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Review

Endocannabinoid system and opioid addiction: Behavioural aspects

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Abstract

Cannabinoids produce a variety of pharmacological effects very similar to those elicited by opioids. Direct and indirect interactions with opioid system have been proposed to explain some cannabinoid effects such as analgesia and attenuation of opioid-withdrawal syndrome, and evidence has been provided in support to the notion that rewarding properties of cannabinoids and opioids might be functionally linked. In particular, a growing body of studies points to an important role of the endogenous cannabinoid system in the modulation of opioid rewarding and addictive effects. The current review examines progresses in the past few years in the elucidation of cannabinoid–opioid interactions in drug abuse and dependence, focusing on recent findings from behavioural studies using different animal models of addiction. Specifically, here we review data on the behavioural aspects (i.e., drug abuse, dependence, tolerance, sensitization, relapse and drug vulnerability) of the specific, often reciprocal, cross-talk between cannabinoids and opioids with particular reference to the role of the endocannabinoid system in opioid addiction. The potential biochemical mechanisms involved in these pharmacological interactions are discussed together with possible therapeutic implications in the pharmacotherapy of opioid dependence. However, individuation of the precise anatomical substrates and molecular mechanisms of such interaction still remains a complex and challenging field for future research.

Keywords: Cannabinoids; Opioids; Addiction; Reward; Drug abuse; Dependence; Withdrawal; Tolerance; Sensitization; Relapse; Knockout mice

Contents

Abbreviations: AC, Adenylyl cyclase (AC); ACTH, Adrenocorticotrophic hormone; cAMP, Adenosine 3′-5′-cyclic monophosphate; CB_1, Central cannabinoid (receptor); CP, Caudate–putamen; CP 55,940, (−)-*cis*-3-[2-hydroxy-4(1,1-dimethyl-heptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)cyclohexanol; CPA, Conditioned place aversion; CPP, Conditioned place preference; CRF, Corticotrophin releasing factor; DS, Discriminative stimulus; DA, Dopamine; Δ^9-THC, Delta-9-tetrahydrocannabinol; FR, Fixed ratio; HPA axis, Hypothalamic–pituitary–adrenal axis; HU 210, *R*(−)-7-hydroxy-delta-6-tetra-hydro-cannabinol-dimethyl-heptyl; ICSS, Intracranial self-stimulation; i.c.v., Intracerebroventricularly; i.p., Intraperitoneally; i.v., Intravenously; KO, Knockout (mice); LC, Locus coeruleus; NAcc, Nucleus accumbens; SA, Self administration; SR 141716A, *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; WIN 55,212-2, *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo-[1,2,3-*de*]-1,4-benzoxazinyl](1-naphthalenyl) methanone mesylate; WT, Wild-type.

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1. Introduction

Cannabinoids and opioids share many pharmacological properties, including antinociception (Bloom and Dewey, 1978; Bhargava and Matwyshyn, 1980; Fuentes et al., 1999), hypothermia (Bhargava, 1980), sedation/catalepsy (Narimatsu et al., 1987; Pontieri et al., 2001a,b) and inhibition of intestinal motility (Dewey, 1986). Chronic administration of both agents produces tolerance to their analgesic and hypothermic effects (Pertwee, 1988; Bhargava, 1991; Rubino et al., 1997b) and leads to the development of physical dependence, although with different intensities (Bhargava, 1991; Tsou et al., 1995; Aceto et al., 1996, 1998, 2001). Cannabinoids are historically used in combination with opioids for the treatment of different types of pain in humans due to their synergistic interactions in the modulation of noxious stimuli (Welch, 1993; Welch and Eads, 1999; Cichewicz, 2004).

Besides analgesia, endogenous cannabinoids interact with the opioid system in a variety of biological functions, including emesis (Simoneau et al., 2001), intestinal motility (Frederickson et al., 1976; Basilico et al., 1999; Kulkarni-Narla and Brown, 2001 but see also Izzo et al., 1999, 2000) and immune activity (Massi et al., 2001, 2003) as well as modulation of anxiety (Berrendero and Maldonado, 2002; Gaveriaux-Ruff and Kieffer, 2002; Marìn et al., 2003), stress (Corchero et al., 1999a; Valverde et al., 2000a), emotion (Costanzi et al., 2003), exploratory behaviour (Poncelet et al., 1999) and locomotion (Ayhan et al., 1979; Ulku et al., 1980; Tulunay et al., 1981, 1982; Buttarelli et al., 2002).

Notably, in the caudate–putamen (CP) of rats treated with repeated administration of the central cannabinoid (CB_1) receptor ligand, delta9-tetrahydrocannabinol (Δ^9-THC), it has been observed (i) an increase in proenkephalin gene expression and μ-opioid receptor activation of G-proteins, (ii) a time-related decrease in central cannabinoid (CB_1) receptor gene expression and (iii) a reduction in CB_1 receptor activation of G-proteins. These findings suggest a possible interaction between the cannabinoid and opioid systems in a brain area (i.e., CP) potentially relevant in the understanding of the alterations of motor behaviour that occur after prolonged exposure to cannabinoids (Corchero et al., 1999b).

Cannabinoid–opioid interactions also exist in the control of hunger through the hyperphagic effects of exogenous and endogenous cannabinoids (Pietras and Rowland, 2002; Chen et al., 2004). Indeed, Δ^9-THC stimulates food consumption, an effect possibly involving activation of the reward pathways and mediated, at least in part, by opioidergic processes (Trojniar and Wise, 1991). Finding that neither naloxone nor SR 141716A reliably affect feeding when administered alone, but suppress food intake when combined together (Rowland et al., 2001), reveals a synergistic interaction between cannabinoids and opioids on feeding behaviour, thus strengthening the postulated role for endocannabinoids in reward processes contributing to the normal control of appetite (Kirkham and Williams, 2001; Solinas and Goldberg, in press).

Considering the long history of abuse of Cannabis derivatives over centuries, they undoubtedly possess positive reinforcing properties; however, cannabinoid rewarding effects in humans have not been readily detected in standard experimental settings (Chait, 1989; Chait et al., 1988; Chait and Zacny, 1992).

In the past, cannabinoids have long been considered 'anomalous' drugs of abuse, with a low abuse potential; nevertheless, over the last decades unambiguous evidence has been provided suggesting that their rewarding effects are mediated through the same brain reward systems shared by more 'classical' drugs of abuse. In addition, preclinical studies showed that endogenous and exogenous cannabinoids interfere with the reinforcing effect of most of the commonly abused drug, such as nicotine (Cohen et al., 2002), alcohol (Mechoulam and Parker, 2003), cocaine (Fattore et al., 1999), MDMA (Braida and Sala, 2002) or phencyclidine (Doty et al., 1994). Involvement of the CB_1 receptor in mediating reinforcing and physical dependence-producing effects of opioids has also been suggested, with the former being considered the result of interaction with the dopaminergic neurotransmission in the midbrain dopamine (DA) system (Chen et al., 1990). That is, stimulation of the

CB_1 receptor alters opioid rewarding properties as well as blockage or absence of such receptors does not allow many opioid pharmacological effects to be manifest, indicating a permissive role for the endocannabinoid system in the expression of opioid reinforcing effects.

Aim of the present work is to review and discuss behavioural data on the interaction between cannabinoid and opioid systems in drug addiction-related phenomena, ranging from modulation of drug intake, as revealed by behavioural animal models of reward, to drug dependence, tolerance and sensitization, up to relapse to drug-seeking after a period of abstinence and possible development of vulnerability to subsequent drug abuse.

2. Behavioural animal models of reward

It is generally appreciated that the recreational use of cannabinoids is related to their positive modulatory effects on brain-rewarding processes along with their ability to positively influence emotional states and remove stress responses to environmental stimuli (Rodriguez de Fonseca et al., 1997; Gardner and Vorel, 1998). Cannabinoids have been tested on a variety of behavioural models of addiction, most of which revealed functional interactions between the endocannabinoid and the opioid systems in the modulation of reciprocal rewarding and addictive effects (Self and Stein, 1992; Yamamoto and Takada, 2000; Fattore et al., 2004).

2.1. Drug discrimination

The drug discrimination procedure is based on the ability of a drug to induce a specific interoceptive stimulus in laboratory animals and thus exert subjective/discriminative effects likely resembling subjective perceptions produced by the same drug in human beings. In this paradigm, animals are trained to make different responses for obtaining a reward according to a priming injection of either a training drug or a vehicle. Once discrimination developed, several drugs are tested for their ability to substitute the effect of the training drug, or to antagonize the effects. Since the discriminative stimulus (DS) effects of a drug in animals have been considered analogous to the subjective drug effects in humans, drug discrimination is a widely used procedure in behavioural pharmacology (Holtzman, 1985; Appel et al., 1991; Preston, 1991; Stolerman, 1993; Colpaert, 1999).

Cannabis derivatives have proved to exert highly specific DS effects, which are not substituted by other classes of drugs (i.e., opioids or more direct dopaminergic compounds) nor are they reversed by antagonists of various neurotransmission systems (Järbe and Ohlin, 1977), supporting the idea that the DS effects only involve the cannabinoid system (Yamamoto and Takada, 2000). Accordingly, morphine does not substitute for Δ^9-THC (Järbe et al., 1998), whereas compounds acting on the CB_1 receptor fully generalize to the Δ^9-THC training stimulus (Barrett et al., 1995; Gold et al., 1992). In support to the notion that cannabinoid DS is mediated by CB_1 receptors only, SR 141716A has been found to completely abolish the DS effects of Δ^9-THC or other CB_1 receptor agonists (De Vry and Jentzsch, 2002, 2003).

However, benzodiazepines (i.e., diazepam) have also been reported to partially generalize to Δ^9-THC training stimulus. This effect is thought to be mediated by benzodiazepine receptors (Järbe and Hiltunen, 1988; Mokler et al., 1986) as it is antagonized by a specific benzodiazepine receptor antagonist (Mokler et al., 1986) but not SR 141716A (Wiley and Martin, 1999).

2.2. Conditioned place preference

Among the different experimental protocols that are typically used to measure drug reward in laboratory animals, the conditioned place preference (CPP) is one of the most widespread (for a comprehensive review see Tzschentke, 1998). Based on pavlovian conditioning principles, CPP reflects a preference for a context due to the contiguous association between the context and a drug-associated stimulus. It also presents important advantages, among which the possibility to reveal both reward and aversion, to test animals in a drug-free state and to allow simultaneous determination of locomotor activity. In this model, animals are trained to receive saline (or vehicle) injections in one compartment of the experimental box and the drug in another one: the two environments have equal size but different visual and tactile (or even olfactive) stimuli. If the drug is rewarding, by virtue of contiguous pairings, the environment develops the capability to elicit approach: a CPP is manifested by a tendency to approach, enter and remain within the drug-associated environment.

While almost all drugs of abuse are able to increase the time spent in the drug-paired compartment, in this protocol cannabinoids have revealed effects not always consistent between studies, and a cannabinoid CPP in the rat, when obtained, has proved to be highly dependent on the timing of injections as well as on the range of doses used. This is well described in a study by Lepore et al. (1995) illustrating the dose-dependent nature of Δ^9-THC effects in CPP under different methodological conditions. In fact, Δ^9-THC was found to induce conditioned place aversion (CPA) at a low dose (1.0 mg/kg) while CPP at higher doses (2.0 and 4.0 mg/kg) when a standard protocol is used. However, when the schedule is modified by allowing a longer wash out time period between drug injections, Δ^9-THC induces CPP at the lowest dose and a CPA at higher doses, suggesting that a possible post-drug dysphoric rebound effect may be overcame by increasing the interval between successive drug injections.

Noteworthy, the ability of Δ^9-THC to induce CPP or CPA seems to be not related to its effect on spontaneous motor activity, since at doses causing hypomotility (Sanudo-

Pena et al., 2000; Järbe et al., 2002) Δ^9-THC has been reported to induce both CPP (Braida et al., 2004) and CPA (Sanudo-Pena et al., 1997).

When the hypothesis that blockade of CB_1 receptors could interfere with the rewarding properties of opioids was evaluated in this paradigm (Table 1), it turned out that the acquisition of CPP induced by morphine (4 mg/kg) is dose-dependently blocked by pre-pairing administration of SR 141716A (0.03–3 mg/kg) in rats (Chaperon et al., 1998; Singh et al., 2004). Accordingly, the CB_1 receptor antagonist is able to antagonize the acquisition of morphine-induced CPP in mice at doses which per se support neither CPP nor CPA (Mas-Nieto et al., 2001). Subsequently, CP 55,940 was found to elicit CPP at a dose of 20 μg/kg, which in turn is fully antagonized by pretreatment with either SR 141716A and naloxone (Braida et al., 2001a). In the same year, it was also reported that repetitive administration of Δ^9-THC reduces morphine withdrawal syndrome but does not modify or even decrease the rewarding responses produced by morphine in the CPP paradigm, thus rendering unlike the possibility that chronic use of high doses of cannabinoids may potentiate the psychological dependence to opioids (Valverde et al., 2001).

To investigate deeper the role of the CB_1 receptor in the establishment of conditioned responses, Martin and colleagues used genetically selected mice lacking the CB_1 receptor gene (CB_1 KO mice). Authors reported that these animals display CPP and sensitization to locomotor responses only following cocaine, but not morphine, administration, thus highlighting the permissive role of the CB_1 receptor in the expression of behavioural responses of opioids (Martin et al., 2000). Furthermore, involvement of dynorphin on Δ^9-THC- and morphine-induced behavioural responses has been investigated by using mice with a targeted inactivation of the prodynorphin gene. Dynorphin-deficient mice display normal acute and chronic opioid effects but reduced Δ^9-THC-induced analgesia and fail to develop CPA (Zimmer et al., 2001). The lack of negative motivational effects of Δ^9-THC in the absence of dynorphin indicates that this endogenous opioid peptide mediates the dysphoric effects of marijuana.

More recently, it was shown that μ-opioid receptors KO mice do not show Δ^9-THC-induced CPP, while κ-opioid receptor KO mice do not show CPA to Δ^9-THC but reveal Δ^9-THC place preference (Ghozland et al., 2002). Accordingly, Δ^9-THC-induced CPP is reduced in double μ- and δ-opioid receptor KO mice (Castañé et al., 2003). The dual euphoric–dysphoric activity of cannabinoids seems therefore arise from an opposing activity of μ- and κ-opioid receptors in modulating reward pathways. A recent study gives support to this notion by showing a selective involvement of the κ-opioid receptor in the anxiogenic-like effect of CP 55,940 in rats (Marìn et al., 2003). Finally, a study by Gaveriaux-Ruff and Kieffer (2002) reveals a critical role of μ-opioid receptor in cannabinoid reinforcement and definitely confirms the involvement of κ-opioid receptor in several dysphoric responses.

However, similarly to findings from other behavioural procedures, also those from CPP studies comprise controversial data on the existence of a mutual interaction between cannabinoid and opioid system in the expression of reciprocal rewarding properties. To this regard, CB_1 KO mice were also found to develop a strong CPP to 4 and 8 mg/kg morphine (Rice et al., 2002).

2.3. Intracranial self-stimulation

Intracranial self-stimulation (ICSS) is a phenomenon whereby an animal (including a human being) will repeatedly stimulate its brain electrically, sometimes to the point of exhaustion. This phenomenon is robust and readily reproducible in many areas of the brain involved in reward processes. For example, rats will repetitively press a lever if it results in electrical stimulation of the medial forebrain bundle, a major element of the brain reward pathway (Olds and Milner, 1954). Thus, ICSS has been used to localize the 'chemical trigger zones' where drugs have habit-forming consequences (Wise and Hoffman, 1992).

Cannabinoids share with other drugs of abuse the ability to facilitate ICSS (Pradhan et al., 1978), even at a dose pharmacologically relevant to moderate human use of marijuana (Gardner et al., 1988). For example, it has been reported that Δ^9-THC (1.0–1.5 mg/kg) and SR 141716A (1, 3 and 10 mg/kg, i.p.) are able to lower and increase, respectively, the brain stimulation threshold in rats (Lepore et al., 1996; Gardner and Vorel, 1998; Deroche-Gamonet et

Table 1
Cannabinoid–opioid interactions in CPP protocols

Animals	Drug tested	Dose	CPP	Response	Reference
Rats	SR 141716A	0.1–0.3 mg/kg, i.p.	Morphine (acquisition)	Blockade	Chaperon et al., 1998
Rats	SR 141716A	0.5 mg/kg, i.p.	Heroin	Blockade	Braida et al., 2001a
Rats	Naloxone	2 mg/kg, i.p.	CP 55,940	Blockade	Braida et al., 2001a
Rats	SR 141716A	3 mg/kg, i.p.	Morphine (expression)	Blockade	Navarro et al., 2001
Rats	Naloxone	0.5–2 mg/kg, i.p.	Δ^9-THC	Blockade	Braida et al., 2004
Mice	SR 141716A	5–10 mg/kg, i.p.	Morphine (acquisition)	Blockade	Mas-Nieto et al., 2001
CB_1 KO mice			Morphine	Failure	Martin et al., 2000
δ and κ KO mice			Δ^9-THC	Development	Ghozland et al., 2002
μ KO mice	–	–	Δ^9-THC	Failure	Ghozland et al., 2002

al., 2001), although some discrepant observations have also been reported (Arnold et al., 2001). However, so far only very few investigations evaluated cannabinoid–opioid interactions by using this behavioural procedure. One of these rare exceptions is the study of Gardner et al. (1989) which reports a naloxone blockade of Δ^9-THC facilitating effect on ICSS.

2.4. Self-administration

Full characterization of the rewarding properties of a drug is best accomplished by the study of its effects on drug self administration (SA) behaviour, in which the rewarding properties of a drug are inferred by the extent to which it can establish and maintain a response habit, such as lever-pressing or nose-poking. In general, drugs that are abused by humans also serve to establish response habits in animals. Indeed, if a reward is given contingently upon an arbitrary behaviour of the animal (i.e., a lever-press or a nose-poke) the probability is increased that the behaviour will re-occur under the same set of circumstances.

Although reliable SA behaviour has been demonstrated in laboratory animals for almost all drugs abused by humans (Wise and Bozarth, 1981; Yokel, 1986; Young et al., 1981), for long time the absence of any classical models of cannabinoid SA led to a delay in the progress of such a topic. After repeated unsuccessful attempts to asses SA protocols in monkeys, rats or mice (Pickens, 1968; Cappell and Pliner, 1974; Carney et al., 1977; van Ree et al., 1978; Takahashi and Singer, 1979; Mansbach et al., 1994), first successful self-administration behaviour was observed only following the occurrence of physical dependence on the cannabinoid (Deneau and Kaymakcalan, 1971) or after exposing animals to phencyclidine (Pickens et al., 1973) or cocaine (Tanda et al., 2000). Only in the last few years, reliable cannabinoid SA protocols have been assessed in both drug-naïve rodents and monkeys, being available for evaluating a possible role of the endocannabinoid system in the modulation of the rewarding properties of drugs of abuse (Table 2).

2.4.1. Acute intravenous SA in drug-naïve mice

Previous studies from our and other laboratories validated this model of acute intravenous SA in drug-naïve mice as a consistent and suitable animal model for the study of rewarding effects of many drugs abused by humans such as morphine, cocaine, amphetamine, nicotine and gamma-hydroxybutyric acid (Kuzmin et al., 1992; Martellotta et al., 1995; Fattore et al., 2000a). In this paradigm, mice are tested in pairs in identical test cages, each presenting a frontal hole provided with an infrared detector that activates a cumulative recorder and operates a syringe pump connected to the lateral tail veins which deliver drug solution contingently on a nose-poke response. Mice are first placed in the test cage for 10 min of habituation with no needle inserted. Basing on the similarity in the baseline activity, mice are then paired, one defined as active and the other passive, and needles inserted in the lateral tail veins. Each nose-poke (NP) of the active mouse results in a contingent drug injection, delivered both to the active and the yoked passive mouse, so that both animals receive the same amount of drug at the same time intervals. NPs of the yoked control mouse are counted but had no programmed consequences. As a measure of the reinforcing effect of a drug, the ratio between the cumulative nose-pokes of the active and passive mouse during 30-min session is used.

In 1998, Martellotta et al. demonstrated that the CB_1 receptor agonist WIN 55,212-2 is intravenously self-administered by mice exposed to the drug for the first time. Subsequently, it was reported that (i) other two synthetic CB_1 receptor agonists, CP 55,940 and HU 210, are able to sustain acute SA behaviour in drug-naïve mice, which is prevented by pretreatment with SR 141716A, (ii) pretreatment with naloxone also reduces cannabinoid SA while (iii) SR 141716A antagonizes morphine SA behaviour in drug-naïve mice (Fratta et al., 1999; Navarro et al., 2001; Fattore et al., 2000b, 2002).

Importantly, mice lacking the CB_1 receptors fail to self-administer cannabinoids or morphine and do not develop morphine dependence (Ledent et al., 1999). As acute morphine-induced analgesia and development of tolerance

Table 2
Cannabinoid–opioid interactions in SA protocols

Animals	Drug tested	Dose	Intracerebral (i.c.)/intravenous (i.v.) SA	Response	Reference
Rats	SR 141716A	0.5 mg/kg, i.p.	Heroin (i.c.)	Reduction	Braida et al., 2001b
Rats	Naloxone	2 mg/kg, i.p.	CP 55,940 (i.c.)	Reduction	Braida et al., 2001b
Rats	SR 141716A	3 mg/kg, i.p.	Heroin (i.v.)—FR5	Reduction	Navarro et al., 2001
Rats	Heroin	0.1–0.5 mg/kg, i.v.	WIN 55,212-2 (i.v.)	Reduction	Fattore et al., 2002
Rats	WIN 55,212-2	0.25–1 mg/kg, i.p.	Heroin (i.v.)—FR1	Reduction	Fattore et al., 2002
Rats	SR 141716A	1–3 mg/kg, i.p.	Heroin (i.v.)—PR	Reduction	De Vries et al., 2003; Solinas et al., 2003
Rats	Naloxone	2 mg/kg, i.p.	Δ^9-THC (i.c.)	Reduction	Braida et al., 2004
Drug-naïve mice	SR 141716A	0.25 mg/kg, i.p.	Morphine (i.v.)	Reduction	Navarro et al., 2001
Drug-naïve mice	Naloxone	0.1–1.0 mg/kg, i.p.	Cannabinoid (i.v.)	Reduction	Fratta et al., 1999; Fattore et al., 2002
CB_1 KO mice	–	–	Morphine (i.v.)	Failure	Ledent et al., 1999
Squirrel monkeys	Naltrexone	0.03–0.3 mg/kg, i.m.	Δ^9-THC (i.v.)	Reduction	Justinova et al., 2004

to chronic morphine-induced analgesia were similar both in CB_1 KO mice and their relative wild-type (WT) control mice, the results of Ledent et al. imply a permissive role of the CB_1 receptor for the expression of the reinforcing effects of morphine. Accordingly, CB_1 KO mice do self-administer cocaine, D-amphetamine and nicotine to the same extent of WT mice (Cossu et al., 2001), pointing to a specific role of the CB_1 receptor in the opioid motivational and rewarding properties.

2.4.2. Chronic intravenous SA in trained rats

This model of chronic intravenous SA in animals trained to operate for obtaining a drug infusion represents the most reliable measure of drug abuse liability. It closely resembles most phases of human addictive behaviour, starting from the acquisition of the SA behaviour, to the retention of a stable drug intake, up to the extinction of such a behaviour and subsequent relapse to drug-seeking following a period, even prolonged, of drug abstinence. Since almost all drugs abused by humans are easily self-administered by rats (Collins et al., 1984), drug SA studies in rats have greatly contributed towards our understanding of central mechanisms involved in drug-taking and drug-seeking behaviour (Koob, 1992a,b).

Although cannabinoids, similarly to other drugs of abuse, serve as positive reinforcers in several animal species including humans (Chait and Zacny, 1992), it has been rather difficult to demonstrate their rewarding properties in this model of SA. The first animal models of chronic cannabinoid SA in animals without a previous history of drug abuse were developed by Fattore et al. (2001) and Justinova et al. (2003) in Long–Evans rats and squirrel monkeys, respectively. Thereafter, these methods have been used for investigating the possibility that CB_1 and opioid receptors may interact in modulating acquired SA behaviour. That is, both heroin and naloxone have been found to alter cannabinoid SA (Fattore et al., 2002; Spano et al., 2004) while pretreatment with WIN 55,212-2 (0.25, 0.5 and 1 mg/kg, i.p.) dose-dependently attenuates heroin SA (0.03 mg/kg/inj) in rats under a continuous (FR-1) schedule of reinforcement and nose-poking as operandum (Fattore et al., 2002). Intriguingly, an acute injection of SR 141716A (3 mg/kg, i.p.) has been reported to reduce heroin SA (0.06 mg/inj) in rats under a fixed (FR-5) schedule of reinforcement and lever-pressing as operandum (Navarro et al., 2001). Accordingly, SR 141716A (1 and 3 mg/kg, i.p.) dose-dependently reduces nose-poke responding for heroin (0.05 mg/kg/inj) on the FR-5 schedule and to a greater extent on the progressive ratio (PR) schedule in rats (De Vries et al., 2003).

Help for understanding the specific effect of the blockade of CB_1 receptors on heroin SA arises from a well-designed study of Solinas et al. (2003) demonstrating how the reinforcing efficacy of heroin are differentially decreased by SR 141716A depending on the number of responses required for each injection (i.e., price for the drug). Indeed, SR 141716A markedly decreases heroin intake under the PR schedule at heroin doses ranging from 12.5 to 100 μg/kg/inj, has no effect on heroin SA under the FR-1 schedule at heroin doses of 50 or 100 μg/kg/inj and only slightly decreases responding rate at 25 and 12.5 μg/kg/inj heroin. Finally, Δ^9-THC SA in squirrel monkeys is significantly reduced by daily pre-session treatment with 0.1 mg/kg naltrexone under a fixed-ratio FR-10 schedule of reinforcement (Justinova et al., 2004).

2.4.3. Intracerebral self-administration

Drug intracerebral self-administration (ICSA) is one of the most direct approaches for studying the abuse liability and the rewarding properties of abused drugs, as animals self-administer the drug directly into selected brain areas if it possesses positive reinforcing effect. Although this model provides the possibility of simultaneous choice between the addicting drug and its vehicle as well as the avoidance of peripheral effects, only two studies used this methodological approach for investigating cannabinoid–opioid interaction. The first one demonstrated that the CB_1 receptor agonist CP 55,940 and heroin are intracerebroventricularly (i.c.v.) self-administered in a free-choice procedure by rats (Braida et al., 2001b) and that pretreatment with SR 141716A (0.5 mg/kg, i.p.) or naloxone (2 mg/kg, i.p.) reduces ICSA of both CP 55,940 and heroin. The combination of CP 55,940 with heroin reduces the mean number of drug-associated lever presses compared to that obtained with the maximal reinforcing unit dose of each drug alone. A very recent study from the same group (Braida et al., 2004) further extended these findings by demonstrating that also i.c.v. SA of Δ^9-THC (0.01–0.05 μg/inf) is significantly reduced by both SR 141716A (0.5 mg/kg, i.p.) and naloxone (2 mg/kg, i.p.), thus confirming the existence of a functional cross-talk between the endocannabinoid and opioid systems in reward-related behaviour.

3. Drug dependence

The effects of long-term exposure to cannabinoids have been extensively investigated and the consequences in terms of tolerance, sensitization and dependence are now well known (for a recent review see Tanda and Goldberg, 2003). Contrary to opioids, a clear-cut abstinence syndrome has been rarely reported for Cannabis, presumably because of the long half-life of cannabinoids, which precludes the emergence of abrupt abstinence symptoms (Compton et al., 1990, 1996; Smith, 2002). Somatic signs of spontaneous withdrawal from chronic Δ^9-THC are difficult to observe in rodents, pigeons, dogs and monkeys, even at high cannabinoid doses (Diana et al., 1998; Aceto et al., 2001), although a distinct abstinence syndrome, characterized in rodents by increased grooming, wet dog and head shakes, hunched-back posture, front paw or body tremor,

hypolocomotion, ataxia, ptosis, piloerection, mastication, licking, rubbing and scratching can be precipitated in animals treated with cannabinoids over a long period (Aceto et al., 1996, 2001; Hutcheson et al., 1998; Tzavara et al., 2000).

Indeed, SR 141716A precipitates both paw tremors and head shakes in four different mouse strains repeatedly exposed to Δ^9-THC (Lichtman et al., 2001). In humans, spontaneous abstinence signs, such as nervousness, tension, restlessness, sleep disturbances and anxiety, have been observed after abrupt termination of long-term cannabinoid administration (Mendelson et al., 1984; Wiesbeck et al., 1996). However, all these symptoms are of smaller intensity than those observed with opioids, since neither dominant behavioural signs, such as jumping, or autonomic signs, such as lacrimation or diarrhoea, which are considered highly indicative of the severity of the withdrawal response, are observed in SR 141716A-precipitated withdrawal syndrome (Tsou et al., 1995; Hutcheson et al., 1998).

First evidence for similarities and interactions between central opioid and endocannabinoid systems with reference to dependence-related phenomena (i.e., withdrawal, tolerance, sensitization) dates middle '70, when it was reported that administration of Δ^9-THC attenuates naloxone-induced abstinence in morphine-dependent rats (Hine et al., 1975) and mice (Bhargava, 1976), whereas rats chronically treated with cannabinoids show opioid-like withdrawal signs following acute naloxone administration (Kaymakcalan et al., 1977).

Since then, numerous research groups demonstrated a reciprocal relationship between cannabinoid and opioid systems in drug dependence (Lichtman and Martin, 2002). For example, an important study by Lichtman et al. (2001) showed that SR 141716A-precipitated Δ^9-THC withdrawal is ameliorated in μ-opioid KO mice compared with the WT control animals and fails to occur in CB_1 KO mice. Moreover, a single administration of morphine dose-dependently decreases both paw tremors and head shakes in Δ^9-THC-dependent mice undergoing SR 141716A-precipitated withdrawal.

The same authors also reported that Δ^9-THC dose-dependently blocks paw tremors and head shakes in morphine-dependent mice undergoing naloxone-precipitated withdrawal, and that naloxone-precipitated morphine withdrawal is significantly decreased in CB_1 KO mice and fails to occur in μ-opioid KO morphine-dependent mice (Lichtman et al., 2001).

Among the evidence for a role of opioids in cannabinoid dependence, of particular relevance is the finding that SR 141716A-precipitated withdrawal syndrome in Δ^9-THC-dependent mice is significantly attenuated in mutant pre-proenkephalin-deficient mice (Valverde et al., 2000b), indicating that the endogenous enkephalinergic system is involved in the expression of cannabinoid abstinence. Moreover, the somatic manifestations of Δ^9-THC withdrawal syndrome has been reported to be reduced in double μ- and δ-opioid receptor KO mice, suggesting that a cooperative action of μ- and δ-opioid is essential for the entire expression of cannabinoid dependence (Castañé et al., 2003).

A large body of evidence points to a role of the endocannabinoid system in opioid dependence, the first being those demonstrating an attenuation of precipitated abstinence in methadone-dependent rats by Δ^9-THC (Deikel and Carder, 1976; Hine et al., 1975). Noteworthy are also the findings that anandamide (5 mg/kg, i.v.) decreases naloxone-precipitated withdrawal signs (i.e., jumping and body weight loss) in morphine-dependent mice (Vela et al., 1995a) and that morphine-dependent rats show withdrawal signs following SR 141716A administration (Navarro et al., 1998). This latter effect is not elicited through a direct interaction of SR 141716A with the μ-opioid receptor, this compound being unable to displace opioid receptor ligands in rat brain membranes (Rinaldi-Carmona et al., 1996). More probably, it is related to the reported convergence of signal transduction mechanisms coupled to both receptors systems (Reisine and Brownstein, 1994; Howlett, 1995; Sim et al., 1996a,b). Furthermore, the teeth chattering sign, which is of maximum intensity when an opiate antagonist is injected in the locus coeruleus (LC) (Maldonado et al., 1992), an area virtually devoid of cannabinoid receptors (Herkenham et al., 1991; Matsuda et al., 1993), is not observed after the SR 141716A-induced withdrawal.

In addition, the role of the endocannabinoid system in naloxone-precipitated morphine withdrawal has been examined through both the use of mutant mice and long-term CB_1 receptor antagonist administration in morphine pellet implanted rats. Thus, it was demonstrated that (i) the severity of the morphine withdrawal syndrome is strongly reduced in CB_1 KO mice (Ledent et al., 1999), (ii) Δ^9-THC withdrawal signs are minimally modified in mice lacking μ-, δ- or κ-opioid receptor genes, whereas (iii) SR 141716A chronic treatment does not influence the development of tolerance to the morphine analgesic effect but significantly reduces the intensity of naloxone-induced opiate withdrawal in tolerant rats (Rubino et al., 2000).

These results suggest that the pharmacological treatment with SR 141716A could be of some interest in ameliorating opiate withdrawal syndrome. Accordingly, changes in the specific binding for CB_1 receptors in the brain of morphine-dependent rats occur in regions, such as the midbrain and the cerebral cortex (Gonzalez et al., 2003), strongly implicated in drug dependence, thus ventilating the hypothesis that pharmacological manipulation of the endocannabinoid system might be of help in reducing opioid addiction.

Finally, a reduction in the incidence of two main signs of abstinence, wet dog shakes and jumping, was found when SR 141716A was co-administered with morphine for 5 days and the withdrawal syndrome precipitated by naloxone administration (Mas-Nieto et al., 2001). In contrast, an acute

injection of the CB_1 antagonist just before naloxone administration is unable to modify the incidence of withdrawal signs, suggesting that only chronic blockade of CB_1 receptors is able to reduce morphine-induced physical dependence. The lack of a complete spectrum of opiate abstinence signs after acute SR 141716A injection in opiate-dependent animals may be related to the fact that the central and peripheral distribution of CB_1 receptors does not exactly match that of the μ-receptor (Matsuda et al., 1993; Delfs et al., 1994). Thus, the peripheral secretory signs, especially diarrhoea, which are characteristic features of opiate withdrawal, do not appear after acute CB_1 receptor blockage, suggesting that CB_1 receptors do not interact with peripheral opioid receptors.

The neuroadaptive processes that contribute to the development of cannabinoid dependence remain to be elucidated, although they are likely to involve activation of the hypothalamic–pituitary–adrenal (HPA) axis (Kubena et al., 1971; Rodriguez de Fonseca et al., 1995). Remarkably, CB_1 and μ-opioid receptor mRNAs are co-localized in brain areas relevant for opiate withdrawal such as the nucleus accumbens (NAcc), septum, dorsal striatum, the central amygdaloid nucleus and the habenular complex (Navarro et al., 1998). In addition, naloxone significantly diminishes the increase of adrenocorticotrophic hormone (ACTH) and corticosterone induced by Δ^9-THC (Manzanares et al., 1999), leading to the proposal that CB_1 cannabinoid receptors may play a role in the neuroadaptive processes associated with opiate dependence. Finally, an increased adenylyl cyclase (AC) activity after chronic treatment with agonist of $G_{i/o}$-coupled receptors, a phenomenon referred to as AC superactivation or sensitization, has been described for both the opioid and CB_1 receptors (Chan and Wong, 1999; Rhee et al., 2000).

Since chronic opiate treatment desensitizes μ-opioid receptor coupling to G-proteins and up-regulates adenylate cyclase (Reisine and Brownstein, 1994; Sim et al., 1996a), it is reasonable to hypothesize that CB_1 receptor blockage might indirectly activate the production of cAMP through the release of the inhibitory endogenous cannabinoid tone acting on this transduction system.

Accordingly, Rubino et al. (1997b) show that chronic morphine results in increased expression of CB_1 mRNA and binding sites in the rat dorsal striatum, supporting the hypothesis of dynamic changes occurring in the CB_1 receptors as a result of the development of opiate dependence. Indeed, the morphine-induced up-regulation of the CB_1 receptor may lead to the enhanced response to SR 141716A-induced CB_1 blockage in opiate-dependent animals, and may result in the manifestation of the withdrawal signs described by Navarro et al. (1998).

However, a second hypothesis may be proposed for functional consequences of a co-expression of CB_1 and μ receptors, which is that such co-localization in the striatum might account for motor signs of opiate withdrawal, whereas that in limbic areas (i.e., hippocampus, amygdala) might account for negative, autonomic and endocrine effects of SR 141716A-induced withdrawal.

As already described for other drugs of abuse (Koob, 1996), elevation in extracellular corticotrophin-releasing factor (CRF) levels and Fos immunoreactivity in the mesolimbic system has been reported during precipitated cannabinoid withdrawal (Rodriguez de Fonseca et al., 1997), and are supposed to mediate the stress-like symptoms and negative effects that accompany cannabinoid abstinence. Moreover, a marked inhibition of mesolimbic DA activity, which is likely related to the aversive and dysphoric consequences of cannabinoid withdrawal, has also been described during cannabinoid abstinence (Diana et al., 1998). Similar to opioids, cannabinoid withdrawal is associated with compensatory changes in the cAMP pathway (Hutcheson et al., 1998), but seems to involve different brain areas, brainstem structures (i.e., LC) being responsible for the somatic signs of opioid withdrawal (Maldonado et al., 1992) while cerebellum the most involved in those of cannabinoid withdrawal (Hutcheson et al., 1998).

Whatever the hypotheses, all these findings unequivocally implicate a reciprocal relationship between the cannabinoid and opioid systems in drug dependence processes.

4. Tolerance

For long time it has been considered that Cannabis does not produce tolerance. Lemberger et al. (1971), after injecting radioactively labelled (C^{14})Δ^9-THC intravenously to chronic marijuana smokers and naïve subjects, found that non-smokers did not report any pharmacological effect while all of the long-term marijuana smokers reported effects lasting up to 90 min.

However, it is now widely accepted the view that tolerance develops to many effects of cannabinoids in both laboratory animal and human beings. Indeed, ataxia in the dog, ptosis of eyelids in the monkey and tachycardia in man (which are the most characteristic effects of Cannabis use) loose their intensity after repeated administration indicating development of tolerance (for reviews see Compton et al., 1990; Adams and Martin, 1996; Ameri, 1999).

The first report of tolerance to the discriminative stimulus effect of Δ^9-THC is far as 1974, where it was reported a reduced degree of discrimination in rats following 2 months of discrimination task (Hirschhorn and Rosecrans, 1974). Several studies also showed development of tolerance to cannabinoid effects on antinociception, hypothermia, gastrointestinal transit, body weight, anticonvulsant activity and corticosterone release (Abood and Martin, 1992). However, tolerance is maximal after short-term cannabinoid treatment (Bass and Martin, 2000).

In cannabinoid tolerance, only a minor role seems to be played by pharmacokinetic factors, such as changes in drug absorption, distribution and excretion (Dewey et al., 1972;

Siemens and Kalant, 1974; Martin et al., 1976). On the contrary, more important appear to be some pharmacodynamic parameters, such as a down-regulation of CB_1 receptors (Rodriguez de Fonseca et al., 1994) and a decrease in mRNA levels for CB_1 receptors (Romero et al., 1998a) or $G_{\alpha i}$- and $G_{\alpha s}$-proteins (Rubino et al., 1997a), these latter being related to desensitization of CB_1 receptors (Sim et al., 1996a). Autoradiographic studies revealed a time-dependent down-regulation and desensitization of CB_1 receptors following chronic Δ^9-THC or CP 55,940 treatment, with decreases in ligand binding and receptor binding being related to a reduction in the number of binding sites and CB_1-activated G-proteins (Oviedo et al., 1993; Breivogel et al., 1999). Interestingly, the pattern of this down-regulation process displays significant regional differences with regard to the onset, the rate of development and the magnitude of the adaptive responses (Romero et al., 1998b; Sim-Selley, 2003). However, somewhat contradictory results have also been reported, such as an increased binding following chronic Δ^9-THC administration (Romero et al., 1995) and the absence of either increased cannabinoid binding or mRNA levels (Abood et al., 1993), although this latter study was conducted in whole brain cells instead of specific brain regions.

Very recently, an involvement of protein kinase A and Src family kinases pathways in cannabinoid tolerance has also been postulated (Martin et al., 2004), although it is still to be verified whether these kinases contribute to the development of tolerance by a direct regulation of CB_1 receptors rather than a modulation of additional signalling pathways.

Cross-tolerance between cannabinoids and opioids is well documented as well, although data are somewhere discordant (Thorat and Bhargava, 1994). Δ^9-THC and morphine show cross-tolerance in nociception and cardiac rhythm in mice (Hine, 1985); accordingly, a cross-tolerance between CB_1 and κ-opioid receptor has been described (Rowen et al., 1998).

Interestingly, the development of tolerance to the analgesic responses induced by Δ^9-THC was slower in pre-proenkephalin KO mice in respect to WT controls (Valverde et al., 2000b). When antinociceptive and hypothermic effects of morphine and Δ^9-THC were examined, Δ^9-THC-tolerant animals were found tolerant to the hypothermic but not antinociceptive action of morphine whereas morphine-tolerant animals were tolerant to the antinociceptive but not hypothermic action of the cannabinoid (Bloom and Dewey, 1978). However, other studies reported no modification (Martin, 1985) or even a potentiation (Melvin et al., 1993) of cannabinoid antinociception in morphine-dependent rats.

Moreover, no cross-tolerance between the antinociceptive effects of morphine and Δ^9-THC was detected in pathological pain states (Mao et al., 2000). Finally, cross-tolerance between morphine and the CB_1 agonist WIN 55,212-2 was observed in the guinea pig ileum, the myenteric plexus-longitudinal muscle exposed to WIN 55,212-2 being less sensitive to the inhibitory effect of morphine on the electrically evoked contractions (Basilico et al., 1999). Vice versa, following incubation with morphine, the myenteric plexus-longitudinal muscle was less sensitive to the inhibitory effect of WIN 55,212-2.

An autoradiographic study of CB_1 receptor binding and WIN 55,212-2-stimulated [^{35}S]GTPγS binding in morphine-dependent mice supports the potential existence of a specific effect of morphine in the coupling of CB_1 receptors to GTP-binding proteins, rather than on receptor binding, with the only exception observed in the substantia nigra and central grey substance (Romero et al., 1998c).

Interaction between CB_1 cannabinoid and κ_1-opioid receptors has been proposed as part of the processes underlying cross-tolerance expression between cannabinoids and opioids (Rowen et al., 1998), at least in the production of antinociception, although the exact mechanisms still remain to be elucidated. To this regard, a preliminary investigation on the mechanisms underlying cannabinoid tolerance in the mouse vas deferens excluded the hypothesis of the occurrence of a down-regulation of μ-, κ- or δ-opioid receptors (Pertwee and Griffin, 1995).

5. Behavioural sensitization

Besides tolerance and dependence, repeated exposure to cannabinoids induces behavioural sensitization (Cadoni et al., 2001; Rubino et al., 2001), which cellular mechanisms started to be clarified only recently (Rubino et al., 2003).

Chronic cannabinoid administration also produces cross-sensitization to the locomotor effects of psychostimulants (Gorriti et al., 1999) and opioids (Pontieri et al., 2001a,b). Indeed, pre-exposure to the CB_1 receptor agonist CP 55,940 enhances morphine behavioural sensitization in rats (Norwood et al., 2003). Cross-sensitization between opioids and cannabinoids is rather symmetrical since rats behaviourally sensitized to morphine are also sensitized to cannabinoids (Cadoni et al., 2001). Indeed, it has been reported that rats previously exposed to Δ^9-THC show a greater behavioural activation characterized by stereotyped activity compared to controls in response to challenge with both Δ^9-THC and morphine. On the other hand, animals behaviourally sensitized to morphine also show a behavioural sensitization to challenge with Δ^9-THC and WIN 55,212-2, an effect which is prevented by SR 141716A administration.

Moreover, heroin administration to vehicle-treated rats produced catalepsy, while the same dose of heroin in WIN 55,212-2-treated rats is followed by a marked increase of locomotor activity with stereotyped and non-stereotyped behaviours (Pontieri et al., 2001a,b). Both SR 141716A and naloxone reverse these effects (Pontieri et al., 2001a,b). These findings indicate that repeated exposure to heroin

produces neuroadaptative changes in brain circuits that contribute to mediate the behavioural consequences of acute administration of WIN 55,212-2.

It has been also reported that in mice lacking the CB_1 receptor, the hyperlocomotion induced by acute morphine administration is preserved, but the sensitization to this locomotor response induced by chronic morphine treatment is abolished (Martin et al., 2000). In addition, chronic treatment with Δ^9-THC results not only in tolerance to the initial hypothermic and anorexic effects, but also increases the locomotor responses to amphetamine and heroin. This cross-sensitization is time-dependent as it is observed 3 days after the last injection of Δ^9-THC for amphetamine, and a relatively long time after the end of chronic treatment for heroin (Lamarque et al., 2001).

6. Relapse to drug-seeking

Very recently, unambiguous evidence for a functional link between cannabinoid and opioid endogenous systems has been provided in relapse to drug-seeking behaviour in rats following a prolonged period of drug abstinence (Fattore et al., 2003; De Vries et al., 2003). This topic is widely reviewed by De Vries and colleagues elsewhere in this issue.

However, a latest study by Spano et al. (2004) further extended previous findings by demonstrating that cannabinoid-seeking behaviour is reinstated following long drug abstinence by an acute injection with heroin. Indeed, in rats previously trained to intravenously self-administer the synthetic CB_1 receptor agonist WIN 55,212-2 (12.5 μg/kg/inf) under a fixed ratio (FR-1) schedule of reinforcement, non-contingent non-reinforced intraperitoneal (i.p.) priming injections of heroin (0.5 mg/kg), but not cocaine (10 mg/kg), effectively reinstated cannabinoid-seeking behaviour following 3 weeks of extinction.

Importantly, SR 141716A (0.3 mg/kg, i.p.) did not reinstate responding when given alone but completely prevented cannabinoid-seeking behaviour triggered by heroin primings. Similarly, naloxone (1 mg/kg, i.p.) had no effect on operant behaviour per se but significantly blocked cannabinoid-induced reinstatement of cannabinoid-seeking behaviour. Thus, this latter study (Spano et al., 2004) corroborates the role of the endocannabinoid system in the central mechanisms triggering reinstatement of extinguished drug-seeking behaviour and provides evidence for the bidirectionality of cannabinoid–opioid interactions in modulating central mechanisms underlying relapse.

7. Does prenatal and perinatal cannabinoid exposure render an individual more vulnerable to opioid abuse?

In laboratory animals, exposure to cannabinoids at foetal stage or during the earliest days of life affects several behavioural responses, such as opiate self-administration behaviour or pain sensitivity, which can be directly related to changes in opioidergic neurotransmission. For example, administration of naloxone to rats perinatally exposed to Δ^9-THC produced withdrawal symptoms resembling those observed in opiate-dependent rats (Vela et al., 1995a). Accordingly, perinatal exposure to cannabinoids might have long-term behavioural consequences on the endogenous opioid system lasting into adulthood (Ambrosio et al., 1999), such as an altered functioning of the endogenous opioid system (Kumar et al., 1990) and an increased susceptibility to the reinforcing properties of morphine (Martin et al., 1996; Rubio et al., 1998; Vela et al., 1995b, 1998).

These findings are of great importance considering that Cannabis preparations (hashish, marijuana) still remain the most widely used illicit drugs during pregnancy in western countries (Day et al., 1994; Fried, 1995a,b). Cannabinoids can be transferred from the mother to the offspring through placental blood during gestation (Hutchings et al., 1989) and through maternal milk during lactation (Jakubovic et al., 1977). Therefore, they may interfere as epigenetic factors with the rigidly ordered temporal sequences of events that occur during the ontogeny of the brain, leading to the onset of neurodevelopmental alterations (Mirmiran and Swaab, 1987).

Several mechanisms have been proposed in the elucidation of the cannabinoid behavioural teratology, including changes in opioid peptides and their receptors (Kumar et al., 1990), reduction of proenkephalin gene expression (Corchero et al., 1998; Perez-Rosado et al., 2000), prenatal stress-like effects (Rubio et al., 1995), direct effects on developing monoaminergic systems (Walters and Carr, 1986, 1988; Rodriguez de Fonseca et al., 1991; Bonnin et al., 1994; Navarro et al., 1996) or activation of brain cannabinoid receptors that are present at birth (Rodriguez de Fonseca et al., 1993).

Similarly to pre/perinatal exposure to cannabinoids, pre-exposure to CP 55,940 during adulthood results in enhanced morphine behavioural sensitization and altered morphine self-administration (Norwood et al., 2003). Repeated administration of Δ^9-THC alters μ-opioid receptor density in several brain areas (Corchero et al., 2004) as well as ACTH and corticosterone plasma concentrations (Manzanares et al., 1999). Following chronic cannabinoid exposure, sex differences have been reported in the expression of several pharmacological and behavioural effects of opioids (Ambrosio et al., 1999; Gonzalez et al., 2003). Among them, different proenkephalin gene expressions in the CP, hypothalamic nuclei and cerebral cortex of rat foetuses (Perez-Rosado et al., 2000) and adults (Corchero et al., 2002) were observed.

Basing on preclinical evidence, recent studies suggested that cannabinoids might initiate the consumption of other highly addictive substances, including opiates. However, chronic use of high doses of cannabinoids does not seem to

potentiate the psychic dependence to opioids (Valverde et al., 2001; Gonzalez et al., 2004). From clinical studies it is known that oral or smoked Δ^9-THC consistently induces changes in mood, usually euphoria, while higher doses are psychotomimetic and may produce marked distortion in visual and auditory perception (Isbell et al., 1967). However, only two clinical trials have been conducted to date for verifying the effect of opioid treatment on subjective responses to Δ^9-THC, but they reported opposite results and therefore do not help to unravel such an intricate issue (Wachtel and de Wit, 2000; Haney et al., 2003).

8. Cannabinoid–opioid interaction: possible mechanisms of action

To explain the possible link in the mechanisms of action of opiates and cannabinoids, several explanations have been proposed, the first of which hypothesizes that cannabinoids and opioids may interact at post-receptorial level. This hypothesis is based on the fact that receptors for both opioids and cannabinoids are coupled to similar intracellular signalling mechanisms, mainly through a decrease in cAMP production through G_i-proteins. Thus, when CB_1 and opioid receptors co-localize on the same neurones (i.e., in the CP, dorsal hippocampus, substantia nigra), they might compete for the same pool of G_i-proteins (Bidaut-Russell et al., 1990; Childers et al., 1992; Shapira et al., 2000). Hence, despite the absence of a decrease in receptor binding, cross-tolerance might be possible through a decrease in the efficiency of agonist-induced receptor activation, thus involving alterations in signal transduction.

In support of this idea is the fact that CB_1 cannabinoid receptors can sequester G-proteins from a common pool and prevent other G-protein-coupled receptors from signalling (Vasquez and Lewis, 1999). Accordingly, animals chronically exposed to morphine exhibit adaptative changes in adenylate cyclase-coupled G-proteins (Nestler et al., 1989; De Vries et al., 1991; Nestler, 1992). This could affect the efficiency of the activation of other receptors also coupled to G_i- and G_o-proteins as cannabinoids, thus explaining the attenuating effect of Δ^9-THC on naloxone-precipitated withdrawal signs in morphine-dependent animals (Hine et al., 1975a,b; Bhargava, 1976).

Alternatively, it has been proposed that cannabinoids may stimulate synthesis and release of endogenous opioid peptides. If true, it would explain the antinociceptive effects of cannabinoids and the ability of opioid receptor antagonists to block some effects of Δ^9-THC (Gardner and Lowinson, 1991) as well as to induce withdrawal signs in Δ^9-THC tolerant rats (Kaymakcalan et al., 1977). Among numerous evidence supporting this hypothesis are studies demonstrating that (i) Δ^9-THC increases the expression of opioid peptide precursors (prodynorphin and proenkephalin) in the spinal cord and proopiomelanocortin in the hypothalamus (Corchero et al., 1997a,b); (ii) administration of CP 55,940 through spinal catheter enhances the release of dynorphin B concurrent with the production of the antinociceptive effect in rats (Pugh et al., 1996, 1997; Houser et al., 2000); (iii) perinatal cannabinoid exposure induces long-lasting functional effects on the endogenous opioid system, in particular changes in the levels of met-enkephalin and β-endorphin (Kumar et al., 1990); (iv) Δ^9-THC increases the release of endogenous enkephalins in the NAcc of awake, freely moving rats (Valverde et al., 2001).

9. Conclusions

The reviewed data clearly demonstrate the existence of a specific functional interaction between cannabinoids and opioids in the modulation of behavioural responses linked to reward- and relapse-related phenomena. Because the interplay between endogenous cannabinoid and opioid systems is complex, the present review may obviously still not fully explain the effects of cannabinoids on inputs to, processing within, and output from the opioid circuit. A complete picture will emerge only once the effects of cannabinoids on each brain area and their relative contribution on behavioural output are elucidated.

To date, against the growing number of preclinical researches investigating cannabinoid–opioid interaction stands the paucity of clinical studies, which renders understanding of endocannabinoid system involvement in opioid addiction more intricate. Another limiting point in relating preclinical data to human conditions is given by the fact that most of the behavioural animals models employs synthetic CB_1 receptor agonists (i.e., WIN 55,212-2, CP 55,940, HU 210), which displayed higher potency and affinity than the natural compound. In addition, Δ^9-THC possesses a specific and particular pharmacokinetic and pharmacodynamic profile, and often fails in sustaining operant or conditioned behaviours in animal models, thus complicating comparison between preclinical results and human situations.

However, evidence that cannabinoids can readily interact with the opioid system in the modulation of drug reward and abuse is therapeutically promising and opens new strategies for the treatment of opiate abuse and dependence. The individuation of central mechanisms underlying reciprocal modulation of pharmacological effects induced by these two classes of drug is the next challenge for the field.

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