Neurotoxicity Research, 2004, VOL. 6(3). pp. 001-012

F.P. Graham Publishing Co.

Role of Endocannabinoid System in Mental Diseases

JORGE MANZANARES*, LEYRE URIGÜEN, GABRIEL RUBIO and TOMÁS PALOMO

Servicio de Psiquiatría y Unidad de Investigación, Hospital 12 de Octubre, Edificio Materno-Infantil, Planta 6a, 613-A, Avenida de Córdoba s/n, 28041 Madrid, Spain. jorgemr@h12o.es

(Received 25 February 2004; Revised 23 March 2004; In final form 23 March 2004)

In the last decade, a large number of studies using Δ⁹-tetrahydrocannabinol (THC), the main active principle derivative of the marijuana plant, or cannabinoid synthetic derivatives have substantially contributed to advance in the understanding of the pharmacology and neurobiological mechanisms produced by cannabinoid receptor activation.

Cannabis has been historically used to relieve some of the symptoms associated with central nervous system disorders. Nowadays, there are anecdotal evidences for the use of cannabis in many patients suffering from multiple sclerosis or chronic pain. Following the historical reports on the use of cannabis for medicinal purposes, recent research has highlighted the potential of cannabinoids to treat a wide variety of clinical disorders. Some of these disorders that are being investigating are pain, motor dysfunctions, or psychiatric illness. On the other hand, cannabis abuse has been related to several psychiatric disorders such as dependence, anxiety, depression, cognitive impairment, and psychosis.

Considering that cannabis or cannabinoid pharmaceutical preparations may no longer be exclusively recreational drugs but may also present potential therapeutic uses, it has become of great interest to analyze the neurobiological and behavioral consequences of their administration.

This review attempts to link current understanding of the basic neurobiology of the endocannabinoid system to novel opportunities for therapeutic intervention and its effects on the central nervous system.

Keywords: Δ⁹-Tetrahydrocannabinol Endocannabinoid; Neuropsychiatry; Neurology; Brain; Marijuana; Cannabinoid receptor; CB_1 receptor; CB_2 receptor;

INTRODUCTION

In the last decade, a large number of studies using Δ⁹-tetrahydrocannabinol (THC), the main active principle derivative of the marijuana plant, or cannabinoid synthetic derivatives have substantially contributed to advance in the understanding of the pharmacology and neurobiological mechanisms produced by cannabinoid receptor activation. This significant advance in cannabinoid pharmacology has identified an endogenous cannabinoid neuronal system and the necessary tools (selective cannabinoid receptor agonists and antagonists) to characterize a number of physiological functions induced by activation of their cannabinoid receptors CB_1 and CB_2. This effort has been driven in part by the increasing interest of medical societies from European and North American countries to consider cannabinoid pharmaceutical preparations as potential therapeutic drugs in a variety of clinical conditions including pain (post-surgical pain, arthritis, cancer) multiple sclerosis, movement disorders, psychiatric diseases and obesity among others. Considering that cannabis or cannabinoid pharmaceutical preparations may no longer be exclusively recreational drugs but may also present potential therapeutic uses, it has become of great interest to analyze the neurobiological and behavioral consequences of their administration.

This review attempts to link current understanding of

*Corresponding author. Tel.: 34-91-390 8252; Fax: 34-91-390 8538; E-mail: jorgemr@h12o.es
ISSN 1029 8428 print/ ISSN 1476-3524 online.

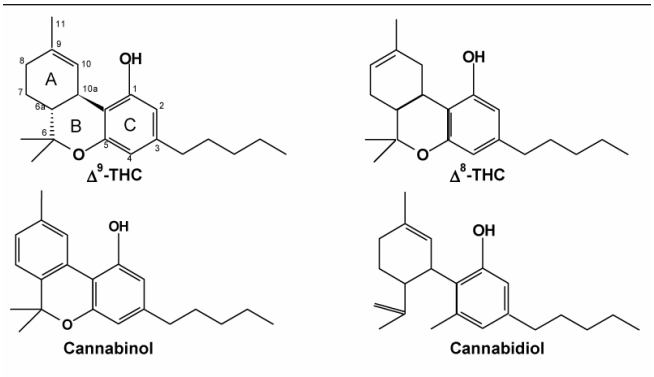

FIGURE 1 THC and other natural cannabinoids.

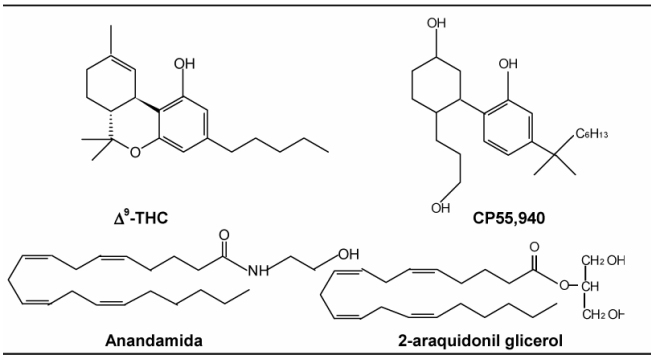

FIGURE 2 Structural comparison between exogenous and endogenous cannabinoids.

the basic neurobiology of the endocannabinoid system to novel opportunities for therapeutic intervention and its effects on the central nervous system (CNS).

HISTORICAL BACKGROUND

The first formal report of cannabis as a medicine appeared in China nearly 5000 years ago when it was recommended for malaria, constipation, rheumatic pains and childbirth and, mixed with wine as a surgical analgesic (Mechoulam, 1986). There are subsequent records of its use throughout Asia, the Middle East, Southern Africa and South America.

It was not until the 19th century that cannabis became a mainstream medicine in Britain. W.B. O´Shaughnessy, an Irish scientist and physician, observed its use in India as an analgesic, anticonvulsant, antispasmodic, anti-emetic and hypnotic. After toxicity experiments on goats and dogs, he gave it to patients and was impressed with its muscle relaxant, anticonvulsant and analgesic properties, and recorded its usefulness as an anti-emetic (Robson, 2001). All these observations were published in 1842 and medicinal use of cannabis expanded rapidly. In 1890, the personal physician of Queen Victoria wrote about the properties of cannabis, on the basis of more than 30 years of experience: "Indian hemp, when pure and administered carefully, is one of the most valuable medicines we possess". He observed its usefulness in the treatment of "senile insomnia" and "night restlessness" but not in the treatment of melancholia, alcoholic delirium or in mania. It was very effective in neuralgia, period pains, asthma and migraine. After the zenith of cannabis as prescribed medicine, in 1928 it was outlawed by ratification of the 1925 Geneva Convention on the manufacture, sale and movement of dangerous drugs. In the United States, medical use was definitively ruled out by the Marijuana Tax Act of 1937.

The State of California permits cultivation and consumption of cannabis for medical purposes, if a doctor provides a written endorsement. Similar law exists in Italy and Australia.

THE ENDOCANNABINOID SYSTEM

Exogenous Cannabinoids

The principal active component of the plant *Cannabis sativa*, Δ^9-tetrahydrocannabinol (THC) (Mechoulam, 1970), is present in an oily resin in the leaves and flowers of the plant. Because of the hydrophobic properties of the drug, smoking remains the most efficient means of delivering the drug and experienced users can control the dose by adjusting the depth and frequency of inhalation (Iversen, 2000). In the last few years a wide number of laboratories have developed synthetic cannabinoids, some of which are more potent than those present in the plant. All of these compounds act as agonists at the CB_1 cannabinoid receptor (Matsuda *et al.*, 1990) (FIG. 1).

The CB_1 receptor is widely distributed in the brain and the spinal cord, and is the only known subtype found to date in the central nervous system. A second cannabinoid receptor (CB_2) has been identified and is present in peripheral tissues, mainly in the immune system (Munro *et al.*, 1993; Felder and Glass, 1998; Pertwee, 1999). Both CB_1 and CB_2 receptors are members of the G-protein coupled receptors family and their activation leads to inhibition of adenylate cyclase (AC) activity (Howlett *et al.*, 1988). CB_1 is predominantly expressed presynaptically, and its primary action is to decrease the release of neurotransmitters including dopamine, norepinephrine, glutamate and serotonin (Ishac *et al.*, 1996; Shen *et al.*, 1996; Kathman *et al.*, 1999; Szabo *et al.*, 1999; Nakazi *et al.*, 2000).

Endogenous Cannabinoids

The discovery of specific cannabinoid receptors led to the search of naturally occurring ligands of these receptors in mammalian tissues. In the early 1990s the first endogenous cannabinoid, the *N*-arachidonylethanolamine, derived of arachidonic acid, named *anandamide* was isolated (Devane *et al.*, 1992). Other endogenous cannabinoids have been identified: 2-arachidonylglycerol (2-AG) (Mechoulam *et al.*, 1995) and 2-arachidonylgliceryl ether (Hanus *et al.*, 2001). These endogenous cannabinoids known as "endocannabinoids" are present only in small amounts in the brain or other tissues. Like other lipid mediators they appear to be synthesized and released locally on demand. The low concentrations of anandamide in serum, plasma and CSF (Felder *et al.*, 1996) and the short duration and magnitude of its effects suggest that this compound is inactivated rapidly at the site of action. Indeed, it has now been shown that anandamide is inactivated by a two-step mechanism. First, a high affinity specific transporter transports it across the plasma membrane (Di Marzo *et al.*, 1994). Re-uptake of endocannabinoids has been shown in both rat neurons and astrocytes (Beltramo *et al.*, 1997). In addition, peripheral mechanisms of anandamide re-uptake also exist in macrophages and human endothelial cells (Bisogno *et al.*, 1997; Maccarrone *et al.*, 2000).

Following the transportation of anandamide across the plasma membrane, it is rapidly metabolized to arachidonic acid and ethanolamine by a specific enzyme fatty acid amido-hydrolase, FAAH (Cravatt *et al.*, 1996; Deutsch *et al.*, 2001). FAAH has been identified in both neurons and astrocytes in the CNS (Beltramo *et al.*, 1997; Egertova *et al.*, 1998) and furthermore, mice lacking FAAH exhibit intense behavioral effects after administration of anandamide, such as hypomotility and analgesia (Cravatt *et al.*, 2001). This provides further evidence of a specific endogenous system for release, action and inactivation of endocannabinoids (FIG. 2).

Distribution of CB_1 Receptors in the Brain

The mapping studies in rat brain showed that CB_1 receptors are mainly localized in axons and nerve terminals. The finding that cannabinoid receptors are predominantly presynaptic rather than postsynaptic is consistent with the postulated role of cannabinoids in modulating neurotransmitter release. Frontal regions of cerebral cortex contain high densities of CB_1 receptors in both animals and humans. There are also high densities in the basal ganglia and cerebellum. In the limbic forebrain CB_1 receptors are found particularly abundant in the hypothalamus and in the anterior cingulate cortex. The hippocampus also contains a high density of CB_1 (Herkenham, 1991) receptors (Table I). The relative absence of cannabinoid receptors from brainstem nuclei may account for the low toxicity of cannabinoids when given in overdose (Iversen, 2003). The regional distribution of the CB_1 receptor in brain correlates only poorly with the levels of anandamide and other endocannabinoids in different brain regions (Felder *et al.*, 1996; Bisogno *et al.*, 1999). However, there is a better correlation between the regional distribution of CB_1 receptors and the enzyme FAAH. This enzyme is widely distributed in the CNS and other tissues suggesting that its role is not limited to inactivation of endogenous cannabinoids, although particularly high levels of the enzyme were found in brain regions enriched in CB_1 receptors. The close and complementary relationship between CB_1 receptors and FAAH led to the hypothesis that FAAH may participate in the inactivation of endogenous cannabinoids released

TABLE I Distribution and density of cannabinoid CB_1 receptor in rat brain revealed by autoradiography.

BRAIN SECTION	CB_1 RECEPTOR DENSITY
Cerebellum	+ + + + +
Deep cerebellar nucleus	-
Corpus callosum	-
Entopeduncular nucleus	+ + + +
Fimbria hippocampus	+
Frontal cortex	+ +
Frontoparietal cortex motor area	+ + +
Globus pallidus	+ + + +
Hippocampus	+ + + +
Inferior colliculus	-
Lateral posterior thalamus	+
Medial amygdaloid nucleus	+ +
Primary olfactory cortex	+ + +
Parvocellular reticular nucleus	+
Substantia nigra reticulate	+ + + + +
Olfactory tubercle	+ + +
Ventroposterior thalamus	+ +

locally at synapses (Elphick and Egertová, 2001). The presence of cannabinoid receptors in important brain structures and the inhibitory effects of cannabinoids on neuropeptide secretion (Howlett *et al.*, 2002) suggest that cannabinoids may have potential as therapeutic agents in a wide variety of disorders in the CNS (Croxford, 2003).

CB_1 and CB_2 are $G_{i/o}$-protein-coupled receptors that following cannabinoid agonist binding and signalling, inhibit AC activity. CB_1 receptor signalling also leads to down-stream activation of mitogen-activated protein kinase (Bouaboula *et al.*, 1995), p38 and *c-jun* amino terminal kinase (Liu *et al.*, 2000), which are involved in cellular regulation of proliferation and differentiation.

The presynaptic localization of CB_1 receptors suggests a role for cannabinoids in modulating the release of neurotransmitters from axon terminals. Early reports showed that administration of THC inhibits acetylcholine release in guinea pig ileum (Roth, 1978). Similar inhibitory effects of THC and other cannabinoids on the release of a variety of neurotransmitters have been observed in subsequent studies. The neurotransmitters involved include L-glutamate, GABA, norepinephrine, dopamine, serotonin (5-hydroxytryptamine, 5-HT) and acetylcholine. The brain regions often studied *in vitro*, usually in tissue slices preparations, include cerebellum, hippocampus and neocortex. Although most of these studies involved rat or mouse brain, a few studies have shown similar results using human brain tissue (Katona *et al.*, 2000; Schlicker and Kathmann, 2001). The specificity of cannabinoid actions were confirmed by demonstrating that the inhibitory effects of agonists were completely blocked by the CB_1-selective antagonist rimonabant (SR141716A).

THERAPEUTIC ASPECTS OF CANNABINOIDS

Cannabis has been historically used to relieve some of the symptoms associated with CNS disorders. Nowadays, there are anecdotal evidences for the use of cannabis in many patients suffering from multiple sclerosis or chronic pain (Croxford, 2003). Following the historical reports of the use of cannabis for medicinal purposes, recent research has highlighted the potential of cannabinoids to treat a wide variety of clinical disorders. Some of these disorders that are being investigated are pain, motor dysfunctions, or psychiatric illness.

Analgesia

Endogenous cannabinoids and cannabinoid receptors exist at various levels in the pain pathways, from peripheral sensory nerve endings to spinal cord and supraspinal centers, in a system that is parallel to but different from the classical pain-related opioid system. THC and synthetic derivatives of cannabinoids exert antinociceptive and anti-hyperalgesic properties when systemically administered in different animal models of acute and inflammatory pain (reviews by Pertwee, 2001; Iversen and Chapman, 2002). Cannabinoids also display antinociceptive activity in animal models of inflammatory pain when injected directly into the spinal cord, brain stem or thalamus (Pertwee, 2001). Noxious stimulation evokes an increased release of anandamide in the periaqueductal gray region of brainstem, a key site for modulating nociceptive information (Walker *et al.*, 1999). The antinociceptive actions of cannabinoids are blocked by CB_1 receptor antagonists (Manzanares *et al.*, 1999a), but the antagonist itself does not alter basal thresholds, suggesting that these are not controlled by tonic activity on the endocannabinoid system. Furthermore, there is evidence for an interaction between opioid and cannabinoid mechanisms. THC and morphine act synergistically, one increasing the anti-nociceptive actions of the other, in two different models of pain, acute pain (Fuentes *et al.*, 1999) and chronic inflammatory pain (Welch and Stevens, 1992). Naloxone and SR141716A were able to block this synergistic action, indicating that both opioid and cannabinoid receptors are involved in the control of pain (Fuentes *et al.*, 1999). Cannabinoids may produce analgesia through activation of a brainstem circuit that is also required for opiate analgesia, although the pharmacology of these two mechanisms is different.

Anxiety

Several studies suggest that cannabinoids regulate anxiety-related behaviors in humans and animals. These effects appear to be dose dependent, with low doses being anxiolytic and higher doses being anxiogenic. Indeed, Rodriguez de Fonseca *et al.* (1997) showed the anxiolytic properties of HU210 at low doses whereas with increasing doses animals experienced anxiety-related behaviors. The non-psychoactive natural component of the marijuana plant, cannabidiol, also displayed anxiolytic activity in rats although over a relatively limited dose range. Similarly, SR141716A induced anxiety-like behaviors in rats (Navarro *et al.*, 1997) probably caused by inhibition of the endogenous tonic level of CB_1 receptor in the absence of agonists. The synthetic cannabinoid nabilone has been included

in a clinical study to test its anxiolytic activity. In one double-blind placebo-controlled study, patients suffering from anxiety showed a "dramatic improvement" after nabilone treatment for 28 days. The only clinically significant adverse effect was postural hypotension with related dizziness or weakness. This was dose-related, experienced by most patients and tended to tolerate out over time (Robson, 2001).

Feeding Behavior

Many reports suggest that cannabis intake is associated with an increased appetite, particularly for sweet foods. This effect has been demonstrated under laboratory conditions (Mattes *et al.*, 1994), although results from studies in human subjects are variable. Nevertheless, therapeutic manipulation of the endogenous cannabinoid system has the potential to increase or decrease feeding. Controlled clinical trials showed that THC had significant beneficial effects in gain of weight in patients suffering from the AIDS-related wasting syndrome and this is one of the medical indications for which the drug has official approval in the United States. On the other hand, the CB_1 cannabinoid receptor selective antagonist SR141716A causes appetite suppression and weight loss, probably due to the activity of SR141716A as an inverse agonist at the CB_1 receptor. At the present time, SR141716A (rimonabant) is included in a clinical trial to evaluate its usefulness in the treatment of obesity.

Movement Disorders

A high density of CB_1 receptors in basal ganglia, an area closely involved in the regulation of motor control, suggests an important role of the endogenous cannabinoid system in movement disorders. It has been proposed that the endocannabinoid system may establish a "set point" of excitatory and inhibitory inputs within the basal ganglia. Parkinson´s disease, dyskinesias, dystonias, Hungtinton´s chorea, Tourette´s syndrome and multiple sclerosis should be considered candidates for cannabinoid-based therapy (Rodriguez de Fonseca *et al.*, 2001; Lastres-Becker *et al.*, 2002; Baker *et al.*, 2003; van der Stelt *et al.*, 2003).

CANNABIS AND PSYCHIATRIC ILLNESS

Prevalence of Consumption

The prevalence of cannabis use has increased in many countries over recent years (Hall and Babor, 2000; Strang *et al.*, 2000; Swift *et al.*, 2001a). At present 40-60% of young people aged 18-25 years in Spain, United Kingdom, United States, Australia, and some European countries have some experience of cannabis use (Hall *et al.*, 1999). Furthermore, cannabis use is starting at a younger age and continuing for longer so that many people in their 20s and 30s are already long-term users (Hall and Swift, 2000).

Psychiatric Disorders and Cannabis

Cannabis abuse has been related to several psychiatric disorders as: dependence, anxiety, depression, cognitive impairment and psychosis.

Dependence

The ability of cannabis to produce a pleasurable 'high' is probably the most important single factor sustaining its widespread and often chronic use. There is much evidence that this rewarding or reinforcing effect, which is shared by animals (Martellota *et al.*, 1998; Tanda *et al.*, 2000), is due to stimulation of limbic system reward pathways mediated by dopamine and endogenous opioids, resulting in increased release of dopamine from the nucleus accumbens (Gardner, 1991). The same action is exerted by other rewarding (and addictive) drugs including opiates, amphetamine, cocaine and nicotine (Koob, 1992).

Repeated or chronic use of cannabis induces considerable tolerance to the behavioral and pharmacological effects, largely due to down-regulation of cannabinoid CB_1 receptors (Oviedo *et al.*, 1993; Rodriguez de Fonseca *et al.*, 1994). Of particular interest is the development of tolerance to the recreationally desired 'high' in humans which has been demonstrated with oral cannabis and THC and cigarettes containing 1.8-3.1% THC (Haney *et al.*, 1999a,b). Such tolerance encourages escalation of dosage or increased frequency of use, as observed both experimentally and among a proportion of users in longitudinal community studies (Coffey *et al.*, 2000; Swift *et al.*, 2000; von Sydow *et al.*, 2001). Although many cannabis users quit in their mid-twenties (von Sydow *et al.*, 2001), Hall and Solowij (1998) note that about 10% of those who ever use cannabis become daily users and another 20-30% use the drug weekly. Escalation to potentially harmful use appears to be most common in those who start cannabis use early in adolescence (age 14-15) (Coffey *et al.*, 2000). A cannabis withdrawal syndrome has been clearly demonstrated in both animals and humans after chronic cannabis administration or use

(Hutcheson *et al.*, 1998; Haney *et al.*, 1999a,b; Kouri *et al.*, 1999). In animals this reaction is accompanied by brain changes similar to those of opiate, cocaine and alcohol withdrawal, including increased release of corticotropin releasing factor and increased stress responses (Rodriguez de Fonseca *et al.*, 1997, Oliva *et al.*, 2003). Recent studies have shown that cessation of cannabinoid treatment in mice induce a behavioral withdrawal syndrome characterized by a pronounced increase in ambulatory activity and rearings. Changes in secretion of circulating hormones and in brain related genes are also related to cannabinoid withdrawal. In mice, corticosterone plasma concentrations dramatically increased 24 and 72 h after cessation of cannabinoid treatment. Spontaneous cannabinoid withdrawal produced time related significant alterations in transcription of genes related to drug dependence such as tyrosine hydroxylase (TH), proenkephalin (PENK) or proopiomelanocortin (POMC) genes (Oliva *et al.*, 2003).

Abstinence symptoms from cannabis in human placebo controlled studies include restlessness, anxiety, dysphoria, irritability, aggression, insomnia, tremor, increased reflexes and several autonomic effects (Johns, 2001). Community surveys show that many people have difficulty in stopping cannabis use, and the prevalence of withdrawal symptoms in chronic cannabis users has been estimated as 16-29% (Thomas, 1996; Wiesbeck *et al.*, 1996). Withdrawal symptoms are likely to contribute to continued cannabis use and are an indication of drug dependence.

The DSM IV (Americal Psychiatric Association, 1994) criteria of drug dependence include tolerance, a withdrawal syndrome, difficulty in controlling consumption, and a pattern of drug use which reduces other important activities. By these or similar criteria cannabis dependence has been demonstrated in many studies (Fergusson *et al.*, 2000; Swift *et al.*, 2000; 2001a,b; von Sydow *et al.*, 2001). Hall and Solowij (1998) estimate that the risk of cannabis dependence (10% of those who ever use it) is of the same order as that of alcohol dependence (15%). Dependence prolongs the use of cannabis, increasing the risks of adverse physical and mental effects. One group of 198 self-defined problem cannabis users (Copeland *et al.*, 2001b) were typically (70%) males, mean age 32.8 years, who smoked cannabis several times weekly. Complaints ascribed to cannabis included lack of motivation (84%), feeling paranoid (77.7%) and respiratory complaints (62.6%). In addition, 86.2% reported driving while 'stoned'. There is an increasing demand for professional assistance in withdrawal (Wickelgren, 1997; Copeland *et al.*, 2001a), but little experience of treatment. Three recent studies have reported significant but modest beneficial results with cognitive behavioral approaches (Lang *et al.*, 2000; Copeland *et al.*, 2001a,b). Numerous studies have shown a highly significant association between cannabis use and alcohol consumption, tobacco smoking and use of illicit drugs (Fergusson and Horwood, 2000; Degenhardt *et al.* 2001b; Swift *et al.*, 2001a; von Sydow *et al.*, 2001) with a progression from smoking and drinking to cannabis and thence to other illicit drugs. Arguments have raged about whether cannabis is a causal factor encouraging progression to 'hard' drugs or whether the association is explained by other factors such as personality characteristics, sociological and demographic differences and drug availability (Hall *et al.*, 1999). Such factors have been investigated in two recent studies (Fergusson and Horwood, 2000; Degenhardt *et al.*, 2001b). Both studies showed that the association between cannabis and other illicit drug use, though reduced, persisted after controlling for a large number of covariates and was related to the amount of cannabis used. For example, cannabis dependent participants were still 28 times more likely than non-users to be dependent on other illicit drugs (Degenhardt *et al.*, 2001b) and those who had used cannabis at least 50 times in a year were still 60 times more likely than non-users to have taken other illicit drugs (Fergusson and Horwood, 2000). Mechanisms which might favor a causal relationship between cannabis use and progression to other illicit drugs, at least in some users, include (1) tolerance to the cannabis 'high' leading users to seek more potent drugs; (2) cannabis withdrawal associated with increased corticotropin releasing factor concentrations and glucocorticoid concentrations which enhance the reinforcing effects of amphetamines and opioids and the vulnerability to drug dependence, as shown in animals (Rodriguez de Fonseca *et al.*, 1997; Piazza and Le Moal, 1998); and (3) an interaction between cannabis and endogenous opioid systems which has been shown in young animals to increase the reinforcing properties of opiates (Ledent *et al.*, 1999; Manzanares *et al.*, 1999a).

Panic Attacks, Anxiety

Cannabis-induced anxiety usually occurs at high doses. The 50-60% of cannabis users experience anxiety symptoms in their life (Grispoon and Bakalar, 1997). The clinical picture typically is an exaggeration of the usual cannabis effects including anxiety, the fear of losing control or going crazy, depersonalization and desre-

TABLE II Cannabis and schizophrenia: clinical implications (adapted from Rubio, 1999).

VARIABLE	EFFECT
GENDER	**Male**
FIRST EPISODE	**Acute, earlier, cannabis abuse appears before schizophrenia.**
FOLLOW-UP COMPLICATIONS	**More hospitalizations, more relapses, less therapeutic compliance, neuroleptic resistance, tardive dyskinesia, more legal problems.**
SYMPTOMS EXACERBATED DUE TO CANNABIS USE	**More positive and affective symptoms and less or few differences on negative symptoms.**
FAMILY HISTORY	**More relatives with drug abuse.**
PERSONALITY TRAIT	**Antisocial personality traits.**
PREMORBID ADJUST	**Better in cannabis abusers.**
OUTCOME	**Worst global outcome.**

alization. These symptoms can be experienced by individuals with no previous psychopathology as well as those who have a history of maladaptive behavior. These episodes usually occur in individuals with pre-existing anxiety about drug use, especially novice users, or in experienced users who have taken more than their usual dose.

Depression

Evidence for an association between cannabis use and depression has grown (Degenhardt *et al.*, 2001a). The comorbid presentation of cannabis abuse and depression is relatively common in clinical and community populations. However, the degree to which psychiatric disorders such as depression are predisposing risk factors for substance abuse, or vice versa, is a subject of controversy. Individuals may use cannabis to self-medicate their dysphoria. On the other hand, chronic cannabis use may exacerbate or induce dysphoria. Longitudinal studies suggest that cannabis abuse in adults increases depressive symptoms, but depressive symptoms do not predict later cannabis abuse (Bovasso, 2001). A recent cohort study has demonstrated a strong association between daily use of cannabis and depression and anxiety symptoms in young women. Frequency of cannabis use in teenage girls predicted later higher rates of depression, but depression and anxiety in teenagers did not predict later cannabis use (Patton *et al.*, 2002). Depressive symptoms observed in cannabis users can be experienced as brief reactions (Thomas, 1993).

Recent studies have reported the up-regulation of CB_1 cannabinoid receptor in prefrontal cortex of depressed suicide victims (Hungund *et al.*, 2004). It may be assumed that the observed elevated CB_1 receptor may be a pathological consequence of depression and/or schizophrenia (Dean *et al.*, 2001).

Cognitive Impairment

Neurocognitive deficits induced by cannabis are dose-related and may persist for some time (up to 2 years) after the last dose in heavy users (Solowij, 1998). These impairments may be subtle but could affect performance in demanding jobs that pose a risk to public safety (Hall, 2001). Recent studies with improved methods have demonstrated changes in cognition and brain function associated with long-term or frequent use of cannabis. Specific impairments of attention, memory, and executive function have been found in cannabis users in the non-intoxicated state in controlled studies using brain event-related potential techniques and neuropsychological assessments (Solowij *et al.*, 2002). Adolescents may be particularly vulnerable to the adverse effects of cannabis which may interfere with personal and emotional development and increase risks of dependence, mental disorders and progression to other drugs (Fergusson and Horwood, 2000; McGee *et al.*, 2000; Johns, 2001). No histological or structural changes have been reported in the brains of human cannabis users, although theoretically long-lasting changes could occur at the level of CB_1 receptors.

Probably, the so-called "amotivational" syndrome could correspond to a cognitive impairment syndrome. This syndrome has been described in the Middle East, Orient and the United States. It is characterized by apathy and diminished interest in activities and goals.

Controversy exists about the importance of preexisting psychopathology in patients such as those with preexisting passive/dependent personality traits (McKenna, 1997).

Psychosis

The prevalence of cannabis use is high among schizophrenic patients (Johns, 2001) and in individuals with schizotypic traits (Mass *et al.*, 2001). Cannabis can cause a schizophreniform psychosis in normal individuals, may precipitate schizophrenia in predisposed persons, and can exacerbate positive symptoms in schizophrenics (Negrete and Gill, 1999; Johns, 2001; Voruganti *et al.*, 2001). As shown in Table II, cannabis use worsens the outcome in schizophrenia. First episodes occur earlier, hospitalizations and relapses appear more frequently, and there is less therapeutic compliance, neuroleptic resistance and tardive dyskinesia (Rubio, 1999).

New findings suggest that the endocannabinoid system may be involved in the psychopathology of schizophrenia. Elevated cerebrospinal concentrations of anandamide have been found in schizophrenic patients (Leweke *et al.*, 1999) and increased cannabinoid CB_1 receptor binding in the dorsolateral prefrontal cortex has been observed *postmortem* (Dean *et al.*, 2001). Neither of these changes was related to psychotropic medication or previous cannabis consumption. Cannabis may aggravate schizophrenia by releasing dopamine from limbic areas and also by further perturbing an underlying imbalance in endocannabinoid signalling in the brain (Leweke *et al.*, 1999).

There are several common aspects between schizophrenia and cannabis abuse: a) Cannabis and alcohol are the most common drugs used by individuals with schizophrenia. It is estimated that 40-60% of schizophrenic patients meet cannabis abuse criteria; b) positive symptoms of schizophrenia may be observed in cannabis intoxication states and symptoms of "amotivational" syndrome exhibit a great overlapping with negative schizophrenia syndrome; c) substance abuse and schizophrenia could share some common neurobiological pathways and putatively some common predisposing genes. Modulation of the dopaminergic mesolimbic system is thought to be involved both in addiction and in schizophrenia, as showed some preclinical pharmacological evidence (Di Chiara and Imperato, 1988), *postmortem* (Mash, 1997) and genetic studies. Several studies have investigated the association between CB_1 polymorphism and schizophrenia. All but one did not find differences between patients and controls (Tsai *et al.*, 2000, Leroy *et al.*, 2001). Ujike *et al.* (2002) pointed out that hebephrenic schizophrenia was associated with an increased rate of the 9 repeat allele (1359G/A) in the Japanese population. Two studies found significant differences in the frequency of the gg genotype (Leroy *et al.*, 2001), and AAT polymorphism (Ponce *et al.*, 2003) in substance-abusing patients compared to non-substance-abusing schizophrenic patients. These results suggest that gene variants of CB_1 receptor are associated with different risks of substance abuse in schizophrenia. Since the distributions in substance-abusing patients is very similar to that in the control population, this may suggest that the gg and AAT genotypes were associated with substance abuse in schizophrenia only. A functional modification of the CB_1 receptor could thus have indirect consequences on dopaminergic-related adaptative regulation and in turn could influence the vulnerability to substance abuse (Table II).

CANNABINOID RESEARCH IN EXPERIMENTAL PSYCHIATRY

The fact that the classical known actions of cannabinoids in brain are related to its psychotropic effects and that cannabinoid receptors are distributed across important emotional circuits in the brain, led to the study of the potential involvement of the endocannabinoid system in psychiatry diseases.

Although the control of emotional states in the brain is under multiheterogenous regulation of several neurotransmitters and hormones, a number of studies suggest a role of cannabinoid receptors in the development of mental disorders. Basic research is based mainly on the role of the endogenous cannabinoid system in the regulation of emotional states in experimental animals. THC or cannabinoid receptor agonists produce pronounced alterations in the endogenous opioid system (Manzanares *et al.*, 1999a; Valverde *et al.*, 2000; Navarro *et al.*, 2001), when administered to rats. In addition, it has been proposed that the endogenous cannabinoid system plays an important physiological role in the control of the hypothalamic-pituitary-adrenal (HPA) axis function, which regulates stress responses. It has been demonstrated that:

1- THC or anandamide increases plasma corticotropin and corticosterone concentrations (Weidenfeld *et al.*, 1994; Manzanares *et al.*, 1999b)

2- THC or cannabinoid receptor agonists increase corticotropin releasing factor gene

expression in the paraventricular nucleus of the hypothalamus (Corchero *et al.*, 1999)

3- An increase in the release of CRF or in the secretion of corticosterone in the plasma occurred after spontaneous (Oliva *et al.*, 2003) or antagonist-precipitated cannabinoid withdrawal syndrome (Rodríguez de Fonseca *et al.*, 1997).

Recent advances in genetics have led to the development of transgenic and genetically manipulated animals that represent an excellent tool to identify specific functions of the proteins that have been altered. Cannabinoid CB_1 knock out mice (deletion of CB_1 receptor gene) may contribute to an understanding of the role of cannabinoid receptors in the neurobiology on mental disorders. Three types of cannabinoid CB_1 mice have been developed to date (Ledent *et al.*, 1999; Zimmer *et al.*, 1999; Marsicano *et al.*, 2002) which differ in the genetic way that they have been generated and in the strain of animals that were finally backcrossed. These differences may account for subtle distinct behavioral, endocrine and neurochemical responses. Recent findings supporting the role of cannabinoid CB_1 receptors in the regulation of anxiety include studies showing that mutant mice exhibited an anxiogenic-like response in the light/dark box, increased anhedonia in chronic unpredictable mild stress procedure (Martin *et al.*, 2002), and reduced exploration in elevated plus maze (Haller *et al.*, 2002). In contrast, Marsicano *et al.* (2002), found no alterations in the time on open arms in the elevated maze between mutant and wild type animals. Recent studies reveal that restraint stress induces a greater increase in plasma corticosterone concentrations in mutant compared to wild type animals (Urigüen *et al.*, 2004). These results suggest hypersensitivity to stress in mice deficient in CB_1 receptors, further supporting a homeostatic function for cannabinoid receptors in the control of anxiety- and mood-related behaviors. In addition the results of this study revealed that benzodiazepines such as bromazepam lack anxiolytic action in mice deficient in CB_1 receptors, suggesting that the presence of these receptors is required to achieve an anxiolytic response. Taking into account that the anxiolytic action of benzodiazepines appears to be mediated by activation of $GABA_A$ receptors, disruption of cannabinoid receptors may alter functionally any of the different α, β or γ receptor subunits (Löw *et al.*, 2000). The mechanisms involved in the regulation between CB_1 receptors and $GABA_A$ receptor subunits remain to be determined. Further studies on $GABA_A$ receptor autoradiography and *in situ* hybridization of $GABA_A$ receptor subunits are in progress to clarify this hypothesis.

CONCLUDING REMARKS

The considerable progress in the pharmacological and physiological actions of THC or cannabinoid synthetic derivatives (agonists and antagonists) has led to the identification of an endogenous cannabinoid system in the central nervous system. The neuromodulatory role of this new neuronal system strongly suggests that direct or indirect manipulations of this cannabinoid system using receptor agonists, antagonists or selective inhibitors of reuptake systems of the endogenous ligands may be potentially useful in the treatment of a wide variety of neuropsychiatric disorders.

References

American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* (DSM-IV) (APA: Washington, DC).

Baker D, G Pryce, G Giovannoni and AJ Thompson (2003) The therapeutic potential of cannabis. *Lancet Neurol.* **2**, 291-298.

Beltramo M, N Stella, A Calignano, SY Lin, A Makriyannis and D Piomelli (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**, 1094-1097.

Bisogno T, S Maurelli, D Melck, L De Petrocellis and V Di Marzo (1997) Biosynthesis, uptake, and degradation of anandamide and palmitoylethanolamide in leukocytes. *J. Biol. Chem.* **272**, 3315-3323.

Bisogno T, F Berrendero, G Ambrosino, M Cebeira, JA Ramos, JJ Fernandez Ruiz and V Di Marzo (1999) Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem. Biophys. Res. Commun.* **256**, 377-380.

Bouaboula M, C Poinot-Chazel, B Bourrie, X Canat, B Calandra, M Rinaldi-Carmona, G Le Fur and P Casellas (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB_1. *Biochem. J.* **312**, 637-641.

Bovasso GB (2001) Cannabis abuse as a risk factor for depressive symptoms. *Am. J. Psychiatry* **158**, 2033-2037.

Coffey C, M Lynskey, R Wolfe and GC Patton (2000) Initiation and progression of cannabis use in a population-based Australian adolescent longitudinal study. *Addiction* **95**, 1679-1690.

Copeland J, W Swift and V Rees (2001a) Clinical profile of participants in a brief intervention program for cannabis use disorders. *J. Subst. Abuse Treat.* **20**, 45-52.

Copeland J, W Swift, R Roffman and R Stephens (2001b) A randomized controlled trial of brief cognitive-behavioral interventions for cannabis use disorder. *J. Subst. Abuse Treat.* **21**, 55-64.

Corchero J, JA Fuentes and J Manzanares (1999) Chronic administration with the cannabinoid receptor agonist CP-55,940 regulates corticotropin releasing factor and proopiomelanocortin gene expression in the hypothalamus and pituitary gland of the rat. *Life Sci.* **64**, 905-911.

Cravatt BF, DK Giang, SP Mayfield, DL Boger, RA Lerner and NB

Gilula (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83-87.

Cravatt BF, K Demarest, MP Patricelli, MH Bracey, DK Giang, BR Martin and AH Lichtman (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signalling in mice lacking fatty acid amide hydrolase. *Proc. Natl. Acad. Sci. USA* **98**, 9371-9376.Croxford JL (2003) Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs* **17**, 179-202.

Dean B, S Sundram, R Bradbury, E Scarr and D Copolov (2001) Studies on [^{3}H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience* **103**, 9-15.

Degenhardt L, W Hall and MT Lynskey (2001a) Alcohol, cannabis and tobacco use among Australians: a comparison of their associations with other drug use and use disorders, affective and anxiety disorders, and psychosis. *Addiction* **96**, 1603-1614.

Degenhardt L, W Hall and M Lynskey (2001b) The relationships between cannabis use and other substance use in the general population. *Drug Alcohol Depend.* **64**, 319-327.

Deutsch DG, ST Glaser, JM Howell, JS Kunz, RA Puffenbarger, CJ Hillard and N Abumrad (2001) The cellular uptake of anandamide is coupled to its breakdown by fatty-acid amide hydrolase. *J. Biol. Chem.* **276**, 6967-6973.

Devane WA, L Hanus, A Breuer, RG Pertwee, LA Stevenson, G Griffin, D Gibson, A Mandelbaum, A Etiger and R Mechoulam (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946-1949.

Di Chiara G and A Imperato (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. USA* **85**, 5274-5278.

Di Marzo V, A Fontana, H Cadas, S Schinelli, G Cimino, JC Schwartz and D Piomelli (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**, 686-691.

Egertova M, DK Giang, BF Cravatt and MR Elphick (1998) A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB_1 receptor in rat brain. *Proc. R Soc. Lond. Biol. Sci.* **265**, 2081-2085.

Elphick MR and M Egertova (2001) The neurobiology and evolution of cannabinoid signalling. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **356**, 381-408.

Felder CC and M Glass (1998) Cannabinoid receptors and their endogenous agonists. *Annu. Rev. Pharmacol. Toxicol.* **38**, 179-200.

Felder CC, A Nielsen, EM Briley, M Palkovits, J Priller, J Axelrod, DN Nguyen, JM Richardson, RM Riggin, GA Koppel, SM Paul and GW Becker (1996) Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide in brain and peripheral tissues of human and rat. *FEBS Lett.* **393**, 231-235.

Fergusson DM and LJ Horwood (2000) Does cannabis use encourage other forms of illicit drug use? Addiction 95, 505-520.

Fuentes JA, M Ruiz-Gayo, J Manzanares, G Vela, I Reche and J Corchero (1999) Cannabinoids as potential new analgesics. *Life Sci.* **65**, 675-685.

Gardner EL and JH Lowinson (1991) Marijuana's interaction with brain reward systems: update 1991. *Pharmacol. Biochem. Behav.* **40**, 571-580.

Grispoon L and JB Bakalar (1997) Marihuana, In: *Substance Abuse. A Comprehensive Texbook (3rd Edition)* (Lovinson JH, P Ruiz, RB Millman and JG Langrod, Eds.) (Williams and Wilkins: Baltimore, MD), pp 199-206.

Hall W (2001) Reducing the harms caused by cannabis use: the policy debate in Australia. *Drug Alcohol Depend.* **62**, 163-174.

Hall W and TF Babor (2000) Cannabis use and public health: assessing the burden. *Addiction* **95**, 485-490.

Hall W and N Solowij (1998) Adverse effects of cannabis. *Lancet* **352**, 1611-1616.

Hall W and W Swift (2000) The THC content of cannabis in Australia: evidence and implications. *Austr. NZ J. Publ. Health* **24**, 503-507.

Hall W, L Johnston and N Donnelly (1999) Epidemiology of cannabis use and its consequences, In: *Health Effects of Cannabis* (Kalant H, WA Corrigall, W Hall and RG Smart, Eds.) (Centre for Addiction and Mental Health: Toronto, Canada), pp 69-125.

Haller J, N Bakos, M Szirmay, C Ledent and TF Freund (2002) The effects of genetic and pharmacological blockade of the CB_1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* **16**, 1395-1398.

Haney M, AS Ward, SD Comer, RW Foltin and MW Fischman (1999a) Abstinence symptoms following oral THC administration to humans. *Psychopharmacology* **141**, 385-394.

Haney M, AS Ward, SD Comer, RW Foltin and MW Fischman (1999b) Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology* **141**, 395-404.

Hanus L, S Abu-Lafi, E Fride, A Breuer, Z Vogel, DE Shalev, I Kustanovich and R Mechoulam (2001) 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB_1 receptor. *Proc. Natl. Acad. Sci. USA* **98**, 3662-3665.

Herkenham M, AB Lynn, MR Johnson, LS Melvin, BR de Costa and KC Rice (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.* **11**, 563-583.

Howlett AC, MR Johnson, LS Melvin and GM Milne (1988) Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Mol. Pharmacol.* **33**, 297-302.

Howlett AC, F Barth, TI Bonner, G Cabral, P Casellas, WA Devane, CC Felder, M Herkenham, K Mackie, BR Martin, R Mechoulam and RG Pertwee (2002) International union of pharmacology. XXVII: Classification of cannabinoid receptors. *Pharmacol. Rev.* **54**, 161-202.

Hungund BL, KY Vinod, SA Kassir, BS Basavarajappa, R Yalamanchili, TB Cooper, JJ Mann and V Arango (2004) Upregulation of CB(1) receptors and agonist-stimulated [^{35}S] GTPγS binding in the prefrontal cortex of depressed suicide victims. *Mol. Psychiatry* **9**, 184-190.

Hutcheson DM, ET Tzavara, C Smadja, E Valjent, BP Roques, J Hanoune and R Maldonado (1998) Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with Δ^9-tetrahydrocannabinol. *Br. J. Pharmacol.* **125**, 1567-1577.

Ishac EJ, L Jiang, KD Lake, K Varga, ME Abood and G Kunos (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.* **118**, 2023-2028.

Iversen LL (2000) *The Science of Marijuana* (Oxford University Press: Oxford, England).

Iversen LL (2003) Cannabis and the brain. Brain 126, 1252-1270

Iversen LL and V Chapman (2002) Cannabinoids : a real prospect for pain relief ? *Curr. Opin. Pharmacol.* **2**, 50-55.

Johns A (2001) Psychiatric effects of cannabis. *Br. J. Psychiatry* **178**, 116-122.

Kathmann M, U Bauer, E Schlicker and M Gothert (1999)

Cannabinoid CB_1 receptor-mediated inhibition of NMDA- and kainate-stimulated noradrenaline and and dopamine release in the brain. *Naunyn Schmiedebergs Arch. Pharmacol.* **359**, 466-470.

Katona I, B Sperlagh, Z Magloczky, E Santha, A Kofalvi, S Czirjak, K Mackie, ES Vizi and TF Freund (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* **100**, 797-804.

Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* **13**, 177-184.

Kouri EM, HG Pope and SE Lukas (1999) Changes in aggressive behavior during withdrawal from long-term marijuana use. *Psychopharmacology* **143**, 302-308.

Lang E, M Engelander and T Brooke (2000) Report of an integrated brief intervention with self-defined problem cannabis users. J. *Subst. Abuse Treat.* **19**, 111-116.

Lastres-Becker I, M Gomez, R de Miguel, JA Ramos and J Fernandez-Ruiz (2002) Loss of cannabinoid CB_1 receptors in the basal ganglia in the late akinetic phase of rats with experimental Huntington's disease. *Neurotoxicity Res.* **4**, 601-608.

Ledent C, O Valverde, G Cossu, F Petitet, JF Aubert, F Beslot, GA Bohme, A Imperato, T Pedrazzini, BP Roques, G Vassart, W Fratta and M Parmentier (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. *Science* **283**, 401-404.

Leroy S, N Griffon, MC Bourdel, JP Olié, MF Poirier and MO Krebs (2001) Schizophrenia and the cannabinoid receptor type 1 (CB_1): association study using a single-base polymorphism in coding exon 1. *Am. J. Med. Gen.* **105**, 749-752.

Leweke FM, A Giuffrida, U Wurster, HM Emrich and D Piomelli (1999) Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* **10**, 1665-1669.

Liu J, B Gao, F Mirshahi, AJ Sanyal, AD Khanolkar, A Makriyannis and G Kunos (2000) Functional CB_1 cannabinoid receptors in human vascular endothelial cells. *Biochem. J.* **346**, 835-840.

Low K, F Crestani, R Keist, D Benke, I Brunig, JA Benson, JM Fritschy, T Rulicke, H Bluethmann, H Mohler and U Rudolph (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**, 131-134.

Maccarrone M, M Bari, T Lorenzon, T Bisogno, V Di Marzo and A Finazzi-Agro (2000) Anandamide uptake by human endothelial cells and its regulation by nitric oxide. *J. Biol. Chem.* **275**, 13484-13492.

Manzanares J, J Corchero, J Romero, JJ Fernandez-Ruiz, JA Ramos and JA Fuentes (1999a) Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol. Sci.* **20**, 287-294.

Manzanares J, J Corchero and JA Fuentes (1999b) Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of delta(9)-tetrahydrocannabinol in rats. *Brain Res.* **21**, 173-179.

Marsicano G, CT Wotjak, SC Azad, T Bisogno, G Rammes, MG Cascio, H Hermann, J Tang, C Hofmann, W Zieglgansberger, V Di Marzo and B Lutz (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530-534.

Martellotta MC, G Cossu, L Fattore, GL Gessa and W Fratta (1998) Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. *Neuroscience* **85**, 327-330.

Martin M, C Ledent, M Parmentier, R Maldonado and O Valverde (2002) Involvement of CB_1 cannabinoid receptors in emotional behaviour. *Psychopharmacology* **159**, 379-387.

Mash DC (1997) D_3 receptor binding in human brain during cocaine overdose. *Mol. Psychiatry* **2**, 5-6.

Mass R, C Bardong, K Kindl and B Dahme (2001) Relationship between cannabis use, schizotypal traits, and cognitive function in healthy subjects. *Psychopathology* **34**, 209-214.

Matsuda LA, SJ Lolait, MJ Brownstein, AC Young and TI Bonner (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561-564.

Mattes RD, K Engelman, LM Shaw and MA Elsohly (1994) Cannabinoids and appetite stimulation. *Pharmacol. Biochem. Behav.* **49**, 187-195.

McGee R, S Williams, R Poulton and T Moffitt (2000) A longitudinal study of cannabis use and mental health from adolescence to early adulthood. *Addiction* **95**, 491-503.

McKenna C (1997) Substance-induced psychiatric disorders, In *The Principles and Practice of Addictions in Psychiatry* (Miller NS, Ed.) (Saunder Company: Philadelphia), pp 103-112.

Mechoulam R (1970) Marihuana chemistry. *Science* **168**, 1159-1166.

Mechoulam R (1986) The pharmacohistory of *Cannabis sativa*, In: *Cannabinoids as Therapeutic Agents* (Mechoulam R, Ed.) (CRC Press: Boca Raton, FL) pp 1-19.

Mechoulam R, S Ben-Shabat, L Hanus, M Ligumsky, NE Kaminski, AR Schatz, A Gopher, S Almog, BR Martin, DR Compton *et al.* (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83-90.

Munro S, KL Thomas and M Abu-Sharr (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61-65.

Nakazi M, U Bauer, T Nickel, M Kathmann and E Schlicker (2000) Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB_1 receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **361**, 19-24.

Navarro M, E Hernandez, RM Munoz, I del Arco, MA Villanua, MR Carrera and F Rodríguez de Fonseca (1997) Acute administration of the CB_1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. *Neuroreport* **8**, 491-496.

Navarro M, MRA Carrera, W Fratta, O Valverde, G Cossu, L Fattore, JA Chowen, R Gómez, I del Arco, MA Villanua, R Maldonado, GF Koob and F Rodríguez de Fonseca (2001) Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J. Neurosci.* **21**, 5344-5350.

Negrete JC and K Gill (1999) Cannabis and schizophrenia: an overview of the evidence to date, In: *Marihuana and Medicine* (Nahas GG, KM Sutin, D Harvey and S Agurell, Eds.) (Humana Press: Totowa, NJ), pp 671-682.

Oliva JM, S Ortiz, T Palomo and J Manzanares (2003) Behavioural and gene transcription alterations induced by spontaneous cannabinoid withdrawal in mice. *J. Neurochem.* **85**, 94-104.

Oviedo A, J Glowa and M Herkenham (1993) Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: a quantitative autoradiographic study. *Brain Res.* **616**, 293-302.

Patton GC, C Coffey, JB Carlin, L Degenhardt, M Lynskey and W Hall (2002) Cannabis use and mental health in young people: cohort study. *BMJ* **325**, 1195-1198.

Pertwee RG (1999) Pharmacology of cannabinoid receptor ligands. *Curr. Med. Chem.* **6**, 635-664.

Pertwee RG (2001) Cannabinoid receptors and pain. *Prog. Neurobiol.* **63**, 569-611.

Piazza PV and M Le Moal (1998) The role of stress in drug self-administration. *Trends Pharmacol. Sci.* **19**, 67-74.

Ponce G, J Hoenicka, G Rubio, I Ampuero, MA Jimenez-Arriero, R Rodriguez-Jimenez, T Palomo and JA Ramos (2003) Association between cannabinoid receptor gene (CNR1) and childhood attention deficit/hyperactivity disorder in Spanish male alcoholic patients. *Mol. Psychiatry* **8**, 466-467.

Robson P (2001) Therapeutic aspects of cannabis and cannabinoids. *Br. J. Psychiatry* **178**, 107-115.

Rodriguez de Fonseca F, MA Gorriti, JJ Fernandez-Ruiz, T Palomo and JA Ramos (1994) Down regulation of rat brain cannabinoid binding sites after chronic Δ^9-tetrahydrocannabinol treatment. *Pharmacol. Biochem. Behav.* **47**, 33-40.

Rodriguez de Fonseca F, MR Carrera, M Navarro, GF Koob and F Weiss (1997) Activation of corticotrophin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* **276**, 2050-2054.

Rodriguez de Fonseca F, MA Gorriti, A Bilbao, L Escuredo, LM Garcia-Segura, D Piomelli and M Navarro (2001) Role of the endogenous cannabinoid system as a modulator of dopamine transmission: implications for Parkinson's disease and schizophrenia. *Neurotoxicity Res.* **3**, 23-35.

Roth SH (1978) Stereospecific presynaptic inhibitory effect of Δ^9-tetrahydrocannabinol on cholinergic transmission in the myenteric plexus of the guinea pig. *Can. J. Physiol. Pharmacol.* **56**, 968-975.

Schlicker E and M Kathmann (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* **22**, 565-572.

Shen M, TM Piser, VS Seybold and SA Thayer (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J. Neurosci.* **16**, 4322-4334.

Solowij N (1998) *Cannabis and Cognitive Functioning* (Cambridge University Press: Cambridge, UK).

Solowij N, RS Stephens, RA Roffman, TF Babor, R Kadden, M Miller, K Christiansen, B McRee and J Vendetti (2002) Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA* **287**, 1123-1131.

Strang J, J Witton and W Hall (2000) Improving the quality of the cannabis debate: defining the different domains. *BMJ* **320**, 108-110.

Swift W, W Hall and J Copeland (2000) One year follow-up of cannabis dependence among long-term users in Sydney, Australia. *Drug Alcohol Depend.* **59**, 309-318.

Swift W, W Hall and M Teesson (2001a) Cannabis use and dependence among Australian adults: results from the National Survey of Mental Health and Wellbeing. *Addiction* **96**, 737-748.

Swift W, W Hall and M Teeson (2001b) Characteristics of DSM-IV and ICD-10 cannabis dependence among Australian adults: results from the National Survey of Mental Health and Wellbeing. *Drug Alcohol Depend.* **63**, 147-153.

Szabo B, T Muller and H Koch (1999) Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens *in vitro*. *J. Neurochem.* **73**, 1084-1089.

Tanda G, P Munzar and SR Goldberg (2000) Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nat. Neurosci.* **3**, 1073-1074.

Thomas H (1993) Psychiatric symptoms in cannabis users. *Br. J. Psychiatry* **163**, 141-149.

Thomas H (1996) A community survey of adverse effects of cannabis use. *Drug Alcohol Depend.* **42**, 201-207.

Tsai SJ, YC Wang and CJ Hong (2000) Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. *Psychiatr. Genet.* **10**, 149-151.

Ujike H, M Takai, K Nakata, Y Tanaka, T Takeda, M Kodoma, Y Fujiwara, A Sakai and S Kuroda (2002) CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. *Mol. Psychiatry* **7**, 515-518.

Urigüen L, S Perez-Rial, CL Ledent, T Palomo and J Manzanares (2004) Impaired action of anxiolytics in mice deficient in cannabinoid CB_1 receptors. *Neuropharmacology* (in press)

Valverde O, C Ledent, F Beslot, M Parmentier and BP Roques (2000) Reduction of stress-induced analgesia but not of exogenous opioid effects in mice lacking CB_1 receptors. *Eur. J. Neurosci.* **12**, 533-539.

van der Stelt M, HH Hansen, WB Veldhuis, PR Bar, K Nicolay, GA Veldink, JFG Vliegenhart and HS Hansen (2003) Biosynthesis of endocannabinoids and their modes of action in neurodegenerative diseases. *Neurotoxicity Res.* **5**, 183-199.

von Sydow K, R Lieb, H Pfister, M Hofler, H Sonntag and HU Wittchen (2001) The natural course of cannabis use, abuse and dependence over four years: a longitudinal community study of adolescents and young adults. *Drug Alcohol Depend.* **64**, 347-361.

Voruganti LN, P Slomka, P Zabel, A Mattar and AG Awad (2001) Cannabis induced dopamine release: an *in-vivo* SPECT study. *Psychiatry Res. Neuroimaging* **107**, 173-177.

Walker JM, AG Hohmann, WJ Martin, NM Strangman, SM Huang and K Tsou (1999) The neurobiology of cannabinoid analgesia. *Life Sci.* **65**, 665-673.

Weidenfeld J, S Feldman and R Mechoulam (1994) Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo-pituitary-adrenal axis in the rat. *Neuroendocrinology* **59**, 110-112.

Welch SP and DL Stevens (1992) Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. *J. Pharm. Exp. Ther.* **262**, 10-18.

Wickelgren I (1997) Marijuana: harder than thought. *Science* **276**, 1967-1970.

Wiesbeck GA, MA Schuckit, JA Kalmijn, JE Tipp, KK Bucholz and TL Smith (1996) An evaluation of the history of marijuana withdrawal syndrome in a large population. *Addiction* **91**, 1469-1478.

Zimmer A, AM Zimmer, AG Hohmann, M Herkenham and TI Bonner (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB_1 receptor knockout mice. *Proc. Natl. Acad. Sci. USA* **96**, 5780-5785.