INTRODUCTION
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BASIC SCIENCE


Theiler's virus infection of the CNS induces an immune-mediated demyelinating disease in susceptible mouse strains and serves as a relevant infection model for human multiple sclerosis (MS). Cannabinoids may act as immunosuppressive compounds that have shown therapeutic potential in chronic inflammatory disorders. Using the Theiler's murine encephalomyelitis virus model, we report here that treatment with the synthetic cannabinoids WIN 55,212-2, ACEA, and JWH-015 during established disease significantly improved the neurological deficits in a long-lasting way. At a histological level, cannabinoids reduced microglial activation, abrogated major histocompatibility complex class II antigen expression, and decreased the number of CD4+ infiltrating T cells in the spinal cord. Both recovery of motor function and diminution of inflammation paralleled extensive remyelination. Overall, the data presented may have potential therapeutic implications in demyelinating pathologies such as MS; in particular, the possible involvement of cannabinoid receptor CB2 would enable nonpsychoactive therapy suitable for long-term use.


The endogenous cannabinoid system has been shown recently to play a crucial role in the extinction of aversive memories. As the amygdala is presumably involved in this process, we investigated the effects of the cannabinoid receptor agonist WIN 55,212-2 (WIN-2) on synaptic transmission in the lateral amygdala (LA) of wild-type and cannabinoid receptor type 1 (CB1)-deficient mice. Extracellular field potential recordings and patch-clamp experiments were performed in an in vitro slice preparation. We found that WIN-2 reduces basal synaptic transmission and pharmacologically isolated AMPA receptor- and GABA(A) receptor-mediated postsynaptic currents in wild-type, but not in CB1-deficient mice. These results indicate that, in the LA, cannabinoids modulate both excitatory and inhibitory synaptic transmission via CB1. WIN-2-induced changes of paired-pulse ratio and of spontaneous and miniature postsynaptic currents suggest a presynaptic site of action. Inhibition of G(i/o) proteins and blockade of voltage-dependent and G protein-gated inwardly rectifying K(+) channels inhibited WIN-2 action on basal synaptic transmission. In contrast, modulation of the adenylyl cyclase-protein kinase A pathway, and blockade of presynaptic N- and P/Q- or of postsynaptic L- and R/T-type voltage-gated Ca(2+) channels did not affect WIN-2 effects. Our results indicate that the mechanisms underlying cannabinoid action in the LA partly resemble those observed in the nucleus accumbens and differ from those described for the hippocampus.

Ethanol increases extracellular anandamide levels in neuronal cells. However, the molecular mechanisms by which this occurs are unknown. Chronic exposure of cerebellar granule neurons to ethanol increased the levels of anandamide accumulated in the cellular medium. Anandamide uptake was saturable and was inhibited (30% at 3 min) in response to chronic exposure to ethanol. Chronic ethanol treatment did not alter the K(m), but significantly decreased V(max) of anandamide transport (33%) (P<0.0001). Fatty acid amide hydrolase activity was not affected by chronic ethanol treatment. Anandamide transport processes are independent of cannabinoid CB1 receptor, as cannabinoid CB1 receptor knockout mice exhibited time-dependent anandamide transport and cannabinoid CB1 receptor antagonists did not alter the effects of chronic ethanol on anandamide transport. Furthermore, anandamide transport was inhibited by acute ethanol in a time- and dose-dependent manner. Interestingly, acute ethanol-induced inhibition of anandamide transport was abolished in neurons exposed to chronic ethanol, suggesting that the anandamide transport processes may play a role in the development of long-term cellular tolerance to ethanol.


Cannabinoids elicit hypotension mainly via activated CB(1) receptors and show complex cardiovascular actions. Effects on human heart muscle have not been studied yet. Isolated human atrial heart muscle preparations were stimulated by electrical field with 1 Hz to contract isometrically at optimal length and were challenged with the endogenous cannabinoid arachidonyl ethanolamide (anandamide), the metabolically stable analogue R-methanandamide, and the potent synthetic CB(1) receptor agonist HU-210. Anandamide dose-dependently decreased systolic force (82.2 +/- 4.8% and 60.8 +/- 6.8% of maximal systolic force for 0.1 and 1 &mgr;M, respectively, P < 0.05). The selective CB(1) receptor antagonist AM-251 (1 &mgr;M, P < 0.05), but not the CB(2) receptor antagonist, AM-630 (1 &mgr;M), the nitric oxide synthase inhibitor Nomega-nitro-l-arginine methyl ester (l-NAME) (500 &mgr;M), or the cyclooxygenase inhibitor indomethacin (100 &mgr;M), prevented the effect. Contrary to indomethacin, l-NAME alone showed negative inotropic effects (72.1 +/- 3.54%, P < 0.001). The R-methanandamide (1 &mgr;M: 50.4 +/- 3.5%, P < 0.001) and HU-210 (1 &mgr;M: 60.1 +/- 3.8%, P < 0.001) had similar negative inotropic effects. The existence of CB(1) receptors on heart muscle was verified using Western blot analysis and immunofluorescence staining. The conclusion is that anandamide, R-methanandamide, and HU-210 decrease contractile performance in human atrial muscle via CB(1) receptors.


Cannabinoids are known to exert mainly excitatory effects on dopaminergic cells of the ventral tegmental area (VTA). We have utilized an in vivo multiple-single unit electrophysiological approach to assess different neuronal contributions that may ultimately lead to excitation in this area. Baseline neuron recordings, using low impedance microwires, showed a variety of waveforms with a wide range of durations (0.8-3.2 ms). In the first experiment systemic injection of the potent cannabinoid agonist HU210 (100 &mgr;g/kg, i.p.) led predominantly to an increase in firing rate (~214%, compared to pre-drug) in slowly firing cells with broad action potentials, possibly driven by a majority of presumed dopaminergic neurons (n = 31). However, the firing rate of some units was either unaffected (<25%, n = 9) or even decreased (~67%, n = 9) following cannabinoid injection concomitantly with excitation. Apomorphine (75 &mgr;g/kg, i.p.) injected following HU210 produced a marked inhibition of both responses (~76%) in 39 out of 49 cells. The second group of animals was treated with the CB(1) receptor antagonist SR141716A (1 mg/kg, i.p.), which had no effect when injected alone but prevented all HU210-evoked changes in firing rate suggesting that cannabinoid receptors mediated the observed responses (n = 39). Taken together, the present results suggest that the observed actions of cannabinoids may
involve complex neurotransmitter interactions leading to differential effects on dopamine release. These heterogeneous neuronal responses are likely to underly the behavioural discrepancies reported in animal models of cannabinoid reinforcement.


Five sets of experiments were carried out with CD1 mice tested in a one-trial inhibitory avoidance task. In a first set, immediately posttraining administrations of the endogenous ligand for the cannabinoid CB1 receptor anandamide (arachidonylethanolamide) (3 or 6mg/kg) dose-dependently impaired memory consolidation in mice. A lower dose (1.5mg/kg) was ineffective. In a second set of experiments, which was carried out at the same time of the first set, preexposure of the animals to the testing apparatus decreased the effect of the drug, as compared with non-preexposed mice. In a third set of experiments, administration of anandamide (3 or 6mg/kg) prior to the retention test did not affect the retention performance of mice given posttraining injections of either saline or anandamide. These findings indicate that the memory-impairing effects of posttraining administration of anandamide are not state-dependent. In the fourth and fifth series of experiments, carried out with non-preexposed mice, an otherwise ineffective immobilization stress (15min) enhanced the memory-impairing effect of anandamide, and an otherwise ineffective dose of naltrexone (0.1mg/kg) completely antagonized the effect. The results are discussed in terms of attenuation of emotionality, resulting in impaired retention, following anandamide administration, and of involvement of opioid system in the effect of this drug.


Theiler murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) is a mouse model of chronic-progressive multiple sclerosis (MS) characterized by Th1-mediated CNS demyelination and spastic hindlimb paralysis. Existing MS therapies reduce relapse rates in 30% of relapsing-remitting MS patients, but are ineffective in chronic-progressive disease, and their effects on disability progression are unclear. Experimental studies demonstrate cannabinoids are useful for symptomatic treatment of spasticity and tremor in chronic-relapsing experimental autoimmune encephalomyelitis. Cannabinoids, however, have reported immunosuppressive properties. We show that the cannabinoid receptor agonist, R(+)-WIN55,212, ameliorates progression of clinical disease symptoms in mice with preexisting TMEV-IDD. Amelioration of clinical disease is associated with downregulation of both virus and myelin epitope-specific Th1 effector functions (delayed-type hypersensitivity and IFN-gamma production) and the inhibition of CNS mRNA expression coding for the proinflammatory cytokines, TNF-alpha, IL1-beta, and IL-6. Clinical trials investigating the therapeutic potential of cannabinoids for the symptomatic treatment of MS are ongoing, and this study demonstrates that they may also have potent immunoregulatory properties.


An accurate, reproducible, and validated gas chromatography-mass spectrometry (GC-MS) method for the quantitation of 11 -nor-delta9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the major metabolite of delta9-tetrahydrocannabinol, in urine is described. Extraction was performed with n-hexane/ethyl acetate. Deuterated THC-COOH was used as the internal standard. The GC-MS analysis was done by selected ion monitoring. No interferences were detected in 20 blank urine samples of different origin. The calibration curve was found to be linear over the range of 10-100 ng/mL. The calculated limits of detection and quantitation were 1.0 ng/mL and 1.7 ng/mL, respectively. Results of positive findings for cannabis use in doping control in Flanders and Portugal in the period of 1997-2000 are commented.

RATIONALE. Recently, we provided evidence for a cannabinoid mechanism in relapse to cocaine seeking in rats. There is also increasing evidence for functional cross-talk between cannabinoid and opioid systems in several physiological processes. OBJECTIVES. This study was designed to evaluate whether the cannabinoid system plays a role in mediating the reinforcing and motivational effects of heroin and heroin-paired stimuli. METHODS. Male Wistar rats were trained to self-administer heroin (50 &mgr;g/kg per infusion) on fixed (FR5) or progressive ratio schedules of reinforcement in the presence of a discriminative and discrete heroin-associated cue. The selective cannabinoid CB1 antagonist SR141716A was given 30 min before the session to determine its effect on responding for heroin. Separate groups of rats were subjected to extinction training during which heroin-associated cues were absent and no heroin was delivered. During subsequent reinstatement tests, the effects of the cannabinoid agonist HU210 and the antagonist SR141716A on reinstatement of heroin seeking were evaluated. RESULTS. The cannabinoid antagonist dose-dependently reduced responding for heroin on the FR5 schedule and to a greater extent on the progressive ratio schedule. HU210 (20 &mgr;g/kg) reinstated heroin seeking behaviour following a 2-week extinction period, whereas SR141716A dose-dependently attenuated heroin seeking that was provoked by a priming injection of heroin (0.25 mg/kg) and heroin seeking that was triggered by re-exposure to heroin paired stimuli. CONCLUSIONS. The results show that the reinforcing and motivational effects of heroin and heroin-paired stimuli are mediated, at least in part, by activation of cannabinoid CB1 receptors. Therefore, the present study provides a rationale for the use of cannabinoid antagonists in the treatment of opiate addiction.


Endocannabinoids form a novel class of intercellular messengers, the functions of which include retrograde signaling in the brain and mediation or modulation of several types of synaptic plasticity. Yet, the signaling mechanisms and long-term effects of the stimulation of CB1 cannabinoid receptors (CB1-R) are poorly understood. We show that anandamide, 2-arachidonoyl-glycerol, and Delta9-tetrahydrocannabinol (THC) activated extracellular signal-regulated kinase (ERK) in hippocampal slices. In living mice, THC activated ERK in hippocampal neurons and induced its accumulation in the nuclei of pyramidal cells in CA1 and CA3. Both effects were attributable to stimulation of CB1-R and activation of MAP kinase/ERK kinase (MEK). In hippocampal slices, the stimulation of ERK was independent of phosphatidyl-inositol-3-kinase but was regulated by cAMP. The endocannabinoid-induced stimulation of ERK was lost in Fyn knock-out mice, in slices and in vivo, although it was insensitive to inhibitors of Src-family tyrosine kinases in vitro, suggesting a noncatalytic role of Fyn. Finally, the effects of cannabinoids on ERK activation were dependent on the activity of glutamate NMDA receptors in vivo, but not in hippocampal slices, indicating the existence of several pathways linking CB1-R to the ERK cascade. In vivo THC induced the expression of immediate-early genes products (c-Fos protein, Zif268, and BDNF mRNAs), and this induction was prevented by an inhibitor of MEK. The strong potential of cannabinoids for inducing long-term alterations in hippocampal neurons through the activation of the ERK pathway may be important for the physiological control of synaptic plasticity and for the general effects of THC in the context of drug abuse.


In several G protein-coupled receptors (GPCRs), the Asp-Arg-Tyr (DRY) motif at the bottom of third transmembrane domain and the amino acid at position 6.34 in the sixth transmembrane domain have been shown to play important roles in signal transduction. In this study, we propose that in the cannabinoid-2 (CB2) receptor, R3.50 in the DRY motif may be crucial for interacting with G proteins, and D3.49 and A6.34 may be important for constraining the receptor in an inactive conformation. To test our hypothesis, R3.50A, D3.49A, and A6.34E mutations of the human CB2 receptor were made by site-directed mutagenesis. These mutant receptors were stably transfected into human embryonic 293 cells, and their ligand binding and signal transduction properties were analyzed. Similar to other GPCRs, R3.50 of the CB2 receptor is crucial for signal transduction. Unlike other GPCRs, D3.49 and A6.34 of the CB2 receptor do
not seem to be important for keeping the receptor in an inactive state. Furthermore, D3.49A and A6.34E mutations abolished ligand binding, and all three mutations abolished constitutive activity of the wild-type CB2 receptor.


Oleoylethanolamide (OEA) is a structural analog of the endogenous cannabinoid anandamide, which does not activate cannabinoid receptors. The biosynthesis of OEA in rat small intestine is increased by feeding and reduced by fasting. Moreover, OEA decreases food intake in food-deprived rats via a mechanism that requires intact sensory fibers (Rodriguez de Fonseca, 2001). These results suggest that OEA may contribute to the peripheral regulation of feeding. In the present study, we have investigated the effects of systemic OEA administration (1-20 mg/kg, intraperitoneal) on meal pattern in free-feeding and food-deprived rats. In free-feeding animals, OEA delayed feeding onset in a dose-dependent manner, but had no effect on meal size or postmeal interval. In food-deprived animals, OEA both delayed feeding onset and reduced meal size. The selective effects of OEA in free-feeding rats are strikingly different from those of the serotonergic anorexiant d-fenfluramine (which delayed feeding and reduced meal size) and the intestinal peptide cholecystokinin (which reduced meal size). These results suggest that OEA may participate in the regulation of satiety and may provide a chemical scaffold for the design of novel appetite-suppressing medications. Neuropsychopharmacology advance online publication, 2 April 2003; doi:10.1038/sj.npp.1300166

Hilairet, S., M. Bouaboula, et al. (2003). "Hypersensitization of the orexin 1 receptor by the CB1 receptor : Evidence for cross-talk blocked by the specific CB1 antagonist, SR141716." J Biol Chem.

In the present study, we observed evidence of cross-talk between the cannabinoid receptor CB1 and the orexin 1 receptor, OX1R, using a heterologous system. When the two receptors are co-expressed, we observed a major CB1-dependent enhancement of the orexin A potency to activate the MAPK pathway; dose-responses curves indicated a 100-fold increase in the potency of orexin-mediated MAPK activation. This effect required a functional CB1 receptor as evidenced by the blockade of the orexin response by the specific CB1 antagonist, SR141716, but also by PTX, suggesting that this potentiation is Gi-mediated. In contrast to OX1R, the potency of direct activation of CB1 was not affected by co-expression with OX1R. In addition, electron microscopy experiments revealed that CB1 and OX1R are closely apposed at the plasma membrane level; they are close enough to form hetero-oligomers. Altogether, for the first time our data provide evidence that CB1 is able to potentiate an orexigenic receptor. Considering the anti-obesity effect of SR141716, these results open new avenues to understand the mechanism by which the molecule may prevent weight gain through functional interaction between CB1 and other receptors involved in the control of appetite.


T cells are sensitive to modulation by cannabinoids as evidenced by their ability to inhibit expression of cytokines, including interleukin (IL)-2 and IL-4. Because T cells play a key role in the pathophysiology of allergic asthma by expressing T helper cell (Th)2 cytokines, the objective of the present studies was to examine the effect of cannabinoids on immunologic and pathologic features associated with the allergic airway response induced by ovalbumin (Ova). A/J mice were systemically sensitized with Ova and subsequently challenged with aerosolized Ova. The steady-state mRNA expression of IL-2 and Th2 cytokines (IL-4, IL-5, and IL-13) was markedly increased in the lungs of Ova-sensitized mice 24 h after a single Ova challenge. Concordantly, the level of total and Ova-specific serum immunoglobulin (Ig)E and intraepithelial mucosubstances in the axial intrapulmonary airway of Ova-sensitized mice was robustly elevated 96 h after the second Ova challenge. Cannabinol (CBN) or Delta(9)-tetrahydrocannabinol (Delta(9)-THC; 50 mg/kg, ip), administered daily for 3 consecutive days before sensitization and then before challenge, significantly attenuated the elevation of IL-2, IL-4, IL-5, and IL-13 steady-state mRNA expression.
elicited by Ova challenge in the lungs. In addition, the elevation of serum IgE and the mucus overproduction induced by Ova challenge was also markedly attenuated by CBN or Delta(9)-THC administration in Ova-sensitized mice. These results suggest that plant-derived immunomodulatory cannabinoids exhibit potential therapeutic utility in the treatment of allergic airway disease by inhibiting the expression of critical T cell cytokines and the associated inflammatory response.


The current study examined the interaction between the cannabinoid CB(1) receptor agonist Delta(9)-tetrahydrocannabinol and (R)-methanandamide in combination with the cannabinoid CB(1) receptor antagonist SR-141716A (N-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H -pyrazole-3-carboxamide HCl) in rats responding for food on a fixed ratio (FR-10) schedule of food reinforcement. The study provided only limited evidence for antagonism by SR-141716A (at 1 mg/kg but not with 0.3, 3 and 10 mg/kg) of the rate suppressant effects induced by the cannabinoid CB(1) receptor agonist Delta(9)-tetrahydrocannabinol (and only at the single dose of 5.6 mg/kg Delta(9)-tetrahydrocannabinol). (R)-Methanandamide in combination with SR-141716A resulted in a greater rate suppression compared to that induced by (R)-methanandamide alone. Thus, SR-141716A augmented the rate-decreasing effects of (R)-methanandamide and only minimally altered the rate-decreasing effects of Delta(9)-tetrahydrocannabinol. Additionally, high doses (10 and 30 mg/kg) of SR-141716 singly consistently suppressed the rate of responding. The current results coupled with our previous data examining combinations of Delta(9)-tetrahydrocannabinol or (R)-methanandamide and SR-141716 (see text) underscore pharmacological/behavioral differences (whether quantitative or qualitative) between the cannabinoid CB(1) agonists (R)-methanandamide and Delta(9)-tetrahydrocannabinol revealed by their interactions with the cannabinoid CB(1) antagonist SR-141716.


AM404 [ N-(4-hydroxyphenyl)arachidonylamide] and VDM 11 [(5 Z,8 Z,11 Z,14 Z)- N-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide] are commonly used to prevent the cellular accumulation of the endocannabinoid anandamide, and thereby to potentiate its actions. However, it has been reported that AM404 can produce an influx of calcium into cells, which might be expected to have deleterious effects on cell proliferation. In the present study, AM404 and VDM 11 were found to reduce C6 glioma cell proliferation with IC(50) values of 4.9 and 2.7 micro M, respectively. The inhibition of cell proliferation following a 96-h exposure was not accompanied by dramatic caspase activation, and was not prevented by either a combination of cannabinoid and vanilloid receptor antagonists, or by the antioxidant alpha-tocopherol, suggestive of a non-specific mode of action. Similar results were seen with palmitoyleisopropylamide, although this compound only produced significant inhibition of cell proliferation at 30 micro M concentrations. AM404 (1 micro M), VDM 11 (1 micro M) and palmitoyleisopropylamide (3-30 micro M), i.e. concentrations producing relatively modest effects on cell proliferation per se, reduced the vanilloid receptor-mediated antiproliferative effects of anandamide, as would be expected for compounds preventing the cellular accumulation of anandamide (and thereby access to its binding site on the vanilloid receptor). It is concluded that concentrations of AM404 and VDM 11 that are generally used to reduce the cellular accumulation of anandamide have deleterious effects upon cell proliferation, and that lower concentrations of these compounds may be more appropriate to use in vitro.

We have recently examined the status of the endocannabinoid transmission in the basal ganglia in Huntington's disease (HD) using a rat model generated by bilateral intrastriatal injections of 3-nitropropionic acid (3-NP). In these previous studies, we focused on the early phase of hyperactivity that occurs 1-2 weeks after the lesion, comparable to early grades of the human disease, while in the present study, we wanted to explore the late akinetic phase observed 3-4 weeks after the lesion (similar to advanced grades). First, we confirmed that 3-NP-lesioned rats exhibited a marked akinesia tested at 4 weeks post-lesion. We observed a marked reduction in ambulatory and exploratory activities and a trend towards a decrease in stereotypies, paralleled by a strong increase in the time spent in inactivity. There was also a profound reduction in GABA contents and glutamic acid decarboxylase activity, particularly in the caudate-putamen and the globus pallidus. Dopamine and DOPAC contents, as well as the activity of tyrosine hydroxylase, were also reduced, particularly in the caudate-putamen. mRNA levels for neuronal-specific enolase, proenkephalin and substance P were also dramatically reduced in the caudate-putamen, thus indicating a death of both the direct (striatonigral) and the indirect (striatopallidal) GABAergic projection pathways, which corresponded with a marked loss of CB(1) receptor-mRNA levels observed in both parts, lateral and medial, of the caudate-putamen. However, losses of CB(1) receptor binding were confined to the globus pallidus and the caudate-putamen, whereas there were no changes in the substantia nigra and the entopeduncular nucleus. Finally, we failed to reduce the marked akinesia found in these animals by administering SR141716A, a selective antagonist of CB(1) receptors, which had exhibited hyperlocomotor effects in previous studies with naive animals. In summary, behavioral and biochemical changes observed in rats intrastriatally lesioned with 3-NP were compatible with a profound degeneration of striatal efferent GABAergic neurons, similar to those occurring in advanced stages of the human disease. As expected, a loss of CB(1) receptors was evident in the basal ganglia of these rats during the late akinetic stage of the disease. Further studies should demonstrate whether these receptors might be a target for a new therapy in HD, a disease with a poor pharmacological outcome.


Ajulemic acid (AJA) is a synthetic analog of the tetrahydrocannabinol (THC) metabolite THC-11-oic acid: THC is a major active ingredient of the drug marijuana derived from the plant cannabis. AJA has potent analgesic and anti-inflammatory activity without the psychotropic action of THC. Unlike the nonsteroidal anti-inflammatory drugs, AJA is not ulcerogenic at therapeutic doses, making it a promising anti-inflammatory drug. However, the mechanism of AJA action remains unknown. Here we report that AJA binds directly and specifically to the peroxisome proliferator-activated receptor gamma (PPARgamma), a pharmacologically important member of the nuclear receptor superfamily. Functional assay indicates that AJA activates the transcriptional activity of both human and mouse PPARgamma at pharmacological concentrations. Activation of PPARgamma by AJA requires the AF-2 helix of the receptor, suggesting that AJA activates PPARgamma through the ligand-dependent AF-2 function. AJA binding consistently enables PPARgamma to recruit nuclear receptor coactivators. In addition, we show that AJA inhibits interleukin-8 promoter activity in a PPARgamma-dependent manner, suggesting a link between the anti-inflammatory action of AJA and the activation of PPARgamma. Finally, we find that AJA treatment induces differentiation of 3T3 L1 fibroblasts into adipocytes, a process mediated by PPARgamma. Together, these data indicate that PPARgamma may be a molecular target for AJA, providing a potential mechanism for the anti-inflammatory action of AJA, and possibly other cannabinoids. These studies also implicate other potential therapeutic actions of AJA through PPARgamma activation in multiple signaling pathways.


Dietary long-chain polyunsaturated fatty acids are known to influence brain levels of the endocannabinoid anandamide in newborn pigs and mice. Furthermore, endocannabinoids were shown to control pup suckling and body weight in mice, and food intake in adult rodents. Here we determined the effect of maternal under-nutrition during gestation, lactation, or both, on body weight, and on the levels of endocannabinoids and expression of cannabinoid CB1 receptors and
fatty acid amide hydrolase in the hypothalamus of rat pups at weaning (21 days old) or adult rats (4 months old). Maternal under-nutrition resulted in a striking decrease in body weight of weaning rats, paralleled by a decrease in the hypothalamic levels of the endocannabinoid anandamide, but not of 2-arachidonoylglycerol. No significant change in the hypothalamic expression of either cannabinoid CB1 receptors or fatty acid amide hydrolase mRNA was detected in any of the three groups of weaned pups. The decrease in pup body weight and hypothalamic anandamide levels was not observable in 4-month-old rats from any of the three groups. These data suggest that maternal under-nutrition causes a decrease in hypothalamic anandamide levels and loss of body weight, and confirm a crucial role for endocannabinoid signalling in neonatal development.


Mereu, G., M. Fa, et al. (2003). "Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release." Proc Natl Acad Sci U S A.

To investigate the possible long-term consequences of gestational exposure to cannabinoids on cognitive functions, pregnant rats were administered with the CB1 receptor agonist WIN 55,212-2 (WIN), at a dose (0.5 mg/kg) that causes neither malformations nor overt signs of toxicity. Prenatal WIN exposure induced a disruption of memory retention in 40- and 80-day-old offspring subjected to a passive avoidance task. A hyperactive behavior at the ages of 12 and 40 days was also found. The memory impairment caused by the gestational exposure to WIN was correlated with alterations of hippocampal long-term potentiation (LTP) and glutamate release. LTP induced in CA3-CA1 synapses decayed faster in brain slices of rats born from WIN-treated dams, whereas posttetanic and short-term potentiation were similar to the control group. In line with LTP shortening, in vivo microdialysis showed a significant decrease in basal and K(+) evoked extracellular glutamate levels in the hippocampus of juvenile and adult rats born from WIN-treated dams. A similar reduction in glutamate outflow was also observed in primary cell cultures of hippocampus obtained from pups born from mothers exposed to WIN. The decrease in hippocampal glutamate outflow appears to be the cause of LTP disruption, which in turn might underlie, at least in part, the long-lasting impairment of cognitive functions caused by the gestational exposure to this cannabinoid agonist. These findings could provide an explanation of cognitive alterations observed in children born from women who use marijuana during pregnancy.


Our objective was to identify the sites of interaction of cannabinoids with cardiovascular sympathetic regulation in the rat. Effects on sympathetic tone were first determined in anaesthetised animals following i.v. administration of the drugs. Central effects were evaluated in anaesthetised rats receiving microinjections of cannabinoids into brain stem nuclei. Peripheral effects were identified in pithed rats with electrically stimulated sympathetic outflow. In anaesthetised and artificially ventilated rats, i.v. injection of the cannabinoid agonists WIN55212-2 and CP55940 decreased mean arterial pressure, heart rate and the plasma noradrenaline concentration. These effects were antagonized by the CB(1) cannabinoid receptor antagonist SR141716A. The bradycardia was abolished by the muscarinic acetylcholine receptor antagonist methylnaloxone. The decreases in mean arterial pressure and heart rate caused by cannabinoids in ventilated rats were much less pronounced than in spontaneously breathing rats. Microinjection of WIN55212-2 into the nucleus tractus solitarius had no effect. Microinjected into the rostral ventrolateral medulla oblongata, WIN55212-2 lowered mean arterial pressure slightly without changing other parameters. In pithed rats, WIN55212-2 inhibited the increases in mean arterial pressure, heart rate and the plasma noradrenaline concentration evoked by electrical stimulation of the sympathetic outflow. Our results show that activation of CB(1) cannabinoid receptors induces sympathoinhibition and enhancement of cardiac vagal tone, leading to hypotension and bradycardia. Presynaptic inhibition of noradrenaline release from terminals of postganglionic sympathetic neurons is the major component of the sympathoinhibition, but an effect in the rostral
ventrolateral medulla oblongata may also contribute. The cannabinoid-evoked cardiovascular depression depends strongly on the respiratory state of the animals.


The cannabinoid system is a regulator of neurotransmission and is linked with hormonal control. We have found in experimental mouse studies that the progesterone receptor inhibitor mifepristone (RU38486, 80 mg/kg i.p.) or the 11beta-hydroxylase inhibitor metyrapone (100 mg/kg i.p.) when administered in combination with cannabinoids potentiates the transient-sedating cannabinoid receptor-1 effects of a high-dose Delta(9)-tetrahydrocannabinol (25 mg/kg i.p.), causing severe prolonged sedation associated with hypomotility, catalepsy and hypothermia. This observation has implications for human subjects taking these drugs and related compounds particularly because of the ubiquitous use of cannabis and the high potential for mifepristone and related compounds to become available on the 'black-market' as abortifacients.


Although many people drink alcohol regularly, only some become addicted. Several studies have shown that genetic and environmental factors contribute to individual differences in the vulnerability to the effects of alcohol (Nestler, 2000; Kreek, 2001; Crabbe, 2002). Among the environmental factors, stress is perhaps the most important trigger for relapse after a period of abstinence (Koob and Nestler, 1997; Piazza and Le Moal, 1998; Koob and Le Moal, 2001; Weiss et al., 2001). Here we show that ethanol withdrawal symptoms were completely absent in cannabinoid CB1 receptor-deficient mice, although acute effects of ethanol and ethanol tolerance and preference were basically normal. Furthermore, foot-shock stress had no effect on alcohol preference in Cnr1-/- mice, although it induced a dramatic increase in Cnr1+/+ animals. These results reveal a critical role for the CB1 receptor in clinically important aspects of alcohol dependence and provide a rationale for the use of CB1 receptor antagonists in the treatment of alcohol addiction.


Significant variability in the effects of cannabinoid CB1 receptor ligands on emotional reactivity in animals and humans suggests that the endocannabinoid system may selectively modulate certain types of anxiety. In view of substantial evidence for qualitative differences in the nature of anxiety elicited on initial and subsequent exposures to the elevated plus-maze, the present studies contrasted the behavioural effects of the selective CB1 receptor antagonist SR141716A (0.1-10.0 mg/kg) and the reference benzodiazepine chlordiazepoxide (CDP, 15 mg/kg) both in maze-naive mice (trial 1) and in mice that had been given a single undrugged exposure to the maze 24 h prior to testing (trial 2). Results confirmed the anxioselective effect of CDP on trial 1 but a complete absence of such activity on trial 2 (i.e. one trial tolerance). In marked contrast, SR141716A had no behavioural effects in maze-naive mice but, at doses of 1.0-3.0 mg/kg (effect maximal at 1.0 mg/kg), significantly reduced anxiety-like responses in maze-experienced animals. Like the effect of CDP on trial 1, the antianxiety profile of SR141716A on plus-maze trial 2 was observed in the absence of any change in general activity levels. The apparent experientially induced 'sensitization' to the anxiolytic-like effects of SR141716A in the plus-maze contrasts markedly with the widely reported loss of benzodiazepine efficacy in test-experienced animals. Data are discussed in relation to the recently described phenotypes of CB1 receptor knockout mice and, in particular, to mounting evidence for the existence of a novel SR141716A-sensitive neuronal cannabinoid receptor.

The compound N-piperidinyl-[8-chloro-1-(2,4-dichlorophenyl)-1,4, 5,6-tetrahydrobenzo [6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide] (NESS 0327) was synthesized and evaluated for binding affinity towards cannabinoid CB1 and CB2 receptor. NESS 0327 exhibited a stronger selectivity for CB1 receptor when compared with N-piperidinyl-5-(4-chlorophenyl)-1-(2,4- dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR 141716A) showing a much higher affinity for CB1 receptor (Ki = 350 +/- 5 fM and 1.8 +/- 0.075 nM, respectively) and a higher affinity for the CB2 receptor (Ki = 21 +/- 0.5 nM and 514 +/- 30 nM, respectively). Affinity ratios demonstrated that NESS 0327 was more than 60,000-fold selective for the CB1 receptor, while SR 141716A only 285 fold. NESS 0327 alone did not produce concentration-dependent stimulation of guanosine 5'-O-(3-[(35)S]thio)-triphosphate ([(35)S]GTPgammaS) binding in rat cerebella membranes. Conversely, NESS 0327 antagonized [R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl] pyrrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalanyl) methanone mesylate] (WIN 55,212-2)-stimulated [(35)S]GTPgammaS binding. In functional assay, NESS 0327 antagonized the inhibitory effects of WIN 55,212-2 on electrically evoked contractions in mouse isolated vas deferens preparations with pA2 values of 12.46 +/- 0.23. In vivo studies indicated that NESS 0327 antagonized the antinociceptive effect produced by WIN 55,212-2 (2 mg/kg, s.c.) in both tail flick (ID50 = 0.042 +/- 0.01 mg/kg i.p.) and hot plate test (ID50 = 0.018 +/- 0.006 mg/kg i.p.). These results indicated that NESS 0327 is a novel cannabinoid antagonist with high selectivity for the cannabinoid CB1 receptor.


Endocannabinoid signaling processes are present in diverse organisms and in organisms 500 million years divergent in evolution. Cannabinoid receptor-1 expression (CB1), anandamide, and anandamide amidase have been found in invertebrates. Furthermore, this signaling system is coupled to constitutive nitric oxide synthase (cNOS)-derived nitric oxide (NO) release in both vertebrates and invertebrates, thereby regulating neural, immune, and vascular-like functions in these divergent organisms. In human endothelial cells from various blood vessels, CB1 immunoreactive components are present as is its coupling to anandamide-stimulated cNOS-derived NO production, which exerts an autoregulatory role on cNOS release. The modulation of vascular diameter and vascular tone represents a crucial point of interest in these pathways, and interactions between NO and the sympathetic nerve system are of importance, i.e, norepinephrine. Here, a possible association of NO and endocannabinoid signaling with the relaxation response, a physiological counterpart of the stress response, may exist.


In the neocortex, inhibitory interneurons tightly regulate the firing patterns and integrative properties of pyramidal neurons (PNs). The endocannabinoid system of the neocortex may play an important role in the activity-dependent regulation of inhibitory (i.e., GABAergic) inputs received by PNs. In the present study, using whole cell recordings from layer 2/3 PNs in slices of mouse sensory cortex, we have identified a role for PN-derived endocannabinoids in the control of afferent inhibitory strength. Pairing evoked inhibitory currents with repeated epochs of postsynaptic depolarization led to a transient suppression of inhibition that was induced by a rise in postsynaptic Ca(2+) and was expressed as a reduction in presynaptic GABA release. An antagonist (AM251) of the type-1 cannabinoid receptor blocked the depolarization-induced suppression of evoked inhibitory postsynaptic currents (eIPSCs), and the cannabinoid WIN55,212-2 reduced eIPSC amplitude and occluded suppression. The degree of WIN55,212-2-mediated inhibition of eIPSCs was strongly correlated with the magnitude of depolarization-induced suppression of the eIPSCs, suggesting that the WIN-sensitive afferents are suppressed by PN depolarization. Moreover, blocking endocannabinoid uptake with AM404 strongly modulated the kinetics and magnitude of eIPSC suppression. We conclude that the release of endocannabinoids from PNs allows for the postsynaptic control of presynaptic inhibition and could have profound consequences for the integrative properties of neocortical PNs.

N-Acylethanolamines (NAEs) are fatty acid derivatives found as minor constituents of animal and plant tissues, and their levels increase 10- to 50-fold in tobacco (Nicotiana tabacum) leaves treated with fungal elicitors. Infiltration of tobacco leaves with submicromolar to micromolar concentrations of N-myristoylethanolamine (NAE 14:0) resulted in an increase in relative phenylalanine ammonia-lyase (PAL) transcript abundance within 8 h after infiltration, and this PAL activation was reduced after co-infiltration with cannabinoid receptor antagonists (AM 281 and SR 144528). A saturable, high-affinity specific binding activity for [(3)H]NAE 14:0 was identified in suspension-cultured tobacco cells and in microsomes from tobacco leaves (apparent K(d) of 74 and 35 nM, respectively); cannabinoid receptor antagonists reduced or eliminated specific [(3)H]NAE 14:0 binding, consistent with the physiological response. N-Oleoylethanolamine activated PAL2 expression in leaves and diminished [(3)H]NAE 14:0 binding in microsomes, whereas N-linoleoylethanolamine did not activate PAL2 expression in leaves, and did not affect [(3)H]NAE 14:0 binding in microsomes. The nonionic detergent dodecylmaltoside solubilized functional [(3)H]NAE 14:0-binding activity from tobacco microsomal membranes. The dodecylmaltoside-solubilized NAE-binding activity retained similar, but not identical, binding properties to the NAE-binding protein(s) in intact tobacco microsomes. Additionally, high-affinity saturable NAE-binding proteins were identified in microsomes isolated from Arabidopsis and Medicago truncatula tissues, indicating the general prevalence of these binding proteins in plant membranes. We propose that plants possess an NAE-signaling pathway with functional similarities to the "endocannabinoid" pathway of animal systems and that this pathway, in part, participates in xylanase elicitor perception in tobacco.


The endogenous fatty acid amide anandamide (AEA) has, as a result of its actions on cannabinoid and vanilloid receptors, a number of important pharmacological properties including effects on nociception, memory processes, spasticity, and cell proliferation. Inhibition of the metabolism of AEA, catalyzed by fatty acid amide hydrolase (FAAH), potentiates the actions of AEA in vivo and therefore may be a useful target for drug development. In the present study, we have investigated whether substitution of the headgroup of the endogenous alternative FAAH substrate palmitoylethanolamide (PEA) can result in the identification of novel compounds preventing AEA metabolism. Thirty-seven derivatives of PEA were synthesized, with the C16 long chain of palmitic acid kept intact, and comprising 20 alkylated, 12 aromatic, and 4 halogenated amides. The ability of the PEA derivatives to inhibit FAAH-catalyzed hydrolysis of [(3)H]AEA was investigated using rat brain homogenates as a source of FAAH. Inhibition curves were analyzed to determine the potency of the inhibitable fraction (pI(50) values) and the maximal attained inhibition for the compound, given that solubility in an aqueous environment is a major issue for these compounds. In the alkylamide family, palmitoylethylamide and palmitoylallylamide were inhibitors of AEA metabolism with pI(50) values of 5.45 and 5.47, respectively. Halogenated derivatives (Cl and Br) exhibit pI(50) values of approximately 5.5 but rather low percentages of maximal inhibition. The -OH group of the ethyl head chain of N-palmitoylethanolamine was not necessary for interaction with FAAH. Amides containing aromatic moieties were less potent inhibitors of AEA metabolism. Compounds containing amide and ester bonds, 13 and 37, showed pI(50) values of 4.99 and 5.08, respectively. None of the compounds showed obvious affinity for CB(1) or CB(2) receptors expressed on Chinese hamster ovary (CHO) cells. It is concluded that although none of the compounds were dramatically more potent than PEA itself at reducing the metabolism of AEA, the lack of effect of the compounds at CB(1) and CB(2) receptors makes them useful templates for development of possible therapeutic FAAH inhibitors.

Drugs acting on brain cannabinoid CB(1) receptors exert complex actions on modulatory transmitters that are involved in attention and cognition; however, little is known about the precise pharmacological and anatomical mechanisms that govern these effects. Previously demonstrated effects of cannabinoids on acetylcholine (ACh) in the hippocampus prompted us to evaluate changes in the prefrontal cortex, a site associated with mnemonic and attentional functions. We utilized in vivo microdialysis, coupled with direct reverse perfusion of agents, to study the actions on cannabinoergic drugs on ACh release within the rat frontal cortex. Systemic administration of the CB(1) receptor agonists Delta(9)-tetrahydrocannabinol (THC) or WIN 55,212-2 (WIN) dose- and time-dependently increased ACh release; these effects were blocked by pretreatment with the selective CB(1) receptor antagonist / partial inverse agonist SR141716A (SR). THC applied by reverse dialysis in the frontal cortex caused no change in ACh release, although intrastriatal infusions of THC decreased ACh efflux. These data indicate that cannabinoid agonists potentiate ACh release in the frontal cortex by activating cannabinoid receptors in brain regions other than the frontal cortex. Synapse 48:178-183, 2003.


Long-term abuse of delta-9-tetrahydrocannabinol (THC), the major psychoactive constituent of marijuana, produces behavioral and metabolic signs of frontal cortical dysfunction in humans; these effects persist even after short-term abstinence. Based on a preliminary finding that repeated administration of THC to rats reduces basal frontal cortical dopamine turnover (Jentsch et al. [1998] Neurosci Lett 246:169-172), we further investigated the effects of repeated administrations of THC or WIN 55,212-2 (WIN), a synthetic cannabinoid receptor agonist, on dopamine turnover in the prefrontal cortex, striatum, and nucleus accumbens. THC or WIN (twice daily for 7 or 14 days) caused a persistent and selective reduction in medial prefrontal cortical dopamine turnover; no significant alterations of dopamine metabolism were observed in the nucleus accumbens or striatum. Importantly, these dopaminergic deficits in the prefrontal cortex were observed after a drug-free period of up to 14 days. Thus, the cognitive dysfunction produced by heavy, long-term cannabis use may be suberved, in part, by drug-induced alterations in frontal cortical dopamine turnover. Synapse 49:61-66, 2003.


RATIONALE. Considerable interplay exists between the brain's opioid and cannabinoid systems. These systems are both involved in the control of appetite and research supports the notion that the opioid system modulates the role of the cannabinoid system on appetite. However, the ability of the cannabinoid system to modulate the opioid system's control over appetite has not been well studied. OBJECTIVES. The present study examined the role of cannabinoid CB(1) receptors in the control of opioid-induced feeding, and sought to identify specific brain regions underlying this role. METHODS. After being habituated to the test environment and injection procedure, sated rats were injected with the cannabinoid CB(1) receptor antagonist SR 141716 (0.03-3.0 mg/kg, IP). Thirty minutes later, morphine or its vehicle were administered systemically (2.5 mg/kg SC, experiments 1 and 2) or intracranially into the nucleus accumbens (nAcc, experiment 3) or paraventricular nucleus of the hypothalamus (PVN, experiment 4). Food intake and locomotor activity was then recorded for 120 min. RESULTS. A significant increase in food intake was observed following systemic and intracranial (10 nmol) application of morphine in all experiments. SR 141716 suppressed systemic and intra-PVN morphine induced feeding (experiments 2 and 4), but did not attenuate food intake induced by intra-nAcc application of morphine (experiment 3). CONCLUSIONS. Because SR 141716 had no effect on intra-nAcc morphine-stimulated feeding, it would appear that cannabinoid receptors do not modify opioid-mediated hedonic responses to food. Rather, we conclude that cannabinoid CB(1) receptor
blockade may suppress opioid-induced feeding by stimulating the release of satiety-related peptides within the hypothalamus. Further, because SR 141716 did not block morphine induced locomotor activity, the observed effects on feeding do not appear to be due to a non-specific reduction in motivated behaviour.

CLINICAL SCIENCE

There has been renewed interest in the therapeutic applications of cannabis, and people, particularly those with multiple sclerosis, claim that it may offer benefit in symptom control. Cannabis exerts many of its effects because it taps into an endogenous cannabinoid system. Recent advances have begun to shine light on the biology of this system and may support some of the anecdotal medical claims. The problem with cannabis as a drug is that both the positive and negative aspects are largely the work of the same receptor. However, it may be possible to avoid these through modulation of the endogenous system. Cannabinoids provide a novel therapeutic target, not only for controlling symptoms, but also slowing disease progression through inhibition of neurodegeneration, which is the cause of accumulating irreversible disability.


This study investigated if changes in pre-synaptic markers on dopaminergic neurons (dopamine transporter [DAT], tyrosine hydroxylase [TH]) were present in the caudate from subjects with schizophrenia who had Delta(9)tetrahydrocannabinol (THC) in their blood at autopsy. These changes were posited because animal studies show that treatment with THC decreases dopamine uptake and TH in the striatum. Studies utilized caudate, obtained postmortem, from 14 schizophrenic and 14 control subjects. [(3)H]mazindol binding to caudate, measured using autoradiography, was taken as a measure of DAT; TH levels were estimated using an antihuman TH antibody and Western blotting. There was decreased [(3)H]mazindol binding to DAT in the caudate from the schizophrenic subjects with no detectable blood THC levels (THC(-)) compared with THC(-) control subjects (mean +/- SEM: 240 +/- 19 vs. 296 +/- 14 fmol/mg estimated tissue equivalents, p = .01). There were no significant differences between levels of DAT in the caudate from schizophrenic and control subjects that had THC in their blood. Tyrosine hydroxylase was not different in any diagnostic cohort. Our data suggests that DAT is decreased in the caudate from THC(-) subjects with schizophrenia, a change that may be reversed by ingesting THC from cannabis.


The induction of hyperalgesia upon capsaicin administration requires activation of specific sub-classes of nociceptive afferent C-fibres providing nociceptive input to the central nervous system. It has been demonstrated in animal models that the endocannabinoid anandamide has anti-hyperalgesic properties upon capsaicin stimulation, albeit it also binds to vanilloid receptors. In the present study we topically administered the cannabinoid receptor ligand HU210 to human skin and investigated its effects on capsaicin-induced pain and hyperalgesia. We demonstrated that pre-treatment with HU210 significantly reduced the perception of pain following the administration of capsaicin. Heat pain thresholds were significantly reduced by capsaicin application measured 5 and 30min after administration. In contrast, at the HU210 pre-treated skin sites capsaicin failed to induce heat hyperalgesia during the fifth minute of administration. Secondary mechanical hyperalgesia to touch (allodynia) was measured during the fifth, 15th and 30th minute after capsaicin administration. In comparison to the ethanol control site, the area of touch-evoked allodynia was significantly reduced at the HU210 skin site during the first two measures. However, 30min after the administration of capsaicin no significant differences of allodynia were observed between the HU210 and ethanol pre-treated skin. The present study provided evidence for analgesic and anti-hyperalgesic properties of a topically applied cannabinoid receptor ligand, which might have important therapeutic implications in humans.

Cannabinoids can modulate the function of immune cells. We here present the first human in vivo study measuring immune function in 16 MS patients treated with oral cannabinoids. A modest increase of TNF-alpha in LPS-stimulated whole blood was found during cannabis plant-extract treatment (p=0.037), with no change in other cytokines. In the subgroup of patients with high adverse event scores, we found an increase in plasma IL-12p40 (p=0.002). The results suggest pro-inflammatory disease-modifying potential of cannabinoids in MS.


BEHAVIOURAL SCIENCE


BACKGROUND: Dependence increases the likelihood of adverse consequences of cannabis use, but its aetiology is poorly understood. Aims To examine adolescent precursors of young-adult cannabis dependence. METHOD: Putative risk factors were measured in a representative sample (n=2032) of secondary students in the State of Victoria, Australia, six times between 1992 and 1995. Cannabis dependence was assessed in 1998, at age 20-21 years. RESULTS: Of 1601 young adults, 115 met criteria for cannabis dependence. Male gender (OR=2.6, P < 0.01), regular cannabis use (weekly: OR=4.9; daily: OR=4.6, P=0.02), persistent antisocial behaviour (linear effect P=0.03) and persistent cigarette smoking (linear effect P=0.02) independently predicted cannabis dependence. Neither smoking severity (P=0.83) nor persistent psychiatric morbidity (linear effect P=0.26) independently predicted dependence. Regular cannabis use increased risk only in the absence of persistent problematic alcohol use. CONCLUSIONS: Weekly cannabis use marks a threshold for increased risk of later dependence, with selection of cannabis in preference to alcohol possibly indicating an early addiction process.


PURPOSE: Information on the potential relation between marijuana use and the incidence of hospitalized injury is extremely limited. The purpose of this effort was to investigate the potential for this association. METHODS: A retrospective study was conducted in a large prepaid Northern California health care program cohort (n = 64,657) that completed baseline questionnaires about health behaviors, including marijuana use, and health status between 1979 and 1985. All injury hospitalizations through December 31, 1991 (n = 965) were identified and validated. RESULTS: Using Poisson regression modeling, increased rate-ratios and 95% confidence intervals were identified for all-cause injury hospitalizations for both men and women among current users (1.58; 1.29 to 1.94 and 1.55; 1.12 to 2.10, respectively) relative to nonusers, adjusted for age, cigarette and alcohol use, and other potential confounders. Increased rates of motor vehicle (2.31, 1.44 to 3.72), assault (2.63, 1.56 to 4.46), and self-inflicted (3.43, 1.54 to 7.87) injuries were identified among men who were current users; an increased rate of self-inflicted injuries (2.13, 1.05 to 4.10) was also identified in women who were current users. CONCLUSIONS: Though the results must be viewed cautiously, they suggest that marijuana use may be independently associated with increased risk of hospitalized injury. Further study of the physiological and behavioral mechanisms is warranted.


**OBJECTIVE:** Data on use and misuse of six classes of illicit substances by male twin pairs were used to examine whether genetic and shared environmental risk factors for substance use disorders are substance-specific or -nonspecific in their effect. **METHOD:** Lifetime history of use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates was assessed at personal interview in both members of 1,196 male-male twin pairs ascertained by the Virginia Twin Registry. Multivariate twin modeling of substance-nonspecific (common) and substance-specific genetic, shared environmental, and unique environmental risk factors was performed by using the program Mx. **RESULTS:** High levels of comorbidity involving the different substance categories were observed for both use and abuse/dependence. One common genetic factor was found to have a strong influence on risk for illicit use and abuse/dependence for all six substance classes. A modest influence of substance-specific genetic factors was seen for use but not for abuse/dependence. Shared environmental factors were more important for use than for abuse/dependence and were mediated entirely through a single common factor. **CONCLUSIONS:** In an adult population-based sample of male twins, both the genetic and the shared environmental effects on risk for the use and misuse of six classes of illicit substances were largely or entirely nonspecific in their effect. Environmental experiences unique to the person largely determine whether predisposed individuals will use or misuse one class of psychoactive substances rather than another.