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Regulation of Brain Reward by the Endocannabinoid System: A Critical Review of Behavioral Studies in Animals

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Abstract: The endocannabinoid system has been implicated in the regulation of a variety of physiological processes, including a crucial involvement in brain reward systems and the regulation of motivational processes. Behavioral studies have shown that cannabinoid reward may involve the same brain circuits and similar brain mechanisms with other drugs of abuse, such as nicotine, cocaine, alcohol and heroin, as well as natural rewards, such as food, water and sucrose, although the conditions under which cannabinoids exert their rewarding effects may be more limited. The purpose of the present review is to briefly describe and evaluate the behavioral and pharmacological research concerning the major components of the endocannabinoid system and reward processes. Special emphasis is placed on data received from four procedures used to test the effects of the endocannabinoid system on brain reward in animals; namely, the intracranial self-stimulation paradigm, the self-administration procedure, the conditioned place preference procedure and the drug-discrimination procedure. The effects of cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2) receptor agonists, antagonists and endocannabinoid modulators in these procedures are examined. Further, the involvement of CB_1 and CB_2 receptors, as well the fatty acid amid hydrolase (FAAH) enzyme in reward processes is investigated through presentation of respective genetic ablation studies in mice. We suggest that the endocannabinoid system plays a major role in modulating motivation and reward processes. Further research will provide us with a better understanding of these processes and, thus, could lead to the development of potential therapeutic compounds for the treatment of reward-related disorders.

Keywords: Cannabinoids, addiction, anandamide, 2-arachidonoylglycerol, intracranial self-stimulation, self-administration, conditioned place preference, drug discrimination.

INTRODUCTION

Although the use of *cannabis sativa* preparations as recreational drugs can be traced back to the earliest civilizations, its abuse has dramatically increased in many countries over the past few decades [1, 2]. Among the best known pharmacological properties of this plant are the euphorigenic, relaxing and well-being feelings that have been attributed to Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of the plant [3-5]. These feelings may play a central role in the reinforcement of repeated use and abuse of cannabis and, in some cases, the development of dependence. Despite the evidence for rewarding effects of cannabis preparations and Δ^9-THC in humans, rewarding effects of Δ^9-THC or other synthetic cannabinoid analogs in animal models of drug abuse and dependence have been rather difficult to demonstrate [6, 7]. Within the last two decades cannabinoid research has progressed rapidly, primarily due to the discovery of an endogenous cannabinoid (endocannabinoid) system in the brain, which mediates the behavioral and neurobiological effects of cannabis. Endocannabinoids are thought to regulate motivational processes and reward-seeking behaviors. Indeed, during the last decade human neuroimaging studies investigating the effects of cannabis and Δ^9-THC suggest that cannabinoids act on widely distributed neural networks that include limbic and cortical areas (i.e., medial temporal and prefrontal cortex, hippocampus, striatum), that are rich in cannabinoid receptors and implicated in the regulation of emotion and brain reward [8-14]. Thus, the present review summarizes animal studies focused on the involvement of the endocannabinoid system in brain reward processes.

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THE ENDOCANNABINOID SYSTEM

Definition. The endocannabinoid system was first identified in the early 1990s during investigations on the mechanism of action of Δ^9-THC. This system consists of cannabinoid (CB) receptors (CB_1, CB_2, and possibly others), endogenous ligands for these receptors and enzymes/proteins responsible for the synthesis, reuptake and degradation of these endogenous ligands [15-17]. The endocannabinoid system has been studied using genetic, pharmacological and behavioral methods. Its better studied function is the modulation of neurotransmission [18].

Cannabinoid receptors. After the discovery of Δ^9-THC, researchers focused their efforts into finding its receptors. Thus, in the late 1980s, due to the availability of new synthetic cannabinoid agonists able to exert similar effects with Δ^9-THC, the existence of specific CB receptors was suggested [19]. Two types of CB receptors have been characterized to date, CB_1 [20, 21] and CB_2 [22] receptors. Both are metabotropic receptors coupled to Gi/o proteins. CB_1 receptors are expressed ubiquitously, but they are preferentially located in the brain and the spinal cord [23]. The localization of CB_1 receptors in the brain is consistent with the known central effects of cannabinoids. Thus, they can be found in the highest concentrations in areas involved in motor function and coordination (e.g., basal ganglia and cerebellum), memory (e.g., hippocampus) and emotionality (e.g., neocortex, and amygdala) [24]. Notably, high levels of CB_1 receptors are present in brain structures of the mesolimbocortical dopaminergic pathway which are related to motivation and reward, such as the prefrontal cortex, the nucleus accumbens, the olfactory tubercle, the hippocampus and the amygdala [25, 26]. CB_1 receptors are localized preferentially at the presynaptic level and it is believed that they mediate inhibition of ongoing release of glutamate, GABA and other neurotransmitters, including dopamine [27]. Until recently, it was thought that CB_2 receptors were present only in the periphery and did not mediate any central effects of cannabinoids. However, recent findings suggest that CB_2

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receptors are also expressed in the brain under pathological conditions, such as tumors [28] or Alzheimer's disease [29], as well as under normal physiological conditions [30-34]. On the basis of numerous studies showing certain behavioral and pharmacological effects of cannabinoid compounds that can not be explained exclusively by their action on CB_1 and CB_2 receptors, the existence of additional CB receptors has also been hypothesized [35].

Endocannabinoids. The endocannabinoids are non-classical lipid neurotransmitters/neuromodulators that serve as the endogenous ligands for the CB receptors. They were discovered in the early 1990s and are named so because they were first found to activate the same receptors as natural cannabinoids. The two most widely studied endocannabinoids are N-arachidonoylethanolamide (AEA), also called anandamide [36], and 2-arachidonoylglycerol (2-AG) [37, 38]. It should be noted that AEA can also activate transient receptor potential vanilloid type 1 (TRPV1) receptors [39]. However, the role of these receptors in motivation and brain reward processes remains largely unknown. In general, brain tissue levels of 2-AG are higher than those of AEA [40]. More recently, other molecules with cannabinoid receptor binding activity termed noladine ether, virodhamine and N-arachidonoyldopamine (NADA) have been discovered, but their roles are still unclear [41, 42]. However, since the majority of studies concerning endocannabinoids have examined only AEA and 2-AG, these two endogenous ligands will be the focus of our review. Endocannabinoids are present in brain regions of the mesolimbocortical dopaminergic system [15] and seem to interact with it and thus regulate motivation and reward [43]. The control of this reward tone is mainly mediated by CB_1 receptors [44].

Endocannabinoid synthesis, release and inactivation. The synthesis, release and degradation of endocannabinoids has been extensively reviewed elsewhere [45, 46], and so will only be dealt with briefly here. AEA and 2-AG have different structures, as well as different biosynthesis and degradation pathways. Structurally, endocannabinoids are lipophylic compounds that are derived from arachidonic acid. Their precursors exist in cell membranes and are cleaved by specific enzymes. They are not synthesized in advance and are not stored in synaptic vesicles, but are rather formed and released "on demand" (i.e., when and where necessary) in response to diverse physiological and pathological stimuli following an increase of the intracellular concentration of Ca^{2+}. Endocannabinoids can passively diffuse through lipid membranes, but a high affinity transporter, which is not yet identified, seems to accelerate this process. Finally, a fatty acid amid hydrolase (FAAH) is the main hydrolase for AEA, whereas 2-AG inactivation is mainly degraded by two other enzymes, called monoacyl-glycerol lipases (MAGLs).

Functions of the endocannabinoid system. Endocannabinoids modulate various physiological functions not only in the central nervous system, but also in the autonomic nervous system, and in the endocrine, the reproductive and the immune systems, as well as in microcirculation [47]. In the central nervous system, endocannabinoids are involved in processes such as plasticity and neurogenesis [48], neuroprotection [49], antinociception and pain [50], appetite [51], emotions [52, 53], reward [6], stress and anxiety [54, 55], memory [56], motor function and coordination [57, 58]. Moreover, the endocannabinoid system has been implicated in the pathophysiology of neurological and psychiatric disorders, such as multiple sclerosis [59], Huntington's disease [60], Parkinson's disease [61], drug addiction [62], depression [63] and schizophrenia [64]. Indeed, there are many studies and clinical trials that indicate the potential therapeutic application of various cannabinoid compounds (for a review, see [17]).

PHARMACOLOGICAL AND MOLECULAR TOOLS TO STUDY ENDOCANNABINOID NEUROTRANSMISSION

Pharmacological approach. The development of potent and selective agonists and antagonists for the cannabinoid receptors, or agents that block the reuptake or degradation of endocannabinoids, has played a major role in the recent advances in behavioral and pharmacological research of the endocannabinoid system [65, 66].

Currently, several cannabinoid receptor agonists possess similar affinity for CB_1 and CB_2 receptors. Based on their chemical structure, they fall into four major classes: classical, nonclassical, aminoalkylindoles and eicosanoids (please see Table **1**).

Classical cannabinoids are tricyclic terpenoid derivatives bearing a benzopyran moiety. This group includes the main psychoactive constituent of cannabis Δ^9-THC, the phytocannabinoid (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC) and synthetic analogues. From the synthetic analogues notable examples are 11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210), levonantradol and AMG-3.

Non-classical cannabinoids consist of bicyclic and tricyclic analogues of Δ^9-THC lacking the pyran ring of classical cannabinoids. Notable examples include the potent non-selective CB_1/CB_2 receptor agonists CP-55,940, CP-47,497 and CP-55,244.

The class of aminoalkylindoles has a completely different structure from both classical and nonclassical cannabinoids. They also differ in their lipophilicity (they are considerably less lipophilic), as well as in the way they activate cannabinoid receptors [30]. The most widely investigated compound from this group is the potent CB_1/CB_2 receptor agonist WIN55,212-2. This compound has high stereoselectivity but low affinity for the CB_2 receptor.

The eicosanoid group consists of CB_1 and CB_2 receptor agonists that have markedly different structures not only from aminoalkylindoles, but also from classical and non-classical cannabinoids. Notable members of this group are the endocannabinoids AEA and 2-AG.

Several compounds are more potent at activating CB_1 than CB_2 receptors. These include several synthetic analogues of AEA, including R-(+)-methanandamide, arachidonyl-2'-chloroethylamide (ACEA), and arachidonylcyclopropylamide (ACPA). Noladin ether (2-arachidonyl glyceryl ether) is also a CB_1 receptor agonist. Selective CB_2 receptor agonists have also been synthesized and these include the classical cannabinoids JWH-133, L-759633 and L-759656, and the non-classical cannabinoid HU-308. Other selective CB_2 receptor agonists are the aminoalkylindoles JWH-015 and AM1241.

Several selective agents have been used extensively as CB_1 receptor competitive antagonists. Two prominent members of this group are the diarylpyrazole SR141716A (rimonabant), AM-251, AM-281 and LY320135. These compounds bind with significantly greater affinity to CB_1 than to CB_2 receptors and they can block agonist-induced activation of CB_1 receptors in a competitive manner. Moreover, although they don't activate CB_1 receptors when administered alone, there is evidence that in some cases they can act as inverse agonists [67]. Some CB_1 receptor competitive antagonists that have recently been developed do not induce signs of inverse agonism at the CB_1 receptor. These include NESS O327, O-2050 and AM4113.

Furthermore, the compounds AM630 and SR144528 are more potent in blocking CB_2- than CB_1-receptor activation. However, both compounds are thought to be CB_2-receptor inverse agonists, because when administered alone they can induce inverse cannabimimetic effects in CB_2 receptor-expressing tissues [68].

Other cannabinoids that target CB_1 and/or CB_2 receptors are the phytocannabinoids cannabinol, cannabidiol and cannabigerol. Cannabinol seems to be a CB_1 receptor partial agonist, although there is evidence that it can also act as a CB_2 receptor agonist/inverse agonist [69]. Cannabidiol and cannabigerol were found to behave as CB_1 receptor antagonists/inverse agonists. However, it has also been reported that cannabidiol has significant potency *in vitro* as a CB_2 receptor antagonist/inverse agonist [70].

Table 1. Cannabinoid CB_1 and CB_2 receptor ligands.

Agonists that target CB_1 and CB_2 receptors with similar potency:	
Classical cannabinoids	Δ^9-THC, Δ^8-THC, HU-210, levonantradol, AMG-3
Non-classical cannabinoids	CP-55,940, CP-47,497, CP-55,244
Aminoalkylindoles	WIN55,212-2
Eicosanoids	AEA, 2-AG
CB_1- selective receptor agonists	R-(+)-methanandamide, ACEA, ACPA, noladin ether
CB_2-selective receptor agonists	JWH-133, L-759633, L-759656, HU-308, JWH-015, AM1241
CB_1-selective competitive antagonists	SR141716A (rimonabant), AM-251, AM-281, LY320135, NESS O327, O-2050, AM4113
CB_2-selective competitive antagonists	AM630, SR144528
Other compounds	cannabinol, cannabidiol, cannabigerol
Indirect agonists:	
FAAH inhibitors	PMSF, AM374, AM381, O-1887, OL-135, URB-532, URB-597, URB-602
Reuptake inhibitors	AM-404, LY 2183240, OMDM-1, OMDM-2, VDM-11, UCM 707, AM-1172

The finding that the actions of AEA and 2-AG are terminated by cellular uptake and intracellular enzymatic hydrolysis has been supported by the development of several drugs that inhibit these processes [71-73]. Many of these drugs have been used as pharmacological tools in animal experiments aimed at elucidating the pathophysiological roles of endocannabinoids. Important members of this group are the FAAH inhibitors/indirect agonists PMSF, palmitylsulphonyl fluoride (AM374), stearylsulphonyl fluoride (AM381), O-1887, OL-135, URB-532, URB-597 and URB-602. The reuptake transporter of endocannabinoids has not been discovered yet, but there is pharmacological evidence of its existence through the use of specific reuptake inhibitors. Among the AEA reuptake inhibitors, AM-404 is the most extensively studied. However, AM-404 is not selective, as it also inhibits FAAH and binds to CB_1 receptors. Other compounds of this group, with a more selective action in the transporter than AM-404 are LY 2183240, OMDM-1, OMDM-2, VDM-11, UCM 707 and AM-1172.

Molecular approach. Genetically modified mice have also been used as research tools to study the behavioral and pharmacological actions of cannabinoids [74-76]. The availability of transgenic mice that lack CB_1, CB_2 or both CB_1 and CB_2 receptors has provided a useful additional tool to study whether or not responses to cannabinoid compounds are CB_1 receptor and/or CB_2 receptor mediated as well as the physiological roles of CB_1 and CB_2 receptors [74, 75]. FAAH-deficient mice represent another powerful model system in the study of the neurochemical and behavioral consequences of endocannabinoid catabolism [76].

ROLE OF CB RECEPTORS IN REWARD

Behavioral procedures for studying reward processes. The main procedures used to examine the effects of CB receptors in reward processes, and the ones presented in this review, are the intracranial self-stimulation paradigm (ICSS), the conditioned place preference (CPP) procedure, the self-administration procedure and drug-discrimination studies [77].

Shortly, the ICSS is an operant behavioral paradigm offering a direct measurement of drug effects on brain reward substrates [78, 79]. In this paradigm, animals learn to deliver brief electrical pulses into specific parts of their own brain hypothesized to be part of the reward pathways that mediate both natural and ICSS reward [80]. Numerous studies have shown that most drugs of abuse are able to lower reward stimulation thresholds in the brain, indicating an increase in the reward value of the stimulation, because less electrical stimulation is required for the subject to perceive the stimulation as rewarding, and supporting the notion that they activate the same substrates with electrical stimulation in a synergistic manner [79, 81].

Further, the intravenous self-administration procedure provides us with the opportunity to study the rewarding properties of abused drugs in experimental animals. In this behavioral paradigm, animals are trained to intravenously self-administer a drug through a catheter inserted in the jugular vein, by making an operant response, such as lever-pressing or nose-poking. This procedure resembles drug-taking behavior in human subjects [82]. Reinforcing effects of a drug assessed by intravenous self-administration procedures in experimental animals, such as rodents and primates, are considered one of the most reliable predictors of abuse potential in human subjects.

The CPP procedure provides an animal model of the subjective, motivational effects of drugs as well as non-drug stimuli [83]. A drug is injected and the animal is placed in a test chamber with distinctive environmental cues (e.g., tactile, visual, and/or olfactory). This procedure is repeated for several days. During these conditioning trials the animal learns an association between the subjective state produced by the drug (e.g., a heightened feeling of euphoria comparable to pleasure in humans) and the environmental cues present during the drug state. When the subject is tested in an apparatus that contains the drug-related environmental cues in one compartment and neutral cues in another, it voluntarily spends more time in the compartment associated with the drug-related cues. In other words, in the CPP procedure neutral environmental cues acquire incentive salience which is closely related with the subjective state produced by the drug. Although the CPP paradigm does not directly measure drug reinforcement, it can be used to infer the motivational values of addictive drugs. Indeed, there is a fairly good concordance with intracranial self-stimulation and self-administration studies [84]. Thus, CPP produced by a drug indicates that it exhibits rewarding properties and that it might therefore have

Table 2. Cannabinoid effect on intracranial self-stimulation in experimental animals.

CANNABINOID DRUG	Dose	EFFECT	Species	REFERENCES
Δ^9-THC, nabilone, canbisol	0.12-10 mg/kg	↑ threshold	Long-Evans rats	*Stark and Dews 1980*[94]
levonantradol	0.2, 0.3 mg/kg	↑ threshold	albino CDF rats	*Kucharski et al. 1983*[93]
Δ^9-THC	1.5 mg/kg	↓ threshold	Lewis rats	*Gardner et al. 1988*[90]
Δ^9-THC	1 and 1.5 mg/kg	↓ threshold	Lewis rats	*Gardner et al. 1989*[88]
Δ^9-THC	1 mg/kg	- - ↓ threshold	Sprague-Dawley rats Fischer 344 rats Lewis rats	*Lepore et al. 1996*[92]
CP 55,940	10, 25, 50 μg/kg	-	Lewis rats	*Arnold et al. 2001*[138]
SR141716A	1, 3, 10 mg/kg	↑ threshold	Sprague-Dawley rats	*Deroche-Gamonet et al. 2001*[175]
WIN 55,212-2	0.1, 0.3 and 1 mg/kg	↑ threshold	Sprague-Dawley rats	*Vlachou et al. 2003*[135]
WIN 55,212-2 CP 55,940 HU-210 SR141716A	0.1, 0.3, 1, 3 mg/kg 10, 30, 56, 100 μg/kg 10, 30, 100 μg/kg 0.02 mg/kg	↑ threshold (reversing effect on agonists)	Sprague-Dawley rats	*Vlachou et al. 2005*[136]
AMG-3	1, 2, 4, 8 mg/kg	↑ threshold	Sprague-Dawley rats	*Antoniou et al. 2005*[134]
PMSF OMDM-2 URB-597 SR141716A	15, 30, 60 mg/kg 3, 10, 30 mg/kg 0.3, 1, 3 mg/kg 0.02 mg/kg	↑ threshold (reversing effect on modulators)	Sprague-Dawley rats	*Vlachou et al. 2006*[137]
Δ^9-THC	1-2 mg/kg	↑ threshold	Sprague-Dawley rats	*Vlachou et al. 2007*[95]
SR141716A	0.02 mg/kg	(reversing effect on Δ^9-THC)		*Vlachou et al. 2007*[95]
Δ^9-THC	0.5, 1 mg/kg	-	Sprague-Dawley rats	*Fokos and Panagis 2010*[96]

- no effect on threshold, ↑ increase, ↓ decrease

abuse potential in humans [84]. A similar place conditioning procedure can also be used to study the aversive properties of a drug or a non-drug stimulus. In this case, the animal will avoid staying in a compartment previously associated with a drug producing aversive or dysphoric effects.

The pharmacological effects of a drug can include distinct interoceptive cueing effects. These cueing effects can play an important role in the addiction process, such as leading to additional drug-seeking behavior. The standard preclinical rodent model used to study the cueing (discriminative stimulus) effects of a drug is referred to as drug discrimination [85]. In this procedure, animals are faced with two possible responses one of which is reinforced (usually with a natural reinforcer, such as food, under a specific schedule), while the other is not reinforced. The animals are trained to detect whether they received an active drug or vehicle (no drug) injection, in order to determine through the specific drug effects which response is the correct. Rats can readily learn to discriminate between a drug *versus* a non-drug state pressing the reinforcement-associated lever. This resembles the same ability in humans to distinguish between drug-induced or non-drug induced conditions. The discrimination between drug and non-drug conditions is based upon the presence or absence of subjective and central effects. Although the drug discrimination procedure does not measure directly the reinforcing/rewarding effects of drugs, it can be used to infer if an experimental drug may have abuse potential in humans [86, 87].

INVOLVEMENT OF CB_1 RECEPTORS IN REWARD

The Role of CB_1 Receptor Agonists

Involvement of Δ^9-THC in reward. Importantly, opposite effects have been observed with different strains of animals and in different procedures used, after the administration of Δ^9-THC or other CB_1 and/or non-selective CB_1/CB_2 receptor agonists. In the ICSS paradigm, some studies have shown that Δ^9-THC, administered at low doses, lowers the ICSS threshold [88-92], while other studies, including one from our research group, have failed to see a Δ^9-THC-induced reward-facilitating effect in the ICSS [93-95] under baseline conditions or in animals pre-exposed to stress [96]. These seemingly contrasting findings could be attributed to the different Δ^9-THC doses used, the strain of the animals used and the methods followed (Table **2**).

Similarly, self-administration of Δ^9-THC or other cannabinoid receptor agonists in rodents or primates has been difficult to achieve [97-101]. In the first Δ^9-THC self-administration studies, Δ^9-THC would either not be self-administered (in naïve animals) [102, 103], or it would only be self-administered by experimental animals previously food-restricted [104-106] or treated with other

Table 3. Cannabinoid effects on self-administration in experimental animals.

CANNABINOID DRUG	Dose	EFFECT	SPECIES	References
WIN 55,212-2	0.05-0.1 mg/kg 0.5 mg/kg	↑ SA ↓ SA	CD1 mice	*Martelotta et al. 1998*[143]
Δ^9-THC	2 and 4 mg/kg/injection	↑ SA	Squirrel monkeys	*Tanda et al. 2000*[108]
WIN 55,212-2	6.25-50 µg/kg/injection	↑ SA	Long-Evans rats	*Fattore et al. 2001*[139]
CP 55,940	0.1-1.6 mg/2µl/infusion	SA	Wistar rats	*Braida et al. 2001*[145]
Δ^9-THC	2, 4, 8 mg/kg/injection	↑ SA	Squirrel monkeys	*Justinova et al. 2003*[109]
Anandamide (AEA)	40 mg/kg/injection	↑ SA	Squirrel monkeys	*Justinova et al. 2005*[173]
Methanandamide	10 20, 40 µg/kg/injection	↑ SA	Squirrel monkeys	*Justinova et al. 2005*[173]
Δ^9-THC	100nl injection of 66 or 200pmol	SA	Sprague-Dawley rats	*Zangen et al. 2006*[112]
WIN 55,212-2	12.5 µg/kg/infusion	SA	Sprague-Dawley rats	*Lecca et al. 2006*[142]
WIN 55,212-2	12.5 µg/kg/infusion	↑ SA	Lister Hooded and Long Evans rats	*Fadda et al. 2006*[140]
WIN 55,212-2	12.5 µg/kg/infusion	SA	Lister Hooded rats	*Fattore et al. 2007*[141]
2-Arachinonoylglycerol (2-AG)	0.1-100 µg/kg	↑ SA	Squirrel monkeys	*Justinova et al. 2011*[229]

↑ increase, ↓ decrease in Self-Administration (SA)

drugs of abuse, such as phencyclidine [107] or cocaine [108]. A clear self-administration procedure was mainly achieved in monkeys [109, 110], while the successful studies on rodents were either using intacerebroventricular self-administration of Δ^9-THC sometimes combined with food/water deprivation [111]. In some cases, even in some of these pre-exposure studies, rates of responding have been relatively low [107] in animals pre-exposed to other drugs of abuse, such as cocaine, phenobarbital, phencyclidine, ethanol or amphetamine [98, 99].

The first studies showing that low doses of Δ^9-THC can initiate and sustain high rates of intravenous self-administration in drug-naïve squirrel monkeys were conducted by Justinova and colleagues [109, 110] (Table **3**). In the first of these studies, the self-administration behavior was rapidly extinguished either by substituting vehicle injections for Δ^9-THC injections or by administering the CB_1 receptor antagonist SR141716A before the session, demonstrating that this effect was mediated by direct stimulation of the CB_1 receptors. Importantly, Braida and colleagues also showed intracerebroventricular self-administration of Δ^9-THC in rats under water-deprived conditions [111], while Zangen and colleagues [112] identified the posterior ventral tegmental area and the shell of the nucleus accumbens as two possible brain sites for the rewarding effects of the reported intracerebral self-administration of Δ^9-THC. In the same study, rats did not self-administer Δ^9-THC intracerebrally in the core of the nucleus accumbens or the anterior ventral tegmental area or the region dorsal to this [112].

Δ^9-THC and other synthetic CB_1 receptor agonists can induce both appetitive and aversive effects in the CPP paradigm under various experimental manipulations [113] (Table **4**). Importantly, in most of the CPP studies the different effects (appetitive or aversive) of Δ^9-THC and other synthetic CB_1 receptor agonists are mainly attributed to pharmacokinetic factors (i.e., factors related to bioavailability of the drug formulation used, lipophilicity, distribution and storage in fat tissue, metabolism and elimination rate); thus, the doses used –with usually only a single dose inducing CPP- and the timing between injections [114] are the main factors affecting the results.

As in the case of the self-administration studies, the first studies in rodents either failed to show Δ^9-THC-induced CPP, or they showed conditioned place aversion [115-122]. Δ^9-THC-induced CPP was first presented by Lepore and colleagues [114]. Interestingly, in this study, only the higher doses of Δ^9-THC induced CPP in rats, when the CPP pairing interval was 24 h, while the lowest dose of Δ^9-THC did not have any effect. However, in the same study, when the schedule between injections was changed to 48 h, contrastingly, Δ^9-THC produced a clear place aversion in the high doses, but place preference in the low dose. Similar results were also presented by Braida and colleagues [111] and Le Foll and colleagues [123]. In the latter study, a low 0.1 mg/kg dose of Δ^9-THC produced CPP. Doses lower or higher than this did not produce any preference [123]. As previously indicated, these differential responses can be attributed to the different methodological conditions followed, such as the duration and the number of sessions, once again suggesting a biphasic effect of Δ^9-THC highly depending on pharmacokinetic factors.

Studies in mice have also shown inconsistent results. Δ^9-THC induced clear CPP in mice only when the animals were previously administered with a priming Δ^9-THC injection 24h before the first conditioning [124], although in a later study by the same group 5 mg/kg Δ^9-THC produced conditioned place aversion [125]. However, in a more recent study using the same experimental manipulations (Δ^9-THC doses and experimental design) [95] no conditioned place preference was observed. This discrepancy could be attributable to the different strain of animals used, the number of pairings or the periods of conditioning and administration of the drugs.

Importantly, a significant number of drug discrimination studies have shown that cannabinoids produce subjective effects in experimental animals [126-132] (Table **5**). More specifically, cannabinoids show a pharmacological specificity in drug discrimination studies. In animals trained to discriminate injections of Δ^9-THC

Table 4. Cannabinoid effects on conditioned place preference in experimental animals.

CANNABINOID DRUG	Dose	EFFECT	SPECIES	References
Δ^9-THC	1mg/kg 2 and 4 mg/kg 2 and 4 mg/kg (wash-out period) 1 mg/kg	CPA CPP CPA CPP	Long-Evans rats	*Lepore et al. 1995*[114]
Δ^9-THC	1 mg/ml	CPA	Lewis and Sprague-Dawley rats	*Parker and Gillies 1995*[115]
CP 55,940	100 μg/kg	CPA	Wistar rats	*McGregor et al. 1996*[146]
Δ^9-THC	1.5 mg/kg 15 mg/kg	- CPA	Sprague-Dawley rats	*Sanudo-Pena et al. 1997*[121]
WIN 55,212-2 SR141716A	0.3-1 mg/kg up to 10mg/kg	CPA - (reversing effect on WIN 55,212-2)	Wistar rats	*Chaperon et al. 1998*[149]
Δ^9-THC	20 mg/kg	CPA	CD1 mice	*Hutcheson et al. 1998*[119]
Δ^9-THC Anandamide (AEA)	1 and 1.5 mg/kg up to 16 mg/kg	CPA -	Wistar rats	*Mallet and Beninger 1998*[117]
HU-210 Δ^9-THC	20, 60, 100 μg/kg 1.5 mg/kg	CPA CPA	Lister Hooded rats	*Cheer et al. 2000*[116]
Δ^9-THC	5 mg/kg 1 mg/kg 5 mg/kg (not standard protocol-pretreatment) 1 mg/kg	CPA - - CPP	CD1 mice	*Valjent and Maldonado 2000*[124]
CP 55,940 SR141716A	20 μg/kg 0.5 mg/kg	CPP - (reversing effect on CP 55,940)	Wistar rats	*Braida et al. 2001*[147]
Δ^9-THC	5 mg/kg	-	dynorphin deficient mice	*Zimmer et al. 2001*[122]
Δ^9-THC SR141716A	0.075-0.75 mg/kg 0.25-1 mg/kg	CPP (reversing effect on Δ^9-THC)	Wistar rats	*Braida et al. 2004*[111]
Δ^9-THC	1 mg/kg 5 mg/kg	CPP CPA	A_{2A}KO and wild-type mice	*Soria et al. 2004*[125]
URB-597	0.03-0.3 mg/kg	-	Wistar, Sprague-Dawley rats	*Gobbi et al. 2005*[243]
AM-404	1.25-10 mg/kg	CPP -	Rats (anxiety models)	*Bortolato et al. 2006*[242]
Δ^9-THC	0.1 mg/kg	CPP	Sprague-Dawley rats	*Le Foll et al. 2006*[123]
Δ^9-THC	1 mg/kg	-	Sprague-Dawley rats	*Vlachou et al. 2007*[95]
WIN 55,212-2	0.1-1 mg/kg	-	Sprague-Dawley rats	*Pollissidis et al. 2009*[148]

CPA conditioned place aversion (avoidance), - no effect, ↑ increase, ↓ decrease

Table 5. Cannabinoid effects on drug discrimination studies in experimental animals.

TRAINING CANNABINOID	DOSE	SUBSTITUTION DRUG	ED_{50}	EFFECT	SPECIES	References
Δ^9-THC	3 mg/kg	Δ^9-THC CP 55,940	0.7 0.03	Dose dependent Generalization CP55,940 more potent	Sprague-Dawley rats and rhesus monkeys	*Gold et al. 1992*[131]
Δ^9-THC	3 mg/kg	Δ^{9-11}-THC Δ^9-THC	3.2 1.0	Generalization Complete substitution	Sprague-Dawley rats and rhesus monkeys	*Wiley et al. 1993*[129,132]
Δ^9-THC	3 mg/kg	vehicle		Generalization	Sprague-Dawley rats	*Barrett et al. 1995*[128]
CP 55,940 SR141716A	0.1 mg/kg	Δ^9-THC WIN 55,212-2 Cannabinol	0.08	Generalization, complete substitution by CB_1 agonists, Antagonism by SR	Sprague-Dawley rats	*Wiley et al. 1995*[151]
WIN 55,212-2	0.3 mg/kg	CP 55,940 Δ^9-THC SR141716A SR140098	0.007 0.64 1.6 -	Generalization by CP 55,940 and Δ^9-THC, antagonism only by SR141716A	Sprague-Dawley rats	*Perio et al. 1996*[155]
R)-Methanandamide Δ^9-THC	10 mg/kg 3 mg/kg	SR141716A Anandamide	0.63, 4.83 0.46, 5.10	Generalization, reversal by SR	Sprague Dawley rats	*Järbe et al. 2001*[152]
CP 55,940	0.03, 0.014 mg/kg	Δ^9-THC SR141716A	0.16, 0.92 0.45, 0.50	Generalization Reversal	Wistar rats	*De Vry and Jentzsch 2003*[126]
Δ^9-THC	3.2 mg/kg	Anandamide R)-Methanandamide	- 1.316	Partial substitution Complete substitution	Sprague Dawley rats	*Alici and Appel 2004*[238]
BAY-593074 SR141716A	0.5 mg/kg	HU-210 CP 55,940 WIN 55,212-2 Δ^9-THC	0.0003 1.79	Generalization Reversal by SR141716A	Wistar rats	*De Vry and Jentzsch 2004*[130]
Δ^9-THC	3, 10 mg/kg	B-endorphin SR141716A	1.137	Potentiation of the discriminative effects of Δ^9-THC, reversal by SR	Sprague Dawley rats	*Solinas et al. 2004*[163]
Δ^9-THC O-1812	3 mg/kg 0.3 mg/kg	Δ^9-THC O-1812	1.08 0.16	Substitution of each other	Sprague Dawley rats	*Wiley et al. 2004*[240]
Δ^9-THC SR141716A	0.3-5.6 mg/kg 0.3-3 mg/kg	SR141716A		Discrimination Reversive effect by SR141716A	Sprague Dawley rats	*Solinas and Goldberg 2005*[164]
Δ^9-THC (R)-Methanandamide	1.8, 3, 5.6 mg/kg 10 mg/kg	SR141716A AM251 SR144528	0.67, 0.87	Discrimination through CB_1 receptors	Sprague Dawley rats	*Järbe et al. 2006*[153]
AM-1346 Δ^9-THC (R)-Methanandamide	1.8, 5.6 mg/kg 10 mg/kg	Δ^9-THC	1.05, 4.10 0.94, 3.79 7.48, ND	Generalization	Sprague Dawley rats	*Järbe et al. 2006*[239]

(Table 5) Contd.…

TRAINING CANNABINOID	DOSE	SUBSTITUTION DRUG	ED_{50}	EFFECT	SPECIES	References
Δ^9-THC	0.32 mg/kg	SR141716A AM251	0.33 0.98	Discrimination of SR vs. vehicle Mimicked SR141716A	Rhesus monkeys	*McMahon 2006*[133]
Δ^9-THC	0.1 mg/kg	CP 55,940 WIN 55,212-2 (R)-Methanandamide AM1241 SR141716A AM251 etc.	0.0025 0.035 0.89	Generalization High levels of Δ^9-THC lever responding Low generalization Antagonistic effects Antagonistic effects	Rhesus monkeys (macaca mulatta)	*McMahon 2006*[133]
Δ^9-THC	3 mg/kg	Nicotine 0.1 mg/kg	0.46	Potentiation	Sprague-Dawley rats	*Solinas et al. 2007*[165,241]

ND: Not Determined

from injections of saline, only drugs that activate CB_1 receptors generalize to the Δ^9-THC training stimulus [126-133].

Involvement of synthetic CB_1 receptor agonists in reward. Except for the studies focusing on Δ^9-THC, numerous studies have investigated the effects of various synthetic cannabinoid agonists in reward mechanisms. In a series of studies from our laboratory we have shown that the potent non-selective CB_1/CB_2 receptor agonists WIN55,212-2 and CP-55,940, and the Δ^9-THC derivatives HU-210 and AMG-3 either do not affect or elevate the ICSS threshold, depending on the dose used [134-137]. Arnold and colleagues [138] have also reported that the CB_1 receptor agonist CP-55,940 did not have an effect on brain stimulation reward thresholds.

Most of the studies testing the reinforcing properties of synthetic cannabinoid analogs in the self-administration procedure have been conducted with the potent non-selective CB_1/CB_2 receptor agonist WIN55,212-2. In a number of studies, Fattore and colleagues [139-141] showed that, under food deprivation, rats could intravenously self-administer different doses of WIN55,212-2. This effect was blocked by the CB_1 receptor antagonist SR141716A, indicating that the self-administration of WIN55,212-2 was mediated by activation of the CB_1 receptors. More recently, Lecca and colleagues [142] also reported self-administration of WIN55,212-2 in rats that were food restricted. Similar results on WIN55,212-2 self-administration and its reinforcing effects blocked by SR141716A were also found in mice by a different research group [143]. One more study has shown that drug naïve mice self-administer WIN55,212-2 and the Δ^9-THC derivative HU-210 [144]. It should be noted that in these studies the experimental design used (i.e., only 1-day experimental tests in severely restrained animals) does not allow for correlation with data received from chronic self-administration studies or under baseline conditions. Another potent non-selective CB_1/CB_2 receptor agonist, CP-55,940, was not self-administered by rhesus monkeys in the study by Mansbach and colleagues [98]. Importantly, CP-55,940 was self-administered intracerebrally by rats in a free-choice procedure [145], an effect that was antagonized by the CB_1 receptor antagonist SR141716A, indicating that this effect was specifically mediated by CB_1 receptors. However, animals used in this study were water-deprived and water was concurrently delivered with each infusion, possibly altering the motivational state of the animals and provoking the self-administration response.

In the CPP paradigm, results are also contradictory and a modified CPP procedure is used in most studies that achieved CPP. In one of these studies, CP-55,940 exhibited both conditioned place and conditioned taste aversion in low and/or high doses [146], while in another study [147] using a broader time interval between the cannabinoid injections, compared with the study by McGregor and colleagues [146], CP-55,940 elicited CPP only in the dose of 20 μg/kg. These findings agree with those observed in the study by Lepore and colleagues with Δ^9-THC [114]. The CPP induced by CP55,940 was fully antagonized by pretreatment with the CB_1 receptor antagonist SR141716A. Further, animals administered with WIN55,212-2 either did not exhibit CPP [148], or they exhibited a clear conditioned place aversion, which was reversed by pretreatment with the CB_1 receptor antagonist SR141716A [149]. Interestingly, in the study by Polissidis and colleagues [148], there was a lack of CPP after the administration of a low dose of WIN 55,212-2, while the same low dose showed an increase in dopaminergic activity in specific areas of the brain, such as the dorsal striatum and the amygdala. These findings contribute to the concept of an atypical and "weak" profile induced by cannabinoids (compared to other drugs of abuse, such as psychostimulants), especially in rodents. Additionally, WIN55,212-2 did not exhibit any preference or aversion when a CPP protocol in the open-field water-maze was used [120], in accordance with the findings of the study by Chaperon and colleagues, while Castané and colleagues [150] showed that WIN55,212-2 produced CPP in mice pre-exposed to a priming injection of the drug. Interestingly, in another study by Cheer and colleagues [116], not only Δ^9-THC, but also its analog HU-210 produced conditioned place aversion in rats, although cocaine and the CB_1 receptor antagonist SR141716A produced a clear CPP.

Synthetic CB_1 receptor agonists generalize to the Δ^9-THC training stimulus with a potency that corresponds with their *in vitro* affinity for the CB_1 receptors [127, 131, 151]. These actions are blocked by SR141716A administration, indicating that CB_1 receptors are selectively involved in these actions [126-133, 152-155]. Importantly, Pério and colleagues [155] have shown that the CB_1 receptor antagonist SR140098, which does not cross the blood brain barrier, could not antagonize the discriminative stimulus properties of cannabinoids. Furthermore, in a previous study by Kallman and colleagues [156] intracerebral administration of Δ^9-THC induced drug appropriate responding in rats trained to discriminate it from vehicle after systemic injections. Both studies suggest that the cannabinoid cue effect seems to be centrally mediated through the CB_1 receptor subtype. Most recently, using drug-discrimination proce-

dures in humans, Lile and colleagues showed that nabilone, a synthetic CB_1 receptor agonist shared agonist effects with Δ^9-THC in cannabis users, and was well-tolerated when co-administered with Δ^9-THC [157].

Most importantly, animals trained to discriminate a CB_1 receptor agonist from saline showed complete generalization only if they were tested with other CB_1 receptor agonists and not compounds from other pharmacological classes, such as opioids, barbiturates, psychostimulants, hallucinogens, anticonvulsants, neuroleptics and antidepressants [127, 128, 130, 158-160]. Only a partial overlap in the discriminative stimulus properties between Δ^9-THC and diazepam, phencyclidine or 3,4-methylenedioxymethamphetamine has been reported [128, 158, 161, 162]. However, in a more recent study the substitutions of both pentobarbital and diazepam for Δ^9-THC were found to be considerably less pronounced than those reported previously [128, 161]. Solinas and colleagues [163] demonstrated that the discriminative stimulus effects of Δ^9-THC are potentiated by morphine and decreased by naloxone. In a follow-up study, Solinas and Goldberg [164] confirmed and extended those findings with heroin and also found that mu, but not delta or kappa, opioid receptors are involved in the discriminative effects of Δ^9-THC. Nicotine also potentiated the discriminative effects of low doses of Δ^9-THC, although it did not produce Δ^9-THC-like effects [165]. An assessment of the separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9-THC using more pharmacologically specific drug-discrimination procedures also indicated that GABA is involved in the clinical effects of Δ^9-THC [166].

Importantly, a large number of studies have found that the endocannabinoid system and, more specifically, cannabinoid compounds acting on the CB_1 receptor activate brain reward circuits also activated by other drugs of abuse, such as nicotine, ethanol and opioids, and they are implicated in their rewarding effects [167]. However, this review does not discuss this literature. Relevant extensive information can be found elsewhere (e.g. [167-172].

The Role of CB_1 Receptor Antagonists

Most of the studies have used CB_1 receptor antagonists to test for CB_1-receptor selectivity of cannabinoid compounds on brain reward. Indeed, in many of these studies, CB_1 receptor antagonists block the rewarding [e.g.[108, 110, 173] and/or reward-facilitating or reward-attenuating effects [e.g.[95, 135-137] of Δ^9-THC and other endogenous or synthetic cannabinoids, or they block the drug-induced reinstatement of Δ^9-THC-seeking behavior [174]. Still, a few studies have tested the effects of CB_1 receptor antagonists on reward *per se*. Cheer and colleagues showed that cocaine and the CB_1 receptor antagonist/inverse agonist SR141716A produced a clear CPP [116], indicating the possibility of the existence of an endogenous cannabinoid tone in the brain, as a physiological system to suppress reward or induce aversion [116]. However, in other studies SR141716A failed to produce either place conditioning or place aversion [147, 149].

Low doses of the CB_1 receptor antagonists SR141716A and AM-251 did not affect ICSS thresholds [134, 136, 137], while high and possibly non-selective doses of SR141716A increased brain stimulation reward thresholds, indicating an anhedonic-like effect [138, 175]. However, in such high doses it is possible that SR141716A acts as a partial or inverse agonist at cannabinoid receptors, as it has been observed in other studies [126, 176, 177]. Furthermore, in another study it was shown that blockade of CB_1 receptors by relatively low doses of AM 251 dose-dependently inhibited cocaine's rewarding effects, whereas SR141716 was largely ineffective, as assessed by both cocaine self-administration under a progressive-ratio schedule of reinforcement and brain stimulation reward thresholds. This study suggested that AM 251 or other more potent CB_1 receptor antagonists could potentially be used as effective anti-cocaine medications [178]. Interestingly, preclinical and clinical studies indicated that SR141716A (rimonabant) could be effective as a therapeutic agent for obesity, cardiometabolic problems [179-183] and smoking cessation [for review, see [184], but it also induced anhedonic states and depressed mood [185-192], which has now lead to efforts for the development of CB_1 receptor antagonists that only act peripherally and do not cross the blood-brain barrier [193], with a good example being the neutral CB_1 receptor antagonist AM6545 a compound with a relatively poor penetrability into the central nervous system [194].

Brain Reward in CB_1 knock-out (KO) Mice

A number of studies have examined the role of CB_1 receptors in reward and in the rewarding effects of opiates [195] and psychostimulants [196]. Ledent and colleagues studied the reinforcing effects of WIN55,212-2 and opiates [197]. In this study, CB_1 knockout (KO) mice did not self-administer WIN55,212-2 and the acute effects of opiates (i.e., tolerance to morphine's antinociceptive effects) were unaffected, but the reinforcing effects of morphine and the morphine withdrawal symptoms were reduced [197]. In a relevant study by the same group [198], morphine did not induce intravenous self-administration in mutant CB_1 receptor knockout mice, while cocaine, amphetamine and nicotine were self-administered to the same extent by both wild-type and CB_1 receptor knockout mice. Both these studies indicated that the CB_1 cannabinoid receptor is essential not only for the expression of cannabinoid reinforcing effects, but also for the modulation of morphine rewarding effects. These data also confirmed previous findings showing that cocaine, but not morphine, induced CPP and sensitization to locomotor responses in CB_1 KO mice [199], whereas another study by Rice and colleagues showed that CB_1 receptor KO mice developed a strong place preference to 4 and 8 mg/kg of morphine, not supporting a contribution of the endocannabinoid system to morphine reward [200]. Interestingly, in contrast to the study by Martin and colleagues [199], Soria and colleagues showed that CB_1 KO mice exhibited a clear reduction in cocaine self-administration, i.e., only 25% of CB_1 KO mice compared to 75% of their wild-type littermates acquired a reliable responding for cocaine self-administration and also after more training sessions, a result which was similar to that obtained after the pharmacological blockade of CB1 receptors with SR141716A in wild-type mice [201].

Studies have also been conducted on the role of CB_1 receptors in the rewarding effects of other drugs of abuse, such as nicotine [202, 203] and alcohol [204-206]. Based on the study by Castañé and colleagues, some acute effects (i.e., antinociception) and motivational responses (i.e. CPP) elicited by nicotine can be modulated by CB_1 receptors in the endogenous cannabinoid system, supporting the existence of a physiological interaction between nicotinic receptors and the endocannabinoid system [202]. Further, high ethanol preference of young C57BL6J mice is reduced by the CB_1 receptor antagonist/inverse agonist SR141716A to levels observed in their CB_1 knockout littermates or in old wild-type mice [206], while CB_1 knockout mice also display reduced ethanol-induced CPP and these reduced rewarding effects of ethanol are correlated to an overexpression of striatal dopamine D_2 receptors [204]. Similar results have also shown not only reduced ethanol CPP, but also ethanol self-administration in CB_1 knockout mice [205].

Involvement of CB_1 Receptors in Natural Reward

Studies have shown that endocannabinoid functions control brain reward processes related not only to drug abuse, but also to appetite regulation [207] and natural rewards, such as food, water and sucrose [208]. Results from a great number of related studies led to the development of CB_1 receptor antagonists as a possible treatment for obesity [179-182]. However, the focus of this review is not on the consummatory aspects of the cannabinoid effects in natural reward [209-212], but on the motivational and hedonic aspects of it.

In a study testing the capacity of explicit stimuli to precipitate food-seeking behavior, SR141716 prevented the enhancement by quinelorane, a dopamine D_3-prefering receptor agonist, of the appetitive value of food pellets unexpectedly delivered during extinction, suggesting that the effects of SR141716 might involve D_3 receptor-mediated processes. Further, SR141716A decreased lever-pressing for a 5% sucrose solution under both fixed-ratio 1 and progressive-ratio schedules of reinforcement, suggesting that CB_1 receptors are involved in the reinforcing and motivational properties of not only drugs of abuse, but also natural rewards, such as sucrose [213]. Most recently, and confirming previous findings [214], stimulation of CB_1 receptors by Δ^9-THC specifically increased the palatability of hedonic taste (i.e., sucrose solutions, sweet chocolate) without affecting that of aversive tastes (i.e., quinine and saturated NaCl solutions). Consistent with these findings was the observation that under Δ^9-THC pretreatment sucrose acquired the ability to induce a release of dopamine in the shell of the nucleus accumbens, a property which underwent adaptation after repeated exposure to the taste [215].

Interestingly, in a recent study, Fischer rats were found to express lower reward sensitivity observed for consummatory, motivational and hedonic aspects, towards a palatable food reward (i.e., sweetened condensed milk) compared to Wistar rats [216]. In the same study, Western blot analysis for the CB_1 receptor and the endocannabinoid degrading enzyme FAAH revealed a lower expression of both proteins in the hippocampus of Fischer rats compared to the Wistar strain [216]. Furthermore, increased cannabinoid-stimulated extracellular-regulated kinase (ERK) phosphorylation was detected in Wistar rats compared to the Fischer strain, indicating alterations in endocannabninoid signaling. These findings were further supported by the pharmacological results, where Fischer rats were found to be less sensitive towards the effects of the CB_1 receptor antagonist/inverse agonist SR141716 and the cannabinoid agonist WIN55,212-2 [216]. Data from this study indicate differences in the expression of the CB_1 receptor and FAAH, as well as the activation of endocannabinoid signaling pathways between Fischer and Wistar rats, which could partly explain the discrepancy of results between studies using different rat strains to test for the rewarding effects of cannabinoid compounds.

Moreover, data from an electrophysiological study showed that depolarization of perifornical lateral hypothalamus (LH) neurons elicits a CB_1 receptor-mediated suppression of inhibition in local circuits thought to be involved in appetite and "natural reward", and the motivational aspects of feeding behavior [217]. CB_1 KO mice consumed less sucrose under operant conditions or when using a two-bottle free choice procedure [218]. Moreover, CB_1 KO mice exhibited a decreased sensitivity to the rewarding properties of sucrose [218]. In another study, it took CB_1 KO mice significantly longer to acquire operant responding maintained by a sweet reinforcer (i.e., a liquid nutritional drink), and responding for the sweet reinforcer under a progressive-ratio schedule was significantly reduced in CB_1 KO, as well as in wild-type mice pretreated with SR141716A, as compared to wild-type controls [219]. In the same study, pretreatment with the potent non-selective CB_1/CB_2 receptor agonist CP 55940 increased responding for the sweet reinforcer in CB_1 KO compared to wild-type mice. In contrast, responding for the fat reinforcer (i.e., corn oil) during acquisition and under the progressive-ratio schedule was not significantly different in CB_1 KO compared to wild-type mice. Taken together, these results suggest that CB_1 receptors are preferentially involved in the reinforcing effects of a complex sweet, as compared to a pure fat, reinforcer [219].

Overall, these findings show that the endocannabinoid system is implicated in the regulation of brain processes related to natural rewards, such as food and sucrose. The endocannabinoid system is not only involved in the consummatory aspects of the natural rewards' control and regulation, as proven by the relevant clinically therapeutic effects of CB_1 receptor antagonists in obesity, but it is also involved in the pleasurable and motivational aspects of this process.

INVOLVEMENT OF CB_2 RECEPTORS IN REWARD

As previously mentioned, CB_2 receptors have been detected in various brain regions including the cerebral cortex, caudate-putamen, nucleus accumbens, hippocampus, hypothalamus, amygdale and the ventral tegmental area, thus suggesting their potential involvement in emotion, motivation and reward. Indeed, it has been suggested that CB_2 receptors may be involved in drug addiction [220]. Most of the studies conducted so far have explored the effects of activation or inactivation of CB_2 receptors on drug-taking and drug-seeking behavior for various drugs of abuse, including alcohol, nicotine and cocaine. However, to the best of our knowledge, no studies have examined the effects of CB_2 receptor agonism/antagonism or CB_2 receptor deletion on brain stimulation reward, self-administration, conditioned place preference and drug discrimination.

Onaivi and colleagues have reported increased CB_2 receptor gene expression in the brain of mice after chronic treatment with heroin or cocaine [221]. On the contrary, mice that developed alcohol preference had reduced CB_2 receptor gene expression in the striatum and midbrain [221]. Interestingly, chronic treatment with the CB_2 receptor agonist JWH015 enhanced alcohol consumption in stressed, but not in control, mice [220, 221]. A recent study has shown that systemic administration of the CB_2 receptor agonist JWH133 inhibits self-administration of cocaine in wild-type and CB_1-deficient mice, but not CB_2-deficient mice [222]. In the same study, pretreatment with the CB_2 receptor antagonist AM630 prevented this effect in the wild type mice. These results indicate that the anti-addictive effect of JWH133 is mediated by CB_2 receptors. Similar results have been obtained with GW405833, another CB_2 receptor agonist with a different chemical structure [222]. In addition, mice overexpressing CB_2 receptors showed reduced behavioral sensitization induced by chronic cocaine, reduced cocaine self-administration and conditioned place aversion [223]. Moreover, according to the results of a recent study, the CB_2 receptor antagonist SR144528 did not affect cocaine self-administration, but inhibited cocaine-induced reinstatement of cocaine-seeking behavior [224]. On the other hand, neither activation of CB_2 receptors by AM1241, nor blockade of them using the CB_2 receptor antagonist AM630 affected nicotine-taking behavior under fixed-ratio or progressive-ratio schedules of reinforcement [225]. Furthermore, CB_2 receptors are not involved in cue-induced or nicotine-induced reinstatement of nicotine-seeking behavior [225].

Experimental findings suggest that the brain CB_2 receptors modulate the rewarding effects of cocaine via a dopamine-dependent mechanism. More specifically, local administration of the CB_2 receptor agonist JWH133 into the nucleus accumbens resulted in attenuation of cocaine self-administration in wild-type mice, but not in CB_2-deficient mice. This effect was blocked by intra-accumbens administration of the CB_2 receptor antagonist AM630 [222]. Moreover, intra-accumbens administration of JWH133 reduced dopamine release in the nucleus accumbens of wild-type mice and CB_1-deficient mice. Intra-accumbens administration of the CB_2 receptor antagonist AM630 had the opposite effect [222]. Since these effects have not been observed in mice lacking CB_2 receptors, we can speculate that activation of CB_2 receptors mediates the inhibitory actions of JWH133 on dopamine release.

In summary, the data presented here provide evidence for interesting putative role of CB_2 receptors in reward and the rewarding/addicted properties of cocaine. Apparently, however, further studies are necessary to explore the involvement of CB_2 receptors in brain reward *per se*, as well as in the actions of other rewarding stimuli or drugs of abuse.

ENDOCANNABINOID MODULATION OF REWARD

Involvement of endocannabinoids in reward. Both AEA and 2-AG increase extracellular levels of dopamine in the nucleus accumbens, an effect which is common among many rewarding stimuli, including food, sex and drugs of abuse [226]. Therefore, it has been hypothesized that Δ^9-THC from smoked marijuana produces rewarding effects affecting brain circuits that normally use endocannabinoids, such as AEA and 2-AG [184, 227, 228]. This hypothesis is also supported by the finding that both AEA (as well as its metabolically stable synthetic analogue methanandamide) [173] and 2-AG [229] are intravenously self-administered by squirrel monkeys. In both studies the authors report rates of responding comparable with those maintained under the same conditions by cocaine or Δ^9-THC. However, we should mention that in the first study which provided evidence of AEA self-administration, 4 out of 6 squirrel monkeys used had a history of Δ^9-THC or methohexital self-administration [173]. Similarly, in the more recent study indicating 2-AG self-administration, the researchers used monkeys with either a history of AEA self-administration or a history of nicotine self-administration [229]. Interestingly, however, the reinforcing effects of AEA and 2-AG appear to be mediated by cannabinoid CB_1 receptors, since daily pre-treatment with SR141716A resulted in complete blockade of AEA or 2-AG self-administration behavior. More importantly, there is also evidence that treatment with the FAAH inhibitor URB597 shifts the AEA self-administration dose-response curve to the left, indicating that AEA has rewarding effects even in lower doses [230].

Two studies have examined so far the effects of the endogenous cannabinoid AEA in the CPP procedure [117, 231]. According to the results of Mallet and Beninger, the intraperitoneal administration of AEA did not produce any significant effects in place conditioning, although using the same experimental protocol Δ^9-THC produced conditioned place aversion. In the more recent study of Scherma *et al.* [231], intravenous administration of AEA produced neither conditioned place preference nor aversion. However, when rats were pretreated with the FAAH inhibitor URB597 AEA produced dose-related conditioned place aversion.

Consistent with the hypothesis implicating endocannabinoids on brain reward, AEA can potentiate the effects of food reward [232] and also appears to increase the palatability of food [233]. More specifically, intracerebral injection of AEA into the nucleus accumbens of rats not only increased food intake [234], but also amplified the hedonic impact of a natural sensory reward, such as sweetness, by increasing the positive liking reactions to sucrose taste [234]. Similarly, intracerebral injection of 2-AG into the shell of the nucleus accumbens increases food intake [235].

Several studies have investigated whether endocannabinoids produce Δ^9-THC-like discriminative effects when administered systemically. AEA, which is rapidly inactivated in the body, either does not produce Δ^9-THC-like discriminative effects or it only produces them at very high doses that suppress the rates of responding [152, 236, 237]. However, under the same conditions, the FAAH-resistant analogue of AEA methanandamide produces Δ^9-THC-like discriminative effects [152, 236, 238]. Since Δ^9-THC-like discriminative effects have been also observed with other metabolically stable synthetic analogues of AEA, such as O-1812 and AM-1346 [239, 240], it is likely that AEA's fast metabolic inactivation is responsible for the weak effects observed with the main endocannabinoid. This is supported by the finding that AEA produces Δ^9-THC-like discriminative effects when its breakdown is inhibited by the FAAH inhibitor URB597 [241].

In summary, endocannabinoids appear to play an important role in motivational processes and regulate brain reward. However, the effects of endocannabinoids on self-administration and CPP are somewhat controversial. These discrepancies, which are likely due to different methodologies and procedural differences, remain to be resolved.

Effects of endocannabinoid modulators in reward. As previously described, AEA is quickly degraded in the body. Therefore, studying its role in reward processes can be more readily investigated when its transport is inhibited or when FAAH, the enzyme which regulates its degradation, is blocked. There are contradictory reports on the effects of the AEA transport inhibitor AM-404 on reward processes. Bortolato *et al.* showed that AM-404 produces CPP only in rats housed under enriched conditions, but not in rats kept in standard cages [242]. However, AM-404 induced CPP at a dose that did not increase tissue levels of AEA or 2-AG in the brain areas investigated [242]. Thus, the involvement of the endocannabinoid system in AM-404-induced place preference remains doubtful. On the other hand, we have shown that AM-404 either does not affect or increases ICSS thresholds, depending on the dose used [137]. Similar results on brain stimulation reward have been obtained with OMDM-2, a more selective transport inhibitor of AEA [137]. Likewise, blockade of AEA transport with VDM-11 does not alter food intake in rats. Notably, AM-404 does not produce Δ^9-THC-like discriminative effects [241]. Finally, AM-404 does not increase dopamine levels in the nucleus accumbens, an effect which is common among various rewarding stimuli, including food, sex and drugs of abuse [241].

Drugs that inhibit AEA degradation by FAAH, such as URB-597, are not self-administered [230] and do not have rewarding effects by themselves in the CPP [243] or the ICSS [137] paradigms. Moreover, URB-597 does not produce Δ^9-THC-like discriminative effects [243] and does not alter dopamine levels in the nucleus accumbens [226]. Although CB_1 receptor agonists, such as Δ^9-THC and WIN55,212-2 can potentiate heroin self-administration, neither URB-597, nor AM-404 produced similar effects [244]. On the contrary, AM-404 induced a small decrease in heroin self-administration under a progressive-ratio schedule of reinforcement. According to these results we can speculate that the endocannabinoid tone has a neutral or slightly inhibitory effect on heroin reward, whereas direct activation of CB_1 receptors can have a facilitatory effect. Interestingly, pretreatment with URB-597 although did not modify self-administration of Δ^9-THC, it significantly potentiated AEA self-administration [230]. In mice, dual FAAH/MAGL blockade, but not selective FAAH or MAGL inhibition, was found to cause profound Δ^9-THC-like discriminative effects that were reversed by the CB_1 antagonist SR141716A. This finding further indicates that CB_1 receptors in both the AEA and 2-AG pathways might be important in the regulation of cannabinoid reward.

In summary, drugs that increase and prolong the effects of endocannabinoids, such as URB-597, do not possess reinforcing properties and do not seem to have the abuse liability that has been associated with the direct CB_1 receptor agonists.

Effects of genetic ablation of the FAAH enzyme in reward. In parallel with studies examining the effects of FAAH inhibition on brain reward, the availability of FAAH-deficient mice has facilitated the assessment of AEA's role in brain reward processes. Mice lacking the enzyme FAAH do not show significant changes in food consumption or body weight [76]. This may suggest that the endocannabinoid system plays only a modulatory role in basic reward functions. Interestingly, genetic ablation of FAAH enhances nicotine reward through a CB_1 mechanism that is most likely due to elevated levels of AEA [245]. To the best of our knowledge, no study has investigated the effects of FAAH deletion on brain stimulation reward. Future studies using conditional FAAH-deficient mice will improve our understanding for the role of the endocannabinoid system in brain reward functions and may delineate its interaction with classic brain reward circuits of the brain.

SUMMARY - CONCLUSIONS

As seen with four of the main procedures used to examine the reward-facilitating or reward-attenuating, the reinforcing and the motivational properties of cannabinoid compounds (i.e., intracranial self-stimulation, self-administration, conditioned place preferences and discrimination studies), the cannabinoid compounds (i.e., CB_1 and CB_2 receptor agonists and antagonists, endocannabinoids, and endocannabinoid modulators) have produced diverge findings, with many studies showing a biphasic effect of these compounds.

Most of the studies using the ICSS paradigm failed to show reward-facilitating effects for Δ^9-THC or other cannabinoids, while others present data for anhedonic actions of these compounds. Data from these studies seem to be dose-, as well as strain-dependent, suggesting an important genetic component in the direction of these effects. In the first self-administration studies, it was difficult to accomplish self-administration of Δ^9-THC or other synthetic cannabinoids without specific experimental manipulations (i.e., without previous drug exposure or food restriction/deprivation). However, recent studies have reported a robust procedure for cannabinoid self-administration either in a limited number of squirrel monkeys or intracerebrally in rodents. Similarly to the self-administration studies, Δ^9-THC and other cannabinoids have not always shown consistent results in the CPP procedure, where CPP has only been presented when specific experimental methodology has been used. Further, drug discrimination studies have shown that cannabinoid compounds are pharmacologically selective, with Δ^9-THC appearing to be fully substituted for other synthetic cannabinoids with affinity for the CB_1 receptor, while compounds with anandamide-like structure exhibiting differences from classical cannabinoids, as in different studies they failed to substitute for Δ^9-THC or other CB_1 receptor agonists. This latter finding suggests that anandamide-like compounds and drugs that directly affect the endocannabinoid levels may prove promising therapeutics in the future, with less unwanted side-effects and minimal abuse potential.

Most recently, an increasing number of studies has focused on the effects of cannabinoid compounds acting on the CB_2 receptor or on the effects of genetic ablation of either the FAAH enzyme or CB_1 and/or CB_2 receptors. Results from these studies have proved to highly contribute to our understanding of the effects of the endocannabinoid system on brain reward and how we can use these findings for the development of potentially therapeutic cannabinoid compounds for the treatment of reward-related disorders and drug abuse. However, there is still a long way to go to gain further insight into the physiological role of the endogenous cannabinoid system in brain reward.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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