Addiction


In this review we will critically assess the hypothesis that the reinforcing effect of virtually all drugs of abuse is primarily dependent on activation of the mesolimbic dopamine system. The focus is on five classes of abused drugs: psychostimulants, opiates, ethanol, cannabinoids and nicotine. For each of these drug classes, the pharmacological and physiological mechanisms underlying the direct or indirect influence on mesolimbic dopamine transmission will be reviewed. Next, we evaluate behavioral pharmacological experiments that specifically assess the influence of activation of the mesolimbic dopamine system on drug reinforcement, with particular emphasis on animal experiments using drug self-administration paradigms. There is overwhelming evidence that all five classes of abused drugs increase dopamine transmission in limbic regions of the brain through interactions with a variety of transporters, ionotropic receptors and
metabotropic receptors. Behavioral pharmacological experiments indicate that increased dopamine transmission is clearly both necessary and sufficient to promote psychostimulant reinforcement. For the other four classes of abused substances, self-administration experiments suggest that although increasing mesolimbic dopamine transmission plays an important role in the reinforcing effects of opiates, ethanol, cannabinoids and nicotine, there are also dopamine-independent processes that contribute significantly to the reinforcing effects of these compounds.

**Cardiovascular**


In the isolated rat mesenteric bed, the 1 min perfusion with 100 nM anandamide, a concentration that did not evoke vasorelaxation, elicited an acute release of 165.1+/−9.2 pmol nitric oxide (NO) that was paralleled by a 2-fold increase in cGMP tissue levels. The rise in NO released was mimicked by either (R)-(−)-methanandamide or the vanilloid receptor agonists resiniferatoxin and (E)-capsaicin but not by its inactive cis-isomer (Z)-capsaicin. The NO release elicited by either anandamide or capsaicin was reduced by the TRPV1 receptor antagonists 5′-iodoresiniferatoxin, SB 366791 and capsazepine as well as by the cannabinoid CB1 receptor antagonists SR141716A or AM251. The outflow of NO elicited by anandamide and capsaicin was also reduced by endothelium removal or NO synthase inhibition, suggesting the specific participation of endothelial TRPV1 receptors, rather than the novel endothelial TRPV4 receptors. Consistently, RT-PCR showed the expression of the mRNA coding for the rat TRPV1 receptor in the endothelial cell layer, in addition to its expression in sensory nerves. The participation of sensory nerves on the release of NO was precluded on the basis that neonatal denervation of the myenteric plexus sensory nerves did not modify the pattern of NO release induced by anandamide and capsaicin. We propose that low concentrations of anandamide, devoid of vasorelaxing effects, elicit an acute release of NO mediated predominantly by the activation of endothelial TRPV1 receptors whose physiological significance remains still elusive.


The mechanisms by which cannabinoids alter coronary vascular tone and cardiac performance are controversial. We investigated the effects of various cannabinoids in spontaneously beating Langendorff-perfused rat hearts. Bolus injections of anandamide (0.1-1 mumol) caused no change in coronary flow (CF) or left ventricular systolic pressure (LVSP). In hearts preperfused with vasopressin to induce vasoconstrictor tone, anandamide or the selective CB1 receptor agonist ACEA (1-100 nmol) dose-dependently increased CF by up to 267% and LVSP by 20 mm Hg. The metabolically stable endocannabinoid derivatives, R-methanandamide and noladin ether, displayed similar effects. In contrast, Delta-THC (10-100 nmol), the major psychoactive ingredient of cannabis, strongly decreased CF and LVSP. The CB2 receptor agonist JWH-133 (10-100 nmol) elicited vasodilator and positive inotropic effects only at higher doses. The CB1 antagonists SR141716A and AM-251 as well as the potassium channel inhibitors tetraethylammonium and iberiotoxin blocked the anandamide-induced increases in CF and LVSP, whereas the CB2 antagonist SR144528 and the putative "CB3 antagonist" O-1918 did not have an inhibitory effect. Immunohistochemistry revealed the presence of cardiac CB1 but no CB2 receptors. Anandamide and 2-arachidonoylglycerol were detected in heart tissue. However, combined application of fatty acid amidohydrolase inhibitors and the transport inhibitor AM-404 to augment tissue levels of endocannabinoids was without effect on CF or LVSP. We conclude that in the rat isolated heart with reestablished vasoconstrictor tone, cannabinoids including anandamide elicit coronary vasodilation and a secondary increase in contractility via CB1 receptors and potassium channels.

Several cannabinoids elicit systemic vasodilation mainly via CB1 cannabinoid and vanilloid receptors. However, effects in the pulmonary circulation are unknown. Using the isolated, ventilated, buffer-perfused rabbit lung, we show that the endocannabinoids arachidonyl ethanolamide (anandamide) and 2-arachidonyl-glycerol (2-AG) dose-dependently increase pulmonary arterial pressure (+19.9 +/- 3.4 mmHg, 5 microM; and +39.5 +/- 10.8 mmHg, 0.4 microM, respectively). 2-AG induced lung edema. The CB1 receptor antagonist AM-251 (0.1 and 5 microM) and the VR1 vanilloid receptor antagonist capsazepine (10 microM) failed to reduce anandamides effects. The metabolically stable anandamide- and 2-AG-analogues, R-methanandamide and noladin ether, Delta(9)-tetrahydrocannabinol and the synthetic cannabinoid HU-210, which is no arachidonic acid product, were without effect. The unspecific cyclooxygenase (COX) inhibitor aspirin (100 microM, p<0.001) and the specific COX-2 inhibitor nimesulide (10 microM, p<0.01), completely prevented pulmonary hypertension following 5 microM anandamide. COX-2 RNA was detected in rabbit lungs. The synthetic thromboxane receptor antagonist SQ 29,548 was without effect but the specific EP1 prostanoid receptor antagonist SC 19220 (0.1 microM) inhibited the pressure increase following anandamide (p<0.05). PCR analysis detected fatty acid amidohydrolase (FAAH), an enzyme that degrades endocannabinoids, in rabbit lung tissue. Furthermore, the specific FAAH inhibitor methyl arachidonyl fluorophosphonate (0.1 microM) blocked pressure effects of anandamide (p<0.01). Finally, anandamide (99 +/- 55 pmol/g) and 2-AG (19.6 +/- 8.4 nmol/g), were found in native lungs. We conclude that anandamide increases pulmonary arterial pressure via COX-2 metabolites following enzymatic degradation by the FAAH into arachidonic acid products.

**Endocrinology**


The combined resorptive activity of osteoclasts and the bone-generating function of osteoblasts result in the constant renewal of this vital tissue. It has long been appreciated that the coupled degradation and formation of bone is coordinately regulated by a complex interplay between endocrine and paracrine effectors; a recent report by now documents the possibility that cannabinoid receptors may also impact bone mass.

**Neuroscience**


This review covers the main features of a newly discovered intercellular signaling system in which endogenous ligands of the brain's cannabinoid receptors, or endocannabinoids, serve as retrograde messengers that enable a cell to control the strength of its own synaptic inputs. Endocannabinoids are released by bursts of action potentials, including events resembling interictal spikes, and probably by seizures as well. Activation of cannabinoid receptors has been implicated in neuroprotection against excitotoxicity and can help explain the anticonvulsant properties of cannabinoids that have been known since antiquity.


Normal tissue toxicity limits the efficacy of current treatment modalities for glioblastoma multiforme (GBM). We evaluated the influence of cannabinoids on cell proliferation, death, and morphology of human GBM cell lines and in primary human glial cultures, the normal cells from which GBM tumors arise. The influence of a plant derived cannabinoid agonist, Delta(9)-tetrahydrocannabinol Delta(9)-THC), and a potent synthetic cannabinoid agonist, WIN 55,212-2, were compared using time lapse microscopy. We discovered that Delta(9)-THC decreases cell proliferation and increases cell death of human GBM cells more rapidly than WIN 55,212-2. Delta(9)-THC was also more potent at inhibiting the proliferation of GBM cells compared to WIN 55,212-2. The effects of Delta(9)-THC and WIN 55,212-2 on the GBM cells were partially the result of cannabinoid receptor activation. The same concentration of Delta(9)-THC that significantly inhibits proliferation and increases death of human GBM cells has no significant
impact on human primary glial cultures. Evidence of selective efficacy with WIN 55,212-2 was also observed but the selectivity was less profound, and the synthetic agonist produced a greater disruption of normal cell morphology compared to Delta(9)-THC.


The in vivo effect of inhibitors of fatty acid amide hydrolase (FAAH) upon oedema volume and FAAH activity was evaluated in the carrageenan induced hind paw inflammation model in the mouse. Oedema was measured at two time points, 2 and 4 h, after intraplantar injection of carrageenan to anaesthetised mice. Intraperitoneal (i.p.) injections of the FAAH inhibitor URB597 (0.1, 0.3, 1 and 3 mg kg(-1)) 30 min prior to carrageenan administration, dose-dependently reduced oedema formation. At the 4 h time point, the ED(50) for URB597 was approximately 0.3 mg kg(-1). Indomethacin (5 mg kg(-1) i.p.) completely prevented the oedema response to carrageenan. The antioedema effects of indomethacin and URB597 were blocked by 3 mg kg(-1) i.p. of the CB(2) receptor antagonist SR144528. The effect of URB597 was not affected by pretreatment with the peroxisome proliferator-activated receptor gamma antagonist bisphenol A diglycidyl ether (30 mg kg(-1) i.p.) or the TRPV1 antagonist capsazepine (10 mg kg(-1) i.p.), when oedema was assessed 4 h after carrageenan administration. The CB(1) receptor antagonists AM251 (3 mg kg(-1) i.p.) and rimonabant (0.5 mg kg(-1) i.p.) gave inconsistent effects upon the antioedema effect of URB597. FAAH measurements were conducted ex vivo in the paws, spinal cords and brains of the mice. The activities of FAAH in the paws and spinal cords of the inflamed vehicle-treated mice were significantly lower than the corresponding activities in the noninflamed mice. PMSF treatment almost completely inhibited the FAAH activity in all three tissues, as did the highest dose of URB597 (3 mg kg(-1)) in spinal cord samples, whereas no obvious changes were seen ex vivo for the other treatments. In conclusion, the results show that in mice, treatment with indomethacin and URB597 produce SR144528-sensitive anti-inflammatory effects in the carrageenan model of acute inflammation.


Cannabidiol, a nonpsychoactive constituent of the Cannabis sativa plant, has been reported to act as an agonist of the vanilloid 1 channel in the transient receptor potential family (TRPV1) and also to inhibit the hydrolysis and cellular uptake of the endogenous cannabinoid anandamide. Cannabidiol has also been reported to have potential as an antipsychotic. We investigated the effect of cannabidiol on sensorimotor gating deficits in mice induced by the noncompetitive NMDA receptor antagonist, MK-801. Sensorimotor gating is deficient in psychotic disorders such as schizophrenia and may be reliably measured by prepulse inhibition (PPI) of the startle response in rodents and humans. MK-801 (0.3-1 mg/kg i.p.) dose dependently disrupted PPI while cannabidiol (1-15 mg/kg i.p.), when administered with vehicle, had no effect on PPI. Cannabidiol (5 mg/kg i.p.) successfully reversed disruptions in PPI induced by MK-801 (1 mg/kg i.p.), as did the atypical antipsychotic clozapine (4 mg/kg i.p.). Pretreatment with capsazepine (20 mg/kg i.p.) prevented the reversal of MK-801-induced disruption of PPI by cannabidiol, providing preliminary evidence that TRPV1 receptors are involved in the reversal of MK-801-induced sensorimotor gating deficits by cannabidiol.


At many central synapses, endocannabinoids released by postsynaptic cells act retrogradely on presynaptic G-protein-coupled cannabinoid receptors to inhibit neurotransmitter release. Here, we demonstrate that cannabinoids may directly affect the functioning of inhibitory glycine receptor (GlyR) channels. In isolated hippocampal pyramidal and Purkinje cerebellar neurons, endogenous cannabinoids anandamide and 2-arachidonylglycerol, applied at physiological concentrations, inhibited the amplitude and altered the kinetics of rise time, desensitization, and deactivation of the glycine-activated current (I(Gly)) in a concentration-dependent manner. These effects of cannabinoids were observed in the presence of cannabinoid
CB1/CB3, vanilloid receptor 1 antagonists, and the G-protein inhibitor GDPβS, suggesting a direct action of cannabinoids on GlyRs. The effect of cannabinoids on I(Gly) desensitization was strongly voltage dependent. We also demonstrate that, in the presence of a GABA(A) receptor antagonist, GlyRs may contribute to the generation of seizure-like activity induced by short bursts (seven stimuli) of high-frequency stimulation of inputs to hippocampal CA1 region, because this activity was diminished by selective GlyR antagonists (strychnine and ginkgolides B and J). The GlyR-mediated rhythmic activity was also reduced by cannabinoids (anandamide) in the presence of a CB1 receptor antagonist. These results suggest that the direct inhibition of GlyRs by endocannabinoids can modulate the hippocampal network activity.


We examined the effects of the cannabinoid anandamide (AEA) and its stable analogue, methanandamide (methAEA), on large-conductance, Ca(2+)-activated K(+) (BK) channels using HEK293 cells, in which the alpha-subunit of the BK channel (BKalpha), both alpha and beta1 (BKalphabeta1), or both alpha and beta4 (BKbeta4) subunits were heterologously expressed. In a whole-cell voltage-clamp configuration, each cannabinoid activated BKalphabeta in a similar concentration range. Because methAEA could potentiate BKalpha, BKalphabeta1, and BKalphabeta4 with similar efficacy, the beta subunits may not be involved in the site of action for cannabinoids. Under cell-attached patch-clamp conditions, application of methAEA to the bathing solution increased BK channel activity; however, methAEA did not alter channel activity in the excised inside-out patch mode even when ATP was present on the cytoplasmic side of the membrane. Application of methAEA to HEKBKalpha and HEKBKalphabeta did not change intracellular Ca(2+) concentration. Moreover, methAEA-induced potentiation of BK channel currents was not affected by pretreatment with a CB1 receptor antagonist (AM251), modulators of G proteins (cholera and pertussis toxins), or application of a selective CB2 agonist (JWH133). Inhibitors of calmodulin, protein kinase G, and MAP kinases (W7, KT5823, and PD98059) did not affect the potentiation. Application of methAEA to mouse aortic myocytes significantly increased BK channel currents. This study provides the first direct evidence that unknown factor(s) in the cytoplasm mediate the ability of endogenous cannabinoids to activate BK channel currents. Cannabinoids may be hyperpolarizing factors in cells, such as arterial myocytes, where BK channels are highly expressed.


Abstract In the present study, we investigated the effects of the cannabinoid receptor agonist CP55,940 on excitatory and inhibitory synaptic transmission in the rat supraoptic nucleus. Whole-cell patch clamp recordings were performed on supraoptic neurones in in vitro brain slice preparations. CP55,940 significantly reduced the frequency of spontaneous excitatory and inhibitory postsynaptic currents in a concentration-dependent manner. These changes were potently reversed by the CB1 receptor antagonist AM251. The results indicate that cannabinoids modulate the activity of magnocellular neurosecretory neurones by presynaptic inhibition of both excitatory and inhibitory synaptic transmission.


The endocannabinoid anandamide (N-arachidonoylthanolamine) was proposed to be an extracellular retrograde messenger, which regulates excitability of neurons by cannabinoid CB(1) receptor-dependent inhibition of neurotransmitter release. Recent findings indicate that the neuromodulatory actions of anandamide might be more complex. Anandamide has been shown to directly modulate various ion channels, such as alpha7-nicotinic acetylcholine receptors, T-type Ca(2+) channels, voltage-gated and background K(+)-channels and Transient Receptor Potential Vanilloid type 1 (TRPV1) channels. The binding site of anandamide at some of these ion channels appears to be intracellular or at the bilayer interface. This rises the intriguing possibility
that anandamide, prior to its release into the synaptic cleft, may regulate ion homeostasis and excitability of neurons as an intracellular modulator of ion channels independent of its action at cannabinoid CB(1) receptors. This possibility might extend the concept of anandamide as an endocannabinoid retrograde messenger and may have profound implications for its role in neurotransmission and neuronal function. Here, we will review the evidence for this hypothesis.


Two G protein-coupled receptors for marijuana's psychoactive component, Delta9-tetrahydrocannabinol, have been cloned to date, the cannabinoid CB1 and CB2 receptors. These two proteins, the endogenous lipids that activate them, also known as endocannabinoids, and the proteins for the biosynthesis and inactivation of these ligands constitute the endocannabinoid system. Evidence has accumulated over the last few years suggesting that endocannabinoid-based drugs may potentially be useful to reduce the effects of neurodegeneration. In fact, exogenous and endogenous cannabinoids were shown to exert neuroprotection in a variety of in vitro and in vivo models of neuronal injury via different mechanisms, such as prevention of excitotoxicity by cannabinoid CB1-mediated inhibition of glutamatergic transmission, reduction of calcium influx, anti-oxidant activity, activation of the phosphatidylinositol 3-kinase/protein kinase B pathway, induction of phosphorylation of extracellular regulated kinases and the expression of transcription factors and neurotrophins, lowering of cerebrovasoconstriction and induction of hypothermia. The release of endocannabinoids during neuronal injury may constitute a protective response. If this neuroprotective function of cannabinoid receptor activation can be transferred to the clinic, it might represent an interesting target to develop neuroprotective agents.


The endocannabinoid anandamide is able to interact with the transient receptor potential vanilloid 1 (TRPV1) channels at a molecular level. As yet, endogenously produced anandamide has not been shown to activate TRPV1, but this is of importance to understand the physiological function of this interaction. Here, we show that intracellular Ca(2+) mobilization via the purinergic receptor agonist ATP, the muscarinic receptor agonist carbachol or the Ca(2+)-ATPase inhibitor thapsigargin leads to formation of anandamide, and subsequent TRPV1-dependent Ca(2+) influx in transfected cells and sensory neurons of rat dorsal root ganglia (DRG). Anandamide metabolism and efflux from the cell tonically limit TRPV1-mediated Ca(2+) entry. In DRG neurons, this mechanism was found to lead to TRPV1-mediated currents that were enhanced by selective blockade of anandamide cellular efflux. Thus, endogenous anandamide is formed on stimulation of metabotropic receptors coupled to the phospholipase C/inositol 1,4,5-triphosphate pathway and then signals to TRPV1 channels. This novel intracellular function of anandamide may precede its action at cannabinoid receptors, and might be relevant to its control over neurotransmitter release.


BACKGROUND/AIMS: The paraventricular nucleus of the hypothalamus (PVN) is the target of converging orexigenic and anorexigenic pathways originating from various hypothalamic sites and is, therefore, considered to be the chief site mediating hypothalamic regulation of energy homeostasis. Although a large body of evidence suggests that central CB(1) cannabinoid receptors mediate food intake, it is not clear whether PVN CB(1) receptors are involved in the control of feeding behaviour. The present study therefore examined the effects of intra-PVN administration of Delta(9)-tetrahydrocannabinol (THC) and the cannabinoid receptor antagonist SR 141716 on feeding. METHODS: After being habituated to the test environment and injection procedure, sated rats were injected with SR 141716 (0.03-3.0μg, Experiment 1) alone or in combination with THC (5.0μg, Experiment 2) into the PVN. Food intake and locomotor activity then were recorded for 120min. RESULTS: Intra-PVN administration of THC produced a significant increase in food intake that was attenuated by SR 141716. Administration of SR
141716 alone did not affect feeding. Locomotor activity was not significantly affected by any drug treatments, suggesting that effects on feeding were not due to a non-specific reduction in motivated behaviour. These findings suggest an important role for PVN cannabinoid signalling in mediating THC-induced feeding behaviour. These results also demonstrate that the blockade of PVN CB(1) receptors alone is insufficient to reduce baseline feeding behaviour under these conditions.


The aim of this work was to study the mechanism of cross-modulation between cannabinoid and opioid systems for analgesia during acute and chronic exposure. Acute coadministration of ineffectual subanalgesic doses of the synthetic cannabinoid CP-55,940 (0.2 mg/kg i.p.) and morphine (2.5 mg/kg i.p.) resulted in significant antinociception. In chronic studies, a low dose of CP-55,940 (0.2 mg/kg, i.p.) that per se did not induce analgesia in naive animals produced a significant degree of antinociception in rats made tolerant to morphine, whereas in rats made tolerant to CP-55,940, morphine challenge did not produce any analgesic response. To identify the mechanism of these asymmetric interactions during chronic treatment, we investigated the functional activity of cannabinoid and mu opioid receptors and their effects on the cyclic AMP (cAMP) cascade. Autoradiographic-binding studies indicated a slight but significant reduction in cannabinoid receptor levels in the hippocampus and cerebellum of morphine-tolerant rats, whereas CP-55,940-stimulated [(35)S]GTPgammaS binding showed a significant decrease in receptor/G protein coupling in the limbic area. In CP-55,940 exposed rats, mu opioid receptor binding was significantly raised in the lateral thalamus and periaqueductal gray (PAG), with an increase in DAMGO-stimulated [(35)S]GTPgammaS binding in the nucleus accumbens. Finally, we tested the cAMP system's responsiveness to the cannabinoid and opioid in the striatum and dorsal mesencephalon. In vivo chronic morphine did not affect CP-55,940's ability to inhibit forskolin-stimulated cAMP production in vitro and actually induced sensitization in striatal membranes. In contrast, in vivo chronic CP-55,940 desensitized DAMGO's efficacy in inhibiting forskolin-stimulated cAMP production in vitro. The alterations to the cAMP system seem to mirror the behavioral responses, indicating that the two systems may interact at the postreceptor level. This might open up new therapeutic opportunities for relief of chronic pain through cannabinoid-opioid coadministration.


We have localized cannabinoid receptor 2 protein in rat and mouse somatic sensory nervous system, using an antibody that recognizes mouse cannabinoid receptor 2. Little or no cannabinoid receptor 2 immunoreactivity was found in sections of naive rat or mouse dorsal root ganglia or spinal cord. This was in accord with the lack of detectable cannabinoid receptor 2 mRNA in (dorsal root ganglion) neurons by in situ hybridization experiments described in the literature. However, we could detect cannabinoid receptor 2 immunoreactivity following unilateral nerve damage—either by sciatic nerve section, or by spinal nerve ligation. It was localized to the superficial laminae of the dorsal horn of the spinal cord, ipsilateral to the nerve damage, coincident with the area of termination of damaged afferents which was marked by loss of isolecitin B4 binding. This upregulation was not seen in cannabinoid receptor 2 null mice. The cannabinoid receptor 2 protein in spinal cord appeared to be expressed on sensory neuron afferent terminals as it colocalized with two markers of damaged afferents, namely growth associated protein-43 and the neuropeptide galanin. Moreover, it did not colocalize with markers of activated microglial cells (OX-42) or astroglial cells (glial fibrillary acidic protein) in rat spinal cord. In the peripheral nerve, accumulation of cannabinoid receptor 2 immunoreactivity was seen in nerve sections proximal, but not distal, to the ligation site, suggesting transport down the nerve from the cell bodies. Although convincing cannabinoid receptor 2 immunoreactivity was seen in neither uninjured nor injured dorsal root ganglion neuron cell bodies in tissue sections, expression was detectable in isolated, cultured neurons that had received a prior axotomy in vivo. This clear
demonstration of CB(2) receptors on sensory neurons suggests an additional cellular target for CB(2) agonist induced analgesia, at least in neuropathic models.

**Gastroenterology**


**BACKGROUND AND AIMS:** Cyclooxygenase-2 (COX-2) is up-regulated in most colorectal cancers and is responsible for metabolism of the endogenous cannabinoid, anandamide, into prostaglandin-ethanolamides (PG-EAs). The aims of this study were to determine whether anandamide and PG-EAs induce cell death in colorectal carcinoma (CRC) cells, and whether the high levels of COX-2 in CRC cells could be utilized for their specific targeting for cell death by anandamide. METHODS: We determined the effect of anandamide on human CRC cell growth by measuring cell growth and cell death, whether this was dependent on COX-2 protein expression or enzyme activity, and the potential involvement of PG-EAs in the induction of cell death. RESULTS: Anandamide inhibited the growth of CRC cell lines HT29 and HCA7/C29; moderate and high COX-2 expressors respectively, but had little effect on very low COX-2 expressing CRC cell line, SW480. Induction of cell death in HT29 and HCA7/C29 cell lines was partially rescued by the COX-2 selective inhibitor NS398. Cell death induced by anandamide was neither apoptosis nor necrosis. Furthermore, inhibition of fatty acid amide hydrolase (FAAH) potentiated the non-apoptotic cell death, indicating that anandamide induced cell death was mediated via the metabolism of anandamide by COX-2, rather than its degradation into arachidonic acid and ethanolamine. Interestingly, both PGE2-EA and PGD2-EA induced classical apoptosis. CONCLUSIONS: These findings suggest anandamide may be a useful chemopreventive/therapeutic agent for colorectal cancer, since anandamide targets cells that are high expressors of COX-2, and may also be used in the eradication of tumour cells that have become resistant to apoptosis.


**Background & Aims:** Two G-protein-coupled cannabinoid receptors, termed CB1 and CB2, have been identified and several mammalian enteric nervous systems express CB1 receptors and produce endocannabinoids. An immunomodulatory role for the endocannabinoid system in gastrointestinal inflammatory disorders has been proposed and this study sought to determine the location of both cannabinoid receptors in human colon and to investigate epithelial receptor function. Methods: The location of CB1 and CB2 receptors in human colonic tissue was determined by immunohistochemistry. Primary colonic epithelial cells were treated with both synthetic and endogenous cannabinoids in vitro, and biochemical coupling of the receptors to known signaling events was determined by immunoblotting. Human colonic epithelial cell lines were used in cannabinoid-binding studies and as a model for in vitro wound-healing experiments. Results: CB1-receptor immunoreactivity was evident in normal colonic epithelium, smooth muscle, and the submucosal myenteric plexus. CB1- and CB2-receptor expression was present on plasma cells in the lamina propria, whereas only CB2 was present on macrophages. CB2 immunoreactivity was seen in the epithelium of colonic tissue characteristic of inflammatory bowel disease. Cannabinoids enhanced epithelial wound closure either alone or in combination with lysophosphatidic acid through a CB1-lysophosphatidic acid 1 heteromeric receptor complex. Conclusions: CB1 receptors are expressed in normal human colon and colonic epithelium is responsive biochemically and functionally to cannabinoids. Increased epithelial CB2-receptor expression in human inflammatory bowel disease tissue implies an immunomodulatory role that may impact on mucosal immunity.

**Genetics**

Genome sequencing projects, and their available resources, have revealed two distinct genes encoding cannabinoid receptors, CB(1) and CB(2). Biochemical evidence in support of a third cannabinoid receptor includes signal transduction events and vasodilation in the vasculature of cannabinoid receptor knockout mice after exposure to the endogenous cannabinoid, anandamide. In addition, a nonpsychoactive ingredient in marijuana, abnormal cannabidiol, which does not activate the two characterized cannabinoid receptor homologues, has been shown to induce vasodilation in the endothelium. Our work distinguishes the biochemical differences by way of a phylogenetic analysis of cannabinoid receptors. Recently a putative orthologue to CB(1) and CB(2) has been identified in the urochordate, Ciona intestinalis, indicating the presence of cannabinoid receptors previous to the evolution of vertebrates. Moreover, the Ciona sequence shares equal identity to both cannabinoid paralogous sequences and no other GPCR sequence identified in an exhaustive database search is as similar. We propose that, although an alternate cannabnergic-activating pathway may be present, it does not include a GPCR (or other receptor type) phylogenetically related to the CB(1)/CB(2)Ciona lineage.

**Immunology**


In a recent study so far published in abstract form, it was reported that the CB(2) receptor selective agonist AM1241 diminishes oedema produced as a result of mast cell degranulation in vivo. It is, however, not known whether other structurally different CB(2) agonists share this effect, and whether this is due to a direct effect on mast cell function. In the present study, we have investigated the effects of JWH133, a CB(2) receptor selective agonist, together with the anti-inflammatory agent palmitoylethanolamide and its analogue palmitoylisopropylamide, on compound 48/80-induced oedema and degranulation in vivo and in vitro. JWH133 (20 and 200 mug/mouse i.p.) significantly reduced the ability of compound 48/80 to induce oedema in vivo in the anaesthetised mouse following its injection into the ear pinna. Palmitoylethanolamide (200 mug/mouse i.p.) also reduced the response to compound 48/80, whereas no firm conclusions could be drawn for palmitoylisopropylamide (20 and 200 mug/mouse i.p.). The CB(2) selective antagonist/inverse agonist SR144528 (60 mug/mouse i.p.) appeared to produce anti-inflammatory effects per se in this model, making it hard to interpret the effects of JWH133 in terms of CB(2) receptor mediated activation. In contrast to the situation in vivo, neither JWH133 (0.3 and 3 muM) nor palmitoylethanolamide (10 muM) affected mast cell degranulation, measured by following the release of the granular protein beta-hexosaminidase, produced by compound 48/80 in vitro in mouse skin slices. The two compounds were also ineffective in inhibiting the binding of [(3)H]pyrilamine to histamine H(1) receptors in vitro. It is concluded that the ability of JWH133 to affect mast cell dependent inflammation in vivo may be mediated by an indirect action upon the mast cells.


Immunomodulatory effects of endogenous and exogenous cannabinoids have been investigated in numerous studies, mostly performed with isolated cells or transformed cell lines, but only sparse data exist on human polymorphonuclear neutrophils (PMN). We therefore investigated the respiratory burst reaction of human whole blood PMN under the influence of cannabinoids using flow cytometry. In their natural whole blood milieu, a CB2 receptor-dependent stimulation of the PMN respiratory burst was found at nanomolar concentrations of CP55 940 and methanandamide after a 3h incubation period, whereas the short living and rapidly hydrolyzed endogenous ligand anandamide did not alter the burst reaction of whole blood PMN under the same experimental conditions. The stimulatory cannabinoid effect was totally absent in isolated PMN, but could be transferred onto isolated PMN by adding the cell-free low molecular weight plasma fraction (<5000 D) of cannabinoid incubated blood, indicating an indirect mechanism depending on humoral products or mediators. Results of our further experiments
suggest that products of the arachidonic acid metabolism are mediators of the cannabinoid-induced enhancement of the respiratory burst reaction of whole blood PMN.


The cannabinoid system is known to be important in neuronal regulation, but is also capable of modulating immune function. Although the CNS resident microglial cells have been shown to express the CB(2) subtype of cannabinoid receptor during non-immune-mediated pathological conditions, little is known about the expression of the cannabinoid system during immune-mediated CNS pathology. To examine this question, we measured CB(2) receptor mRNA expression in the CNS of mice with experimental autoimmune encephalomyelitis (EAE) and, by real-time PCR, found a 100-fold increase in CB(2) receptor mRNA expression during EAE onset. We next determined whether microglial cells specifically express the CB(2) receptor during EAE, and found that activated microglial cells expressed 10-fold more CB(2) receptor than microglia in the resting state. To determine the signals required for the up-regulation of the CB(2) receptor, we cultured microglial cells with combinations of gamma-interferon (IFN-gamma) and granulocyte/macrophage-colony stimulating factor (GM-CSF), which both promote microglial cell activation and are expressed in the CNS during EAE, and found that they synergized, resulting in an eight to 10-fold increase in the CB(2) receptor. We found no difference in the amount of the CB(2) receptor ligand, 2-arachidonoylglycerol (2-AG), in the spinal cord during EAE. These data demonstrate that microglial cell activation is accompanied by CB(2) receptor up-regulation, suggesting that this receptor plays an important role in microglial cell function in the CNS during autoimmune-induced inflammation.


Topically administered cannabinoids have been shown to reduce intraocular pressure by interacting with the ocular cannabinoid receptor. Most cannabinoids have very poor aqueous solubility, which limits their pharmaceutical development and usefulness. In this study, permeation of three cannabinoids (arachidonylethanolamide, R-methanandamide and noladin ether) and their water-soluble phosphate ester prodrugs across isolated rabbit cornea was investigated in vitro. Hydroxypropyl-beta-cyclodextrin (HP-beta-CD) was used to solubilize the parent cannabinoids in permeation studies to achieve the required concentration in donor and receiving cells. Highest fluxes were obtained with lipophilic parent compounds administered with HP-beta-CD, and the fluxes of phosphate esters were 45-70% that of their corresponding parent compounds. Phosphate esters hydrolysed on the surface of the cornea or during the permeation to release the lipophilic parent compound, which further permeated the cornea. No phosphate esters were detected on the endothelial side of the cornea. Although the phosphate esters had lower fluxes than their corresponding parent compounds in these HP-beta-CD formulations, the results are promising and the fluxes of phosphate esters are significantly higher than the fluxes of parent compounds administered as a suspension (due to their low aqueous solubility) without HP-beta-CD.


The cannabinoid system has been suggested to participate in processes such as antinociception, cognition, motor control, and, more recently, development of the nervous system. This study describes the expression of the CB(1) cannabinoid receptor in the developing chick retina and optic tectum by means of conventional immunoperoxidase protocols. CB(1) immunoreactivity was initially detected around the embryonic day 4 (E4) in both the retina and tectum. In the retina, CB(1) immunoreactivity was first observed in presumptive ganglion cells and, subsequently, in the inner plexiform layer and two populations of neurons of the inner nuclear layer. The post-hatched chick exhibited a pattern of staining that included four sublayers of the inner plexiform layer, a few stained cells in the ganglion cell layer, and labeled neurons.
both in the inner and central parts of the inner nuclear layer. The latter two types of neurons appear to be amacrine and bipolar cells, respectively. In the tectum, CB(1) first appeared in its most superficial zone and later in several tectal laminae, including a white matter layer (stratum album centrale; Cajal's layer 14). There was a remarkable and transient increase of labeling at E10, followed by a continuous reduction of staining until E18. In the post-hatched chick, tectal staining was mostly confined to layers 2-3 and 5-6. Stained perikarya were seldom observed in the tectum at any stage. These data are in agreement with a possible developmental function of CB(1), as it is expressed several days before synaptogenesis ensues and exhibits transient expression in the optic tectum.

Pharmacology


delta(9)-Tetrahydrocannabinol (Delta(9)-THC), the primary psychoactive constituent of marijuana, is subject to first pass hepatic metabolism primarily by hydroxylation to yield active and inactive oxygenated products. The primary metabolite is formed via oxidation of the allylic methyl group to yield 11-hydroxy-Delta(9)-THC, which is oxidized further to 11-nor-9-carboxy-Delta(9)-THC. The hydroxylation is thought to be mediated by CYP2C9. The present study was designed to address the kinetics and pharmacogenetics of CYP2C-mediated metabolism of (Delta(9))-THC by studying its metabolism in human liver microsomes and expressed enzymes. Expressed CYP2C9.1 exhibited high affinity for the hydroxylation of Delta(9)-THC (apparent K(m), 2muM), similar to that observed in human liver microsomes (apparent K(m,) 0.8muM). In contrast, the calculated intrinsic clearance (apparent V(m)/K(m)) for CYP2C9.2 and CYP2C9.3 was approximately 30% that of the wild type, CYP2C9.1. Given the high affinity of CYP2C9 for the hydroxylation of Delta(9)-THC, we evaluated the potential for an interaction between Delta(9)-THC, 11-hydroxy-Delta(9)-THC, or 11-nor-9-carboxy-Delta(9)-THC and the CYP2C9 substrate, phenytoin. Surprisingly, Delta(9)-THC increased the rate of phenytoin hydroxylation in human liver microsomes and expressed CYP2C9 enzyme. Similar increases in rate were observed with co-incubation of 11-hydroxy-Delta(9)-THC and 11-nor-9-carboxy-Delta(9)-THC with phenytoin. These in vitro data suggest the potential for an interaction from the concomitant administration of Delta(9)-THC and phenytoin that could result in decreased phenytoin concentrations in vivo.


CB1 and CB2 cannabinoid receptors are the primary targets of endogenous cannabinoids (endocannabinoids). These G protein-coupled receptors play an important role in many processes, including metabolic regulation, craving, pain, anxiety, bone growth, and immune function. Cannabinoid receptors can be engaged directly by agonists or antagonists, or indirectly by manipulating endocannabinoid metabolism. In the past several years, it has become apparent from preclinical studies that therapies either directly or indirectly influencing cannabinoid receptors might be clinically useful. This review considers the components of the endocannabinoid system and discusses some of the most promising endocannabinoid-based therapies.


We investigated the pharmacology of three novel compounds, Org 27569, Org 27759 and Org 29647, at the cannabinoid CB1 receptor. In equilibrium binding assays the Org compounds significantly increased the binding of the CB1 receptor agonist [(3)H]CP55940, indicative of a positively cooperative allosteric effect. The same compounds caused a significant, but incomplete, decrease in the specific binding of the CB1 receptor inverse agonist, [(3)H]SR141716A, indicative of a limited negative binding cooperativity. Analysis of the data according to an allosteric ternary complex model revealed that the estimated affinity of each Org compound was not significantly different when the radioligand was [(3)H]CP55940 or
However, the estimated co-operatively factor for the interaction between modulator and radioligand was greater than 1 when determined against [(3)H]CP55940 and less than 1 when determined against [(3)H]SR141716A. [(3)H]CP55940 dissociation kinetic studies also validated the allosteric nature of the Org compounds, since they all significantly decreased radioligand dissociation. These data suggest that the Org compounds bind allosterically to the CB1 receptor and elicit a conformational change that increases agonist affinity for the orthosteric binding site. In contrast to the binding assays, however, the Org compounds behaved as insurmountable antagonists of receptor function; in the reporter gene assay, the [(35)S]GTPgammaS binding assay and the mouse vas deferens assay they elicited a significant reduction in the Emax value for CB1 receptor agonists. The data presented clearly demonstrate, for the first time, that the cannabinoid CB1 receptor contains an allosteric binding site that can be recognized by synthetic small molecule ligands.


We recently reported that compound 1 is a potent inhibitor of the CB2 receptor with high selectivity over CB1. This paper describes the SAR development for this class of compounds. Variation of the substitution pattern on the aromatic rings, as well as the groups linking them together, led to sub-nanomolar inhibitors of the CB2 receptor, with high selectivity over CB1.


This study examined the ability of the endocannabinoids, 2-arachidonoyl glycerol (2-AG) and noladin ether, as well as the synthetic cannabinoid CP-55,940 to regulate three intracellular effectors via CB2 receptors in transfected CHO cells. Although the three agonists regulate all effectors with equivalent efficacy, the rank order of potencies differs depending on which effector is evaluated. Noladin ether and CP-55,940 most potently inhibit adenylyl cyclase, requiring higher concentrations to stimulate the extracellular signal-regulated kinase subgroup of the mitogen-activated protein kinases (ERK-MAPK) and Ca(2+)-transients. In contrast, 2-AG most potently activates ERK-MAPK, necessitating greater concentrations to inhibit adenylyl cyclase, and even higher amounts to stimulate Ca(2+)-transients. Endocannabinoids also appear to be more "efficient" agonists at CB2 receptors relative to synthetic agonists. 2-AG and noladin ether require occupancy of less than half the number of receptors to produce comparable regulation of adenylyl cyclase and ERK-MAPK, relative to the synthetic cannabinoid CP-55,940. The CB2 antagonist AM630 reverses the actions of all agonists except Ca(2+)-transient stimulation by 2-AG. However, the effect of 2-AG on Ca(2+)-transients is attenuated by a second CB2 antagonist SR144528. This suggests that 2-AG stimulates Ca(2+)-transients by binding to sites on CB2 receptors distinct from those occupied by AM630 and the other cannabinoids examined. Agonists produce no effects in pertussis toxin-treated cells. In summary, cannabinoid agonists distinctly bind to CB2 receptors and display different rank order of potencies and fractional receptor occupancies for regulation of intracellular effectors. These data provide direct evidence for agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors.


The family of endocannabinoids (i.e., the endogenous agonists of cannabinoid receptors) contains several polyunsaturated fatty acid amides such as anandamide (AEA) and oleamide but also esters such as 2-arachidonoylglycerol (2-AG). These compounds are the subject of growing interest in pharmacology for their multiple therapeutic potentials. Unfortunately, they are rapidly inactivated by enzymatic hydrolysis, which prevents their effective medical use. Inhibitors of endocannabinoid degradation seem to be necessary tools for the development of endocannabinoid therapeutics. But hitting this target is inconceivable without good knowledge of the enzymes. Fatty acid amide hydrolase (FAAH) is the oldest and the best characterised enzyme involved in the degradation of endocannabinoids. Cloning, distribution in the body and crystal structure of FAAH have been described. A large number of FAAH inhibitors have also been synthesised and tested. For a long time, FAAH was considered as the only key enzyme
hydrolysing endocannabinoids. But recent findings indicate that at least two other enzymes have critical role in the endocannabinoids degradation. Monoglyceride lipase participates in 2-AG degradation and some data indicate that it is the primary mechanism for 2-AG inactivation in intact neurons. N-palmitoylethanolamine-selective acid amidase (NPAA) is a secondary fatty acid amide hydrolase more active with N-palmitoylethanolamine, an anti-inflammatory substance. The purpose of this review is to collect and compare the catalytic properties of these 3 key enzymes hydrolysing endocannabinoids.

**Respirology**


Background: Although neurogenic inflammation via the activation of C fibers in the airway must have an important role in the pathogenesis of asthma, their regulatory mechanism remains uncertain. Objective: The pharmacological profiles of endogenous cannabinoid receptor agonists on the activation of C fibers in airway tissues were investigated and the mechanisms how cannabinoids regulate inflammatory reactions were clarified. Methods: The effects of endogenous cannabinoid receptor agonists on electrical field stimulation-induced bronchial smooth muscle contraction, capsaicin-induced bronchoconstriction and capsaicin-induced substance P release in guinea pig airway tissues were investigated. The influences of cannabinoid receptor antagonists and K(+) channel blockers to the effects of cannabinoid receptor agonists on these respiratory reactions were examined. Results: Both endogenous cannabinoid receptor agonists, anandamide and palmitoylethanolamide, inhibited electrical field stimulation-induced guinea pig bronchial smooth muscle contraction, but not neurokinin A-induced contraction. A cannabinoid CB2 antagonist, SR 144528, reduced the inhibitory effect of endogenous agonists, but not a cannabinoid CB1 antagonist, SR 141716A. Inhibitory effects of agonists were also reduced by the pretreatment of large conductance Ca(2+)-activated K(+) channel blockers, iberiotoxin and charybdotoxin, but not by other K(+) channel blockers, dendrotoxin or glibenclamide. Anandamide and palmitoylethanolamide blocked the capsaicin-induced release of substance P-like immunoreactivity from guinea pig airway tissues. Additionally, intravenous injection of palmitoylethanolamide dose-dependently inhibited capsaicin-induced guinea pig bronchoconstriction, but not neurokinin A-induced reaction. However, anandamide did not reduce capsaicin-induced guinea pig bronchoconstriction. Conclusions: These findings suggest that endogenous cannabinoid receptor agonists inhibit the activation of C fibers via cannabinoid CB2 receptors and maxi-K(+) channels in guinea pig airways.

**Molecular biology**


R(+)WIN55,212 is a synthetic cannabinoid that controls disease progression in models of multiple sclerosis. This is associated with its ability to reduce migration of leukocytes into the CNS. Since leukocyte migration is dependent on induction of adhesion molecules and chemokines by proinflammatory cytokines we examined the effects of R(+)WIN55,212 on their expression. Using 1321N1 astrocytoma and A-172 glioblastoma as cell models we show that R(+)WIN55,212, but not its inactive chiral form S(-)WIN55,212, strongly inhibits the IL-1 induction of the adhesion molecules ICAM-1 and VCAM-1 and the chemokine IL-8. This inhibition is not mediated via the CB1 or CB2 cannabinoid receptors since their selective antagonists and pertussis toxin failed to affect the inhibitory effects of R(+)WIN55,212. Furthermore RT-PCR analysis did not detect the expression of either receptor in 1321N1 cells. R(+)WIN55,212 was shown to inhibit adhesion molecule and chemokine expression at the level of transcription since it strongly inhibited the IL-1 induction of ICAM-1, VCAM-1 and IL-8 mRNAs and blocked the IL-1 activation of their promoters. The NFKB pathway was then assessed as a lead target for R(+)WIN55,212. NFKB was measured by expression of a transfected NFKB-regulated reporter.
gene. Using this assay, R(+)WIN55,212 strongly inhibited IL-1 activation of NFkB. Furthermore R(+)WIN55,212 inhibited the ability of overexpressed Myd88, Tak-1 and IKK-2 to induce the reporter gene suggesting that R(+)WIN55,212 acts at or downstream of IKK-2 in the IL-1 pathway. However R(+)WIN55,212 failed to inhibit IL-1-induced degradation of IkBa, excluding IKK-2 as a direct target. In addition electrophoretic mobility shift and chromatin immunoprecipitation assays showed that R(+)WIN55,212 does not regulate the IL-1-induced nuclear translocation of NFkB or the ability of the latter to bind to promoters regulating expression of ICAM-1 and IL-8. These data suggest that R(+)WIN55,212 blocks IL-1 signaling by inhibiting the transactivation potential of NFkB.


After their discovery, the two known cannabinoid receptors, CB(1) and CB(2), have been the focus of research into the cellular signalling mechanisms of cannabinoids. The initial assessment, mainly derived from expression studies, was that cannabinoids, via G(i/o) proteins, negatively modulate cyclic AMP levels, and activate inward rectifying K(+) channels. Recent findings have complicated this assessment on different levels: (1) cannabinoids include a wide range of compounds with varying profiles of affinity and efficacy at the known CB receptors, and these profiles do not necessarily match their biological activity; (2) CB receptors appear to be intrinsically active and possibly coupled to more than one type of G protein; (3) CB receptor signalling mechanisms are diverse and dependent on the system studied; (4) cannabinoids have other targets than CB receptors. The aim of this mini review is to discuss the current literature regarding CB receptor signalling pathways. These include regulation of adenylyl cyclase, MAP kinase, intracellular Ca(2+), and ion channels. In addition, actions of cannabinoids that are not mediated by CB(1) or CB(2) receptors are discussed.


It is postulated that lipophilic ligands reach their sites of action on membrane-bound functional proteins through fast lateral diffusion across the membrane bilayer. We have shown using NMR experiments that such ligands when incorporated in a membrane system assume a preferred orientation and conformation. While occupying a specific location within the bilayer, these molecules undergo fast lateral diffusion which allows them to engage in productive interactions with their respective protein sites of action. The proposed model is discussed using a group of classical and non-classical cannabinoids as well as the endogenous cannabinoid ligand anandamide.


Postsynaptic Ca(2+) signal influences synaptic transmission through multiple mechanisms. Some of them involve retrograde messengers that are released from postsynaptic neurons in a Ca(2+)-dependent manner and modulate transmitter release through activation of presynaptic receptors. Recent studies have revealed essential roles of endocannabinoids in retrograde modulation of synaptic transmission. Endocannabinoid release is induced by either postsynaptic Ca(2+) elevation alone or activation of postsynaptic G(q/11)-coupled receptors with or without Ca(2+) elevation. The former pathway is independent of phospholipase Cbeta (PLCbeta) and requires a large Ca(2+) elevation to a micromolar range. The latter pathway requires PLCbeta and is facilitated by a moderate Ca(2+) elevation to a submicromolar range. This facilitation is caused by Ca(2+)-dependency of receptor-driven PLCbeta activation. The released endocannabinoids then activate presynaptic cannabinoid receptor type 1 (CB1), and suppress transmitter release from presynaptic terminals. Both CB1 receptors and G(q/11)-coupled receptors are widely distributed in the brain. Thus, the endocannabinoid-mediated retrograde modulation may be an important and widespread mechanism in the brain, by which postsynaptic events including G(q/11)-coupled receptor activation and Ca(2+) elevation can retrogradely influence presynaptic function.

The cannabinoid receptor 1 (CB1) cannabinoid receptor is an essential component of the cannabinergic system. It has been recognized as a therapeutic target for treating numerous diseases and is currently receiving considerable attention by the pharmaceutical community. Target-based drug design, utilizing three-dimensional information of receptor structure and ligand-binding motifs, requires significant amounts of purified protein. To facilitate the purification of CB1, we have expressed the receptor fused to various epitope tags using the baculovirus expression system. In addition, expression levels and ligand-binding profiles corresponding to the expressed fusion proteins have been compared. C-terminal histidine (His)-tagged CB1 gave a B(max) higher than most other systems previously reported in the literature, and was selected for subsequent metal affinity chromatography purification and mass spectroscopic (MS) analysis. Moreover, cells expressing C-terminal His-tagged CB1 were shown to inhibit forskolin-stimulated cyclic adenosine 3',5'-monophosphate (cAMP) production in a concentration-dependent manner in the presence of CP-55,940, confirming the expressed receptor's functional characteristics. A Western blot analysis of the purified receptor showed several forms of CB1, the most abundant being a 57 kDa monomeric protein. The purified CB1 preparations were subjected to protein digestion followed by MS. Fragments corresponding to >70% of the receptor were identified by this method, confirming the identity and purity of the expressed protein. The work presented here demonstrates that epitope-tagged CB1 can be expressed in sufficient amounts and purified to homogeneity for MS analysis. Moreover, these results will serve as a basis for future experiments aimed at characterizing the ligand-binding domains using covalently reacting receptor probes.

CLINICAL SCIENCE

Clinical trials

Endogenous cannabinoids activate cannabinoid receptors in the brain and elicit mood-altering effects. Parallel effects (e.g., anxiolysis, analgesia, sedation) may be elicited by osteopathic manipulative treatment (OMT), and previous research has shown that the endorphin system is not responsible for OMT’s mood-altering effects. The authors investigate whether OMT generated cannabimimetic effects for 31 healthy subjects in a dual-blind, randomized controlled trial that measured changes in subjects’ scores on the 67-item Drug Reaction Scale (DRS). Chemical ionization gas chromatography and mass spectrometry were also used to determine changes in serum levels of anandamide (AEA), 2-arachidonoylglycerol (2-AG), and oleylthanolamide (OEA). In subjects receiving OMT, posttreatment DRS scores increased significantly for the cannabimimetic descriptors good, high, hungry, light-headed, and stoned, with significant score decreases for the descriptors inhibited, sober, and uncomfortable. Mean posttreatment AEA levels (8.01 pmol/mL) increased 168% over pretreatment levels (2.99 pmol/mL), mean OEA levels decreased 27%, and no changes occurred in 2-AG levels in the group receiving OMT. Subjects in the sham manipulative treatment group recorded mixed DRS responses, with both increases and decreases in scores for cannabimimetic and noncannabimimetic descriptors and no changes in sera levels. When changes in serum AEA were correlated with changes in subjects’ DRS scores, increased AEA correlated best with an increase for the descriptors cold and rational, and decreased sensations for the descriptors bad, paranoid, and warm. The authors propose that healing modalities popularly associated with changes in the endorphin system, such as OMT, may actually be mediated by the endocannabinoid system.

OBJECTIVE: Marijuana abuse, primarily a disorder of adolescents and young adults, is highly prevalent among patients with severely ill psychiatric population, especially those with bipolar disorder. Additional marijuana abuse may impact on the clinical presentation of bipolar illness and may potentially act as mediator of treatment response in this population. However, the characterization of bipolar disorder patients with additional marijuana abuse and the impact of such abuse on treatment outcome has been rarely examined. The aim of this study was to characterize bipolar alcoholic patients with comorbid marijuana abuse and test the impact of marijuana abuse on alcohol and mood outcome of patients with bipolar disorder and comorbid alcohol dependence. METHOD: We conducted secondary analyses of a randomized, double blind, placebo-controlled trial testing valproate in 52 bipolar alcoholics. Subjects had a comprehensive assessment at baseline using structured diagnostic assessments, and they were then assessed every 2 weeks for 24 weeks. RESULTS: Twenty-five subjects (48%) reported marijuana abuse. Those with co-occurring marijuana abuse were younger, had fewer years of education, and had significantly higher number of additional psychiatric comorbidity. They also had more severe alcohol and other drug use and were significantly more likely to present in the manic phase. The mixed model indicated that the placebo-treated marijuana abuse group had the worst alcohol use outcome. CONCLUSIONS: Marijuana abuse among patients with bipolar disorder and alcohol dependence is associated with higher degree of severity of alcohol and other drugs of abuse and may negatively impact on alcohol treatment outcome.

Adverse events


Cannabis, commonly known as marijuana, is the most frequently used illicit drug in Australia. Therefore, oral health care providers are likely to encounter patients who are regular users. An upward trend in cannabis use is occurring in Australia, with 40 per cent of the population aged 14 and above having used the drug. There are three main forms of cannabis: marijuana, hash and hash oil, all of which contain the main psychoactive constituent delta-9-tetrahydrocannabinol (THC). Cannabis is most commonly smoked, however it can be added to foods. THC from cannabis enters the bloodstream and exerts its effects on the body via interaction with endogenous receptors. Cannabis affects almost every system of the body, particularly the cardiovascular, respiratory and immune systems. It also has acute and chronic effects on the mental health of some users. Therefore, chronic abuse is a concern because of its negative effects on general physical and mental health. Cannabis abusers generally have poorer oral health than non-users, with an increased risk of dental caries and periodontal diseases. Cannabis smoke acts as a carcinogen and is associated with dysplastic changes and premalignant lesions within the oral mucosa. Users are also prone to oral infections, possibly due to the immunosuppressive effects. Dental treatment on patients intoxicated on cannabis can result in the patient experiencing acute anxiety, dysphoria and psychotic-like paranoid thoughts. The use of local anaesthetic containing epinephrine may seriously prolong tachycardia already induced by an acute dose of cannabis. Oral health care providers should be aware of the diverse adverse effects of cannabis on general and oral health and incorporate questions about patients' patterns of use in the medical history.


Cannabis is generally considered a drug of low toxicity. Although attention has focused on its neuropsychiatric effects, little has been given to cardiovascular side effects. Here we report a case of atrial tachycardryas following cannabis use, and review the literature on its cardiovascular effects and complications.

Marijuana is the most commonly used illegal drug in the United States and is considered by young adults to be the illicit drug with the least risk. On the other hand, marijuana smoke contains several of the same carcinogens and co-carcinogens as the tar from tobacco, raising concerns that smoking of marijuana may be a risk factor for tobacco-related cancers. We reviewed two cohort studies and 14 case-control studies with assessment of the association of marijuana use and cancer risk. In the cohort studies, increased risks of lung or colorectal cancer due to marijuana smoking were not observed, but increased risks of prostate and cervical cancers among non-tobacco smokers, as well as adult-onset glioma among tobacco and non-tobacco smokers, were observed. The 14 case-control studies included four studies on head and neck cancers, two studies on lung cancer, two studies on non-Hodgkin’s lymphoma, one study on anal cancer, one study on penile cancer, and four studies on childhood cancers with assessment of parental exposures. Zhang and colleagues reported that marijuana use may increase risk of head and neck cancers in a hospital-based case-control study in the United States, with dose-response relations for both frequency and duration of use. However, Rosenblatt and co-workers reported no association between oral cancer and marijuana use in a population-based case-control study. An eightfold increase in risk among marijuana users was observed in a lung cancer study in Tunisia. However, there was no assessment of the dose response, and marijuana may have been mixed with tobacco. Parental marijuana use during gestation was associated with increased risks of childhood leukemia, astrocytoma, and rhabdomyosarcoma, but dose-response relations were not assessed. In summary, sufficient studies are not available to adequately evaluate marijuana impact on cancer risk. Several limitations of previous studies include possible underreporting where marijuana use is illegal, small sample sizes, and too few heavy marijuana users in the study sample. Recommendations for future studies are to (1) focus on tobacco-related cancer sites; (2) obtain detailed marijuana exposure assessment, including frequency, duration, and amount of personal use as well as mode of use (smoked in a cigarette, pipe, or bong; taken orally); (3) adjust for tobacco smoking and conduct analyses on nonusers of tobacco; and (4) conduct larger studies, meta-analyses, or pooled analyses to maximize statistical precision and investigate sources of differences in results. Despite the challenges, elucidation of the association between marijuana use and cancer risk is important in weighing the benefits and risks of medical marijuana use and to clarify the impact of marijuana use on public health.


Teratological investigations have demonstrated that agents that are relatively harmless to the mother may have significant negative consequences to the fetus. Among these agents, prenatal alcohol, nicotine or cannabis exposure have been related to adverse offspring outcomes. Although there is a relatively extensive body of literature that has focused upon birth and behavioral outcomes in newborns and infants after prenatal exposure to maternal smoking, drinking and, to a lesser extent, cannabis use, information on neurobehavioral and cognitive teratogenic findings beyond these early ages is still quite limited. Furthermore, most studies have focused on prenatal exposure to heavy levels of smoking, drinking or cannabis use. Few recent studies have paid attention to low or moderate levels of exposure to these substances. This review endeavors to provide an overview of such studies, and includes animal findings and potential mechanisms that may explain the mostly subtle effects found on neurobehavioral and cognitive outcomes. It is concluded that prenatal exposure to either maternal smoking, alcohol or cannabis use is related to some common neurobehavioral and cognitive outcomes, including symptoms of ADHD (inattention, impulsivity), increased externalizing behavior, decreased general cognitive functioning, and deficits in learning and memory tasks.


Recent longitudinal studies from Sweden, the Netherlands, New Zealand, and Israel report that cannabis use during childhood and adolescence doubles the risk of later appearance
of psychosis or schizophrenia. These data have been interpreted as indicating that cannabis has a causal effect along the pathway to psychosis. In this paper, we will offer an alternative explanation of these data. Recent investigations of patients with schizophrenia found increased density of cannabinoid receptors in the dorso-lateral prefrontal cortex and the anterior cingulate cortex. Others reported higher levels of endogenous cannabinoids in the blood and cerebrospinal fluid of patients; these findings were independent of possible cannabis use. Several genetic studies have reported an association between genes encoding the cannabinoid receptor and schizophrenia. Thus, an alternative explanation of the association between cannabis use and schizophrenia might be that pathology of the cannabinoid system in schizophrenia patients is associated with both increased rates of cannabis use and increased risk for schizophrenia, without cannabis being a causal factor for schizophrenia.

Policy


Reviews


For centuries extracts from the Cannabis sativa plant have been used for recreational use and as remedies. Anecdotal reports from patients with multiple sclerosis (MS) experiencing relief of their spasticity and pain after smoking marihuana have prompted discussions about a potential therapeutic application of cannabis preparations in MS. Only recently the first large, multicenter, double-blind, placebo controlled study was conducted evaluating the use of cannabinoids for treatment of spasticity and other symptoms related to MS. Based on this trial and previous uncontrolled observations together with insights from basic research and animal experiments there is reasonable evidence for the therapeutical employment of cannabinoids in the treatment of MS related symptoms. Furthermore, data are arising that cannabinoids have immunomodulatory and neuroprotective properties. However, results from clinical trials do not allow the recommendation for the general use of cannabinoids in MS. This article summarizes the present knowledge of clinical and experimental research regarding the therapeutic potential of cannabinoids for the treatment of MS.

BEHAVIOURAL SCIENCE

Driving studies

Objective. Although studies have demonstrated that clients in treatment for alcohol abuse are more at risk of driving while impaired (DWI) by alcohol than normal licensed drivers from the general population, no research was found on DWI convictions among those in treatment for abusing cannabis or cocaine. The purpose of this article is to compare DWI convictions among clients in treatment for alcohol, cannabis, cocaine, or various combinations of these substances,
compared to a matched population control group. Method. A stratified random sample of driver records was drawn from seven client groups who sought treatment in 1994 for alcohol, cannabis, cocaine, or any combination of these substances (n = 445). A random sample of drivers, frequency matched by age and sex (n = 566), served as control subjects. Results. Logistic regression analysis, controlling for sex and age, was conducted to assess whether DWI convictions were elevated for each of the client groups, compared to controls. Two sets of analyses were conducted, before treatment (from 1985 to 1993) and after treatment (from 1995 to 2000). In the time period before treatment, every drug group except the "cannabis only" group had significantly more DWI convictions than controls (p < .05). In the period after treatment, the "alcohol only," "cocaine only," "alcohol and cocaine," and the "cannabis and cocaine" groups still had significantly more DWI convictions than controls (p < .05). Conclusion. The results show that DWI convictions are elevated among those who abused cocaine but not among those who abused cannabis. The results suggest that cross-addiction of alcohol and cocaine is common, and problematic drinking among cocaine clients can go undetected when clients are being diagnosed for treatment.


Delta9-Tetrahydrocannabinol (THC) is frequently found in the blood of drivers suspected of driving under the influence of cannabis or involved in traffic crashes. The present study used a double-blind crossover design to compare the effects of medium (16.5 mg THC) and high doses (45.7 mg THC) of hemp milk decoctions or of a medium dose of dronabinol (20 mg synthetic THC, Marinol(R)) on several skills required for safe driving. Forensic interpretation of cannabinoids blood concentrations were attempted using the models proposed by Daldrup (cannabis influencing factor or CIF) and Huestis and coworkers. First, the time concentration-profiles of THC, 11-hydroxy-Delta9-tetrahydrocannabinol (11-OH-THC) (active metabolite of THC), and 11-nor-9-carboxy-Delta9-tetrahydrocannabinol (THCCOOH) in whole blood were determined by gas chromatography-mass spectrometry-negative ion chemical ionization. Compared to smoking studies, relatively low concentrations were measured in blood. The highest mean THC concentration (8.4 ng/mL) was achieved 1 h after ingestion of the strongest decoction. Mean maximum 11-OH-THC level (12.3 ng/mL) slightly exceeded that of THC. THCCOOH reached its highest mean concentration (66.2 ng/mL) 2.5-5.5 h after intake. Individual blood levels showed considerable intersubject variability. The willingness to drive was influenced by the importance of the requested task. Under significant cannabinoids influence, the participants refused to drive when they were asked whether they would agree to accomplish several unimportant tasks, (e.g., driving a friend to a party). Most of the participants reported a significant feeling of intoxication and did not appreciate the effects, notably those felt after drinking the strongest decoction. Road sign and tracking testing revealed obvious and statistically significant differences between placebo and treatments. A marked impairment was detected after ingestion of the strongest decoction. A CIF value, which relies on the molar ratio of main active to inactive cannabinoids, greater than 10 was found to correlate with a strong feeling of intoxication. It also matched with a significant decrease in the willingness to drive, and it matched also with a significant impairment in tracking performances. The mathematic model II proposed by Huestis et al. (1992) provided at best a rough estimate of the time of oral administration with 27% of actual values being out of range of the 95% confidence interval. The sum of THC and 11-OH-THC blood concentrations provided a better estimate of impairment than THC alone. This controlled clinical study points out the negative influence on fitness to drive after medium or high dose oral THC or dronabinol.

Population studies

OBJECTIVE: Research on the effects of cannabis on the brain and behavior has been surprisingly scarce. In humans, laboratory studies document toxicity and psychoactive effects of cannabinoids. However, among substance abuse patients, only a few studies have prospectively examined the relationship of cannabis use to remission or relapse of use of other substances. Because cannabis is a widely used substance, the authors examined whether cannabis use during follow-up after discharge from inpatient treatment affected cocaine, alcohol, and/or heroin use. 

METHOD: Two hundred fifty patients 18 years old or older from an inpatient psychiatric/substance abuse setting participated in a Psychiatric Research Interview for Substance and Mental Disorders. All patients were diagnosed according to DSM-IV as having current alcohol, cocaine, and/or heroin dependence. Sustained remission was defined as at least 26 weeks without use following hospital discharge. Data were analyzed with Cox proportional hazards models. 

RESULTS: About one-third of the patients (N=73) used cannabis after hospital discharge. Postdischarge cannabis use substantially and significantly increased the hazard of first use of any substance and strongly reduced the likelihood of stable remission from use of any substance. Examination of specific substances indicated that cannabis use affected first use of alcohol, stable remission, and subsequent relapse of alcohol use as well as first use of cocaine and stable remission but was unrelated to heroin outcomes. 

CONCLUSIONS: Potential negative clinical implications of cannabis use should be considered when treating dependence on other substances and planning aftercare. Clinical and laboratory research is needed to provide understanding of the mechanisms of cannabinoids in relapse to alcohol and cocaine use.


This study utilized data on a treatment cohort from a randomized clinical trial that recruited adolescents with co-occurring major depression and substance use disorder (N=126). The purpose of this study was to compare adolescents for whom the onset of depression was first versus those for whom the onset of substance use disorder was first or in the same year as depression. Intake clinical evaluations were abstracted to yield common stressors that included childhood abuse, early loss or death, exposure to violence, and attachment problems. Tobacco, alcohol, and cannabis initiation and dependence were compared for the depression first and substance use disorder first groups, and within those groups by gender. Among the substances studied, only cannabis dependence was significantly more prevalent among those with depression first. Comparisons suggest some differences in the developmental path toward comorbid depression and substance use disorders, but remarkable similarity in measures of dependence and severity. Although small samples limited statistical significance, observed differences suggest possible avenues for prevention or intervention.


Cannabis use and suicidal behaviour are causes of adolescent morbidity and mortality worldwide. Changing trends in these behaviours in younger age groups, higher incidence, gender differences and sociocultural variations present an enormous challenge. There is no consensus whether these complex relationships are either a direct or an indirect effect due to other mental disorders, or a social response of disclosure of drug taking habits to family members and school authorities. This paper reviews the epidemiology of suicidal behaviour and cannabis use among adolescents and looks at the relationship of these behaviours regionally and internationally. The Caribbean islands have an established use of cannabis with higher suicidal rates, which provides an ideal setting to investigate the interrelationship of these disorders. Preliminary research findings in Trinidad indicate high rates of cannabis use among school students with higher rates in vocational schools compared to grammar schools. Utilising the CAPE questionnaire, depressive and psychotic experiences were common findings in adolescent cannabis users with a significant preponderance of depressive experiences (p<0.01). Our findings suggest that there is a convincing relationship between suicidal behaviour and cannabis use, the latter awakening depressive experiences. Suicidal behaviour and cannabis use are major public health problems and require a multidimensional approach with culturally competent preventive interactions. School
based prevention programmes are necessary at the levels of parent-teacher partnership and classroom intervention. The treatment of adolescent disorders remains a major challenge of the future. Double disorders such as cannabis use and suicidal behaviour are uncharted areas and need novel approaches.


OBJECTIVE: To assess the extent of cannabis and other drug use among patients presenting with recent injuries at trauma units in Cape Town, Port Elizabeth and Durban from 1999 to 2001. DESIGN: Cross-sectional surveys were conducted during a 4-week period at each of the above sites in 1999, 2000 and 2001. The concept of an idealised week was used to render representative samples. OUTCOME MEASURES: Cause of injury and biological markers to assess use of cannabis, methaqualone (Mandrax), opiates, cocaine, amphetamine, and methamphetamine. RESULTS: Over half of all patients tested experienced violent injuries. Excluding opiates, across sites and over time between 33% and 62% of patients tested positive for at least one drug (N = 1565). In most cases the drugs were cannabis and/or methaqualone. While no inter-city differences were found, male patients were typically more likely to test positive for drugs in general and specific drugs such as cannabis and the cannabis/methaqualone ('white pipe') combination than female patients. Drug positivity was higher in 2001 than in the previous 2 years in Cape Town, and patients injured as a result of violence in Cape Town and Durban were more likely to test positive for drugs than patients with certain other types of injuries. CONCLUSIONS: Drug use among trauma patients has remained consistently high for each of the 3 study periods. Efforts to combat the abuse of drugs such as cannabis and methaqualone would appear to be paramount in reducing the burden of injuries on health care services. The study has raised numerous issues requiring further research.


In a study of young cannabis users attending further education colleges across London which specifically excluded young heroin users or injecting drug users, 35% were found to have been offered heroin, 36% had been present during heroin smoking, and 12% present at injecting. Factors associated with these exposure opportunities were investigated. The proportion of friends who use drugs other than cannabis was also considered, both as an indicator of risk in its own right and as a possible mechanism for high-risk drug exposure opportunities involving heroin and/or injecting. Alcohol variables and interactional problems perceived by the study subjects to be caused by their own drug use were found to be predictive of the involvement of friends in drug use other than cannabis and of exposure to heroin and injecting drug use. Non-cannabis illicit drug use among friends was also found to be associated with offers of heroin and with having been present during injecting drug use by others. Interpretations of these data are considered and the need for more detailed study with an area of increasing public policy significance is discussed. [Strang J, McCambridge J. Are cannabis users exposed to other drug use opportunities? Investigation of high-risk drug exposure opportunities among young cannabis users in London. Drug Alcohol Rev 2005;24:185 - 191].


Working knowledge or vocabulary of drug slang, as a manifestation of learned behavior, might help predict or explain risk of starting to use cannabis in early adolescence. To study this issue, an epidemiologic sample of 1,255 11- to 12-year-olds was assessed for knowledge of cannabis slang terms in 1992, with follow-up in 1993 and 1994. The basic design is that of a prospective and longitudinal study, with recruitment of an epidemiologic sample of children as they entered primary school in a single metropolitan area, also with baseline assessments and randomization to intervention conditions, and subsequent multiple waves of follow-up assessment. Youths assessed in 1992 and who were familiar with terms such as blunts, Mary Jane, roach, and herb were more likely to start using cannabis within the subsequent two years of
the follow-up interval, as compared to other youths (estimated relative risk, RR=11.0; 95% CI 3.6-33.7; P<.001). A youth's working vocabulary may signal important variations in health- and disease-related behavior, such as illegal drug use.


New data collected on the procurement and purchase of cannabis in the 2001 New Zealand National Drug Survey are used to estimate the dollar value of the illicit market for cannabis as well conduct other economic analyses of the illicit purchase of cannabis in New Zealand. Eighty-four per cent of last-year cannabis users received at least some of their cannabis 'free', 38% 'bought' at least some of their cannabis, while only 8% grew any of their supply. By proportion of the total dollar value of the cannabis market, sales of 'tinnies' (1.5 g) had the biggest share of the market (30%), followed closely by $50 bags (4 g) (29%) and ounces (28 g) (26%). The average price paid per gram of cannabis fell steadily from $20.50 per gram for a joint to $5.63 per gram for a pound ($NZ). The distribution of spending on cannabis was positively skewed (average $1313, median $400, mode $100, range $5 - 55 200, interquartile range $100 - 1200) ($NZ). The bottom 50% of cannabis buyers spent a median amount on cannabis of $100 a year while the top 5% spent a median amount of $7425 a year ($NZ). The dollar value of the illicit cannabis market was estimated to be $190 million ($131 - 249 million) ($NZ). Three limitations are acknowledged with the market estimate calculated: (i) the truncated age range of the survey data (13 - 45 years); (ii) the likely underestimation of heavy cannabis use in household drug surveys; (iii) a degree of 'double-counting' when the same cannabis is bought and sold a number of times. [Wilkins C, Reilly JL, Pledger M, Casswell S. Estimating the dollar value of the illicit market for cannabis in New Zealand. Drug Alcohol Rev 2005; 24:227 - 234].

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