BASIC SCIENCE

Addiction

Cannabinoid withdrawal has been indicated in both human and animal subjects. One of pathways proposed to facilitate cannabinoid action is the arachidonic acid cascade. Previously, we have shown that prostaglandin attenuated the expression of withdrawal signs in tetrahydrocannabinol-dependent mice. It follows that the cascade might participate in the expression of cannabinoid withdrawal. We utilized a quasi abstinence approach (the induction of a state of cannabinoid withdrawal without giving any cannabinoid substances in a naive animal) to describe the relationship between the change in prostaglandin level, an end product of the arachidonic acid cascade, and the expression of cannabinoid withdrawal. Administration of 10 mg/kg diclofenac, a prostaglandin synthesis inhibitor, i.p. 30 min before SR 141716A induced cannabinoid withdrawal signs in naive mice, which were comparable to the true abstinence in cannabinoid-tolerant mice. In turn, 10 mg/kg Delta(8)-THC i.p., given 15 min prior to SR 141716A, blocked the expression of these signs. These results suggested that the decrease in prostaglandin level is a prerequisite for the expression of cannabinoid withdrawal.

Cardiovascular

We have previously shown that over time (2 h), the active ingredient of cannabis, Delta(9)-tetrahydrocannabinol (THC) produces peroxisome proliferator-activated receptor gamma (PPARgamma-mediated vasorelaxation of conduit arteries. We have now investigated whether incubation with THC affects agonist-stimulated contractile (methoxamine) and endothelium-dependent vasorelaxant (acetylcholine) responses in the rat superior mesenteric artery (G0) and aorta by myography. We have also investigated whether similar responses are observed in isolated resistance (G3) vessels of the mesenteric bed. In both the aorta and G0, incubation with THC (10 microM) for 2 h, but not 10 min, significantly attenuated the contractile responses to methoxamine. This effect of THC was abolished in the presence of the enzyme catalase, which breaks down hydrogen peroxide (H2O2), and was reduced in the presence of the superoxide dismutase (SOD) inhibitor, DETCA, but was not PPARgamma-mediated. THC also inhibited calcium influx in a H2O2-dependent manner. In G0, but not the aorta, incubation with THC (10 microM, 2 h) significantly enhanced endothelium-dependent vasorelaxation. This was inhibited by a PPARgamma antagonist (GW9662), catalase and DETCA, but not by the NO synthase inhibitor L-NAME. By contrast, in G3, no time-dependent vasorelaxation of precontracted arteries to THC was observed, and incubation with THC led to potentiation of contractile responses and blunting of vasorelaxation to acetylcholine, which appears to involve inhibition of endothelium-derived hyperpolarising factor (EDHF) production, and agonist-stimulated production of EDHF. These data demonstrate further the time-dependent vascular actions of THC, and also highlight the heterogenous effects of THC in different arterial types.
**Embryology**

**Endocrinology**


This research was designed to examine the effect of three weeks of administration of corticosterone (20 mg/kg) on endocannabinoid content and cannabinoid CB(1) receptor binding in the amygdala. It was found that the endocannabinoid 2-arachidonylglycerol was significantly increased in the amygdala following chronic corticosterone treatment. However, there was no change in either the maximal binding (B(max)) or binding affinity (K(D)) of [(3)H]-CP 55,940 to the CB(1) receptor in the amygdala. Given the role of amygdalar endocannabinoids in the regulation of emotionality, this suggests that the ability of glucocorticoids to influence affective behavior may involve interactions with regulation of endocannabinoid content.


This study compared the effects of the putative cannabinoid receptor 'silent antagonist' O-2050 with the cannabinoid receptor inverse agonist SR 141716 on food and water consumption, and locomotor activity. Non-deprived male Wistar rats were habituated to the apparatus and testing procedures, then injected intraperitoneally with vehicle, O-2050 (0.03-3.0 mg/kg), or SR 141716 (3.0 mg/kg) prior to 4-h test sessions. Food consumption was significantly reduced by both drugs. Water intake and locomotor activity were significantly reduced only by O-2050. Results support the notion that cannabinoid receptor antagonists suppress feeding behaviour by blocking an endogenous cannabinoid orexigenic signal, rather than by inverse agonism at cannabinoid receptors. However, further studies are needed to confirm the status of O-2050 as a cannabinoid CB(1) receptor antagonist devoid of inverse agonist properties.


Recently developed therapeutics for obesity, targeted against cannabinoid receptors, result in decreased appetite and sustained weight loss. Prior studies have demonstrated CB1 receptors (CB1Rs) and leptin modulation of cannabinoid synthesis in hypothalamic neurons. Here, we show that depolarization of perifornical lateral hypothalamus (LH) neurons elicits a CB1R-mediated suppression of inhibition in local circuits thought to be involved in appetite and "natural reward." The depolarization-induced decrease in inhibitory tone to LH neurons is blocked by leptin. Leptin inhibits voltage-gated calcium channels in LH neurons via the activation of janus kinase 2 (JAK2) and of mitogen-activated protein kinase (MAPK). Leptin-deficient mice are characterized by both an increase in steady-state voltage-gated calcium currents in LH neurons and a CB1R-mediated depolarization-induced suppression of inhibition that is 6-fold longer than that in littermate controls. Our data provide direct electrophysiological support for the involvement of endocannabinoids and leptin as modulators of hypothalamic circuits underlying motivational aspects of feeding behavior.


Insulin is the main hormone involved in the regulation of glycaemia, its impaired secretion is a hallmark of type I and type II diabetic individuals. Additionally, insulin is involved in lipogenesis and weight gain, provoking an anorexigenic action. The endocannabinoid system contributes to the physiological regulation of energy balance, food intake and lipid and glucose metabolisms. Despite that, an experimental link between the endocannabinoid system and the endocrine pancreas has not yet been described. Using quantitative real-time PCR and immunocytochemistry, we have demonstrated the existence of both CB1 and CB2 receptors in the endocrine pancreas. While the CB1 receptor is mainly expressed in non-beta-cells, the CB2 type exists in beta- and non-beta-cells within the islet. The endocannabinoid 2-
arachidonylglycerol (2-AG) through CB2 receptors regulates [Ca(2+)](i) signals in beta-cells and as a consequence, it decreases insulin secretion. This effect may be a new component involved in the orexigenic effect of endocannabinoids and constitutes a potential target for pharmacologic manipulation of the energy balance.

**Gastrointestinal**

**Genetics**


Owing to their agonist action on dopaminergic systems, cannabinoids may play a major role in substance dependency and schizophrenia. We examined the (AAT)n triplet repeat polymorphism nearby the CNR1 gene, which encodes human cannabinoid (CB1) receptor, in a male Afro-Caribbean population. The allelic and genotypic distributions were significantly different in non-schizophrenic cocaine dependents (n=97), schizophrenic cocaine dependents (n=45) and matched controls (n=88) (P<10(-4)). The frequency of the (AAT)12 repeat allele was increased in non-schizophrenic cocaine dependents and schizophrenic cocaine dependents vs controls (25.3 and 26.7 vs 5.7%) (P<10(-4)). Our results support that the (AAT)n polymorphism nearby the CNR1 gene could be associated with predisposition to cocaine dependency. The Pharmacogenomics Journal advance online publication, 29 November 2005; doi:10.1038/sj.tpj.6500352.

**Infectious Diseases**

**Immunology**


Two topics are presented in this review. In the first section, we review data regarding the effects of the endocannabinoids (eCBs) and cannabinoid receptors on neuroimmune function. The function of eCBs in the interaction between the immune system and the central nervous system (CNS) is of particular interest, since the CNS itself is a rich source of eCBs while being exquisitely sensitive to inflammation. There are several sites at which cannabinoids can influence neuroinflammation. Microglial cells express both CB receptors and make eCBs. Activation of CB receptors on these cells seems to promote migration and proliferation but to reduce activation to macrophages. In several neurodegenerative diseases, up-regulation of microglial CB2 receptors have been observed. It is our hypothesis that microglial CB receptor activity is anti-inflammatory and could be exploited to manipulate neuroinflammatory processes with a minimum of unwanted effects. The second topic discussed suggests that the eCB/CB1 receptor pair is involved in the responses of animals to acute, repeated and variable stress. The roles of this pair are complex and dependent upon previous stress, among other things. Dysfunctional responding to stress is a component of several human neuropsychiatric disorders, including anxiety and panic disorders, post-traumatic stress disorders, premenstrual dysphoria and quite possibly, drug abuse. While it is too early to say with certainty, it is very possible that either inhibition or potentiation of endocannabinoid signaling will be an efficacious novel therapeutic approach to more than one human psychiatric disease.


BACKGROUND: Activated microglial cells have been implicated in a number of neurodegenerative disorders, including Alzheimer's disease (AD), multiple sclerosis (MS), and HIV dementia. It is well known that inflammatory mediators such as nitric oxide (NO), cytokines, and chemokines play an important role in microglial cell-associated neuron cell damage. Our previous studies have shown that CD40 signaling is involved in pathological activation of
microglial cells. Many data reveal that cannabinoids mediate suppression of inflammation in vitro and in vivo through stimulation of cannabinoid receptor 2 (CB2). METHODS: In this study, we investigated the effects of a cannabinoid agonist on CD40 expression and function by cultured microglial cells activated by IFN-gamma using RT-PCR, Western immunoblotting, flow cytometry, and anti-CB2 small interfering RNA (siRNA) analyses. Furthermore, we examined if the stimulation of CB2 could modulate the capacity of microglial cells to phagocytise A-beta1-42 peptide using a phagocytosis assay. RESULTS: We found that the selective stimulation of cannabinoid receptor CB2 by JWH-015 suppressed IFN-gamma-induced CD40 expression. In addition, this CB2 agonist markedly inhibited IFN-gamma-induced phosphorylation of JAK/STAT1. Further, this stimulation was also able to suppress microglial TNF-alpha and nitric oxide production induced either by IFN-gamma or A-beta peptide challenge in the presence of CD40 ligation. Finally, we showed that CB2 activation by JWH-015 markedly attenuated CD40-mediated inhibition of microglial phagocytosis of A-beta1-42 peptide. Taken together, these results provide mechanistic insight into beneficial effects provided by cannabinoid receptor CB2 modulation in neurodegenerative diseases, particularly AD.


Endocannabinoids are involved in neuroprotection through numerous biochemical pathways. We have shown that the endocannabinoid 2-arachidonoyl glycerol (2-AG) is released in mouse brain after closed head injury (CHI), and treatment with exogenous 2-AG exerts neuroprotection via the central cannabinoid receptor CB(1). This process involves inhibition of inflammatory signals that are mediated by activation of the transcription factor NF-kB. The present study was designed to examine the effect of 2-AG on the blood-brain barrier (BBB) and the possible inhibition of the early expression of proinflammatory cytokines, which are implicated in BBB disruption. We found that 2-AG decreased BBB permeability and inhibited the acute expression of the main proinflammatory cytokines: TNF-alpha, IL-1beta and IL-6. It also augmented the levels of endogenous antioxidants. We suggest that 2-AG exerts neuroprotection in part by inhibition of the early (1-4 h) inflammatory response and augmentation of the brain reducing power.


Endocannabinoids have analgesic/anti-inflammatory properties. The biology of endocannabinoids, their receptors, signalling mechanisms and role in the regulation of physiological processes have been extensively reviewed. This review focuses on the role of palmitoylethanolamide (PEA), an endogenous fatty acid amide analogue of the endocannabinoid anandamide, in tissue protective mechanisms. PEA was first identified almost five decades ago in lipid extracts of various natural products, and its anti-inflammatory and antinociceptive effects were established later. Evidence exists that PEA is synthesised during inflammation and tissue damage and a number of beneficial effects, including the relief of inflammation and pruritus, have been shown to be useful in the control of neurogenic and neuropathic pain. The postulated hypotheses as to the mode of action of PEA include a possible local autacoid-like mediator activity regulating mast-cell activity and putative activation of cannabinoids and vanilloid TRPV1 receptors via "entourage" effects. The large number of scientific investigations into the effects of PEA and PEA-related compounds has given rise to new therapeutic opportunities. In spite of the multitude of therapies currently employed to control inflammation, pain, pruritus and tissue damage, the possibility of using a natural compound, such as PEA to manipulate endogenous protective mechanisms may be considered a beneficial novel therapeutic strategy in veterinary medicine.
**Molecular biology**


Increasing evidence suggests that some cannabinoids mediate their effects independently of the known cannabinoid CB(1) and CB(2) receptors. Two recently published patents indicate that several cannabinoid receptor ligands also bind to the orphan G-protein-coupled receptor GPR55. This receptor is reported to be expressed in several tissues and might function in lipid or vascular biology. Thus, GPR55 might represent a new cannabinoid receptor.


Fatty acid amide hydrolase (FAAH) is a serine hydrolase responsible for the degradation of anandamide, an endogenous cannabinoid agonist, and oleamide, a sleep-inducing lipid. Recently, Boger and co-workers reported a potent, selective, and efficacious class of reversible alpha-ketoheterocycle inhibitors of FAAH that produce analgesia in animal models (J. Med. Chem. 2005, 48, 1849-1856; Bioorg. Med. Chem. Lett. 2005, 15, 1423-1428). Key aspects of the structure-activity data are addressed here through computational analysis of FAAH inhibition using Monte Carlo (MC) simulations in conjunction with free energy perturbation (FEP) calculations. The MC/FEP simulations demonstrate that incorporation of pyridine at the C5 position of the 2-keto-oxazole and 2-keto-1,3,4-oxadiazole derivatives significantly enhances binding affinity by formation of a hydrogen-bonded array between the pyridyl nitrogen and Lys142 and Thr236. The results also attribute the activity boost upon substitution of oxazole by oxadiazole to reduced steric interactions in the active site and a lower torsional energy penalty upon binding.


Central nervous system responses to cannabis are primarily mediated by CB(1) receptors, which couple preferentially to G(i/o) G proteins. Here, we used calcium photometry to monitor the effect of CB(1) activation on intracellular calcium concentration. Perfusion with 5 μM CB(1) aminoalkylindole agonist, WIN55,212-2 (WIN), increased intracellular calcium by several hundred nanomolar in human embryonic kidney 293 cells stably expressing CB(1) and in cultured hippocampal neurons. The increase was blocked by coincubation with the CB(1) antagonist, SR141716A, and was absent in nontransfected human embryonic kidney 293 cells. The calcium rise was WIN-specific, being essentially absent in cells treated with other classes of cannabinoid agonists, including Delta(9)-tetrahydrocannabinol, HU-210, CP55,940, 2-arachidonoylglycerol, methanandamide, and cannabidiol. The increase in calcium elicited by WIN was independent of G(i/o), because it was present in pertussis toxin-treated cells. Indeed, pertussis toxin pretreatment enhanced the potency and efficacy of WIN to increase intracellular calcium. The calcium increases appeared to be mediated by G(q) G proteins and phospholipase C, because they were markedly attenuated in cells expressing dominant-negative G(q) or treated with the phospholipase C inhibitors U73122 and ET-18-OCH(3) and were accompanied by an increase in inositol phosphates. The calcium increase was blocked by the sarco/endoplasmic reticulum Ca(2+) pump inhibitor thapsigargin, the inositol trisphosphate receptor inhibitor xestospongin D, and the ryanodine receptor inhibitors dantrolene and 1,1'-dihyptyl-4,4'-bipyridinium dibromide, but not by removal of extracellular calcium, showing that WIN releases calcium from intracellular stores. In summary, these results suggest that WIN stabilizes CB(1) receptors in a conformation that enables G(q) signaling, thus shifting the G protein specificity of the receptor.


The membrane properties of isolated frog parathyroid cells were studied using perforated and conventional whole-cell patch-clamp techniques. Frog parathyroid cells displayed transient
inward currents in response to depolarizing pulses from a holding potential of -84 mV. We analyzed the biophysical properties of the inward currents. The inward currents disappeared by the replacement of external Na(+) with NMDG(+) and were reversibly inhibited by 3 mumol l(-1) TTX, indicating that the currents occur through the TTX-sensitive voltage-gated Na(+) channels. Current density elicited by a voltage step from -84 mV to -24 mV was -80 pA pF(-1) in perforated mode and -55 pA pF(-1) in conventional mode. Current density was decreased to -12 pA pF(-1) by internal GTPgammaS (0.5 mmol l(-1)), but not affected by internal GDPbetaS (1 mmol l(-1)). The voltage of half-maximum (V(1/2)) activation was -46 mV in both perforated and conventional modes. V(1/2) of inactivation was -80 mV in perforated mode and -86 mV in conventional mode. Internal GTPgammaS (0.5 mmol l(-1)) shifted the V(1/2) for activation to -36 mV and for inactivation to -98 mV. A putative endocannabinoid, 2-arachidonoylglycerol ether (2-AG ether, 50 mumol l(-1)) and a cannabinomimetic aminoalkylindole, WIN 55,212-2 (10 mumol l(-1)) also greatly reduced the Na(+) current and shifted the V(1/2) for activation and inactivation. The results suggest that the Na(+) currents in frog parathyroid cells can be modulated by cannabinoids via a G protein-dependent mechanism.

Neuroscience

Reports of cannabinoid CB2 receptor protein in the brain have been ambiguous. We therefore tested for CB2 immunoreactivity in the rat brain using immunofluorescence. We detected CB2 labeling in fine fibers in the granule layer. This CB2 labeling did not co-localise with the astrocyte marker glial fibrillary acidic protein (GFAP) and, therefore, the CB2-positive fibers were not astrocytes and were possibly microglial or neuronal. Additionally, strong CB2 labeling was detected in capillary endothelia in the granule, Purkinje cell, and molecular layers. Our results suggest that the role of CB2 receptors in the brain may have been previously underestimated.


AIM: The aim of this study was to evaluate the role of the endogenous cannabinoid system in controlling neuroplasticity. METHODS: The pain threshold for electrical stimuli was determined in transgenic mice lacking the cannabinoid receptor type 1 (CB1(-/-)) and in the corresponding respective wild-type animals. Electrophysiological experiments were performed in prepared brain slices to test the effect of endogenous and exogenous cannabinoids on synaptic transmission and long-term potentiation (LTP) in the amygdala. RESULTS: The pain threshold was nearly identical in both groups for the first pain induction; however, with repeated pain induction it decreased to a significantly greater extent in the CB1(-/-) mice than in the wild-type animals. Synaptic transmission and the inducibility of LTP were not influenced by the acute pharmacological blockade of CB1 receptors, but inhibited by the CB1 agonist WIN55,212-2. CONCLUSION: The endogenous cannabinoid system is involved in the control of neuroplasticity as part of pain processing. Cannabinoids prevent the formation of LTP in the amygdala via activation of CB1 receptors. Synaptic transmission and the inducibility of LTP were not influenced by the acute pharmacological blockade of CB1 receptors, but inhibited by the CB1 agonist Win55,212-2.


In utero exposure to Delta(9)-tetrahydrocannabinol (Delta(9)-THC), the active component from marijuana, induces cognitive deficits enduring into adulthood. Although changes in synaptic structure and plasticity may underlie Delta(9)-THC-induced cognitive impairments, the neuronal basis of Delta(9)-THC-related developmental deficits remains unknown. Using a Boyden chamber assay, we show that agonist stimulation of the CB(1) cannabinoid receptor (CB(1)R) on cholecystokinin-expressing interneurons induces chemotaxis that is additive with brain-derived
neurotrophic factor (BDNF)-induced interneuron migration. We find that Src kinase-dependent TrkB receptor transactivation mediates endocannabinoid (eCB)-induced chemotaxis in the absence of BDNF. Simultaneously, eCBs suppress the BDNF-dependent morphogenesis of interneurons, and this suppression is abolished by Src kinase inhibition in vitro. Because sustained prenatal Delta(9)-THC stimulation of CB(1)Rs selectively increases the density of cholecystokinin-expressing interneurons in the hippocampus in vivo, we conclude that prenatal CB(1)R activity governs proper interneuron placement and integration during corticogenesis. Moreover, eCBs use TrkB receptor-dependent signaling pathways to regulate subtype-selective interneuron migration and specification.


We examined the effects of the endocannabinoid anandamide (AEA), the synthetic cannabinoid, WIN55,212-2, and the active phorbol ester, 4-beta-phorbol 12-myristate 13-acetate (4-beta-PMA), on the release of [(3)H]d-Aspartate ([3H]d-ASP) from rat hippocampal synaptosomes. Release was evoked with three different stimuli: (1) KCl-induced membrane depolarization, which activates voltage-dependent Ca(2+) channels and causes limited neurotransmitter exocytosis, presumably from ready-releasable vesicles docked in the active zone; (2) exposure to the Ca(2+) ionophore-A23187, which causes more extensive transmitter release, presumably from intracellular reserve vesicles; and (3) K(+) channel blockade by 4-aminopyridine (4-AP), which generates repetitive depolarization that stimulates release from both ready-releasable and reserve vesicles. AEA produced concentration-dependent inhibition of [3H]d-ASP release stimulated with 15mM KCl (E(max)=47.4+/-2.8; EC(50)=0.8muM) but potentiated the release induced by 4-AP (1muM) (+22.0+/-1.3% at 1muM) and by A23187 (1muM) (+98.0+/-5.9% at 1muM). AEA’s enhancement of the [3H]d-ASP release induced by the Ca(2+) ionophore was mimicked by 4-beta-PMA, which is known to activate protein kinase C (PKC), and the increases produced by both compounds were completely reversed by synaptosome treatment with staurosporine (1muM), a potent PKC blocker. In contrast, WIN55,212-2 inhibited the release of [3H]d-ASP evoked by KCl (E(max)=47.1+/-2.8; EC(50)=0.9muM) and that produced by 4-AP (-26.0+/-1.5% at 1muM) and had no significant effect of the release induced by Ca(2+) ionophore treatment. AEA thus appears to exert a dual effect on hippocampal glutamatergic nerve terminals. It inhibits release from ready-releasable vesicles and potentiates the release observed during high-frequency stimulation, which also involves the reserve vesicles. The latter effect is mediated by PKC. These findings reveal novel effects of AEA on glutamatergic nerve terminals and demonstrate that the effects of endogenous and synthetic cannabinoids are not always identical.


Recent work in our laboratories has demonstrated that an opioid-independent form of stress-induced analgesia (SIA) is mediated by endogenous ligands for cannabinoid receptors-anandamide and 2-arachidonoylglycerol (2-AG) [A.G. Hohmann, R.L. Suplita, N.M. Bolton, M.H. Neely, D. Fegley, R. Mangieri, J.F. Krey, J.M. Walker, P.V. Holmes, J.D. Crystal, A. Duranti, A. Tontini, M. Mor, G. Tarzia, D. Piomelli, An endocannabinoid mechanism for stress-induced analgesia, Nature 435 (2005) 1108-1112]. The present study was conducted to examine the contribution of cannabinoid CB(1) receptors in the basolateral nucleus of the amygdala (BLA) and central nucleus of the amygdala (CeA) to nonopioid SIA. SIA was induced by continuous footshock (3min 0.9mA) and quantified behaviorally using the tail-flick test. Microinjection of the CB(1) antagonist/inverse agonist rimonabant (SR141716A) into the BLA, a limbic forebrain region with high densities of CB(1) receptors, suppressed SIA relative to control conditions. By contrast, the same dose administered into the CeA, where CB(1) immunoreactivity is largely absent, or outside the amygdala did not alter SIA. To examine the contribution of endocannabinoids in the BLA to SIA, we used selective pharmacological inhibitors of the anandamide-degrading enzyme fatty-acid amide hydrolase (FAAH) and the 2-arachidonoylglycerol-degrading enzyme monoacylglycerol lipase (MGL). The FAAH inhibitor URB597 and MGL inhibitor URB602, at doses that enhanced SIA following microinjection in the midbrain periaqueductal gray, did not alter SIA relative to control conditions. Our findings suggest that CB(1) receptors in the BLA but
not the CeA contribute to SIA, but pharmacological inhibition of endocannabinoid degradation at these sites does not affect the expression of stress antinociception.


BACKGROUND: Activated microglial cells have been implicated in a number of neurodegenerative disorders, including Alzheimer's disease (AD), multiple sclerosis (MS), and HIV dementia. It is well known that inflammatory mediators such as nitric oxide (NO), cytokines, and chemokines play an important role in microglial cell-associated neuron cell damage. Our previous studies have shown that CD40 signaling is involved in pathological activation of microglial cells. Many data reveal that cannabinoids mediate suppression of inflammation in vitro and in vivo through stimulation of cannabinoid receptor 2 (CB2). METHODS: In this study, we investigated the effects of a cannabinoid agonist on CD40 expression and function by cultured microglial cells activated by IFN-gamma using RT-PCR, Western immunoblotting, flow cytometry, and anti-CB2 small interfering RNA (siRNA) analyses. Furthermore, we examined if the stimulation of CB2 could modulate the capacity of microglial cells to phagocytise A-beta1-42 peptide using a phagocytosis assay. RESULTS: We found that the selective stimulation of cannabinoid receptor CB2 by JWH-015 suppressed IFN-gamma-induced CD40 expression. In addition, this CB2 agonist markedly inhibited IFN-gamma-induced phosphorylation of JAK/STAT1. Further, this stimulation was also able to suppress microglial TNF-alpha and nitric oxide production induced either by IFN-gamma or A-beta peptide challenge in the presence of CD40 ligation. Finally, we showed that CB2 activation by JWH-015 markedly attenuated CD40-mediated inhibition of microglial phagocytosis of A-beta1-42 peptide. Taken together, these results provide mechanistic insight into beneficial effects provided by cannabinoid receptor CB2 modulation in neurodegenerative diseases, particularly AD.


Although anecdotal reports suggest that cannabis may be used to alleviate symptoms of depression, the psychotrophic effects and abuse liability of this drug prevent its therapeutic application. The active constituent of cannabis, Delta(9)-tetrahydrocannabinol, acts by binding to brain CB(1) cannabinoid receptors, but an alternative approach might be to develop agents that amplify the actions of endogenous cannabinoids by blocking their deactivation. Here, we show that URB597, a selective inhibitor of the enzyme fatty-acid amide hydrolase, which catalyzes the intracellular hydrolysis of the endocannabinoid anandamide, exerts potent antidepressant-like effects in the mouse tail-suspension test and the rat forced-swim test. Moreover, URB597 increases firing activity of serotonergic neurons in the dorsal raphe nucleus and noradrenergic neurons in the nucleus locus ceruleus. These actions are prevented by the CB(1) antagonist rimonabant, are accompanied by increased brain anandamide levels, and are maintained upon repeated URB597 administration. Unlike direct CB(1) agonists, URB597 does not exert rewarding effects in the conditioned place preference test or produce generalization to the discriminative effects of Delta(9)-tetrahydrocannabinol in rats. The findings support a role for anandamide in mood regulation and point to fatty-acid amide hydrolase as a previously uncharacterized target for antidepressant drugs.


This research was designed to examine the effect of three weeks of administration of corticosterone (20 mg/kg) on endocannabinoid content and cannabinoid CB(1) receptor binding in the amygdala. It was found that the endocannabinoid 2-arachidonoylglycerol was significantly increased in the amygdala following chronic corticosterone treatment. However, there was no change in either the maximal binding (B(max)) or binding affinity (K(D)) of [(3)H]-CP 55,940 to the CB(1) receptor in the amygdala. Given the role of amygdalar endocannabinoids in the regulation of emotionality, this suggests that the ability of glucocorticoids to influence affective behavior may involve interactions with regulation of endocannabinoid content.

Recently developed therapeutics for obesity, targeted against cannabinoid receptors, result in decreased appetite and sustained weight loss. Prior studies have demonstrated CB1 receptors (CB1Rs) and leptin modulation of cannabinoid synthesis in hypothalamic neurons. Here, we show that depolarization of perifornical lateral hypothalamus (LH) neurons elicits a CB1R-mediated suppression of inhibition in local circuits thought to be involved in appetite and "natural reward." The depolarization-induced decrease in inhibitory tone to LH neurons is blocked by leptin. Leptin inhibits voltage-gated calcium channels in LH neurons via the activation of janus kinase 2 (JAK2) and of mitogen-activated protein kinase (MAPK). Leptin-deficient mice are characterized by both an increase in steady-state voltage-gated calcium currents in LH neurons and a CB1R-mediated depolarization-induced suppression of inhibition that is 6-fold longer than that in littermate controls. Our data provide direct electrophysiological support for the involvement of endocannabinoids and leptin as modulators of hypothalamic circuits underlying motivational aspects of feeding behavior.


The identification of peripherally expressed CB(2) receptors and reports that the selective activation of cannabinoid CB(2) receptors produces antinociception without traditional cannabinergic side effects suggests that selective cannabinoid CB(2) receptor agonists might be useful in the management of pain. In a rat hindpaw incision model, we examined the antiallodynic activity of the selective cannabinoid CB(2) receptor agonists AM1241 (3-30 mg/kg i.p.), GW405833 (3-30 mg/kg i.p.), and HU-308 (0.3-30 mg/kg i.p.). The rank order for efficacy in the hindpaw incision model following a dose of 10 mg/kg, i.p. was AM1241 > GW405833 = HU-308, and the selective cannabinoid CB(2) receptor antagonist, SR144528, reversed the antiallodynic effect of HU-308. Together, these data suggest that selective cannabinoid CB(2) receptor agonists might represent a new class of postoperative analgesics.


The cannabinoid receptor 1 (Cb1) mediates the psychoactive effect of marijuana. In mammals, there is abundant evidence advocating the importance of cannabinoid signaling; activation of Cb1 exerts diverse functions, chiefly by its ability to modulate neurotransmission. Thus, much attention has been devoted to understand its role in health and disease and to evaluate its therapeutic potential. Here, we have cloned zebrafish cb1 and investigated its expression in developing and adult zebrafish brain. Sequence analysis showed that there is a high degree of conservation, especially in residues demonstrated to be critical for function in mammals. In situ hybridization revealed that zebrafish cb1 appears first in the preoptic area at 24 hours post-fertilization. Subsequently, transcripts are detected in the dorsal telencephalon, hypothalamus, pretectum and torus longitudinalis. A similar pattern of expression is recapitulated in the adult brain. While cb1 is intensively stained in the medial zone of the dorsal telencephalon, expression elsewhere is weak by comparison. In particular, localization of cb1 in the telencephalic periventricular matrix is suggestive of the involvement of Cb1 in neurogenesis, bearing strong resemblance in terms of expression and function to the proliferative mammalian hippocampal formation. In addition, a gradient-like expression of cb1 is detected in the torus longitudinalis, a teleost specific neural tissue. In relation to dopaminergic neurons in the diencephalic posterior tuberculum (considered to be the teleostean homologue of the mammalian midbrain dopaminergic system), both cb1 and tyrosine hydroxylase-expressing cells occupy non-overlapping domains. However there is evidence that they are co-localized in the caudal zone of the hypothalamus, implying a direct modulation of dopamine release in this particular region. Collectively, our data indicate the propensity of zebrafish cb1 to participate in multiple neurological processes.

Alterations of long-term synaptic plasticity have been proposed to participate in the development of addiction. To preserve synaptic functions, homeostatic processes must be engaged after exposure to abused drugs. At the mouse cortico-accumbens synapses, a single in vivo injection of Delta9-tetrahydrocannabinol (THC) suppresses endocannabinoid-mediated long-term depression. Using biochemical and electrophysiological approaches, we now report that 1 week of repeated in vivo THC treatment reduces the coupling efficiency of cannabinoid CB1 receptors (CB1Rs) to G(i/o) transduction proteins, as well as CB1R-mediated inhibition of excitatory synaptic transmission at the excitatory synapses between the prefrontal cortex and the nucleus accumbens (NAc). Nonetheless, we found that cortico-accumbens synapses unexpectedly express normal long-term depression because of a reversible switch in its underlying mechanisms. The present data show that, in THC-treated mice, long-term depression is expressed because a presynaptic mGluR2/3 (metabotropic glutamate receptor 2/3)-dependent mechanism replaces the impaired endocannabinoid system. Thus, in the NAc, a novel form of presynaptic homeostasis rescues synaptic plasticity from THC-induced deficits.


RATIONALE: Cannabinoid type 1 (CB(1)) receptor antagonists are reportedly effective in reducing food intake both preclinically and clinically. This may be due in part to their effects on monoamine release in the brain. The level of central CB(1) receptor occupancy underlying these neurobiological effects is unclear. OBJECTIVES: We explored the relationship between in vivo CB(1) receptor occupancy in the frontal cortex and changes in both monoamine release in the medial prefrontal cortex (mPFC) and feeding behavior in rats in response to two orally administered CB(1) receptor antagonists presently in clinical trials, SR141716A (rimonabant) and SLV319. METHODS: CB(1) receptor occupancy was measured using [3H] SR141716A, and these occupancies were related to potencies to mediate increases in dopamine (DA) and norepinephrine (NE) release measured with microdialysis and decreases in consumption of a highly palatable diet (HP). RESULTS: High receptor occupancy levels (>65%) were required to detect increases in monoamine release that were achieved with 3 and 10 mg/kg of SR141716A and 10 mg/kg of SLV319 for DA and 10 mg/kg of SR141716A for NE. Decreases in HP consumption were seen at occupancies higher than 65% for SR141716A that were achieved with 3 and 10 mg/kg. In contrast, decreases in HP consumption were seen at relatively low CB(1) receptor occupancies (11%) for SLV319. CONCLUSIONS: The occupancy method described here is an effective tool for interrelating central CB(1) receptor occupancy with neurobiological actions of CB(1) receptor antagonists and for furthering our understanding of the role of CB(1) receptors in central nervous system physiology and pathology.


Cannabinoids have been shown to modulate the inhibitory effect of cholecystokinin-containing GABAergic interneurons in the hippocampus via type 1 cannabinoid receptors (CB(1) receptor). Although immunohistochemical studies, using pre-embedding techniques, have demonstrated that these receptors are abundant on GABAergic axon terminals, little is known about their exact location relative to the synapse. Here we used two recently developed antibodies against the CB(1) receptor to study this question with the postembedding immunogold method, which allows the quantitative examination of receptor distribution along the axonal membrane, even within the synaptic active zone. CB(1) receptor positive terminals target both the dendritic and somatic surface of neurons in the CA1 area of the rat hippocampus. We found no difference between these two populations of terminals either in their CB(1) receptor density or in the distribution of receptors on their membrane. Recent studies suggest that endocannabinoids play a role in retrograde signaling at these synapses, i.e. signaling molecules diffuse from the postsynaptic membrane to nearby presynaptic terminals. Therefore, we examined the distribution
of CB(1) receptors on the terminal membranes. We found that they are rare in the synaptic active zone, but are enriched in the perisynaptic annulus, where they can directly influence synaptic calcium channels. Perisynaptic CB(1) receptors represent about one tenth of all CB(1) receptors in a terminal. In contrast, CB(1) receptors have a lower density on the extrasynaptic membrane of terminals far from the postsynaptic cell. We estimated that these terminals contain exceptionally large numbers of CB(1) receptors, i.e. a single axon terminal was usually labeled with more than 450 particles. An unexpected finding was that the density of CB(1) receptors was significantly higher on preterminal axons than on synaptic terminals. These observations suggest that endocannabinoid signaling may subserve roles other than simply reducing transmitter release from axon terminals.


Endocannabinoids are involved in neuroprotection through numerous biochemical pathways. We have shown that the endocannabinoid 2-arachidonoyl glycerol (2-AG) is released in mouse brain after closed head injury (CHI), and treatment with exogenous 2-AG exerts neuroprotection via the central cannabinoid receptor CB(1). This process involves inhibition of inflammatory signals that are mediated by activation of the transcription factor NF-kB. The present study was designed to examine the effect of 2-AG on the blood-brain barrier (BBB) and the possible inhibition of the early expression of proinflammatory cytokines, which are implicated in BBB disruption. We found that 2-AG decreased BBB permeability and inhibited the acute expression of the main proinflammatory cytokines: TNF-alpha, IL-1beta and IL-6. It also augmented the levels of endogenous antioxidants. We suggest that 2-AG exerts neuroprotection in part by inhibition of the early (1-4 h) inflammatory response and augmentation of the brain reducing power.


The development of neuropathic pain is associated with multiple changes in gene expression occurring in the dorsal root ganglia (DRG) and spinal cord. The goal of this study was to evaluate whether the disruption of CB1 cannabinoid receptor gene modulates the changes induced by neuropathic pain in the expression of mu- (MOR), delta- (DOR) and kappa-opioid receptors (KOR) mRNA levels in the DRG and spinal cord. The induction of c-fos expression in the lumbar and sacral regions of the spinal cord was also evaluated in these animals. Opioid receptors mRNA levels were determined by using real-time PCR and Fos protein levels by immunohistochemistry. Nerve injury significantly reduced the expression of MOR in the DRG and the lumbar section of the spinal cord from CB1 cannabinoid knockout (KO) mice and wild-type littermates (WT). In contrast, mRNA levels of DOR and KOR were not significantly changed in any of the different sections analysed. Furthermore, sciatic nerve injury evoked a similar increase of c-fos expression in lumbar and sacral regions of the spinal cord of both KO and WT. In all instances, no significant differences were observed between WT and KO mice. These data revealed specific changes induced by neuropathic pain in MOR expression and c-fos levels in the DRG and/or spinal cord that were not modified by the genetic disruption of CB1 cannabinoid receptors.


Cannabinoids, such as the delta9-tetrahydrocannabinol (THC), present in the cannabis plant, as well as anandamide and 2-arachidonoyl glycerol, produced by the mammalian body, have been shown to protect the brain from various insults and to improve several neurodegenerative diseases. The current review summarizes the evidence for cannabinoid neuroprotection in vivo, and refers to recent in vitro studies, which help elucidate possible molecular mechanisms underlying this protective effect. Some of these mechanisms involve the activation of CB1 and CB2 cannabinoid receptors, while others are not dependent on them. In some cases, protection is due to a direct effect of the cannabinoids on neuronal cells, while in
others, it results from their effects on non-neuronal elements within the brain. In many experimental set-ups, cannabinoid neurotoxicity, particularly by THC, resides side by side with neuroprotection. The current review attempts to shed light on this dual activity, and to dissociate between the two contradictory effects.


Transient forebrain ischemia of 5-min duration causes delayed neuronal death (DND) of vulnerable CA1 neurons in the gerbil hippocampus, which can be prevented by "preconditioning" with a short ischemic stimulus of 2.5-min duration. While a key role of excitatory glutamate receptors for both phenomena has been widely accepted, little is known about the postischemic regulation of central cannabinoid (CB1) receptors. The present study was designed to test whether ischemic preconditioning is associated with specific alterations of protein expression and/or ligand binding of these receptors compared to ischemia severe enough to induce DND. Gerbils were subjected to either a 5-min ischemic period resulting in DND of CA1 neurons, or a 2.5-min period of ischemia usually used for preconditioning. Postischemic hippocampal CB1 receptor protein expression was investigated immunohistochemically, while postischemic ligand binding of [(3)H]CP 55940 to CB1 receptors was analyzed by quantitative receptor autoradiography in both experimental groups after 24, 48, and 96 h (n=4-5 per time point), respectively, and compared to sham-treated gerbils (n=10). Short-term ischemia of 2.5-min duration caused a transient reduction of hippocampal CB1 receptor protein expression, while receptor binding density was permanently decreased. In contrast, 5-min ischemia did not alter protein expression or ligand binding up to 48 h. Based on these data, postischemic down-regulation of hippocampal CB1 receptors, specifically seen after short-term ischemia usually used for preconditioning, may participate in the mechanisms of endogenous postischemic neuroprotection.


Recent work in our laboratories has demonstrated that an opioid-independent form of stress-induced analgesia (SIA) is mediated by endogenous cannabinoids [Hohmann et al., 2005. Nature 435, 1108]. Non-opioid SIA, induced by a 3-min continuous foot shock, is characterized by the mobilization of two endocannabinoid lipids-2-arachidonoylglycerol (2-AG) and anandamide-in the midbrain periaqueductal gray (PAG). The present studies were conducted to examine the contributions of spinal endocannabinoids to nonopioid SIA. Time-dependent increases in levels of 2-AG, but not anandamide, were observed in lumbar spinal cord extracts derived from shocked relative to non-shocked rats. Notably, 2-AG accumulation was of smaller magnitude than that observed previously in the dorsal midbrain following foot shock. 2-AG is preferentially degraded by monoacylglycerol lipase (MGL), whereas anandamide is hydrolyzed primarily by fatty-acid amide hydrolase (FAAH). This metabolic segregation enabled us to manipulate endocannabinoid tone at the spinal level to further evaluate the roles of 2-AG and anandamide in nonopioid SIA. Intrathecal administration of the competitive CB(1) antagonist SR141716A (rimonabant) failed to suppress nonopioid SIA, suggesting that supraspinal rather than spinal CB(1) receptor activation plays a pivotal role in endocannabinoid-mediated SIA. By contrast, spinal inhibition of MGL using URB602, which selectively inhibits 2-AG hydrolysis in the PAG, enhanced SIA through a CB(1)-selective mechanism. Spinal inhibition of FAAH, with either URB597 or arachidonoyl serotonin (AA-5-HT), also enhanced SIA through a CB(1)-mediated mechanism, presumably by increasing accumulation of tonically released anandamide. Our results suggest that endocannabinoids in the spinal cord regulate, but do not mediate, nonopioid SIA.


Endothelin (ETB)-receptors mediate anti-apoptotic actions. Lack of functional ETB-receptors leads to increased neuronal apoptosis in the hippocampus. The increased apoptosis
must be compensated by other mechanisms, however, as ETB-deficient rats display normal overall brain morphology. To illuminate on brain plasticity in ETB-receptor deficiency, we studied the expression and function of another neuroprotective system, the cannabinoid CB1-receptors, in ETB-deficient hippocampus. We show that CB1 expression in hippocampus increases postnatally in all rats but that the increase in CB1-receptor expression is significantly higher in ETB-deficient compared to wildtype littermates. Neuronal apoptosis decreases during brain maturation but remains on a significantly higher level in the ETB-deficient compared to wildtype dentate. When investigating survival of hippocampal neurons in culture, we found significant protection against hypoxia-induced cell death with CB1-analogs (noladin, (9-tetrahydrocannabinol) only in ETB-deficient neurons. We suggest that CB1-receptor upregulation in the ETB-mutant hippocampus reflects an attempt to compensate for the lack of ETB-receptors.


While recent evidence suggests that fatty acid amide hydrolase (FAAH) may represent a potential therapeutic target, few published studies have investigated FAAH or its fatty acid amide substrates (FAAs) in animal models of learning and memory. Therefore, our primary goal was to determine whether FAAH (-/-) mice, which possess elevated levels of anandamide and other FAAs, would display altered performance in four Morris water maze tasks: 1) acquisition of a hidden fixed platform, 2) reversal learning, 3) working memory, and 4) probe trials. FAAH (-/-) mice failed to exhibit deficits in any task; in fact they initially acquired the working memory task more rapidly than FAAH (+/+) mice. The second goal of this study was to investigate whether the FAAH inhibitor OL-135, anandamide, other FAAs, and methanandamide would affect working memory in both genotypes. FAAH (-/-), but not (+/+) mice displayed working memory impairments following exogenous administration of anandamide (ED50=6 mg/kg) or oleamide (50 mg/kg). However, the CB1 receptor antagonist SR141716 (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide-HCl) only blocked the disruptive effects of anandamide. Methanandamide, which is not metabolized by FAAH, disrupted working memory performance in both genotypes (ED50=10 mg/kg), suggesting that CB1 receptor signaling is unaltered by FAAH deletion. In contrast, OL-135 and other FAAs failed to affect working memory in either genotype. These results suggest that FAAH deletion does not impair spatial learning, but may enhance acquisition under certain conditions. More generally, FAAH may represent a novel therapeutic target that circumvents the undesirable cognitive side effects commonly associated with direct-acting cannabinoid agonists.


A variety of physical and psychological stressors induce analgesia by activating descending systems that project from the brain to the spinal cord. This stress-induced analgesia (SIA) is mediated by distinct opioid- and non-opioid-dependent mechanisms. New evidence suggests that non-opioid SIA is mediated by two independent endocannabinoids within the midbrain. Furthermore, novel agents that disrupt breakdown of these endocannabinoids enhance non-opioid SIA and pave the way for novel therapies.

Oncology


OBJECTIVE: To test in vitro and in vivo the safety and efficacy of a novel chemotherapeutic agent, KM-233, for the treatment of glioma. METHODS: In vitro cell cytotoxicity assays were used to measure and compare the cytotoxic effects of KM-233, Delta(8)-tetrahydrocannabinol (THC), and bis-chloroethyl-nitrosurea (BCNU) against human U87 glioma cells. An organotypic brain slice culture model was used for safety and toxicity studies. A human
glioma-SCID mouse side-pocket tumor model was used to test in vivo the safety and efficacy of KM-233 with intratumoral and intra-peritoneal administration. RESULTS: KM-233 is a classical cannabinoid with good blood brain barrier penetration that possesses a selective affinity for the CB2 receptors relative to THC. KM-233 was as efficacious in its cytotoxicity against human U87 glioma as Delta(8)-tetrahydrocannabinol, and superior to the commonly used anti-glioma chemotherapeutic agent, BCNU. The cytotoxic effects of KM-233 against human glioma cells in vitro occur as early as two hours after administration, and dosing of KM-233 can be cycled without compromising cytotoxic efficacy and while improving safety. Cyclical dosing of KM-233 to treat U87 glioma in a SCID mouse xenograft side pocket model was effective at reducing the tumor burden with both systemic and intratumoral administration. CONCLUSION: These studies provide both in vitro and in vivo evidence that KM-233 shows promising efficacy against human glioma cell lines in both in vitro and in vivo studies, minimal toxicity to healthy cultured brain tissue, and should be considered for definitive preclinical development in animal models of glioma.


We have earlier reported overexpression of the central and peripheral cannabinoid receptors CB1 and CB2 in mantle cell lymphoma (MCL), a B cell non-Hodgkin lymphoma. In this study, treatment with cannabinoid receptor ligands caused a decrease in viability of MCL cells, while control cells lacking CB1 were not affected. Interestingly, equipotent doses of the CB1 antagonist SR141716A and the CB1/CB2 agonist anandamide inflicted additive negative effects on viability. Moreover, treatment with the CB1/CB2 agonist Win-55,212-2 caused a decrease in long-term growth of MCL cells in culture. Induction of apoptosis, as measured by FACS/Annexin V-FITC, contributed to the growth suppressive effect of Win-55,212-2. Our data suggest that cannabinoid receptors may be considered as potential therapeutic targets in MCL.


The endocannabinoid system regulates cell proliferation in human breast cancer cells. We reasoned that stimulation of cannabinoid CB(1) receptors could induce a non-invasive phenotype in breast metastatic cells. In a model of metastatic spreading in vivo, the metabolically stable anandamide analogue, 2-methyl-2'-F-anandamide (Met-F-AEA), significantly reduced the number and dimension of metastatic nodes, this effect being antagonized by the selective CB(1) antagonist SR141716A. In MDA-MB-231 cells, a highly invasive human breast cancer cell line, and in TSA-E1 cells, a murine breast cancer cell line, Met-F-AEA inhibited adhesion and migration on type IV collagen in vitro without modifying integrin expression: both these effects were antagonized by SR141716A. In order to understand the molecular mechanism involved in these processes, we analyzed the phosphorylation of FAK and Src, two tyrosine kinases involved in migration and adhesion. In Met-F-AEA-treated cells, we observed a decreased tyrosine phosphorylation of both FAK and Src, this effect being attenuated by SR141716A. We propose that CB(1) receptor agonists inhibit tumor cell invasion and metastasis by modulating FAK phosphorylation, and that CB(1) receptor activation might represent a novel therapeutic strategy to slow down the growth of breast carcinoma and to inhibit its metastatic diffusion in vivo.

Ophthalmology


Prostaglandins (PGs) and matrix metalloproteinases (MMP) have been implicated in lowering intraocular pressure (IOP) by facilitating aqueous humor outflow. A possible role of cyclooxygenase-2 (COX-2) in this process was emphasized by findings showing an impaired COX-2 expression in the non-pigmented ciliary epithelium (NPE) of patients with primary open-angle glaucoma. Using human NPE cells, the present study therefore investigated the effect of the IOP-lowering cannabinoid R(+)-methanandamide (R(+)-MA) on the expression of COX-2 and
different MMPs and tissue inhibitors of MMPs (TIMPs). R(+)‐MA led to a concentration- and time-dependent increase of COX-2 mRNA expression. R(+)‐MA‐induced COX-2 expression was accompanied by time‐dependent phosphorylations of p38 mitogen‐activated protein kinase (MAPK) and p42/44 MAPK, and was abrogated by inhibitors of both pathways. Moreover, R(+)‐MA increased the mRNA and protein expression of MMP‐1, ‐3, ‐9 and TIMP‐1, but not that of MMP‐2 and TIMP‐2. Inhibition of COX‐2 activity with NS‐398 was associated with a virtually complete suppression of R(+)‐MA‐induced MMP‐9 and TIMP‐1 expression. Consistent with this data, MMP‐9 and TIMP‐1 expression was also induced by PGE2, a major COX‐2 product. Two other COX‐2‐inducing cannabinoids, anandamide and Delta(9)‐tetrahydrocannabinol, caused the same pattern of MMP‐ and TIMP expression as R(+)‐MA both in the absence and presence of NS‐398. Altogether, cannabinoids induce the production of several outflow‐facilitating mediators in the human NPE. Our results further imply an involvement of COX‐2‐dependent PGs in MMP‐9 and TIMP‐1 expression. In conclusion, stimulation of intraocular COX‐2 and MMP expression may represent a potential mechanism contributing to the IOP‐lowering action of different cannabinoids.

**Pain**


AIM: The aim of this study was to evaluate the role of the endogenous cannabinoid system in controlling neuroplasticity. METHODS: The pain threshold for electrical stimuli was determined in transgenic mice lacking the cannabinoid receptor type 1 (CB1(−/−)) and in the corresponding respective wild‐type animals. Electrophysiological experiments were performed in prepared brain slices to test the effect of endogenous and exogenous cannabinoids on synaptic transmission and long‐term potentiation (LTP) in the amygdala. RESULTS: The pain threshold was nearly identical in both groups for the first pain induction; however, with repeated pain induction it decreased to a significantly greater extent in the CB1(−/−) mice than in the wild‐type animals. Synaptic transmission and the inducibility of LTP were not influenced by the acute pharmacological blockade of CB1 receptors, but inhibited by the CB1 agonist WIN55,212‐2. CONCLUSION: The endogenous cannabinoid system is involved in the control of neuroplasticity as part of pain processing. Cannabinoids prevent the formation of LTP in the amygdala via activation of CB1 receptors. Synaptic transmission and the inducibility of LTP were not influenced by the acute pharmacological blockade of CB1 receptors, but inhibited by the CB1 agonist Win55,212‐2.


Recent work in our laboratories has demonstrated that an opioid‐independent form of stress‐induced analgesia (SIA) is mediated by endogenous ligands for cannabinoid receptors‐anandamide and 2‐arachidonoylglycerol (2‐AG) [A.G. Hohmann, R.L. Suplita, N.M. Bolton, M.H. Neely, D. Fegley, R. Mangieri, J.F. Krey, J.M. Walker, P.V. Holmes, J.D. Crystal, A. Durant, A. Tontini, M. Mor, G. Tarzia, D. Piomelli, An endocannabinoid mechanism for stress‐induced analgesia, Nature 435 (2005) 1108‐1112]. The present study was conducted to examine the contribution of cannabinoid CB(1) receptors in the basolateral nucleus of the amygdala (BLA) and central nucleus of the amygdala (CeA) to nonopioid SIA. SIA was induced by continuous footshock (3min 0.9mA) and quantified behaviorally using the tail‐flick test. Microinjection of the CB(1) antagonist/inverse agonist rimonabant (SR141716A) into the BLA, a limbic forebrain region with high densities of CB(1) receptors, suppressed SIA relative to control conditions. By contrast, the same dose administered into the CeA, where CB(1) immunoreactivity is largely absent, or outside the amygdala did not alter SIA. To examine the contribution of endocannabinoids in the BLA to SIA, we used selective pharmacological inhibitors of the anandamide‐degrading enzyme fatty‐acid amide hydrolase (FAAH) and the 2‐arachidonoylglycerol‐degrading enzyme monoacylglycerol lipase (MGL). The FAAH inhibitor URB597 and MGL inhibitor URB602, at doses that enhanced SIA following microinjection in the midbrain periaqueductal gray, did not alter SIA relative to control conditions. Our findings suggest that CB(1) receptors in the BLA but
not the CeA contribute to SIA, but pharmacological inhibition of endocannabinoid degradation at these sites does not affect the expression of stress antinociception.


While cannabinoid receptor agonists have analgesic activity in chronic pain states, they produce a spectrum of central CB(1) receptor-mediated motor and psychotropic side effects. The actions of endocannabinoids, such as anandamide are terminated by removal from the extracellular space, then subsequent enzymatic degradation by fatty-acid amide hydrolase (FAAH). In the present study, we compared the effect of a selective FAAH inhibitor, URB597, to that of a pan-cannabinoid receptor agonist HU210 in rat models of chronic inflammatory and neuropathic pain. Systemic administration of URB597 (0.3 mg kg(-1)) and HU210 (0.03 mg kg(-1)) both reduced the mechanical allodynia and thermal hyperalgesia in the CFA model of inflammatory pain. In contrast, HU210, but not URB597, reduced mechanical allodynia in the partial sciatic nerve-ligation model of neuropathic pain. UH210, but not URB597, produced a reduction in motor performance in unoperated rats. The effects of URB597 in the CFA model were dose dependent and were reduced by coadministration with the cannabinoid CB(1) antagonist AM251 (1 mg kg(-1)), or the CB(2) and SR144528 (1 mg kg(-1)). Coadministration with AM251 plus SR144528 completely reversed the effects of URB597. These findings suggest that the FAAH inhibitor URB597 produces cannabinoid CB(1) and CB(2) receptor-mediated analgesia in inflammatory pain states, without causing the undesirable side effects associated with cannabinoid receptor activation. British Journal of Pharmacology advance online publication, 5 December 2005; doi:10.1038/sj.bjp.0706510.


The identification of peripherally expressed CB(2) receptors and reports that the selective activation of cannabinoid CB(2) receptors produces antinociception without traditional cannabinergic side effects suggests that selective cannabinoid CB(2) receptor agonists might be useful in the management of pain. In a rat hindpaw incision model, we examined the antiallodynic activity of the selective cannabinoid CB(2) receptor agonists AM1241 (3-30 mg/kg i.p.), GW405833 (3-30 mg/kg i.p.), and HU-308 (0.3-30 mg/kg i.p.). The rank order for efficacy in the hindpaw incision model following a dose of 10 mg/kg, i.p. was AM1241>GW405833=HU-308, and the selective cannabinoid CB(2) receptor antagonist, SR144528, reversed the antiallodynic effect of HU-308. Together, these data suggest that selective cannabinoid CB(2) receptor agonists might represent a new class of postoperative analgesics.


The development of neuropathic pain is associated with multiple changes in gene expression occurring in the dorsal root ganglia (DRG) and spinal cord. The goal of this study was to evaluate whether the disruption of CB1 cannabinoid receptor gene modulates the changes induced by neuropathic pain in the expression of mu- (MOR), delta- (DOR) and kappa-opioid receptors (KOR) mRNA levels in the DRG and spinal cord. The induction of c-fos expression in the lumbar and sacral regions of the spinal cord was also evaluated in these animals. Opioid receptors mRNA levels were determined by using real-time PCR and Fos protein levels by immunohistochemistry. Nerve injury significantly reduced the expression of MOR in the DRG and the lumbar section of the spinal cord from CB1 cannabinoid knockout (KO) mice and wild-type littermates (WT). In contrast, mRNA levels of DOR and KOR were not significantly changed in any of the different sections analysed. Furthermore, sciatic nerve injury evoked a similar increase of c-fos expression in lumbar and sacral regions of the spinal cord of both KO and WT. In all instances, no significant differences were observed between WT and KO mice. These data revealed specific changes induced by neuropathic pain in MOR expression and c-fos levels in the DRG and/or spinal cord that were not modified by the genetic disruption of CB1 cannabinoid receptors.

Endocannabinoids have analgesic/anti-inflammatory properties. The biology of endocannabinoids, their receptors, signalling mechanisms and role in the regulation of physiological processes have been extensively reviewed. This review focuses on the role of palmitoylethanolamide (PEA), an endogenous fatty acid amide analogue of the endocannabinoid anandamide, in tissue protective mechanisms. PEA was first identified almost five decades ago in lipid extracts of various natural products, and its anti-inflammatory and antinociceptive effects were established later. Evidence exists that PEA is synthesised during inflammation and tissue damage and a number of beneficial effects, including the relief of inflammation and pruritus, have been shown to be useful in the control of neurogenic and neuropathic pain. The postulated hypotheses as to the mode of action of PEA include a possible local autacoid-like mediator activity regulating mast-cell activity and putative activation of cannabinoids and vanilloid TRPV1 receptors via "entourage" effects. The large number of scientific investigations into the effects of PEA and PEA-related compounds has given rise to new therapeutic opportunities. In spite of the multitude of therapies currently employed to control inflammation, pain, pruritus and tissue damage, the possibility of using a natural compound, such as PEA to manipulate endogenous protective mechanisms may be considered a beneficial novel therapeutic strategy in veterinary medicine.


Recent work in our laboratories has demonstrated that an opioid-independent form of stress-induced analgesia (SIA) is mediated by endogenous cannabinoids [Hohmann et al., 2005. Nature 435, 1108]. Non-opioid SIA, induced by a 3-min continuous foot shock, is characterized by the mobilization of two endocannabinoid lipids-2-arachidonoylglycerol (2-AG) and anandamide-in the midbrain periaqueductal gray (PAG). The present studies were conducted to examine the contributions of spinal endocannabinoids to nonopioid SIA. Time-dependent increases in levels of 2-AG, but not anandamide, were observed in lumbar spinal cord extracts derived from shocked relative to non-shocked rats. Notably, 2-AG accumulation was of smaller magnitude than that observed previously in the dorsal midbrain following foot shock. 2-AG is preferentially degraded by monoacylglycerol lipase (MGL), whereas anandamide is hydrolyzed primarily by fatty-acid amide hydrolase (FAAH). This metabolic segregation enabled us to manipulate endocannabinoid tone at the spinal level to further evaluate the roles of 2-AG and anandamide in nonopioid SIA. Intrathecal administration of the competitive CB(1) antagonist SR141716A (rimonabant) failed to suppress nonopioid SIA, suggesting that supraspinal rather than spinal CB(1) receptor activation plays a pivotal role in endocannabinoid-mediated SIA. By contrast, spinal inhibition of MGL using URB602, which selectively inhibits 2-AG hydrolysis in the PAG, enhanced SIA through a CB(1)-selective mechanism. Spinal inhibition of FAAH, with either URB597 or arachidonoyl serotonin (AA-5-HT), also enhanced SIA through a CB(1)-mediated mechanism, presumably by increasing accumulation of tonically released anandamide. Our results suggest that endocannabinoids in the spinal cord regulate, but do not mediate, nonopioid SIA.


A variety of physical and psychological stressors induce analgesia by activating descending systems that project from the brain to the spinal cord. This stress-induced analgesia (SIA) is mediated by distinct opioid- and non-opioid-dependent mechanisms. New evidence suggests that non-opioid SIA is mediated by two independent endocannabinoids within the midbrain. Furthermore, novel agents that disrupt breakdown of these endocannabinoids enhance non-opioid SIA and pave the way for novel therapies.

Several recent reports have demonstrated a role for selective cannabinoid CB(2) receptor agonists in pain modulation, showing both analgesic and antihyperalgesic activities. While the mechanism of action is poorly understood, it has been postulated that these effects may be indirect, involving release of endogenous opioids. We have previously reported that administration of the selective cannabinoid CB(2) receptor agonist GW405833 (2,3-dichlorophenyl)-[5-methoxy-2-methyl-3-(2-morpholin-4-yl-ethyl)-indol -1-yl]-methanone) to rats elicits potent and efficacious antihyperalgesic effects against neuropathic and inflammatory pain and, at high dose (100 mg/kg), is analgesic and ataxic [Valenzano, K.J., Tafesse, L., Lee, G., Harrison, J.E., Boulet, J., Gottshall, S.L., Mark, L., Pearson, M.S., Miller, W., Shan, S., Rabadi, L., Rotstheyn, Y., Chaffer, S.M., Turchin, P.I., Elsemore, D.A., Toth, M., Koetzner, L., Whiteside, G.T., 2005. Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology 48, 658-672]. In the current study, we confirm these properties using mouse models and investigate the role of cannabinoid CB(2) receptors using knockout animals. Furthermore, we provide evidence that the antinociceptive properties of GW405833 are opioid independent. GW405833 elicited robust antihyperalgesic effects in mouse models of inflammatory (Freud's complete adjuvant) and neuropathic (Seltzer) pain. In contrast, GW405833 showed no antihyperalgesic activity against Freud's complete adjuvant-mediated inflammatory pain in cannabinoid CB(2) receptor knockout mice. As in rats, high-dose GW405833 (100 mg/kg) showed both analgesic and sedative activities in wild-type mice, activities that were also apparent in cannabinoid CB(2) receptor knockout mice. In rats, neither the antihyperalgesic effect in the Freud's complete adjuvant model nor the analgesic effects in tail flick and hot plate assays were inhibited by pre-treatment with the non-selective opioid receptor antagonist, naltrexone. These data demonstrate that the antihyperalgesic effects of GW405833 are mediated via the cannabinoid CB(2) receptor, whereas the analgesic and sedative effects are not. Furthermore, these data suggest that the mechanism of action for GW405833 does not depend on the release of endogenous opioids.

**Pharmacology**


Cyclosporine, beside its immunosuppressive action, has several effects on different neuronal functions, such as modulation of neurotransmitter release, the inhibition of nitric oxide synthesis and release, the reduction of cAMP production and inhibition of morphine-induced tolerance. In the present study, the effect of cyclosporine on the expression and development of tolerance to WIN 55,212-2, a cannabinoid receptor agonist, was studied. Intra peritoneal (i.p.) injection of WIN 55,212-2 (2-6 mg/kg) induced time-dependent and dose-dependent analgesia and catalepsy in mice. Administration of cyclosporine (20 mg/kg i.p.), 30 min before WIN 55,212-2 (6 mg/kg i.p.), did not change the analgesic and cataleptic effects of WIN 55,212-2. When WIN 55,212-2 (6 mg/kg i.p.) was injected once a day, animals became completely tolerant to the analgesic and cataleptic effects within five and nine days respectively. Cyclosporine (20 mg/kg i.p.) injected once daily, 30 min before WIN 55,212-2, attenuated the development of tolerance to the analgesic and cataleptic effects of WIN 55,212-2 but did not affect the expression of tolerance. Since cyclosporine given chronically by itself did not alter the analgesia and catalepsy induced by acute administration of WIN 55,212-2, our findings suggest cyclosporine may act with some selectivity on the mechanisms involved in development of cannabinoid tolerance.


The endogenous cannabinoids (endocannabinoids) are bioactive signaling molecules, that show diverse cellular and physiological effects and play various roles in the central nervous system, as well as in the periphery. The discovery of N-arachidonoylthanolamine (anandamide, AEA) and of the enzyme that terminates its signaling, i. e. fatty acid amide hydrolase (FAAH), has inspired pharmacological strategies to augment endocannabinoid tone and biological activity
through inhibition of FAAH. Here we discuss the role of natural endocannabinoid derivatives, like the hydroxy-anandamides (OH-AEAs) generated from AEA via lipoxygenase activity, as powerful inhibitors of FAAH. We propose that these compounds, by reversibly inhibiting FAAH, may control in vivo the endocannabinoid tone. We consider the theoretical structural properties of OH-AEAs and other natural inhibitors of FAAH, based on the calculation of theoretical molecular descriptors commonly used in Quantitative Structure Activity Relationship (QSAR) studies. The QSAR properties of OH-AEAs and congeners suggest that they could act at different specific sites of FAAH, thus confirming their potential value as templates for the development of next-generation therapeutics.


This study compared the effects of the putative cannabinoid receptor 'silent antagonist' O-2050 with the cannabinoid receptor inverse agonist SR 141716 on food and water consumption, and locomotor activity. Non-deprived male Wistar rats were habituated to the apparatus and testing procedures, then injected intraperitoneally with vehicle, O-2050 (0.03-3.0 mg/kg), or SR 141716 (3.0 mg/kg) prior to 4-h test sessions. Food consumption was significantly reduced by both drugs. Water intake and locomotor activity were significantly reduced only by O-2050. Results support the notion that cannabinoid receptor antagonists suppress feeding behaviour by blocking an endogenous cannabinoid orexigenic signal, rather than by inverse agonism at cannabinoid receptors. However, further studies are needed to confirm the status of O-2050 as a cannabinoid CB(1) receptor antagonist devoid of inverse agonist properties.


Anandamide (AEA) and Delta(9)-tetrahydrocannabinol (THC) are endogenous and exogenous ligands, respectively, for cannabinoid receptors. While most of the pharmacological actions of cannabinoids are mediated by CB1 receptors, there is also evidence that these compounds can produce effects that are not mediated by the activation of identified cannabinoid receptors. Here, we report that THC and AEA, in a CB1 receptor-independent manner, cause a significant potentiation of the amplitudes of glycine-activated currents (IGly) in acutely isolated neurons from rat ventral tegmental area (VTA) and in Xenopus oocytes expressing human homomeric (alpha1) and heteromeric (alpha1beta1) subunits of glycine receptors (GlyRs). The potentiation of IGly by THC and AEA is concentration-dependent with respective EC50 values of 86 +/- 9 nM and 319 +/- 31 nM for alpha1 homomeric receptors, 73 +/- 8 nM and 318 +/- 24 nM for alpha1beta1 heteromeric receptors, and 115 +/- 13 nM and 230 +/- 29 nM for native GlyRs in VTA neurons. The effects of THC and AEA are selective for IGly, since GABA-activated current in VTA neurons or in Xenopus oocytes expressing alpha2beta3gamma2 GABAA receptor subunits were unaffected by these compounds. The maximal potentiation by THC and AEA was observed at the lowest concentration of glycine; with increasing concentrations of glycine, the potentiation significantly decreased. The site for THC and AEA seems to be distinct from that of the alcohol and volatile anesthetics. The results indicate that THC and AEA, in pharmacologically relevant concentrations, directly potentiate the function of GlyRs through an allosteric mechanism.


While cannabinoid receptor agonists have analgesic activity in chronic pain states, they produce a spectrum of central CB(1) receptor-mediated motor and psychotropic side effects. The actions of endocannabinoids, such as anandamide are terminated by removal from the extracellular space, then subsequent enzymatic degradation by fatty-acid amide hydrolase (FAAH). In the present study, we compared the effect of a selective FAAH inhibitor, URB597, to that of a pan-cannabinoid receptor agonist HU210 in rat models of chronic inflammatory and neuropathic pain. Systemic administration of URB597 (0.3 mg kg(-1)) and HU210 (0.03 mg kg(-1)) both reduced the mechanical allodynia and thermal hyperalgesia in the CFA model of inflammatory pain. In contrast, HU210, but not URB597, reduced mechanical allodynia in the partial sciatic nerve-ligation model of neuropathic pain. HU210, but not URB597, produced a
reduction in motor performance in unoperated rats. The effects of URB597 in the CFA model were dose dependent and were reduced by coadministration with the cannabinoid CB(1) antagonist AM251 (1 mg kg(-1)), or the CB(2) and SR144528 (1 mg kg(-1)). Coadministration with AM251 plus SR144528 completely reversed the effects of URB597. These findings suggest that the FAAH inhibitor URB597 produces cannabinoid CB(1) and CB(2) receptor-mediated analgesia in inflammatory pain states, without causing the undesirable side effects associated with cannabinoid receptor activation. British Journal of Pharmacology advance online publication, 5 December 2005; doi:10.1038/sj.bjp.0706510.


Fourteen novel CB(2) receptor selective cannabinoids were synthesized via initial Lewis acid catalyzed rearrangement of resorcinol precursors to obtain the cannabinoid moiety. These are the 1-methoxy-9-hydroxyhexahydrocannabinols and the 1-deoxy-9-hydroxyhexahydrocannabinols, with 1', 1'-dimethylalkyl side chains of four to seven carbon atoms at C-3 of the cannabinoid nucleus. The cannabinoids synthesized and described in this paper all exhibit greater affinity for the CB(2) receptor than for the CB(1) receptor. Exceptionally high CB(2) affinity was observed for 1-deoxy-9beta-hydroxy-dimethylhexahydrocannabinol (JWH-361, 9, n=3) K(i)=2.7nM and 1-deoxy-9beta-hydroxydimethylpentylhexahydrocannabinol (JWH-300, 9, n=2) K(i)=5.3nM. In general, the stereochemistry of the 9-hydroxy group is important and the beta-orientation enhances both CB(2) receptor affinity and selectivity.


Several recent reports have demonstrated a role for selective cannabinoid CB(2) receptor agonists in pain modulation, showing both analgesic and antihyperalgesic activities. While the mechanism of action is poorly understood, it has been postulated that these effects may be indirect, involving release of endogenous opioids. We have previously reported that administration of the selective cannabinoid CB(2) receptor agonist GW405833 (2,3-dichlorophenyl)-[5-methoxy-2-methyl-3-(2-morpholin-4-yl-ethyl)-indol -1-yl]-methanone) to rats elicits potent and efficacious antihyperalgesic effects against neuropathic and inflammatory pain and, at high dose (100 mg/kg), is analgesic and ataxic [Valenzano, K.J., Tafesse, L., Lee, G., Harrison, J.E., Boulet, J., Gottshall, S.L., Mark, L., Pearson, M.S., Miller, W., Shan, S., Rabadi, L., Rotstheyn, Y., Chaffer, S.M., Turchin, P.I., Elsemore, D.A., Toth, M., Koetzner, L., Whiteside, G.T., 2005. Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology 48, 658-672]. In the current study, we confirm these properties using mouse models and investigate the role of cannabinoid CB(2) receptors using knockout animals. Furthermore, we provide evidence that the antinociceptive properties of GW405833 are opioid independent. GW405833 elicited robust antihyperalgesic effects in mouse models of inflammatory (Freund's complete adjuvant) and neuropathic (Seltzer) pain. In contrast, GW405833 showed no antihyperalgesic activity against Freund's complete adjuvant-mediated inflammatory pain in cannabinoid CB(2) receptor knockout mice. As in rats, high-dose GW405833 (100 mg/kg) showed both analgesic and sedative activities in wild-type mice, activities that were also apparent in cannabinoid CB(2) receptor knockout mice. In rats, neither the antihyperalgesic effect in the Freund's complete adjuvant model nor the analgesic effects in tail flick and hot plate assays were inhibited by pre-treatment with the non-selective opioid receptor antagonist, naltrexone. These data demonstrate that the antihyperalgesic effects of GW405833 are mediated via the cannabinoid CB(2) receptor, whereas the analgesic and sedative effects are not. Furthermore, these data suggest that the mechanism of action for GW405833 does not depend on the release of endogenous opioids.
Physiology

Plant Sciences

A headspace solid-phase microextraction combined with GC-MS method was developed for the extraction and analysis of cannabinoids from Cannabis samples. Different commercially available fibres were evaluated; polydimethylsiloxane 100 microm was selected as the most efficient one. In order to enhance sensitivity and reduce analysis time, the sampling temperature was studied and it showed that extraction should be performed at a high temperature (150 degrees C). In relation with the high lipophilicity of cannabinoids, a relatively long desorption time (3 min) was necessary to ensure a total transfer from the fibre into the injection port of the gas chromatograph. The method was finally applied to the extraction of Swiss marijuana samples from different regions. Data treatment by principal component analysis and hierarchical cluster analysis allowed a discrimination of the different batches.

Psychiatry

Two topics are presented in this review. In the first section, we review data regarding the effects of the endocannabinoids (eCBs) and cannabinoid receptors on neuroimmune function. The function of eCBs in the interaction between the immune system and the central nervous system (CNS) is of particular interest, since the CNS itself is a rich source of eCBs while being exquisitely sensitive to inflammation. There are several sites at which cannabinoids can influence neuroinflammation. Microglial cells express both CB receptors and make eCBs. Activation of CB receptors on these cells seems to promote migration and proliferation but to reduce activation to macrophages. In several neurodegenerative diseases, up-regulation of microglial CB2 receptors have been observed. It is our hypothesis that microglial CB receptor activity is anti-inflammatory and could be exploited to manipulate neuroinflammatory processes with a minimum of unwanted effects. The second topic discussed suggests that the eCB/CB1 receptor pair is involved in the responses of animals to acute, repeated and variable stress. The roles of this pair are complex and dependent upon previous stress, among other things. Dysfunctional responding to stress is a component of several human neuropsychiatric disorders, including anxiety and panic disorders, post-traumatic stress disorders, premenstrual dysphoria and quite possibly, drug abuse. While it is too early to say with certainty, it is very possible that either inhibition or potentiation of endocannabinoid signaling will be an efficacious novel therapeutic approach to more than one human psychiatric disease.


Although anecdotal reports suggest that cannabis may be used to alleviate symptoms of depression, the psychotropic effects and abuse liability of this drug prevent its therapeutic application. The active constituent of cannabis, Delta(9)-tetrahydrocannabinol, acts by binding to brain CB(1) cannabinoid receptors, but an alternative approach might be to develop agents that amplify the actions of endogenous cannabinoids by blocking their deactivation. Here, we show that URB597, a selective inhibitor of the enzyme fatty-acid amide hydrolase, which catalyzes the intracellular hydrolysis of the endocannabinoid anandamide, exerts potent antidepressant-like effects in the mouse tail-suspension test and the rat forced-swim test. Moreover, URB597 increases firing activity of serotonergic neurons in the dorsal raphe nucleus and noradrenergic neurons in the nucleus locus ceruleus. These actions are prevented by the CB(1) antagonist rimonabant, are accompanied by increased brain anandamide levels, and are maintained upon repeated URB597 administration. Unlike direct CB(1) agonists, URB597 does not exert rewarding
effects in the conditioned place preference test or produce generalization to the discriminative effects of Delta(9)-tetrahydrocannabinol in rats. The findings support a role for anandamide in mood regulation and point to fatty-acid amide hydrolase as a previously uncharacterized target for antidepressant drugs.

Reproductive Biology

Respiratory

Toxicology

CLINICAL SCIENCE

Adverse events

BACKGROUND: Few studies have examined samples of people with cannabis-induced psychotic symptoms. AIMS: To establish whether cannabis-induced psychotic disorders are followed by development of persistent psychotic conditions, and the timing of their onset. METHOD: Data on patients treated for cannabis-induced psychotic symptoms between 1994 and 1999 were extracted from the Danish Psychiatric Central Register. Those previously treated for any psychotic symptoms were excluded. The remaining 535 patients were followed for at least 3 years. In a separate analysis, the sample was compared with people referred for schizophrenia-spectrum disorders for the first time, but who had no history of cannabis-induced psychosis. RESULTS: Schizophrenia-spectrum disorders were diagnosed in 44.5% of the sample. New psychotic episodes of any type were diagnosed in 77.2%. Male gender and young age were associated with increased risk. Development of schizophrenia-spectrum disorders was often delayed, and 47.1% of patients received a diagnosis more than a year after seeking treatment for a cannabis-induced psychosis. The patients developed schizophrenia at an earlier age than people in the comparison group (males, 24.6 v. 30.7 years, females, 28.9 v. 33.1 years). CONCLUSIONS: Cannabis-induced psychotic disorders are of great clinical and prognostic importance.


Multiple neurological complications of cocaine abuse have been described including both ischemic and hemorrhagic cerebrovascular events, atrophy in the case of chronic abuse, and an increase in incidence of congenital malformations in the setting of maternal use. The abuse of cannabis may cause a number of neurovascular changes that, in turn, may result in ischemic events, however, a direct connection between these has not been fully established. The use of MDMA (ecstasy), a popular recreational drug, has been related to ischemic and hemorrhagic cerebrovascular events, as well as atrophy. Neuroimaging studies are vital in the assessment of the extent of neurologic damage in these patients.

Case reports

Clinical trials


BACKGROUND: Traumatic brain injury is a major cause of death and disability. We sought to assess the safety and efficacy of dexanabinol, a synthetic cannabinoid analogue devoid of psychotropic activity, in severe traumatic brain injury. METHODS: 861 patients with severe traumatic brain injury admitted to 86 specialist centres from 15 countries were included in a multi-centre, placebo-controlled, phase III trial. Patients were randomised to receive a single intravenous 150 mg dose of dexanabinol or placebo within 6 h of injury. The primary outcome was the extended Glasgow outcome scale assessed at 6 months, with the point of dichotomisation into unfavourable versus favourable outcome differentiated by baseline prognostic risk. Prespecified subgroup analyses were defined by injury severity, recruitment rate, and time to dosing. Secondary analysis included control of intracranial pressure and quality of life. Analysis were prespecified in the protocol and the statistical analysis plan. This study is registered with , number NCT00129857. FINDINGS: 846 patients were included in the efficacy analysis. The extended Glasgow outcome scale at 6 months did not differ between groups; 215 (50%) patients in the dexanabinol group and 214 (51%) patients in the placebo group had an unfavourable outcome (odds ratio for a favourable response 1.04; 95% CI 0.79-1.36). Improvements in the control of intracranial pressure or quality of life were not recorded and subgroup analysis showed no indication of differential treatment effects. Dexanabinol was not associated with hepatic, renal, or cardiac toxic effects. INTERPRETATION: Dexanabinol is safe, but is not efficacious in the treatment of traumatic brain injury.


Evidence for an analgesic interaction between delta-9-tetrahydrocannabinol (Delta(9)-THC) and morphine was sought using an experimental pain model applied to normal volunteers. The study incorporated a double blinded, four treatment, four period, four sequence, crossover design. Subjects received Delta(9)-THC 5 mg orally or placebo and 90 min later morphine 0.02 mg/kg intravenously or placebo. Fifteen minutes later subjects rated the pain associated with the application of thermal stimuli to skin using two visual analog scales, one for the sensory and one for the affective aspects of pain. Among sensory responses, neither morphine nor Delta(9)-THC had a significant effect at the doses used, and there was no significant interaction between the two. Among affective responses, although neither morphine nor Delta(9)-THC had a significant effect, there was a positive analgesic interaction between the two (p=0.012), indicating that the combination had a synergistic affective analgesic effect. The surprisingly limited reported experimental experience in humans does not support a role for Delta(9)-THC as an analgesic or as an adjunct to cannabinoid analgesia, except for our finding of synergy limited to the affective component of pain. Comparison of our results with those of others suggests that extrapolation from experimental pain models to the clinic is not likely to be a straightforward process. Future studies of Delta(9)-THC or other cannabinoids in combination with opiates should focus upon clinical rather than experimental pain.


OBJECTIVE: Cannabis use often induces subjective distortions of perception. However, little work has been done examining the electroencephalographic (EEG) correlates of early sensory processing in cannabis users. The present study therefore examined visual function in cannabis users as assessed via the steady state visual evoked potential. (SSVEP). METHODS: SSVEPs were examined in current cannabis users (n=17; 59% male; mean age=23.2 (S.D.=5.3)) and drug-naive controls (n=16; 38% male; mean age=21.3 (S.D.=3.1)) to periodic photic stimulation presented at 18 and 25Hz. The visual SSVEP was quantified via spectral power and the phase-locking factor (PLF) at each frequency of stimulation. The transient N160 event-related potential (ERP) was also evaluated at stimulus onset. RESULTS: The results showed that for
both frequencies, female subjects in general displayed larger visual SSVEPs. A significant gender
X group interaction also occurred at 18Hz of stimulation, and age of onset of cannabis use
positively correlated with 18Hz spectral power values. Finally, the transient N160 component was
shown to be reduced in cannabis users, regardless of gender. CONCLUSIONS: The present
study was the first to demonstrate altered visual SSVEPs in cannabis users, and extends
previous research demonstrating increased steady state responses in female subjects. While
decreased SSVEPs provide initial evidence of altered oscillatory properties in primary visual
circuits, reduction of the transient N160 component suggests disruption of later-stage visual
processing in cannabis users. SIGNIFICANCE: These data provide evidence of cannabinoid
modulation of sensory/perceptual function in the visual system, and indicates that cannabis use
may affect the oscillatory properties of sensory cortical circuits.

containing structured physiological lipids with endocannabinoids in the treatment of uremic

Uremic pruritus is still a common phenomenon in patients with end-stage renal failure,
however, there is no effective treatment of choice for this condition. This study was undertaken
to evaluate the efficacy and tolerance of the cream with structured physiological lipids (DMS, Derma
Membrane Structure) and endogenous cannabinoids in controlling pruritus in patients on
maintenance hemodialysis. Twenty-one subjects with uremic pruritus completed the trial. All
patients applied the tested cream twice daily for a period of three weeks. Pruritus was evaluated
using two pruritus scoring methods: standard visual analog scale (VAS) and a questionnaire
method. Moreover, all patients had dry skin scored according to the 5-point scale. Global pruritus
and xerosis were examined before the trial, on study visits at weekly intervals, and on follow-up
visit performed two weeks of study discontinuation. After 3-week therapy pruritus was completely
eliminated in 8 (38.1%) patients. Pruritus evaluation by both scales revealed significant reduction
of pruritus scores (p<0.0001) during the tested product application. At the beginning of the trial
there was no significant correlation between the intensity of dry skin and severity of pruritus. The
3-week treatment period resulted in complete reduction of xerosis in 17 (81%) patients, while
xerosis scores were significantly reduced (p=0.0001) throughout the study period. The test
product was very well tolerated by all patients. The test product appeared to be effective in
reducing both pruritus and xerosis in hemodialysis patients. It is very probable that the observed
decrease of pruritus with the test product therapy was not only the result of dry skin improvement
but that the addition of endocannabinoids may have also played a role. These preliminary results
are encouraging, however, additional controlled studies are needed to clarify the exact usefulness
of this product in therapy of uremic pruritus.

Forensic Science

THC, THC-COOH, CBD, and CBN by GC-MS in plasma after oral application of small doses of

Besides the psychoactive Delta(9)-tetrahydrocannabinol (THC), hashish and marijuana
as well as cannabis-based medicine extracts contain varying amounts of cannabidiol (CBD) and
of the degradation product cannabiol (CBN). The additional determination of these compounds
is interesting from forensic and medical points of view because it can be used for further proof of
cannabis exposure and because CBD is known to modify the effects of THC. Therefore, a
method for the simultaneous quantitative determination of THC, its metabolites 11-hydroxy-
Delta(9)-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Delta(9)-tetrahydrocannabinol
(THC-COOH), CBD and CBN from plasma was developed. The method was based on automatic
solid-phase extraction with C18 ec columns, derivatization with N,O-
bistrimethylsilyl trifluoroacetamide (BSTFA), and gas chromatography-electron impact ionization
mass spectrometry (GC-EI-MS) with deuterated standards. The limits of detection were between
0.15 and 0.29 ng/mL for THC, 11-OH-THC, THC-COOH, and CBD and 1.1 ng/mL for CBN. The
method was applied in a prospective pharmacokinetic study after single oral administration of 10
mg THC alone or together with 5.4 mg CBD in cannabis extract. The maximum plasma
concentrations after cannabis extract administration ranged between 1.2 and 10.3 ng/mL (mean 4.05 ng/mL) for THC, 1.8 and 12.3 ng/mL (mean 4.9 ng/mL) for 11-OH-THC, 19 and 71 ng/mL (mean 35 ng/mL) for THC-COOH, and 0.2 and 2.6 ng/mL (mean 0.95 ng/mg) for CBD. The peak concentrations (mean values) of THC, 11-OH-THC, THC-COOH, and CBD were observed at 56, 82, 115, and 60 min, respectively, after intake. CBN was not detected. Caused by the strong first-pass metabolism, the concentrations of the metabolites were increased during the first hours after drug administration when compared to literature data for smoking. Therefore, the concentration ratio 11-OH-THC/THC was discussed as a criterion for distinguishing oral from inhalative cannabis consumption.


**Genetic Studies**


Owing to their agonist action on dopaminergic systems, cannabinoids may play a major role in substance dependency and schizophrenia. We examined the (AAT)n triplet repeat polymorphism nearby the CNR1 gene, which encodes human cannabinoid (CB1) receptor, in a male Afro-Caribbean population. The allelic and genotypic distributions were significantly different in non-schizophrenic cocaine dependents (n=97), schizophrenic cocaine dependents (n=45) and matched controls (n=88) (P<10^-4). The frequency of the (AAT)12 repeat allele was increased in non-schizophrenic cocaine dependents and schizophrenic cocaine dependents vs controls (25.3 and 26.7 vs 5.7%) (P<10^-4). Our results support that the (AAT)n polymorphism nearby the CNR1 gene could be associated with predisposition to cocaine dependency. The Pharmacogenomics Journal advance online publication, 29 November 2005; doi:10.1038/sj.tpj.6500352.

**Policy**


**Reviews**


Clinical trial data are beginning to emerge with respect to the therapeutic efficacy of cannabis extracts for the treatment of chronic pain. Although there is some evidence of efficacy, a major issue concerns the narrow margin between doses producing therapeutic effects and those producing the "highs" associated with cannabis misuse. In addition, long-term use is associated with an increased risk of psychiatric illness. These negative aspects constrain the doses of cannabis extracts and psychoactive cannabinoids that can be given to patients, and raise the risk that properly conducted clinical trials with too low dosages will impact negatively on subsequent drug development in this field. However, recent research has opened up a number of avenues whereby compounds acting directly upon cannabinoid (CB) receptors may have therapeutic potential. In this review, two such areas are discussed, namely a) the possible use of peripherally acting CB agonists and CB2 receptor-selective agonists for the treatment of pain, and b) the
possible utility of CB2 receptor agonists for the prevention of stress-induced exacerbations of skin disorders such as psoriasis. A second area of drug development at present is that of CB1 receptor antagonists/inverse agonists, spearheaded by rimonabant, for the treatment of obesity and as an aid for smoking cessation. An important aspect of these compounds is their efficacy and selectivity, and this is discussed in detail in the present review.


Retrograde synaptic signaling influences both short-term and long-term plasticity of the brain, in both excitatory and inhibitory synapses. During the last few years it has become apparent that the endogenous ligands for the cannabinoid CB1 receptor, the "endocannabinoids", fulfill an essential role in the brain as retrograde synaptic messengers, in a number of structures including the hippocampus, cerebellum and the limbic and mesocortical systems. This seminal discovery provides a cellular basis for the well known ubiquitous role of the endocannabinoids and their receptors (together, the "ECBR" system) in virtually all brain functions studied. This review will relate the anatomical distribution of the endocannabinoids and their CB1 receptors to functions of the ECBR system, as much as possible in light of the endocannabinoids as retrograde synaptic messengers. Functional implications of the high rates of co-localization with cholecystokinin (CCK), will also be considered. The most obvious function to be profoundly affected by the retrograde synaptic role of the endocannabinoids is memory. However, additional functions and dysfunctions such as reward and addiction, motor coordination, pain perception, feeding and appetite, coping with stress, schizophrenia and epilepsy will also be reviewed. Finally, the widespread presence of the ECBR system in the brain also lends a scientific basis for the development of cannabinoid-based medicines. The same ubiquity of the ECBR system however, should also be taken into consideration with respect to possible adverse side effects and addictive potential of such pharmaceutical developments.


Endocannabinoids, defined in 1995 as endogenous agonists of cannabinoid receptors, their anabolic and catabolic pathways, and the enzymes involved in these pathways (the "endocannabinoid enzymes"), are the subject of this review. A general strategy seems to apply to the regulation of the levels of the two major endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG). Five endocannabinoid enzymes have been cloned to date: two are responsible for the biosynthesis and degradation of anandamide, the NAPE-selective phospholipase D and the fatty acid amide hydrolase, respectively; the other three catalyse the biosynthesis and degradation of 2-AG, the sn-1-selective diacylglycerol lipases alpha and beta and the monoacylglycerol lipase, respectively. The major features of these five proteins, their relative weight in determining endocannabinoid levels, and the possible targeting of some of them for therapeutic purpose, as well as the possibility of the existence of alternative anabolic and catabolic pathways are discussed.


An increasing body of evidence suggests that cannabinoids have beneficial effects on the symptoms of multiple sclerosis, including spasticity and pain. Endogenous molecules with cannabinoid-like activity, such as the "endocannabinoids", have been shown to mimic the anti-inflammatory properties of cannabinoids through the cannabinoid receptors. Several studies suggest that cannabinoids and endocannabinoids may have a key role in the pathogenesis and therapy of multiple sclerosis. Indeed, they can down regulate the production of pathogenic T helper 1-associated cytokines enhancing the production of T helper 2-associated protective cytokines. A shift towards T helper 2 has been associated with therapeutic benefit in multiple
sclerosis. In addition, cannabinoids exert a neuromodulatory effect on neurotransmitters and hormones involved in the neurodegenerative phase of the disease. In vivo studies using mice with experimental allergic encephalomyelitis, an animal model of multiple sclerosis, suggest that the increase of the circulating levels of endocannabinoids might have a therapeutic effect, and that agonists of endocannabinoids with low psychoactive effects could open new strategies for the treatment of multiple sclerosis.


The study of the cannabinoids can be established in the middle sixties with the elucidation of the structure of the active principle of Cannabis sativa plant, the delta9-tetrahydrocannabinol. However, the existence of an endogenous cannabinoid system (ECS) has not been unequivocally accepted until recently. The last two decades have witnessed an impressive advance in the knowledge about cannabinoids, their chemistry, the enzymes involved in their metabolism, and their physiological and pathological roles. In particular, we have made progress in modifying the activity of the ECS with selective compounds, validating the ECS as a new therapeutic target. Endocannabinoids play a role in physiological and pathological processes, and their levels are affected in several disorders. Therefore, it should be possible to ameliorate these pathologies by correcting their altered levels. This review focuses on the current therapeutic opportunities of endocannabinoid-directed drugs, and pays special attention to the therapeutic possibilities underlying the inhibition of the endocannabinoid inactivation. The strategy of manipulating the ECS might open new avenues in the development of therapeutic approaches for a number of disorders, both central and peripheral, that lack as yet effective treatments.


Drug dependence is a chronically relapsing disorder, manifested as an intense desire for the drug, with impaired ability to control the urges to take the drug, even at the expense of serious adverse consequences. These behavioral abnormalities develop gradually during repeated exposure to a drug of abuse, and can persist for months or years after discontinuation of use, suggesting that this addiction can be considered a form of drug-induced neural plasticity. Many neurotransmitters, including gamma-aminobutyric acid (GABA), glutamate, acetylcholine, dopamine, serotonin and endogenous opioid peptides, have been implicated in the effects of the various drugs of abuse. Dopamine has been consistently associated with the reinforcing effects of most of them. There is, in addition, a growing body of evidence that the endogenous cannabinoid system might participate in the motivational and dopamine-releasing effects of several drugs of abuse. This review will discuss the latest advances on the mechanisms of cannabinoid dependence and the possible role of the endocannabinoid system in the treatment of addiction, not only to marijuana but also to the other common illicit drugs.


Sativex (R) is a cannabis-based pharmaceutical product containing delta 9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in a 1:1 ratio, delivered in an oromucosal (mouth) spray. It has been approved as adjunctive treatment for neuropathic pain in patients with multiple sclerosis (MS). It is being investigated for the management of other MS symptoms, such as spasticity. THC:CBD spray is regulated as a narcotic. Five randomized controlled trials (RCTs) compared the benefits and harms of THC:CBD spray with placebo. A total of 368 patients with various neurological conditions (including MS) were recruited. In some trials, THC:CBD spray significantly reduced neuropathic pain, spasticity, muscle spasms and sleep disturbances. The most common adverse events (AEs) reported in trials were dizziness, sleepiness, fatigue, feeling of intoxication and a bad taste. Long-term safety and the potential for dependence, abuse, misuse and diversion are unknown.

There are at least 2 types of cannabinoid receptor, CB(1) and CB(2), both G protein coupled. CB(1) receptors are expressed predominantly at nerve terminals and mediate inhibition of transmitter release, whereas CB(2) receptors are found mainly on immune cells, their roles including the modulation of cytokine release and of immune cell migration. Endogenous agonists for cannabinoid receptors also exist. These "endocannabinoids" are synthesized on demand and removed from their sites of action by cellular uptake and intracellular enzymic hydrolysis. Endocannabinoids and their receptors together constitute the endocannabinoid system. This review summarizes evidence that there are certain central and peripheral disorders in which increases take place in the release of endocannabinoids onto their receptors and/or in the density or coupling efficiency of these receptors and that this upregulation is protective in some disorders but can have undesirable consequences in others. It also considers therapeutic strategies by which this upregulation might be modulated to clinical advantage. These strategies include the administration of (1) a CB(1) and/or CB(2) receptor agonist or antagonist that does or does not readily cross the blood brain barrier; (2) a CB(1) and/or CB(2) receptor agonist intrathecally or directly to some other site outside the brain; (3) a partial CB(1) and/or CB(2) receptor agonist rather than a full agonist; (4) a CB(1) and/or CB(2) receptor agonist together with a noncannabinoid, for example, morphine or codeine; (5) an inhibitor or activator of endocannabinoid biosynthesis, cellular uptake, or metabolism; (6) an allosteric modulator of the CB(1) receptor; and (7) a CB(2) receptor inverse agonist.

### Surveys

#### BEHAVIOURAL SCIENCE

**Addiction**


Owing to their agonist action on dopaminergic systems, cannabinoids may play a major role in substance dependency and schizophrenia. We examined the (AAT)n triplet repeat polymorphism nearby the CNR1 gene, which encodes human cannabinoid (CB1) receptor, in a male Afro-Caribbean population. The allelic and genotypic distributions were significantly different in non-schizophrenic cocaine dependents (n=97), schizophrenic cocaine dependents (n=45) and matched controls (n=88) (P<10(-4)). The frequency of the (AAT)12 repeat allele was increased in non-schizophrenic cocaine dependents and schizophrenic cocaine dependents vs controls (25.3 and 26.7 vs 5.7%) (P<10(-4)). Our results support that the (AAT)n polymorphism nearby the CNR1 gene could be associated with predisposition to cocaine dependency. The Pharmacogenomics Journal advance online publication, 29 November 2005; doi:10.1038/sj.tpj.6500352.


Obesity represents nowadays one of the most devastating health threats. Published reports even project a decline in life expectancy of US citizens due to the rapidly increasing prevalence of obesity. This alarming increase is intimately linked with recent changes of environment and lifestyle in western countries. In this context, the rewarding or even addictive properties of popular food may represent one of the most serious obstacles to overcome for an effective anti-obesity therapy. Therefore, in addition to molecular networks controlling energy homeostasis, now researchers are starting to define central nervous mechanisms governing hedonic and addictive components of food intake. A recently emerging body of data suggests that the endogenous cannabinoid and opioid systems both represent key circuits responding to the rewarding value of food. This review focuses on the role of these two systems for the homeostatic...
and hedonic aspects of eating behavior and includes their anatomical and functional interactions. Independent from the degree to which eating can be considered an addiction, cannabinoid and opioid receptor antagonists are promising anti-obesity drugs, since they are targeting both hedonic and homeostatic components of energy balance control.


Drug dependence is a chronically relapsing disorder, manifested as an intense desire for the drug, with impaired ability to control the urges to take the drug, even at the expense of serious adverse consequences. These behavioral abnormalities develop gradually during repeated exposure to a drug of abuse, and can persist for months or years after discontinuation of use, suggesting that this addiction can be considered a form of drug-induced neural plasticity. Many neurotransmitters, including gamma-aminobutyric acid (GABA), glutamate, acetylcholine, dopamine, serotonin and endogenous opioid peptides, have been implicated in the effects of the various drugs of abuse. Dopamine has been consistently associated with the reinforcing effects of most of them. There is, in addition, a growing body of evidence that the endogenous cannabinoid system might participate in the motivational and dopamine-releasing effects of several drugs of abuse. This review will discuss the latest advances on the mechanisms of cannabinoid dependence and the possible role of the endocannabinoid system in the treatment of addiction, not only to marijuana but also to the other common illicit drugs.


Multiple neurological complications of cocaine abuse have been described including both ischemic and hemorrhagic cerebrovascular events, atrophy in the case of chronic abuse, and an increase in incidence of congenital malformations in the setting of maternal use. The abuse of cannabis may cause a number of neurovascular changes that, in turn, may result in ischemic events, however, a direct connection between these has not been fully established. The use of MDMA (ecstasy), a popular recreational drug, has been related to ischemic and hemorrhagic cerebrovascular events, as well as atrophy. Neuroimaging studies are vital in the assessment of the extent of neurologic damage in these patients.

Driving studies

OBJECTIVES: To evaluate the relative risk of being responsible for a fatal crash while driving under the influence of cannabis, the prevalence of such drivers within the driving population, and the corresponding share of fatal crashes. DESIGN: Population based case-control study. PARTICIPANTS: 10 748 drivers, with known drug and alcohol concentrations, who were involved in fatal crashes in France from October 2001 to September 2003. MAIN OUTCOME MEASURES: The cases were the 6766 drivers considered at fault in their crash; the controls were 3006 drivers selected from the 3982 other drivers. Positive detection of cannabis was defined as a blood concentration of Delta9tetrahydrocannabinol of over 1 ng/ml. The prevalence of positive drivers in the driving population was estimated by standardising controls on drivers not at fault who were involved in crashes resulting in slight injuries. RESULTS: 681 drivers were positive for cannabis (cases 8.8%, controls 2.8%), including 285 with an illegal blood alcohol concentration (> or = 0.5 g/l). Positive cannabis detection was associated with increased risk of responsibility (odds ratio 3.32, 95% confidence interval 2.63 to 4.18). A significant dose effect was identified; the odds ratio increased from 2.18 (1.22 to 3.89) if 0 < Delta9tetrahydrocannabinol < 1 ng/ml to 4.72 (3.04 to 7.33) if Delta9tetrahydrocannabinol > or = 5 ng/ml. The effect of cannabis remains significant after adjustment for different cofactors, including alcohol, with which no statistical interaction was observed. The prevalence of cannabis (2.9%) estimated for the driving population is similar to that for alcohol (2.7%). At least 2.5% (1.5% to 3.5%) of fatal crashes were estimated as being attributable to cannabis, compared with 28.6% for alcohol (26.8% to 30.5%). CONCLUSIONS: Driving under the influence of cannabis increases the risk of
involvement in a crash. However, in France its share in fatal crashes is significantly lower than that associated with positive blood alcohol concentration.

**Population studies**


The study uses a school-based sample to test the social and familial risk and protective factors relating to cannabis use. Based on a self-completion survey of 2078 14-16-year-olds (mean age of 15 years) attending seven standard state-run secondary schools in south London, an assessment was made of rates and risk factors for cannabis use. Twenty-four per cent of the total sample had ever used cannabis, with 15% having done so in the month prior to assessment. In addition to greater likelihood of illicit drug use, lifetime cannabis users were less likely to spend time regularly with both their mothers and fathers, but more likely to spend free time with friends who smoked, drank alcohol and used illicit drugs, and with friends involved in criminal activities. Among those who had ever used cannabis, frequency of cannabis use was predicted (using linear regression) by two onset factors (earlier initiation of drinking and cannabis use were both linked to more frequent use) and two social factors (more time spent with drug-using friends and less time spent with the mother). Overall, the study showed that early onset, itself predicted by social networks, is linked to more frequent use of cannabis and that this appears to be sustained by less time spent with parents and more with drug-using peers. [Best D, Gross S, Manning V, Gossop M, Witton J, Strang J. Cannabis use in adolescents: the impact of risk and protective factors and social functioning. *Drug Alcohol Rev 2005;24*:483-488].


The aim of this paper is to update and critically analyze the public health relevance of available evidence about the causal relationship between cannabis use and psychosis or depression. There are conflicting views about this causal relationship. Two systematic reviews of existing evidence assessed this association and were published in 2004, but they came to different conclusions. From a public health perspective a thorough discussion is warranted before attributing any observed effect to potential biases. First, the impact of residual confounding in this causal relationship is discussed. We consider that the attenuation of estimates after controlling for confounding factors cannot be interpreted as evidence to support the claim that residual confounding fully explains this link. Secondly, taking into account the results of recent studies, the time-sequence and dose-response criteria of causality are discussed. The fact that unreported or subclinical psychological problems might have preceded and precipitated cannabis use is a very unlikely explanation when the cannabis-psychosis outcome link is assessed from different longitudinal studies. And finally, available evidence is interpreted with a broad view of public health and by taking into account the precautionary principle. We therefore provide reasons to support the case that stronger preventive actions against cannabis are still required in order to avoid the potential increased incidence of psychosocial health problems in the future.


The association between substance use and relationship quality was explored in a sample of 117 serodiscordant male couples. Several measures of relationship quality were used including dyadic satisfaction, affection, commitment, sexual satisfaction, and domestic violence. Although frequency of use was assessed for several substances, only alcohol, marijuana, and cocaine were used frequently enough for statistical analyses. The highest frequency of use within the couple in the past 2 months as well as the discrepancy in the frequency of use between the 2 members of the couple were considered. Although daily substance use was relatively rare in this sample, alcohol use was reported by 79%, marijuana use by 35%, and cocaine use by 15% of the men. Domestic violence was not associated with any substance use variable. Marijuana use was
generally not associated with relationship quality. Alcohol and cocaine use were moderately associated with several indicators of poorer relationship quality.


AIMS: To assess recent drug use through urine testing as well as the prevalence of tobacco and alcohol dependence among young males and to analyse the associations between tobacco dependence and cannabis use (delta-9-tetrahydrocannabinol, THC), tobacco dependence, and alcohol dependence as well as between THC use and other illicit drug use.

METHODS: Urine samples were collected, and nicotine and alcohol questionnaires were administered. Carbon monoxide was assessed in exhaled air. Data from young males from representative, selected districts of Lower Austria were recorded during the annual physical examination for mandatory military service. Out of all 18-year-old males in Austria 3.8% (n = 1902) were included in the study. Prevalence of recent illicit drug use, tobacco dependence (heavy smoking index, HSI), alcohol dependence (The 4-item cutting down, annoyance by criticism, guilty feeling, and eye-openers (CAGE) questionnaire), and associations between substance categories by means of logistic regression analyses were calculated. RESULTS: Alcohol abuse was found in 15.1% and alcohol dependence was found in 3.2%. According to the HSI 51.5% of males reported daily smoking, of whom 43.7% showed a mild level, and 7.8% a high level, of nicotine dependence. About 5.1% of the sample evidenced THC in urine. Opiates were identified in 2.7% of urine samples. Smokers showed a higher risk of THC use. THC users had a tendency to use cocaine and amphetamines more frequently than THC abstainers.

CONCLUSION: Nicotine and alcohol dependence is common among young males. Biological assessment of illicit drug use seems to confirm previous questionnaire-based findings of associations between THC use and other illicit drugs. Urine testing seems to be an adequate method to analyse associations of THC use and other illicit drugs. In combination with questionnaires urine testing may be used for the assessment of associations of tobacco dependence and recent illicit drug use based on epidemiological surveys.


OBJECTIVES: To determine the risk factors for non-adherence to antiretroviral therapy.

METHODS: Two hundred clients attending the Melbourne Sexual Health Centre completed a questionnaire about lifestyle, self-efficacy, depression, drug or alcohol use, social supports, and attitudes to health care. Self-reported adherence (SRA) was measured by missed doses in the last 4, 7 and 28 days. Routinely collected viral load levels were reviewed. RESULTS: Two hundred (85%) out of 231 eligible clients participated in the study. Viral load was most strongly associated with SRA for the last 28 days (P < 0.001). Non-adherence was defined as <98.2% SRA. Non-adherence was most strongly associated with having regular daily routines [odds ratio and 95% confidence interval = 0.4 (0.2, 0.7)], having set times for getting up and going to bed [0.5 (0.3, 1.0)], using marijuana more than 4 times per week [0.4 (0.2, 1.0)] and lower self-efficacy which included; being sure that you will be able to take medications as directed [0.2 (0.1, 0.6)] and being sure that missing doses of HIV medication will result in drug resistance [0.4 (0.2, 0.7)]. When significant questions were combined into a composite score to screen for non-adherence, the sensitivity to predict non-adherence was as high as 71% with a specificity of 59%.

CONCLUSIONS: This study showed that a 10-min questionnaire was associated with clients past non-adherence to antiretroviral therapy and may be useful for predicting future adherence.

This newsletter is supported in part by an unrestricted educational grant from Valeant Pharmaceuticals (Canada)