INTRODUCTION
Here is the latest summary of research abstracts.

BASIC SCIENCE

The human cannabinoid receptor 1 (CB1) belongs to the G protein-coupled receptor (GPCR) family. Among the members of GPCR family, it has an exceptionally long extracellular N-terminal domain (N-tail) of 116 amino acids but has no typical signal sequence. This poses questions of how the long N-tail affects the biosynthesis of the receptor and of how it is inserted into the endoplasmic reticulum (ER) membrane. Here we have examined the process of membrane assembly of CB1 in the ER membrane and the maturation of the receptor from the ER to the plasma membrane. We find that the long N-tail cannot be efficiently translocated across the ER membrane, causing the rapid degradation of CB1 by proteasomes; this leads to a low level of expression of the receptor at the plasma membrane. The addition of a signal peptide at the N terminus of CB1 or shortening of the long N-tail greatly enhances the stability and cell surface expression of the receptor without affecting receptor binding to a cannabinoid ligand, CP-55,940. We propose that the N-tail translocation is a crucial early step in biosynthesis of the receptor and may play a role in regulating the stability and surface expression of CB1.


Oral administration of ajulemic acid (AjA), a synthetic nonpsychoactive cannabinoid acid, prevents joint cartilage and bone damage in an experimental model of arthritis in rats. Joint tissue injury in patients with rheumatoid arthritis (RA) is due in part to activation of T lymphocytes in the synovium, and T lymphocytes in synovium of RA patients are resistant to apoptosis. Thus, a potential mechanism whereby AjA prevents joint tissue injury in the animal model might be enhanced apoptosis of T lymphocytes. Apoptosis of human T cells in vitro was assessed by Annexin V expression, caspase-3 activity, DNA fragmentation, and microscopy. AjA induced apoptosis of T cells in a dose- and time-dependent manner. Apoptosis preceded loss of cell viability by trypan blue dye exclusion, confirming that cell loss was due to programmed cell death rather than necrosis. A nontoxic compound such as AjA may be a useful therapeutic agent for patients with diseases such as RA which are characterized by T-cell-driven chronic inflammation and tissue injury.


This review article draws the attention to the many species of plants possessing activity on the central nervous system (CNS). In fact, they cover the whole spectrum of central activity such as psychoanaleptic, psycholeptic and psychodysleptic effects, and several of these plants are currently used in therapeutics to treat human ailments.Among the psychoanaleptic (stimulant) plants, those utilized by human beings to reduce body weight [Ephedra spp. (Ma Huang), Paullinia spp. (guarana), Catha edulis Forssk. (khat)] and plants used to improve general health...
conditions (plant adaptogens) were scrutinized. Many species of hallucinogenic (psychodysleptic) plants are used by humans throughout the world to achieve states of mind distortions; among those, a few have been used for therapeutic purposes, such as Cannabis sativa L., Tabernanthe iboga Baill. and the mixture of Psychotria viridis Ruiz and Pav. and Banisteriopsis caapi (Spruce ex Griseb.) C.V. Morton. Plants showing central psycholeptic activities, such as analgesic or anxiolytic actions (Passiflora incarnata L., Valeriana spp. and Piper methysticum G. Forst.), were also analysed. Finally, the use of crude or semipurified extracts of such plants instead of the active substances seemingly responsible for their therapeutic effect is discussed.


Febrile (fever-induced) seizures are the most common form of childhood seizures, affecting 3%-5% of infants and young children. Here we show that the activity-dependent, retrograde inhibition of GABA release by endogenous cannabinoids is persistently enhanced in the rat hippocampus following a single episode of experimental prolonged febrile seizures during early postnatal development. The potentiation of endocannabinoid signaling results from an increase in the number of presynaptic cannabinoid type 1 receptors associated with cholecystokinin-containing perisomatic inhibitory inputs, without an effect on the endocannabinoid-mediated inhibition of glutamate release. These results demonstrate a selective, long-term increase in the gain of endocannabinoid-mediated retrograde signaling at GABAergic synapses in a model of a human neurological disease.


The cannabinoid receptor type 1 (CB1) and its endogenous ligands, the endocannabinoids, are involved in the regulation of food intake. Here we show that the lack of CB1 in mice with a disrupted CB1 gene causes hypophagia and leanness. As compared with WT (CB1+/+) littermates, mice lacking CB1 (CB1-/-) exhibited reduced spontaneous caloric intake and, as a consequence of reduced total fat mass, decreased body weight. In young CB1-/- mice, the lean phenotype is predominantly caused by decreased caloric intake, whereas in adult CB1-/- mice, metabolic factors appear to contribute to the lean phenotype. No significant differences between genotypes were detected regarding locomotor activity, body temperature, or energy expenditure. Hypothalamic CB1 mRNA was found to be coexpressed with neuropeptides known to modulate food intake, such as corticotropin-releasing hormone (CRH), cocaine-amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), and prepro-orexin, indicating a possible role for endocannabinoid receptors within central networks governing appetite. CB1-/- mice showed significantly increased CRH mRNA levels in the paraventricular nucleus and reduced CART mRNA levels in the dorsomedial and lateral hypothalamic areas. CB1 was also detected in epidydimal mouse adipocytes, and CB1-specific activation enhanced lipogenesis in primary adipocyte cultures. Our results indicate that the cannabinoid system is an essential endogenous regulator of energy homeostasis via central orexigenic as well as peripheral lipogenic mechanisms and might therefore represent a promising target to treat diseases characterized by impaired energy balance.


In the present research we isolated and characterized Xenopus laevis CB1 cannabinoid receptor mRNA. The CB1 coding sequence shows a high degree of identity with those of other vertebrates, mammals included, confirming that CB1 receptor is conserved over the course of vertebrate evolution. Notably, the similarity between the X. laevis CB1 sequence and that of the urodele amphibian Taricha granulosa is not higher than the similarity existing between Xenopus and mammals, thus supporting phylogenetic distance between anurans and urodeles. By means of in situ hybridization histochemistry, CB1 mRNA expression and distribution was investigated in the X. laevis central nervous system. As revealed, CB1 mRNA-containing neurons are numerous in the prosencephalon, especially in the olfactory bulbs, telencephalic pallium, and hypothalamus. In the midbrain and hindbrain, labeled cells were observed in the mesencephalic tegmentum and
dorsolateral romboencephalon. Abundant CB1 mRNA positive neurons are localized throughout the gray matter of the spinal cord, in particular in the dorsal and ventral fields, where labeled motor neurons are also observed. The distribution of CB1 mRNA in the Xenopus CNS is generally consistent with the CB1-like-immunohistochemistry results we have previously obtained, showing in amphibians a well developed cannabinergic system almost comparable to that described in mammals. However, some differences, such as the abundance of CB1 mRNA-containing neurons in the olfactory system and the rich CB1 spinal innervation, are found. J. Comp. Neurol. 464:487-496, 2003.


Cannabinoids, the active components of marijuana, affect memory and hippocampal neurotransmission. It has been claimed that nabilone, a synthetic cannabinoid endowed with antiemetic properties, has a peculiar profile of actions. We studied the effects of the drug on spatial learning and in vitro hippocampal CA1 electrophysiology in the rat. Nabilone (0.1, 0.5, and 1.0 mg/kg ip) does not impair place learning in a water maze task, whereas Delta(8)-tetrahydrocannabinol (Delta(8)-THC) disrupts this function. At concentrations ranging from 1 nM to 10 microM nabilone does not influence basal glutamatergic neurotransmission, which is decreased by Delta(8)-THC. Although cannabinoids have been consistently reported to affect synaptic plasticity, nabilone 1 microM does not change paired-pulse facilitation, long-term potentiation and the magnitude of long-term depression. However, the time course of the latter phenomenon is significantly changed by the drug, the depression being lower than in control experiments from 7 to 35 min postinduction. Altogether, our data indicate that there might be differences in the effects of agonists for central cannabinoid receptors, which could help to understand the pharmacology of this class of molecules. The results also suggest that amnesia induced by cannabinoids be possibly related to their effects on hippocampal neurotransmission. The study supports the use of nabilone in conditions the course of which is complicated by cognitive impairment.


Background. Anandamide induces not only endothelium-dependent vasodilatation through cannabinoid receptors but also some endothelium-independent vasodilator effect by calcitonin gene-related peptide release through vanilloid receptors. Endothelin-1, a powerful vasoconstrictive peptide derived from endothelial cells, has been shown to be converted to its active form after cleaving by a vascular matrix metalloproteinase which is also involved in inactivation of calcitonin gene-related peptide. The purpose of this study was to investigate whether anandamide inhibits the acute vascular and morphological effects of Endothelin-1 applied intra-arterially on rabbit basilar arteries. Method. Fifteen albino rabbits were anaesthetised and underwent placement of a vertebral artery catheter for angiography of the basilar artery. Animals were divided, arbitrarily, into animals in which there was either intra-arterial injection of saline (Group I, n=5), Endothelin-1 (Group II, n=5) and Endothelin-1 and anandamide (Group III, n=5). The diameter of the basilar artery between the pre and post injection angiograms was measured in each of the three groups and transmission electron microscopic investigations on basilar arteries were performed. Findings. Angiographic studies showed that simultaneous administration of anandamide significantly attenuated Endothelin-1 induced vasoconstriction. Furthermore, it was demonstrated that anandamide reversed the morphological changes induced by Endothelin-1 on the vessel wall. Interpretation. These results indicated that anandamide overcomes the angiographic and morphological effects of intraratively administered ET-1 induced vasospasm in rabbit basilar arteries probably by induction of CGRP related vasodilatation through vanilloid receptors and prevents the acute ET-1 induced ultrastructural vessel wall damage.

Endogenous cannabinoids modulate neurotransmitter action and release in the brain. The effects are exerted on membrane permeability to Ca^{2+} and K^{+} via protein kinase A (PKA). Cannabinoid CB1 receptors are present at the synaptic terminals of cones in goldfish retina. We investigated the effects of CB1 receptor agonist WIN 55212-2 on voltage-gated currents of goldfish cones. Whole-cell currents were recorded with conventional-patch-clamp methods in goldfish retinal slices. Depolarizing pulses elicited inward I(Ca) and I(outward) that contained several components: I(K), I(A), and I(Cl). WIN 55212-2 (< 1 microM) enhanced I(K), I(Cl), and I(Ca), while at > 1 microM, I(K), I(Cl), and I(Ca) were suppressed. The voltage-activation ranges of these currents were not affected. All effects of WIN 55212-2 were blocked by the CB1 receptor antagonist SR 141716A as well as the PKA inhibitor Wiptide. The enhancing effect of WIN 55212-2 was blocked selectively by 0.5 nM cholera toxin and the suppressive effect was blocked by pertussis toxin. The results obtained from long and short single cones and double cones were basically the same. Cannabinoids, via CB1 receptor and PKA, dose-dependently enhance I(K), I(Cl), and I(Ca) by a pertussis-toxin insensitive Gs and suppress these currents by a pertussis-toxin sensitive Gi/o in cones. This biphasic regulation may provide a mechanism to inhibit constitutively active CB1 receptors in the presence of a high concentration of ligand. Thus, neuronal excitability appears to be affected by cannabinoids at the first synapse of the visual pathway and could account for some of the visual effects of marijuana.


Anandamide is an endogenous ligand for cannabinoid receptor and its protein-mediated transport across cellular membranes has been demonstrated in cells derived from brain as well as in cells of the immune system. This lipid is inactivated via intracellular degradation by a fatty acid amidohydrolase (FAAH). In the present study, we report that rabbit platelets, in contrast to human platelets, do not possess a carrier-mediated mechanism for the transport of [3H]anandamide into the cell, i.e. cellular uptake was not temperature dependent and its accumulation was not saturable. This endocannabinoid appears to enter the cell by simple diffusion. Once taken up by rabbit platelets, [3H]anandamide was rapidly metabolized into compounds which were secreted into the medium. Small amounts of free arachidonic acid as well as phospholipids were amongst the metabolic products. FAAH inhibitors did not decrease anandamide uptake, whereas these compounds inhibited anandamide metabolism. In conclusion, anandamide is rapidly taken up by rabbit platelets and metabolized mainly into water-soluble metabolites. Interestingly, the present study also suggests the absence of a transporter for anandamide in these cells.


Microglial cells, the macrophages of the brain, express low, yet detectable levels of cannabinoid CB(1) receptors, which are known to modulate cell migration. To determine if cannabinoid CB(1) receptors expressed by microglial cells modulate their migration, we assessed whether arachidonylcyclopropylamide (ACPA, an agonist shown to selectively activate CB(1) receptors) affects the migration of BV-2 cells, a mouse microglial cell line. We found that ACPA induced a dose-dependent increase in BV-2 cell migration (EC(50)=2.2 nM). This ACPA response was blocked by pertussis toxin pretreatment, suggesting the involvement of G(i/o) protein-coupled receptors. However, the cannabinoid CB(1) receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) did not prevent ACPA-induced BV-2 cell migration. Two antagonists of cannabinoid CB(2) receptors N-(1,S)-endo-1,3,3-trimethyl bicyclo(2,2,1)heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) and cannabidiol, as well as two antagonists of the newly identified "abnormal-cannabidiol-sensitive" (abn-CBD) receptors (O-1918 and cannabidiol) prevented this response. Our results suggest that cannabinoid CB(2) receptors and abn-CBD receptors, rather than cannabinoid CB(1) receptors, regulate microglial cell migration, and that ACPA is a broad cannabinoid receptor agonist.

Parkinson's disease is a chronic neurodegenerative disease of the extrapyramidal system associated with dopaminergic neuronal loss in the basal ganglia. However, several other neurotransmitters, such as serotonin, gamma-aminobutyric acid and glutamate, are also related to the symptoms of Parkinson's disease patients and their response to levodopa treatment. The co-expression of cannabinoid and dopamine receptors in the basal ganglia suggests a potential role for endocannabinoids in the control of voluntary movement in Parkinson's disease. In the present study we treated unilaterally 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine)-lesioned rats with the enantiomers of the synthetic cannabinoid 7-hydroxy-Delta6-tetrahydrocannabinol 1,1-dimethylheptyl. Treatment with its (-)-(3R, 4R) enantiomer (code-name HU-210), a potent cannabinoid receptor type 1 agonist, reduced the rotations induced by levodopa/carbidopa or apomorphine by 34% and 44%, respectively. In contrast, treatment with the (+)-(3S, 4S) enantiomer (code-name HU-211), an N-methyl-D-aspartate antagonist, as well as the psychotropically inactive cannabis constituent: cannabidiol and its primary metabolite, 7-hydroxy-cannabinol, did not show any reduction of rotational behavior. Our results indicate that activation of the CB1 stimulates the dopaminergic system ipsilaterally to the lesion, and may have implications in the treatment of Parkinson's disease.


The effects of chronic exposure to cannabinoids on short-term memory in rats were assessed during repeated daily injections of an initially debilitating dose (3.75 mg/kg) of the potent CB1 cannabinoid receptor ligand, WIN 55,212-2. Delayed nonmatch to sample (DNMS) performance was assessed over a 35-day exposure period in which performance was initially disrupted during the first 21 days of exposure but recovered by day 30 and was stable at pre-drug levels for 5 days thereafter. Withdrawal was precipitated by injections of the CB1 receptor antagonist SR141716A and transiently reduced performance for 2 days but was restabilized to pre-drug levels within 3-4 days. Concomitant recording from identified CA1 and CA3 hippocampal neurons demonstrated a marked correspondence in the time course of suppression of peak firing in the sample and delay phases of the task to the drug-induced performance deficits over the same days of exposure. Hippocampal encoding of task-relevant events and performance levels "tracked" each other on a daily basis throughout the chronic cannabinoid treatment and withdrawal regimen. However, hippocampal neuronal activity in the nonmatch phase of the task was unaffected by the chronic cannabinoid treatment or withdrawal, suggesting that only a select population of hippocampal neurons and synapses are involved in cannabinoid-sensitive short-term memory processes.


The possible localization of cannabinoid (CB) receptors to glutamatergic and GABAergic synaptic terminals impinging upon GABAergic interneurons in the CA1 region of the rat hippocampus was examined using the electrophysiological measurement of neurotransmitter release in brain slices. Whereas activation of cannabinoid receptors via the application of the cannabinoid agonist WIN55,212-2 significantly and dose-dependently reduced evoked IPSCs recorded from interneurons possessing somata located in the stratum radiatum (S.R.) and stratum oriens (S.O.) lamellae, evoked glutamatergic EPSCs were unaffected in both neuronal populations. However, in agreement with previous reports, WIN55,212-2 significantly reduced EPSCs recorded from CA1 pyramidal neurons. Additional experiments confirmed that the effects of WIN55,212-2 on IPSCs were presynaptic and that they could be blocked by the CB1 receptor antagonist SR141716A. The involvement of endogenous cannabinoids in the presynaptic inhibition of GABA release was also examined in the interneurons and pyramidal cells using a depolarization-induced suppression of inhibition (DSI) paradigm. DSI was observed in CA1 pyramidal neurons under control conditions, and its incidence was greatly increased by the
cholinergic agonist carbachol. However, DSI was not observed in the S.R. or S.O. interneuron populations, in either the presence or absence of carbachol. Whereas DSI was not present in these interneurons, the inhibitory inputs to these cells were modulated by the synthetic cannabinoid WIN55,212-2. These data support the hypothesis that cannabinoid receptors are located on inhibitory, but not excitatory, axon terminals impinging upon hippocampal interneurons, and that CA1 pyramidal neurons, and not interneurons, are capable of generating endogenous cannabinoids during prolonged states of depolarization.


Endocannabinoids, endogenous ligands of cannabinoid receptor type 1 (CB1), have emerged as novel and important regulators of energy homeostasis. A report in this issue demonstrates reduced body weight, fat mass, and appetite in CB1-/- mice. Examination of the underlying mechanisms reveals a dual role for endocannabinoids as they affect both appetite and peripheral lipolysis.


We designed AM1241, a selective CB2 cannabinoid receptor agonist, and used it to test the hypothesis that CB2 receptor activation would reverse the sensory hypersensitivity observed in neuropathic pain states. AM1241 exhibits high affinity and selectivity for CB2 receptors. It also exhibits high potency in vivo. AM1241 dose-dependently reversed tactile and thermal hypersensitivity produced by ligation of the L5 and L6 spinal nerves in rats. These effects were selectively antagonized by a CB2 but not by a CB1 receptor antagonist, suggesting that they were produced by actions of AM1241 at CB2 receptors. AM1241 was also active in blocking spinal nerve ligation-induced tactile and thermal hypersensitivity in mice lacking CB1 receptors (CB1(-/-) mice), confirming that AM1241 reverses sensory hypersensitivity independent of actions at CB1 receptors. These findings demonstrate a mechanism leading to the inhibition of pain, one that targets receptors localized exclusively outside the CNS. Further, they suggest the potential use of CB2 receptor-selective agonists for treatment of human neuropathic pain, a condition currently without consistently effective therapies. CB2 receptor-selective agonist medications are predicted to be without the CNS side effects that limit the effectiveness of currently available medications.


Spinally administered cannabinoid receptor agonists are anti-nociceptive in a variety of models of acute and persistent pain. The present study investigated the effects of activation of spinal cannabinoid CB(1) receptors on mechanically evoked responses of spinal neurones in acute and inflammatory pain states. In vivo electrophysiology studies were carried out in anaesthetised rats. Effects of spinal administration of a selective cannabinoid CB(1) receptor agonist, arachidonyl-2-chloroethylamide (ACEA), on mechanically evoked responses of dorsal horn neurones in control rats and rats with peripheral hindpaw carrageenan-induced inflammation were compared. ACEA (0.27 nM-27 microM) significantly inhibited innocuous and noxious mechanically evoked responses of dorsal horn neurones in control rats. Pre-administration of the CB(1) receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1-H-p yrazole-3-carboxyamide, SR141716A, (0.43 microM) attenuated the inhibitory effects of ACEA (27 microM). ACEA did not alter mechanically evoked responses of dorsal horn neurones in rats with hindpaw carrageenan-induced inflammation. Following peripheral inflammation, there is a loss of spinal CB(1) receptor-mediated inhibition of mechanically evoked responses, which is suggestive of a functional down-regulation of CB(1) receptors under these conditions.


Endocannabinoids are a new class of lipid mediators, which include amides, esters and ethers of long-chain polyunsaturated fatty acids. Anandamide (N-arachidonylethanolamine; AEA) and 2-arachidonoylglycerol (2-AG) are the main endogenous agonists of cannabinoid receptors able to mimic several pharmacological effects of Delta-9-tetrahydrocannabinol, the active principle of Cannabis sativa preparations like hashish and marijuana. The pathways leading to the synthesis and release of AEA and 2-AG from neuronal and non-neuronal cells are still rather uncertain. Instead, it is known that the activity of AEA is limited by cellular uptake through a specific membrane transporter, followed by intracellular degradation by a fatty acid amide hydrolase. Together with AEA and congeners these proteins form the ‘endocannabinoid system’. Here, the involvement of AEA in apoptosis and the underlying signal transduction pathways will be reviewed, along with the metabolic routes and the molecular targets of this endocannabinoid. Also, recent findings on the apoptotic potential of AEA for neuronal cell differentiation and brain development will be discussed.Cell Death and Differentiation (2003) 10, 946-955. doi:10.1038/sj.cdd.4401284


The amygdala is a temporal lobe region that is implicated in emotional information processing. The amygdala also is associated with the processing and modulation of pain sensation. Recently, we demonstrated that in nonhuman primates, the amygdala is necessary for the full expression of cannabinoid-induced antinociception [J Neurosci 21 (2001) 8238]. The antinociceptive effect of the cannabinoid receptor agonist (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo(1,2,3-de)-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55,212-2) was significantly reduced in rhesus monkeys with large bilateral lesions of the amygdaloid complex. In the present study, we investigated the contribution of the amygdala to cannabinoid-induced antinociception in the rat. Using bilateral local microinjections of the GABA(A) receptor agonist muscimol, we inactivated neurons originating from the central nucleus of the amygdala (CeA) or basolateral nucleus of the amygdala (BLA). In rats injected with intra-CeA saline, the cannabinoid receptor agonist WIN55,212-2 produced dose-dependent antinociception on the noxious heat-evoked tail flick assay. In rats treated with intra-CeA muscimol, however, the antinociceptive effect of WIN55,212-2 was significantly reduced. Rats treated with intra-BLA muscimol showed no deficit in WIN55,212-2-induced antinociception. The effect of CeA inactivation on WIN55,212-2-induced suppression of prolonged pain in the formalin test also was tested. In rats treated with intra-CeA saline, WIN55,212-2 reduced the incidence of formalin-induced nociceptive behaviors and also reduced formalin-evoked c-fos expression in both superficial and deep laminae of the spinal cord dorsal horn. In rats treated with intra-CeA muscimol, however, these effects of WIN55,212-2 were significantly reduced. The results constitute the first causal data demonstrating the necessity of descending pain-modulatory circuitry (of which the CeA is a component) for the full expression of cannabinoid-induced antinociception in the rat. Furthermore, the results complement previous findings suggesting an overlap in neural circuitry activated by opioids and cannabinoids.


Over the past decade, several putative homologs of cannabinoid receptors (CBRs) have been identified by homology screening. Homology screening utilizes sequence alignment search engines to recognize homologs. We investigated these putative CBR homologs further by ‘functional mapping’ of their deduced amino acid sequences. The entire pharmacophore of a CBR has not yet been elucidated, but point-mutation studies have identified over 20 amino acid residues that impart CBR specificity for ligand recognition and/or signal transduction. Twenty point-mutation studies were used to construct a CBR functionality matrix. Sixteen putative CBR homologs were then mapped over the matrix. Several putative homologs did not hold up to this analysis: human GPR3, GPR6, GPR12, and Caenorhabditis elegans C02H7.2 expressed a
series of crippling substitutions in the matrix, strongly suggesting they do not encode functional CBRs. Mapping the contested leech (Hirudo medicinalis) CBR sequence suggests that it encodes a functional CB1; it expresses fewer substitutions than the sea squirt (Ciona intestinalis) CB1 sequence. Mapping a putative CB2 ortholog in the puffer fish (Fugu rubripes T012234) suggests it may encode a CBR other than CB2. These findings are consistent with the lack of experimental data proving these putative CBRs have affinity for cannabinoid ligands. Matrix analysis also reveals that SR144528, a 'CB2-specific' synthetic antagonist, has affinity for non-mammalian CB1 receptors, and that L3.45 appears to be CB2-specific, its cognate in CB1 receptors is F3.45. In conclusion, functional mapping, utilizing point-mutation studies, may improve the specificity of homology screening performed by sequence alignment search engines.


The endogenous cannabinoid system is a relevant modulator of dopaminergic synapses in dorsal striatum. Perinatal exposure to cannabinoid receptor agonists has been described to affect the development of dopaminergic circuits in rat brain. The epigenetic alterations described affected both dopamine neurons and dopamine receptor-expressing neurons. The present work has been designed to explore the effects of maternal exposure to orally delivered Delta(9)-tetrahydrocannabinol, (Delta(9)-THC 0.1, 0.5, 2 mg/kg) on the behavioural responses to the dopamine receptor agonists apomorphine (0.1 mg/kg) and quinpirole (0.5 mg/kg), at doses that target presynaptic dopamine D2 receptors. Maternal exposure to Delta(9)-THC affected both the developmental pattern of motor behaviours, and the behavioural responses to acute injections of apomorphine and quinpirole, tested in an open field. The effects were sex dimorphic, being more intense in male animals. Perinatal exposure to Delta(9)-THC resulted in enhanced presynaptic dopamine D2 receptor-mediated responses such as immobility and inhibition of locomotion. Additionally, postsynaptic dopamine D2 receptor agonist-induced stereotypes were reduced in the group exposed to the highest dose of Delta(9)-THC (2 mg/kg). However, the late-onset pattern of behavioural activation observed after acute quinpirole exposure was equal in vehicle- and cannabinoid-treated animals. These effects suggest that perinatal exposure to Delta(9)-THC affects the functionality of dopaminergic autoreceptors, inducing a greater sensitivity to the presynaptic actions of dopamine D(2) receptor agonists.


Several lines of evidence indicate that the opioid and cannabinoid systems produce synergistic interactions. The present study examined the opioid receptors involved in the antitussive effect of WIN 55212-2 ((R)-(+)2,3-dihydro-5-methyl-3-[4-morpholinylmethyl]-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl)(1-naphthy) methane mesylate), a high-affinity cannabinoid receptor agonist, in mice. WIN 55212-2, at doses of 0.3-3 mg/kg ip, produced a dose-dependent antitussive effect. This antitussive effect of WIN 55212-2 was antagonized by pretreatment with either methysergide (3 mg/kg ip), a 5-HT receptor antagonist, or naloxone (1 mg/kg ip), an opioid receptor antagonist. Furthermore, pretreatment with N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-p yrazole-3-carboxamide hydrochloride (SR141716A, 3 mg/kg ip), a cannabinoid CB(1) receptor antagonist, also significantly reduced the antitussive effect of WIN 55212-2. Blockade of mu-opioid receptors by pretreatment with beta-funaltrexamine (40 mg/kg sc) significantly reduced the antitussive effect of WIN 55212-2. However, pretreatment with norbinaltorphimine (20 mg/kg sc), a kappa-opioid receptor antagonist, did not affect the antitussive effect of WIN 55212-2. Pretreatment with naloxonazine (35 mg/kg sc), a mu(1)-opioid receptor antagonist, also did not affect the antitussive effect of WIN 55212-2. These results indicate that the antitussive effect of WIN 55212-2 is mediated by the activation of cannabinoid CB(1) receptors and mu(2) (naloxonazine-insensitive)-opioid receptors, but not mu(1) (naloxonazine-sensitive)- or kappa-opioid receptors.

Sleep is an unavoidable activity of the brain. The delay of the time to sleep (sleep deprivation), induces an increase of slow-wave sleep and rapid-eye-movement (REM) sleep (rebound) once the subject is allowed to sleep. This drive to sleep has been hypothesized to be dependent on the accumulation of sleep-inducing molecules and on the high expression of these molecule receptors. In this study we selectively deprived rats of REM sleep for 24 h by using the flowerpot technique. One group deprived of REM sleep was treated with SR141716A, a cannabinoid receptor 1 (CB1) receptor antagonist and then allowed to sleep for the next 4 h. Two other groups were killed, one immediately after the REM sleep deprivation period and the other after 2 h of REM sleep rebound (REM sleep deprivation plus 2 h of rebound). In both groups we determined the expression of the CB1 receptor and its mRNA. Results indicated that SR141716A prevents REM sleep rebound and REM sleep deprivation does not modify the expression of the CB1 protein or mRNA. However, REM sleep deprivation plus 2 h of sleep rebound increased the CB1 receptor protein and, slightly but significantly, decreased mRNA expression. These results suggest that endocannabinoids may be participating in the expression of REM sleep rebound.


Activation of postsynaptic group 1 metabotropic glutamate receptors (mGluRs) by the agonist DHPG causes a long-term depression (DHPG-LTD) of excitatory transmission in the CA1 region of the hippocampus, as well as causing the release of endocannabinoids from pyramidal cells. As cannabinoid agonists cause a presynaptic inhibition at these synapses and DHPG-LTD is thought to be expressed, at least in part, by a presynaptic mechanism, we examined the possibility that endocannabinoids mediated DHPG-LTD. We find that antagonists of cannabinoid receptors reduce the acute depression induced by DHPG, but have no effect on the lasting depression. Furthermore, both the acute and the lasting effects of DHPG were unaffected in the CB1 knockout mouse. These findings suggest that endocannabinoids, acting on a non-CB1 cannabinoid receptor, contribute to the acute depression but not to DHPG-LTD. Presumably some other retrograde signalling mechanism is responsible for DHPG-LTD.


There is evidence from studies in humans and animals that a vulnerable period for chronic cannabinoid administration exists during certain phases of development. The present study tested the hypothesis that long-lasting interference of cannabinoids with the developing endogenous cannabinoid system during puberty causes persistent behavioral alterations in adult rats. Chronic treatment with the synthetic cannabinoid agonist WIN 55,212-2 (WIN) (1.2 mg/kg) or vehicle was extended over 25 days either throughout the rats’ puberty or for a similar time period in adult rats. The rats received 20 injections intraperitoneally (i.p.), which were not delivered regularly. Adult rats were tested for object recognition memory, performance in a progressive ratio (PR) operant behavior task, locomotor activity, and prepulse inhibition (PPI) of the acoustic startle response (ASR). PPI was significantly disrupted only by chronic peripubertal cannabinoid treatment. This long-lasting PPI deficit was reversed by the acute administration of the dopamine antagonist haloperidol. Furthermore, we found deficits in recognition memory of pubertal-treated rats and these animals showed lower break points in a PR schedule, whereas food preference and locomotion were not affected. Adult chronic cannabinoid treatment had no effect on the behaviors tested. Therefore, we conclude that puberty in rats is a vulnerable period with respect to the adverse effects of cannabinoid treatment. Since PPI deficits, object recognition memory impairments, and anhedonia/avolition are among the endophenotypes of schizophrenia, we propose chronic cannabinoid administration during pubertal development as an animal model for some aspects of the etiology of schizophrenia.*Neuropsychopharmacology* advance online publication, 23 July 2003; doi:10.1038/sj.npp.1300225

There is a consensus that NMDA receptors (NMDARs) detect coincident pre- and postsynaptic activity during induction of long-term potentiation (LTP), but their role in timing-dependent long-term depression (tLTD) is unclear. We examine tLTD in neocortical layer 5 (L5) pyramidal pairs and find that tLTD is expressed presynaptically, implying retrograde signaling. CB1 agonists produce depression that mimics and occludes tLTD. This agonist-induced LTD requires presynaptic activity and NMDAR activation, but not postsynaptic Ca(2+) influx. Further experiments demonstrate the existence of presynaptic NMDARs that underlie the presynaptic activity dependence. Finally, manipulating cannabinoid breakdown alters the temporal window for tLTD. In conclusion, tLTD requires simultaneous activation of presynaptic NMDA and CB1 receptors. This novel form of coincidence detection may explain the temporal window of tLTD and may also impart synapse specificity to cannabinoid retrograde signaling.


The present study evaluated the potential mechanism involved in the hypotensive effect induced by ET-1 in rats treated with the NO synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) in the drinking water during 7 days. Hypertension developed in the L-NAME-treated rats (164±3 versus 112±1 mm Hg in untreated control rats), and the hypotensive effect of ET-1 (100 pmol/kg IV) was significantly enhanced compared with control rats (32±2% versus 20±1% fall in mean arterial pressure). The enhanced ET-1 hypotensive effect in L-NAME-treated rats was abolished by the ETB receptor antagonist BQ-788 but was unaltered by the cyclooxygenase inhibitor diclofenac, the cytochrome P450 inhibitor fluconazole, or the potassium channel blockers apamin, glibenclamide, tetraethylammonium, and 4-aminopyridine. Pretreatment with the cannabinoid CB1 receptor antagonist SR141716A significantly reduced the hypotensive response to ET-1 in L-NAME-treated rats (20±1%), although it did not modify the response in untreated control rats (17±1%). These findings indicate that in rats under chronic NOS inhibition, the hypotensive effect of ET-1 is unexpectedly enhanced and appears to be mediated by a non-NO/non-prostanoid mechanism and involves an SR141716A-sensitive mechanism triggered by ETB receptor activation.


Cannabinoids are known to inhibit neurotransmitter release in the CNS through CB(1) receptors. The present study compares the effects of synthetic cannabinoids on acetylcholine (ACh) release in human and mice neocortex. We further investigated a possible endocannabinoid tone on CB(1) receptors in human neocortex caused by endogenous agonists like anandamide or 2-arachidonoylglycerol. Brain slices, incubated with [3H]-choline, were superfused and stimulated electrically under autoinhibition-free conditions to evoke tritium overflow assumed to represent ACh release. The first series of experiments was performed with 26 pulses, 60 mA, at 0.1 Hz. In mice neocortical slices, the cannabinoid receptor agonist WIN55212-2 decreased ACh release (pIC50=6.68, l(max)=67%). In the human neocortex the concentration-response curve of WIN55212-2 was bell-shaped and flat (l(max observed) approximately 30%). The estimated maximum possible inhibition, however, was much larger: l(max derived)=79%. Lec, the negative logarithm (lg) of the biophase concentration of endocannabinoids in 'WIN55212-2 units,' was -6.52, the pK(d) of WIN55212-2 was 7.47. The CB(1) receptor antagonist/inverse agonist SR141716A enhanced ACh release in the human neocortex (by 38%) and prevented the inhibitory effect of WIN55212-2. The concentration-response curve of WIN55212-2 was changed in its shape including a shift to the right due to the presence of SR141716A. A pA2(2) of this antagonist between 11.60 and 11.18 was obtained. SR141716 alone had no effect in mice neocortical slices. A partial agonist without inverse agonistic activity, O-1184, enhanced ACh release in the human neocortex. The endocannabinoid uptake-inhibitor AM404 decreased ACh release in human, but not in mice, neocortical slices. Change of the stimulation parameters (eight trains of pseudo-one-pulse bursts (4 pulses, 76 mA, 100 Hz), spaced by 45 s intervals) led to a stronger inhibitory effect of WIN55212-2, and abolished the disinhibitory effect of SR141716 and O-1184. The results show that activation of CB(1) cannabinoid receptors leads to inhibition of ACh release in the
human and mouse neocortex. The endocannabinoid tone is high in the human, but not in the mouse neocortex and is dependent on neuronal activity. SR141716 acts as a competitive CB(1) receptor antagonist.


Recent evidence obtained from in vitro experiments indicates that cannabinoid receptor type 1 (CB1) modulates neurotransmission in the gastrointestinal tract of rodents. Standard intracellular recording techniques were applied to study the effects of cannabinoid receptor agonists and antagonists on smooth muscle resting membrane potentials and on membrane potentials following electrical neuronal stimulation in a myenteric neuron/smooth muscle preparation of wild-type and CB1-deficient mice in vitro. Double staining for CB1 and nitric oxide synthase (nNOS) was performed to identify the myenteric CB1-expressing neurons. Focal electrical stimulation of the myenteric plexus induced a fast excitatory junction potential (EJP; abolished by atropine, an inhibitor of cholinergic transmission) followed by a fast inhibitory junction potential (fIJP; reduced by apamin, a K(+) channel blocker) and a slow inhibitory junction potential (sIJP; abolished by L-N(G)-nitroarginine, a NO synthase inhibitor). Treatment of wild-type mice with the endogenous CB1 receptor agonist anandamide significantly reduced EJP, while not affecting fIJP and sIJP. EJP was significantly higher in CB1-deficient mice than in wild-type littermate controls, and anandamide induced no effects in CB1-deficient mice. WIN 55,212-2, a synthetic CB1 receptor agonist, nearly abolished EJP and significantly reduced the fIJP in wild-type mice, while not affecting sIJP. SR141716A, a CB1 specific receptor antagonist, was able to reverse all the effects induced in wild-type mice by anandamide and WIN 55,212-2. SR141716A, when given alone, significantly increased EJP in wild-type mice without affecting fIJP in wild-type and EJP in CB1-deficient mice. Interestingly, SR141716A reduced fIJP in CB1-deficient mice but not in wild-type mice. In the mouse colon, nitricergic myenteric neurons do not express CB1, implying that CB1 is expressed in cholinergic neurons. Excitatory and inhibitory neurotransmission in the mouse colon is modulated by activation of CB1 receptors. The significant increase in EJP in CB1-deficient mice strongly suggests a physiological involvement of CB1 in excitatory cholinergic neurotransmission. These electrophysiological findings suggesting the involvement of cholinergic neurons are in line with the immunocytochemical data indicating that CB1 receptors are expressed in non-nitricergic myenteric neurons. Finally, our study supports the notion that CB1 is the only receptor involved in the action of the endogenous agonist anandamide on the mouse proximal colon, since anandamide had no effect on excitatory or inhibitory neurotransmission in CB1-deficient mice.


We designed and synthesized a series of pyrrole derivatives with the aim of investigating the structure-activity relationship (SAR) for the binding of non-classical agonists to CB(1) and CB(2) cannabinoid receptors. Superposition of two pyrrole-containing cannabinoid agonists, JWH-007 and JWH-161, allowed us to identify positions 1, 3 and 4 of the pyrrole nucleus as amenable to additional investigation. We prepared the 1-alkyl-2,5-dimethyl-3,4-substituted pyroles 10a-e, 11a-d, 17, 21, 25 and the tetrahydroindole 15, and evaluated their ability to bind to and activate cannabinoid receptors. Noteworthy in this set of compounds are the 4-bromopyrrole 11a, which has an affinity for CB(1) and CB(2) receptors comparable to that of well-characterized heterocyclic cannabimimetics such as Win-55,212-2; the amide 25, which, although possessing a moderate affinity for cannabinoid receptors, demonstrates that the 3-naphthoyl group, commonly present in indole and pyrrole cannabimimetics, can be substituted by alternative moieties; and compounds 10d, 11d, showing CB(1) partial agonist properties.


The repeated administration of Delta(9)-tetrahydrocannabinol (THC) results in tolerance to many of its behavioral and physiological effects. It also produces changes in the functionality of cannabinoid receptors. What is not completely understood is how these cellular events translate...
into the behavioral and physiological changes that are associated with repeated cannabinoid agonist treatment. The purpose of these studies was to determine the development of changes in the patterns of functional activity, as measured by the 2-\(\{14C\}\)deoxyglucose method (2DG), associated with repeated THC exposure. Male Sprague-Dawley rats (n=4-5) were administered THC (vehicle or 10 mg/kg, intraperitoneally), daily for 7 or 21 days. Fifteen minutes following the final THC treatment the 2DG procedure was initiated. In separate sets of rats similarly treated with THC, locomotor activity and core body temperature were measured at corresponding time points in order to establish the behavioral profile of repeated THC administration. The acute administration of THC following 7 or 21 days of drug exposure resulted in a significant attenuation of changes in rates of glucose utilization throughout the majority of brain regions analyzed when compared to the large global decreases observed following a single administration of THC. After 7 and 21 days of treatment, cerebral metabolic rates were no longer different from vehicle-treated controls in most cortical, thalamic and basal ganglia regions. This attenuation closely paralleled the development of tolerance to the effects of THC on locomotor activity and core body temperature. However, glucose utilization remained altered in the nucleus accumbens, mediodorsal thalamus, basolateral amygdala, portions of the hippocampus and median raphe. These data suggest that the development of tolerance to the cerebral metabolic effects of THC is regionally specific and temporally distinct. The persistence of effects in limbic areas as well as portions of the hippocampal complex, however, suggests that processes such as stress, reward, and aspects of memory mediated by these brain regions may continue to be affected by THC even after prolonged THC exposure.

**CLINICAL SCIENCE**


The possible medicinal use of cannabinoids for chronic diseases emphasizes the need to understand the long-term effects of these compounds on the central nervous system. We provide a quantitative synthesis of empirical research pertaining to the non-acute (residual) effects of cannabis on the neurocognitive performance of adult human subjects. Out of 1,014 studies retrieved using a thorough search strategy, only 11 studies met essential a priori inclusion criteria, providing data for a total of 623 cannabis users and 409 non- or minimal users. Neuropsychological results were grouped into 8 ability domains, and effect sizes were calculated by domain for each study individually, and combined for the full set of studies. Using slightly liberalized criteria, an additional four studies were included in a second analysis, bringing the total number of subjects to 1,188 (i.e., 704 cannabis users and 484 non-users). With the exception of both the learning and forgetting domains, effect size confidence intervals for the remaining 6 domains included zero, suggesting a lack of effect. Few studies on the non-acute neurocognitive effects of cannabis meet current research standards; nevertheless, our results indicate that there might be decrements in the ability to learn and remember new information in chronic users, whereas other cognitive abilities are unaffected. However, from a neurocognitive standpoint, the small magnitude of these effect sizes suggests that if cannabis compounds are found to have therapeutic value, they may have an acceptable margin of safety under the more limited conditions of exposure that would likely obtain in a medical setting. (JINS, 2003, 9, 679-689.)

Cannabis is by far the illegal substance the most widely used by youth aged 12-25 years. One out of five persons living in France has already tried it once in his lifetime. Although the psychiatric symptoms as well as the cognitive and acute or chronic behavioral effects linked to repeated use of cannabis are well documented in the literature, search for persistent cognitive effects amongst chronic users has not provided convincing evidence because of methodological biases. The issue is nevertheless most crucial, especially amongst teenagers, due to the potential risks of deteriorated academic, social and occupational performance. The longer the exposure to the effects of the substance, the greater the risk of complications. Based on data reported in the literature, we report the cognitive effects associated with chronic use of cannabis as well as its social and educational consequences.


In the last few years, the use of cannabinoids has been advocated for several indications, and evaluation of the side effect profile is necessary. Euphoric mood changes are among the most frequent side effects, while dysphoric reactions are less frequent. Triggering of acute psychotic episodes has been reported. Cannabinoids can initiate or exacerbate schizophrenic psychosis in predisposed persons. Cannabinoids impede cognitive and psychomotor performance, resulting in impaired driving ability. Chronic use can lead to the development of tolerance. Tachycardia and hypotension frequently are documented as adverse events in the cardiovascular system. A few cases of myocardial ischemia have been reported in young and previously healthy patients. Side effects on the respiratory system are induced by inhaling the smoke of cannabis cigarettes. Some reports have indicated a carcinogenic risk for the children when cannabis was used during pregnancy. In summary, a low risk profile is evident from the literature available. Lifethreatening complications are very rare and were not reported after use of cannabinoids for medical indications. Cannabinoids are contraindicated during pregnancy or for patients with a history of cardiac ischemias.


Cannabis and marihuana are psychoactive substances. Beside alcohol they belong to the most widely consumed drugs in most western countries. Psychiatric complications are frequent: substance use, dependence, possibly withdrawal, intoxications and cognitive dysfunction. There is substantial evidence for an increased risk for psychosis in chronic cannabis consumers. Recent data also indicate a high comorbidity with affective disorder. Chronic pain is only rarely mentioned as reasons for cannabis use, psychological or social reasons prevail. Psychiatric complications in cannabis consumers and its relevance for mental health are discussed.


**BEHAVIOURAL SCIENCE**


AIMS: To study the relationship between sporting activity and alcohol, cigarette and cannabis use among adolescents and young adults, by focusing on elite student athletes (ESAs). DESIGN, SETTING, SUBJECTS: Cross-sectional survey (Spring 2002), in a sample of 460 ESAs (ages 16-24 years) recruited at 40 public centres gathering the young sporting elite from 30 different sports in South-Eastern France, comparison with samples of the general population of adolescents in South-Eastern France. MEASURES: Respondents were asked confidentially by a self-administered questionnaire about their use of licit and illicit drugs, their sporting activity and other aspects of their life-style. FINDINGS: Prevalences of cigarette, alcohol and cannabis use were markedly lower for ESAs than for other adolescents (generally twice or three times as low).
Among ESAs, when compared with the practice of an individual sport, the practice of a team sport was correlated positively with alcohol use (OR = 2.7 for girls, OR = 1.8 for boys), and the practice of a sliding sport was correlated positively with cannabis use (for girls: OR = 2.3) and with alcohol use (for boys: 4.3). Girls who entered competition at international level were more prone to smoke cigarettes and cannabis (OR = 6.1 and 2.4, respectively). CONCLUSIONS: As a whole, practising sports as an elite student-athlete can be considered as correlated negatively with cigarette, alcohol and cannabis use. Nevertheless, this relationship depends on the kind of sport practised as well as the level of competition, and further research is needed to understand specific elite athletes' motives for use.


Parents and their teenage children were questioned about medical marijuana and whether they believed that passage of medical marijuana laws in their states would increase teenage use of marijuana for non-medical purposes. A 24-question written survey was distributed separately to teenager/parent pairs who visited 1 of 2 suburban general pediatric offices located in Vienna, Virginia or Mason, Ohio. Completed surveys were collected from 393 parent-teen pairs. Only 13% of the teenagers admitted to ever smoking marijuana while 6% admitted smoking it in the past 30 days. There was good agreement between parents and teens (81% of parents and 76% of their teenagers who responded to the survey) that regular use of marijuana causes harm to many or most users, not just "potheads." Although there was close agreement in the range of opinions about medical marijuana (i.e., from liberal use to no use) by parents as a group and the teens as a group, agreement between the answers of parents matched with their own children was poor based on K-coefficient analysis (K = 0.20). Twenty-eight percent of the parent group and 55% of the teenagers believed that passage of state laws for medical marijuana would make it easier for teens to smoke marijuana for medical purposes.


This study examined relations between perceived conflict/utility of marijuana use in achieving valued personal goals and marijuana use initiation, marijuana use frequency, and marijuana-related problems. Personal strivings are higher order goals that may influence marijuana use to the extent that they are congruent or incongruent with use. Participants were 592 young adults who generated lists of personal strivings independent of the substance use assessment. They then evaluated their 10 most important strivings with regard to the perceived conflict/utility of several levels of marijuana use in achieving their most important strivings. Less marijuana use-striving conflict was positively associated with use initiation and frequency. A significant gender interaction emerged in the prediction of use frequency; marijuana use-striving conflict was more strongly associated with use frequency for men than women. The relationship between use-striving conflict and marijuana-related problems was mediated fully by use frequency.


This study examined how aggregate levels of news coverage about marijuana have impacted adolescents' marijuana behavior generally, and through the intervening variables of personal disapproval and perceived harmfulness of marijuana, two variables that existing research has identified as significant predictors of adolescent marijuana use at the aggregate level. It was hypothesized that news coverage of reasons why people should not use marijuana would cause increase in aggregate marijuana abstinence, perceived harmfulness, and personal disapproval. Conversely, news coverage of positive aspects of marijuana use would cause decreases in marijuana abstinence, perceived harmfulness, and personal disapproval. Results of distributed lagged time-series regression and non-linear modeling offered support for two of the three proposed hypotheses. Aggregate media coverage explained a significant portion of the variation in adolescents' abstinence from marijuana use over time. It also explained a significant
portion of the variation in personal disapproval of marijuana. Personal disapproval was found to partially mediate the relationship between media coverage and marijuana abstinence. Implications for the conceptualization of media effects on health behaviors are discussed.

This newsletter is supported in part by unrestricted educational grants from GW Pharmaceuticals and ICN Pharmaceuticals (Canada)