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REVIEW

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Cannabinoids: reward, dependence, and underlying neurochemical mechanisms—a review of recent preclinical data

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Abstract *Background and rationale:* Starting with the discovery of an endogenous brain cannabinoid system with specific receptors and endogenous ligands, research in the cannabinoid field has accelerated dramatically over the last 15 years. Cannabis is the most used illicit psychotropic substance in the world but only recently have reliable preclinical models become available for investigating the rewarding and dependence-producing actions of its psychoactive constituent, Δ^9-tetrahydrocannabinol (THC). *Objectives:* The goal of this review is to examine the various animal models currently available that are being used to facilitate our understanding of the rewarding and dependence-producing actions of cannabinoids, which are central to their abuse liability, and of the neurochemical mechanisms that may underlie these actions of cannabinoids. *Results and conclusions:* Recent demonstrations that strong and persistent intravenous self-administration behavior can be obtained in squirrel monkeys using a range of THC doses that are in agreement with the total intake and the single doses of THC normally self-administered by humans smoking marijuana cigarettes provides a reliable and direct tool for assessing the reinforcing effects of THC that are central to its abuse liability. In addition, recent demonstrations of persistent intravenous self-administration of synthetic cannabinoid CB1 receptor agonists by rats and mice and the development of genetically modified mice lacking specific cannabinoid receptors provide convenient rodent models for exploring underlying neurochemical mechanisms. Repeated demonstrations in rats that THC and synthetic CB1 agonists can induce conditioned place preferences or aversions, depending on details of dose and spacing, can reduce the threshold for intracranial self-stimulation behavior under certain conditions, and can serve as effective discriminative stimuli for operant behavior provide less direct, but more rapidly established, measures for investigating the rewarding effects of cannabinoids. Finally, there have been numerous recent reports of major functional interactions between endogenous cannabinoid, opioid, and dopaminergic neurotransmitter systems in areas such as analgesia, physical dependence and tolerance development, and drug reinforcement or reward. This provides an opportunity to search for drugs with the beneficial therapeutic effects of currently available cannabinoids or opioids but without undesirable adverse effects such as abuse liability.

Keywords THC · Marijuana · Drug self-administration · Conditioned place preference · Drug discrimination · Cannabinoid dependence · In vivo microdialysis · Opioid-cannabinoid interactions

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Introduction

In spite of the well-established abuse properties of *Cannabis sativa* (marijuana) in humans, knowledge of the abuse liability of cannabis and of the reinforcing and dependence-producing effects of its psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), is still limited. Indeed, until the last few years, only indirect evidence for positive-reinforcing and dependence-producing actions of THC had been obtained with laboratory animals. Description of the chemical structure and synthesis of THC did not occur until the 1960s (Gaoni and Mechoulam 1964). As recently as the early 1980s, the behavioral effects of THC were believed to be mediated by the

ability of the drug to alter the fluidity and permeability of a cell's membrane through a nonspecific mechanism, based on its very high lipophilicity (Paton 1975; Leuschner et al. 1984).

Starting in 1988, with evidence for the existence of a cannabinoid receptor in the brain (Devane et al. 1988) and the subsequent cloning of such a receptor (Matsuda et al. 1990), cannabinoid research accelerated. There is now evidence for at least two different cannabinoid receptors, CB1 and CB2 (Pertwee 1999), and likely for a CB3 receptor (Járai et al. 1999; Breivogel et al. 2001; Zimmer et al. 2001; Zygmunt et al. 2002), different endogenous ligands for these receptors, including anandamide (Devane et al. 1992), 2-arachidonylglycerol (2AG) (Mechoulam et al. 1995), 2-arachidonyl-glyceril-ether (noladine ether) (Hanus et al. 2001), virodhamine (Porter et al. 2002), and N-arachidonyl-dopamine (NADA) (Huang et al. 2002; see also Walker et al. 2002), and other endogenous compounds whose effects have been correlated with activation of cannabinoid receptors (entourage effect), such as palmitoylethanolamide and oleamide (Lambert and Di Marzo 1999; Calignano et al. 2001; Walker et al. 2002). Moreover, the synthetic and degradative pathways for these endocannabinoids were elucidated and a reuptake carrier protein for endocannabinoids was identified (Di Marzo 1999; Hillard and Jarrahian 2000; Giuffrida et al. 2001). To date, CB1 receptors are the only cannabinoid receptors that have been found in the brain. A large body of scientific evidence has accumulated indicating that brain CB1 receptors mediate almost all the behavioral and neurochemical properties of cannabinoids, including rewarding effects (Tanda et al. 1997, 2000), tolerance (Di Marzo et al. 2000), and physical dependence (De Fonseca et al. 1997; Tanda et al. 1999). Altogether these discoveries have led to the notion of a true brain endocannabinoid system upon which cannabinoids can act to produce dependence and abuse.

During the last decade new synthetic drugs have also become available which facilitate behavioral and neurobiological research with cannabinoids. It is now possible to utilize selective synthetic agonists and antagonists for cannabinoid receptors (Pertwee 1999; Palmer et al. 2002) and block the degradation of the endogenous cannabinoid ligand, anandamide (Cravatt et al. 1996; Di Marzo 1999; Gifford et al. 1999), or its reuptake carrier (Hillard and Jarrahian 2000; Kathuria et al 2003; Palmer et al. 2002). Genetically modified mice can also be utilized as research tools to study the behavioral and neurochemical actions of cannabinoids (Ledent et al. 1999; Zimmer et al. 1999). Finally, during the last decade, numerous interactions between cannabinoid transmission and other neuronal systems, including dopaminergic, glutamatergic, GABAergic, and opioidergic systems in the brain, have been discovered which can profoundly modulate the behavioral and neurochemical effects of cannabinoids (Ameri 1999; Manzanares et al. 1999; Romero et al. 2002).

Thus, there has been rapid progress over the past decade in the understanding of the pharmacology and neurochemistry of cannabinoid dependence and abuse. This review will focus on recent developments that facilitate an understanding of the rewarding and dependence-producing actions of cannabinoids, which are central to its abuse, and on neurochemical mechanisms that may underlie these actions of cannabinoids.

Cannabinoid drug discrimination

Due to the difficulties involved in obtaining a reliable animal model of the rewarding effects produced by cannabinoids in humans, a preclinical model of subjective response to systemic administration of THC (two-lever, choice, drug discrimination procedure) has long been the primary animal model available for evaluating the potential abuse liability of cannabinoids (Balster and Prescott 1992; Wiley 1999). The drug discrimination procedure is a behavioral procedure based on the ability of a drug to induce a specific set of interoceptive stimulus conditions perceived by animals that might be predictive of the subjective reports of perceptions/feelings induced by the same drug in humans. Thus, the drug discrimination procedure does not measure reinforcing/rewarding effects of drugs. Indeed, certain drugs belonging to different pharmacological classes (e.g., antipsychotics, serotonergic antidepressants), which do not possess any demonstrated reinforcing/rewarding effects in animals or humans, can produce a specific set of interoceptive stimulus conditions that can be discriminated by animals and humans. On the other hand, there is usually a good correlation between those drugs belonging to a particular pharmacological class that serve as reinforcers and those that produce subjective/discriminative effects characteristic of that pharmacological class (e.g., Woods et al. 1982; Griffiths et al. 1985; Katz and Goldberg 1988). As a result, studies of the subjective effects of new drugs in both humans and animals have been relatively good predictors of whether or not a drug will be abused.

Animal drug discrimination procedures consist of an initial period of training, with alternating exposure either to a specific drug dose or to the drug's vehicle, before each session. With the most common procedure, subjects are placed for daily sessions inside an experimental cage, which has two different levers. They learn to press one of the levers during sessions preceded by injection of the training drug and the other lever during sessions preceded by a vehicle injection. Lever pressing is intermittently reinforced by food pellets or by postponement or escape from electric shocks. Once subjects reach a criterion level of correct performance, test sessions are occasionally scheduled, during which they receive either the training drug at a different dose or a different drug, to test whether or not such a drug/dose will produce a discriminative stimulus effect similar to the training drug. Generally, if the training drug is an agonist, only agonist drugs belonging to the same pharmacological class will produce

full generalization to the training stimulus and only antagonists belonging to the same pharmacological class will selectively block the training stimulus.

Cannabinoid drugs, indeed, show a pharmacological specificity in this behavioral procedure. Thus, in animals trained to discriminate injections of THC from injections of saline, only drugs that possess the ability to activate CB1 cannabinoid receptors fully generalize to the THC training stimulus (Wiley et al. 1993a, 1993b, 1995c; Barret et al. 1995). For example, synthetic CB1 receptor agonists generalized to the THC training stimulus in the drug discrimination procedure, with a potency that was consistent with their in vitro affinity for the CB1 receptors (Balster and Prescott 1992; Gold et al. 1992; Wiley et al. 1995b).

The specificity of the cannabinoid discrimination has been assessed using other cannabinoid CB1 receptor agonists as training drugs (Wiley et al. 1995a, 1995b; Perio et al. 1996; Järbe et al. 2001). For example, in rats trained to discriminate the CB1 receptor agonist CP 55,940 from saline, the potencies of the cannabinoid agonists THC, WIN 55,212-2, cannabinol and CP 55,940 in generalizing to the CP 55,940 training stimulus were in agreement with their potencies in displacing CP 55,940 in binding studies (Wiley et al. 1995b). Rats also can learn to discriminate WIN 55,212-2 from vehicle (Perio et al. 1996), and THC and CP 55,940 then produce complete generalization. Moreover, the discriminative stimulus effects of THC and other synthetic CB1 agonists can be completely blocked by pretreatment with the selective CB1 receptor antagonist SR 141716A (Rinaldi-Carmona et al. 1994), further demonstrating that the cannabinoid discrimination is mediated by CB1 receptors (Mansbach et al. 1996; Perio et al. 1996). Finally, failure of SR 140098, a selective antagonist at cannabinoid CB1 receptors that does not cross the blood-brain-barrier, to antagonize the discriminative stimulus effects of cannabinoids (Perio et al. 1996) points toward central CB1 receptors in the mediation of the discriminative stimulus effects of cannabinoids.

In contrast to other CB1 receptor agonists, anandamide, an endogenous ligand for cannabinoid CB1 receptors, has generally failed to produce consistent generalization to a THC training stimulus in monkeys (Wiley et al. 1997) and rats (Burkey and Nation 1997; Wiley et al. 1998; Järbe et al. 2001), or has done so only at doses that severely decrease food-maintained responding (Wiley et al. 1995a). However, fast reuptake and rapid metabolism of anandamide by the fatty acid amide hydrolase (FAAH) enzyme is a likely explanation for why anandamide, which is a partial agonist of CB1 receptors, just like THC, usually fails to produce THC-like discriminative-stimulus effects. As one might expect, the metabolically more stable analogs of anandamide, 2-methyl-arachidonyl-2-fluoroethylamide and R-methanandamide, can produce complete generalization to a THC training stimulus in rats (Burkey and Nation 1997; Järbe et al. 1998, 2000, 2001) and monkeys (Wiley et al. 1997), although the training dose of THC can be an important determinant in obtaining such a generalization. For example, methanandamide produced only partial generalization to THC in rats trained with higher doses of THC (Järbe et al. 1998, 2000) but produced full generalization to THC in rats trained to discriminate lower doses of THC (Järbe et al. 1998, 2000).

Anandamide has been shown to have cannabinoid-like discriminative stimulus effects under some situations. In one recent study, methanandamide was successfully used as a training stimulus in rats, and THC produced complete generalization (Järbe et al. 2001). A high, 18-mg/kg dose of anandamide, produced approximately 80% responding on the methanandamide lever when administered 3 min before the session but not when administered 15 min before the session. A lower, 10-mg/kg dose of anandamide was ineffective. In contrast, 18 mg/kg of anandamide failed to produce generalization to a THC training stimulus at both pretreatment times (3 and 15 min) in the same study (Järbe et al. 2001). This apparent discrepancy between the ability of anandamide to produce generalization to the methanandamide but not to the THC training stimulus might be related to the different affinity of THC and methanandamide for cannabinoid CB1 receptors, which might result in a discriminative stimulus for methanandamide showing an intensity and a quality closer to the anandamide stimulus as compared to the THC stimulus. It could also be the case that anandamide and methanandamide, but not THC, possess affinity for a subpopulation of receptors, other than the CB1. Unfortunately, the ability of the CB1 cannabinoid antagonist SR 141716A to block the generalization of anandamide to the methanandamide training stimulus was not tested.

Among noncannabinoid drugs, only the benzodiazepine diazepam has been found to produce partial generalization to a cannabinoid training stimulus (Mokler et al. 1986; Barrett et al. 1995; Wiley et al. 1995b, 1995c; Wiley and Martin. 1999). Since the CB1 cannabinoid antagonist SR 141716A did not block such partial generalization by diazepam, it has been suggested this effect is mediated by an interaction through the GABAergic system (Wiley and Martin 1999). Also, THC has been reported to partially attenuate the discriminative stimulus effects of phencyclidine in rats (Doty et al. 1994).

Cannabinoids and conditioned place preference/aversion

The conditioned place-preference procedure is a classical conditioning procedure that provides an indication of drug-related reward/aversion effects in animals but interpretations of results obtained with this behavioral procedure are limited due to the frequency of false positives and the difficulties encountered in obtaining systematic dose-response relationships (for reviews, see Bardo and Bevins 2000; Tzschentke 1998). Methodological details differ among laboratories, but generally animals are first allowed to freely explore two distinct environments, which differ in color, size, or texture, and are connected

by an open door. Time spent in each compartment is recorded. During conditioning sessions, a drug injection (unconditioned stimulus) is repeatedly paired with one of the two compartments. During these conditioning sessions, the connecting door is closed and animals cannot move to the other compartment. On alternate sessions, vehicle injections are paired with the other compartment. On the final test day, no injections are given and the animal is allowed to explore the two compartments with the connecting door open and time spent in each compartment is again recorded. The difference between time spent in the drug-paired vs the saline-paired compartment during pretest and test sessions, provides an indication of the rewarding or aversive effects of a drug at a given dose.

While almost all drugs abused by humans are able to increase the time spent in the drug-paired compartment (i.e., produce a place preference) over a limited range of doses (Bardo and Bevins 2000; Tzschentke 1998), results with cannabinoids have not always been consistent between studies, and positive place preferences, when found, usually are highly dose dependent, often occurring at only a single dose. This leads to difficulties in interpreting and comparing findings from different studies, particularly when only a limited range or a limited number of cannabinoid doses has been used (McGregor et al. 1996; Sanudo-Pena et al. 1997; Cheer et al. 2000a). In addition, the ability of THC and the potent synthetic CB1 receptor agonist, CP 55,940 to produce a positive place preference in rats and mice depends on the timing of injections as well as on the range of doses used (Lepore et al. 1995; Valjent and 2000; Braida et al. 2001a; Ghozland et al. 2002) as it does with other drugs (Bardo and Bevins 2000; Tzschentke 1998). Figure 1 shows data from Lepore et al. (1995), illustrating the dose-dependent nature of THC effects in place-preference studies under different methodological conditions. When a standard schedule of daily injections (i.e., vehicle, drug, vehicle, drug, etc., before consecutive daily sessions) was used, THC produced a conditioned place aversion for the compartment associated with its administration at a low 1-mg/kg dose but positive place preferences at higher 2- and 4-mg/kg doses. When the schedule of daily injections was changed, allowing a longer wash-out time period between drug injections (i.e.: vehicle, day off, drug, day off, vehicle, day off, drug, etc.), THC produced a conditioned place preference at a low 1-mg/kg dose but produced place aversions at higher 2- and 4-mg/kg doses. Lepore et al. (1995) suggested that increasing the interval of time between THC injections might qualitatively change the effects of THC in this behavioral test, due to a postdrug dysphoric rebound (e.g., suggested not less than 48–84 h spacing between successive drug injections, depending on dose). This may help to explain the apparently conflicting findings that THC and synthetic cannabinoids often produce conditioned place aversions, rather than place preferences, in rats and mice using similar dose ranges and standard place-preference conditioning procedures (Lepore et al. 1995; McGregor et al. 1996; Sanudo-Pena et al. 1997; Mallet and Beninger 1998; Chaperon et al. 1998; Cheer et al. 2000a; Zimmer et al. 2001). It should also be noted that differences in rat strains, duration and number of sessions, and other methodological details might play a role in the contrasting findings by different research groups.

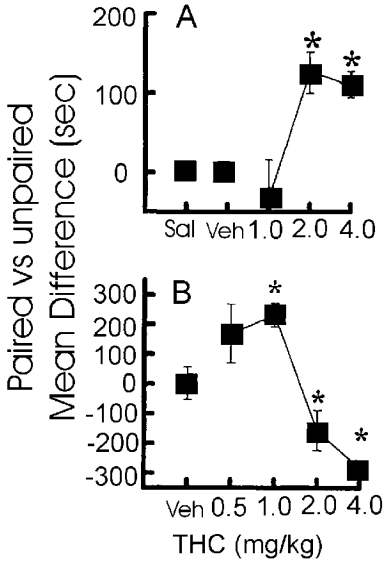

Fig 1A, B THC-induced place preference. Means and standard errors for conditioned place-preference scores obtained with two different THC place-conditioning procedures. **A** Data obtained using a standard schedule of daily injections, i.e., vehicle, THC, vehicle, THC, etc., over consecutive daily sessions. THC administration produced a conditioned place aversion at a low 1 mg/kg IP dose and conditioned place preferences at higher 2 and 4 mg/kg IP doses. **B** Data obtained when the schedule of daily injections was changed to allow a longer wash-out time period between THC injections, i.e., vehicle, day off, THC, day off, vehicle, day off, THC, etc., over consecutive daily sessions. Using this schedule, THC produced a conditioned place preference at a low 1-mg/kg dose and dose-dependent conditioned place aversions at higher 2- and 4-mg/kg doses. *Sal* saline administration, *Veh* THC vehicle (*β*-cyclodextrin) administration. See Lepore et al. (1995) for more detailed information on methods and statistics. Modified from Lepore et al. (1995)

Another example of the qualitatively different effects of THC that may occur in place-conditioning studies is provided in a recent report by Valjent and Maldonado (2000). Using a standard place-conditioning protocol, they found that a high, 5-mg/kg dose of THC produced place aversion in mice, while a lower 1-mg/kg dose produced neither place aversion nor preference. However, when mice received a previous priming injection of THC (1 mg/kg in their home cage 24 h before the start of the experiment) and were exposed to the drug-paired conditioning chamber for a longer period of time (45 min instead of 15–30 min), the lower dose of 1-mg/kg THC produced a positive place preference while the higher dose of 5 mg/kg produced neither place preference nor aversion. The authors suggest that there may be an initial dysphoric/anxiogenic effect of cannabinoid agonists during their first administration, which could mask the development of positive place preferences. This finding

has been replicated (Ghozland et al. 2002), establishing the ability of this procedural variation to reveal a positive place preference with THC. It is also interesting to note that the selective CB1 receptor antagonist SR 141716A has been reported to produce a positive place preference in rats, suggesting the possibility that an endogenous cannabinoid tone might be present in the brain, as a physiologic system to suppress reward or to induce aversion (Sanudo-Pena et al. 1997; Cheer et al. 2000a). However, in other place-conditioning studies, SR 141716A administration failed to produce either place preferences or place aversions (Chaperon et al. 1998; Braida et al. 2001a).

Cannabinoid effects on intracranial self-stimulation

Another methodology that has been employed to indirectly study the reinforcing/rewarding effects of cannabinoids in vivo is the intracranial self-stimulation (ICSS) procedure (Wise 1996; Gardner and Vorel 1998). The method is based on the discovery by Olds and Milner (1954) that rats will repeatedly press a lever when lever presses result in electrical stimulation of the medial forebrain bundle, a component of the reward circuit in the brain. It has been repeatedly shown that most drugs of abuse are able to lower brain-stimulation reward thresholds in the ICSS procedure in a dose-dependent fashion (see Olds and Fobes 1981; Wise 1996).

Findings with cannabinoid drugs using ICSS procedures during the last decade have not been consistent. Thus, low doses of THC, 1.0–1.5 mg/kg, lowered the brain-stimulation reward threshold in Lewis rats (see Fig. 2) (Gardner et al. 1989) and Sprague Dawley rats, but not in the Fisher strain of rats (Lepore et al. 1996; Gardner and Vorel 1998). Moreover, it was shown that withdrawal from a single, acute, 1.0-mg/kg dose of THC induced a significant elevation in brain-stimulation reward thresholds (Gardner and Vorel 1998). On the other hand, the synthetic CB1 receptor agonist CP 55,940, in a range of doses comparable to those of THC used in the paper by Lepore et al. (1996), had no effect on brain-stimulation reward thresholds during either acute or withdrawal experiments (Arnold et al. 2001). In this paper it was also reported that the selective CB1 receptor antagonist SR 141716A increased brain-stimulation reward thresholds, but only when administered at very high and likely nonselective doses at which inverse agonist effects of SR 141716A, which have been found in in vitro studies (Landsman et al. 1997), might occur.

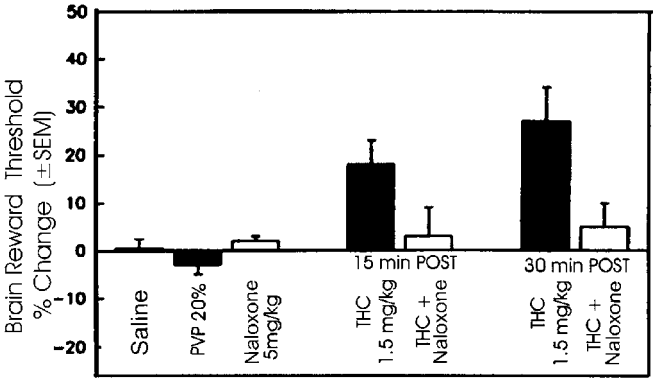

Fig. 2 THC-induced decrease in the brain reward threshold. Enhancement of brain stimulation reward (decrease in electrical brain stimulation threshold) produced by THC, 1.5 mg/kg IP, and antagonism of this effect by acute naloxone administration. Data are expressed as percentage change in electrical brain stimulation threshold in the rat medial forebrain bundle. See Gardner et al. (1989) and Gardner and Vorel (1998) for more detailed information on methods and statistics. Modified from Gardner et al. (1989) and from Gardner and Vorel (1998)

Cannabinoid self-administration

Drug self-administration behavior has been one of the most direct and productive approaches for studying the rewarding properties of abused drugs. With this procedure, animals are given the opportunity to self-administer a drug by making an operant response such as pressing a lever or inserting their nose into a hole (a "nosepoke"), which activates a syringe to intravenously deliver the drug. Reliable and persistent self-administration behavior has been demonstrated in laboratory animals for almost all drugs abused by humans, including psychostimulants, narcotics, ethanol, and nicotine (Young and Herling 1986; Yokel 1987). Using this methodology, it has been possible to study neuropharmacological mechanisms involved in such behaviors and preclinically evaluate therapeutic strategies for treatment of drug abuse (see Katz and Goldberg 1988).

During the past 3 decades, different research groups have varied the parameters of self-administration procedures in unsuccessful attempts to demonstrate reliable and persistent reinforcing effects of THC or synthetic cannabinoids in experimental animals (Kaymakcalan 1972, 1973; Pickens et al. 1973; Harris et al. 1974; Leite and Carlini 1974; Carney et al. 1977; Van Ree et al. 1978; Mansbach et al. 1994). In none of these studies were THC or synthetic cannabinoids clearly shown to maintain self-administration behavior that was persistent, dose-related, and susceptible to vehicle extinction and subsequent reinstatement. Only a few studies reported self-administration of THC at levels higher than vehicle controls. In one of these studies (Kaymakcalan 1973), two rhesus monkeys out of six acquired THC self-administration behavior, but only after 36 days of forced automatic injections of IV THC at doses as high as 0.4 mg/kg/injection and only after the automatic injections of THC were withdrawn and overt signs of physical dependence (withdrawal) occurred.

Previous drug exposure and experience can be an important factor in modulating acquisition and maintenance of self-administration of various drugs in animals (Schlichting et al. 1971; Young et al. 1981a, 1981b; Bergman and Johnson 1985) and this has frequently been

used in attempts to facilitate the acquisition of THC self-administration. In an early self-administration study with rhesus monkeys, for example, Harris et al. (1974) failed to obtain THC self-administration responding above vehicle-control levels in drug-naïve as well as in cocaine-experienced monkeys and in monkeys that had learned to self-administer a mixture of THC and cocaine followed by a progressive decrease of cocaine concentration in the mixture. They also failed to obtain THC self-administration responding above vehicle-control levels in monkeys with histories of phenobarbital, PCP, ethanol, or amphetamine self-administration. In another early study with rhesus monkeys (Pickens 1973), THC was self-administered when it was substituted for phencyclidine, but rates of responding were low and there was no clear evidence that responding for THC could persist above vehicle-control levels over repeated daily sessions. A recent attempt to replicate these findings under a more rigorous set of experimental conditions in rhesus monkeys that had learned to self-administer phencyclidine was unsuccessful (Mansbach et al. 1994). The monkeys in this study also failed to self-administer the synthetic CB1 cannabinoid agonist CP 55,940.

Within the last few years, however, reinforcing effects of some synthetic CB1 cannabinoid agonists have been reported using IV self-administration procedures in rats and mice. In one study, the cannabinoid CB1 receptor agonist WIN 55,212-2 showed reinforcing effects in naïve mice by means of a one-session self-administration procedure (Martellotta et al. 1998). In this behavioral test, paired mice were restricted in two different cages each equipped with a nosepoke operandum. Only one cage had the nosepoke connected to the syringe delivering the drug. In this active cage, the mouse received an IV injection of drug each time it inserted its head in the nosepoke hole. The yoked mouse received the same injections of drug at the same time as the mouse in the active cage, but in a noncontingent manner. The difference, or the ratio, between the number of reinforced nosepokes by the mouse in the active cage and the noncontingent nosepokes by the mouse in the yoked control cage provided an index of the reinforcing effects of the drug. Pretreatment with the CB1 cannabinoid receptor antagonist SR 141716A completely prevented the self-administration behavior maintained by WIN 55,212-2 in mice (Martellotta et al. 1998). The same procedure has been employed to assess the reinforcing effects of the synthetic cannabinoid agonist WIN 55,212-2 in genetically modified mice lacking the CB1 receptors (Ledent et al. 1999). As might be expected, CB1 receptors knock-out mice did not self-administer WIN 55,212-2.

The studies with mice by Martellotta et al. (1998) and Ledent et al. (1999) raise a number of methodological and conceptual issues. Among them is the fact that it employed 1-day experimental tests. Since drug abuse and addiction are chronic or subchronic phenomena, it is hard to correlate this behavior with such time-related conditions. Also, this procedure provides little, if any, information about acquisition, extinction, and relapse to self-administration behavior. Finally, the known analgesic properties of cannabinoids (Martin and Lichtman 1998) could have been responsible for the increased number of self-injections with their procedure, since the mice were severely restrained for acute intravenous administration through the tail vein. It is possible that self-administration of WIN 55,212-2 was maintained due to its analgesic activity resulting in a reduction in the levels of pain or stress produced by the restraint, rather than to direct positive reinforcement effects of WIN 55,212-2 injections. This is further indicated by the finding that, although spontaneous nociceptive thresholds were not different in CB1 receptors knock-out mice and in the wild-type mice, antinociceptive properties of THC were lost in the CB1 receptors knock-out mice (Ledent et al. 1999).

A more reliable and frequently used procedure has been employed by these and other investigators to study self-administration of cannabinoids by unrestrained, freely moving rats over repeated daily sessions. In these studies, chronically catheterized rats were given the opportunity to IV self-administer different doses of THC or synthetic CB1 agonists, such as WIN 55,212-2 and CP 55,940, and self-administration of cannabinoids above placebo levels was sometimes reported. A limitation of these studies, however, is that food restriction was often used to initiate and subsequently maintain drug self-administration, and food deprivation has been shown to markedly increase self-administration of certain drugs (Campbell and Carroll 2000; Carr 2002). For example, in one early series of studies (Takahashi and Singer 1979, 1980), THC self-administration behavior above placebo levels was found in food-deprived rats when a pellet of food was automatically delivered at 1-min intervals. However, this behavior immediately disappeared when either food deprivation was discontinued or the automatic food presentation was discontinued. In a more recent study by Fattore et al. (2001), chronically catheterized rats had the opportunity to IV self-administer different doses of the CB1 agonist WIN 55,212-2. Figure 3 shows the acquisition phase of self-administration behavior in rats exposed to different doses of WIN 55,212-2, when a single nosepoke response was required to produce each injection. The number of injections the rats self-administered each session depended on dose and a classic, inverted-U-shaped, dose-response curve was reported. Also, self-administration responding was blocked by the CB1 receptor antagonist SR 141716A, indicating that self-administration of WIN 55,212-2 was mediated by activation of CB1 receptors (Fattore et al. 2001). A limitation of this study, as in the earlier studies by Takahashi and Singer (1979, 1980), is that food restriction was used to initiate and maintain cannabinoid self-administration. Finally, there is a recent report (Braida et al. 2001b) that CP 55,940, another synthetic CB1 cannabinoid receptor agonist, can maintain intracerebroventricular (ICV) self-administration in rats. Although the rats in this study had food available ad libitum, they were water deprived, with access to water only during

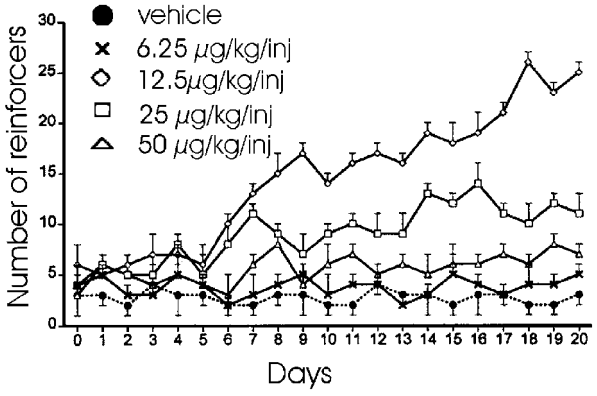

Fig. 3 Self-administration behavior maintained by WIN 55,212-2 in rats. Acquisition of self-administration behavior reinforced by different intravenous doses of the CB1 cannabinoid receptor agonist WIN 55,212-2 in rats. Values indicate the mean number of infusions (± S.E.M.) self-injected by rats per 3-h session during consecutive daily sessions. See Fattore et al. (2001) for more detailed information on methods and statistics. Modified from Fattore et al. (2001)

daily 1-h sessions and for 10 min after each daily session. During each session, two levers were available and a response on either lever initially delivered a small amount of water. Subsequently, each response on one lever produced water delivery paired with ICV injection of CP 55,940 while each response on the other lever produced water delivery paired with ICV injection of cannabinoid vehicle. Responding was consistently greater on the lever producing water delivery paired with ICV CP 55,940 injection and was dose dependent, with an inverted-U-shaped, dose-response curve. Also, pretreatment with SR 141716A reduced responding for water and CP 55,940.

Many factors have been considered over the years to explain the repeated failure of THC to function as a reinforcer in experimental animals in most studies. Among these factors, discussion focused most often on (1) the delayed onset of pleasurable neuropharmacological effects of THC that can prevent the necessary contingent association with operant tasks that provide the self-administered drug, (2) the apparent aversive properties of THC in experimental animals, (3) the depressant effects of cannabinoids on operant lever pressing, and (4) the long duration of THC's pharmacological and behavioral effects. Additional factors that should be taken into consideration include the dose range of THC made available for self-administration, the formulation of THC solutions for intravenous injection, and the speed of intravenous injection. As discussed below, most attempts to obtain THC self-administration used relatively high doses of the drug. Moreover, due to the lipophylic nature of THC, its very low solubility in water, and the use of high THC doses, many studies were performed using the drug in suspension and not in a clear solution. This resulted in low concentrations of the drug solution, large amounts of injected/infused solution, relatively low injection/infusion speeds, and relatively long durations of injection/infusion.

In recent studies, the possibility that monkeys will actively self-administer IV THC was reexamined using a primate species, dosage, and vehicle and injection speed parameters not previously employed (Tanda et al. 2000). Low clinically relevant doses of THC were employed and THC was dissolved in a vehicle containing 0.4–1.0% Tween-80 and 0.4–1.0% ethanol in saline to produce a clear solution which could be injected rapidly (0.2 ml/200 ms). Subjects were squirrel monkeys, which had unrestricted access to food and water in their individual living cages. During daily 1-h experimental sessions, monkeys had the opportunity to intravenously self-administer THC over a wide range of doses (1.0–8.0 μg/kg/injection) using a fixed-ratio 10 lever presses/60-s time-out schedule of reinforcement (10 lever presses were required to produce each 200-ms injection of THC and there was a 60-s time-out after each injection, during which lever presses had no specified consequences). Peak rates of responding and maximal numbers of injections per session were maintained by intermediate, 2.0–4.0 μg/kg IV, injection doses of THC (Fig. 4A). In contrast to previous studies, monkeys were only given access to THC after at least 1 week of saline extinction wash-out from other drugs and 1 week of vehicle extinction was always kept between self-administration of different THC doses. Thus, direct substitution for other psychoactive drugs was not used to induce acquisition of THC self-administration in these monkeys and their THC self-administration behavior was repeatedly shown to undergo rapid extinction with vehicle substitution, and rapid recovery when THC injections were reinstated. However, monkeys did have a history of cocaine self-administration, which had been extinguished by saline substitution before the THC self-administration studies were started.

Since the squirrel monkeys used by Tanda et al. (2000) had a history of cocaine self-administration, it is possible this played a role in the subsequent successful demonstration of reinforcing effects of THC, although previous attempts to use a history of self-administration of cocaine or other drugs to facilitate self-administration of THC were unsuccessful. As recently pointed out by Maldonado (2002), the cocaine self-administration history in the study by Tanda et al. (2000) might have induced long-term neurobiological adaptations that facilitated subsequent acquisition and maintenance of THC self-administration behavior. In a recent study from our lab (Justinova et al. 2003), however, THC was able to initiate and maintain self-administration behavior in drug-naïve squirrel monkeys and both rates of responding and numbers of injections per session were greater than those in the previous study where monkeys had a history of cocaine self-administration.

The reinforcing effects of THC demonstrated in the squirrel monkey study by Tanda et al. (2000) appeared to be due to direct actions on cannabinoid CB1 receptors. When the selective CB1 receptor antagonist SR 141716A was administered before each self-administration session

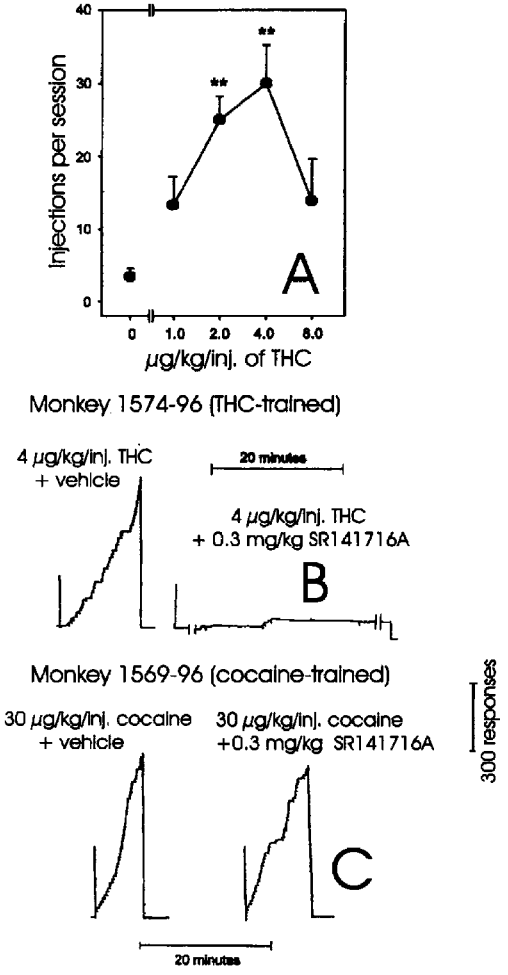

Fig. 4A–C Self-administration behavior maintained by THC in squirrel monkeys. **A** Number of intravenous injections of different doses of THC self-administered by squirrel monkeys during 1-h sessions. Values represent the mean (± S.E.M.) of the last three of five consecutive daily sessions at each dose. **B** and **C** Cumulative-response records of representative performance of monkey 1574–96 (**B**) under a 10-response, fixed-ratio schedule FR 10 of intravenous THC injection and of monkey 1569–96 (**C**) under an identical FR 10 schedule of intravenous cocaine injection, before the start of SR 141716A pretreatment (*left side*) and on the 5th day of SR 141716A pretreatment (*right side*). *Abscissas* time; *ordinates* cumulative lever-press responses. *Short diagonal marks* on the cumulative records indicate drug injections. See Tanda et al. (2000) for more detailed information on methods and statistics. Modified from Tanda et al. (2000)

for five consecutive sessions, THC self-administration responding decreased to vehicle-control levels within a few sessions, (Fig. 4B), but rapidly recovered when SR 141716A treatment was stopped. In contrast, in monkeys responding for cocaine rather than THC under identical conditions and with comparable rates and pattern of self-administration behavior, there was no effect of SR 141716A pretreatment (Fig. 4C). Thus, the effect of SR 141716A in suppressing THC self-administration responding by squirrel monkeys did not appear to be due to any nonselective depressant effect on behavior.

Taken together, the results of previous studies on cannabinoid self-administration by animals suggest that many factors should be taken into consideration in designing future studies. First, one must carefully consider the range of doses to be used. As noted above, most attempts to obtain THC self-administration used relatively high doses of the drug and THC administration may produce aversive or anxiogenic reactions or may impair operant behavior (Carriero et al. 1998) if doses administered are too high. It is interesting to note that in previous self-administration studies using larger injection doses of 25 to 100 μg/kg (Pickens et al. 1973; Harris et al. 1974; Mansbach et al. 1994), monkeys appeared intoxicated at the end of the session in spite of the low number of THC injections self-administered during the session. In contrast, in the study by Tanda et al. (2000), monkeys self-administered lower injection doses of THC, 2–8 μg/kg, and no behavioral changes indicative of intoxication were noted at the end of experimental sessions.

It should also be noted that the IV injection doses used in most previous studies of THC self-administration by monkeys were higher than those calculated from clinical marijuana smoking studies in humans. The bioavailability of THC from smoking marijuana cigarettes is, on average, only about 20% (Agurell et al. 1986). Thus, the actual intake of THC would be about 3 mg in human subjects with a cigarette containing about 15 mg of THC. Assuming that people smoke a marijuana cigarette in 10 to 15 puffs, each puff would contain 200 to 300 μg of THC, resulting in 2.9 to 4.3 μg/kg of THC delivered per puff (for an average body weight of 70 kg). This is in perfect agreement with the 2 to 4 μg/kg/injection doses that maintained peak levels of THC self-administration by squirrel monkeys in the Tanda et al. study (2000). Thus, it is likely that the doses of THC used in previous self-administration studies may have been too high and may have produced a degree of intoxication that could have negatively influenced the development of self-administration behavior.

Finally, in designing THC self-administration studies, the speed at which THC is injected and the bioavailability of THC from solution should be taken into consideration. Differences in speed of injection have been shown to influence self-administration of cocaine and other drugs (e.g., Panlilio et al. 1998; Wakasa et al. 1995; Kato et al. 1987; Balster and Schuster 1973), with slower speeds of injection resulting in decreased self-administration responding. In contrast, rapid IV injection of a bolus of THC solution, as in the report by Tanda et al. (2000), can result in a nonhomogeneous pattern of distribution of the drug in the circulatory system, with higher concentrations of the drug in organs, such as the lungs and the brain, and this may more closely resemble the intake and successive delivery of THC in humans smoking marijuana cigarettes.

Tolerance to cannabinoids

Repeated administration of THC or cannabinoid agonists in different animal species induces tolerance to many behavioral effects of cannabinoids (Bass and Martin 2000; Abood et al. 1993; Oviedo et al. 1993; Pertwee et al. 1993). While after chronic treatment with the CB1 agonist CP 55,940 in rats, a pharmacokinetic involvement has been demonstrated in the development of tolerance to the effects of its acute administration (Costa et al. 1996), there is a general agreement that tolerance to the behavioral effects of cannabinoids primarily involves pharmacodynamic mechanisms (Romero et al. 1997, 1998; Childers and Breivogel 1998; see also Ameri 1999, for review).

The importance of pharmacodynamic processes in tolerance development to the effects of cannabinoids is supported by many recent molecular and neurochemical studies. For example, CB1 receptors are down-regulated after chronic exposure to THC (Oviedo et al. 1993; De Fonseca et al. 1994; Romero et al. 1997, 1998; Breivogel et al. 1999) but expression of cannabinoid receptor mRNA is either increased, decreased, or unchanged, depending on the duration of treatment and the brain area studied (Romero et al. 1997, 1998; Zhuang et al. 1998). In addition, chronic exposure to the synthetic cannabinoid receptor agonists WIN 55,212-2 and methanandamide results in internalization of G-protein-coupled receptors in the surface of plasma membranes (Coutts et al. 2001), which is blocked by the cannabinoid antagonist SR 141716A. There are also changes in G-protein expression in THC-tolerant rats (Rubino et al. 1997a; Breivogel et al. 1999). Finally, chronic exposure to THC produces region-dependent alterations in brain endocannabinoid levels in rats. Di Marzo et al. (2000) reported that anandamide and 2AG levels significantly decreased in the striatum, and a statistically nonsignificant trend to decrease was found in brain stem and cerebellum, especially for 2AG. In contrast, anandamide levels increased in the limbic forebrain without any significant change in 2AG content. The limbic forebrain area region is also the only brain area where no changes in either CB1 receptor binding or agonist stimulated G-protein have been observed. Region-dependent changes in endogenous cannabinoid levels in THC-tolerant rats suggest an adaptation of regulatory mechanisms that control biosynthesis of endocannabinoids in the brain. All of these effects are likely to reduce the ability of cannabinoid agonists to exert their effects through activation of receptors and subsequent intracellular signaling.

Rapid tolerance develops to the hypothermic effects of THC in mice after only 2 days of treatment (Pertwee et al. 1993). Tolerance also develops to the hypothermic effects of other cannabinoid agonists, such as WIN 55,212-2 and CP 55,940, but not to the hypothermic effects of the endogenous cannabinoid anandamide. This suggests the existence of a subset of effects of the endogenous compound (including hypothermia), not mediated by CB1 receptors (Pertwee et al. 1993). Rapid tolerance also develops (in 24 h) to the disrupting effects of THC on motor coordination in the rota-rod test with mice (Da Silva et al. 2001), an effect that is not antagonized by SR 141716A. An explanation for this inability of SR 141716A to antagonize the rapid tolerance induced by THC could be found in the long duration of action of THC, or, as speculated by the authors, in the ability of SR 141716A to improve learning of animals during the practicing of the test.

Although tolerance develops to the hypothermic, antinociceptive, locomotor, and immune functions of cannabinoids (Bass and Martin 2000; Massi et al. 1998; Pertwee et al. 1993; Welch 1997), there are some effects of THC to which tolerance does not appear to develop. For example, Nava et al. (2001) reported that there was no tolerance to the behavioral effects of THC in a working-memory test in rats (T-maze performance) or to its effects in reducing extracellular levels of hyppocampal acetylcholine. In another study (Wu and French 2000), it has been reported that tolerance developed to THC-induced hypothermia, catalepsy, and locomotion in rats after repeated administration of 5-mg/kg THC twice a day, for 14 days. These rats also developed tolerance to THC-induced increases in firing rate of dopamine neurons in substantia nigra pars reticulata neurons, but dopamine neurons in the ventral tegmental area (VTA) continued to show a significant increase in firing rate when THC was administered (Wu and French 2000). These results are in agreement with those from an in vitro study (Cheer et al. 2000b) in which no tolerance developed to increases in firing rate of VTA dopamine neurons produced by the CB1 receptor agonist HU210 after repeated addition to a brain-slice preparation containing VTA neurons.

Altogether these findings indicate that tolerance to the effects of THC can differentially develop, depending on the brain area, the function, and the behavioral effects under consideration. It is important to note that the absence of tolerance to the effects of THC in preclinical models of cognition may help to account for the persistent effects of repeated THC exposure on human learning and memory (Pope and Yurgelun-Todd 1996; Pope et al. 2001). Moreover, since VTA dopamine neurons are considered a primary brain substrate in mediating drug abuse and addiction (Koob 1992; Di Chiara et al. 1999), failure of dopamine neuron firing activity in the VTA to undergo tolerance after long term THC exposure is in agreement with reports that the pleasurable effects of marijuana in humans (the subjective "high") do not undergo tolerance after chronic consumption of the drug (Lindgren et al. 1981; Perez-Reyes et al. 1991; Kirk and De Wit 1999), although small reductions in subjective effects after long-term exposure to THC has been reported by some investigators (Nowlan and Cohen 1977; Haney et al. 1997).

Sensitization to cannabinoids

Another adaptive neurobiological alteration that occurs after repeated exposure to drugs is behavioral sensitization. This phenomenon is shared by many drugs abused by humans and is likely to play a role in drug abuse and addiction (Robinson and Berridge 1993, 2001; De Vries et al. 1998). Repeated exposure to cannabinoid agonists can induce behavioral sensitization (Pontieri et al. 2001a; Rubino et al. 2001; Cadoni et al. 2001). Rats repeatedly treated with THC for several days (usually 3 or 5 days) and then challenged with THC 2 or more weeks after the last THC injection show a greater behavioral activation than rats repeatedly treated with vehicle. The behavioral activation after THC challenge in THC-sensitized rats was mainly characterized by increased stereotyped behavior. Cross-sensitization may occur between cannabinoid agonists and other drugs abused by humans, including heroin (Pontieri et al. 2001b; Lamarque et al. 2001), morphine (Cadoni et al. 2001), and amphetamine (Gorriti et al. 1999; Lamarque et al. 2001). In one study (Arnold et al. 1998), the CB1 receptor agonist CP 55,940 did not induce behavioral sensitization in rats, but the dose range and the spaced time between injections may have contributed to the negative results. In another study (Martin et al. 2000), cocaine, but not morphine, was able to induce behavioral sensitization in genetically modified mice lacking the CB1 cannabinoid receptor. The adaptive neurobiological changes underlying sensitization could play a role not only in the development of vulnerability to marijuana addiction but also in the vulnerability to addiction to other drugs.

Cannabinoid dependence

Abstinence from cannabis use by chronic users seldom results in development of pronounced signs of withdrawal, indicative of physical dependence, although there are a growing number of reports of spontaneously occurring signs of withdrawal from THC in humans (Weisbeck et al. 1996; Crowley et al. 1998; Budney et al. 1999, 2001; Haney et al. 1999; Smith 2002) and animals (Costa et al. 2000; Aceto et al. 2001). Due to its high lipophilicity, THC is easily stored in fat tissue and then slowly released. This "depot" effect likely prevents the development of a pronounced withdrawal syndrome when chronic cannabis use is abruptly discontinued. However, administration of the CB1 selective cannabinoid receptor antagonist SR 141716A generally precipitates a pronounced withdrawal syndrome in animals that have been chronically treated with cannabinoids (Aceto et al. 1995, 1996, 2001; Tsou et al. 1995; De Fonseca et al. 1997; Hutcheson et al. 1998; Tanda et al. 1999; Costa et al. 2000). The repertoire of behavioral signs of antagonist-precipitated cannabinoid withdrawal in rats and mice includes, increased grooming, wet-dog shakes, hunched-back posture, piloerection, tremors, paw tremors, and ptosis. At variance with findings of Costa et al. (2000) of both spontaneous and SR 141716A–precipitated withdrawal symptoms in anandamide dependent rats, Aceto et al. (1998) found no signs of withdrawal in rats subchronically infused with IP anandamide. The different findings in these two reports (Aceto et al. 1998; Costa et al. 2000) are likely due to different parameters of subchronic administration of anandamide in terms of dose range, length of exposure (in time), and timing of injections. All of these factors can play a role in the degree of dependence obtained at the end of chronic treatment. Other methodological differences that could account for the different findings are differences are the range of doses of the CB1 antagonist used to precipitate withdrawal signs and the different behavioral tests used to measure withdrawal.

Biochemical indexes of adaptive changes have also been demonstrated during antagonist-precipitated cannabinoid withdrawal in rats and mice, including activation of corticotropin releasing factor (De Fonseca et al. 1997), pronounced increases in adenyl-cyclase activity in the cerebellum (Hutcheson et al. 1998), reduced activity of dopamine neurons in the VTA (Diana et al. 1998), and dose-related decreases in dopamine transmission in the shell of the nucleus accumbens (Tanda et al. 1999). Some of these neurochemical signs also occur during withdrawal from other drugs of abuse, such as alcohol (Rossetti et al. 1991, 1992a, 1992b; Merlo Pich et al. 1995), cocaine (Richter et al. 1995; Richter and Weiss 1999) and morphine (Rossetti et al. 1991, 1992a, 1992b; Acquas et al. 1991; Acquas and Di Chiara 1992).

Acute administration of THC provides excitatory input to dopaminergic neurons in the VTA through activation of CB1 cannabinoid receptors (French et al. 1997). It also increases extracellular levels of dopamine in the nucleus accumbens shell (Tanda et al. 1997), a brain area involved in the reinforcing and addictive actions of drugs abused by humans (Pontieri et al. 1995, 1996; Di Chiara et al. 1999). In rats treated with increasing doses of THC over a period of 8 days, a single challenge with SR 141716A produced behavioral signs of withdrawal, the intensity of which was time- and dose-dependently associated with decreases in extracellular levels of dopamine in the shell of the nucleus accumbens (Tanda et al. 1999) (see Fig. 5). However, extracellular levels of dopamine were not significantly different between chronic THC- and saline-treated rats. This suggests that THC-induced increases in dopaminergic firing (French et al. 1997) and dopamine release (Tanda et al. 1997) progressively substituted for the tonic excitatory inputs to dopamine neurons projecting to the nucleus accumbens shell (Diana et al. 1998), a phenomenon revealed by administration of SR 141716A, which abruptly precipitated a physical withdrawal syndrome together with a decrease in mesolimbic dopamine transmission. As with other drugs abused by humans, these adaptive responses of the body to chronic cannabinoid exposure likely contribute to the abuse liability of cannabis. Indeed, reduction in dopamine levels in the nucleus accumbens, which is associated with aversive states, has been suggested to play an important role in

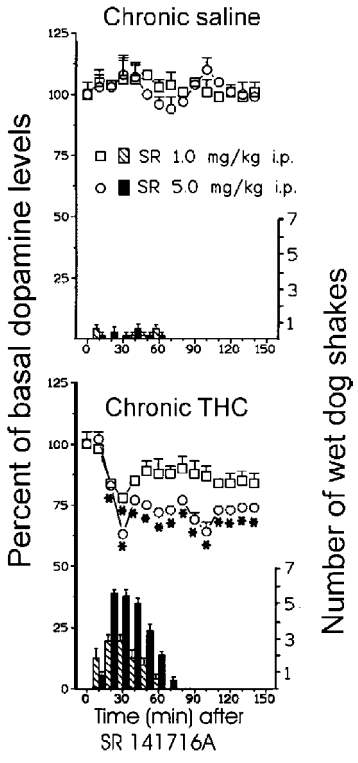

Fig. 5 THC-induced dependence in rats. Time course of effects of acute administration of the cannabinoid CB1 receptor antagonist SR 141716A on dopamine neurotransmission in the shell of the nucleus accumbens and on behavioral signs of a withdrawal syndrome in rats made dependent on THC by repeated daily THC injections (*lower panel*) or in control rats injected daily with saline (*upper panel*). Results are expressed as the percentage change (mean ± SEM) in dopamine levels from basal values (*open square* = 1.0 and *open circle* = 5.0 mg/kg IP dose of SR 141716A), or as the number of wet-dog-shakes (*hatched bars* 1.0 and *solid bars* 5.0 mg/kg IP dose of SR 141716A) observed every 10 min, for a total of 150 min, after administration of SR 141716A. *Filled symbols* $p<0.05$ compared with basal values. *$p<0.05$ compared with the corresponding time point of chronic saline treated rats challenged with SR 141716A. See Tanda et al. (1999) for more detailed information on methods and statistics. Modified from Tanda et al. (1999)

compulsive drug use and addiction (Koob and Le Moal 1997, 2001; Kreek and Koob 1998; Di Chiara et al. 1999).

Interactions between cannabinoid, opioid, and dopaminergic systems

The cannabinoid system functionally interacts with many other neurotransmitter systems in the brain, including GABAergic, glutaminergic, and cholinergic systems (Ameri 1999). However, interactions with dopaminergic and opioid systems are believed to be of primary importance for the expression of rewarding effects of cannabinoids and development of cannabinoid physical dependence (Tanda et al. 1997, 1999; Ledent et al. 1999; Navarro et al. 2001; Zimmer et al. 2001; Ghozland et al. 2002; also see reviews by Gardner and Vorel 1998; Maldonado and de Fonseca 2002) and have been the focus of most recent research in this area. One study demonstrating such interactions was described by Gardner et al. (1989) (see also Gardner and Vorel 1998), in which THC's effect of reducing the threshold for intracranial self-stimulation was blocked by naloxone, a nonselective, opioid-receptor antagonist (Fig. 2). In another recent study (Mas-Nieto et al. 2001), it was found that the cannabinoid CB1 receptor antagonist SR 141716A blocked acquisition of a morphine-induced place-preference and reduced the incidence of withdrawal signs precipitated by acutely administered naloxone when coadministered with morphine for 5 days, but not when acutely injected before naloxone. Interactions between opioid and cannabinoid systems have also been found in recent drug self-administration studies. For example, the cannabinoid antagonist SR 141716A reduced self-administration of heroin by rats (Braida et al. 2001b; Navarro et al. 2001), and, in turn, the opioid antagonists naloxone or naltrexone reduced self-administration of THC by squirrel monkeys (Goldberg et al. 2001) or self-administration of the CB1 agonist CP 55,940 by rats (Braida et al. 2001b).

The mesolimbic dopamine system is part of a brain reward circuit that has long been thought to play a major role in mediating reinforcing/rewarding effects of drugs of abuse (Koob 1992; Wise 1987, 1996; Di Chiara et al. 1999). Many drugs abused by humans share the common property of selectively increasing dopamine release in the nucleus accumbens, the major terminal area of the mesolimbic dopamine system (Prado-Alcala and Wise 1984; Wise 1987; Di Chiara and Imperato 1986, 1988; Hernandez and Hoebel 1988; Kalivas and Duffy 1990; Carboni et al. 1989; Damsma et al. 1989; Kuczenski et al. 1991; Chang et al. 1994), but this has been a matter of debate with regard to THC and other cannabinoids.

The first demonstration that cannabinoids could increase dopamine levels in the accumbens was by Gardner and colleagues (Chen et al. 1990, 1991). They found that low doses of THC (0.5 and 1.0 mg/kg IP) elevated dopamine levels in the nucleus accumbens with Lewis rats and, to a lesser extent, with Sprague Dawley rats (there was a smaller increase and only at a single dose of 1.0 mg/kg IP), but no increase was found with Fisher rats. However, in another study by Castaneda et al. (1991), oral doses of 1–10-mg/kg THC failed to increase dopamine levels in either the caudate putamen or the nucleus accumbens of Long Evans rats. Conflicting findings have also been obtained in in vitro studies with THC in Sprague Dawley rats. In one study (De Fonseca et al. 1992), THC administration did not change dopamine or L-3,4-dihydroxyphenylacetic acid (DOPAC) content, or DOPAC/DA ratios, in either the striatum or limbic forebrain areas. In a subsequent study (Navarro et al. 1993), however, THC administration increased dopamine and DOPAC content in the same brain areas. In a more recent study, Tanda et al. (1997), both THC and the synthetic CB1 receptor agonist WIN 55212-2 dose dependently and selectively increased dopamine levels in the shell compared with the core of the nucleus

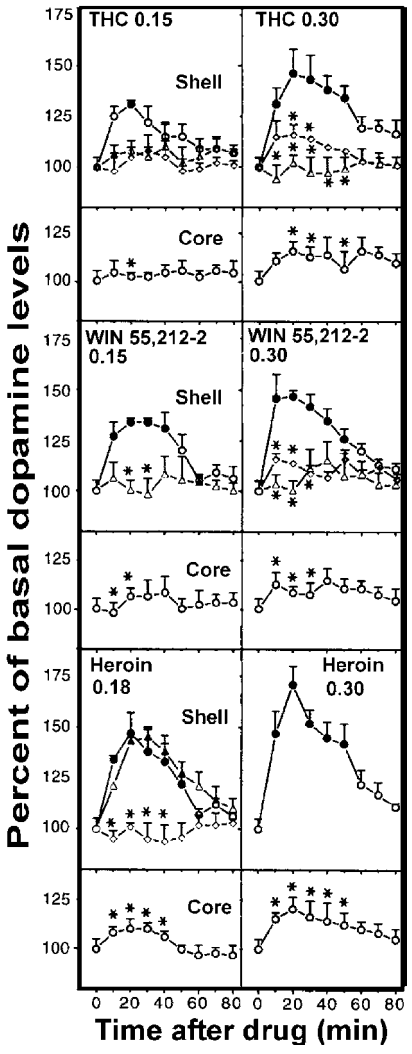

Fig. 6 Effects of cannabinoids and heroin on mesolimbic dopamine transmission. Time course of the effects of acute administration of SR 141716A (*open triangle*), naloxone (*open diamond*), or vehicle (*open circle*) on increases in extracellular levels of dopamine in the nucleus accumbens shell and core elicited by intravenous administration of THC (0.15 and 0.30 mg/kg, *four upper panels*), WIN 55,212-2 (0.15 and 0.30 mg/kg, *four middle panels*), or heroin (0.018 and 0.03 mg/kg, *four lower panels*). Results are expressed as the percentage change (mean ± SEM) in dopamine levels from basal values. *Filled symbols* $p<0.05$ compared with basal values. *$p<0.05$ compared with the corresponding time point obtained in the shell of saline pretreated rats. See Tanda et al. (1997) for more detailed information on methods and statistics. Modified from Tanda et al. (1997)

accumbens in Sprague Dawley rats (see Fig. 6). This strict topographical selectivity of THC's effects on dopamine transmission demonstrated by Tanda et al. (1997) might be responsible for previous difficulties in obtaining reliable THC-induced elevations in dopamine levels in the nucleus accumbens when the shell and core were not differentiated (e.g., Chen et al. 1991; Castaneda et al. 1991).

The ability of THC to selectively increase dopamine release in the shell versus the core of the nucleus accumbens is shared by most other drugs abused by humans, including cocaine, amphetamine, morphine, nicotine and heroin (Pontieri et al. 1995,1996; Tanda et al. 1997). Moreover, it has been shown that THC and opioid agonists, like morphine and heroin, increase dopamine transmission in the nucleus accumbens through a common opioid receptor-mediated mechanism (Chen et al. 1990; Tanda et al. 1997) (Fig. 6). Indeed, dopamine elevations in the accumbens that are produced by both THC and opioids are blocked by either systemic administration of naloxone (Chen et al. 1990; Tanda et al. 1997) (Fig. 6), a nonselective antagonist of opioid receptors, or by local infusion of naloxonazine, a selective antagonist of μ1-opioid receptors, directly into the VTA where dopaminergic neurons projecting to limbic areas are located (Tanda et al. 1997). In the VTA, opioid receptors are located on the cell membranes of non-dopaminergic neurons that tonically inhibit dopaminergic neurons. Administration of opioid agonists like heroin can reduce the activity of these non-dopaminergic neurons (Johnson and North 1992), resulting in increased firing activity of dopaminergic neurons and increased levels of dopamine in terminal areas such as the shell of the nucleus accumbens (Di Chiara and North 1992). Thus, THC acting on CB1 cannabinoid receptors in the brain (but not necessarily in the VTA) might elicit the release of endogenous opioids, which then act on opioid receptors in the VTA resulting in increased levels of dopamine in the nucleus accumbens (Tanda et al. 1997).

An alternative mechanism for the ability of cannabinoid agonists to increase dopamine release in the mesolimbic dopamine system has been proposed by Gardner and Vorel (1998). In in vivo microdialysis studies by Gardner and colleagues, THC increased extracellular dopamine levels in the nucleus accumbens when it was injected directly into this dopaminergic terminal area, but not when it was injected into the VTA (Chen et al. 1993). Moreover, in electrophysiology experiments with rats, Gifford et al. (1997) found that microinjections of THC into the VTA did not increase the firing rate of dopamine neurons projecting to the nucleus accumbens (a finding at variance with that of French et al. 1997, who found that THC increased firing rate of dopaminergic neurons). Gardner and Vorel (1998) suggested that cannabinoid agonists exert their rewarding effects through direct activation of dopaminergic neurotransmission in the nucleus accumbens.

Antinociception and physical dependence development are two other areas, in addition to drug reinforcement or reward, in which major interactions between opioid neurotransmitter systems and cannabinoid neurotransmitter systems have been found. For example, opioid-receptor antagonists block the antinociceptive activity of cannabinoids (Smith et al. 1994) and cross-tolerance exists between morphine and THC for antinociception (Thorat and Bhargava 1994). These effects are potentiated in morphine-dependent rats treated with THC (Rubino et al. 1997b). It has also been reported that acute administration of naloxone to cannabinoid-dependent rats

produces an opioid-like withdrawal syndrome (Kaymakcalan et al. 1977; Navarro et al. 2001), which suggests adaptive alterations of brain opioid systems after prolonged exposure to THC. This is further suggested by recent findings that young rats treated with THC during the age of brain development are subsequently more sensitive to the reinforcing effects of opioids once mature (Rubio et al. 1998; Vela et al. 1998). Interactions between opioid and cannabinoid systems also occur in the opposite direction. For example, in opioid-dependent rats, administration of the cannabinoid CB1 receptor antagonist SR 141716A can precipitate an opioid-like withdrawal syndrome (Navarro et al. 1998), and reduction of the severity of behavioral withdrawal signs in opioid-dependent animals has been obtained by administration of cannabinoid agonists (Hine et al. 1975; Vela et al. 1995; Valverde et al. 2001).

The absence of changes in cannabinoid-receptor binding in animals tolerant to morphine or in opioid-receptor binding in animals tolerant to THC (Thorat and Bhargava 1994; Romero et al. 1998) suggests that these systems might interact at the level of their signal transduction mechanisms. This hypothesis is supported by the colocalization of these receptors in different brain areas mediating antinociception (Mansour et al. 1988; Thorat and Bhargava 1994; Mailleux and Vanderhaegen 1992). An alternate hypothesis for the interactions between the cannabinoid and the opioid systems comes from studies demonstrating that cannabinoids can increase the synthesis and induce the release of endogenous opioids (Corchero et al. 1997a, 1997b; Manzanares et al. 1998).

Cannabinoid actions in genetically modified mice

The recent introduction of genetically modified mice in neuroscience has been a useful tool for studying the physiological role and the possible involvement in neurological disease and therapy of different neurotransmitter systems and receptors. A limitation of these kind of studies has usually been the difficulty of interpreting results obtained in an organism in which development of alternative pathways and/or different activity of other systems and receptors might occur as a result of deletion of receptors or other enzymatic proteins in the brain. It has been shown, for example, that CB1 receptors knock-out mice, although apparently healthy and without obvious morphological abnormalities, have an increased mortality when compared with heterozygous control mice (Zimmer et al. 1999). Such an effect was not mentioned in a report on studies with another line of CB1 receptors knock-out mice (Ledent et al. 1999). The mice died without any obvious cause of death that could be determined from pathological examination. These mice also showed behavioral and physiological alterations that might reflect the lack of CB1 receptors in the brain (Zimmer et al. 1999). Interestingly, Zimmer et al. (1999) reported that CB1 receptors knock-out mice showed reduced spontaneous activity in an open field test and reduced analgesia in a hot plate test when compared with heterozygous control mice. In contrast, Ledent et al. (1999) reported increased locomotor activity and exploratory behavior in an open field test and no significant difference in the nociceptive thresholds in a hot plate test in CB1 receptors knock-out mice when compared with heterozygous control mice. From these results it appears that different compensative adaptations to the lack of CB1 receptors occurred in the different lines of CB1 receptors knock-out mice.

As pointed out above, there are important interactions between cannabinoid and opioid systems that exist with drug reinforcement or reward. These interactions are increasingly being studied through the use of genetically modified mice. For example, CB1 cannabinoid-receptor knock-out mice do not appear to self-administer morphine (Ledent et al. 1999; Cossu et al. 2001), although they do self-administer amphetamine and cocaine (Cossu et al. 2001). Moreover, in these mice, morphine did not significantly increase dopamine release in reward-related areas (Mascia et al. 1999) and the opioid withdrawal syndrome was less severe (Ledent et al. 1999). CB1 receptors knock-out mice, also, do not appear to develop conditioned place aversions to compartments associated with the effects of the K-opioid agonist U-50488, as compared with wild-type mice (Ledent et al. 1999).

The effects of cannabinoids have also been recently investigated using mice with genetically modified opioid systems, including pre-proenkephalin deficient mice, pre-dynorphin deficient mice, and mice lacking μ-, K-, or δ-opioid receptors. In pre-proenkephalin deficient mice, the THC withdrawal syndrome induced by SR 141716A was less pronounced (Valverde et al. 2000), suggesting a role for the endogenous enkephalinergic system in the development of cannabinoid physical dependence. In other studies, dynorphin-deficient mice did not develop conditioned place aversions after repeated treatment with THC, suggesting the involvement of K-opioid receptors in the aversive effects of cannabinoids (Zimmer et al. 2001). Also, mice lacking μ-opioid receptors did not develop positive place preferences for compartments associated with THC, while mice lacking K-opioid receptors developed positive place preferences with THC, in contrast to the place aversion that developed in wild-type mice (Ghozland et al. 2002). Thus, there is a rapidly growing body of evidence from preclinical studies with experimental animals that the cannabinoid and opioid systems in the brain are able to interact and modulate each other's functional activity. The relevance of this to the clinical situation with humans remains to be established. It is interesting to note, however, that in recent human studies, naloxone treatment failed to produce any significant change in a range of subjective and physiological responses to oral or smoked marijuana (Wachtel and de Wit 2000; Greenwald and Stitzer 2000).

Summary

It has now been demonstrated that strong and persistent intravenous self-administration behavior can be obtained in squirrel monkeys using a range of THC doses that are in agreement with the total intake and the single doses of THC normally self-administered by humans smoking marijuana cigarettes. This provides a reliable and direct tool for assessing the reinforcing effects of THC that are central to its abuse liability. In addition, recent demonstrations of persistent intravenous self-administration of synthetic cannabinoid CB1 receptor agonists by rats and mice and the development of genetically modified mice lacking specific cannabinoid receptors provide convenient rodent models for exploring underlying neurochemical mechanisms. Repeated demonstrations in rats that THC and synthetic CB1 agonists can induce conditioned place preferences or aversions, depending on details of dose and spacing, can reduce the threshold for intracranial self-stimulation behavior under certain conditions, and can serve as effective discriminative stimuli for operant behavior, provide less direct, but more rapidly established, measures for investigating the rewarding effects of cannabinoids.

Also, compounds acting at different levels on the brain cannabinoid system have been discovered and are now available as research tools (Di Marzo et al. 1994; Piomelli et al. 1998, 2000; Felder and Glass 1998; Martin et al. 1999; Martin 2002; Kathuria et al. 2003; Palmer et al. 2002). Endogenous ligands for brain cannabinoid receptors, like anandamide, 2AG, and (possibly) noladine ether have been isolated. Synthetic drugs able to interact as agonists at cannabinoid CB1 receptors, like WIN 55,212 and CP 55,940, or as antagonists, like SR 141716A and SR 144528, have been developed. Finally, drugs able to block the reuptake mechanism for endogenous cannabinoids, like AM 404, and drugs able to block the metabolic pathways for endogenous cannabinoids, like AM 374, AM 381, and the more recently developed fenoxybutamate and carbifenamate (Kathuria et al. 2003) are now available.

As in the case with other drug classes, such as psychostimulants and opiates, the development of reliable methodologies for measuring the reinforcing effects of cannabinoids and their underlying neurochemical mechanisms and the availability of new generations of compounds that act on the cannabinoid system provide exciting opportunities to search for drugs that possess the therapeutic potentials attributed to THC, alone or in the form of marijuana, but lack the potentially harmful behavioral adverse effects of abuse liability. The recent discoveries of major functional interactions between endogenous cannabinoid, opioid, and dopaminergic neurotransmitter systems in areas such as analgesia, physical dependence, and tolerance development and drug reinforcement or reward provide another opportunity to search for drugs with the beneficial therapeutic actions of currently available cannabinoids or opioids but without their undesirable adverse effects such as abuse liability.

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