Role of endocannabinoids in regulating drug dependence

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Abstract: This review will discuss the latest knowledge of how the endocannabinoid system might be involved in treating addiction to the most common illicit drugs. Experimental models are providing increasing evidence for the pharmacological management of endocannabinoid signaling not only to block the direct reinforcing effects of cannabis, opioids, nicotine and ethanol, but also for preventing relapse to the various drugs of abuse, including opioids, cocaine, nicotine, alcohol and metamphetamine. Preclinical and clinical studies suggest that the endocannabinoid system can be manipulated by the CB1 receptor antagonist SR141716A, that might constitute a new generation of compounds for treating addiction across different classes of abused drugs.
Keywords: Endocannabinoids, drug dependence, opioids, nicotine, alcohol, psychostimulants

Introduction

Drug addiction is a chronic relapsing brain disorder, manifested as an intense desire for the drug, with impairment of the ability to control the urges to take the drug, even at the expense of serious adverse consequences (Camì and Farré 2003). These behavioral abnormalities develop gradually with repeated exposure to a drug of abuse, and can persist for months or years after discontinuation of use, suggesting that addiction can be considered a form of drug-induced neural plasticity (Nestler 2004).

Several compounds can lead to addictive behavior including opioids, psychostimulants, cannabinoids, alcohol and nicotine, and although their initial mechanism of action affects different neurochemical targets, the resulting neural dysregulation involves similar neurochemical and neuroanatomical pathways (Hyman and Malenka 2001). The limbic component of basal ganglia pathways, the endogenous opioid system and the brain-pituitary stress system are all essential for the addictive properties of most drugs of abuse, whose interaction with these circuits leads to a common dysregulation of brain motivational and reward pathways (Maldonado et al 2006).

The limbic component of the basal ganglia pathway is a common neuronal substrate for the reinforcing properties of drugs of abuse and drives the motivational, emotional and affective information on behavior (see for review Koob 1992; Di Chiara 1999; Koob et al 2004; Pierce et al 2006). The mesocorticolimbic dopaminergic pathway (comprising dopaminergic neurons in the ventral tegmental area – VTA – innervating the nucleus accumbens – NAc – hippocampus, amygdala, medial prefrontal cortex and ventral pallidum), is a vital factor governing the flow of information through the limbic circuit comprising the interconnected nuclei. Thus dopamine is considered one of the most important actors in the rewarding effects of drugs of abuse, as suggested by the finding that most of the drugs abused by humans raise dopamine levels in the NAc, and blockade of dopamine transmission reduces the rewarding effect of psychostimulants (see for review Pierce and Kumaresan 2006). Moreover, mesolimbic dopaminergic neurons communicate with cerebral areas involved in cognitive functions and dopamine release in the forebrain can be considered a learning signal. In the NAc

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glutamatergic projections from the cerebral cortex, amygdala and hippocampus drive information about external situations and internal emotional and physiological states, thus contributing to addiction by consolidating reward-driven behavior (Hyman and Malenka 2001; Kauer 2004).

The endocannabinoid system and addictive behavior

Besides the importance of the mesocorticolimbic dopaminergic system in addiction, the shared mechanisms in the development of addictive behavior have not yet been fully identified so this review will focus on recent findings pointing towards a role of the endocannabinoid system in the circuitry underlying drug addiction.

Knowledge of the endocannabinoid system has been largely boosted since the CB1 receptor was cloned in 1990 and we now understand that the endocannabinoid system consists of cannabinoid receptors, endogenous ligands and several proteins responsible for their synthesis and degradation (see for review Bisogno et al 2005). Two cannabinoid receptors, CB1 and CB2 have been cloned and characterized, both belonging to the class of G protein-coupled receptors. CB1 has been located in the central nervous system and peripheral tissues and CB2 appeared mainly in the cells of the immune system (Fride and Mechoulam 2003) although it has now also been identified in brainstem, cortex and cerebellum neurons (Van Sickle et al 2005).

The most fully characterized endocannabinoid substances isolated from brain tissue are anandamide (AEA) and 2-arachidonylglycerol (2-AG) (Fride and Mechoulam 2003). Endocannabinoids serve as neuromodulators in many physiological processes and once released from postsynaptic neurons upon depolarization, they activate presynaptic receptors, resulting in inhibition of the release of both excitatory and inhibitory transmitters (see for review Fride 2005). In this capacity the endocannabinoid system may have important additional roles in the regulation of synaptic brain function.

CB1 receptors are abundant in the brain reward circuitry, and the dopaminergic neurons of the mesocorticolimbic pathway are regulated by excitatory and inhibitory inputs influenced by activation of cannabinoid receptors (see for review Gardner 2005). Endocannabinoids released after depolarization in the NAc and from dopaminergic neurons in the VTA may possibly influence GABAergic and glutamatergic afferents by acting as retrograde messengers on CB1 receptors.

Wenger et al (2003) reported the presence of cannabinoid receptors in tyrosine hydroxylase-expressing neurons (most probably dopaminergic neurons) of the NAc, VTA, striatum and pyriform cortex, suggesting the endocannabinoid system might directly influence dopaminergic reward mechanisms. CB1 receptors are present in other areas related to reward and motivation (such as the basolateral amygdala and hippocampus) and endocannabinoids induce long-term depression (LTD) of the inhibitory synapses in the hippocampus, contributing to the synaptic plasticity involved in the learning processes related to addictive behavior (De Vries and Schoffelmeer 2005).

The endocannabinoid system is certainly the primary site of action for cannabinoid addiction and in fact cannabinoids, like other drugs of abuse, induce tolerance and physical dependence and activate a rewarding system (see for review Parolaro et al 2005; Fattore et al 2005; Gonzalez et al 2005). The exact sites and substrates of cannabinoid action in the core VTA-medial forebrain bundle (MFB)-NAc reward axis and on reward-related behaviors are still not clear but there is evidence that cannabinoids enhance brain reward substrates, acting on both dopamine-dependent substrates in the VTA and dopamine-dependent/independent ones in the NAc (Lupica et al 2004).

However, the endocannabinoid system certainly has an overall effect on the reward circuitry and participates in the rewarding and addictive properties of all prototypical drugs of abuse such as opioids, nicotine, alcohol and psychostimulants (cocaine and amphetamine). Animal models of drug reward provide evidence of the endogenous cannabinoids' role in the rewarding effects of several addictive drugs, and pharmacological manipulation of endocannabinoid tone with SR141716A (rimonabant, a specific CB1 receptor antagonist) in humans gave positive results (Anthenelli and Despres 2004).

Two complementary approaches have been used to demonstrate the endocannabinoid system's role in addictive behavior; the first is a genetic approach evaluating changes to the addictive properties of several drugs of abuse in CB1 knockout mice, and the second is a pharmacological approach looking at the effect of SR141716A on drugs' addictive properties. Research in this field has also gained from the use of validated experimental models for the subjective effects of drugs (drug discrimination), their rewarding/reinforcing properties (intravenous self-administration, conditioned place preference – CPP – and intracranial self-stimulation), the influence of environmental factors on drug-seeking behavior (CPP, second-order schedules of self-administration, reinstatement of extinguished drug-seeking behavior and other relapse models), and the withdrawal states associated

with abrupt termination of drug action (administration of a selective antagonist after chronic exposure).

The endocannabinoid system in opioid addiction

Opioids and cannabinoids have several similar pharmacological effects, including analgesia and stimulation of brain circuitry, that are believed to underlie drug addiction and reward. There is ample evidence of a role for the endocannabinoid system in opioid dependence (Table 1). Cannabinoids attenuated morphine and methadone withdrawal signs (Hine et al 1975; Deikel and Carder 1976; Vela et al 1995; Yamaguchi et al 2001; Del Arco et al 2002) and the cannabinoid antagonist SR141716A precipitated abstinence in morphine-dependent rats (Navarro et al 1998; Maldonado 2002). The severity of naloxone-precipitated morphine withdrawal was robustly attenuated in CB1 ko mice (Ledent et al 1999; Lichtman et al 2001) and long-term treatment with SR141716A reduced the intensity of naloxone-precipitated opioid withdrawal (Rubino et al 2000; Mas Nieto et al 2001); however an acute dose of SR141716A just before naloxone did not affect the incidence of withdrawal signs (Mas-Nieto et al 2001).

Thus it appears that chronic blockade of CB1 receptors is needed to alleviate the main signs of morphine abstinence, suggesting that chronic treatment with SR141716A might be useful in the opiate withdrawal syndrome. In addition

changes in CB1 receptor function (in terms of receptor binding and coupling with G protein) and/or in endocannabinoid levels were observed in specific brain areas of morphine-dependent animals, although the results ranged from no change to a decrease or even an increase (Romero et al 1998; Gonzalez et al 2003; Viganò et al 2005).

The opioid antagonist naloxone precipitated abstinence symptoms in rats tolerant to Δ^9-tetrahydrocannabinol (Δ^9-THC) (Kaymakcalan et al 1977) and SR141716A-precipitated withdrawal was dose-dependently reduced by morphine (Lichtman et al 2001). The somatic expression of cannabinoid withdrawal was attenuated in mice lacking pre-pro-enkephalin or mu opioid receptor genes (Valverde et al 2000; Lichtman et al 2001) whereas the deletion of mu, kappa and delta opioid receptors did not affect cannabinoid withdrawal (Ghozland et al 2002). In contrast, in double ko mice deficient in mu and delta opioid receptors, cannabinoid withdrawal was significantly reduced (Castane et al 2003), suggesting that a cooperative action of both receptors was required for the expression of Δ^9-THC dependence.

The traditional animal paradigm of drug dependence further confirmed the importance of the endocannabinoid system in opioid addiction. Manzanedo et al (2004) reported that pre-exposure to the synthetic cannabinoid agonist WIN55212-2 increased the rewarding effect of morphine evaluated in a place preference paradigm, supporting the idea

Table 1 Cannabinoid system in opioid addiction

	Drug	**Model**	**Effect**	**Refs**
CB1 agonist	Morphine, Methadone	Withdrawal	Attenuation	Hine 1975; Deikel and Carder1976; Vela 1995; Ymaguchi 2001; Del Arco 2002
	Morphine	CPP	Increase	Manzanedo 2004
	Heroin	SA	Increase	Solinas 2005
		SA (relapse)	Reinstatement	De Vries 2003; Fattore 2003
	Morphine	Withdrawal	Precipitated abstinence	Navarro 1998; Maldonado 2002
			Attenuation, no change	Rubino 2000; MasNieto 2001
CB1 antagonist	Morphine, heroin	CPP, SA	Attenuation	Chaperon 1999; Braida 2001; Navarro 2001; MasNieto 2001; Caille and Parsons 2003; De Vries 2003; Fattore 2003, 2005; Navarro 2004; Cohen 2002; Solinas 2003; Maldonado 2006
	Heroin	SA (relapse)	Reinstatement attenuation	De Vries 2003; Fattore 2003
CB1 KO	Morphine	Withdrawal	Attenuation	Ledent 1999; Lichtman 2001
		CPP, SA	Suppression, attenuation, no change	Ledent 1999; Cossu 2001 Martin 2000; Rice 2002

that using cannabis might make an individual more vulnerable to opiate addiction. More recently, Goldberg's group (Solinas et al 2005) reported that the reinforcing efficacy of heroin, measured in a progressive ratio schedule of i.v. heroin self-administration, was significantly enhanced by Δ⁹-THC and WIN55212-2 but not by compounds that raise the levels of endocannabinoids by blocking their uptake or metabolism; this suggested that the interaction between cannabinoids and opioids might be mediated by their receptors and signaling pathways rather than by opioid-induced release of endogenous cannabinoids.

In contrast, approaches involving the CB1 receptor antagonist SR141716A suggested the endocannabinoids had a facilitating effect on opioid reinforcement that was unmasked by CB1 receptor blockade. SR141716A reduced opioid self-administration and conditioned place preference in rodents (Chaperon et al 1999; Braida et al 2001; Navarro et al 2001, 2004; Mas Nieto et al 2001; Caille and Parsons 2003; De Vries et al 2003; Fattore et al 2003; see for review Fattore et al 2005 and Maldonado et al 2006). SR141716A had more effect on heroin self-administration when more effort was required to obtain a heroin infusion; for example it was efficacious under a progressive ratio schedule of reinforcement (high price of drug), weaker under a fixed ratio schedule 5 (low price of drug) and null under a fixed ratio schedule 1 (very low price) (Solinas et al 2003).

These results strengthen the idea that SR141716A attenuates the reinforcing effects of heroin and provide support for the potential efficacy of cannabinoid CB1 antagonists in the prevention and treatment of opioid reward. Thus, morphine conditioned place preference (Martin et al 2000) and self-administration (Ledent et al 1999; Cossu et al 2001) were abolished in CB ko mice although the results on morphine CPP in CB1 ko mice tended to vary. Martin et al (2000) found morphine induced CPP in wild-type mice but there was no such response in ko mice, indicating that the drug had no rewarding effects in the absence of CB1 cannabinoid receptors. Rice et al in 2002, reported that CB1 receptor ko mice developed a strong place preference to morphine, similar to that in wild-type Swiss-Webster mice; this indicated that the brain cannabinoid system made no contribution to morphine reward.

One explanation of these conflicting results might be found in the slightly more intensive conditioning paradigm and differences in the conditioning chambers used in the last study.

In summary, the cannabinoid system plays a permissive role in opioid motivational and rewarding properties. The rewarding effects of Δ⁹-THC were suppressed in opioid-receptor ko mice (Ghozland et al 2002; Castane et al 2003) and attenuated by opioid antagonists (Braida et al 2001; Justinova et al 2004).

It is interesting that SR141716A did not modify the dopamine releasing effect of heroin in the NAc (Tanda and Di Chiara 1997; Caille and Parsons 2003). Caille and Parsons (2006) have subsequently focused on an additional substrate in opiate reward, namely the ventromedial pallidum (VP), a cerebral area that receives dense GABAergic input from the NAc. Opiates strongly reduced VP GABA efflux and the resulting disinhibition of the VP is thought to contribute to the positive reinforcing effects of opiates (Caille and Parsons 2004). SR141716A caused a dose-dependent blockade of the effect of morphine on VP GABA efflux without influencing morphine's dopamine-releasing effect. However, SR14716A did not alter cocaine self-administration, or cocaine-induced decrements in VP GABA efflux and increases in NAc dopamine. This is consistent with evidence that selective inactivation of CB1 receptors reduces opiate-, but not psychostimulant-maintained self-administration. However, the CB1 receptor agonist WIN55212-2 reduced VP GABA efflux in a manner similar to morphine, and this effect was reversed by the opiate receptor antagonist naloxone. These results point to an interaction between cannabinoids and opioids in their effects on VP activity, and suggest that SR141716A attenuates opiate reward by reducing the opiates' inhibitory influence on NAc medium spiny neurons.

Finally, endocannabinoid tone seems to have a particularly interesting role in relapse to opiate abuse, especially in humans. Detoxification from opiate addiction has been a medical problem for as long as opiates have been abused, as relapses occur even after long drug-free periods. The endocannabinoid system almost certainly plays a part in triggering or preventing reinstatement of drug-seeking behavior (see for review Fattore et al 2006).

The three synthetic CB1 receptor agonists WIN55212-2, CP55940 and HU210 promptly reinstate heroin-seeking after a long drug-free period (De Vries et al 2003; Fattore et al 2003). Rimonabant, however, attenuates heroin-induced reinstatement of heroin-seeking behavior (De Vries et al 2003; Fattore et al 2003) suggesting that CB1 receptor blockade alters heroin's reinforcing consequences.

Further supporting the notion of a close reciprocal relationship between cannabinoid and opioid systems in relapse, blockade of opioid receptors by naloxone prevented relapse to cannabinoids (Spano et al 2004) and a priming injection

of heroin reinstated cannabinoid-seeking behavior after three weeks of extinction. Interestingly, SR141716A per se did not reinstate responding but did prevent cannabinoid-seeking behavior triggered by heroin (Spano et al 2004).

The endocannabinoid system in nicotine addiction

There appears to be a functional interaction between the endogenous cannabinoid system and nicotine addiction (Table 2). In the mouse CPP paradigm, co-administration of sub-threshold doses of Δ^9-THC and nicotine induced rewarding effects (Valjent et al 2002). Acute Δ^9-THC significantly lowered the incidence of several precipitated nicotine withdrawal signs and improved the aversive motivational

consequences of nicotine withdrawal in mice (Balerio et al 2004). These findings suggest that each drug enhances the rewarding effect of the other, and that cannabis might be used to reduce aversive reactions resulting from nicotine withdrawal. In contrast, Valjent et al (2002) showed an enhancement in the somatic expression of withdrawal in animals co-treated with nicotine and Δ^9-THC, suggesting an asymmetric relationship between the two compounds. Nicotine had no rewarding effect in CB1 ko mice in a place preference paradigm (Castane et al 2002). By contrast, Cossu et al (2001) reported that the absence of CB1 receptors did not affect nicotine self-administration. Methodological aspects such as the specific paradigm and the dose used may partly explain this discrepancy.

Table 2 Cannabinoid system in nicotine and alcohol addiction

Nicotine	Model	Effect	Refs
CB1 agonist	Withdrawal	Attenuation, increase	Balerio 2004; Valjent 2002
CB1 antagonist	CPP	Increase	Valjent 2002
	CPP	Attenuation	Le Foll and Goldberg 2004
	CPP	Suppression short-term expression	Forget 2005
	SA	Attenuation	Cohen 2002
	SA (relapse)	Attenuation nicotine-associated cues	Cohen 2005
	STRATUS-US trial	Increase quit rate in humans	Anthenelli and Despres 2004
CB1 KO	CPP	Suppression	Castane 2002
	SA	No change	Cossu 2001
Alcohol			
CB1 agonist	Voluntary consumption	Increase intake in alcohol-preferring rats and mice	Colombo 2002; Wang 2003
		Increase break point in rats (beer)	Gallate 1999
	Voluntary consumption	Prevents acquisition of drinking behaviour in alcohol-preferring rats	Serra 2001; Gessa 2005
		Attenuation of ethanol consumption in mice, in alcohol-preferring rats and Wistar rats	Arnone 1997; Colombo 1998; Lallemand 2001; Gessa 2005; Cippitelli 2005;
CB1 antagonist	SA	Reduction in unselected rats	Gallate and Mc Gregor 1999; Freedland 2001; Colombo 2004
	SA (relapse)	Suppression of alcohol deprivation effect	Serra 2002
CB1 KO	Withdrawal	Increase	Naassila 2004
	Voluntary consumption	Attenuation	Naassila 2004
FAAH KO, FAAH inhibitor	SA, Voluntary consumption	Increase motivation to drink	Basavarajappa 2006; Hansson 2006

The second approach used to demonstrate the endocannabinoid system's role in nicotine addiction involves SR141716A. Pretreatment with this specific CB1 receptor antagonist reduced nicotine self-administration, nicotine-seeking behavior induced by conditioned cues in rats (Le Foll and Goldberg 2004; Cohen et al 2005), nicotine-induced dopamine release in the NAc (Cohen et al 2002) and the dopaminergic component of nicotine discrimination (Cohen et al 2002).

To conclude, since dopamine release in the NAc is thought to play a major role in the positive reinforcement of nicotine, and although findings in CB1 receptor ko mice are inconsistent, SR141716A may well have some antismoking activity, by reducing the rewarding/reinforcing effect of nicotine.

Some recent papers have looked at the utility of SR141716A for reducing cues associated with nicotine-seeking behavior, one of the major causes of relapse in former smokers. Forget et al (2005) showed that SR141716A impaired both the establishment and the short-term expression of CPP induced by nicotine, suggesting that endogenous cannabinoids are implicated in nicotine's motivational effects. Interestingly, rimonabant did not affect the long-term expression of the incentive learning, suggesting other cannabinoid-independent mechanisms are involved (Forget et al 2005). However, Cohen et al (2005) found that rimonabant attenuated the long-term influence of environmental stimuli responsible for relapse in nicotine-seeking behavior. After demonstrating the persistence of conditioned behavior in response to nicotine-related cues several weeks after nicotine withdrawal, Cohen et al showed that rimonabant could reduce the responding maintained by these cues three months after stopping smoking. Thus rimonabant may not only help stop people smoking but may also help them remain abstinent.

Biochemical investigations found altered levels of AEA and 2-AG in several brain areas in animals chronically treated with nicotine. AEA was elevated in the limbic area and brainstem, and 2-AG in the brainstem, but one or both were reduced in other regions such as hippocampus, striatum and cerebral cortex. CB1 receptor levels were not altered in any of these brain areas (Gonzalez et al 2002; Balerio et al 2004).

To summarize, the pharmacological and cellular studies described indicate that the endogenous cannabinoid system has a specific role in nicotine responses related to its addictive behavior and open up new possibilities for the treatment of this major public health disorder.

There are already some preliminary data from the STRATUS-US trial (smoking cessation in smokers motivated to quit) on the efficacy of rimonabant in humans (Anthenelli and Despres 2004). This clinical trial enrolled 787 smokers in the United States. The participants were randomized to rimonabant 5 mg or 20 mg, or placebo. The study lasted ten weeks and the smokers were allowed to smoke during the first two weeks but were asked to abstain after this. The quitting rate in the 2-mg rimonabant group was double that with placebo and they showed markedly less weight gain during the ten-week study period (Anthenelli and Despres 2004).

The endocannabinoid system in alcohol addiction

Recent studies suggest that the endocannabinoid system has a major part among the neurotransmitter systems involved in regulating different alcohol-related phenomena, including tolerance, vulnerability, reinforcement, consumption and metabolism (Table 2). Thus acute administration of synthetic or endogenous cannabinoid agonists stimulated alcohol intake in Sardinian alcohol-preferring rats (Colombo et al 2002) and C57BL/6J mice (Wang et al 2003), and dose-dependently increased break points (an indicator of motivation to drink alcohol) for beer in Wistar rats (Gallate et al 1999). All these effects were completely prevented by pre-treatment with the cannabinoid antagonist SR141716A. Furthermore, genetic deletion of the FAAH enzyme (Basavarajappa et al 2006) or an injection of URB597 (an irreversible FAAH inhibitor) into the prefrontal cortex enhanced motivation to drink alcohol (Hansson et al 2006), pointing clearly to a role for the endocannabinoid system in alcohol addiction. It is in fact now established that SR141716A, given alone, has effects on alcohol-related behavior opposite to those of the CB1 receptor agonists. For example, SR141716A prevented the acquisition of alcohol drinking behavior in alcohol-naïve Sardinian alcohol-preferring rats with a free choice between alcohol (10%, v/v) and water (Serra et al 2001). It also reduced ethanol consumption in C57Bl/6 mice at doses only marginally affecting regular chow intake or water drinking; this suggests that the endogenous cannabinoid system may affect the appetitive value of ethanol (Arnone et al 1997).

Similar reductions in voluntary alcohol intake were obtained in Sardinian alcohol-preferring rats (Colombo et al 1998), and Wistar rats (Lallemand et al 2001) after chronic alcoholization (a model of the "maintenance" phase of human alcoholism). In the Sardinian alcohol-preferring rats the CB1 receptor antagonist completely abolished the effect of alcohol deprivation (ie, the temporary increase in alcohol intake after a period of withdrawal, a model for relapse episodes in human alcoholics). This suggested that the cannabinoid CB1

receptor might be part of the neural substrate of the alcohol deprivation effect and that SR141716A may have anti-relapse properties (Serra et al 2002).

Finally, SR141716A reduced oral self-administration and attenuated the appetitive properties of alcohol in unselected rats under operant procedures (Gallate and McGregor 1999; Freedland et al 2001; Colombo et al 2004). These data were confirmed by Gessa et al (2005) using a second CB1 receptor antagonist, the newly synthesized SR147778, which suppressed acquisition and maintenance of alcohol drinking, relapse-like drinking and motivation to consume alcohol in Sardinian alcohol-preferring rats. Cippitelli et al (2005) provided clear evidence that blockade of CB1 receptors reduced both ethanol self-administration and conditioned reinstatement of alcohol-seeking behavior in Marchigian-Sardinian alcohol-preferring rats and Wistar rats, the genetically selected rats showing higher sensitivity to rimonabant. These researchers also reported that at least in the strain of rats bred for its ethanol preference, the Marchigian-Sardinian alcohol-preferring rats, CB1 cannabinoid receptor mRNA expression was increased in brain areas relevant for processing reward and reward-associated behavior, suggesting that alterations in the function of the CB1 receptor system may be linked to genetic vulnerability to alcohol misuse (Cippitelli et al 2005).

All these results further reinforce the concept that pharmacological blockade of the CB1 receptor may offer a novel approach to the treatment of alcoholism, not only for consumption but also for context-induced relapses to alcohol, one of the main problems in the treatment of this disorder.

The CB1 receptor signaling system's participation in alcohol drinking and alcohol sensitivity was confirmed using CB1 receptor ko mice. CB1 -/- mice with CD1 background showed lower ethanol intake and less preference, effects associated with dramatic sensitivity to the hypothermic and hypolocomotor effects in response to low doses of ethanol (Naassila et al 2004). These mice also had more severe withdrawal-induced convulsions. Since previous studies in rodents have suggested that high levels of ethanol drinking are often associated with resistance to its intoxicant effects (Schuckit 1994; Kurtz et al 1996), the lower ethanol consumption in CB1 ko mice might be due to their greater sensitivity to ethanol's acute effects.

Chronic in vivo (Gonzalez et al 2002) or in vitro (Basavarajappa and Hungund, 1999a; Basavarajappa et al 2003) ethanol exposure increased accumulation of AEA. This was inhibited by pertussis toxin and the CB1 receptor antagonist SR141716A, and paralleled by the activation of Ca^{2+}-dependent and arachidonic acid-specific phospholipase A2, a key enzyme in the formation of endocannabinoids in neuronal cells and brain (Basavarajappa et al 1997, 1998). The mechanism by which chronic ethanol exposure increases the endocannabinoid content remains to be established, though Basavarajappa et al (2003) have reported that in cerebellar granular neurons chronic exposure to alcohol led to an increase in extracellular AEA by inhibiting its uptake. This event was CB1 receptor-independent since it also occurred in CB1 ko mice and cannabinoid CB1 receptor antagonists did not alter the effects of chronic ethanol on AEA transport. Moreover, in rodents chronic exposure to alcohol impairs CB1 receptor function, lowering the levels of CB1 receptor binding, expression and CB1 receptor/G protein coupling (Basavarajappa and Hungund 1999b; Ortiz et al 2004). This might be due to overstimulation of receptors through increased endocannabinoid synthesis. These studies suggest that during the development of alcohol tolerance there are changes in the steady state in the endogenous cannabinoid system, and this might explain the altered response to alcohol.

These converging findings suggest that cannabinoid CB1 receptor blockade may be an effective therapeutic approach for alcohol dependence in humans but information on the clinical efficacy of rimonabant is still lacking.

The endocannabinoid system in addiction to psychostimulants

Psychostimulants differ from the drugs of abuse since they affect mesolimbic dopaminergic terminals directly, raising dopamine levels in the NAc by direct action on dopaminergic axon terminals; the other drugs seem to induce rewarding effects by increasing dopaminergic neuron firing rates, acting in the VTA, possibly through the release of endocannabinoids (Lupica and Riegel 2005).

This may help us understand why endocannabinoids are not involved in cocaine's or amphetamine's primary reinforcing effects. In fact, there are several reports (Table 3) that genetic deletion or pharmacological blockade of CB1 receptors does not alter cocaine or amphetamine self-administration (Cossu et al 2001; De Vries et al 2001; Braida et al 2005; Lesscher et al 2005) or the neurochemical correlate of this behavior, namely dopamine release in the NAc (Cossu, unpublished results, Soria et al 2005). Similarly, cocaine-induced CPP was not modified in CB1 ko mice (Martin et al 2000; Houchi et al 2005). These results clearly indicate that CB1 receptors are probably not involved in the primary reinforcing effects of psychostimulants.

Table 3 Cannabinoid system in psychostimulant addiction

	Drug	Model	Effect	Refs
CB1 agonist	Cocaine	CPP	Decrease	Fattore 1999
	Amphetamine	SA	Decrease	Braida and Sala 2002
	Cocaine	SA (relapse)	Increase	De Vries 2001
CB1 antagonist	Amphetamine	CPP	Attenuation	Braida 2005
	Cocaine	SA	No change	Lesscher 2005
		SA	Attenuation	Soria 2005
		SA (relapse)	Attenuation	De Vries 2001
	Amphetamine, Cocaine	SA (relapse)	Inhibits reinstatement of reward-seeking behavior	Xi 2006; Anggadiredja 2004
CB1 KO	Cocaine	CPP	No change	Martin 2000; Houchi 2005
		SA	Attenuation	Soria 2005
	Amphetamine, Cocaine	SA	No change	Cossu 2001

Although acute reinforcing properties are essential for the establishment of drug addiction, other complex behavioral processes are involved in consolidating this chronic relapsing disorder (Koob and LeMoal 2001), so the acute reinforcing effects of the drug are only the first step in the acquisition of stable operant self-administration responding. Besides the mesolimbic dopaminergic system, particularly the NAc, it has been proposed that dopamine-independent neuronal circuits are involved in regulating reward-related processes (Lupica et al 2004). CB1 cannabinoid receptors are highly expressed in other brain regions involved in the rewarding circuitry such as the basolateral amygdala, medial prefrontal cortex, and hippocampus, so mechanisms involving CB1 receptors in these structures might well participate in other aspects such as consolidation and relapse of cocaine-seeking behavior. In line with this hypothesis, Soria et al (2005) found that only 25% of CB1 ko mice, compared with 75% of the wild-type, acquired reliable operant responding to self-administer the most effective dose of cocaine (1 mg/kg/infusion), and needed more sessions to attain this behavior. Moreover, the maximal effort to obtain a cocaine infusion was significantly lower after genetic ablation of CB1 receptors, indicating decreased motivation for maintaining cocaine-seeking behavior. Results were similar after pharmacological blockade of CB1 receptors with SR141716A in wild-type litter mates (Soria et al 2005). The lack of motivation for cocaine in CB1 ko or SR141716A-pretreated mice might be due to impaired detection, association, and representation of the reward signal or to inadequate responding to the rewarding stimuli (Soria et al 2005).

In line with the idea of CB1 receptors involved in these other aspects of reward, Fattore et al (1999) found that WIN 55212-2 reduced intravenous cocaine self-administration, suggesting that stimulation of CB1 receptors may potentiate cocaine's reinforcing effects. Similar findings were reported with amphetamine (Braida and Sala 2002): the combination of CP-55,940 with the maximal reinforcing unit dose of 3,4-methylendioxymethamphetamine (MDMA) significantly lowered the mean number of drug-associated lever pressings in comparison with the drug alone, suggesting a synergistic action of cannabinoid agonists on MDMA's reinforcing properties.

Finally, CB1 receptors play an important role in relapse to psychostimulants. De Vries et al (2001) found that the cannabinoid agonist HU210 provoked relapse to drug-seeking in animals after prolonged withdrawal from cocaine self-administration, whereas blockade of the CB1 receptor by SR141716A attenuated the relapse induced by re-exposure to cocaine-associated cues or the drug itself. Xi et al (2006) have now shown that the CB1 antagonist AM251, administered systemically, selectively inhibited cocaine-induced reinstatement of reward-seeking behavior by a glutamate-dependent mechanism. CB1 receptor-mediated disinhibition of NAc glutamate release could activate presynaptic mGluR2/3 receptors, which then inhibited cocaine-enhanced NAc glutamate release and cocaine-triggered reinstatement of drug-seeking behavior (Xi et al 2006). Along the same lines, SR141716A blocked the reinstatement of methamphetamine-seeking behavior in rats (Anggadidiredja et al 2004).

Given the paucity of effective medications to treat psychostimulant addiction, and although the endocannabinoid system does not participate in the primary reinforcing effects of this class of drugs, CB1 receptor antagonists may offer hope for treating consolidated psychostimulant-seeking behavior and relapse.

Conclusions and future directions

In the last 25 years the neurobiological and behavioral mechanisms that lead to drug dependence have been extensively investigated but clinical treatment is still unsatisfactory and ineffective in many subjects.

Experimental models are now providing evidence for the pharmacological management of endocannabinoid signaling not only to block the direct reinforcing effects of cannabis, opioids, nicotine and ethanol, but also to prevent relapse to these various substances of abuse, also including cocaine and metamphetamine. The endocannabinoid system can be manipulated by SR141716A and by all the new compounds that protect AEA and 2-AG from deactivation and prolong the lifespan of these endocannabinoid substances in vivo. Rimonabant reduces the motivational effect of drug-related stimuli and drug re-exposure, probably by altering synaptic plasticity, thus providing an effective means of preventing relapse and a new tool for the treatment of drug abuse.

Although further studies are needed to clarify the precise mechanism underlying the endocannabinoid system's role in addiction, some promising clinical findings have now been presented (eg, smoking cessation). A new question has now arisen from the discovery of CB2 receptors in the brain: are they involved in drug addiction?

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