INTRODUCTION

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BASIC SCIENCE


Cannabinoids have been shown to increase the release of arachadonic acid, whereas nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to decrease the analgesic effects of cannabinoids. We evaluated the antinociceptive effects of chronic administration of Delta(9)-tetrahydrocannabinol (Delta(9)-THC), anandamide (an endogenous cannabinoid), arachadonic acid, ethanolamine, and methanandamide on several NSAIDs via p.o. and/or i.p. routes of administration using the mouse p-phenylquinone (PPQ) test, a test for visceral nociception. Our studies with a cannabinoid receptor (CB1) antagonist [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride (SR141716A)], a CB2 antagonist [N-((1S)-endo-1,3,3-trimethyl-bicyclo-heptan-2-yl]-5-(4-chloro-3-methylphe nyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) (SR144528)], and an another CB2 agonist [1,1-dimethylbutyl-1-deoxy-Delta(9)-THC (JWH-133)] were performed to better characterize PPQ interactions with cannabinoid receptors. The acute affects of Delta(9)-THC were blocked by SR141716A (i.p.) and partially blocked by SR144528 (i.p.). When NSAIDs (p.o.) were administered, the ED(50) values were as follows: 23 mg/kg aspirin, 3 mg/kg indomethacin, 5 mg/kg celecoxib, 3 mg/kg ketorolac, 57 mg/kg acetaminophen (32.3-99.8), and 0.8 mg/kg diclofenac (0.1-4.9). In animals given chronic Delta(9)-THC, only diclofenac and acetaminophen were active. Conversely, chronic methanandamide (i.p.) did not alter the antinociceptive effects of the NSAIDs. Neither the CB1 or CB2 antagonist blocked the effects of the NSAIDs. The effects of chronic arachadonic acid, ethanolamine, and anandamide could not be evaluated. In summary, our data indicate that chronic Delta(9)-THC alters the cyclooxygenase system. Alternatively, the data suggest that this alteration is not due to chronic endogenous cannabinoid release. Based upon these data, we hypothesize that human subjects who are chronic users of Delta(9)-THC may not respond to analgesic treatment with the above NSAIDs.


The cytoplasmic helix domain (fourth cytoplasmic loop, helix 8) of numerous G protein-coupled receptors (GPCRs) such as rhodopsin and the beta-adrenergic receptor exhibit unique structural and functional characteristics. Computer models also predict this structure for the cannabinoid CB2 receptor, another member of the GPCR superfamily. In our study, a peptide corresponding to helix 8 of the CB2 receptor was synthesized chemically and its secondary structure determined by circular dichroism (CD) and 1H NMR spectroscopy. NMR and CD revealed an alpha-helical structure in this region in both dodecylphosphocholine micelles and dimethylsulfoxide, in contrast to a random coil configuration found in aqueous solvent. This finding is in good agreement with other previous GPCR structural studies including X-ray
By combining our finding with other studies, we further hypothesize that the amphipathic nature of helix 8 can play a significant role in the function and regulation of CB receptors as well as other GPCRs in general.


Synchronized activity of neuronal networks has been proposed to be essential for cerebellar function. To examine the occurrence and organization of spontaneous neuronal activity in the cerebellum in vivo, we imaged mouse cerebellar slices loaded with the intracellular Ca2+ concentration indicator, fura-2. Recordings were then analysed statistically to identify correlated network activity. Ca2+ imaging revealed consistent spontaneous correlated network activity of granule cells (GC), which often occurred in clusters of coactivated GC. The number of spontaneously active GC, their activation frequency and correlation, were controlled by glutamate and GABA ionotropic receptors. These findings indicate that distinctive patterns of correlated activity between GC networks may be relevant for cerebellar circuit function. Cannabinoid antagonist-precipitated Delta9-tetrahydrocannabinol (THC) withdrawal impaired motor coordination. Given that the cerebellum has been suggested recently to be a main substrate for cannabinoid withdrawal, we used imaging of spontaneous network activity to examine whether GC, which contain CB1 cannabinoid receptors, respond to chronic THC treatment and withdrawal. Acute administration of THC had no effect on patterns of spontaneous GC network activity. In contrast, chronic THC administration severely impaired GC activity and network coordination. Incubation of cerebellar slices, from chronically THC-treated mice, with the cannabinoid antagonist, SR141716A increased the number and network correlation of active GC. These data provide physiological evidence of the involvement of cerebellar circuits in the adaptive changes occurring during chronic THC exposure and withdrawal.


Improgan, a nonopioid antinociceptive agent, activates descending, pain-relieving mechanisms in the brain stem, but the receptor for this compound has not been identified. Because cannabinoids also activate nonopioid analgesia by a brain stem action, experiments were performed to assess the significance of cannabinoid mechanisms in improgan antinociception. The cannabinoid CB(1) antagonist N-(piperidin-1-yl)-5-(4-chloro phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) induced dose-dependent inhibition of improgan antinociception on the tail-flick test after i.c.v. administration in rats. The same treatments yielded comparable inhibition of cannabinoid [R-(+)-(2,3-dihydro-5-methyl-3-{[(4-mor pholinyl)methyl]pyrrol[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthalenyl)methan one monomethanesulfonate, WIN 55,212-2] analgesia. Inhibition of improgan and WIN 55,212-2 antinociception by SR141716A was also observed in Swiss-Webster mice. Radioligand binding studies showed no appreciable affinity of improgan on rat brain, mouse brain, and human recombinant CB(1) receptors, ruling out a direct action at these sites. To test the hypothesis that CB(1) receptors indirectly participate in improgan signaling, the effects of improgan were assessed in mice with a null mutation of the CB(1) gene with and without SR141716A pretreatment. Surprisingly, improgan induced complete antinociception in both CB(1) (-/-) and wild-type control [CB(1) (+/+) ] mice. Furthermore, SR141716A inhibited improgan antinociception in CB(1) (+/+) mice, but not in CB(1) (-/-) mice. Taken together, the results show that SR141716A reduces improgan antinociception, but neither cannabinoids nor CB(1) receptors seem to play an obligatory role in improgan signaling. Present and previous studies suggest that Delta(9)-tetrahydrocannabinol may act at both CB(1) and other receptors to relieve pain, but no evidence was found indicating that improgan uses either of these mechanisms. SR141716A will facilitate the study of improgan-like analgesics.

This study examined the effects of Delta(9)-tetrahydrocannabinol (Delta(9)-THC) and the CB1 antagonist SR-141716 on open-field behaviors in male Sprague-Dawley rats. Animals were examined after administration of Delta(9)-THC alone (dose range: 0.3-5.6 mg/kg), SR-141716 alone (dose range: 1-5.6 mg/kg) and the two drugs in combination; injections were given intraperitoneally 30 min prior to testing. There was a dose-related suppression of ambulation (horizontal activity) and rearing (vertical activity) after Delta(9)-THC administration. Co-administration of SR-141716 counteracted this suppression; however, antagonism was only partial for rearing. Interestingly, 1 mg/kg SR-141716 was as effective as 3 and 5.6 mg/kg SR-141716 in this antagonist action. Increasing doses of Delta(9)-THC produced an increase in circling behavior; latency to leave the starting area in the center of the field was significantly elevated by 5.6 mg/kg Delta(9)-THC. Those effects were completely blocked by SR-141716. Grooming and scratching showed a dose-related increase following administration of SR-141716 (1-5.6 mg/kg), which were only partially blocked by co-administration of Delta(9)-THC (3 and 5.6 mg/kg). When given alone, only the highest dose of SR-141716 (5.6 mg/kg) depressed ambulation; rearing and latency were not significantly changed, and circling was absent. Differences in the number of vocalizations, urination and defecation generally did not differ clearly among the treatment conditions. These results may show that SR-141716 is acting as (i) an inverse agonist and/or (ii) that the endogenous cannabinoid system is tonically active under certain conditions.


PURPOSE. The endogenous cannabinoids N-arachidonylethanolamide (AEA) and 2-arachidonylglycerol (2-AG) are known to decrease intraocular pressure (IOP). Recently, a novel putative endogenous cannabinoid, noladin ether, was isolated in porcine and rat brains. In the present study, both the degradation of endogenous cannabinoids in ocular tissues and the effect on IOP of 2-AG and noladin ether were compared. METHODS. The rates of enzymatic degradation for AEA, 2-AG, and noladin ether were determined in bovine cornea and iris-ciliary body homogenates. 2-AG and noladin ether were dissolved in either hydroxypropyl-beta-cyclodextrin (HP-beta-CD) or propylene glycol and administered unilaterally to the rabbit eye. IOPs were measured in the treated and untreated eyes. The CB1 receptor antagonist AM251 was administered topically 15 minutes before the cannabinoids to investigate whether CB1 receptors mediate the effect on IOP produced by 2-AG and noladin ether. RESULTS. Noladin ether degraded more slowly than either 2-AG or AEA in the iris-ciliary body and cornea homogenates. The effect on IOP of 2-AG was biphasic (i.e., an initial increase in IOP followed by a reduction in the treated eye). Noladin ether decreased IOP immediately after topical administration, and no initial IOP increase was observed in the treated eye. The CB1 receptor antagonist AM251 (25 micro g) blocked the effect on IOP of noladin ether but did not affect the action of 2-AG. CONCLUSIONS. Topical administration of the novel putative endogenous cannabinoid noladin ether decreased IOP in rabbits. This IOP reduction was most probably mediated through the CB1 receptor. The effect on IOP of noladin ether differed from those of the known endogenous cannabinoids AEA and 2-AG, probably because of its more stable chemical structure.


Background & Aims: Transient lower esophageal sphincter relaxations (TLESRs) are the major cause of gastroesophageal acid reflux, and are triggered by postprandial gastric distention. Stimulation of GABA(B) receptors potently inhibits triggering of TLESR by gastric loads. The functional similarity between GABA(B) and cannabinoid receptors (CBRs) prompted us to study the role of CBRs on mechanisms of gastric distention-induced TLESRs. METHODS: Gastric nutrient infusion and air insufflation was performed during gastroesophageal manometry in conscious dogs. The effects of the CBR agonist WIN 55,212-2 were assessed alone and in
combination with the CBR1 antagonist SR141716A or the CBR2 antagonist SR144528. The effects of WIN 55,212-2 were also studied on firing of gastric vagal mechanosensitive afferents in an isolated preparation of ferret stomach. RESULTS: WIN 55,212-2 (57 nmol/kg) inhibited the occurrence of TLESR after gastric loads by 80% (P < 0.01). The latency to the first TLESR after the load was prolonged (P < 0.001), and the occurrence of swallowing was reduced (P < 0.05). The CBR1 antagonist SR141716A reversed the effects of WIN 55,212-2, whereas the CBR2 antagonist SR144528 did not. The CBR1 antagonist alone increased occurrence of TLESR (P < 0.05). The responses of gastric vagal mechanoreceptors to distention were unaffected by WIN 55,212-2 at a concentration of 3 &mgr;mol/L. CONCLUSIONS: Exogenous and endogenous activation of the CBR1 receptor inhibits TLESRs. The effects of CBR1 are not mediated peripherally on gastric vagal afferents, and therefore are most likely in the brain stem.


Stearoylthanolamide (SEA) is present in human, rat, and mouse brain in amounts comparable to those of the endocannabinoid anandamide (arachidonylethanolamide, AEA). Yet, the biological activity of SEA has never been investigated. We report that SEA has the same effects as AEA on catalepsy, motility, analgesia, and body temperature of mice and that specific binding sites for SEA are present in mouse brain and are most abundant in cortex. Pharmacological experiments and the use of knockout mice demonstrated that these sites are different from cannabinoid receptors, are not coupled to G proteins, and regulate different signaling pathways. Mouse brain has also a specific SEA membrane transporter and a fatty acid amide hydrolase able to cleave SEA, with the same regional distribution as the binding sites of this lipid. Moreover, SEA potentiates the decrease of cAMP induced by AEA in mouse cortical slices, suggesting that SEA might also be an "entourage" compound.


Two radioligands, [11C] SR149080 and its morpholino analog [11C] SR149568, were synthesized by reaction of the respective phenolic precursors with [11C] methyl iodide. Both radioligands had appropriate regional brain distribution for cannabinoid receptors in mice with peak target to non-target ratios of 2.2 for [11C] SR149080 and 1.6 for [11C] SR149568 at 90 and 30 minutes post-injection respectively. The uptake of both tracers was blocked with a 1 mg/kg dose of SR141716A.


The extent to which acute and repeated administration of the CB(1) agonist WIN 55,212-2 would affect the stimulatory properties of amphetamine was assessed in Lewis rats. In the first experiment, Lewis rats were treated with either 1 mg/kg of WIN 55,212-2 or vehicle and subsequently treated with 2 mg/kg amphetamine. Acute treatment with WIN 55,212-2 initially increased locomotor activity and then attenuated the stimulating effect of amphetamine on locomotion and exploration (as measured by rears). In a separate experiment, Lewis rats were given daily injections of either WIN 55,212-2 (1 mg/kg) or vehicle for 10 days and the effects of amphetamine were assessed at 1 and 3 days following the last chronic cannabinoid treatment. Those rats, which had been treated with WIN 55,212-2, had an enhanced response to amphetamine with rearing but not with ambulatory movements, suggesting the occurrence of behavioral cross-sensitization to the ability of amphetamine to increase rearing. These data add to the growing evidence that there is at least some overlap between those neural systems acted upon by cannabinoids and those that are believed to be involved in incentive properties associated with other drugs of abuse.

All of the therapeutic properties of marihuana (analgesic, antiemetic, appetite stimulant, antiglaucoma) have been duplicated by the tetrahydrocannabinol (THC) molecule or its synthetic derivatives. Today, the molecular mechanisms of action of these compounds have led to a general understanding of the pharmacological effects of marihuana and of its therapeutic properties. These mechanisms involve the specific binding of THC to the 7-transmembrane (7TM) domain G protein-linked receptor, a molecular switch which regulates signal transduction in the cell membrane. The natural ligand of the 7TM receptor is an eicosanoid, arachidonylethanolamide (AEA), generated in the membrane and derived from arachidonic acid. THC acts as a substitute ligand to the 7TM receptor site of AEA. THC would deregulate the physiological function of the 7TM receptor and of its ligand AEA. As a result, the therapeutic effects of the drug may not be separated from its adverse psychoactive and cardiovascular effects. The binding of THC to the 7TM receptor site of AEA induces allosteric changes in the receptor sites of neurotransmitter and opiates resulting in variable interactions and pharmacological responses. The pharmacokinetics of THC with its prolonged storage in fat and its slow release result in variable and delayed pharmacological response, which precludes precise dosing to achieve timely therapeutic effects. The experimental use of THC and of its synthetic analogues, agonists, and antagonists has provided novel information in the nature of molecular signaling in the cell membrane. As a result, the relationships between allosteric receptor responsiveness, molecular configuration of proteins, and physiological regulation of cellular and organ function may be further investigated.


To identify novel genes involved in cannabinoid receptor-mediated signaling, we used cDNA microarrays to detect changes in mRNA expression in the forebrains of mice 12 h after they were given a single intraperitoneal dose of the naturally-occurring Cannabis sativa alkaloid Delta(9)-tetrahydrocannabinol (Delta(9)-THC) or the synthetic cannabinoid receptor agonist (R)-(+)-2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-de]-1,4-benzoxazin-yl-1-naphthalenylmethanone mesylate [R(+)-WIN 55,212-2]. Of ~11,000 genes from a mouse brain cDNA library that were probed, 65 showed altered (increased or decreased at least 2-fold) expression after exposure to Delta(9)-THC, 41 after exposure to R(+)-WIN 55,212-2, and 20 genes after exposure to both drugs. Genes affected similarly by Delta(9)-THC and R(+)-WIN 55,212-2 were considered likely to reflect cannabinoid receptor activation, and expression of the protein products of two such genes not previously implicated in cannabinoid signaling—melanocyte-specific gene-related gene 1 (MRG1) and hexokinase 4 (glucokinase, GK)—was measured by Western blotting and immunohistochemistry. Western blots showed ~2-fold increases in the levels of both proteins in mouse forebrain. Immunohistochemistry revealed preferential localization of MRG1 to cerebral blood vessels and of GK to hypothalamic neurons. These findings suggest that MRG1 and GK are cannabinoid-regulated genes and that they may be involved in the vascular and hypothalamic effects of cannabinoids, respectively.


Neuronal elements are increasingly suggested as primary targets of an autoimmune attack in certain neurological and neuropsychiatric diseases. Type 1 cannabinoid receptors (CB1) were selected as autoimmune targets because they are predominantly expressed on neuronal surfaces in brain and display strikingly high protein levels in striatum, hippocampus, and cerebellum. Female Lewis rats were immunized with N-terminally acetylated peptides (50 or 400 &mgr;g per rat) of the extracellular domains of the rat CB1 and killed at various time points. Subsequent evaluation using immunohistochemistry and in situ hybridization showed dense infiltration of immune cells exclusively within the cerebellum, peaking 12-16 days after...
immunization with the CB1 peptide containing amino acids 9-25. The infiltrates clustered in meninges and perivascular locations in molecular and granular cell layers and were also scattered throughout the CB1-rich neuropil. They consisted primarily of CD4(+) and ED1(+) cells, suggestive of cell-mediated autoimmune pathology. There were no inflammatory infiltrates elsewhere in the brain or spinal cord. The results show that neuronal elements, such as neuronal cell-surface receptors, may be recognized as antigenic targets in a cell-mediated autoimmune attack and, therefore, support the hypothesis of cell-mediated antineuronal autoimmune pathology in certain brain disorders. Published 2002 Wiley-Liss, Inc.


Binding of the endocannabinoid anandamide or of Delta(9)-tetrahydrocannabinol to the agonist site of the cannabinoid receptor (CB1) is commonly assayed with [3H]CP 55,940. Potent long-chain alkylfluorophosphonate inhibitors of agonist binding suggest an additional, important and closely-coupled nucleophilic site, possibly undergoing phosphorylation. We find that the CB1 receptor is also sensitive to inhibition in vitro and in vivo by several organophosphorus pesticides and analogs. Binding of [3H]CP 55,940 to mouse brain CB1 receptor in vitro is inhibited 50% by chlorpyrifos oxon at 14 nM, chlorpyrifos methyl oxon at 64 nM and paraoxon, diazoxon and dichlorvos at 1200-4200 nM. Some 15 other organophosphorus pesticides and analogs are less active in vitro. The plant defoliant tribufos inhibits CB1 in vivo, without cholinergic poisoning signs, by 50% at 50 mg/kg intraperitoneally with a recovery half-time of 3-4 days, indicating covalent derivatization. [3H-ethyl]Chlorpyrifos oxon may be suitable for radiolabeling and characterization of this proposed nucleophilic site.

Laboratory research has shown promising results for using plant-derived, synthetic or endogenous cannabinoids for analgesia. However, human studies are few and far between and have been held up by the law and the lack of standardized extracts. This paper considers these drugs' potential uses in pain management.


Capsaicin-sensitive sensory nerves are widely distributed in the cardiovascular system. They are activated by a variety of physical and chemical stimuli, characteristically by capsaicin acting via the vanilloid receptor VR1, and have a role in the regulation of peripheral vascular resistance and maintenance of homeostasis via their afferent and efferent functions. Cannabinoids, a recently discovered family of extracellular signalling molecules, can act at cannabinoid (CB) receptors expressed on sensory nerves, to cause inhibition of sensory neurotransmitter release. There is recent evidence, however, that anandamide, an endogenous cannabinoid, can activate VR1, coexpressed with CB receptors on the same sensory nerve terminals, causing a release of sensory neurotransmitter, vasorelaxation and hypotension. Hence, anandamide can elicit opposite actions, inhibition via CB receptors and excitation via VR1, on sensory neurotransmission. The possible biological significance of this is discussed.


CB(1) cannabinoid receptors mediate profound hypothermia when cannabinoid agonists are administered to rats. Glutamate, the principal excitatory neurotransmitter in the central nervous system (CNS), is thought to tonically increase body temperature by activating N-methyl-D-aspartate (NMDA) receptors. Because NMDA antagonists block cannabinoid-induced antinociception and catalepsy, intimate glutamatergic-cannabinoid interactions may exist in the CNS. The present study investigated the effect of two NMDA antagonists on the hypothermic response to WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6 H-pyrrolo[3,2,1-i,j]quinolin-6-one], a selective cannabinoid agonist, in rats. WIN
55212-2 (1-10 mg/kg i.m.) produced dose-dependent hypothermia that peaked 60 to 180 min postinjection. Dextromethorphan (5-75 mg/kg i.m.), a noncompetitive NMDA antagonist, or LY 235959 [(−)-6-[phosphonomethyl]-1,2,3,4,4a,5,6,7,8a-decahydro-isoquinoline-2-carboxylate](1-4 mg/kg i.m.), a competitive and highly selective NMDA antagonist, evoked hypothermia in a dose-sensitive manner, suggesting that endogenous glutamate exerts a hyperthermic tone on body temperature. A dose of dextromethorphan (10 mg/kg) that did not affect body temperature by itself potentiated the hypothermic response to WIN 55212-2 (1, 2.5, or 5 mg/kg). The enhancement was strongly synergistic, indicated by a 2.7-fold increase in the relative potency of WIN 55212-2. Similarly, a dose of LY 235959 (1 mg/kg) that did not affect body temperature augmented the hypothermia associated with a single dose of WIN 55212-2 (2.5 mg/kg), thus confirming that NMDA receptors mediated the synergy. We have demonstrated previously that CB(1) receptors mediate WIN 55212-2-evoked hypothermia in rats. The present data are the first evidence that NMDA antagonists exert a potentiating effect on cannabinoid-induced hypothermia. Taken together, these data suggest that interactions between NMDA and CB(1) receptors produce synergistic hypothermia.


Recent evidence indicates that 2-arachidonylglycerol (2-AG) is a potent and specific ligand for the central and peripheral cannabinoid receptors. Therefore, the chemical stability of this molecule under biological conditions is of interest. A method for the isolation and detection of 2-AG using HPLC with evaporative light scattering detection is described. The method provides an extraction recovery from aqueous media of 78%, and a limit of detection of 60 ng on column. Incubation of 2-AG in culture medium or biological buffers indicated that it is stable to oxidation and ester hydrolysis for up to 6 h at 37 degrees C. However, gradual disappearance of the compound was noted due to adherence to glass and plastic surfaces. During incubation in RPMI culture medium, 2-AG rearranged to 1(3)-arachidonylglycerol (1(3)-AG) in a first order process with a half-life of 10 min in the absence of serum and 2.3 min in the presence of 10% fetal calf serum. Further studies indicated that the acyl migration reaction is base catalyzed \(k_{cat} = 78000 \text{ min}^{-1} \text{ M}^{-1}\), and that the reaction is affected slightly by changes in buffer (Tris) concentration and not at all by changes in ionic strength. The results indicate that 2-AG is readily converted to 1(3)-AG under conditions commonly used to study receptor-ligand interactions, findings that have significant implications for the interpretation of relative ligand potency between the two isomers.


Endocannabinoids are neuromodulators that act as retrograde synaptic messengers inhibiting the release of different neurotransmitters in cerebral areas such as hippocampus, cortex and striatum. However, little is known about other roles of the endocannabinoid system in brain. In the present work we provide substantial evidence that the endocannabinoid anandamide (AEA) regulates neuronal differentiation both in culture and in vivo. Thus AEA, through the CB1 receptor, inhibited cortical neuron progenitor differentiation to mature neuronal phenotype. In addition, human neural stem cell differentiation and NGF-induced PC12 cell differentiation were also inhibited by cannabinoid challenge. AEA decreased PC12 neuronal-like generation via CB1-mediated inhibition of sustained extracellular signal-regulated kinase (ERK) activation, which is responsible for NGF action. AEA thus inhibited TrkA-induced Rap1/B-Raf/ERK activation. Finally, immunohistochemical analyses by confocal microscopy revealed that adult neurogenesis in dentate gyrus was significantly decreased by the AEA analogue methanandamide and increased by the CB1 antagonist SR141716. These data indicate that endocannabinoids inhibit neuronal progenitor cell differentiation through attenuation of the ERK pathway, and suggest that they constitute a new physiological system involved in the regulation of neurogenesis.

The role of cannabinoids in spinal analgesia has so far been investigated in mammals and the interactions between cannabinoid receptors and markers involved in nociception have been described in the rat spinal cord. An endocannabinoid system is well developed also in the amphibian brain. However, the anatomical substrates of pain modulation have been scarcely investigated in anamniotes, neither is there reference to such a role for cannabinoids in lower vertebrates. In the present paper we employed multiple cytochemical approaches to study the distribution of CB1 cannabinoid receptors and their morphofunctional relationships with some nociception markers (i.e. Substance P, nitric oxide synthase, GABA and μ opioid receptors) in the spinal cord of the anuran amphibian Xenopus laevis. We found a co-distribution of CB1 receptors with the aforementioned signaling molecules, as well as a more limited cellular co-localization, in the dorsal and central fields of the spinal cord. These regions correspond to the mammalian laminae I-IV and X, respectively, areas strongly involved in spinal analgesia. Comparison of these results with those previously obtained in the mammalian spinal cord, reveals a number of similarities between the two systems and suggests that cannabinoids might participate in the control of pain sensitivity also in the amphibian spinal cord.


Ejaculated mammalian sperm require several hours exposure to secretions in female reproductive tracts, or incubation in appropriate culture medium in vitro, before acquiring the capacity to fertilize eggs. Arachidonylethanolamide (AEA), also known as anandamide, is a novel lipid-signal molecule that is an endogenous agonist (endocannabinoid) for cannabinoid receptors. We now report that AEA is present in human seminal plasma, mid-cycle oviductal fluid, and follicular fluid analyzed by high-performance liquid chromatography/mass spectrometry. Sperm are sequentially exposed to these reproductive fluids as they move from the vagina to the site of fertilization in the oviduct. Specific binding of the potent cannabinoid agonist [(3)H]CP-55,940 to human sperm was saturable (K(D) 9.71 +/- 1.04 nM), suggesting that they express cannabinoid receptors. R-methanandamide [AM-356], a potent and metabolically stable AEA analog, and (-)-Delta(9) tetrahydrocannabinol (THC), the major psychoactive constituent of Cannabis, modulated capacitation and fertilizing potential of human sperm in vitro. AM-356 elicited biphasic effects on the incidence of hyperactivated sperm motility (HA) between 1 and 6 hr of incubation: at (2.5 nM) it inhibited HA, while at (0.25 nM) it stimulated HA. Both AM-356 and THC inhibited morphological alterations over acrosomal caps between 2 and 6 hr (IC(50) 5.9 +/- 0.6 pM and 3.5 +/- 1.5 nM, respectively). Sperm fertilizing capacity, measured in the Hemizona Assay, was reduced 50% by (1 nM) AM-356. These findings suggest that AEA-signaling may regulate sperm functions required for fertilization in human reproductive tracts, and imply that smoking of marijuana could impact these processes. This study has potential medical and public policy ramifications because of the incidence of marijuana abuse by adults in our society, previously documented reproductive effects of marijuana and cannabinoids. Mol. Reprod. Dev. 63: 376-387, 2002. Copyright 2002 Wiley-Liss, Inc.

Sim-Selley, L. J. and B. R. Martin (2002). "Effect of Chronic Administration of R(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-b enzoxazinyl]-[1-naphthalenyl]methanone Mesylate (WIN55,212-2) or Delta(9)-Tetrahydrocannabinol on Cannabinoid Receptor Adaptation in Mice." J Pharmacol Exp Ther 303(1): 36-44.

Agonist efficacy may influence the magnitude of neuroadaptation in response to chronic drug exposure. Chronic administration of either Delta(9)-tetrahydrocannabinol (THC), a partial agonist, or R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo-[1,2,3-de]-1,4-b enzoxazinyl]-[1-naphthalenyl]methanone mesylate (WIN55,212-2), a full agonist, for G protein activation produces tolerance to cannabinoid-mediated behaviors. The present study examined whether chronic administration of maximally tolerated doses of Delta(9)-THC and WIN55,212-2 produces similar cannabinoid receptor desensitization and down-regulation. Mice were treated with escalating doses of agonist for 15 days, with final doses of 160 mg/kg Delta(9)-THC and 48 mg/kg WIN55,212-2. Tolerance to cannabinoid-mediated hypoactivity, hypothermia, and antinociception was found after treatment with Delta(9)-THC or WIN55,212-2. In autoradiographic
studies, cannabinoid-stimulated guanosine 5'-O-(3-[35S]thio)triphosphate ([35S]GTPgammaS) binding was significantly decreased in all regions of Delta(9)-THC- and WIN55,212-2-treated brains. In addition, Delta(9)-THC-treated brains showed greater desensitization in some regions than WIN55,212-2-treated brains. Concentration-effect curves for cannabinoid-stimulated [35S]GTPgammaS binding confirmed that decreases in the hippocampus resulted from loss of maximal effect in both WIN55,212-2- and Delta(9)-THC-treated mice. In the substantia nigra, the E(max) decreased and the EC(50) value increased for agonist stimulation of [35S]GTPgammaS binding in Delta(9)-THC-treated mice. [(3)H]N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-IH-pyrazole-3-carboxamide (SR141716A) binding was decreased in all brain regions in Delta(9)-THC- and WIN55,212-2-treated mice, with no difference between treatment groups. These results demonstrate that chronic treatment with either the partial agonist Delta(9)-THC or the full agonist WIN55,212-2 produces tolerance to cannabinoid-mediated behaviors, as well as cannabinoid receptor desensitization and down-regulation. Furthermore, Delta(9)-THC produced greater desensitization than WIN55,212-2 in some regions, indicating that agonist efficacy is one determinant of cannabinoid receptor desensitization in brain.


Cannabinoid receptors are found in moderate density throughout the cerebral cortex. The anterior cingulate cortex (ACC) is of particular interest due to its high level of cannabinoid receptors and role in behaviors known to be modulated by cannabinoids. These studies were conducted to determine the cellular localization of cannabinoid receptors and to compare the level of cannabinoid receptor binding with receptor-mediated G-protein activity in the rat ACC. Either ibotenic acid or undercut lesions were made in ACC, and brains were processed for [3H]WIN 55,212-2 and WIN 55,212-2-stimulated [35S]GTPgammaS autoradiography. Both cannabinoid receptors and receptor-activated G-proteins were highest in laminae I and VI of ACC in control tissue. Although similar levels of receptor binding were found in these laminae, significantly higher levels of receptor-activated G-proteins were found in lamina VI. Ibotenic acid lesions that destroyed ACC neurons decreased [3H]WIN 55,212-2 binding by 60-70% and eliminated WIN 55,212-2-stimulated [35S]GTPgammaS binding. In contrast, deafferentation of the ACC with undercut lesions had no significant effect on cannabinoid receptor binding or G-protein activation. These results indicate that cannabinoid receptors in laminae I and VI of the ACC are located on somatodendritic elements or axons intrinsic to the ACC. In addition, differences in the relative levels of cannabinoid binding sites and activated G-proteins between cortical laminae indicate that the efficiency of cannabinoid receptors for G-protein activation may vary within a specific brain region.


The design, synthesis and biological activities of potent pyrazole-based tricyclic CB(1) receptor antagonists (2) are described. The key synthetic step involves the ring closure of the lithiated alpha, gamma-keto ester adduct (4). The optimal nitroderivative (28) in this series exhibits a high CB(1) receptor affinity (pK(i)=7.2) as well as very potent antagonistic activity (pA(2)=8.8) in vitro. The regioselectivity of the pyrazole ring closure is shown to depend strongly on the aromatic substitution pattern of the applied arylhydrazine.


The structure of the C-terminal region of the third cytoplasmic loop (IC3) of the cannabinoid receptor one (CB1) bound to G(alpha1) has been determined using transferred nuclear Overhauser effects (NOEs). The wild-type IC3 sequence is helical when associated with G(alpha1). In contrast, a peptide containing the amino-acid inversion, Ala(341)-Leu(342) adopts a single turn. These findings correlate with the attenuated G(i) association of CB1 with the Ala(341)-Leu(342) mutation previously observed in vivo and the diminished stimulation of G(alpha1) GTPase activity by the corresponding peptide demonstrated in vitro here. These
results, the first to report the structure of a GPCR domain while associated with G protein, imply the C-terminus of CB1 IC3, a region with high-sequence conservation among G-protein coupled receptors, must be helical for efficient coupling and activation of the G(i) protein.


The third cytoplasmic loop (IC3) is a determinant in the dynamic life cycle of G protein-coupled receptors, including the activation, internalization, desensitization, and resensitization processes. Here, we characterize the structural features of the IC3 of the cannabinoid 1 receptor (CB1) in micelle solution using heteronuclear, (1)H,(15)N-high-resolution NMR methods. The IC3 construct was designed to contain one-third of each of the transmembrane helices (TM 5 and 6) to tether the protein to the hydrophobic portion of the micelle. Indeed, the NMR analysis illustrates prominent alpha-helices at the N-terminus (G 1-R10) and C-terminus (Q37-T47) of the IC3 receptor domain, corresponding to the cytoplasmic termini of TM5 and TM6. The structural features of the central portion of the IC3 consist of a small alpha-helix, adjacent to the terminus of TM5. The remainder is mostly unstructured as indicated by the NMR-based observables (NOEs and chemical shifts). Despite the lack of secondary structure, the hydrophobic triplet of isoleucine residues in the center of the IC3 is found in molecular dynamics simulations to associate with the lipid environment, producing two smaller loops out of the IC3. Previous studies examining mastoparan and related peptides and their ability to activate G proteins have concluded an alpha-helix is required for efficient binding and activation. Our structural results for the IC3 of CB1 would then suggest that in the intact receptor the G protein is activated by the alpha-helices of the cytoplasmic ends of TM5 or TM6 and not the unstructured central region of the IC3.


The anticonvulsant effect of cannabinoids has been shown to be mediated through activation of the cannabinoid CB(1) receptor. This study was initiated to evaluate the effects of endogenously occurring cannabinoids (endocannabinoids) on seizure severity and threshold. The anticonvulsant effect of the endocannabinoid, arachidonylethanolamine (anandamide), was evaluated in the maximal electroshock seizure model using male CF-1 mice and was found to be a fully efficacious anticonvulsant (ED(50)=50 mg/kg i.p.). The metabolically stable analog of anandamide, (R)-(20-cyano-16,16-dimetyldocos cis-5,8,11,14-tetraenoyl)-1'-hydroxy-2'-propylamine (O-1812), was also determined to be a potent anticonvulsant in the maximal electroshock model (ED(50)=1.5 mg/kg i.p.). Furthermore, pretreatment with the cannabinoid CB(1) receptor specific antagonist N-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) completely abolished the anticonvulsant effect of anandamide as well as O-1812 (P<0.01, Fisher exact test), indicating a cannabinoid CB(1) receptor-mediated anticonvulsant mechanism for both endocannabinoid compounds. Additionally, the influence of cannabinoid CB(1) receptor endogenous tone on maximal seizure threshold was assessed using SR141716A alone. Our data show that SR141716A (10 mg/kg i.p.) significantly reduced maximal seizure threshold (CC(50)=14.27 mA) compared to vehicle-treated animals (CC(50)=17.57 mA) (potency ratio=1.23, lower confidence limit=1.06, upper confidence limit=1.43), indicating the presence of an endogenous cannabinoid tone that modulates seizure activity. These data demonstrate that anandamide and its analog, O-1812, are anticonvulsant in a whole animal model and further implicate the cannabinoid CB(1) receptor as a major endogenous site of seizure modulation.


The present study investigated the role of peripheral cannabinoid (CB(2)) receptors in producing hypomobility, antinociception and hypothermia in mice. Results revealed that the CB(2)-selective antagonist, SR144528, did not block cannabimimetic effects of a potent Delta(8)-tetrahydrocannabinol (THC) analog in mice. While most of a series of CB(2)-selective 1-deoxy-THC analogs were active in vivo only if they also had good affinity for CB(1) receptors, four of
these analogs showed in vivo activity even though their affinities for CB(1) receptors were poor. Further, this activity was blocked by the CB(1) antagonist SR141716A, but not by SR144528. One of the deoxy analogs also stimulated [(35)S]GTPgammaS binding, an effect that was blocked by SR141716A. These results provide further evidence that these cannabimimetic effects are not mediated through action at CB(2) receptors. In addition, some of these analogs may be very low efficacy agonists at CB(1) receptors that act as full agonists in vivo, but lack the ability to displace high affinity and high efficacy binding ligands in vitro.

Zhang, Q., P. Ma, et al. (2002). "In Vitro Metabolism of R(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]1,4-benzoxazinyl)-(1-naphthalenyl) methanone mesylate, a Cannabinoid Receptor Agonist." Drug Metab Dispos 30(10): 1077-86.

R(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]1,4-benzoxazinyl)-(1-naphthalenyl)methanone mesylate (WIN55212-2) is a potent cannabinoid receptor agonist that has been found to exhibit antinociceptive activity and to inhibit brain cyclooxygenase. The metabolism of WIN55212-2 has not been reported, and it is unknown whether its metabolites retain any agonist properties. In this study, in vitro metabolism of WIN55212-2 in rat liver microsome was investigated. The metabolic profile was obtained using high-performance liquid chromatography (HPLC) with UV and mass spectrometry detectors. The HPLC chromatogram revealed two major and at least six minor metabolites derived from the parent compound ([M + H](+) = m/z 427). The two major metabolites (structural isomers at m/z 461), constituting 60 to 75% of the total metabolites, were each identified as dihydrodiol metabolites resulting from the arene oxide pathway. The minor metabolites were all detected as protonated molecules, three of which appeared at m/z 477, corresponding to structural isomers of trihydroxylated parent compound; another two appeared at m/z 443, representing monohydroxylated isomers; and another was observed at m/z 425, and was assigned as a dehydrogenation product. These structural assignments are based on HPLC/tandem mass spectrometry and NMR analysis. Metabolic pathways have been proposed to account for the various metabolites observed. Two major metabolites have been isolated in pure form, allowing future receptor binding studies to be conducted.

CLINICAL SCIENCE

A sensitive analytical method was developed for quantitative analysis of Delta(9)-tetrahydrocannabinol (Delta(9)-THC), 11-nor-Delta(9)-tetrahydrocannabinol-carboxylic acid (Delta(9)-THC-COOH), cannabiol (CBN) and cannabidiol (CBD) in human hair. The identification of Delta(9)-THC-COOH in hair would document Cannabis use more effectively than the detection of parent drug (Delta(9)-THC) which might have come from environmental exposure. Ketamine was added to hair samples as internal standard for CBN and CBD. Ketoprofen was added to hair samples as internal standard for the other compounds. Samples were hydrolyzed with beta-glucuronidase/aryl sulfatase for 2h at 40 degrees C. After cooling, samples were extracted with a liquid-liquid extraction procedure (with chloroform/isopropyl alcohol, after alkalization, and n-hexane/ethyl acetate, after acidification), which was developed in our laboratory. The extracts were analysed before and after derivatization with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) using a Hewlett Packard gas chromatographer/mass spectrometer detector, in electron impact mode (GC/MS-EI).Derivedized Delta(9)-THC-COOH was also analysed using a Hewlett Packard gas chromatographer/mass spectrometer detector, in negative ion chemical ionization mode (GC/MS-NCI) using methane as the reagent gas. Responses were linear ranging from 0.10 to 5.00ng/mg hair for Delta(9)-THC and CBN, 0.10-10.00ng/mg hair for CBD, 0.01-5.00ng/mg for Delta(9)-THC-COOH (r(2)>0.99). The intra-assay precisions ranged from <0.01 to 12.40%. Extraction recoveries ranged from 80.9 to 104.0% for Delta(9)-THC, 85.9-100.0% for Delta(9)-THC-COOH, 76.7-95.8% for CBN and 71.0-94.0% for CBD. The analytical method was applied to 87 human hair samples, obtained from individuals who testified in court of having committed drug related crimes. Quantification of Delta(9)-THC-COOH using GC/MS-NCI
was found to be more convenient than GC/MS-EI. The latter may give rise to false negatives due to the detection limit.


AIM: To describe the pulmonary function and prevalence of dyspnoea among methadone patients and to study the relation with exposure to heroin by inhaling. STUDY POPULATION: A sample of 100 patients from methadone maintenance treatment (84% male, average age 42 years). MEASUREMENTS: Questionnaires were used to measure life-time exposure to heroin, cocaine, cannabis, tobacco, and symptoms of dyspnoea. Spirometry was performed and residual difference of measured FEV(1) from the age, sex, height and ethnicity predicted value (DeltaFEV(1)) was used as a main outcome parameter. FINDINGS: The median DeltaFEV(1) was -0.26 l (inter quartile range -0.70; +0.12). Twenty per cent experienced dyspnoea while 'walking at a normal pace with someone of their own age'. History of cigarette smoking was reported by 98%; heroin smoking by 88%. Multiple linear regression analysis showed a statistically significant association between heroin-smoking and DeltaFEV(1), logistic regression analysis showed an association between heroin-smoking and prevalence of dyspnoea. CONCLUSIONS: Chronic heroin smoking seems to be related to an impaired lung function and higher prevalence of dyspnoea. However, part of the observed lung function impairment will be caused by tobacco smoking. Further research is needed to quantify the effect of heroin smoking and disentangle the effect of smoking heroin and tobacco.


Derived indices on the Trail Making Test (TMT), a test often used for screening for cognitive impairment, were examined in a sample of marijuana abusers in drug abuse treatment programs. A mixed-race sample of 259 subjects was drawn from electronic files of data from the Drug Abuse Treatment Outcome Study (DATOS). The DATOS was a naturalistic, prospective cohort study that collected data from 1991 to 1993 in 96 programs in 11 cities in the United States. Data were analyzed to determine the effects of demographic variables on derived indices created by adding, subtracting, multiplying, and dividing Parts A and B of the TMT in this large treatment sample of marijuana abusers. The variables of age, ethnicity, and education were statistically significant for the total (A + B), and interaction (A x B/100) derived indices of the TMT. The difference score (B - A) was significant only for ethnicity and the ratio score (B/A) was not significant for any demographic variable.


AIMS: The study was designed to investigate the acute effects of ingested tetrahydrocannabinol (THC) on auditory function. METHODS: Eight male subjects (aged 22-30 years), who had previous experience of cannabis use, took part in this study. They performed air conduction pure tone audiometry in both ears over 0.5-8 kHz. A simple test of frequency selectivity by detecting a 4-kHz tone under two masking noise conditions was also carried out in one ear. Three test sessions at weekly intervals were carried out, at the start of which they ingested a capsule containing either placebo, or 7.5 or 15 mg of THC. These were administered in a randomized cross-over, double-blind manner. Auditory testing as described above was carried out 2 hours after ingestion. Blood samples were also obtained at this time point and assayed for delta 9- and 11-OH-THC levels. RESULTS: No significant changes in threshold or frequency resolution were seen with the dosages employed in this study. CONCLUSIONS: This suggests that THC at the plasma levels attained in this study does not have profound effect on the processing of elementary stimuli by the auditory pathway.

The significance of cannabinoid signaling for human cognition and motor control is still poorly understood. Here, we have investigated acute behavioral effects of oral delta-9-tetrahydrocannabinol (THC) with oculomotor paradigms in 12 healthy human subjects. Compared to baseline testing: (i) THC increased latencies of reflexive visually guided saccades, while their accuracy was not affected; (ii) latencies of memory-guided saccades were unaffected, but THC modulated accuracy of these eye movements by increasing average gain and gain variability; (iii) frequency of anticipated memory-guided saccades and antisaccade errors was increased; (iv) the saccade amplitude/peak velocity relationships were not affected. These results show that THC acts on selected aspects of saccade control, namely spatial attention shifts, fine tuning of volitional saccades, spatial working memory and inhibition of inappropriate saccades. The pattern of effects suggests modulation of neuronal activity in substantia nigra pars reticulata and/or dorsolateral prefrontal cortex and sparing of the eye fields and the final motor pathway for saccades. Behaviorally, our findings reflect the distribution of CB-1 cannabinoid receptors in the human neocortex, basal ganglia and brainstem and provide evidence for participation of the cannabinoidergic system in high level control of saccades and associated cognitive functions. Saccadic eye movements may provide an objective measure of motor and cognitive effects of cannabinoids.


Witztum, E. and E. Shufman (2002). "[Cerebral and physical risks associated with cannabis use]." *Harefuah* 141(7): 636-41, 665. This article summarizes the results of research studies that indicate that the accepting, permissive approach to cannabis use is not justified. After theoretical introduction and a discussion of the forms currently available, we review research and epidemiological surveys documenting the effects of cannabis on various body systems. The deleterious effects associated with cannabis use and its derivatives are discussed.

**BEHAVIOURAL SCIENCE**


The pattern of tobacco, alcohol, and other substance use was assessed among 1,197 Chinese undergraduates in Hong Kong. Students reported their current and past use of tobacco (13%), alcohol (61%), marijuana (2%), and other illicit drugs (0.4%). Perceptions of risk from the use of different substances were low among those who use substances and among senior students. The rate of substance use was higher among males, residents of university hall, senior students, and among those who possessed a positive attitude towards substance use. There were significant associations between different substance uses among the respondents.


Objective: To provide community-level public health surveillance information on cannabis and Mandrax (methaqualone) use and associated health and social consequences. Design: A descriptive, epidemiological study of cannabis and Mandrax supply and demand indicators based on data gathered from multiple sources, including specialist treatment centres, trauma units, police records, and quantitative and qualitative studies of school students, sex workers, persons attending rave clubs, and arrestees. Networks were established at five sentinel sites to facilitate the collection, interpretation, and dissemination of data. Results: Supply and demand indicators point to the widespread use of cannabis and Mandrax and significant increases in white pipe-related health and social problems. There has been an increase in the demand for treatment related to cannabis/Mandrax use, a high proportion of patients in trauma units who test positive for cannabis and/or Mandrax, and a high proportion of cannabis and Mandrax-positive arrestees. Although the use of cannabis is predominantly a male phenomenon
and is widespread among young people, it occurs in all sectors of South African society. Mandrax users tend to be young, male, and coloured. CONCLUSIONS: Cannabis and Mandrax use has a number of implications for health and social policy, including the need to develop protocols for the identification and management of cannabis/Mandrax-positive trauma patients and arrestees. The study points to the need for further monitoring of cannabis and Mandrax use and the negative consequences associated with their use.


Immigrant parents and their U.S.-born children may experience stressful family conflicts over the disparate sociocultural norms of the United States and their country of origin. Such stresses may heighten adolescents' vulnerability to drug abuse. This article documents the extent of drug use in a sample of 200 U.S.-born Asian Indian adolescents. According to the study participants' self-reports on lifetime use, 28 percent had used alcohol on at least one occasion, 16.5 percent had used cigarettes, and 2.5 percent had used marijuana. Adolescents who placed importance on their parents' drug abuse prevention messages tended not to use drugs. The implications of the study's findings for drug abuse assessment, treatment, and prevention are discussed.


To examine the influence of ecological/cultural factors and family, personality, and peer factors present during early adolescence that influence marijuana use in late adolescence. A community sample of 2226 Colombian adolescents living in mixed urban-rural communities and their mothers were interviewed in their homes by trained Colombian interviewers, first in 1995-1996 and then again 2 years later. The scales used were based on item intercorrelations and grouped into the following categories: (a) adolescent personality, (b) family traits, (c) peer factors, (d) ecological/cultural variables, and (e) marijuana use. Data were examined using hierarchical regression modeling to determine the relationship between each of the domains and late adolescent marijuana use. The findings supported the family interactional theory of adolescent drug use behavior and found that factors in all of the domains had a direct effect on late adolescent marijuana use as well as indirect effects mediated through the more proximal domains in the model. Of particular interest was the strength of the influence of the ecological/cultural factors, which far exceeded that observed in similar studies done in the United States. Owing to the similarity with findings from studies conducted in the United States, interventions designed domestically could effectively be directly applied to adolescents in Colombia. The findings also suggest that prevention programs designed specifically to target ecological or cultural factors may have the most profound influence for reducing marijuana use in late adolescence.


AIMS: To assess the effects of former heavy marijuana use on selected aspects of health. DESIGN: A monozygotic co-twin control design was used to compare the health of former heavy marijuana using male monozygotic twins to that of their co-twins who never used marijuana significantly. SETTING: In-person survey and questionnaires. PARTICIPANTS: Fifty-six marijuana use discordant monozygotic twin pair members of the Vietnam Era Twin (VET) Registry. MEASUREMENTS: Current socio-demographic characteristics; current nicotine and alcohol use; lifetime nicotine and alcohol abuse/dependence; past 5-year physical and mental health services utilization; and health-related quality of life. FINDINGS: The mean number of days on which the marijuana user twin used marijuana in his life-time was 1085, while the non-marijuana user used marijuana a maximum of 5 days. Marijuana was last used a mean of 20 years previously. No significant differences were found between the former marijuana user twins...
and their siblings for current socio-demographic characteristics; current nicotine or alcohol use; life-time nicotine or alcohol abuse/dependence; past 5-year out-patient or emergency room visits, hospitalizations or medication use for medical problems; past 5-year mental health out-patient use or hospitalizations; or health-related quality of life. CONCLUSIONS: Previous heavy marijuana use a mean of 20 years earlier by a group of men who reported no other significant illicit drug use does not appear to be associated with adverse socio-demographic, physical or mental health adverse effects. The conclusions of the study are limited by possible participation and recall biases, relatively small sample size and the absence of a physical health examination.


**AIM:** To examine the associations between frequency of cannabis use and psychosocial outcomes in adolescence/young adulthood. **DESIGN:** A 21-year longitudinal study of the health, development and adjustment of a birth cohort of 1265 New Zealand children. **MEASUREMENTS:** Annual assessments of the frequency of cannabis use were obtained for the period from age 14-21 years, together with measures of psychosocial outcomes including property/violent crime, depression, suicidal ideation, suicide attempt and other illicit drug use. **FINDINGS:** The frequency of cannabis use was associated significantly with all outcomes, and particularly other illicit drug use. Statistical control for confounding by both fixed and time-dynamic factors substantially reduced the strength of association between cannabis use and outcome measures. Nevertheless, cannabis use remained significantly (P < 0.05) associated with all outcomes and particularly other illicit drug use, after adjustment for confounding. For the measures of crime, suicidal behaviours and other illicit drug use there was evidence of age related variation in the strength of association with cannabis use, with younger (14-15 years old) users being more affected by regular cannabis use than older (20-21 years old) regular users. However, the association between cannabis use and depression did not vary with age. **CONCLUSIONS:** Cannabis use, and particularly regular or heavy use, was associated with increased rates of a range of adjustment problems in adolescence/young adulthood—other illicit drug use, crime, depression and suicidal behaviours—with these adverse effects being most evident for school-aged regular users. The findings reinforce public health concerns about minimizing the use of cannabis among school-aged populations.


**BACKGROUND & OBJECTIVES:** There are no reports of incidence studies in the Indian setting on substance use disorders in the general population. This survey-resurvey carried out in metropolis Delhi estimated the incidence rates of substance use disorders. **METHODS:** A cross-sectional survey was carried out at two points of time with an interval of one year in a representative sample from the general population of metropolis, Delhi. The instrument was pre-coded, structured and based on DSM III-R operationalised criteria for use of tobacco, alcohol, cannabis and opioids (past one month). Matched data for two points of time were available for 5414 males and 4898 females. **RESULTS:** In the total cohort, the annual incidence rates (per 100 persons) among males for any drug use, alcohol, tobacco, cannabis and opioids were 5.9, 4.2, 4.9, 0.02 and 0.04 respectively. Among females, incidence of any drug use was 1.2/100 persons. **INTERPRETATION & CONCLUSION:** Results showed that males have higher incidence for both not-dependent and dependent use for all the drug categories. Females had a higher incidence of dependent tobacco use.


The theory of reasoned action (TRA) is used to model decisions about substance use among young mothers who became premaritally pregnant at age 17 or younger. The results of structural equation modeling to test the TRA indicated that most relationships specified by the model were significant and in the predicted direction. Attitude was a stronger predictor of intention than norm, but both were significantly related to intention, and intention was related to actual
marijuana use 6 months later. Outcome beliefs were bidimensional, and positive outcome beliefs, but not negative beliefs, were significantly related to attitude. Prior marijuana use was only partially mediated by the TRA variables; it also was directly related to intentions to use marijuana and to subsequent use.


OBJECTIVE: The 2001 Clients of Treatment Service Agencies (COTSA) census, the fourth since 1990, was conducted to enable a comparison of the drug and alcohol-related problems being treated over an 11-year period. METHOD: The 24-hour census was conducted on Wednesday 2 May 2001 in all Australian States and Territories. All agencies providing treatment for drug and alcohol problems in Australia were asked to provide demographic, treatment and substance use information about all clients treated on census day. The data were analysed with frequencies and basic descriptive statistics. RESULTS: Of the agencies surveyed, 90.3% responded. The census suggests that, among the treatment population, the mean age of substance users has decreased and the proportion of clients who are women has increased. Treatment for opiate, cannabis and amphetamine problems increased; treatment for alcohol problems decreased. Substance use patterns differed according to sex, age, size of the population centre, and Indigenous status. CONCLUSIONS AND IMPLICITATIONS: Changes among the treatment population reflect changes in demographics and substance use among the broader drug-using community, with the exception of the presentation of alcohol problems for treatment. The reasons for the apparent decline in treatment for alcohol problems are not clear, although a number of factors, such as changes in treatment strategies and facilities and relative increases in other substance use problems, are considered. Any decrease in treatment for a significant health problem such as alcohol use disorder will have considerable public health implications.


The prevalence of nicotine dependence among alcohol or other substance abusers is extremely high, and surveys have revealed that many patients in drug or alcohol treatment programs are interested in smoking cessation. However, smoking cessation has not been a traditional focus in clinical interventions for this population. Recent evidence from clinical trials among individuals abusing alcohol, marijuana, cocaine, or opioids have shown the following: 1) smokers with a past but not current history of alcohol dependence have a similar rate of success compared with non-alcoholic smokers; 2) tobacco abstinence does not increase alcohol relapse; 3) continued smoking adversely affects treatment for marijuana dependence; 4) patterns of cocaine and nicotine use are interrelated; 5) smoking cessation rates among opioid-dependent individuals are several times lower than in the general US population. Smoking cessation is indicated for substance dependent persons already in recovery and may protect against relapse to the illicit drug of abuse.


OBJECTIVE: To provide a review of the evidence from 3 experimental trials of Project Towards No Drug Abuse (TND), a senior-high-school-based drug abuse prevention program. METHODS: Theoretical concepts, subjects, designs, hypotheses, findings, and conclusions of these trials are presented. A total of 2,468 high school youth from 42 schools in southern California were surveyed. RESULTS: The Project TND curriculum shows reductions in the use of cigarettes, alcohol, marijuana, hard drugs, weapon carrying, and victimization. Most of these results were replicated across the 3 trials. CONCLUSION: Project TND is an effective drug and violence prevention program for older teens, at least for one-year follow-up.

The dollar value of an illicit drug market is an important statistic in drug policy analysis. It can be used to illustrate the scale of the trade in a drug; evaluate its impact on a local community or nation; provide an indication of the level of criminality related to a drug; and can inform discussions of future drug policy options. This paper calculates the first ever demand side estimates of the New Zealand cannabis black market. The estimates produced are calculated using cannabis consumption data from the Alcohol & Public Health Research Unit's (APHRU) 1998 National Drug Survey. The wholesale value of the market is estimated to be $81.3-104.6 million a year, and the retail value of the market is estimated to be $131.3-168.9 million a year. These demand side estimates are much lower than the existing supply side estimates of the market calculated using police seizures of cannabis plants. The retail figure is four times lower than the lowest national supply side estimate ($636 million) and seven times lower than the highest national supply side estimate ($1.27 billion). The demand side estimates suggest a much smaller cannabis economy to fuel organized criminal activity in New Zealand than previous estimates implied.

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