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Endocannabinoid system involvement in brain reward processes related to drug abuse

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Abstract

Cannabis is the most commonly abused illegal drug in the world and its main psychoactive ingredient, delta-9-tetrahydrocannabinol (THC), produces rewarding effects in humans and non-human primates. Over the last several decades, an endogenous system comprised of cannabinoid receptors, endogenous ligands for these receptors and enzymes responsible for the synthesis and degradation of these endogenous cannabinoid ligands has been discovered and partly characterized. Experimental findings strongly suggest a major involvement of the endocannabinoid system in general brain reward functions and drug abuse. First, natural and synthetic cannabinoids and endocannabinoids can produce rewarding effects in humans and laboratory animals. Second, activation or blockade of the endogenous cannabinoid system has been shown to modulate the rewarding effects of non-cannabinoid psychoactive drugs. Third, most abused drugs alter brain levels of endocannabinoids in the brain. In addition to reward functions, the endocannabinoid cannabinoid system appears to be involved in the ability of drugs and drug-related cues to reinstate drug-seeking behavior in animal models of relapse. Altogether, evidence points to the endocannabinoid system as a promising target for the development of medications for the treatment of drug abuse.

1. Introduction

Although recreational use of cannabis has been known for centuries, its abuse has dramatically increased in Europe and the United States over the last few decades. Cannabis and its derivatives produce feelings of euphoria, “high” and well-being in humans (1) which probably are central in the reinforcement of repeated cannabis use and the development of cannabis dependence. Until recently the brain mechanisms underlying the reinforcing and dependence-producing effects of cannabis remained unclear. However, the characterization of delta-9-tetrahydrocannabinol (THC) as the main active ingredient in cannabis (2), the identification and cloning of the first cannabinoid receptors, and the designing of potent and stereoselective synthetic cannabinoid agonists and antagonists led to the discovery of an entire endogenous

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system, which not only mediates the behavioral and neurobiological effects of cannabis but also plays an important role in the regulation of a wide variety of brain functions (3–7).

The endogenous cannabinoid system consists of cannabinoid (CB) receptors, endogenous ligands for these receptors and enzymes involved in the synthesis and degradation of these endogenous ligands (3–7). Two types of cannabinoid receptors, CB1 and CB2, have been cloned and well characterized and additional cannabinoid receptors have been proposed but not yet identified (8,9). Although it has been recently shown that CB2 receptors are present in the brain (10–12), it appears that the abuse-related effects of cannabinoids are mostly, if not exclusively, mediated by CB1 receptors. The two identified and best characterized endogenous ligands for cannabinoid receptors are anandamide (13) and 2-AG (14,15). These lipophilic compounds are derived from arachidonic acid and are not stored in vesicles but, instead, are formed and released “on demand” and are quickly inactivated by a two-step process involving transport into neurons, presumably by a yet-to-be identified active membrane transport mechanism (16,17), and metabolic inactivation, primarily by fatty acid amide hydrolase (FAAH) in the case of anandamide (18–21) and monoacylglycerol lipase (MGL) in the case of 2-AG (22,23).

Here, we will focus on the involvement of the endogenous cannabinoid system in brain reward processes that are believed to play pivotal roles in the development and maintenance of drug abuse and dependence.

2. Rewarding effects of cannabinoids

Clinical evidence supporting the hypothesized role of the cannabinoid system in brain reward processes (24,25) are the wide spread abuse of cannabis, the self-administration of marijuana and THC by human subjects in experimental settings, and the subjective reports of “high, well being and euphoria” following administration of cannabis extracts or THC in humans (26–28). Despite the clinical evidence for rewarding effects of cannabis and its psychoactive ingredient THC in humans, rewarding effects of THC or other synthetic cannabinoid agonists in animal models of drug abuse have been difficult to demonstrate (24,29–32). It has been long debated whether cannabis is primarily used for its rewarding effects or whether it lacks clear rewarding effects and is, instead, voluntarily administered by humans for its mind-altering effects. However, several recent findings have demonstrated that, cannabinoid agonists can initiate and sustain active self-administration behavior in monkeys and rodents, although the range of conditions under which this occurs is more limited than with drugs such as cocaine and heroin, (33–35). A further step in demonstrating that the endogenous cannabinoid system is involved in brain reward processes is the recent demonstration that the endogenous cannabinoid receptor ligand anandamide can initiate and sustain active self-administration behavior in monkeys (32).

2.1. Self-administration of cannabinoids

Until a decade ago, demonstrations of THC self-administration by experimental animals had been limited and unconvincing (32). Several studies (36–42) failed to demonstrate that persistent, dose-related, intravenous drug self-administration behavior could be maintained by THC or synthetic cannabinoid agonists, in a manner that was susceptible to vehicle extinction and subsequent reinstatement.

In the 1990s, with the expansion in cannabinoid research that occurred after the cloning of cannabinoid receptors, several groups reassessed the rewarding effects of cannabinoids in experimental animals using intravenous drug self-administration techniques. In 1998, Martellotta and colleagues, were the first to report that mice would show some evidence of self-administration behavior with a synthetic CB1 receptor agonist WIN55,212-2 (43).

However, special conditions were required for this demonstration. First, self-administration was inferred from a dose-dependent shift to nose pokes in an active hole associated with a WIN55,212-2 injection from nose pokes in an inactive hole, but total number of nose-pokes did not vary from one condition to the other. In addition, self-administration was studied during a single acute self-administration session and required restraint and acute insertion of a needle into the mouse's tail, raising questions about the involvement of cannabinoid-induced anxiolytic effects relieving restraint-induced stress (44) or cannabinoid-induced analgesic effects (45–47), rather than true brain reward, in the reinforcement of active-hole nose-poke behavior. Finally, the acute nature of the experiment, by itself, made results difficult to interpret and compare with data from self-administration studies in catheterized animals which employed repeated daily sessions.

More recently, self-administration of WIN55,212-2 was demonstrated in catheterized mice during repeated daily testing when they were food-deprived (34). WIN55,212-2 self-administration behavior was rapidly acquired in mice lacking kappa-opioid receptors; however acquisition was markedly delayed in wild-type mice unless they received an intraperitoneal injection of WIN55,212-2 the day before the start of self-administration sessions. This strategy of pre-exposure to cannabinoid was previously used by the same research group to obtain significant conditioned place preference with THC in wild-type mice (48). The authors speculated that rewarding effects of THC and synthetic agonists such as WIN55,212-2 are normally masked in mice by strong aversive effects mediated by kappa-opioid receptors (49), which are preponderant during the first exposure to THC or WIN55,212-2 and can be unmasked by a priming injection of THC or WIN (34,48).

Significant intravenous self-administration of WIN55,212-2 was again reported in food-restricted Long Evans rats by Fattore and colleagues (33). Self-administration of WIN55,212-2 was dose-dependent (with peak responding at 12.5 µg/kg/injection) and behavior extinguished (although very slowly) when vehicle was substituted for WIN55,212-2. Vehicle-like responding was also found when the cannabinoid CB1 receptor antagonist rimonabant (SR-141716, Acomplia) was administered before the session. Self-administration of WIN55,212 in rats has been confirmed several times by the same group as well as other by groups (50–54). Interestingly, whereas Deiana et al., (51) reported that Sprague-Dawley rats (in contrast to Long Evans and Lister Hooded rats) do not acquire WIN55,212-2 self-administration behavior, Lecca et al., (53) reported significant acquisition of self-administration behavior in Sprague-Dawley rats under conditions similar to those used by Deiana et al. (51).

Further investigations are needed to resolve these discrepancies, but, it should be noted, that we and others have been unable to demonstrate reliable intravenous self-administration behavior with THC under similar conditions (unpublished findings). However, we recently demonstrated that THC maintains self-administration behavior in Sprague-Dawley rats when it is directly injected into the ventral tegmental area (VTA) or the nucleus accumbens (55). This intracranial self-administration behavior is acquired quickly, extinguishes within a single session when vehicle is substituted for THC or when rats are injected with the CB1 antagonist rimonabant before the session, and self-administration behavior is immediately re-acquired when THC is again available. Thus, it is possible that THC has negative effects in rats that mask its rewarding effects and that brain mechanisms mediating these negative effects are bypassed when THC is directly injected into the VTA or nucleus accumbens.

In 2000, we were the first to report robust and persistent self-administration of THC in non-human primates (35). Self-administration of THC was maintained at rates as high as those of cocaine in the same experimental conditions, was dose dependent, extinguished rapidly when vehicle was substituted for THC or when rimonabant was administered before the session, and

immediately returned to normal levels when THC was made available again (35). Whereas the monkeys in this study had a history of cocaine self-administration behavior that had been extinguished for several weeks before the study began, (30), we subsequently found that naïve squirrel monkeys, with no drug or experimental history, also learned to self-administer THC and, in fact, the final levels of drug-taking behavior that developed were about two-fold higher than in our earlier study with cocaine-experienced monkeys (56). Interestingly, we have also found that THC can sustain long sequences of drug-seeking behaviour under a second-order schedule of intravenous THC self-administration (Justinova et al., unpublished findings) where responding during experimental sessions is maintained by cues associated with THC injection only at the end of the session (57–59).

Finally, we have recently demonstrated that, like THC, the endogenous cannabinoid anandamide and its synthetic analogue methanandamide maintain high rates of self-administration behavior when they are intravenously administered (32). Self-administration of anandamide and methanandamide was dose dependent (with both compounds being about 10 times less potent than THC), and was sensitive to vehicle extinction and to pharmacological blockade of CB1 receptors by rimonabant (32).

2.2. Conditioned place preference and aversion with cannabinoids

Conditioned place preference (CPP) is an alternative measure of reward in animals. Although CPP provides a less direct estimation of rewarding effects of drugs than intravenous self-administration, it has several advantages. Animals do not need to be catheterized, allowing the use of subcutaneous and intraperitoneal routes of administration, and experiments can usually be completed within one or two weeks. Also, both positive (rewarding) and negative (aversive) effects of drugs can be evaluated within the same experiment, which can be particularly interesting with cannabinoid agonists, where quite contrasting findings ranging from positive place preferences to no effect to place aversions can be found.

Gardner and colleagues were among the first to evaluate the effects of THC on place conditioning (60). In their experiments with Long-Evans rats, THC-induced conditioned place preferences were observed but they depended not only on the dose of THC but also on the regimen of THC administration. With place-conditioning procedures, conditioning sessions with drug and vehicle alternate. Thus, when conditioning sessions were performed every day and THC was given every second day, conditioned place preferences developed at THC doses of 2 and 4 mg/kg with no effect at 1 mg/kg, whereas, when conditioning sessions were performed every second day and THC was given every fourth day, preferences only developed at the 1 mg/kg dose of THC and higher THC doses produced conditioned place aversions (60). Subsequently, a few studies have shown cannabinoid-induced conditioned place preferences in rats and all of them used a standard every day conditioning procedure. One study using the potent synthetic CB1 receptor agonist CP 55, 940 and Wistar rats found conditioned place preferences at a dose of 20 µg/kg but there were no effects at lower or higher doses (61); another study using Sprague-Dawley rats and THC found conditioned place preferences at a dose of 0.1 mg/kg but no effect at lower or higher doses (62); a third study using Wistar rats and WIN55,212-2 found conditioned place preferences at a dose of 1 mg/kg in rats housed in enriched conditions but not in rats housed in standard conditions (63). A number of studies have found that cannabinoid agonists produce conditioned place aversions and not place preferences (61,64–68). These studies used the same compounds, the same rat strains, similar conditioning procedures, and similar dose range as the studies that demonstrated conditioned place preferences. Thus, drawing general conclusions on whether cannabinoids have reinforcing or aversive effects in place conditioning procedures is difficult. If we consider the reinforcing effects of cannabinoids in self-administration procedures, it appears that the rewarding effects of THC in place preference procedures may be masked and, in fact, often

reversed by some aversive effects. It is also possible that differences exist in the rewarding and aversive effects of cannabinoids in mice and rats, since a survey of results with cannabinoid agonists on another emotional measure, anxiety, found that mice show predominantly anxiolytic effects while rats show predominantly anxiogenic effects (69).

Important advances in the characterization and understanding of the rewarding and aversive effects of cannabinoids in mice have been provided by the work of Maldonado and colleagues. These studies suggest that the first administration of THC, even at a low dose (1mg/kg), produces aversive effects that prevent the development of subsequent conditioned place preferences (48). However, these aversive effects appear to undergo fast tolerance, since mice that are given a first injection of THC 24 hours before the start of conditioning go on to develop positive conditioned place preferences (48). In addition, whereas 5 mg/kg of THC induced conditioned place aversions in their experiments under normal conditions, this aversive effect was lost when a priming injection of THC was given 24h before the start of conditioning (48). In subsequent studies, it was found that the THC-induced conditioned place aversions in mice depended on kappa-opioid receptors (49) and endogenous dynorphin (70), whereas THC-induced conditioned place preferences depended on mu-opioid receptors (49).

The activation of these systems by cannabinoid agonists can be region-specific, since we have recently shown that THC induces conditioned place preferences when injected directly into the posterior part of the nucleus accumbens shell or the posterior ventral tegmental area (55). Only one study has addressed the question on whether endogenous cannabinoids have rewarding or aversive effects in place conditioning procedures. Mallet and Beninger (67) compared THC and the endocannabinoid anandamide in Wistar rats and found that THC at doses of 1 and 1.5 mg/kg (but not lower or higher doses), but not anandamide (0–16 mg/kg i.p.) induced significant conditioned place aversions. In this study, anandamide injections (as well as vehicle injections) were preceded by injection of the non-specific protease inhibitor phenylmethylsulfonyl fluoride in order to slow down anandamide's rapid metabolic inactivation. We have recently found that anandamide does not have effects on place conditioning when injected intravenously by itself but it induces dose-related conditioned place aversions when its metabolic inactivation is inhibited with the selective fatty acid amide hydrolase (FAAH) inhibitor, URB-597 (Scherma et al., unpublished findings). URB-597 alone does not produce either conditioned place preferences or aversions (19,71), even at doses 10 to 30-fold higher than the 0.1 to 0.3 mg/kg doses needed to produce significant elevations in brain levels of anandamide (19,71) and potentiate the *in vivo* effects of systemically administered anandamide (72,73). In contrast, the endocannabinoid transport inhibitor, AM-404 induces a small but significant conditioned place preference in rats housed in enriched conditions (63). Interestingly, the dose of 2.5 mg/kg AM-404 that induces conditioned place preferences does not increase either anandamide or 2-AG in the brain areas investigated (63). Thus, the involvement of the endogenous cannabinoid system in AM-404-induced place preferences remains doubtful.

Finally, although most studies have found no effects of the cannabinoid CB1 antagonist rimonabant on place conditioning (74–76), a few studies have found that rimonabant induces conditioned place preferences in rats (66,68). Again, as with AM-404, it is not possible to determine whether rimonabant induces conditioned place preferences in these studies by blocking an endogenous cannabinoid tone or by acting as an inverse agonist (77). These results, together with those showing conditioned place aversions induced by cannabinoid CB1 agonists, have led some authors to hypothesize that the endogenous cannabinoid system is an “aversive or counter-rewarding system” (66).

2.3. Discriminative stimulus effects of cannabinoids

A protocol that we and others have used to characterize the abuse-related effects of cannabinoids is the two-lever choice drug discrimination procedure with THC or other cannabinoid CB1 agonists as the training drug (78–88). Rats or monkeys readily learn to discriminate even relatively low doses of THC from vehicle, although the development of stable discrimination performance usually requires 30 sessions or more. While discriminative stimulus effects of drugs are not a direct measure of reward, it is clear that discriminative effects of drugs play an important role in the initiation and maintenance of drug-taking behavior (89,90). Importantly, the range of effects measured by drug discrimination are wider than those of direct measures of reward and reinforcement and can include aversive, anxiogenic or anxiolytic effects of cannabinoids (89,90). From a practical point of view, discriminative stimulus effects of cannabinoid CB1 receptor agonists are very strong and they provide a behavioral baseline that remains stable over long periods of time (84). The discriminative effects of cannabinoids are also pharmacologically selective so that, generally, only cannabinoid CB1 receptor agonists produce discriminative effects similar to those of THC and only CB1 receptor antagonists block them (72,84–86,91–93). There are examples of non-CB1 agonists or antagonists having THC-like discriminative effects, but when this occurs results often suggest increased or decreased release of endogenous cannabinoid CB1 agonists as a mechanism. For example, we have recently demonstrated that nicotine produces dose related THC-like discriminative effects after FAAH inhibition with URB-597 (93).

Drug discrimination procedures allow for extensive collection of data, for the qualitative and quantitative evaluation of the effects of new cannabinoid compounds and for the evaluation of the influences of an infinite number of compounds on the behavioral effects of cannabinoids. Drug discrimination procedures also appear to have good predictive validity for self-administration of drugs of abuse (89). For example, we have recently demonstrated that the alpha-7 nicotinic receptor antagonist MLA reduce both the discriminative effects of THC and the reinforcing effects of WIN55,212-2 (54). Thus, given the difficulties of having stable and replicable models of cannabinoid self-administration and conditioned place preference in rodents, drug discrimination is an excellent alternative for studying the pharmacology of cannabinoids (29–31).

Several studies have investigated whether endogenous cannabinoid ligands produce THC-like discriminative effects when systemically administered and found that anandamide either does not produce THC-like discriminative effects or it does so only at very high doses that dramatically depress rates of responding (80,91,94). Since, under the same or similar conditions, metabolically stable synthetic analogues of anandamide such as methanandamide, O-1812 and AM-1346, produce complete generalization to a THC training stimulus (80,87, 91,95,96), it is likely that anandamide's fast metabolic inactivation is responsible for the weak effects observed. In fact, in a recent study, we found that anandamide produces dose-related THC-like discriminative effects when its metabolic inactivation by FAAH was blocked by the FAAH inhibitor URB-597 (72). Importantly, when URB-597 was given alone it did not produce any THC-like effects, even at doses 10 times higher than those that potentiated the effects of anandamide (71), supporting the conclusion that this compound has little or no potential for abuse. Interestingly, AM-404, an inhibitor of endocannabinoid transport, produced no THC-like effects itself and, surprisingly, did not potentiate the THC-like effects of anandamide (72), suggesting that, in those brain regions mediating the discriminative effects of THC, membrane transport is not a main mechanism for anandamide inactivation.

2.4. Activation of endogenous dopamine systems involved in brain reward functions

Most psychoactive drugs that have reinforcing or rewarding effects in experimental animals and are abused by humans increase dopamine levels in the nucleus accumbens shell (97–

100). Although dopamine elevations in the nucleus accumbens are not a direct measure of reward, they are often considered an important neurochemical correlate of rewarding and reinforcing effects of abused drugs and a potential mechanism for these effects. Importantly, THC and other cannabinoid CB1 agonists activate dopamine neurotransmission, like other abused drugs. In microdialysis studies, cannabinoid CB1 agonists increase dopamine levels in the nucleus accumbens (61,101–103) and in particular in its shell sub-region (104). Also, in studies using fast-scan cyclic voltammetry, the CB1 agonist WIN55, 212-2 increases the frequency of dopamine concentration transients (105). Finally, burst firing of ventral tegmental area dopaminergic neurons is increased by cannabinoid CB1 agonists (106–109). In contrast, cannabinoid CB1 antagonists are ineffective by themselves on all these measures but they block the effects of cannabinoid CB1 agonists, which is considered a demonstration that the effects of cannabinoid agonists on dopaminergic neurotransmission are mediated through a CB1 receptor mechanism (106,107,109).

We have recently shown that intravenously injected anandamide and methanandamide also increase dopamine levels in the shell of the nucleus accumbens by a CB1 receptor mediated mechanism (73). The FAAH inhibitor URB-597 dramatically enhanced and prolonged the effects of anandamide at doses of URB-597 that had no effect by themselves (73). However, when anandamide was injected intraperitoneally rather than intravenously, it only increased dopamine levels in the nucleus accumbens after FAAH inhibition by URB-597. Surprisingly, AM-404, an inhibitor of endogenous cannabinoid transport, which also did not increase dopamine levels in the nucleus accumbens shell by itself, did not enhance or prolong the effects of intravenously injected anandamide (72). Although dopamine elevations in the nucleus accumbens shell are believed to play an important role in the reinforcing effects of THC (24, 29,31), the fact that a number of differences between the dopamine-increasing profile and the discriminative stimulus profiles of endogenous cannabinoids were found (72) and that dopaminergic compounds do not produce THC-like discriminative effects (88) indicates that the behavioral effects of cannabinoids are only partially mediated by the dopaminergic system.

2.5. Activation of endogenous opioid systems involved in brain reward functions

The endogenous opioid system, like the endogenous dopamine system, appears to play a pivotal role in brain reward and reinforcement processes (110,111). This system is comprised of three main types of receptors, mu-, delta- and kappa-opioid receptors, and of several endogenous ligands. These ligands are peptides that have different affinities for different types of opioid receptors: endomorphins are selective for mu receptors, beta-endorphin preferentially activates mu receptors, but also activates delta receptors, enkephalin preferentially activates delta receptors but also activates mu receptors, and dynorphin selectively activates kappa opioid receptors. Importantly, whereas mu and delta receptors are thought to mediate positive reinforcement and reward processes, kappa opioid receptors appear to mediate negative reinforcement and aversion (110,111). Cannabinoids appear to have very strong interactions with the opioid system and, interestingly, these interactions appear to be important both for the reinforcing and rewarding and for the aversive effects of cannabinoids.

Administration of THC increases levels of enkephalins in the nucleus accumbens (112) and levels of beta-endorphin both in the nucleus accumbens and the VTA, with the VTA playing the more relevant role in the mediation or modulation of the behavioral effects of THC by mu-opioid compounds (86). Pharmacological blockade or genetic ablation of mu-opioid receptors reduces the reinforcing (49,61,113), discriminative (86,92) and dopamine releasing (103, 104) of THC, but not the effects of THC on firing of dopaminergic mesolimbic neurons (107,109). Although these results suggest that many THC effects are mediated by released endopeptides, other mechanisms may participate in these interactions. Cannabinoid and opioid receptors, especially mu-opioid receptors, show similar brain distributions, show at least a

partial degree of co-localization in brain areas involved in motivated behaviors (61,114,115), and share similar second-messenger cascades (116,117), which indicates that cannabinoid and opioid receptors may interact at the level of the cell membrane (direct protein-protein heterodimerization) or at the level of signalling pathways. This is supported by recent demonstrations of allosteric modulation of mu- and delta-opioid receptors (118) and reductions in the signalling strength of cannabinoid CB1 receptor agonists in mu-opioid receptor-deficient “knock-out” mice (119).

As previously discussed, kappa-opioid receptors may mediate the aversive effects of THC and other cannabinoid CB1 agonists (34,49). Although increases in dynorphin levels following injections of cannabinoid CB1 receptor agonists have not been reported, it is likely that dynorphin plays a role in these aversive effects since dynorphin deficient mice do not develop conditioned place aversions with high doses of THC (70) and show a leftward shift in the dose-response curve for WIN55, 212-2 self-administration (34).

2.6. Summary

The existence of reliable models of cannabinoid and endocannabinoid self-administration in squirrel monkeys and of WIN 55,212-2 self-administration in rats and mice, the limited evidence that THC and WIN 55,212-2 can induce the development of conditioned place preferences in rodents under certain conditions, together with of the consistent findings that THC and WIN 55,212-2 can activate dopaminergic brain reward circuits, demonstrates that cannabinoid CB1 agonists have rewarding and reinforcing effects under certain experimental conditions. On the other hand, the clear difficulties encountered in obtaining reliable and persistent self-administration of THC and other CB1 agonists and the frequent reports that they induce dose-related conditioned place aversions under many conditions, demonstrates that cannabinoids can also have aversive effects, particularly in rodents. The balance between these opposing effects (that at the moment appears to be influenced by several factors in unpredictable ways) determines the behavioral output measured in experimental settings. However, in humans, and in the non-human primate (squirrel monkey) model, this balance appears to be shifted to the side of reinforcing effects (1,26–28). Thus, it remains to be established how the large amounts of pharmacological and neurobiological data collected in preclinical rodent models reflects and predicts effects in humans.

3. The endocannabinoid system and non-cannabinoid drugs of abuse

The recent availability of selective CB1 receptor antagonists such as rimonabant (120) and AM-251 (121) and of mice genetically lacking CB1 receptors (122,123) has greatly facilitated the study of cannabinoid system involvement in the rewarding and reinforcing effects of abused and addictive drugs. The involvement of the endogenous cannabinoid system in the modulation or mediation of the rewarding and reinforcing effects of opioids, psychostimulants alcohol and nicotine has now been clearly demonstrated in a series of animal studies.

3.1. Opioids

Opioids and cannabinoids have many similar effects and mu-opioid and cannabinoid-CB1 receptors are similarly expressed in brain areas involved in reward processes (115,124–127) where they share common signalling cascades (116,117). Not surprisingly, an impressive number of interactions have been described to date (128–132). Concerning the reinforcing effects of opioids, it has been demonstrated that pharmacological blockade or genetic deletion of CB1 receptors reduces self-administration of heroin and morphine in rats and mice (85, 122,133–135). Consistent with these findings, the CB1 receptor antagonist rimonabant reduces the reinforcing effects of self-administered heroin in rats (85,133,134). The effects of cannabinoid CB1 receptor antagonists on opioid reinforcement appear to be mediated primarily

by the nucleus accumbens and its projections to the ventral pallidum, since injections of rimonabant directly into the nucleus accumbens decrease heroin self-administration and morphine-induced release of GABA in the ventral pallidum (136). Importantly, the effects of cannabinoid CB1 antagonists appear to be relatively weak when the effort animals have to make to self-administer an opioid is low (i.e. under continuous reinforcement, fixed-ratio 1 schedules) but become more pronounced when the effort is high (i.e. progressive ratio schedules) (85,133,134).

The involvement of the endogenous cannabinoid system in the reinforcing effects of opioids is further supported by the findings that administration of CB1 agonists dramatically increases the motivation for self-administering heroin under a progressive-ratio schedule (137). In that study, however, administration of compounds that should increase endogenous cannabinoid system activity by interfering with the inactivation of endocannabinoids, such as the FAAH inhibitor URB-597 or the endocannabinoid transport inhibitor AM-404, did not increase self-administration of heroin and, instead, at high doses decreased it (137). These results do not provide support for the hypothesis that opioid-induced release of endocannabinoids is a major mechanism underlying the influences of cannabinoids on opioid reinforcement and suggest, instead, that interactions at other levels may be responsible for these effects.

In one study, mice lacking CB1 receptors were shown to develop morphine-induced conditioned place preferences (138). However, another study found that mice lacking CB1 receptors did not develop morphine-induced conditioned place preferences (138). Furthermore, administration of the CB1 receptor antagonist rimonabant blocks the development of morphine-induced conditioned place preferences in mice (135) and rats (74,76). Heroin or morphine-induced elevations in dopamine levels in the nucleus accumbens are not mediated by CB1 receptors, since rimonabant does not block them (104,133) and mice lacking CB1 receptors show normal dopamine accumbal elevations following administration of morphine (139).

3.2. Psychostimulants

The involvement of the endogenous cannabinoid system in psychostimulant reinforcement has also been investigated, but results have not been consistent. For example, self-administration of cocaine under fixed-ratio schedules is not altered by administration of the CB1 antagonist rimonabant in monkeys (35), rats (140,141) and in restrained CB1 knock out mice (142). However, a recent study has shown that non-restrained CB1 knock out mice actually show reduced acquisition of cocaine self-administration and that these mice, or wild type mice treated with rimonabant, show a reduced motivation to self-administer cocaine injections under progressive-ratio schedules (143). In addition, the cannabinoid system appears to be involved in reinstatement of extinguished cocaine self-administration behavior (see next section). In contrast, treatment with CB1 antagonists or genetic ablation of CB1 receptors does not alter cocaine-induced conditioned place preferences (74,142,144) or cocaine-induced elevations in dopamine levels in the nucleus accumbens (133,143). However, dopamine concentration transients in the nucleus accumbens are significantly reduced by the CB1 antagonist rimonabant (145).

The involvement of CB1 receptors in the reinforcing effects of amphetamines has been also studied and, again, results have not been consistent. On one hand, amphetamine is self-administered in CB1 knock out mice (142). On the other hand, the CB1 antagonist AM-251 reduces self-administration of methamphetamine and the endogenous cannabinoid ligand anandamide, and its synthetic analogue methanandamide, increase self-administration of methamphetamine in rats (146).

3.3. Alcohol

The endogenous cannabinoid system also appears to play an important role in the rewarding and reinforcing effects of alcohol (114,147). Pharmacological blockade or genetic ablation of CB1 receptors decrease operant self-administration of ethanol (148,149) and decrease voluntary consumption of ethanol in rats (150–154) and in mice (155–159). Conversely, cannabinoid CB1 receptor agonists increase voluntary consumption of ethanol in rats (160, 161). A caveat for the results obtained with oral self-administration or voluntary consumption of ethanol is that cannabinoid CB1 agonists have facilitating effects and cannabinoid CB1 antagonists have inhibitory effects on food and fluid intake, on the motivation to respond to obtain food, and on ingestive behaviour and palatability (162–165), and these actions may contribute to the behavioral effects of CB1 receptor blockade or deletion on ethanol self-administration and voluntary ethanol consumption. Place conditioning procedures, where ethanol is normally given by the intraperitoneal route and the oral route of administration is bypassed, may provide important information about the role of the cannabinoid system in ethanol reward. Such studies have confirmed that the rewarding effects of ethanol require CB1 receptor activation since CB1 knock out mice do not develop preferences for ethanol-paired compartments (144,159). In addition, pharmacological blockade or genetic ablation of FAAH enzymes in mice increase ethanol preference and intake in a two-bottle free-choice procedure (166,167). The prefrontal cortex and the nucleus accumbens appear two brain regions implicated in the modulation of the reinforcing effects of ethanol by cannabinoids (168,169).

Finally, pharmacological blockade or genetic deletion of CB1 receptors results in the inhibition of ethanol-induced elevations in dopamine extracellular levels as well as dopamine transients in the nucleus accumbens (145,156,170) and inhibition of ethanol-induced increases in firing of dopaminergic neurons in the VTA (171).

3.4. Nicotine

Characterization of the interactions between nicotine and the endogenous cannabinoid system may have very important implications for our understanding of the biological basis for the co-administration of cannabis and tobacco in humans. In addition, preclinical results obtained over the last ten years have led to clinical trials that indicate that cannabinoid CB1 receptor antagonists could have important therapeutic utility as smoking cessation agents (172) (http://en.sanofi-aventis.com/press/ppc_1960.asp).

In preclinical studies, the CB1 antagonist rimonabant reduces or blocks both the intravenous self-administration of nicotine and the development and expression of nicotine-induced conditioned place preferences in rats (170,173). Rimonabant also reduces the motivational impact of drug-related cues or environments on nicotine-seeking behavior (174–176). Although CB1 knock out mice do develop nicotine-induced conditioned place preferences (177), they do not acquire intravenous nicotine self-administration (142). In addition, in place conditioning procedures, doses of THC and nicotine that are ineffective by themselves, induce significant conditioned place preferences in mice when they are given in combination (44) and these effects are paralleled at the molecular level by synergistic effects on the expression of immediate early genes such as c-FOS in several brain areas, including the shell of the nucleus accumbens (44). Thus, the rewarding effects of low doses of THC and nicotine appear to be synergistic. Consistent with these findings, we have recently found that nicotine potentiates the discriminative effects of low doses of THC and that these effects are at least in part mediated by release of the endogenous cannabinoid anandamide (93).

3.6. Summary

Experimental data supports a role for the endocannabinoid system in the rewarding and reinforcing effects of non-cannabinoid drugs of abuse. The facilitating effects of cannabinoids

are clearest for opioids and alcohol, where pharmacological blockade or genetic ablation of cannabinoid receptors have been repeatedly shown to reduce opioid and alcohol self-administration and opioid- and alcohol-induced conditioned place preferences. In the case of nicotine, although the preclinical data is still incomplete, clinical studies strongly support the involvement of endocannabinoids in nicotine reward and cannabinoid antagonists are promising medications for smoking cessation. On the other hand, the endocannabinoid system does not appear to play an important role in the primary reinforcing effects of psychostimulants but they may be involved in incentive motivational effects of these drugs.

4. Release of endogenous cannabinoids by abused drugs

A mechanism that could explain the modulation by the endocannabinoid system of the rewarding effects of non-cannabinoid drugs and that would support the role of the endogenous cannabinoid system in the signalling of rewarding events is the release of endogenous cannabinoids by non-cannabinoid drugs. Unfortunately, because of the high lipophilic nature and instability of endocannabinoids, techniques such as *in vivo* microdialysis that allow monitoring the dynamics of neurotransmitter release have proven very difficult to perform routinely for anandamide and 2-AG. Thus, until very recently, only one microdialysis study existed showing that intrastriatal administrations of the dopamine D2 agonist quinpirole locally elevate levels of anandamide but not those of 2-AG (178). All other studies had investigated tissue levels at a single time point (most often 2 hours) after administration of drugs. Since drugs of abuse produce their reinforcing effects rapidly, it may be difficult to correlate endocannabinoid levels and behavioral consequences in order to establish whether measured levels represent a direct effect of the drug or a reaction to the drug effects. Moreover, most studies have investigated brain levels of endocannabinoids after chronic drug treatment. In these studies one group received drug at all times (chronic) and one group received saline, vehicle or water at all times (control) but no group received saline or vehicle chronically and then drug acutely and vice versa. Thus, it is difficult to establish with certainty whether measured levels of endocannabinoids reflect the consequences of chronic drug administration or of drug withdrawal. For example, it has been shown that chronic administration of THC itself decreases the levels of anandamide and 2-AG in the striatum which contains the nucleus accumbens (179); ethanol decreases the levels of anandamide and 2-AG in the midbrain which contains the VTA (180); nicotine decreases the levels of anandamide in the striatum (180); cocaine does not alter anandamide or 2-AG levels either in the striatum or the midbrain (180); morphine decreases 2-AG levels in the striatum without altering anandamide levels (181). Interestingly, Vigano and colleagues in a more recent paper used a protocol of morphine administration that induced behavioral sensitization and investigated the levels of anandamide and 2-AG at different time points: at the end of induction phase, after fifteen days of withdrawal and after the expression of morphine sensitization by a morphine challenge (182). In addition they had a saline group and an acute morphine group control. They found that chronic morphine administration did not change anandamide levels but decreased 2-AG levels. More importantly, they found that acute administration as well as a challenge administration of morphine increased levels of anandamide and decreased levels of 2-AG in the striatum (182). These findings underscore the critical role of the protocol of administration and the time point at which endocannabinoids are measured in determining the quality and quantity of alterations in endocannabinoid levels observed.

A promising approach to the study of the involvement of released endocannabinoids in the rewarding and reinforcing effects of abused drugs reward has recently been employed by Caille et al. (169), involving the combination of drug self-administration and *in vivo* microdialysis techniques in rats. They found that, in the nucleus accumbens, self-administration of heroin increased anandamide and decreased 2-AG levels, self-administration of alcohol increased 2-AG and did not alter anandamide levels and self-administration of cocaine did not alter either

anandamide or 2-AG levels (169). Hopefully, such a protocol will be soon used to study the effects of self-administration of other drugs and the effects of self-administration of different types of drugs on changes in the levels of endocannabinoids in other brain areas, such as the VTA, that play an important role in the reinforcing effects of cannabinoids (55).

Although our knowledge is still limited and there is a need for further research to investigate conditions more relevant to drug abuse and dependence, it appears likely that the alterations found in the referenced papers reflect the involvement of released endocannabinoids in the effects of non-cannabinoid drugs of abuse. On the other hand, as discussed in the sections describing modulation of the behavioral and neurochemical effects of cannabinoids and opioids (sections 2.4. and 2.5.), other mechanisms, such as receptor-receptor interactions or interactions at the signalling cascade level already described for mu-opioid and dopamine D2 receptors (118,119,183–186), could play a role in the regulation of the rewarding effects of abused drugs by the endogenous cannabinoid system.

5. The endocannabinoid system and reinstatement of drug-seeking

A key feature of drug abuse and dependence is relapse to drug use even after long period of forced or voluntary withdrawal and detoxification (187). A commonly used preclinical model of relapse is reinstatement of extinguished intravenous drug self-administration by a priming injection of drug or by drug-associated stimuli (188–190). In a typical version of this procedure (137), animals learn to intravenously self-administer a drug over repeated daily sessions and then extinction sessions are conducted when responding has no programmed consequences (injections and, in some instances, drug-associated stimuli are not presented) until the animal's responding drops to a low, arbitrary level; reinstatement of operant drug-seeking behavior can then be tested by injecting a drug (drug-induced), by presenting visual or auditory stimuli previously associated with the drug (cue-induced) or by exposing animals to acute stress (stress induced).

The endogenous cannabinoid system appears to be involved in reinstatement of extinguished self-administration of several drugs of abuse and, in particular, appears to be involved in drug- and cue-induced reinstatement (191,192). The first evidence for this role was provided by De Vries et al., (140) in a study that found that the CB1 agonist HU210 could reinstate extinguished cocaine-seeking behaviour. The cannabinoid CB1 antagonist rimonabant not only blocked this effect but also reduced cocaine- and cue-induced reinstatement; however, rimonabant had no effect on stress-induced reinstatement. Cocaine-induced reinstatement of drug-seeking behavior is also blocked by another cannabinoid CB1 antagonist, AM-251, and appears to be mediated by inhibition of glutamate release in the nucleus accumbens (193). Rimonabant also reduces heroin-induced reinstatement (134,194); methamphetamine-induced reinstatement (195), nicotine-induced reinstatement (196) and ethanol-induced reinstatement (148,149). Interestingly, although the endocannabinoid system appears to play an important role in the regulation of stress reactions (197), stress-induced reinstatement does not seem involve endocannabinoid signalling (140,148,149). These preclinical data have recently received strong support by preliminary data from clinical trials (STRATUS, Studies with Rimonabant And Tobacco Use) showing that rimonabant significantly increases the odds of quitting smoking (172) (http://en.sanofi-aventis.com/press/ppc_1960.asp).

6. Conclusions

The endogenous cannabinoid is a recently discovered system that appears to play an important and pervasive role in many types of drug abuse and dependence. Endogenous cannabinoids are neuromodulators that are involved in the signalling of rewarding events and can produce reinforcing and rewarding effects in experimental animals, as they do in humans. Endogenous

cannabinoids can also activate other brain systems involved in reward signalling, can modulate the reinforcing and rewarding effects of other non-cannabinoid abused drugs, and are released by drugs of abuse in brain areas involved in reward and reinforcement processes. Accumulating evidence points to the endocannabinoid system as a major target for the development of new pharmacological agents for the treatment of many different types of drug abuse and dependence.

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