

NEUROCHEMICAL BASIS OF CANNABIS ADDICTION

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Abstract—Cannabis derivatives have become the most widely used illicit substances in developed countries, and constitute a major health concern. The psychoactive compounds contained in cannabis induce their pharmacological effects by the activation of at least two different receptors, CB1 and CB2 cannabinoid receptors. Multiple studies have demonstrated the specific involvement of CB1 cannabinoid receptors in the addictive properties of cannabinoids. Several neurotransmitter systems involved in the addictive effects of other prototypical drugs of abuse, such as the dopaminergic and the opioid system are also involved in cannabis addiction. The participation of other neurochemical systems in behavioural responses of cannabinoids related to their addictive effects has also been reported. This review describes the experimental methods now available to study the pharmacological responses of cannabinoids related to their addictive effects and how these methods have contributed to advance the knowledge of the specific contribution of different neurochemical systems in cannabis addiction. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: THC, reward, tolerance, CB1 receptor, dopamine, opioid.

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Abbreviations: CRF, corticotrophin-releasing factor; DA, dopamine; DAMGO, D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin; FAAH, fatty acid amide hydrolase; [35S]-GTP gamma S, [35S]-guanylyl-5'-O-(gamma-thio)-triphosphate; MGL, monoacylglycerol lipase; NAc, nucleus accumbens; THC, delta9-tetrahydrocannabinol; VTA, ventral tegmental area.

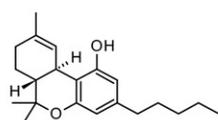
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CANNABINOID COMPOUNDS

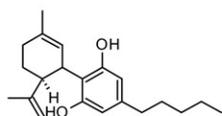
Cannabis sativa derivatives have been known for thousands of years for their recreational use and medicinal properties. The interest for these substances has increased in the last decades due to the identification and characterization of an endogenous cannabinoid system located in the CNS and peripheral tissues that could serve as a therapeutic target. However, cannabis derivatives have become the illegal drugs with the highest consumption rate for recreational purposes, and constitute a major health concern in developed countries especially among the young population (Pope et al., 2002; Johnston et al., 2007).

The main active compounds isolated from cannabis, called phytocannabinoids, are delta9-tetrahydrocannabinol (THC), cannabidiol, delta8-tetrahydrocannabinol and cannabinol (Pertwee, 2005) (Fig. 1). Other cannabinoids are also present in the plants such as cannabichromene, cannabigerol, cannabicyclol and cannabitrilol (Elsohly and Slade, 2005; Mechoulam et al., 1970; Turner et al., 1980). Among them, THC is the main psychoactive component in the cannabis extracts (Gaoni and Mechoulam, 1964), while another abundant phytocannabinoid, cannabidiol, lacks the psychoactive effects and produces antiinflammatory responses (Iuvone et al., 2009). The cannabis plants are classified as drug-type plants depending on their relative content of THC and cannabidiol (ratio THC/cannabidiol much higher than 1), intermediate-type plants (ratio THC/cannabidiol around 1), and fiber-type plants (ratio THC/cannabidiol lower than 1) (Hillig and Mahlberg, 2004). In Europe, the maximum THC content allowed for cannabis cultivation of fiber-type plants is 0.2–0.3% of dry matter weight. In contrast, the new genetically selected plant variants that are cultivated for recreational consumption can reach a content of THC over 20% (Pijlman et al., 2005).

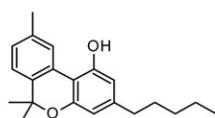
Synthetic cannabinoid agonists have been generated displaying different intrinsic activity and selectivity for the cannabinoid receptors. According to their chemical structure, synthetic cannabinoid agonists can be classified as classical, non-classical and aminoalkylindoles (Pertwee et

Phytocannabinoids

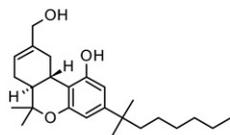
THC



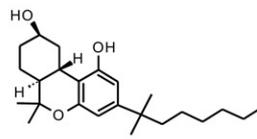
Cannabidiol



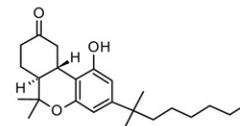
Cannabinol

Synthetic cannabinoids

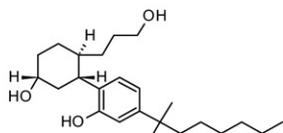
HU-210



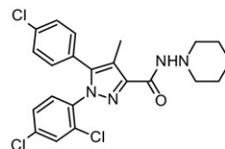
HU-243



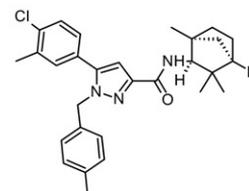
Nabilone



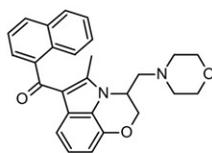
CP 55,940



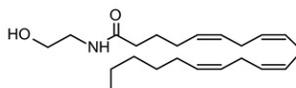
SR141716A (rimonabant)



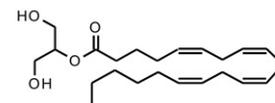
SR144528



WIN 55,212-2

Endocannabinoids

anandamide



2-arachidonoyl glycerol

Fig. 1. Chemical structure of representative phytocannabinoids, synthetic cannabinoids and endocannabinoids.

al., 2010). The classical group consists of dibenzopyran derivatives of THC that include HU-210, HU-243 and nabilone. The non-classical group consists of bicyclic and tricyclic analogs of THC that lack the pyran ring. CP 55,940 would be the most representative compound for this group. The aminoalkylindole group shows a completely different structure to THC, and the best known member in this group is WIN 55,212-2 (Fig. 1) (Pertwee et al., 2010).

Selective antagonists for the different cannabinoid receptors such as SR141716A (rimonabant) (Rinaldi-Carmona et al., 1994) and AM251 (Gatley et al., 1996) for the CB1 cannabinoid receptor subtype, and SR144528 (Rinaldi-Carmona et al., 1998) and AM630 (Pertwee et al., 1995) for the CB2 cannabinoid receptor subtype, have also been generated. These selective antagonists represent excellent tools to advance in the knowledge of the endocannabinoid system. The endogenous ligands for this system are endocannabinoids derived from polyunsaturated fatty acids (Bisogno, 2008), and are described below.

ENDOCANNABINOID SYSTEM

The endocannabinoid system is composed of the cannabinoid receptors, the endogenous ligands or endocannabinoids, and the enzymes participating in the synthesis and degradation of the endocannabinoids. The best character-

ized cannabinoid receptors are CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993) receptors, although other receptors, such as GPR55 (Baker et al., 2006) have also been reported to bind cannabinoid ligands. All these receptors are seven transmembrane domain-G protein-coupled receptors (Pertwee et al., 2010). The CB1 receptor is the most abundant seven-transmembrane domain receptor in the brain, and it is extensively distributed in all the CNS, mainly in the basal ganglia, the cerebellum and the hippocampus (Herkenham et al., 1991). CB1 receptors are mainly confined to the presynaptic terminals where they modulate the release of excitatory and inhibitory neurotransmitters, usually promoting the inhibition of their release (Alger, 2002; Vaughan and Christie, 2005; Szabo and Schlicker, 2005). Thus, CB1 receptors in glutamatergic terminals are neuroprotective against excitotoxicity (Monory et al., 2006) and responsible for the effects of THC on locomotion, hypothermia, analgesia and catalepsy (Monory et al., 2007). On the other hand, CB1 receptors expressed in GABAergic terminals are critical for the memory deficits promoted by THC (Puighermanal et al., 2009), and for stress and natural reward mechanisms (Rossi et al., 2008; De Chiara et al., 2010). The control of CB1 receptors over neurotransmitter release promotes the prominent role of these receptors in anxiety, depression, cognition, addiction, motor function, feeding behaviour and pain (Kano

et al., 2009). The widespread distribution of CB1 receptors in the CNS parallels the psychoactive effects of cannabis. CB1 receptors are also expressed in peripheral tissues, such as the heart, testis, prostate, vascular tissue, immune system and all the tissues involved in the control of metabolism (Pertwee et al., 2010).

CB2 receptors are mainly located in the cells of the immune system (Munro et al., 1993), although a low level of expression has been identified in specific brain areas in neurons. The functional role of these central CB2 receptors has not been yet clarified (Van Sickle et al., 2005; Atwood and Mackie, 2010), while their expression in activated microglia has been shown to modulate neuroinflammatory responses (Stella, 2010; Racz et al., 2008; Atwood and Mackie, 2010).

GPR55 receptors are mainly expressed in adrenal tissue, ileum, jejunum and some brain areas, such as the frontal cortex and striatum. The brain levels for GPR55 are lower than those for CB1 receptors (Ryberg et al., 2007), and its pharmacology and signalling are still rather controversial (Pertwee et al., 2010).

Both CB1 and CB2 receptors inhibit adenylyl cyclase and enhance mitogen-activated protein kinase activity by signalling through Gi/o proteins. CB1 receptors activate outward potassium conductance and inhibit N- and P/Q-type calcium currents (Howlett, 2005). Under some circumstances, such as coupling to dopamine D2 receptors, CB1 receptors were also reported to signal through Gs proteins (Glass and Felder, 1997).

Anandamide, the first endocannabinoid identified (Devane et al., 1992), and 2-arachidonoyl glycerol (Sugiura et al., 1995) are the best-characterized endocannabinoids. However, other endocannabinoids have also been reported, such as noladin ether (Hanus et al., 2001), N-arachidonoyl-dopamine (Huang et al., 2002) and O-arachidonoyl-ethanolamine (virodhamine) (Porter et al., 2002), although their physiological relevance is under study. The endocannabinoid anandamide behaves as a partial agonist at both CB1 and CB2 receptors (Sugiura et al., 2002), and also activates the transient receptor potential vanilloid 1 (TRPV1) (Tóth et al., 2009; Di Marzo and De Petrocellis, 2010). On the other hand, 2-arachidonoylglycerol acts as a full agonist for CB1 and CB2 receptors (Sugiura et al., 2006). Endocannabinoids are thought to act as retrograde messengers in the CNS (Wilson and Nicoll, 2002) behaving as neuromodulators in a great variety of physiological processes. Accordingly, endocannabinoids released from postsynaptic neurons upon depolarization activate presynaptic CB1 receptors, which results in the inhibition of the release of different neurotransmitters (Alger, 2002).

Anandamide and 2-arachidonoylglycerol are synthesized on demand in a Ca²⁺-dependent manner (Wilson and Nicoll, 2001). The enzymes directly involved in the synthesis of anandamide have not been completely clarified, although N-acylphosphatidylethanolamine phospholipase D might play a significant role (Okamoto et al., 2007). On the other hand, the enzyme responsible for 2-arachidonoylglycerol synthesis in the CNS is the diacylglycerol lipase alpha (Tanimura et al., 2010). Anandamide

and 2-arachidonoylglycerol are locally metabolized through specific enzymes. The main anandamide-hydrolyzing enzyme is the fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), while monoacylglycerol lipase (MGL) is the main 2-arachidonoylglycerol hydrolase (Nomura et al., 2008). Thus, the effects of endocannabinoids can be prolonged through the selective inhibition of FAAH (Fegley et al., 2005) and MGL (Straiker et al., 2009). Indeed, FAAH inhibitors such as URB597 (Fegley et al., 2005) or PF-3845 (Ahn et al., 2009) have demonstrated anxiolytic-like properties (Piomelli et al., 2006; Kinsey et al., 2011) and antinociceptive effects (Piomelli et al., 2006; Naidu et al., 2010). In addition, inhibitors of the MGL such as JZL184 produce analgesia, hypothermia and hypomotility (Long et al., 2009). However, chronic exposure to JZL184 causes tolerance to the analgesic effects, physical dependence, desensitization of brain CB1 receptors and impaired endocannabinoid-dependent synaptic plasticity (Schlosburg et al., 2010).

CANNABIS ABUSE IN HUMANS

Cannabis derivatives are the most widely used illicit substance in Europe, USA and Australia (AIHW, 2005; SAMHSA, 2010; EMCDDA, 2009). Notably, recent analyses of cannabis extracts have shown an increase in potency over the last years due to enhanced THC content (Pijlman et al., 2005; McLaren et al., 2008; Mehmedic et al., 2010), which probably leads to an increase in the subjective effects of this drug. Cannabis effects will also depend on the route of administration, previous experience of the user with the drug, and the setting in which the drug is taken. The currently reported subjective effects of the drug are mild euphoria, relaxation, perceptual alterations such as time distortion, and intensification of routine everyday experiences. On the other hand, the most common acute adverse subjective effects are anxiety and panic reactions most often reported by naive users (Green et al., 2003).

Marijuana use has been associated with low academic achievement, legal problems, unemployment and risk for the development of psychotic disorders (Ferdinand et al., 2005; Friedman et al., 2001; Hall and Degenhardt, 2009; Henquet et al., 2005). These negative effects of cannabis are of particular relevance in young consumers considering the early age of starting consumption in developed countries. Early cannabis consumption is often associated with an elevated risk of later problematical use of cannabis and other drugs, poor academic performance, mental health problems and enhanced criminal behaviour (Copeland and Swift, 2009; Lynskey et al., 2003; Hall, 2006). However, other studies could not establish a casual link between cannabis consumption and these psychological and social problems due to the associated consumption of tobacco, alcohol and other drugs of abuse (Macleod et al., 2004).

It is estimated that one out of 12 cannabis users will eventually become dependent on marijuana (Wagner and Anthony, 2002). As with other addictions, cannabis-dependent individuals are characterized by the compulsive seek-

ing, loss of control and reinstatement to use marijuana despite significant problems associated with its consumption. Thus, approximately 90% of those seeking treatment for cannabis-related substance disorders report difficulty achieving and maintaining abstinence (Budney and Hughes, 2006). In general, adults seeking treatment for marijuana dependence average more than 10 years of daily or almost daily use and around six attempts to reduce or stop consumption (Budney and Hughes, 2006; Copeland and Swift, 2009). Cannabis users develop tolerance to many of the effects of THC (Lichtman and Martin, 2005) and some of them report withdrawal symptoms that include anxiety, insomnia, appetite disturbance and depression (Budney and Hughes, 2006).

No specific therapeutic approaches have been yet developed for cannabis dependence and most interventions are based on those used for alcohol dependence (Hart, 2005). Thus, motivational enhancement and cognitive behavioural therapy together with contingency management have shown some effectiveness (Copeland and Swift, 2009). Continuous abstinence is less common than reduced cannabis intake after these different interventions. However, complete abstinence is not absolutely necessary to achieve clinically relevant improvement and a marked reduction in cannabis-related problems (Marijuana Treatment Project Research Group, 2004; Copeland et al., 2001).

Other cannabinoid compounds, like the synthetic cannabinoid nabilone, approved for prescription in Canada since 1981, or Sativex, a combination of THC and cannabidiol approved in several countries for spasticity treatment in multiple sclerosis, are very rarely abused since they do not induce the same level of psychoactive effects than cannabis (Ware and St. Arnaud-Trempe, 2010). Indeed, a recent meta-analysis of the efficacy and safety of Sativex in patients with multiple sclerosis has confirmed the absence of abuse liability of this compound under the appropriate prescription conditions (Wade et al., 2010). In agreement, the sudden stop of chronic Sativex treatment was not associated to any manifestation of withdrawal (Wade et al., 2006). The use of the oromucosal route of administration for Sativex could explain this safety profile considering the slower absorption of cannabinoids and their less rapid delivery to the brain (Karschner et al., 2011). These results confirm the interest of cannabinoids as new therapeutic agents for some specific pathological conditions. As for other drugs of abuse, the therapeutic effects of cannabinoids must be clearly dissociated of the risks of abuse and addiction linked to the recreational use of cannabis derivatives.

ANIMAL MODELS TO EVALUATE PHARMACOLOGICAL RESPONSES OF CANNABINOIDS RELATED TO THEIR ADDICTIVE PROPERTIES

Several animal models are available to study particular responses that are related to cannabis addiction. Thus, predictive models are available in animals to evaluate the

development of cannabinoid tolerance and physical dependence as well as the rewarding/reinforcing effects produced by cannabinoids. These animal models have been extremely useful to understand the neurobiological mechanisms underlying cannabis addiction and can be of great interest to evaluate the effectiveness of any possible new therapeutic strategy.

Tolerance

One criterion for the diagnosis of addiction included in DSM IV-TR, and in the proposed draft of the DSM-5 for “Cannabis-Use Disorder” is the development of tolerance; defined as the decrease in the effects of the drug after the repeated consumption, or the enhancement of the amount of drug consumed in order to maintain the desired effects (American Psychiatric Association, 2000). Following chronic administration, tolerance develops to most pharmacological responses of cannabinoids, including antinociception, locomotor effects, hypothermia, catalepsia, suppression of operant behaviour, anticonvulsivant activity, ataxia, corticosterone release and the effects on gastrointestinal transit, body weight and cardiovascular functions (Maldonado, 2002). This tolerance has been reported in rodents, pigeons, dogs and monkeys (Abood and Martin, 1992). The development of cannabinoid tolerance is particularly rapid, and an important decrease of the acute response has been already observed after the second administration of a cannabinoid agonist (Maldonado, 2002). However, tolerance to THC effects can differentially develop depending on the physiological and behavioural response under consideration (Pope et al., 2001).

Withdrawal syndrome

The presence of a withdrawal syndrome is another criterion for the diagnosis of addiction included in DSM IV-TR, and in the proposed draft of the DSM-5 for “Cannabis-Use Disorder”, defined by the appearance of evident physiological symptoms of abstinence after the withdrawal of the chronic drug consumption, or the consumption of the same or a similar drug to alleviate the manifestations of abstinence (American Psychiatric Association, 2000). Several studies have reported the absence of somatic signs of spontaneous withdrawal after a chronic THC treatment in rodents, pigeons, dogs and monkeys, even after the administration of extremely high doses of THC (Maldonado, 2002). Similarly, no physical manifestations of withdrawal were reported after chronic treatment with other cannabinoid agonists (Young et al., 1981), although somatic signs of spontaneous abstinence were revealed after the abrupt interruption of chronic WIN 55,212-2 treatment, a cannabinoid agonist with shorter half-life than THC (Aceto et al., 2001). In agreement, the administration of CB1 cannabinoid antagonists has been reported to precipitate somatic manifestations of withdrawal in THC-dependent rodents (Maldonado, 2002). In spite of the absence of physical signs of spontaneous withdrawal, a suppression of an operant behaviour maintained by food has been reported in monkeys during spontaneous THC abstinence, which has been interpreted as a behavioural manifestation of spontaneous cannabinoid withdrawal (Beardsley et al.,

1986). These results are in agreement with the presence of behavioural manifestations of withdrawal after discontinuing chronic marijuana use in humans (Budney and Hughes, 2006). Subjective effects associated to a THC withdrawal syndrome have also been recently revealed in rhesus monkeys using the drug discrimination procedure (Stewart and McMahon, 2010), a behavioural paradigm described below.

Rewarding effects

Similarly to other addictive processes, the initiation of cannabis addiction has been related to its capacity to induce rewarding effects. Several predictive animal models are available to study responses related to the rewarding effects produced by cannabinoids. These procedures include the drug discrimination paradigm that evaluates the subjective effects of the drug, the conditioned place preference that assesses conditioned responses related to the rewarding effects, the intracranial electric self-stimulation procedures that evaluate the effects of the drug in the brain reward circuits and the self-administration methods that directly measure drug reinforcing properties. These experimental models have advanced the knowledge of the neurobiological substrate involved in cannabis rewarding effects that are crucial for the addictive process. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed more recently (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Belin et al., 2008; Kasanetz et al., 2010). These models of addiction are extremely complex and have been validated only for cocaine addiction. Due to their complexity, these models have still not been used to investigate the neurobiology of cannabis addiction.

Drug discrimination. Drug discrimination in animals is widely accepted as a model for subjective drug effects in humans. In this model, animals faced with two possible responses, one of which results in reinforcement delivery, are trained to detect whether they received an active drug or vehicle injection in order to determine through drug effects which response is correct. This drug discrimination procedure is based upon the presence or absence of subjective and perceptible CNS effects (Balster, 1990). Drug discrimination studies have revealed that cannabinoid agonists produce subjective drug effects in several animal species including rodents and monkeys (Maldonado, 2002). Cannabinoid agonists can have discriminative effects at doses smaller than those producing some other *in vivo* effects (De Vry et al., 2004). A strong correlation has been found between the drugs that have subjective effects similar to those of THC in animals and those that produce marijuana-like intoxication in humans, which demonstrates that this model is highly predictive of cannabis-like effects in humans (Panlilio et al., 2010). However, it is difficult to ascertain what properties are being detected in the drug discrimination studies, and this procedure is best used as a screen prior to more specific testing with other behavioural paradigms of reward.

Conditioned place preference. Conditioned place preference is a behavioural model currently used to measure the rewarding properties induced by the administration of a drug (Mucha et al., 1982). In this paradigm, the subjective effects of the drug are repeatedly paired to a previously neutral stimulus. Through this conditioning process, the neutral stimulus acquires the ability to act as a conditioned stimulus, and the animal will prefer or avoid the conditioned stimulus depending on the rewarding or aversive effects produced by the drug. The administration of cannabinoid agonists generally produces aversive-like responses in the place conditioning paradigm, mainly when used at high doses, and animals tend to avoid staying in the compartment associated with the cannabinoid administration (Parker and Gillies, 1995; McGregor et al., 1996; Sañudo-Peña et al., 1997; Chaperon et al., 1998; Hutcheson et al., 1998; Mallet and Beninger, 1998). The aversive effects of cannabinoids were less pronounced in adolescent than adult rats (Quinn et al., 2008). This age-specific difference suggests that adolescent rodents are more vulnerable to the addictive properties of cannabinoids (Quinn et al., 2008). Rewarding effects of cannabinoid agonists can also be revealed in the place conditioning paradigm by using particular experimental conditions. Thus, THC produced conditioned place preference in rats when administered at lower doses than those used to induce place aversion and when animals were exposed to a 24 h washout period between the two THC conditioning sessions (Lepore et al., 1995). More recent studies have also reported conditioned place preference after the administration of low doses of THC (Braidia et al., 2004). THC also produced conditioned place preference in mice by using low doses, a long period of conditioning and avoiding the possible dysphoric consequences of the first drug exposure by previous THC priming in the home cage before the conditioning sessions (Valjent and Maldonado, 2000).

Intracranial self-stimulation. Intracranial electric self-stimulation procedures were essential in the discovery of the brain reward circuits (Olds and Milner, 1954), and are now widely used to study the effects of drugs of abuse on the activity of these reward pathways (Sanchis-Segura and Spanagel, 2006). In this paradigm, animals are trained to maintain an operant behaviour in order to obtain an electric pulse through an electrode that has been previously implanted in a reward-related brain site, most frequently the lateral hypothalamic area. The threshold of the minimal current needed to promote intracranial electric self-stimulation is estimated. A drug that stimulates the reward circuit will decrease this threshold, which would be related to its rewarding properties, whereas a drug having aversive effects will enhance the minimal current required to maintain the self-stimulation (Markou et al., 1993). THC acute administration has been reported to lower intracranial self-stimulation thresholds in rats suggesting the activation of central hedonic systems (Gardner et al., 1988; Lepore et al., 1996), while the antagonist/inverse agonist rimonabant can induce an opposite effect (Xi et al., 2007). Doses of THC used in these studies (Gardner et al., 1988) were

similar to those reported to induce conditioning place preference in rodents (Lepore et al., 1995; Valjent and Maldonado, 2000; Braida et al., 2004).

Self-administration. Self-administration methods are widely used to directly evaluate the reinforcing properties of a drug. These procedures are considered by most researchers to be valid and reliable models of drug consumption in humans, and to have a high predictive value. It is assumed that the neurobiological mechanisms involved in drug self-administration in animals are similar to those underlying drug intake in humans (Sanchis-Segura and Spanagel, 2006). Operant and non-operant procedures can be used in these self-administration models. Non-operant paradigms are centred on the amount of drug consumed, are mainly restricted to oral self-administration and are therefore not useful in evaluating cannabis rewarding effects. Operant paradigms require an instrumental response in order to obtain the drug, and the analysis of this response provides valuable information about different behavioural aspects of drug consumption. Operant intravenous self-administration paradigms have been widely used to evaluate the reinforcing effects of all the prototypical drugs of abuse including cannabinoids. Early studies have reported that THC is unable to maintain operant self-administration behaviour in any of the animal species studied, and animals that have already learnt to self-administer other drugs of abuse did not self-infuse THC (Maldonado, 2002). In contrast, recent studies have revealed that squirrel monkeys are able to acquire and maintain an operant intravenous THC self-administration behaviour, for doses much lower than those previously used (Tanda et al., 2000; Justinová et al., 2003). Doses of THC self-administered by monkeys (2–4 $\mu\text{g}/\text{kg}$) are comparable to doses in marijuana smoke inhaled by humans (Tanda et al., 2000). THC self-administration has not been consistently reported in rodents (Maldonado, 2002). However, rodents self-administer the synthetic and short half-life cannabinoid agonist WIN 55,212-2 (Martellotta et al., 1998; Fattore et al., 2001; Mendizabal et al., 2006).

INVOLVEMENT OF CB1 CANNABINOID RECEPTORS IN CANNABINOID ADDICTIVE PROPERTIES

The specific involvement of CB1 cannabinoid receptors in the pharmacological responses of cannabinoids related to their addictive properties has been clearly demonstrated in animal studies using selective CB1 antagonists and knockout mice deficient in CB1 receptors (Maldonado, 2002; Panlilio et al., 2010). Thus, CB1 receptors are selectively involved in the development of tolerance to the different pharmacological responses of cannabinoids and the somatic manifestations of cannabinoid withdrawal, as well as in the behavioural responses induced by cannabinoids in drug discrimination, conditioned place preference and aversion, intracranial self-stimulation and intravenous self-administration paradigms (Maldonado, 2002). However, the recent discovery of CB2 receptors in neurons in the CNS (Van Sickle et al., 2005) and the involvement of these

central CB2 receptors in specific behavioural responses of cannabinoids (García-Gutiérrez et al., 2010; García-Gutiérrez and Manzanares, 2010), open new possibilities to investigate the potential role of CB2 receptors in cannabis addictive properties.

Genetic studies in humans provide additional support for the specific involvement of CB1 receptors in the vulnerability to cannabinoid addiction and probably also to other addictive processes. Indeed, numerous studies have suggested an association between polymorphisms in the CB1 cannabinoid receptor gene (CNR1) and cannabis dependence, as well as dependence to other illicit drugs and alcohol (Benyamina et al., 2011). The association of three different polymorphisms of the CNR1 with drug dependence has been widely studied: rs1049353 and rs806379 single nucleotide polymorphism, and the repetition of the AAT microsatellite (Benyamina et al., 2011). A positive association was initially reported between the rs1049353 polymorphism and alcohol dependence (Schmidt et al., 2002). However, this observation was not replicated in subsequent studies in alcohol dependence (Preuss et al., 2003), although the same association was reported in opioid (Heller et al., 2001) and cannabis (Hartman et al., 2009) addicts. The rs806379 polymorphism was initially associated with dependence and/or abuse of illicit substances (Zhang et al., 2004). However, three subsequent studies failed to replicate this association (Herman et al., 2006; Zuo et al., 2007; Hartman et al., 2009). On the other hand, an initial study associated the AAT repeat with intravenous drug dependence (Comings et al., 1997), although this association was not replicated in later studies (Li et al., 2000; Covault et al., 2001; Heller et al., 2001). More recently, an association between this AAT repeat and cocaine dependence has been reported (Ballon et al., 2006). A recent meta-analysis of all these association studies has revealed that only the AAT repeat showed a modest significant association with illicit drug dependence, but exclusively in the Caucasian population samples (Benyamina et al., 2011).

INVOLVEMENT OF DOPAMINE IN CANNABINOID ADDICTIVE PROPERTIES

Several heterologous systems different from the endocannabinoid system also participate in the addictive properties of cannabinoids. The mesocorticolimbic system mediates the rewarding properties of all the prototypical drugs of abuse (Di Chiara et al., 2004; Fattore et al., 2008). An important component of this system is the dopaminergic projection from the ventral tegmental area (VTA) to the frontal cortex and limbic structures, such as the nucleus accumbens (NAc) and amygdala. Cannabinoids, similarly to the main prototypical drugs of abuse, enhance the firing rate of dopaminergic neurons in the VTA (Gessa et al., 1998). Moreover, THC and the synthetic cannabinoid WIN 55,212-2 increase extracellular dopamine (DA) concentrations in the shell, but not in the core of the NAc (Tanda et al., 1997), which has been related to their reinforcing properties. Indeed, a correlation between self-administered

doses of the synthetic cannabinoid agonist WIN 55,212-2 and increased DA levels in the NAc shell has been described in rats (Fadda et al., 2006; Lecca et al., 2006). Additionally, the endocannabinoid anandamide also enhanced extracellular DA levels in the shell of the NAc when injected intravenously (Solinas et al., 2006). This effect was magnified and prolonged by the FAAH inhibitor, URB597 (Solinas et al., 2006). Besides the NAc, the VTA is another important region involved in the rewarding properties of cannabinoids since local administration of THC in this brain area induces rewarding effects in both the intracranial drug self-administration and the conditioned place-preference paradigm (Zangen et al., 2006).

Although CB1 cannabinoid receptors are abundant in the brain reward circuitry, cannabinoid agonists do not directly activate VTA DA neurons since CB1 receptors are not present in these cells (Herkenham et al., 1991; Matsuda et al., 1993). In fact, CB1 receptors are located in the VTA on presynaptic glutamatergic and GABAergic neurons. Therefore, the dopaminergic neurons of the mesocorticolimbic pathway are under the control of excitatory and inhibitory inputs that are modulated by CB1 receptor activation. The final increase of VTA DA neuronal activity induced by cannabinoids would be the result of a functional balance between the effects on GABAergic inhibitory interneurons and glutamatergic excitatory inputs mainly from the prefrontal cortex (Maldonado et al., 2006; Fattore et al., 2008).

In spite of the role for the dopaminergic system in cannabinoid reward, DA does not seem to play a major role in the discriminative effects of THC. Thus, neither the D1 receptor antagonist SCH-23390 nor the D2 antagonist raclopride reduced the discriminative effects of THC (Solinas et al., 2010). However, cocaine, amphetamine and D2 receptor agonists enhanced the discriminative effects induced by low doses of THC (Solinas et al., 2010), suggesting that activation of the dopaminergic system positively modulates this cannabinoid response. In agreement, repeated treatment with cocaine sensitized striatal GABAergic synapses to the presynaptic effect of cannabinoid CB1 receptor stimulation (Centonze et al., 2007), and haloperidol blocked this effect (De Chiara et al., 2010).

On the other hand, the spontaneous withdrawal from chronic THC administration was associated with reduced dopaminergic transmission in the limbic system (Diana et al., 1998), similar to that observed with other addictive drugs. In agreement, a decrease in extracellular DA in the NAc shell was reported after rimonabant precipitated THC withdrawal (Tanda et al., 1999). This low DA tone during cannabinoid withdrawal has been related to the aversive/dysphoric symptoms of abstinence and seems also to participate in the decreased motivation for non drug-related stimuli and increased sensitivity to the abused drug (Koob and Volkow, 2010). However, the dopaminergic system does not participate in the somatic expression of cannabinoid withdrawal since the treatment with DA antagonists did not attenuate THC physical dependence (Sañudo-Peña et al., 1999).

In agreement with the involvement of the dopaminergic system in cannabis addictive properties, neurochemical studies have reported changes in this system during chronic cannabinoid treatment and withdrawal. Thus, morphometrical reductions of tyrosine hydroxylase positive neurons were revealed in the VTA, but not in the substantia nigra, of cannabinoid withdrawn rats (Spiga et al., 2010), providing direct morphological evidence for functional abnormalities associated with cannabinoid dependence at the level of dopaminergic neurons. In addition, chronic treatment with the cannabinoid agonist WIN 55,212-2 induced tolerance to the increase in L-3,4-dihydroxyphenylalanine (DOPA) synthesis in cortex, hippocampus and cerebellum observed following WIN 55,212-2 acute injection (Moranta et al., 2009).

INVOLVEMENT OF THE ENDOGENOUS OPIOID SYSTEM IN CANNABINOID ADDICTIVE PROPERTIES

Opioid receptors and their endogenous ligands have been demonstrated to play an important role in brain reward processes, and to modulate the behavioural and neurochemical effects of multiple drugs of abuse including cannabinoids (Trigo et al., 2010). A first evidence for this participation is the overlapping distribution of CB1 and mu-opioid receptors revealed by anatomical studies in several areas of the CNS, such as the limbic system, mesencephalon, brain stem and spinal cord (Rodríguez et al., 2001; Salio et al., 2001). In addition, recent reports have shown that CB1 and mu-opioid receptors form heterodimers and may transmit intracellular signals through common G proteins (Rios et al., 2006; Hojo et al., 2008). This direct association between CB1 and mu-opioid receptors could participate in the interactions between the cannabinoid and opioid systems in the regulation of different behavioural responses, including drug addiction.

Cross-tolerance between cannabinoid and opioid agonists has been demonstrated in several studies (Maldonado, 2002). Thus, chronic exposure to THC (Thorat and Bhargava, 1994) or CP 55,940 (Vigano et al., 2005) produced cross-tolerance to morphine antinociception. On the contrary, repeated local administration of the synthetic cannabinoid HU-210 into the rat periaqueductal gray enhanced subsequent morphine antinociception (Wilson et al., 2008). This result could be explained by an up-regulation of mu-opioid receptors observed in the periaqueductal grey matter and lateral thalamus of CP 55,940-tolerant rats (Vigano et al., 2005). In agreement, chronic contingent cannabinoid intake induces adaptive changes in the density and activity of mu-opioid receptors within brain reward circuits, which could contribute to the development of cannabinoid addiction. Thus, an increase in mu-opioid receptor levels was found in different brain areas, such as the prefrontal cortex, NAc, caudate-putamen, hippocampus and amygdala, following self-administration of WIN 55,212-2 in rats (Fattore et al., 2007a). Increases in DAMGO-stimulated [³⁵S]GTPS binding were also found in the hippocampus, hypothalamus and amygdala of rats

self-administering this cannabinoid agonist (Fattore et al., 2007b).

The development of tolerance to the antinociceptive effects of the endocannabinoid anandamide seems to involve different mechanisms than those implicated in tolerance to exogenous cannabinoids. Thus, mice tolerant to anandamide did not show cross-tolerance to the antinociceptive responses induced by mu-, delta- or kappa-opioid agonists, while THC-tolerant mice exhibited cross-tolerance to dynorphin, or the kappa-opioid agonists U-50,488H and CI-977 (Smith et al., 1994; Welch, 1997). In agreement with an interaction between THC and kappa-opioid receptors, the administration of antisense oligodeoxynucleotides directed against kappa-opioid receptors accelerated the development of THC tolerance (Rowen et al., 1998). The development of THC tolerance was also slightly modified in kappa-opioid receptor knockout mice, but was unaltered in either mu- or delta-opioid receptor knockouts (Ghozland et al., 2002). However, mice lacking the pre-proenkephalin gene showed a decrease in the development of tolerance to THC antinociception and a slight attenuation of tolerance to THC hypolocomotion, suggesting the involvement of endogenous opioid peptides derived from this precursor (Valverde et al., 2000).

The role of the endogenous opioid system in cannabinoid physical dependence has been widely demonstrated. The opioid antagonist naloxone precipitated behavioural signs of withdrawal in rats chronically treated with cannabinoid agonists (Kaymakcalan et al., 1977; Navarro et al., 1998). However, this effect was not observed in cannabinoid-dependent mice after naloxone challenge (Lichtman et al., 2001b). In addition, a reduction in the expression of the somatic signs of THC withdrawal was reported in double-knockout mice deficient in mu- and delta-opioid receptors (Castañé et al., 2003), as well as in knockout mice lacking the pre-proenkephalin gene (Valverde et al., 2000), suggesting that the endogenous enkephalin system is involved in the expression of cannabinoid withdrawal. Nevertheless, the severity of cannabinoid withdrawal was not modified in single knockout mice deficient in mu-, delta- or kappa-opioid receptors (Ghozland et al., 2002), or in mice lacking the prodynorphin gene (Zimmer et al., 2001), although a previous study showed reduced somatic manifestations of cannabinoid withdrawal in mu-opioid receptor knockout mice when higher doses of THC were used (Lichtman et al., 2001b).

The involvement of the different components of the opioid system in the rewarding properties of cannabinoids has also been reported in several experimental models using genetic and pharmacological tools. The discriminative effects of THC show a high degree of pharmacological specificity (Fattore et al., 2004), although these THC effects appear also to be modulated by mu-opioid receptors. Thus, intracranial injection of the opioid peptide β -endorphin or systemic administration of heroin or morphine enhanced the discriminative effects of THC, whereas the opioid receptor antagonist naltrexone attenuated these effects (Solinas et al., 2004; Solinas and Goldberg, 2005). In contrast, a recent study performed in monkeys did not

support this interaction since neither heroin nor morphine modified THC discriminative effects (Li et al., 2008). The involvement of the opioid system in cannabinoid reward has also been studied in the place conditioning paradigm. Thus, THC-induced conditioned place preference was abolished in mice lacking mu-opioid receptors (Ghozland et al., 2002), and in double knockout mice lacking both mu- and delta-opioid receptors (Castañé et al., 2003), but was not modified in mice deficient in delta- or kappa-opioid receptors (Ghozland et al., 2002). In agreement, the opioid antagonist naltrexone attenuated CP 55,940-induced conditioned place-preference (Braidá et al., 2001a) and intracerebral self-administration (Braidá et al., 2001b) in rats, and THC self-administration in monkeys (Justinová et al., 2004).

The endogenous opioid system is also involved in the processes driving to reinstatement of cannabinoid-seeking behaviour. Thus, heroin, but not cocaine, reinstated cannabinoid-seeking behaviour in rats following extinction of WIN 55,212-2 self-administration (Spano et al., 2004). In agreement, THC-seeking behaviour in squirrel monkeys was reinstated by cannabinoid agonists and morphine, but not by cocaine (Justinová et al., 2008). The interaction between opioids and cannabinoids in the regulation of craving and relapse processes seems to be reciprocal since rimonabant and naloxone prevented cannabinoid-seeking behaviour induced by heroin and WIN 55,212-2 administration, respectively (Spano et al., 2004).

These behavioural data are in agreement with neurochemical experiments showing an interaction between cannabinoids and opioids in the modulation of the reward circuits. Thus, early studies demonstrated that systemic administration of naloxone (Chen et al., 1990; Tanda et al., 1997) or direct infusion of the mu-opioid antagonist naloxonazine into the VTA (Tanda et al., 1997) blocked the THC-induced increase of extracellular DA levels in the shell of the NAc. In addition, other important targets for the modulation of the opioid system in the rewarding properties of cannabinoids are the glutamatergic and GABAergic systems. In this sense, mu-opioid and CB1 receptors might form heterodimers in the NAc that control the release of GABA (Schoffelmeer et al., 2006). In agreement, the cannabinoid agonist WIN 55,212-2 reduced GABA efflux in the ventral pallidum and this effect was prevented by naloxone (Caillé and Parsons, 2006). Taken together, these results demonstrate the crucial role of mu-opioid receptors in the rewarding properties of cannabinoids.

Finally, the endogenous opioid system participates in the dysphoric effects induced by high doses of THC. Thus, the conditioned place aversion induced by a high dose of THC was abolished in mice lacking either prodynorphin (Zimmer et al., 2001) or kappa-opioid receptors (Ghozland et al., 2002). The pretreatment with the specific kappa-opioid antagonist nor-binaltorphamine also blocked THC dysphoric effects (Zimmer et al., 2001). Consistent with these results, the aversive effects of THC were strengthened in mice lacking the prodynorphin gene transcriptional repressor DREAM (Cheng et al., 2004). The important role played by the kappa-dynorphin system in the aversive

properties of cannabinoids was confirmed in the self-administration paradigm. Thus, the reinforcing effects of WIN 55,212-2 in this paradigm were facilitated in prodynorphin knockout mice (Mendizabal et al., 2006).

OTHER MECHANISMS INVOLVED IN CANNABINOID ADDICTIVE PROPERTIES

Noradrenaline and serotonin

Early studies evaluating the peripheral effects of cannabinoid exposure showed that the acute administration of THC interfered with noradrenaline release following stimulation of the rat isolated *vas deferens* (Graham et al., 1974), whereas the hypotensive effects of noradrenaline were not modified after chronic THC treatment (Adams et al., 1976). Subsequent studies reported that repeated cannabinoid treatment increased noradrenaline efflux in the prefrontal cortex of mice (Hillard and Bloom, 1982) and tyrosine hydroxylase protein content in the locus coeruleus of rats, which was accompanied by anxiety-like behaviour (Page et al., 2008). This evidence implies that noradrenaline may play a role in the mood-altering effects induced by chronic THC administration. In addition, pharmacological studies have provided support for the involvement of noradrenaline in the manifestations of cannabinoid withdrawal. Thus, rimonabant-precipitated cannabinoid withdrawal following repeated THC administration was attenuated by the noradrenergic α_2 -receptor agonist, clonidine (Lichtman et al., 2001a). Additionally, adaptations in presynaptic monoamine receptor function have been shown in noradrenergic and serotonergic brain regions during rimonabant-precipitated cannabinoid withdrawal (Moranta et al., 2009). Hence, desensitization of α_2 -receptors and supersensitivity of 5-HT_{1A} receptors were observed in WIN 55,212-2-dependent rats, suggesting possible changes in the regulation of both serotonin and noradrenaline synthesis during repeated cannabinoid administration (Moranta et al., 2009).

In terms of reward-related mechanisms, the systemic administration of the Tat-3L4F peptide, which prevents the dephosphorylation of 5-HT_{2C} receptors, as well as the serotonin 5-HT_{2C} receptor agonist, Ro600175, suppressed the increased firing rate of VTA dopaminergic neurons induced by THC, and blocked THC-induced conditioned place preference in rats (Ji et al., 2006). These data suggest a role for serotonin 5-HT_{2C} receptors in THC rewarding effects, as it has been described for other drugs of abuse (Higgins and Fletcher, 2003; Ji et al., 2006). In addition, (+)-MDMA, which potently inhibits serotonin uptake, produced significant responding for the THC lever in substitution tests, suggesting that these two substances share discriminative stimulus effects (Barrett et al., 1995).

GABA and glutamate

There is a considerable amount of data demonstrating the functional interaction between cannabinoids and GABAergic/glutamatergic neurotransmission in the reward circuit, including structures such as the VTA, the NAc, the prefrontal cortex and the amygdala. Both endogenous and exoge-

nous cannabinoids decrease GABA_A receptor-mediated inhibitory neurotransmission, and NMDA and AMPA receptor-mediated excitatory effects in the limbic system (López-Moreno et al., 2008). Most of these responses are mediated through the activation of CB1 receptors located presynaptically in GABAergic and glutamatergic terminals (Ferraro et al., 2001). Studies using brain slice preparations containing the VTA as well as the subcortical glutamatergic projections from the pedunculo-pontine nucleus have demonstrated that acute THC exposure selectively activates GluR2-lacking AMPA receptors and allows long-term potentiation only in the pedunculo-pontine nucleus pathway, in a CB1 receptor-dependent manner (Good and Lupica, 2010). These data suggest the possible involvement of glutamatergic transmission in the rewarding effects of THC. Other studies *in vitro* using VTA slice preparations demonstrated that the cannabinoid agonist, HU-210 increased DA cell firing in a dose- and CB1 receptor-dependent manner through a reduction of GABA inhibition of DA neurons, and repeated HU-210 applications failed to show tolerance to this effect (Cheer et al., 2000).

Albeit these data, the involvement of GABA and glutamate in the rewarding effects of cannabinoids has been poorly addressed in behavioural studies. Using drug-discrimination procedures, the interaction between the GABAergic system and cannabinoids has been revealed in studies showing that diazepam produced significant responding on the THC-appropriate lever in substitution tests (Mokler et al., 1986; Barrett et al., 1995; Wiley and Martin, 1999). Rimonabant did not block the partial substitution of diazepam for THC, nor did it decrease the discriminative stimulus effects of diazepam, suggesting a CB1-independent mechanism (Barrett et al., 1995; Wiley and Martin, 1999). Moreover, recent studies have reported that another benzodiazepine, triazolam did not substitute for THC in discrimination studies (Lile et al., 2009). In addition, human laboratory studies have revealed that the GABA_B receptor agonist, baclofen dose-dependently decreased craving for marijuana in withdrawn subjects that smoke marijuana daily. However, baclofen was not effective for relapse prevention (Haney et al., 2010). On the other hand, the involvement of glutamatergic mechanisms in the manifestations of cannabinoid withdrawal syndrome has only been studied in the planarian model. Hence, withdrawal from an acute exposure to WIN 52212-2 in planarians decreases the rate of spontaneous locomotor velocity, and this effect was attenuated by administration of the NMDA antagonist, LY 235959 (Rawls et al., 2007).

Acetylcholine

The participation of cholinergic mechanisms in behaviours related to the addictive properties of THC has been studied using drug discrimination techniques in rats. Thus, both muscarinic and nicotinic receptor activation potentiates the discriminative effects of low doses of THC. However, rimonabant reverses only the nicotine-mediated effect. In addition, nicotine produced THC-like discriminative effects dependent on CB1 receptors when the degradation of anandamide was blocked by the FAAH inhibitor URB597.

Interestingly, neither the non-selective nicotinic antagonist mecamylamine nor the muscarinic antagonist, scopolamine altered THC discriminative effects (Solinas et al., 2007a). In another study however, it was shown that the selective α_7 nicotinic acetylcholine receptor antagonist methyllycaconitine, but not the selective heteromeric non- α_7 nicotinic acetylcholine receptor antagonist dihydro-betaerythroidine, decreased the discriminative effects of THC, reduced intravenous self-administration of WIN 55,212-2, and decreased THC-induced dopamine elevations in the shell of the NAc in rats (Solinas et al., 2007b).

Corticotrophin-releasing factor

Corticotrophin-releasing factor (CRF) has been proposed to be involved in the aversive/dysphoric manifestations of the cannabinoid withdrawal syndrome (Rodríguez de Fonseca et al., 1997). Thus, the administration of the cannabinoid CB1 antagonist, rimonabant to rats treated chronically with the synthetic cannabinoid agonist HU-210, increased extracellular CRF concentrations in the central nucleus of the amygdala. This effect was associated with the progression of behavioural withdrawal symptoms, increased anxiety-like behaviour, and activation of the hypothalamic-pituitary-adrenal axis as shown by an increase in plasma corticosterone levels, and increased Fos immunoreactivity in the central nucleus of the amygdala (Rodríguez de Fonseca et al., 1997). Although these data suggest that the neuroadaptations at the level of CRF neurotransmission in the limbic system are involved in cannabinoid withdrawal, there is no direct basic or clinical evidence for the participation of CRF mechanisms in THC protracted withdrawal and relapse.

Sexual hormones and oxytocin

Sex differences have been observed in relation to the expression and brain location of CB1 cannabinoid receptors, as well as for the emotional and cognitive effects induced by cannabinoids (Viveros et al., in press). In addition, the density of CB1 receptors in the medial basal hypothalamus varies over the course of the oestrous cycle (Rodríguez de Fonseca et al., 1994). However, there is a paucity of studies evaluating the effects of sexual hormones on tolerance and dependence to cannabinoids. On the other hand, the role of ovarian hormones in cannabinoid reinforcement has been demonstrated recently. Female rats acquired operant self-administration of WIN 55,212-2 more robustly and at higher rates than did male rats. Moreover, ovariectomized female rats display lower rates of acquisition and cannabinoid intake than intact females (Fattore et al., 2007b). In addition, drug- and cue-induced reinstatement of extinguished WIN 55,212-2 self-administration was higher in intact female rats than in male or ovariectomized rats (Fattore et al., 2010). These preclinical data show evidence for the modulatory role of ovarian hormones in cannabinoid-seeking behaviour, and are in line with recent epidemiological data showing that the use of marijuana has notably increased during the last years among females, with no significant changes observed for males (SAMHSA, 2008).

Chronic cannabinoid exposure also modulates brain oxytocin, a neuropeptide robustly linked to oestrogen. Thus, repeated THC administration induces a downregulation of oxytocin mRNA in the NAc and VTA (Butovsky et al., 2006). In addition, systemic administration of lithium suppressed cannabinoid withdrawal syndrome in rats, which was associated with increased expression of Fos proteins within most oxytocin-immunoreactive neurons, and increased oxytocin mRNA expression in the hypothalamic paraventricular and supraoptic nuclei (Cui et al., 2001). Moreover, the antagonistic effects of lithium on cannabinoid withdrawal were attenuated by prior administration of the oxytocin antagonist, L-368,899 and mimicked by systemic or i.c.v. injection of oxytocin (Cui et al., 2001).

Adenosine

The contribution of adenosine A_{2A} receptors to cannabinoid tolerance and physical dependence has been studied in mice (Soria et al., 2004). Thus, following chronic THC treatment, no differences were observed in the development of tolerance to its antinociceptive or hypothermic effects between mice lacking A_{2A} receptors and wild-type controls. In contrast, the somatic manifestations of rimonabant-precipitated THC withdrawal were attenuated in A_{2A} mutant mice. Similarly, a reduction of THC-induced rewarding and aversive effects was observed in the place conditioning paradigm in mice lacking A_{2A} receptors. These data reveal the involvement of adenosine A_{2A} receptors in the behavioural responses related to the addictive properties of THC (Soria et al., 2004). In line with this notion, pharmacological studies in squirrel monkeys have recently shown that systemic administration of low doses of the adenosine A_{2A} receptor antagonist, MSX-3 produced a downward shift of the dose–response curve for THC and anandamide self-administration (Justinová et al., in press). This effect was specific to cannabinoid reinforcement since MSX-3 did not modify the reinforcing properties of cocaine or food reward. Moreover, MSX-3 significantly decreased THC-induced increases in extracellular dopamine levels in the NAc shell of rats (Justinová et al., in press). All these results suggest that adenosine A_{2A} antagonists could be potential medications for treatment of cannabis abuse (Justinová et al., in press).

CONCLUDING REMARKS

Specific animal models are now available to evaluate the different behavioural responses induced by cannabinoids that are related to their addictive properties. These experimental models have allowed an important advance in the knowledge of the neurobiological substrate underlying cannabis addiction. These models have clearly revealed that the CB1 cannabinoid receptors are responsible of all the responses related to the addictive properties of cannabinoids. However, the recent discovery of CB2 cannabinoid receptors in neurons in the CNS has open new possibilities. Indeed, these receptors are located in brain areas directly involved in cannabis reward. Although the density of CB2 receptors in these brain areas is low, some

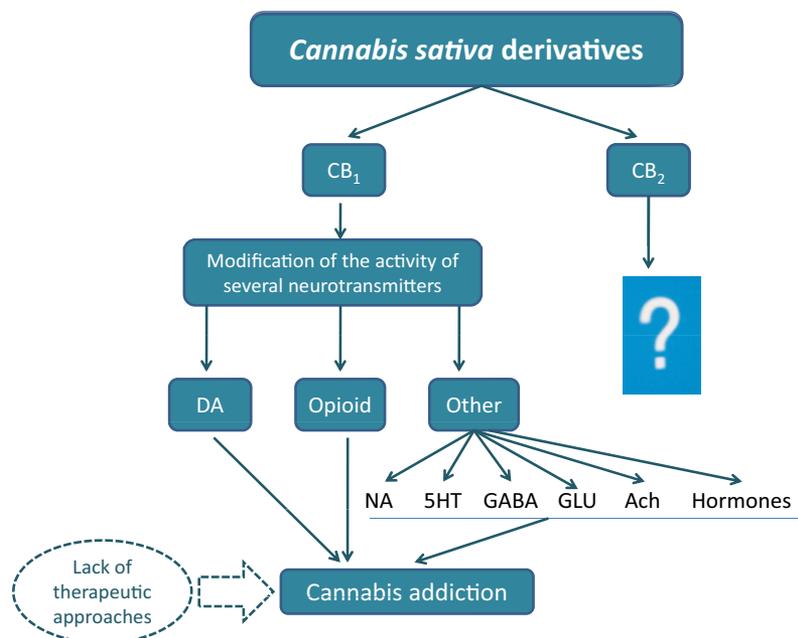


Fig. 2. Schematic summary of the main neurochemical mechanisms involved in cannabis addiction.

studies have recently suggested the possible involvement of central CB2 receptors in the control of several behavioural responses. Therefore, the possibility that central CB2 receptors participate in cannabis addictive properties remains an interesting question to be answered in the next years.

The neurochemical mechanisms underlying the development of cannabinoid addiction are similar to those observed for other drugs of abuse (Fig. 2). Thus, dopaminergic, opioid and cannabinoid systems are involved in the neurochemical substrate of the addictive processes induced by the main prototypical drugs of abuse, including cannabinoids. Other neurochemical systems have also been involved in the addictive effects of cannabinoids, including monoamines, GABA, glutamate, acetylcholine, adenosine and several neuropeptides. In spite of these advances in the understanding of cannabis addiction, no specific therapeutic approaches have been developed yet. The recreational use of marijuana has significantly increased in most developed countries in the last decade, which has generated a major health concern. The number of marijuana users seeking treatment for cannabis-related substance disorders has also dramatically increased in recent years. The lack of specific treatment for these disorders represents today a major limitation. However, the advances in the knowledge of the neurochemical substrate involved in cannabis addiction may provide novel approaches for the treatment of these disorders. This new basic information is highly relevant for developing clinical trials with specific ligands of newly identified targets in order to evaluate their potential application in the treatment of cannabis abuse.

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