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Study of cannabinoid dependence in animals

Rafael Maldonado*

Laboratori de Neurofarmacologia, Facultat de Ciencès de la Salut i de la Vida, Universitat Pompeu Fabra, C/Dr Aiguader 80, 08003 Barcelona, Spain

Abstract

Different animal models have been used to clarify the consequences of chronic exposure to cannabinoid agonists and their abuse liability. Following the chronic administration of cannabinoids, tolerance develops to most of their pharmacological effects. The development of cannabinoid tolerance is particularly rapid, and seems to be due to pharmacodynamic events. A cross-tolerance among different exogenous cannabinoid agonists has been reported. Somatic signs of spontaneous withdrawal have not been reported after chronic Δ^9-tetrahydrocannabinol (THC) treatment, but were observed after chronic treatment with the cannabinoid agonist WIN-55,212-2. The administration of the CB_1 cannabinoid antagonist SR141716A in animals chronically treated with THC and other cannabinoid agonists precipitated somatic manifestations of withdrawal. The potential ability of anandamide to induce physical dependence has not been clarified. Subjective drug effects of cannabinoids have been reported by drug discrimination studies, which show cross discrimination among different natural and synthetic agonists. The rewarding effects of cannabinoids have been revealed by using several paradigms: place conditioning, intracranial self-stimulation, and self-administration. Cannabinoids have been reported to lower intracranial self-stimulation thresholds in rats. However, particular experimental conditions are required to induce conditioned place preference with cannabinoids. Numerous studies have shown that THC is unable to induce a self-administration behaviour in animals. However, WIN-55,212-2 was intravenously self-administered in mice, and monkeys that had a previous history of cocaine self-administration also self-administered THC. The mesolimbic dopaminergic system seems to be the substrate for the rewarding properties of cannabinoids.
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Keywords: Tolerance; Dependence; Withdrawal; Reward; Drug discrimination; Reinforcement

Abbreviations: cAMP, cyclic AMP; CRF, corticotropin-releasing factor; THC, Δ^9-tetrahydrocannabinol.

Contents

1. Introduction

Derivatives of *Cannabis sativa*, such as marijuana and hashish, are the most widely consumed illicit drug in humans.

* Tel.: +34-93-542-2845; fax: +34-93-542-2802.
E-mail address: rafael.maldonado@cexs.upf.es (R. Maldonado).

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A large number of psychoactive compounds have been identified in *C. sativa* preparations, and Δ^9-tetrahydrocannabinol (THC) constitutes its main psychoactive component. Cannabinoid compounds induce their pharmacological effects by activating two different receptors that have been identified and cloned: the CB_1 cannabinoid receptor, which is highly expressed in the CNS (Devane et al., 1988), and the CB_2 cannabinoid receptor, which is localized in the peripheral tissues, mainly at the level of the immune system (Munro et al., 1993). Both are seven transmembrane domain receptors coupled to G-proteins, and through these proteins, cannabinoid receptor activation produces an inhibition of adenylyl cyclase activity (Howlett, 1984; Howlett & Fleming, 1984). The stimulation of cannabinoid receptors also modifies the activity of other second messenger systems producing activation of mitogen-activated protein kinases (Bouaboula et al., 1995) and inhibition of Ca^{2+} influx (Felder et al., 1992). The development of potent and selective synthetic cannabinoid agonists, as well as selective cannabinoid antagonists, has played a major role in the recent advances obtained on cannabinoid pharmacology.

The potential ability of cannabis derivatives to produce dependence in humans is still a controversial issue. Several authors have reported that cannabis derivatives do not induce physical dependence or an abstinence syndrome in humans, while others describe some symptoms of abstinence in heavy users of strong cannabis preparations (Hollister, 1986). The rarity of reports of these withdrawal reactions may reflect the fact that they are mild and seldom observed in humans. However, cannabis derivatives produce clear subjective motivational responses in humans, leading to drug-seeking behaviour and abuse. Many studies have used different animal models to clarify the consequences of chronic exposure to cannabinoid agonists and the abuse liability of these compounds.

Processes involved in substance abuse are neurobiologically and behaviourally complex. Tolerance and withdrawal syndrome are adaptive responses to the prolonged exposure of neurons to different drugs, but they provide only a partial correlate of their addictive properties. The main factor common to all drugs of abuse is their ability to induce drug-seeking behaviour, which is due to the positive reinforcing effects of the drugs. Several behavioural models have been used to evaluate motivational responses of drugs of abuse that could be related to their addictive properties. The first behavioural model allowing the evaluation of the presence or absence of subjective CNS effects of a compound is the drug discrimination paradigm. However, this paradigm does not permit the definition of the characteristics of such a subjective effect. Indirect indices of reinforcement can be evaluated through the ability of a drug to modulate the reinforcing properties of other rewards (e.g., intracranial self-stimulation techniques) or to impart reinforcing properties on previously neutral stimuli or environments (e.g., the place-conditioning paradigm). Drug reinforcement can also be directly evaluated by using operant self-administration paradigms (Schulteis et al., 1997). Biochemical and electrophysiological studies can also be helpful to clarify the potential addictive properties of drugs of abuse. Indeed, these kinds of studies have identified the mesolimbic dopaminergic system as the common neuronal substrate for the motivational and rewarding properties of most drugs of abuse (Koob, 1992). Results obtained by using cannabinoid ligands on all these experimental models have provided important progress toward a better understanding of the potential capability of cannabinoids to induce dependence and the neurobiological mechanisms involved.

2. Tolerance

The chronic administration of different cannabinoid agonists produces the development of tolerance to most of their pharmacological effects. Indeed, several studies have shown tolerance to cannabinoid effects on antinociception (Martin, 1985; Hutcheson et al., 1998), locomotion (Magour et al., 1977; Karler et al., 1984; Abood et al., 1993; Hutcheson et al., 1998), hypothermia (Thompson et al., 1974; Hutcheson et al., 1998), catalepsia (Pertwee, 1974), the suppression of operant behaviour (Kosersky et al., 1974; Lamb et al., 2000), gastrointestinal transit (Anderson et al., 1974), body weight (Hutcheson et al., 1998), the cardiovascular system (Dewey et al., 1972; Birmighan, 1973; Adams et al., 1976), anticonvulsant activity (Colasanti et al., 1982), ataxia (Martin et al., 1976), and corticosterone release (Miczek & Dihit, 1980). This tolerance has been reported in rodents and also in pigeons, dogs, and monkeys (Abood & Martin, 1992). The development of cannabinoid tolerance is particularly rapid, and a marked decrease of the acute response is already observed after the second administration of a cannabinoid agonist (McMillan et al., 1971; Abood & Martin, 1992; Hutcheson et al., 1998). Cannabinoid tolerance peaks soon after the start of chronic cannabinoid treatment. Thus, the degree of tolerance observed after 7 days of treatment with a high dose of THC (10 mg/kg) was higher than the tolerance detected 13 days after chronic THC administration (Bass & Martin, 2000).

Several studies have suggested the involvement of pharmacokinetic mechanisms on the development of cannabinoid tolerance, such as changes in drug absorption, distribution, biotransformation, and excretion. However, the role of such a pharmacokinetic mechanism, if any, seems minor (Dewey et al., 1973; Martin et al., 1976; Magour et al., 1977). In contrast, pharmacodynamic events seem to play a crucial role on cannabinoid tolerance. Indeed, a significant decrease in the total number of CB_1-binding sites (B_{max}) has been reported in several brain structures, such as the striatum, limbic system, cortex, and cerebellum, during chronic administration of cannabinoid agonists (Oviedo et al., 1993; Rodriguez de Fonseca et al., 1994; Fan et al., 1996; Rubino et al., 1998, 2000b, 2000c). Levels of mRNA coding for the CB_1 cannabinoid receptor have also been reported to

decrease in several brain areas, mainly in the anterior portion of the brain, during chronic administration of the cannabinoid agonist CP-55,940 (Rubino et al., 1994) and THC (Romero et al., 1998a). Changes of mRNA levels appeared after the decrease of CB_1 binding, and in several brain structures, loss of CB_1 receptor binding was not accompanied by changes in its mRNA expression (Romero et al., 1998a). This decrease in the density and expression of CB_1 cannabinoid receptors could play an important role in the development of cannabinoid tolerance. However, other studies have reported no changes on CB_1 cannabinoid receptor-binding properties and mRNA expression during chronic THC administration (Abood et al., 1993), and even an increase in the number of CB_1-binding sites has been reported in the hippocampus and the cerebellum (Romero et al., 1995). An increased expression of CB_1 cannabinoid receptor mRNA has also been reported in the cerebellum after chronic THC (Zhuang et al., 1998) or CP-55,940 administration (Fan et al., 1996), but other studies have not found changes in CB_1 cannabinoid receptor mRNA in this brain structure (Romero et al., 1997).

Alteration in G-protein expression or functional activity is another cellular event that can be correlated with cannabinoid tolerance. Thus, a widespread decrease in mRNA levels of $G_{\alpha i}$- and $G_{\alpha s}$-proteins has been reported in rats chronically treated with the cannabinoid agonist CP-55,940. A more localized reduction of $G_{\alpha o}$-proteins was also observed in these tolerant rats. The alterations in G-protein α-subunit gene expression were not followed by any change in the amount of proteins (Rubino et al., 1997). Changes in G-protein expression have been postulated to be related to desensitization of CB_1 cannabinoid receptors. Accordingly, a strong reduction of cannabinoid agonist-stimulated [^{35}S]GTPγS binding has been obtained in most brain regions of rats chronically treated with THC (Sim et al., 1996) or CP-55,940 (Rubino et al., 2000b). A similar desensitization of CB_1 receptors revealed by a decrease in cannabinoid agonist-stimulated [^{35}S]GTPγS binding has been reported during the development of tolerance after chronic anandamide administration. Binding properties of CB_1 cannabinoid receptors were not modified in anandamide-tolerant animals (Rubino et al., 2000a).

Most of the changes induced in mRNA expression of CB_1 cannabinoid receptors and G-protein α-subunit genes by chronic CP-55,940 administration recovered their basal level of expression within the first hours after withdrawal. Only the expression of $G_{\alpha s}$ and $G_{\alpha i}$ remained low after the first hour of withdrawal, but these levels rebounded after 96 and 24 hr, respectively (Rubino et al., 1998). Other studies have also shown that changes induced by chronic THC administration on CB_1 cannabinoid receptors are reversible, even when animals were treated long-term (3 months in rats and 7 months in monkeys) (Westlake et al., 1991). In agreement with these results, tolerance to the pharmacological effects of THC were shown to disappear from 7 to 11 days after the end of a chronic cannabinoid treatment (Bass & Martin, 2000).

Several studies have revealed cross-tolerance for the main pharmacological effects of different exogenous CB_1 cannabinoid agonists, i.e., for antinociception, hypolocomotion, catalepsia, and hypothermia (Pertwee et al., 1993; Fan et al., 1994). Controversial results have been reported on the possible cross-tolerance between anandamide and other cannabinoid agonists. Thus, chronic anandamide administration induced tolerance in female mice and cross-tolerance to the antinociceptive, hypolocomotor, cataleptic, and hypothermic effects of THC (Fride, 1995). However, cross-tolerance between anandamide and several CB_1 cannabinoid agonists, including THC, has not been observed in male mice with respect to the hypothermic effects (Pertwee et al., 1993). Additionally, anandamide and THC did not show cross-tolerance in the rat for the antinociceptive responses (Welch, 1997).

The presence of cross-tolerance between opioid and cannabinoid compounds has also been revealed in several studies. Thus, THC and morphine elicit cross-tolerance in mice for the effects induced on nociception (Hine, 1985; Thorat & Bhargava, 1994) and cardiac rhythm (Hine, 1985). However, a potentiation of the antinociceptive effects induced by cannabinoids has also been reported in morphine-dependent rats (Melvin et al., 1993). Cross-tolerance between CB_1 cannabinoid agonists and κ-opioid agonists on antinociceptive responses has also been reported (Smith et al., 1994; Welch, 1997; Rowen et al., 1998). In the same way, the development of THC tolerance was slightly modified in knockout mice deficient in κ-opioid receptors, but was unaltered in knockout mice lacking μ- or δ-opioid receptors (Ghozland et al., 2002). Administration of antisense oligodeoxynucleotides that selectively blocked the expression of κ-opioid receptors increased the development of tolerance induced by chronic THC (Rowen et al., 1998). These results are in agreement with the release of the endogenous κ-opioid agonist dynorphin induced by the acute administration of THC (Welch & Eads, 1999; Houser et al., 2000). However, no correlation between THC-induced dynorphin A release and the development of tolerance to THC antinociceptive effects has been reported during chronic intrathecal THC administration (Mason et al., 1999). Development of tolerance to the antinociceptive effects of anandamide seems to involve different mechanisms than those involved in tolerance to other cannabinoids. Indeed, anandamide-tolerant animals did not show cross-tolerance to the antinociceptive responses induced by μ-, δ-, or κ-opioid agonists, while THC-tolerant animals showed cross-tolerance to κ-opioid agonists under similar experimental conditions (Welch, 1997).

3. Physical dependence

Several studies have reported the absence of somatic signs of a spontaneous withdrawal syndrome after a chronic THC treatment in rodents, pigeons, dogs, and monkeys,

even after the administration of extremely high doses of this compound (McMillan et al., 1970, 1971; Dewey et al., 1972; Chesher & Jackson, 1974; Harris et al., 1974; Leite & Carlini, 1974; Diana et al., 1998; Aceto et al., 2001). Early studies had suggested the presence of somatic manifestations of spontaneous withdrawal syndrome after chronic intravenous administration of high doses of THC in dogs (Kaymakcalan, 1973) and monkeys (Deneau & Kaymakcalan, 1971). However, more recent studies have questioned these results since the behavioural manifestations of spontaneous cannabinoid abstinence were not reversed by a new THC challenge (Abood & Martin, 1992). In spite of the absence of spontaneous withdrawal, a suppression of operant behaviour 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). An absence of spontaneous manifestations of withdrawal has also been reported after chronic treatment with other cannabinoid agonists (Young et al., 1981). However, a recent study has reported somatic signs of spontaneous abstinence after the abrupt interruption of a chronic treatment with the cannabinoid agonist WIN-55,212-2 (Aceto et al., 2001). Differences in the pharmacokinetic properties of THC and WIN-55,212-2 could explain these results. Indeed, the shorter half-life of WIN-55,212-2 compared with THC could produce a rapid decrease in the plasma levels of WIN-55,212-2 after abrupt cessation of the chronic treatment; this should not occur in the case of chronic THC administration.

The administration of the selective CB_1 cannabinoid receptor antagonist SR141716A in animals (mouse, rat, and dog) chronically treated with THC has been shown to precipitate somatic manifestations of cannabinoid withdrawal. In rodents, this cannabinoid withdrawal syndrome is characterized by the presence of a large number of somatic signs and the absence of vegetative manifestations. The most characteristic somatic manifestations of cannabinoid withdrawal in rodents are wet dog shakes, head shakes, facial rubbing, front paw tremor, ataxia, hunched posture, body tremor, ptosis, piloerection, hypolocomotion, mastication, licking, rubbing, and scratching (Tsou et al., 1995; Aceto et al., 1996, 2001; Hutcheson et al., 1998; Cook et al., 1998; Ledent et al., 1999; Tzavara et al., 2000). It is important to point out the dramatic motor impairment occurring during the cannabinoid withdrawal syndrome (Hutcheson et al., 1998; Tzavara et al., 2000). Doses of THC required to induce dependence in rodents are very high, and SR14161716A-precipitated withdrawal is currently reported after chronic administration of doses from 10 to 100 mg/kg daily of THC (Tsou et al., 1995; Aceto et al., 1996, 2001; Hutcheson et al., 1998; Cook et al., 1998; Ledent et al., 1999; Tzavara et al., 2000). In dogs, both somatic and vegetative signs are expressed during SR141716A-precipitated cannabinoid withdrawal. Thus, signs of cannabinoid withdrawal in this animal species include diarrhoea, vomiting, excessive salivation, decreases in social behaviour, and increases in restless behaviour and trembling (Lichtman et al., 1998). SR141716A administration has also been shown to disrupt operant behaviour in rats chronically treated with THC (Beardsley & Martin, 2000). CB_1 cannabinoid receptors are responsible for the somatic manifestations of cannabinoid withdrawal. Thus, SR141716A administration in CB_1 knockout mice receiving a chronic THC treatment did not precipitate any manifestation of cannabinoid abstinence (Ledent et al., 1999).

The potential ability of anandamide to induce physical dependence has not been clarified, and opposite results have been reported. Abrupt cessation of a chronic treatment with high doses of anandamide or SR141716A challenge in rats receiving chronic anandamide did not produce any manifestation of withdrawal (Aceto et al., 1998, 2001). However, another study that has evaluated a large range of behavioural signs revealed a spontaneous abstinence syndrome and SR141716A-precipitated withdrawal in rats chronically treated with anandamide (Costa et al., 2000).

Cannabinoid withdrawal syndrome is associated with compensatory changes in the cyclic AMP (cAMP) pathway. Initially, acute activation of cannabinoid receptors leads to an inhibition of adenylyl cyclase activity (Howlett & Fleming, 1984). In contrast, during SR141716A-precipitated THC withdrawal in mice, basal-, forskolin-, and Ca^{2+}/calmodulin-stimulated adenylyl cyclase activities were selectively increased in the cerebellum. Adenylyl cyclase activities in the frontal cortex, hippocampus, striatum, and periaqueductal gray matter were not modified during cannabinoid withdrawal (Hutcheson et al., 1998). This selective up-regulation of the cAMP pathway in the cerebellum seems to play an important role for the somatic manifestations of cannabinoid withdrawal. Thus, adenylyl cyclase activity in the cerebellum increases in a transient and reversible manner during SR141716A-precipitated THC withdrawal, with a time course that fits the temporal pattern of behavioural manifestations of withdrawal (Tzavara et al., 2000). Furthermore, the somatic manifestations of cannabinoid abstinence are markedly reduced by preventing the activation of protein kinase A by microinfusion of the selective cAMP inhibitor Rp-8Br-cAMPS onto the surface of the cerebellum. The microinfusion of the selective cAMP activator Sp-8Br-cAMPS mimics some behavioural manifestations of cannabinoid withdrawal in naïve animals (Tzavara et al., 2000). Similar increases in cAMP levels and protein kinase A activity have been reported during chronic THC treatment in the cerebellum, striatum, and cortex (Rubino et al., 2000c).

Common features of the withdrawal syndrome to several drugs of abuse, such as opioids, psychostimulants, and ethanol, include an important elevation in extracellular levels of corticotropin-releasing factor (CRF) in the mesolimbic system (Koob, 1996) and a marked inhibition of mesolimbic dopamine activity (Rossetti et al., 1992). Sim-

ilar changes have been reported during cannabinoid withdrawal. Thus, an increased release of CRF and an enhancement of Fos immunoreactivity have been found in the central amygdala during SR141716A-precipitated cannabinoid withdrawal (Rodriguez de Fonseca et al., 1997). This alteration of CRF function in the limbic system may have a motivational role in mediating the stress-like symptoms and negative affect that accompany cannabinoid withdrawal. In agreement with this hypothesis, the spontaneous firing rate of ventral tegmental area dopamine neurons has been reported to be attenuated during cannabinoid abstinence (Diana et al., 1998). This decreased dopaminergic activity also seems to be related to the aversive/dysphoric consequences of cannabinoid withdrawal. However, changes in the activity of the dopaminergic system are not involved in the somatic expression of cannabinoid withdrawal (Sañudo-Peña et al., 1999).

Bi-directional interactions between cannabinoid and opioid dependence have been reported. Thus, administration of the CB_1 cannabinoid antagonist SR141716A is able to precipitate several behavioural and biochemical manifestations of opioid withdrawal in morphine-dependent rats (Navarro et al., 1998). Additionally, the opioid antagonist naloxone precipitated behavioural signs of cannabinoid withdrawal in rats chronically treated with cannabinoid agonists (Kaymakcalan et al., 1977; Navarro et al., 1998). However, the severity of the abstinence was less intense than the withdrawal syndrome precipitated by the homologous antagonist of each system: SR141716A in cannabinoid-dependent rats and naloxone in opioid-dependent animals (Navarro et al., 1998). Accordingly, SR141716A administration in morphine-dependent mice did not precipitate jumping, the most important behavioural manifestation of opioid withdrawal in this animal species (Romero et al., 1998b). Furthermore, a significant decrease in the severity of the morphine withdrawal syndrome has been reported in knockout mice deficient in CB_1 cannabinoid receptors (Ledent et al., 1999). Besides, acute administration of THC (Hine et al., 1975a, 1975b; Litchtman et al., 2001) or anandamide (Vela et al., 1995) has been reported to attenuate the severity of naloxone-precipitated morphine withdrawal. A similar effect was observed when THC was administered for a long period of time before starting opioid dependence. Thus, chronic pretreatment with THC (10 mg/kg once daily during a period of 3 weeks) before starting chronic opioid administration decreased the somatic manifestations of naloxone-precipitated morphine withdrawal and did not modify morphine rewarding effects on the place-conditioning paradigm (Valverde et al., 2001). Therefore, the long-term pre-exposure to cannabinoids does not seem to modify motivational responses of opioids that are related to their addictive properties.

Recent studies using knockout mice have clarified the involvement of the endogenous opioid system on the somatic expression of cannabinoid abstinence. Thus, the severity of SR141716A-precipitated cannabinoid withdrawal was significantly decreased in THC-dependent knockout mice lacking the pre-proenkephalin gene (Valverde et al., 2000), but was not modified in knockout mice deficient in the prodynorphin gene (Zimmer et al., 2001). The behavioural expression of SR141716A-precipitated THC withdrawal has also been evaluated in knockout mice lacking the different opioid receptors. Cannabinoid withdrawal syndrome precipitated by SR141716A administration was not modified in μ-, δ-, or κ-opioid receptor knockout mice chronically treated with 20 mg/kg (twice daily) of THC (Ghozland et al., 2002). Another recent study has reported a decrease in the severity of the cannabinoid withdrawal syndrome in μ knockout mice chronically treated once daily with 30 and 100 mg/kg of THC, but not those receiving 10 mg/kg of THC (Litchtman et al., 2001). Therefore, endogenous opioid peptides derived from pre-proenkephalin are important for the somatic expression of cannabinoid abstinence by acting on μ-opioids and probably other opioid receptors.

In spite of the interactions between cannabinoid and opioid dependence, different brain structures have been reported to be involved in the physical manifestations of opioid and cannabinoid withdrawal. Thus, the locus coeruleus and other brainstem structures, such as the periaqueductal gray matter, are responsible for the somatic signs of the opioid withdrawal syndrome (Maldonado et al., 1992). In the case of cannabinoid dependence, the cerebellum seems to play a crucial role for the somatic expression of THC withdrawal (Hutcheson et al., 1998; Tzavara et al., 2000).

4. Drug discrimination studies

Drug discrimination studies in animals currently have been considered as a model for subjective drug effects in humans. In drug discrimination research, animals faced with two possible responses, one of which results in reinforcement delivery, are trained to detect whether they received an active drug or a placebo vehicle injection, in order to determine through drug effects which response is correct. The discrimination between drug and vehicle is based upon the presence or absence of subjective and perceptible CNS effects (Balster, 1990; Schuster & Johanson, 1988). Drug discrimination studies have revealed that cannabinoid agonists produce subjective drug effects in rats (Balster & Prescott, 1992; Wiley et al., 1993, 1995a, 1995b; Barret et al., 1995) and monkeys (Wiley et al., 1993, 1995a, 1995b). CB_1 cannabinoid receptors seem to be selectively involved in the discriminative stimulus effects induced by cannabinoid agonists since these responses were blocked by SR141716A administration (Wiley et al., 1995b). Substitution tests have demonstrated the pharmacological specificity of cannabinoid discriminative stimulus responses. Numerous psychoactive compounds have been evaluated in these substitution studies (opioids, barbiturates and other

anticonvulsant agents, neuroleptics, benzodiazepines and other γ-aminobutyric acid agents, adrenergic ligands, serotonergic ligands, cholinergic compounds, antidepressants, psychostimulants, hallucinogens, antihistaminergics, and corticoids), and a large majority of them did not show any cross discrimination with cannabinoids (Barret et al., 1995; Balster & Prescott, 1992; Wiley & Martin, 1999). A partial overlap in the discriminative stimulus effects of THC and diazepam has been reported (Barret et al., 1995; Wiley & Martin, 1999). This partial substitution is not mediated by diazepam action at CB_1 cannabinoid receptors, and is consistent with a γ-aminobutyric acid component to cannabinoid drug discrimination (Wiley & Martin, 1999). Two other psychoactive compounds, phencyclidine and 3,4-methylenedioxymethamphetamine, have also shown some cross-discriminative stimulus effects with cannabinoids, but partial substitution with these compounds and THC was less important than with diazepam (Barret et al., 1995). It is important to point out that there is no cross discrimination between cannabinoid and opioid agonists (Barret et al., 1995; Wiley et al., 1995a; Järbe et al., 1998; Järbe & Lamb, 1999).

A complete cross discrimination has been reported among different natural and synthetic cannabinoid agonists (Wiley et al., 1995a). However, it is difficult to reveal a cross discrimination between exogenous cannabinoid agonists and anandamide (Wiley et al., 1997, 1998). Thus, a very high dose of anandamide (45 mg/kg) is required to show cross-discriminative stimulus effects with THC (3 mg/kg) (Wiley et al., 1998). It must be highlighted that such a high dose of anandamide is able to induce nonspecific discriminative stimulus effects on this behavioural model (Wiley et al., 1998; Wiley, 1999). Metabolically stable analogs of anandamide, such as (*R*)-methanandamide (Burkey & Nation, 1997; Järbe et al., 1998) and methylated-fluoroanandamide (Wiley et al., 1997), show cross discrimination only with low doses of THC. (*R*)-Methanandamide also showed partial overlap in the discriminative stimulus effects with high doses of THC (Järbe et al., 2000). Taken together, these results suggest that the absence of a clear cross discrimination between anandamide-like compounds and THC is not completely due to their pharmacokinetic properties, and both groups of compounds may produce their behavioural actions on the drug discrimination paradigm through different mechanisms of action (Wiley, 1999). The lower intrinsic activity at the CB_1 receptor site of anandamide-like compounds may also account for the lack of cross discrimination with THC (Järbe et al., 1998).

5. Place-conditioning paradigm

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; Schechter & Calcagnetti, 1993). In this paradigm, the rewarding properties of a compound are associated with the particular characteristics of a given environment. After conditioning, the animal will prefer to spend more time in the environment associated with the drug. A similar place-conditioning animal model can also be used to explore the aversive properties of a drug. In this case, the animal will avoid staying in the compartment associated with a compound producing aversive/dysphoric effects. The administration of cannabinoid agonists currently produces aversive-like responses in the place-conditioning paradigm (Parker & Gillies, 1995; McGregor et al., 1996; Sañudo-Peña et al., 1997; Chaperon et al., 1998; Hutcheson et al., 1998; Mallet & Beninger, 1998a). The endogenous cannabinoid anandamide does not induce any behavioural response on this paradigm (Mallet & Beninger, 1998b). Conditioned place aversion induced by cannabinoid agonists is abolished by the co-administration of SR141716A (Chaperon et al., 1998). This selective CB_1 cannabinoid antagonist is also able to block the acquisition of conditioned place preference to other drugs of abuse, such as morphine and cocaine, as well as the rewarding effects of natural stimuli such as food (Chaperon et al., 1998). SR141716A administration can produce some intrinsic motivational responses in this conditioned paradigm. Thus, some specific doses of SR141716A are able to produce conditioned place preference in rats (Sañudo-Peña et al., 1997) and conditioned place aversion in mice (Hutcheson et al., 1998). Rewarding effects of cannabinoid agonists can also be revealed in this paradigm by using particular experimental conditions. 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-hr washout period between the two THC conditioning sessions (Lepore et al., 1995). THC also produces a clear conditioned place preference in mice by using a long period of conditioning and by avoiding the possible dysphoric consequences of the first drug exposure. Indeed, place preference was obtained with 1 mg/kg of THC when mice received a previous priming THC exposure in the home cage before the conditioning sessions (Valjent & Maldonado, 2000). Administration of the CB_1 antagonist SR141716A after each THC conditioning session failed to reveal or to modify any THC-induced conditioned response, supporting that the long duration of cannabinoid effects was not relevant in this paradigm (Valjent & Maldonado, 2000).

6. Intracranial self-stimulation

A common property of most drugs of abuse is to acutely facilitate electrical stimulation of brain reward loci, presumably due to their euphorigenic properties (Esposito & Kornetsky, 1978; Wise, 1980). Acute administration of THC 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). Interestingly, doses of THC used in these studies (1.5 mg/kg) (Gardner et al., 1988) were similar to those reported to induce conditioning place preference in rodents (Lepore et al., 1995; Valjent & Maldonado, 2000). Genetic differences in rats are important for the effects of cannabinoids on intracranial self-stimulation. Thus, Lewis rats showed a pronounced THC-induced enhancement of brain reward functions, whereas Sprague–Dawley rats showed an enhancement of brain reward functions that was approximately one-half of that seen in Lewis rats (Lepore et al., 1996). Similar genetic differences between rat strains have been reported previously on the rewarding properties of other drugs of abuse, including opiates, cocaine, and alcohol (Gardner & Lowinson, 1991). Opioid antagonists are able to block the behavioural effects of THC on the intracranial self-stimulation paradigm, indicating the involvement of the endogenous opioid system in the rewarding effects of cannabinoids (Gardner & Lowinson, 1991).

7. Self-administration studies

The procedure by which animals are permitted to intravenously self-administer drugs has provided a reliable method to directly evaluate the reinforcing properties of a psychoactive compound. The reinforcing potential of a drug, as evaluated in the self-administration paradigms, is probably the clearest indication in animals of its addictive potential in humans (Deneau et al., 1964). Numerous studies have shown that THC is unable to induce a self-administration behaviour in any of the animal species studied (Kaymakcalan, 1973; Pickens et al., 1973; Corcoran & Amit, 1974; Harris et al., 1974; Leite & Carlini, 1974; Carney et al., 1997; Gardner & Lowinson, 1991; Mansbach et al., 1994). Animals that have already learned to self-administer other drugs of abuse did not self-infuse THC (Harris et al., 1974; Carney et al., 1997; Mansbach et al., 1994). An early study (Takahashi & Singer, 1979) had reported self-administration of THC in rats, but this result was not found in more recent studies using similar or different experimental conditions. Several hypothesis can be postulated to explain the ineffectiveness of THC in sustaining self-administration: (1) the relatively delayed onset and lengthy duration of THC effects; (2) THC aversive effects could be predominant, thus masking its appetitive properties; and (3) the abuse liability of THC could be lower than other drugs of abuse (Gardner & Lowinson, 1991; Martellotta et al., 1998). THC pharmacokinetic properties seem to be crucial for the behavioural responses on the self-administration paradigm. Indeed, WIN-55,212-2, a synthetic cannabinoid agonist that has a shorter half-life than THC, was intravenously self-administered in mice in a concentration-dependent manner according to a two-phase ‘‘bell-shaped’’ curve. SR141716A selectively blocked WIN-55,212-2-induced self-administration, indicating the involvement of CB_1 cannabinoid receptors (Martellotta et al., 1998). Another synthetic cannabinoid agonist, CP-55,940, has been reported to sustain intracerebroventricular self-administration in rats. SR141716A completely reversed, and the opioid antagonist naloxone partially blocked, the reinforcing properties induced by CP-55,940 (Braida et al., 2001). Interestingly, SR141716A also partially reversed heroin-induced intracerebroventricular self-administration (Braida et al., 2001). In agreement with this result, morphine-induced intravenous self-administration (Ledent et al., 1999) and conditioned place preference (Martin et al., 2000) were abolished in knockout mice lacking the CB_1 cannabinoid receptors.

A recent study has revealed intravenous THC self-administration behaviour for doses much lower than those previously used by monkeys that have a previous history of cocaine self-administration (Tanda et al., 2000). Doses of THC self-administered by monkeys (2–4 μg/kg) are comparable with doses in marijuana smoke inhaled by humans (Tanda et al., 2000), but are from 5000 to 25,000 times lower than those required daily to induce physical dependence in rats (Tsou et al., 1995; Aceto et al., 1996, 2001; Hutcheson et al., 1998; Cook et al., 1998; Ledent et al., 1999; Tzavara et al., 2000). The administration of SR141716A suppressed THC self-administration behaviour, indicating the selective involvement of CB_1 cannabinoid receptors (Tanda et al., 2000). It is important to emphasize that the functional state of the brain reward circuits in naïve and cocaine self-administering animals is different, and their behavioural responses on this paradigm can also be distinct. THC self-administration behaviour was also attenuated by the administration of the opioid antagonist naltrexone, again indicating the involvement of the endogenous opioid system in cannabinoid rewarding properties (Goldberg et al., 2001).

8. Biochemical and electrophysiological studies

Experimental evidence indicates that the mesolimbic dopaminergic system is the common neuronal substrate for the motivational and rewarding properties of drugs of abuse (Koob, 1992). Indeed, prototypical drugs of abuse, including opioids, psychostimulants, alcohol, and nicotine, increase the discharge rate of mesolimbic dopamine neurons (Matthews & German, 1984; Mereu et al., 1984, 1987). Such activation is associated with increased dopamine output in innervated projection structures (Di Chiara & Imperato, 1988).

In vivo microdialysis studies have revealed that acute THC administration also increases extracellular efflux of dopamine and its metabolites in the nucleus accumbens of freely moving rats (Chen et al., 1990; Tanda et al., 1997). A similar increase in extracellular dopamine concentrations in the nucleus accumbens was observed after acute administration of the synthetic cannabinoid agonist WIN-55,212-2 (Tanda et al., 1997). The biochemical effects of cannabinoids on in vivo dopamine transmission in the nucleus

accumbens were prevented by the administration of the CB_1 antagonist SR141716A (Tanda et al., 1997). Acute THC administration has also been reported to increase the content of dopamine and its metabolites, as well as tyrosine hydroxylase activity in rat limbic forebrain structures (Navarro et al., 1993). In agreement with these biochemical results, THC and other cannabinoid agonists produced a dose-dependent increase in the spontaneous firing rate of ventral tegmental area dopamine neurons. This stimulant response to cannabinoids was also suppressed by SR141716A administration, indicating the selective involvement of CB_1 receptors (Gessa et al., 1998; Gessa & Diana, 2000).

Administration of opioid antagonists was reported to block the increase in extracellular efflux of dopamine in the nucleus accumbens induced by the acute administration of cannabinoid agonists (Chen et al., 1990; Tanda et al., 1997). However, other studies have reported that naloxone did not modify the THC-induced increase of the firing rate of dopamine neurons projecting to the nucleus accumbens (French, 1997; Gessa & Diana, 2000) and the neostriatum (Gessa & Diana, 2000).

9. Conclusions

The effects of cannabinoid agonists on the different animal models available to evaluate abuse liability are now well known. Chronic treatment with cannabinoids leads to the development of tolerance to most of their pharmacological effects and produces physical dependence, as revealed by the presence of a cannabinoid antagonist-precipitated withdrawal syndrome. However, doses of cannabinoid agonists required to induce tolerance and physical dependence in animals are very high, and are not comparable with doses consumed by humans. Spontaneous cannabinoid withdrawal syndrome is even more difficult to reveal in animals. Thus, somatic signs of spontaneous abstinence were only observed after chronic administration of the cannabinoid agonist WIN-55,212-2, but not after chronic treatment with massive doses of THC. Several neurochemical changes observed during cannabinoid abstinence are similar to those reported during withdrawal to other prototypical drugs of abuse. Thus, a selective compensatory up-regulation of the cAMP pathway, as well as a decreased mesolimbic dopaminergic activity and increased CRF release, have been reported during cannabinoid withdrawal.

Subjective effects of cannabinoid agonists have been shown in the drug discrimination paradigm, and are specific to this group of compounds. From the wide range of compounds evaluated, only a partial overlap of discriminative stimulus effects of cannabinoids has been reported with diazepam, and to a lower extent with 3,4-methylenedioxymethamphetamine and phencyclidine. However, this paradigm does not permit the definition of the characteristics of such subjective effects. Several behavioural models have revealed the rewarding properties of cannabinoid agonists. However, the experimental conditions required to reveal cannabinoid-rewarding properties are, in most of the cases, different from those used to exhibit rewarding properties of other drugs of abuse. The rewarding properties of THC are clearly shown by a decrease in the intracranial self-stimulation thresholds. In contrast, cannabinoid agonists currently produce aversive-like responses in the place-conditioning paradigm. Particular experimental conditions are required to induce cannabinoid place preference, such as administering a priming-cannabinoid injection before the conditioning sessions. Self-administration behaviour has been shown for WIN-55,212-2 and CP-55,940, but the reinforcing properties of THC are very difficult to reveal in this paradigm. Indeed, self-administration of THC has been demonstrated only in animals that have a previous history of cocaine self-administration. Cannabinoid rewarding properties, similar to other prototypical drugs of abuse, seem to be due to the activation of the mesolimbic dopaminergic system.

In spite of the large amount of information now available on cannabinoid dependence in animals, several important issues are still not clarified. Indeed, behavioural responses and adaptive changes occurring in the brain during chronic treatment with massive doses of cannabinoids are well known. However, these treatment schedules are not relevant for the habits of cannabis consumption in humans, and the possible adaptive neuronal consequences of chronic exposure to cannabinoids using more realistic doses should be explored. Tolerance, dependence, and motivational responses induced by anandamide seem to be different from the responses induced by exogenous cannabinoid agonists, and would involve other neurobiological mechanisms. The elucidation of the particular mechanisms, and probably the specific receptors, involved in such anandamide responses will represent an important advance in the knowledge of the functional role of the endogenous cannabinoid system. Finally, the lack of experimental models to demonstrate THC self-administration in naïve animals is still an important obstacle in the pathway to the clear definition of the abuse liability of cannabis derivatives. Further studies will probably clarify in the near future these unresolved issues of cannabinoid dependence.

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