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Involvement of the endocannabinoid system in drug addiction

Rafael Maldonado, Olga Valverde and Fernando Berrendero

Laboratori de Neurofarmacologia, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Carrer Dr. Aiguader 80, 08003 Barcelona, Spain

Recent studies have shown that the endocannabinoid system is involved in the common neurobiological mechanism underlying drug addiction. This system participates in the primary rewarding effects of cannabinoids, nicotine, alcohol and opioids, through the release of endocannabinoids in the ventral tegmental area. Endocannabinoids are also involved in the motivation to seek drugs by a dopamine-independent mechanism, demonstrated for psychostimulants and opioids. The endocannabinoid system also participates in the common mechanisms underlying relapse to drug-seeking behaviour by mediating the motivational effects of drug-related environmental stimuli and drug re-exposure. In agreement, clinical trials have suggested that the CB_1 cannabinoid antagonist rimonabant can cause smoking cessation. Thus, CB_1 cannabinoid antagonists could represent a new generation of compounds to treat drug addiction.

Introduction

Drug addiction is a chronic relapsing brain disorder, characterized by neurobiological changes leading to compulsive drug seeking and drug taking despite serious negative consequences, and by loss of control over drug use [1]. Addiction includes complex behavioural and neurobiological processes. All the drugs of abuse produce reinforcing effects that are responsible for the initiation of the addictive disorder. However, other behavioural processes are also crucial for the maintenance of addiction, including the negative consequences of drug abstinence and the different stimuli leading to relapse (e.g. drug-associated cues, stressors and drug re-exposure) [2].

Several groups of compounds that produce different pharmacological effects can lead to addictive behaviour, including opioids, psychostimulants, cannabinoids, alcohol and nicotine. The initial mechanism of action of these drugs implicates different neurochemical targets [3]. However, all these compounds produce neural dysregulations involving similar neurochemical and neuroanatomical pathways [4]. Indeed, multiple studies support the existence of common neurobiological mechanisms for the addictive properties of most drugs of abuse. This information is based on findings showing the crucial role of the mesocorticolimbic dopaminergic pathways, the endogenous opioid system, and the brain and pituitary stress system in the addictive processes. Drugs of abuse interact with these common brain circuits producing adaptive changes leading to a profound dysregulation of brain motivational and reward pathways [2]. The mesocorticolimbic system represents a common neuronal substrate for the reinforcing properties of drugs of abuse, where both dopamine and opioid transmission are crucial [5]. The major components of this drug reward circuit are the ventral tegmental area (VTA), which contains the dopaminergic cell bodies, and the terminal areas in the basal forebrain [the nucleus accumbens (NAc), olfactory tubercle, amygdala, and frontal and limbic cortices] [6]. These neurochemical circuits are also involved in the negative motivational consequences of drug withdrawal [2]. Mesolimbic dopaminergic neurons receive highly processed information from the cerebral cortex and other areas involved in cognitive functions, and dopamine release in the forebrain has been proposed to serve as a learning signal. Dopamine neurons in the NAc interact with glutamatergic projection neurons from the cerebral cortex, hippocampus and amygdala, providing information about external context and about internal emotional and physiological states. Hence, drug-induced plasticity in these NAc projections contributes to addiction by consolidating reward-driven behaviour [3,7]. Recruitment of brain stress pathways has also been reported as a common change during drug abstinence that seems be crucial in the reinstatement of drug seeking behaviour [8]. However, the common mechanisms involved in the development of the addictive processes have not been yet completely identified. This review focuses on the recent findings supporting participation of the endocannabinoid system in the common circuitry underlying drug addiction and proposes a mechanistic explanation for this physiopathological role.

Endocannabinoid system and brain reward circuitry

Knowledge of the endocannabinoid system has been largely improved since the cloning in 1990 of the CB_1 cannabinoid receptor, which is activated by Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of *Cannabis sativa*. This system consists of cannabinoid receptors, endogenous ligands and several proteins responsible for their synthesis and degradation. To date, two subtypes of cannabinoid receptors, CB_1 and CB_2, have been characterized and cloned. CB_1 receptors are the most

Corresponding author: Maldonado, R. (rafael.maldonado@upf.edu).
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abundant G-protein-coupled receptor in the CNS and are also found in peripheral tissues. CB_2 receptors are mainly located in the cells of the immune system [9], but they have also been recently identified in brainstem, cortex and cerebellum neurons [10]. Several endogenous cannabinoids have been isolated from brain tissue, anandamide and 2-arachidonoylglycerol being the best characterized [9]. Endocannabinoids are thought to act as retrograde messengers in the CNS [11] and behave as neuromodulators in many physiological processes. Accordingly, endocannabinoids released from postsynaptic neurons upon depolarization activate presynaptic CB_1 cannabinoid receptors, resulting in inhibition of the release of both excitatory and inhibitory neurotransmitters. This endocannabinoid retrograde control has also been recently demonstrated after synaptic activation of group I metabotropic glutamate receptors [12] and D_2 dopamine receptors [13].

Several studies support the view that the endocannabinoid system represents a new candidate for the control of drug rewarding properties. Indeed, CB_1 cannabinoid receptors are abundant in the brain reward circuitry and participate in the addictive properties induced by different drugs of abuse. The dopaminergic neurons of the mesocorticolimbic pathway are controlled by excitatory and inhibitory inputs that are modulated by CB_1 cannabinoid receptors. Thus, endocannabinoids can be released following depolarization in the NAc [14] and from dopaminergic neurons in the VTA [13,15], and they modulate glutamatergic and GABAergic afferents by acting as retrograde messengers on CB_1 receptors. The presence of CB_1 receptors in other structures related to motivation and reward, such as the basolateral amygdala and the hippocampus, also contributes to this function of the endocannabinoid system [16]. In addition, endocannabinoids participate in synaptic plasticity in the mesolimbic system. The stimulation of prelimbic cortex afferents causes long-term depression (LTD) of NAc glutamatergic synapses that is mediated by endocannabinoid release and presynaptic CB_1 receptors [14,17]. Endocannabinoids also produce LTD of inhibitory synaptic transmission in the hippocampus and prepare excitatory synapses for facilitating subsequent induction of long-term potentiation (LTP) [18], which contributes to the plasticity mechanisms reported in the learning processes related to addictive behaviour.

The endocannabinoid system is certainly the primary site of action for the rewarding and pharmacological responses induced by cannabinoids [19,20]. However, this system plays an overall modulatory effect on the reward circuitry and also participates in the rewarding and addictive properties of all prototypical drugs of abuse.

Endocannabinoid system and nicotine addiction

Nicotine addiction is a complex neurochemical process that involves many neurotransmitters, and the endocannabinoid system is crucial in the addictive effects of this drug. Pharmacological studies revealed that non-effective doses of nicotine and THC produced significant conditioned place preference in mice when administered together [21]. Interestingly, the rewarding properties of nicotine, assessed in a place-conditioning paradigm, were absent in knockout mice lacking CB_1 receptors [22] (Table 1). By contrast, CB_1 knockout mice learned to self-administer nicotine using an acute paradigm in mice that were restrained to avoid their movement [23]. However, this acute paradigm fails to evaluate the maintenance of a stable operant self-administration response, and nicotine effects on anxiety-like behaviour could influence this self-administration response in restrained animals [23]. Pharmacological studies using the selective CB_1 receptor antagonist rimonabant (Box 1) have confirmed the involvement of these receptors in nicotine addiction (Table 2). Thus, rimonabant reduces

Table 1. Changes to the addictive properties of drugs observed in CB_1 cannabinoid receptor knockout mice

Drug	Model	Effect	Refs
Morphine	Conditioned place preference	Suppression	[45]
		No change	[46]
	Behavioural sensitization	Suppression	[45]
	Self-administration in restrained mice	Suppression	[19]
		Suppression	[23]
	Withdrawal syndrome	Attenuation	[19]
		Attenuation	[79]
Ethanol	Conditioned place preference	Attenuation	[40]
	Two-bottle choice (voluntary consumption)	Attenuation	[37]
		No change	[41]
		Attenuation	[38]
		Attenuation	[34]
	Withdrawal syndrome	Suppression	[41]
		Increase	[38]
	Extracellular dopamine levels (*in vivo* microdialysis)	Suppression	[37]
Nicotine	Conditioned place preference	Suppression	[22]
	Self-administration in restrained mice	No change	[23]
	Withdrawal syndrome	No change	[22]
Cocaine	Conditioned place preference	No change	[45]
		No change	[40]
	Behavioural sensitization	No change	[45]
	Self-administration in restrained mice	No change	[23]
	Self-administration	Attenuation	[63]
	Extracellular dopamine levels (*in vivo* microdialysis)	No change	[63]
Amphetamine	Self-administration in restrained mice	No change	[23]

Box 1. Chemical names

AM-251: *N*-(piperidin-1-yl)-5-(4-iodophynyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
HU-210: (6aR)-*trans*-3-(1,1-dimethylhepthyl)-6a, 7, 10, 10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol
Rimonabant: *N*-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide
WIN 55,212-2: (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1,4-benzoxazin-6yl]-1-naphtalenylmethanone mesylate

nicotine operant self-administration [24] and nicotine-induced conditioned place preference in rats [25], although no effect was observed when nicotine place preference was evaluated 3 or 12 weeks after the initial conditioning phase [26]. Nicotine relapse induced by associated environmental stimuli is also mediated by activation of the endocannabinoid system. Thus, rimonabant attenuated the influence of these environmental stimuli on nicotine-seeking behaviour in rats [27,28]. CB_1 receptors do not seem to participate in the development of nicotine physical dependence because rimonabant did not precipitate a withdrawal syndrome in nicotine-dependent mice [29] and the severity of nicotine abstinence was not modified in CB_1 knockout mice [22]. The effects of the endocannabinoid system on the rewarding properties of nicotine are related to modulation of the extent to which nicotine activates the mesolimbic dopaminergic pathway. Thus, *in vivo* microdialysis studies revealed that rimonabant pre-treatment blocks nicotine-enhanced extracellular dopamine levels in the shell of the NAc and in the bed nucleus of the stria terminalis [24]. In agreement with these behavioural and biochemical results in rodents, Phase III clinical trials have revealed that rimonabant is significantly effective in obtaining smoking cessation [Studies with Rimonabant and Tobacco Use in North America (STRATUS-North America)] and can produce a strong tendency for such cessation in a population with a more intense daily tobacco consumption (STRATUS-Europe) [30]. These results suggest that CB_1 cannabinoid receptors represent a promising target for new therapies to treat tobacco addiction.

Endocannabinoid system and alcohol addiction

Cannabinoids and alcohol activate similar reward pathways, and CB_1 receptors also seem to regulate the reinforcing properties of alcohol. Thus, the acute administration of cannabinoid agonists stimulates voluntary alcohol intake in Sardinian alcohol-preferring (sP) and Wistar rats [31,32]. In agreement, blockade of CB_1 receptors reduces alcohol consumption in C57BL/6 mice, and in Wistar and sP rats [33–35] (Table 2). However, part of this effect can be attributed to a more general suppression of food and fluid intake [36]. Genetic inactivation of CB_1 receptors has confirmed these pharmacological data (Table 1). Thus, a decrease of voluntary alcohol intake in CB_1 knockout mice has been shown using a two-bottle free-choice paradigm [34,37–39], and ethanol-induced place preference was reduced in these mutants [39,40]. A role of CB_1 receptors in stress-induced alcohol drinking and ethanol withdrawal has also been reported using knockout mice [41], although the same study showed normal ethanol drinking behaviour under non-stressful conditions in these animals. CB_1 receptors are also involved in the mechanisms mediating alcohol relapse. Accordingly, the exposure to the cannabinoid agonists WIN 55 212-2 (Box 1) or THC promotes the relapse of alcohol use in abstinent rats [42,43], and rimonabant reduces conditioned reinstatement of

Table 2. Effects of rimonabant on drug addictive properties

Drug	Model	Dose (mg kg^{-1})[a]	Effect	Animal	Refs
Morphine	Conditioned place preference	0.1 (ip)	Attenuation	Rat	[80]
		3.0 (ip)	Suppression	Rat	[47]
	Self-administration	0.25 (ip)	Suppression	Rat	[47]
Heroin	Self-administration	3.0 (ip)	Suppression	Rat	[47]
		3.0 (ip)	Suppression	Rat	[81]
		0.3–3.0 (ip)	Attenuation	Rat	[82]
		1.0 and 3.0 (ip)	Attenuation	Rat	[48]
	Self-administration (relapse)	0.3 (ip)	Suppression	Rat	[50]
		1.0 and 3.0 (ip)	Attenuation	Rat	[48]
	Extracellular dopamine levels (*in vivo* microdialysis)	0.3–3.0 (ip)	No change	Rat	[82]
		1.0 (sc)	No change	Rat	[53]
Ethanol	Two-bottle choice (voluntary consumption)	0.3–3.0 (sc)	Attenuation	Rat, mouse	[33]
		2.5–10.0 (ip)	Attenuation	Rat	[83]
		0.3–3.0 (ip)	Attenuation	Rat	[84]
		3.0 (ip)	Attenuation	Mouse	[34]
	Self-administration	0.3–3.0 (ip)	Attenuation	Rat	[35]
	Self-administration (relapse)	1.0 and 3.0 (ip)	Attenuation	Rat	[35]
	Extracellular dopamine levels (*in vivo* microdialysis)	3.0 (ip)	Attenuation	Rat	[24]
		3.0 (ip)	Suppression	Mouse	[37]
Nicotine	Conditioned place preference	1.0 and 3.0 (ip)	Suppression	Rat	[25]
	Self-administration	0.3 and 1.0 (ip)	Attenuation	Rat	[24]
	Self-administration (relapse)	1.0 and 3.0 (ip)	Attenuation	Rat	[28]
		1.0 (ip)	Attenuation	Rat	[27]
	Extracellular dopamine levels (*in vivo* microdialysis)	1.0 and 3.0 (ip)	Attenuation	Rat	[24]
Cocaine	Self-administration in restrained mice	1.0 (ip)	No change	Mouse	[85]
	Self-administration	1.0–3.0 (ip)	Attenuation	Mouse	[63]
	Self-administration (relapse)	0.3–3.0 (sc)	Attenuation	Rat	[64]

[a]Abbreviations: ip, intraperitoneal; sc, subcutaneous.

ethanol-seeking behaviour in rats [35]. The endocannabinoid system seems to participate in alcohol rewarding properties by modulating its effects on the activation of mesolimbic dopamine transmission. *In vivo* microdialysis studies revealed that alcohol did not enhance extracellular levels of dopamine in the NAc in CB_1 knockout mice [37]. A similar result was obtained when wild-type mice were pretreated with rimonabant before alcohol administration [37]. Clinical data on the possible efficacy of CB_1 receptor antagonists in the treatment of alcohol addiction are not still available.

Endocannabinoid system and opioid addiction

Several studies have revealed the existence of functional bidirectional interactions between cannabinoid and opioid systems, and both systems participate in the common circuits involved in the addictive properties of different drugs of abuse [44]. CB_1 cannabinoid receptors have an important role in the rewarding properties of opioids. Thus, morphine-induced conditioned place preference [45] and intravenous self-administration [19] were abolished in knockout mice lacking CB_1 receptors, although contradictory results have been reported on the place-conditioning paradigm [46] (Table 1). In agreement, rimonabant reduced opioid self-administration and conditioned place preference in rodents [47,48] (Table 2). The effects of rimonabant on heroin self-administration were more pronounced when the effort required to obtain a heroin infusion was enhanced. Indeed, rimonabant markedly impaired heroin self-administration under a progressive ratio (PR) schedule of reinforcement, whereas this effect was attenuated under a fixed ratio (FR) schedule of 5 and almost disappeared at a FR1 [49] (Box 2). Rimonabant also prevented heroin-seeking behaviour after a long period of extinction, and the cannabinoid agonist HU-210 (Box 1) reinstated such a seeking behaviour [48–50]. Reciprocally, the rewarding effects induced by THC were suppressed in μ-opioid receptor knockout mice [51], and were attenuated by the opioid receptor antagonist naltrexone in monkeys [52]. Both opioid and cannabinoid rewarding responses are related to their facilitatory effects on mesolimbic dopamine transmission [5]. Rimonabant did not prevent the activation of dopamine transmission induced by heroin, although the opioid receptor antagonist naloxone prevented such a biochemical effect from being produced by cannabinoids [53,54].

Cross-dependence has also been reported between opioid and cannabinoid compounds. Thus, naloxone induced a withdrawal syndrome in THC-dependent rats, whereas rimonabant precipitated abstinence in morphine-dependent animals [55,56]. In agreement, a robust attenuation in the severity of naloxone-precipitated morphine withdrawal was reported in CB_1 knockout mice [19]. Reciprocally, the expression of cannabinoid withdrawal was decreased in knockout mice lacking the gene encoding pre-proenkephalin and in double knockout mice deficient in μ and δ opioid receptors [54]. Both opioid and cannabinoid withdrawal syndromes have been associated with compensatory changes in the cAMP pathway. Thus, enhanced activity of several components of the cAMP pathways has been reported during opioid and cannabinoid abstinence, although different brain structures are involved in these compensatory mechanisms [4,54]. Changes in the cAMP pathway occur mainly in the locus coeruleus and some limbic structures, such as the NAc, during opioid withdrawal, whereas these alterations were selectively located in the cerebellum in the case of cannabinoid withdrawal [4,54]. Changes to the mitogen-activated protein (MAP) kinases cascade seem to be another common compensatory modification during the development of opioid and cannabinoid physical dependence [57]. Therefore, the endocannabinoid system is crucial not only in opioid-induced rewarding effects, but also in development of physical dependence during chronic opioid administration. The existence of bidirectional interactions between the endogenous cannabinoid and opioid systems provides neurobiological support for this role of the endocannabinoid system.

Box 2. Technical terms

Breaking points: the maximal numbers of operant responses that the animal achieves in order to obtain an injection of the drug.

Fixed ratio (FR) schedule: a FR schedule of drug self-administration requires a fixed number of operant responses to obtain a drug injection. Such schedules are used mainly to evaluate the acquisition and maintenance of drug self-administration.

Progressive ratio (PR) schedule: in a PR schedule of drug self-administration, the response requirement to earn a drug injection escalates progressively during the session. This provides information about the reinforcing strength of the drug.

Endocannabinoid system and psychostimulant addiction

The mechanism of action of psychostimulants differs from that of other drugs of abuse in that they affect the mesolimbic dopaminergic terminals directly. Indeed, psychostimulants enhance activity of dopaminergic neurons by directly acting on the reuptake of monoamines, binding to one or multiple monoamine transporters [58]. This mechanism is important for understanding the particular involvement of the endocannabinoid system in psychostimulant rewarding effects. Several behavioural responses induced by acute and chronic administration of psychostimulants were not modified in CB_1 knockout mice (Table 1). Interestingly, cocaine-induced conditioned place preference and locomotor behavioural sensitization were not modified in these mice [45]. These knockout mice also learned to self-administer cocaine and amphetamine when using an acute paradigm in restrained animals [23], and rimonabant did not interfere with cocaine self-administration in rats [48] or monkeys [59] trained under FR schedules of reinforcement (Table 2). These results indicate that CB_1 receptors are not involved in the primary reinforcing effects of psychostimulants. By contrast, rimonabant decreased the acquisition but not the expression of conditioned place preference to cocaine [60], whereas the CB_1 antagonist AM-251 (Box 1) decreased methamphetamine self-administration under a FR schedule in rats [61]. In addition, THC and cannabidiol facilitated the extinction of place preference induced by cocaine and amphetamine, although this effect was not reversed by rimonabant [62]. A recent study using

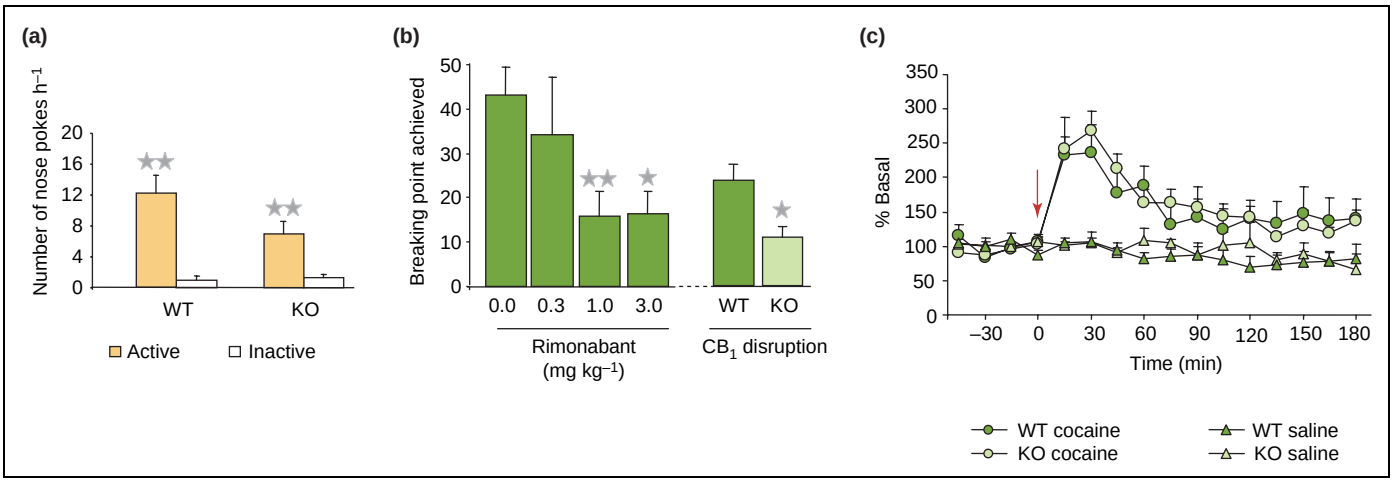

Figure 1. Suppression of CB_1 cannabinoid receptors impairs cocaine self-administration, but does not modify the effects of cocaine on extracellular dopamine levels in the nucleus accumbens (NAc). **(a)** CB_1 knockout (KO) and wild-type (WT) littermates self-administered cocaine (1 mg kg^{-1} per infusion) under a fixed ratio 1 schedule of reinforcement. Bars represent the average of the number of nose pokes into the active and inactive holes required to meet criteria for acquisition of the cocaine self-administration behaviour during three consecutive sessions. **(b)** Effects of rimonabant (0.3, 1.0 and 3.0 mg kg^{-1}, intraperitoneal administration) and genetic disruption of CB_1 receptors on the breaking points achieved under a progressive ratio schedule of reinforcement (Box 2). **(c)** Acute cocaine administration (20 mg kg^{-1}, intraperitoneal administration) enhanced extracellular dopamine levels in the NAc similarly in wild-type and CB_1 knockout mice. Effects on dopamine dialysate were determined by *in vivo* microdialysis from the NAc of wild-type and CB_1 knockout mice. The arrow indicates cocaine or saline administration at time 0. Values are expressed as mean ±SEM. One star indicates $P < 0.05$; two stars indicate $P < 0.01$. Adapted, with permission, from [63].

CB_1 knockout mice has provided new insights on these mechanisms [63]. Indeed, the acquisition of an operant response to self-administer cocaine was impaired in these mutants mainly when the effort required to obtain a cocaine infusion was enhanced. Thus, the breaking point achieved on a PR schedule of reinforcement was significantly reduced in CB_1 knockout mice, whereas self-administration behaviour was only slightly attenuated on a FR1 schedule (Figure 1). A similar result was obtained on the PR schedule after the blockade of these receptors using rimonabant in wild-type mice [63] (Figure 1). This impairment in cocaine self-administration indicates a decreased motivation for maintaining cocaine-seeking behaviour, providing a role for CB_1 receptors in consolidation of the psychostimulant addictive process. Furthermore, CB_1 receptors are also required to reinstate cocaine self-administration. Thus, the cannabinoid agonist HU-210 induced relapse to cocaine seeking after a prolonged withdrawal period, whereas rimonabant attenuated relapse induced by environmental cocaine-associated cues or cocaine re-exposure [64,65].

The precise mechanisms underlying the modulatory role of the endocannabinoid system on psychostimulant rewarding effects remain to be elucidated. These mechanisms seem to be independent from the activating effects on mesolimbic dopamine-mediated transmission. Thus, the enhancement of extracellular dopamine levels produced by cocaine in the NAc was not modified in CB_1 knockout mice [63] (Figure 1). Activation of the mesolimbic circuitry is essential for psychostimulants to induce feelings of reward, and CB_1 receptors are then not required to obtain the primary reinforcing effects of cocaine. Participation of CB_1 receptors in the motivation to maintain cocaine self-administration should therefore involve other neurochemical systems related to this complex addictive behaviour. Thus, amphetamine releases endocannabinoids in the amygdala to produce LTD by a dopamine-independent mechanism mediated by CB_1 receptors [66], and these endocannabinoids participate in the synaptic plasticity produced by psychostimulants in mesocorticolimbic structures [67]. Hence, although the endocannabinoid system does not participate in the primary reinforcing effects of psychostimulants, it is important for maintaining psychostimulant seeking behaviour, probably by modulating synaptic processes induced by these drugs.

Mechanisms involved in modulation of the rewarding circuitry by endocannabinoids

CB_1 cannabinoid receptors are present in the different regions of the brain reward circuitry, including the VTA and the NAc, and also in several areas projecting to these two structures, such as the prefrontal cortex, central amygdala and hippocampus [68]. Acting as a retrograde messenger, endocannabinoids modulate the glutamatergic excitatory and GABAergic inhibitory synaptic inputs into the VTA and the glutamate transmission in the NAc (Figure 2). Thus, the activation of CB_1 receptors present on axon terminals of GABAergic neurons in the VTA would inhibit GABA transmission, removing this inhibitory input on dopaminergic neurons [15,69]. Glutamate synaptic transmission from neurons of the prefrontal cortex in the VTA and NAc is similarly modulated by the activation of CB_1 receptors [13,70]. The final effect on the modulation of VTA dopaminergic activity by endocannabinoids would depend on the functional balance between these inhibitory GABAergic and excitatory glutamatergic inputs, which are both inhibited by endocannabinoids under different physiological conditions.

The modulatory role of the endocannabinoid system on the primary rewarding effects of drugs of abuse might depend on endocannabinoid release in the VTA [69]. Thus, the endocannabinoid system seems to be involved in the primary rewarding effects of cannabinoids, opioids, nicotine and alcohol because these drugs increase dopaminergic neuron firing rates, thus making

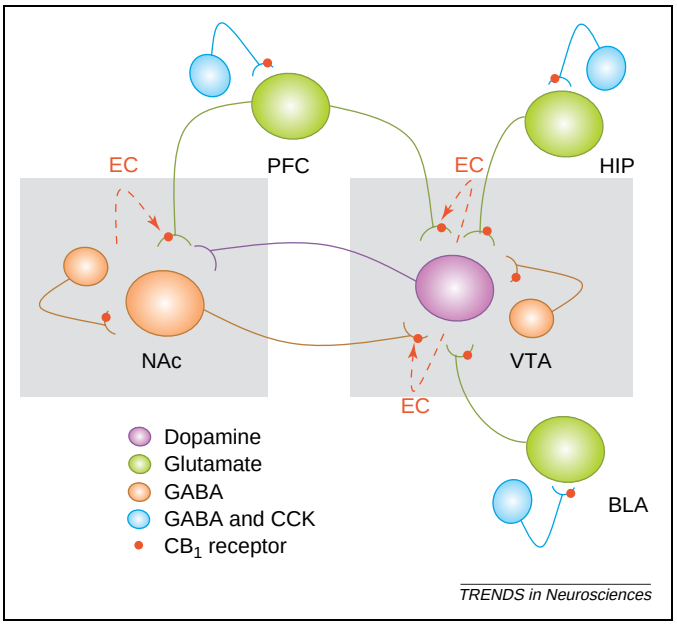

Figure 2. Possible sites of endocannabinoid action in modulation of drug rewarding effects. In the ventral tegmental area (VTA), CB_1 cannabinoid receptors are located on presynaptic glutamatergic and GABAergic neurons. By contrast, VTA dopaminergic neurons do not synthesize CB_1 cannabinoid receptors. Activation of CB_1 receptors in the VTA by endocannabinoids (EC; broken red arrows) produces inhibition of GABA release, thus removing the inhibitory effect of these GABAergic cells on dopaminergic neurons. In addition, the increase of dopaminergic neuron activity induces release from the dopaminergic cells of endocannabinoids that, acting in a retrograde manner on presynaptic CB_1 receptors, inhibit both inhibitory GABAergic and excitatory glutamatergic inputs to VTA dopaminergic neurons. Glutamatergic projections from the basolateral amygdala (BLA) and hippocampus (HIP), which are involved in motivation and memory processes related to drug rewarding effects, are also under the control of CB_1 receptors, through an inhibitory effect on presynaptic inhibitory neurons that release both GABA and cholecystokinin (CCK). In the nucleus accumbens (NAc), endocannabinoids behave as retrograde modulators acting mainly on CB_1 receptors on the axon terminals of glutamatergic neurons. The subsequent inhibition of glutamate release inhibits the GABAergic neurons that originate in the NAc and project to the VTA, thus indirectly activating VTA dopaminergic neurons. Endocannabinoids have also been demonstrated to participate in synaptic plasticity in the NAc. Thus, repetitive activation of prelimbic glutamatergic afferents to the NAc results in long-term depression (LTD) of this excitatory transmission that depends on endocannabinoids and CB_1 receptors [14]. Chronic [77] or even a single [78] THC exposure modifies this form of synaptic plasticity, which is important for the development of the addictive process. Endocannabinoid release in the VTA participates in the modulation of drug rewarding effects [69], which would explain the involvement of CB_1 receptors in the rewarding properties of opiates, ethanol, THC and nicotine. Hence, CB_1 receptors would not participate in the primary rewarding effects of psychostimulants because they essentially act on dopaminergic axon terminals in the NAc. Nevertheless, somatodendritic dopamine release induced by psychostimulants in the VTA could promote endocannabinoid release in this brain area [13]. Finally, CB_1 receptors on the glutamatergic projections from the prefrontal cortex (PFC) would be important to modulate motivation to seek the drug.

possible the release of endocannabinoids in the VTA. However, psychostimulants enhance dopamine levels in the NAc by directly acting on dopaminergic axon terminals. This mechanism of action avoids endocannabinoid release in the VTA and could explain the lack of alteration of primary psychostimulant rewarding effects in the absence of CB_1 receptors [69,71]. In addition, although chronic treatment with THC, nicotine or alcohol increases endocannabinoid content in the limbic forebrain, chronic cocaine reduces 2-arachidonoylglycerol content in these brain structures, indicating that psychostimulants and other drugs of abuse regulate endocannabinoid transmission differently [72]. Similarly, chronic administration of cocaine, but not ethanol or nicotine, decreases mRNA levels for CB_1 receptors in several brain structures [73].

The endocannabinoid system also modulates the motivation to seek psychostimulants and opioids by a mechanism independent from release of dopamine in the NAc. CB_1 receptors are present in the prefrontal cortex, which constitutes a nexus for sensory integration, emotional processing and hedonic experience. This brain area is an important component in the addictive phenomenon because it processes the reward to become a 'hedonic experience' [74]. Hence, endocannabinoids could be involved in the motivation to obtain the drug by linking the reward to a 'hedonic experience' in the prefrontal cortex.

The mechanisms underlying the role of the endocannabinoid system in relapse to drug-seeking behaviour produced by drug-related environmental stimuli and drug re-exposure seem related to modulation of the impact of reward-related memories. Indeed, endocannabinoids acting as retrograde messengers mediate LTP and/or LTD of synaptic transmission in several addiction and memory-related brain areas, including the NAc, prefrontal cortex, amygdala and hippocampus [65]. These effects of endocannabinoids on synaptic plasticity might consolidate the reward-driven behaviour required to establish the addictive processes.

The recent identification of CB_2 receptors in the brain presents an alternative site of action for endocannabinoids [10]. These CB_2 receptors are functionally active because their stimulation, together with CB_1 receptor activation, inhibits morphine-6-glucuronide-induced vomiting at a central level. Therefore, CB_2 receptors are potentially involved in other CNS-mediated effects of cannabinoids that have previously been attributed to CB_1 receptors. Further studies are required to understand the precise role of central CB_2 receptors, and the possible alteration of their physiological activity during drug addictive processes. The possible involvement of other neurochemical circuits in the effects of the endocannabinoid system on reward function cannot be excluded. Thus, endocannabinoids facilitate the effects of orexin-releasing neurons in the hypothalamus, which also project to the NAc and the VTA. Interestingly, hypothalamic orexins, in addition to endocannabinoids, are directly involved in the rewarding effects of drugs of abuse and the relapse to drug-seeking behaviour [75].

Therefore, the endocannabinoid system represents a key component in the common neurobiological substrate of drug addiction, and the CB_1 receptor is a possible candidate to explain genomic variations that might determine human addiction vulnerability [76].

Concluding remarks

The endocannabinoid system participates in the addictive properties of all prototypical drugs of abuse by at least three complementary mechanisms. First, the system is directly involved in the primary rewarding effects of cannabinoids, nicotine, alcohol and opioids by acting on common cellular mechanisms and/or by permitting the effects of these drugs on mesolimbic transmission. Second, the endocannabinoid system is involved in the motivation

to seek the drug by a dopamine-independent mechanism; this has been demonstrated for psychostimulants and opioids and might also be the case for other drugs of abuse. Third, this system is implicated in relapse to drug-seeking behaviour participating in the motivational effects of drug-related environmental stimuli and drug re-exposure, probably by acting on the synaptic plasticity underlying memory processes. Further studies will be required to clarify the precise mechanisms involved in this physiological role of the endocannabinoid system, which has promising clinical consequences. Indeed, CB_1 cannabinoid antagonists might represent a new generation of compounds to treat a wide range of drug addictive processes, as clinical trials have already indicated for smoking cessation. Pharmaceutical companies have now focused the target of these new compounds in the treatment of tobacco dependence and other diseases such as obesity and cardiovascular risk. The possible application of CB_1 antagonists to other addictive processes remains to be demonstrated. Finally, the recent identification of CB_2 receptors in the brain has suggested that they might be a new therapeutic target for treatment of CNS disorders, and possible involvement of these receptors in drug addiction remains open.

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