarction has been reported. However, it is not yet known whether cannabis contributes directly to coronary artery disease. We describe two distinct cases; in the first cannabis use precipitated a malignant arrhythmia in a patient with critical ischaemia from longstanding coronary artery disease. In the second, a young patient presented with an acute myocardial infarction that had started whilst smoking marijuana; subsequently diffuse coronary artery disease was found at angiography despite the patient's low risk factor status. Patients who are known cannabis smokers and who have cardiovascular disease should be warned that it is likely to aggravate coronary ischaemia, and may even trigger myocardial infarction.


In many societies, marijuana is the second most commonly smoked substance after tobacco. While delta9-tetrahydrocannabinol (THC) is unique to marijuana and nicotine to tobacco, the smoke of marijuana, like that of tobacco, consists of a toxic mixture of gases and particulates, many of which are known to be harmful to the lung. Although far fewer marijuana than tobacco cigarettes are generally smoked on a daily basis, the pulmonary consequences of marijuana smoking may be magnified by the greater deposition of smoke particulates in the lung due to the differing manner in which marijuana is smoked. Whereas THC causes modest short-term bronchodilation, regular marijuana smoking produces a number of long-term pulmonary consequences, including chronic cough and sputum, histopathologic evidence of widespread airway inflammation and injury and immunohistochemical evidence of dysregulated growth of respiratory epithelial cells, that may be precursors to lung cancer. The THC in marijuana could contribute to some of these injurious changes through its ability to augment oxidative stress, cause mitochondrial dysfunction, and inhibit apoptosis. On the other hand, physiologic, clinical or epidemiologic evidence that marijuana smoking may lead to chronic obstructive pulmonary disease or respiratory cancer is limited and inconsistent. Habitual use of marijuana is also associated with abnormalities in the structure and function of alveolar macrophages, including impairment in microbial phagocytosis and killing that is associated with defective production of immunostimulatory cytokines and nitric oxide, thereby potentially predisposing to pulmonary
infection. In view of the growing interest in medicinal marijuana, further epidemiologic studies are needed to clarify the true risks of regular marijuana smoking on respiratory health.

**Case reports**


Insight-Adherence-Abstinence focused treatment for first episode of schizophrenia and schizoaffective patients is described using examples from clinical practice with 68 patients, 30 of whom have recent or active cannabis misuse. The treatment model is based on the unique characteristics of first-episode patients, who have little insight or experience with the relapses of chronic patients, demonstrate a great deal of denial, and frequently attribute their illness to cannabis. Treatment focuses on building adherence, abstinence, and insight during the first year of treatment in order to prevent repeated relapse and to optimize recovery. Interventions recognize the many needs of cannabis-using first-episode patients and therefore include supportive, cognitive-behavioral, behavioral, and motivational therapies, as well as skill building and psychoeducation.


Summary A 26-year-old man with a history of heavy marijuana and minimal tobacco use was found to have extensive bilateral lung bullae and interstitial fibrosis, heavily infiltrated by pigmented macrophages. These features can be associated with marijuana smoking. The differential diagnoses in this patient are also discussed.


No abstract.


**Clinical trials**


This study investigated the contribution of different cannabinoids to the subjective, behavioral and neurophysiological effects of smoked marijuana. Healthy marijuana users (12 men, 11 women) participated in four sessions. They were randomly assigned to a low or a high Delta-tetrahydrocannabinol group (THC; 1.8% versus 3.6%). In the four sessions under blinded conditions subjects smoked marijuana cigarettes containing placebo (no active cannabinoids), or cigarettes containing THC with low or high levels of cannabichromene (CBC; 0.1% versus 0.5%) and low or high levels of cannabidiol (CBD; 0.2% versus 1.0%). Dependent measures included subjective reports, measures of cognitive task performance and neurophysiological measures [electroencephalographic (EEG) and event-related potential (ERP)]. Compared to placebo, active THC cigarettes produced expected effects on mood, behavior and brain activity. A decrease in performance, reduction in EEG power and attenuation of ERP components reflecting attentional processes were observed during tests of working memory and episodic memory. Most of these effects were not dose-dependent. Varying the concentrations of CBC and CBD did not change subjects’ responses on any of the outcome measures. These findings are consistent with previous studies indicating that THC and its metabolites are the primary active constituents of marijuana. They also suggest that neurophysiological EEG and ERP measures are useful biomarkers of the effects of THC.

Regardless of a wide research interest the nature of a relationship between cannabis use and schizophrenia is controversial. One of the physiological abnormalities in schizophrenia is attention-modulated deficit in prepulse inhibition (PPI), which is a normal reduction in the startle reflex magnitude when a non-startling stimulus (prepulse) precedes the startling stimulus (pulse). This experiment was designed to determine whether or not otherwise healthy people using cannabis would exhibit attention-modulated deficit in PPI. The startle reflex was recorded in carefully screened healthy humans attending to and ignoring auditory pulse and prepulse stimuli separated by short (20-200 ms) and long prepulse intervals (1600 ms). In contrast to 12 non-using controls, cannabis use in 16 healthy humans was associated with significant reduction in %PPI while attending to auditory stimuli, but not while ignoring them. The PPI was correlated with the duration of cannabis use but not with the concentration of cannabinoid metabolites in urine and the recency of cannabis use in the preceding 24 hours. Cannabis use was not associated with changes in prepulse facilitation of startle reflex magnitude (%PPF) at long prepulse intervals, prepulse facilitation of startle reflex latency and startle reflex magnitude in the absence of prepulses. These results suggest that chronic, but not acute, use of cannabis is associated with schizophrenia-like disruption in PPI in healthy controls. Such reduction in PPI is attention-dependent and does not reflect a global deficit in sensorimotor gating in cannabis users.


BACKGROUND: Central pain in multiple sclerosis (MS) is common and often refractory to treatment. METHODS: We conducted a single-center, 5-week (1-week run-in, 4-week treatment), randomized, double-blind, placebo-controlled, parallel-group trial in 66 patients with MS and central pain states (59 dysesthetic, seven painful spasms) of a whole-plant cannabis-based medicine (CBM), containing delta-9-tetrahydrocannabinol:cannabidiol (THC:CBD) delivered via an oromucosal spray, as adjunctive analgesic treatment. Each spray delivered 2.7 mg of THC and 2.5 of CBD, and patients could gradually self-titrate to a maximum of 48 sprays in 24 hours. RESULTS: Sixty-four patients (97%) completed the trial, 34 received CBM. In week 4, the mean number of daily sprays taken of CBM (n = 32) was 9.6 (range 2 to 25, SD = 6.0) and of placebo (n = 31) was 19.1 (range 1 to 47, SD = 12.9). Pain and sleep disturbance were recorded daily on an 11-point numerical rating scale. CBM was superior to placebo in reducing the mean intensity of pain (CBM mean change -2.7, 95% CI: -3.4 to -2.0, placebo -1.4 95% CI: -2.0 to -0.8, comparison between groups, p = 0.005) and sleep disturbance (CBM mean change -2.5, 95% CI: -3.4 to -1.7, placebo -0.8, 95% CI: -1.5 to -0.1, comparison between groups, p = 0.003). CBM was generally well tolerated, although more patients on CBM than placebo reported dizziness, dry mouth, and somnolence. Cognitive side effects were limited to long-term memory storage. CONCLUSIONS: Cannabis-based medicine is effective in reducing pain and sleep disturbance in patients with multiple sclerosis related central neuropathic pain and is mostly well tolerated.

Reviews


Ajulemic acid (CT-3, IP-751, 1',1'-dimethylheptyl-Delta8-tetrahydrocannabinol-11-oic acid) (AJA) has a cannabinoid-derived structure; however, there is no evidence that it produces psychotropic actions when given at therapeutic doses. In a variety of animal assays, AJA shows efficacy in models for pain and inflammation. Furthermore, in the rat adjuvant arthritis model, it displayed a remarkable action in preventing the destruction of inflamed joints. A phase-2 human
trial with chronic, neuropathic pain patients suggested that AJA could become a useful drug for treating this condition. Its low toxicity, particularly its lack of ulcerogenicity, further suggests that it will have a highly favorable therapeutic index and may replace some of the current anti-inflammatory/analgesic medications. Studies to date indicate a unique mechanism of action for AJA that may explain its lack of adverse side effects.


Although used for more than 4000 years for recreational and medicinal purposes, Cannabis and its best-known pharmacologically active constituents, the cannabinoids, became a protagonist in medical research only recently. This revival of interest is explained by the finding in the 1990s of the mechanism of action of the main psychotropic cannabinoid, Delta(9)-tetrahydrocannabinol (THC), which acts through specific membrane receptors, the cannabinoid receptors. The molecular characterization of these receptors allowed the development of synthetic molecules with cannabinoid and noncannabinoid structure and with higher selectivity, metabolic stability, and efficacy than THC, as well as the development of antagonists that have already found pharmaceutical application. The finding of endogenous agonists at these receptors, the endocannabinoids, opened new therapeutic possibilities through the modulation of the activity of cannabinoid receptors by targeting the biochemical mechanisms controlling endocannabinoid tissue levels. Expected online publication date for the Annual Review of Medicine Volume 57 is January 7, 2006. Please see http://www.annualreviews.org/catalog/pub_dates.asp for revised estimates.


This article will examine harm reduction from a novel perspective. Its central thesis is that harm reduction is not only a social concept, but also a biological one. More specifically, evolution does not make moral distinctions in the selection process, but utilizes a cannabis-based approach to harm reduction in order to promote survival of the fittest. Evidence will be provided from peer-reviewed scientific literature that supports the hypothesis that humans, and all animals, make and use internally produced cannabis-like products (endocannabinoids) as part of the evolutionary harm reduction program.


**Surveys**


Surveys of physicians' attitudes regarding the therapeutic value of marijuana are rare. Drawing on a national sample of family physicians, general internists, obstetrician-gynecologists, psychiatrists, and addiction specialists, 960 (adjusted response rate 66%) offered opinions about the legal prescription of marijuana as medical therapy. Thirty-six percent believed prescribed marijuana should be legal and 26% were neutral to the proposition. Non-moralistic attitudes toward substance use were significantly associated with support for physician prescription, as was internal medicine and obstetrics-gynecology specialization. Physicians are, in general, less supportive than the general American public regarding the use of medical marijuana.


Since September 2003, cannabis is available for medicinal purposes in Dutch pharmacies to. It was anticipated that the medicinal cannabis use via illegal ways would decrease. The objective of this study was to get insight in the use of medicinal cannabis in daily practise as dispensed by community pharmacies and to characterize the users as well as the
symptoms and conditions cannabis is prescribed for. A prospective follow-up study among 200 patients who filled a prescription for medicinal cannabis was performed in the period between September 2003 and January 2004. The patients filled out a structured questionnaire concerning symptoms and conditions and their experience with cannabis. Of all patients, 42% suffered from multiple sclerosis, 11% suffered from rheumatic diseases, and 60% of respondents already used cannabis before the legalization. Cannabis was mainly used for chronic pain and muscle cramp/stiffness. The indication of medicinal cannabis use was in accordance with the labeled indications. However, more than 80% of the patients still obtained cannabis for medicinal purpose from the illegal circuit. Because of the higher prices in pharmacies, ongoing debate on the unproven effectiveness of the drug and the hesitation by physicians to prescribe cannabis.


Cannabinoids are increasingly being considered for the management of various painful conditions, and could be considered as an option for treating acute pain in sickle cell disease (SCD). The objective of this study was to determine the extent of use of cannabis in the community for pain and other symptom relief, and its side effects during self-administration in patients with SCD. Patients attending Central Middlesex Hospital in London were invited to complete a structured self-administered anonymous questionnaire. Eighty-six young adults with HbSS, HbSC and HbSbetathalassaemia disease (median age 30 years) participated in the study. Results showed that 31 (36%) had used cannabis in the previous 12 months to relieve symptoms associated with SCD. The main route in all but two patients was by smoking. The main reasons for use were to reduce pain in 52%, and to induce relaxation or relieve anxiety and depression in 39%. Symptoms related to sedation and mood effects were reported in 77% of patients. The majority of patients (58%) expressed their willingness to participate in studies of cannabis as a medicine. We conclude that research in the use of cannabinoids for pain relief in SCD would be both important and acceptable to adult patients.

BEHAVIOURAL SCIENCE

Addiction


Drug users have attentional biases for drug-related stimuli, and the frequency of drug use and craving are associated with attentional bias. However, research to date has not investigated the relationship between the severity of cannabis dependence, and attentional bias for cannabis-related stimuli. In the present study, 28 recreational cannabis users completed a modified Stroop task with cannabis-related and control words, plus questionnaire measures of cannabis dependence and craving. Participants were split into those who met diagnostic criteria for cannabis dependence based on scores on the Cannabis Severity of Dependence Scale (C-SDS), and those who did not. The cannabis-dependent group had a significant attentional bias for the cannabis-related words, but the non-dependent group did not. Correlations indicated that attentional bias was also associated with the frequency of cannabis use, and with subjective craving.


AIM: To examine prospectively over a period of 4 years the profile of cannabis dependence and the risk of specific dependence criteria in a community sample of adolescents. METHODS: A representative community sample of 2446 young adults aged 14-24 years at baseline was followed up over a period of 4 years. Frequency of use measures and of criteria for DSM-IV dependence were assessed by standardized diagnostic interview measures (CIDI). To explore the nature of this association, frequency of use and concomitant use of other...
psychoactive substances was considered. RESULTS: 30% of the sample were cannabis users. Among all users 35% met at least one dependence criterion. Most frequently reported dependence criteria among all users were withdrawal (17%), tolerance (15%), loss of control (14%) and continued use despite a health problem (13%). Even without concomitant use of other illicit drugs, 22% of low frequency users and 81% of high frequency users met at least one dependence criterion. Symptom patterns were similar in high and low frequency users. The occurrence of a dependence syndrome or of specific dependence criteria could not be attributed to the use of other illicit drugs or to comorbid nicotine and alcohol dependence. CONCLUSIONS: Regular cannabis use in adolescence is associated with the development of a dependence syndrome. This association cannot be explained by the concomitant use of other illicit substances or by comorbid nicotine and alcohol dependence.


**ABSTRACT** Aims To examine the risk posed by cannabis use in young people for tobacco use disorders. Specifically we examined whether cannabis use in non-smokers predicted later initiation of tobacco use and whether cannabis use predicted later nicotine dependence in tobacco users. Design A 10-year eight-wave cohort study. Setting State of Victoria, Australia. Participants A community sample of 1943 participants initially aged 14-15 years. Measurements Self-report of tobacco and cannabis use was assessed in the teens using a computerized interview assessment and in young adulthood with a CATI assessment. The Fagerstrom Test for Nicotine Dependence was used to define nicotine dependence. Findings For teen non-smokers, at least one report of weekly cannabis use in the teens predicted a more than eightfold increase in the odds of later initiation of tobacco use (OR 8.3; 95% CI 1.9-36). For 21-year-old smokers, not yet nicotine-dependent, daily cannabis use raised the odds of nicotine dependence at the age of 24 years more than threefold (OR 3.6; 1.2, 10) after controlling for possible confounders, including level of tobacco use and subsyndromal signs of nicotine dependence. Conclusions Weekly or more cannabis use during the teens and young adulthood is associated with an increased risk of late initiation of tobacco use and progression to nicotine dependence. If this effect is causal, it may be that a heightened risk of nicotine dependence is the most important health consequence of early frequent cannabis use.


The study examined models of marijuana (n = 309) and alcohol (n = 731) problems. Impulsivity was directly associated with both marijuana- and alcohol-related problems. Negative mood regulation expectancies were indirectly associated with marijuana problems through coping motives. Sensation seeking was indirectly associated with alcohol problems through enhancement motives. Affect lability and negative affect were indirectly associated with alcohol problems though coping motives. In both models, coping motives were directly associated with use-related problems. A multigroup analysis indicated that the association between negative affect and coping motives as well as use and problems was stronger among participants using both alcohol and marijuana relative to alcohol only. Enhancement motives were a stronger predictor of alcohol use among participants using alcohol only. ((c) 2005 APA, all rights reserved).


Despite epidemiological reports indicating an association between social anxiety disorder (SAD) and cannabis use disorders (CUD), there is a paucity of research exploring the nature of this relationship. The present investigation examined potential moderators of this relationship that are consistent with a tension-reduction model of addiction. Specifically, physiological reactivity to stress and perceived coping with stress were evaluated as moderators of the relation between symptoms of SAD and CUD. Physiological (SCR) and subjective (perceived coping) responses to unpredictable white noise bursts were collected from non-clinical participants (n=123). Lifetime symptoms of CUD and anxiety disorders were assessed using a structured diagnostic interview. CUD symptomatology was associated with symptoms of SAD but not with symptoms of any other
anxiety disorder. Only perceived coping to unpredictable stimuli moderated the relationship between SAD and CUD symptoms. Findings are discussed in the context of tension-reduction models of co-occurring social anxiety and problematic cannabis use.


Though marijuana has been reported to stimulate appetite, we searched for a correlation between obesity and decreased marijuana use. We examined charts of all females referred for morbid obesity/weight management in a 12-month period. BMI and substance use data were collected from 297 charts. While 29% of the sample with BMI < 30 (n = 7) used marijuana in the past year, only 21% of those with BMI 30-39 (n = 84), 16% of those with BMI 40-49 (n = 110) and 14% (n = 96) of those with BMI > 50 used marijuana in the past year. Linear regression revealed a negative correlation between BMI group and percent marijuana use (R-squared = 0.96; P = 0.0173). These findings provide support for overeating as competition for drugs and alcohol in brain reward sites.


PURPOSE: To investigate relationships between adolescents' current alcohol, tobacco, and marijuana use; perceptions of neighborhood disorder; and sense of hope. DESIGN: Questionnaires were administered to a nonrandom sample of middle school students during the spring of 1999. SUBJECTS: The ethnically and geographically diverse sample (n = 369), from a range of low socioeconomic status backgrounds, was considered to be at high risk for alcohol, tobacco, and other drug use because of previous enrollment in low socioeconomic status elementary schools. MEASURES: Alcohol, tobacco, and marijuana use were dichotomized into current and never/no use. Six variables described neighborhood social disorder. Sense of hope was assessed using the Children's Hope Scale. RESULTS: Statistically significant relationships were found between perceived neighborhood disorder and current alcohol (p = .01), tobacco (p = .001), and marijuana (p < .001) use. A statistically significant and independent relationship was found between sense of hope and current alcohol (p = .02), tobacco (p = .02), and marijuana (p = .06) use. Results indicated linear trends in participants' increased use of alcohol, tobacco, and marijuana and (1) perception of higher neighborhood disorder and (2) lower sense of hope. CONCLUSION: Substance use prevention programs for youth might usefully be directed not only to adolescents but also to the neighborhoods in which they live. Additionally, it would be important to emphasize creating safer neighborhood environments that support the development of a stronger sense of hope for the future.

Driving studies

BACKGROUND: The aim of the study was to map the prevalence of alcohol and other psychotropic substances in deceased participants of traffic accidents in the Czech Republic. METHODS AND RESULTS: The studied sample included persons autopsied in the departments of forensic medicine and forensic toxicology that died during traffic accidents and were toxicologically tested in 2003. Case definition involved alcohol cases with blood alcohol concentration (BAC) 0.2 g/kg and higher, with cannabis, detections of active THC metabolites only were taken into account; in cases where volatile substances (solvents) were detected we included into the definition only cases with substances not produced post mortem or in some physiological or pathological statuses. We identified 554 cases of whom 440 (79.4%) were males and 114 (20.6%) were females. 35.5% were in the age group 20 - 34 years. The sample has been classified into 4 categories (average age, % of males): pedestrians (45.4, 76.2), drivers (36.3, 91.2) and others (36.1, 66.2). Alcohol was tested in 548 cases, 214 (39.1%) of those were found positive. 380 cases were tested for other psychotropic substances than alcohol; samples taken from 25 bodies (6.6%) were found positive for at least for one of
these substances. 8 cases were positive both for alcohol and some other psychotropic substance - i.e. 3.7% out of 214 cases positive for alcohol were positive for other substance and 32.0% out of 25 cases positive at least for any other psychotropic substance were alcohol-positive. When focusing our analysis at the active participants of road traffic accidents only - pedestrians, bicyclists and drivers (altogether 397 cases) - we have found alcohol to be tested in 394 cases, out of which 158 (39.1%) were positive; as for other psychotropic substances, 314 cases were tested and 23 (7.3%) were positive at least for one of them. 7 cases were found positive for alcohol and other psychotropic substance simultaneously; this represents 4.4% out of 158 cases positive for alcohol and 30.4% out of 23 cases positive at least for one psychotropic substance other than alcohol. Average BAC in active participants of road traffic accidents positive for alcohol were 1.81%, (1.98%o in pedestrians, 1.78 %o in bicyclists and 1.64 %o in drivers). Prevalence of either alcohol or any other psychotropic substances is the lowest in the category of drivers - with the exception of active cannabinoid compounds. Alcohol was by far the most prevalent psychotropic substance, also cannabis, benzodiazepines and stimulants have been found in not negligible frequencies. CONCLUSIONS: The study confirms high prevalence of alcohol influence in deceased participants of traffic accidents. Prevalence of other psychotropic substances is lower by order, but it becomes also significant.


ABSTRACT While there is a great deal of data documenting the etiologic role alcohol use plays in crash culpability, there is a dearth of data for other drugs. The purpose of this study was to assess crash culpability for single drug use among injured drivers admitted to a regional trauma center. This study is the largest of its kind involving trauma center patients. Clinical toxicology results obtained for patient care were linked to police crash reports containing a field attributing crash culpability. Drugs studied were alcohol, cocaine, and marijuana. As expected crash culpability was strongly associated with pre-crash alcohol use. In contrast, for both men and women, this study did not find an association between crash culpability and marijuana use. The data documents a significant association between cocaine use and crash culpability for both sexes and for drivers 21 to 40 years of age. This is the first large study to assess for crash culpability among injured drivers relative to cocaine use. Each year approximately 42 to 43,000 people die annually as the result of vehicular crashes. (NHTSA, 2005) For the decade 1994 through 2003, alcohol was a factor in 40-43% fatal injury crashes - the fatally injured person being either a vehicular occupant or pedestrian. Specifically 25 to 29% of drivers of cars and light trucks involved in those crashes were alcohol positive. Further, it is estimated that 80% or more of those drivers had blood alcohol concentrations (BAC) of 80 mg/dl or greater. (NHTSA, 2005).

Population studies


AIM: To test whether a single session of Motivational Interviewing (MI) focussing on drinking alcohol, and cigarette and cannabis smoking, would successfully lead to reductions in use or problems. METHODS: Naturalistic quasi-experimental study, in 162 young people (mean age 17 years) who were daily cigarette smokers, weekly drinkers or weekly cannabis smokers, comparing 59 receiving MI with 103 non-intervention assessment-only controls. MI was delivered in a single session by youth workers or by the first author. Assessment was made of changes in self-reported cigarette, alcohol, cannabis use and related indicators of risk and problems between recruitment and after 3 months by self-completion questionnaire. RESULTS: 87% of subjects (141 of 162) were followed up. The most substantial evidence of benefit was achieved in relation to alcohol consumption, with those receiving MI drinking on average two days per month less than controls after 3 months. Weaker evidences of impact on cigarette smoking, and no evidence of impact on cannabis use, were obtained. CONCLUSIONS: Evidence of effectiveness for the
delivery of MI by youth workers in routine conditions has been identified. However, the extent of benefit is much more modest than previously identified in efficacy studies.


In this study, we assessed the feasibility and effectiveness of the Adolescent Cannabis Check-Up (ACCU), a brief intervention for young cannabis users. For this initial feasibility study, we used an uncontrolled pre-test/post-test design. Participants were cannabis users aged between 14 and 19 years (n = 73) and concerned parents (n = 69). The intervention comprised an individual assessment session followed 1 week later by a session of personalized feedback delivered in a motivational interviewing style. An optional third session that focused on skills and strategies for making behavioral change was offered. Of the entire sample of cannabis users, 78% reported voluntarily reducing or stopping their cannabis use during the 90 days to follow-up and 16.7% reported total abstinence during this time. In addition, significant reductions were found on measures of both quantity and frequency of use and dependence. These reductions were maintained at 6-month follow-up. Clearly, these preliminary findings must be interpreted with caution given the study design and absence of a control group. The ACCU was, however, able to attract and retain young cannabis users who were not necessarily interested in change. The approach was acceptable to young people and associated with reductions in cannabis use. It appears to be a model that warrants further research in early and brief interventions for this population.


This study aims to estimate changes in the prevalence of ecstasy use over time, analyze the overlap of ecstasy use and other drug use, and compare other drug use in ecstasy versus marijuana users. The authors hypothesized that ecstasy users early in the "epidemic" would be polydrug users and that associations between ecstasy and other drug use would diminish as the prevalence of ecstasy use increased. Data were drawn from public use data files from the 1995, 1997, 1999, and 2001 National Household Survey on Drug Abuse. Ecstasy use increased in the U.S. population and the prevalence was greater in younger age groups. Ecstasy users were likely to use a variety of other drugs; however, association of ecstasy use with other drug use was strongest early in the "epidemic," diminishing as the number of new users increased. Later, more drug-naive adolescents and young adults began experimenting with ecstasy. These results can orient prevention strategies that target ecstasy users. ((c) 2005 APA, all rights reserved).


ABSTRACT Aim To assess the impact of telephone audio computer-assisted self-interviewing (T-ACASI) on reporting of alcohol use, alcohol problems and illicit drug use in telephone surveys of the general population. Prior research suggests that illicit drug use is underreported in traditional, interviewer-administered, telephone surveys. Design Randomized experiment embedded in telephone survey of probability samples of populations of USA and Baltimore, MD. Survey respondents were randomly assigned to be interviewed either by human telephone interviewers or by T-ACASI after household screening, recruitment, and informed consent procedures were completed. Setting Respondents were interviewed by telephone in their homes. Participants Probability samples of 1543 English-speaking adults ages 18-45 residing in telephone-accessible households in USA and 744 similarly defined adults residing in Baltimore, MD, USA. Measurements Nine questions on alcohol, marijuana, cocaine, and injection drug use adapted from 1994 NHSDA and four CAGE questions on alcohol problems. Crude odds ratios and odds ratios controlling for demographic factors calculated to test for differences between responses obtained by T-ACASI and human interviewers. Findings T-ACASI had mixed effects on reporting of alcohol use, but it did increase reporting of one of four CAGE alcohol problems: feeling guilty about drinking (23.0% in T-ACASI vs. 17.6% in T-IAQ, OR = 1.4, P < 0.01). T-ACASI also obtained significantly more frequent reporting of marijuana, cocaine, and injection
drug use. The impact of T-ACASI was most pronounced for reporting of recent use of ‘harder’ drugs. Thus T-ACASI respondents were more likely to report marijuana use in the past month (10.0% vs. 5.7%, crude OR = 1.9, P < 0.001), cocaine use in the past month (2.1% vs. 0.7%, crude 3.2, P < 0.001) and injection drug use in the past five years (1.6% vs. 0.3%, crude OR = 4.8, P < 0.01). Conclusions Telephone survey respondents were more likely to report illicit drug use and one alcohol problem when interviewed by T-ACASI rather than by human telephone interviews.