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Endocannabinoid system and drug addiction: new insights from mutant mice approaches

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The involvement of the endocannabinoid system in drug addiction was initially studied by the use of compounds with different affinities for each cannabinoid receptor or for the proteins involved in endocannabinoids inactivation. The generation of genetically modified mice with selective mutations in these endocannabinoid system components has now provided important advances in establishing their specific contribution to drug addiction. These genetic tools have identified the particular interest of CB_1 cannabinoid receptor and endogenous anandamide as potential targets for drug addiction treatment. Novel genetic tools will allow determining if the modulation of CB_2 cannabinoid receptor activity and 2-arachidonoylglycerol tone can also have an important therapeutic relevance for drug addiction.

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Introduction

Drug addiction is a chronic brain disease induced by repeated drug consumption leading to compulsive drug seeking, loss of control over drug use despite negative consequences, and repeated relapse. All drugs of abuse produce similar changes in specific brain pathways, including the reward circuits, which constitute the common neurobiological substrate for this brain disease. The endocannabinoid system has recently emerged as a crucial component of this common circuitry underlying drug addiction [1].

The endocannabinoid system consists of cannabinoid receptors, their endogenous ligands, and the enzymes involved in the synthesis and degradation of these endocannabinoids [2]. Two subtypes of cannabinoid receptors, CB_1 (CB_1R) and CB_2 (CB_2R), have been characterized and cloned, although compelling evidence supports the existence of other receptors that bind cannabinoid ligands, such as GPR55. Both CB_1R and CB_2R are G protein-coupled receptors with quite different distributions in the central nervous system (CNS) and peripheral tissues [2]. CB_1R is highly expressed in the CNS, while CB_2R is mainly localized in immune cells, although it is also expressed in brain neurons [3].

The most relevant endogenous ligands for cannabinoid receptors are *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG). Additional endogenous molecules that bind to the cannabinoid receptors have been identified, although some of them may be artifacts [2]. These endocannabinoids are synthesized on demand, mainly postsynaptically and act as retrograde messengers regulating the presynaptic release of neurotransmitters [4]. Whether both endocannabinoids, or only 2-AG, act as retrograde synaptic messengers remains to be clarified. Anandamide and 2-AG are produced from cell membrane lipids via different biosynthetic pathways. Anandamide acts as a partial agonist at both CB_1R and CB_2R, and also binds to the transient receptor potential vanilloid type 1 channel. 2-AG is the most abundant endocannabinoid in the CNS and activates both CB_1R and CB_2R [5]. Cannabinoid receptor activation by endocannabinoids is rapidly terminated through carrier-mediated uptake into cells followed by intracellular enzymatic degradation. Anandamide is degraded by the fatty acid amide hydrolase (FAAH), whereas 2-AG is primarily metabolized by monoacylglycerol lipase (MAGL) [4]. The molecular entities that transport anandamide and 2-AG into cells have not been yet identified, although this transport has been characterized pharmacologically [6]. The molecular characterization of proteins involved in these re-uptake processes will allow the future generation of genetically modified animals, which may clarify the relevance of these endocannabinoid inactivation mechanisms. In contrast, the genetically modified mice now available with constitutive or conditional mutations of the cannabinoid receptors (CB_1R and CB_2R) and endocannabinoid degrading enzymes (FAAH and MAGL) have provided important advances for understanding the physiological role of these endocannabinoid components in multiple functions, including drug addiction (Table 1).

Studies on CB_1R genetically modified mice

Genetic approaches have provided clear evidence regarding the involvement of CB_1R in drug addiction. CB_1R is

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Table 1

Genetically modified mice used to study the involvement of the endocannabinoid system in the addictive properties of drugs of abuse

Drug	Mutant mice	Behavioral model	Effect	Reference
Morphine	CB_1R KO	Conditioned place preference	Suppression	[18]
			No change	[19]
		Self-administration in restrained mice	Suppression	[20,11]
		Withdrawal syndrome	Attenuation	[20]
Ethanol	CB_1R KO	Conditioned place preference	Attenuation	[12,13]
		Two-bottle voluntary consumption	Attenuation	[14,15]
			No change	[17]
		Withdrawal syndrome	Suppression	[17]
	FAAH KO	Two-bottle voluntary consumption	Increased	[15,34]
		Withdrawal syndrome	Decreased	[15]
		Acute withdrawal	No change	[34]
Nicotine	CB_1R KO	Conditioned place preference	Suppression	[8,9]
		Self-administration in restrained mice	No change	[11]
		Withdrawal syndrome	No change	[8,9]
	FAAH KO	Conditioned place preference	Increased	[9]
		Withdrawal syndrome	Increased	[9]
Cocaine	CB_1R KO	Conditioned place preference	No change	[18,12]
		Self-administration in restrained mice	No change	[11]
		Self-administration in freely moving mice	Attenuation	[23•]
	CB_2R KO	Self-administration in freely moving mice	No change	[28••]
	CB_2R overexpression	Conditioned place preference	Attenuation	[29•]
		Self-administration in freely moving mice	Attenuation	[29•]
Amphetamine	CB_1R KO	Self-administration in restrained mice	No change	[11]
MDMA	CB_1R KO	Conditioned place preference	No change	[25]
		Self-administration in freely moving mice	Suppression	[25]

the primary site of action for the rewarding and pharmacological responses of cannabinoids, although this receptor plays an overall modulatory effect on the addictive properties of all prototypical drugs of abuse [7]. Thus, CB_1R is involved in nicotine rewarding properties, as revealed by the abolishment of nicotine place preference in CB_1R knockout mice (CB_1KO) [8,9], and the reduction of nicotine self-administration by CB_1R antagonists [10]. In contrast, the acquisition of nicotine self-administration in an acute reinforcement paradigm in mice with restrained mobility was not modified in CB_1 KO [11]. However, this acute paradigm fails to evaluate the maintenance of a stable operant self-administration responding, and the effects could be influenced by the stress induced by this restraint procedure. The influence of CB_1R in nicotine physical dependence is less clear. Thus, although the somatic expression of nicotine withdrawal was not modified in CB_1KO [8,9], the CB_1R antagonist rimonabant ameliorated somatic withdrawal in wild-type mice [9].

CB_1R also regulates ethanol-rewarding properties. Thus, CB_1KO show a reduction of ethanol-induced place preference [12,13] and a decrease in voluntary ethanol intake [14,15], in agreement with pharmacological results using CB_1R antagonists [16]. Stress could participate in the regulation that CB_1R exerts on alcohol consumption since stress-induced increase in ethanol preference is blocked in CB_1KO [17]. CB_1R involvement in alcohol reward seems mediated through the modulation of its effects on the activation of mesolimbic dopamine transmission [14].

CB_1R also participates in opiate reward by modulating dopamine transmission. Thus, CB_1KO do not exhibit morphine place preference [18], although this effect was not observed in a later study [19]. Morphine self-administration was also abolished in CB_1KO [20,11]. In addition, morphine-enhanced extracellular dopamine in the nucleus accumbens (NAc) was attenuated in CB_1KO [21], although this effect was not replicated in the case of heroin when using rimonabant [22]. The severity of morphine withdrawal was also attenuated in CB_1KO [20].

In contrast to other drugs of abuse, psychostimulants enhance NAc dopamine levels by acting directly on dopaminergic terminals and do not require the modulatory role of mesolimbic CB_1R activity. Indeed, cocaine-enhanced NAc dopamine was unaltered in CB_1KO [23•], although another study reported a reduction of this cocaine effect [24]. Cocaine [18,12] and MDMA [25] place preference were preserved in CB_1KO. These knockout mice also learn to self-administer cocaine and amphetamine when using an acute paradigm in restrained

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animals [11]. In spite of these unaltered cocaine reward responses, chronic cocaine self-administration was attenuated in freely moving CB_1KO, and the motivation to obtain a cocaine infusion was dramatically decreased in these mutants using a progressive ratio schedule [23•].

Therefore, CB_1R is involved in the primary rewarding effects and the motivation to seek different drugs of abuse. Conditional knockout mice deficient in CB_1R at GABAergic interneurons or glutamatergic principal neurons are now available [26••]. These mice are promising tools to clarify the circuits mediating the effects of CB_1R on drug addiction.

Studies on CB_2R genetically modified mice

The presence of CB_2R in neurons is still a controversial issue mainly due to the difficulties to obtain reliable antibodies. However, previous pharmacological studies have shown an involvement of CB_2R in the modulation of some central effects of alcohol [27] and opioids [3]. A pivotal role of CB_2R in cocaine rewarding effects has been recently reported. Thus, systemic administration of the CB_2R agonist JWH133 decreased cocaine self-administration in wild-type and CB_1KO, but not in CB_2R knockout mice (CB_2KO) [28••]. Similar responses were revealed after intra-NAc infusion of JWH133 in wild-type, but not in CB_2KO [28••]. Cocaine enhanced locomotion and NAc dopamine levels were also inhibited by JWH133 in wild-type mice, and in CB_1KO, but not in CB_2KO [28••]. In agreement, transgenic mice overexpressing CB_2R in the CNS showed a reduction of cocaine-induced place preference, self-administration and locomotor sensitization [29•]. All these new data reveal the interest of CB_2R pharmacological manipulation for cocaine addiction treatment. Future research will be necessary to investigate the possible mechanisms by which CB_2R modulates cocaine reward, and if this effect could be generalizable to other drugs of abuse. In this respect, nicotine self-administration and reinstatement of nicotine-seeking were not modified by the selective CB_2R agonist AM630 in rats [30].

Studies on FAAH genetically modified mice

The behavioral effects of anandamide can be effectively increased by pharmacological blockade of FAAH or by genetic FAAH deletion [5]. The effects of FAAH disruption on drug addictive properties have been evaluated using genetic approaches. Studies investigating the role of FAAH inhibition on THC dependence report that THC withdrawal was not modified in FAAH knockout mice (FAAHKO), although acute administration of the FAAH inhibitor, URB597 attenuated this withdrawal response [31]. These different results could be attributed to possible compensatory changes after the gene deletion, and the authors concluded that endocannabinoid modulation may be an effective treatment for cannabis withdrawal [31].

The enhancement of the anandamide tone increased sensitivity to nicotine reward. Indeed, FAAHKO exhibited place preference at subthreshold doses of nicotine, and this nicotine response was also augmented by repeated URB597 administration [9]. In addition, nicotine withdrawal was enhanced in FAAHKO, and after acute URB597 administration at high doses [9]. Therefore, anandamide may modulate the positive and negative effects of nicotine in opposite ways, that is, increasing reward and worsening aversion. Pharmacological studies in rats have yielded opposite results to those obtained in mice. Thus, URB597 inhibited nicotine reward, decreased reinstatement of nicotine seeking behavior [32], and reversed nicotine withdrawal-induced anxiety in rats, without modifying the somatic manifestations of nicotine withdrawal [33]. These discrepant results observed in mice and rats hinder a definite conclusion as to the role of anandamide on the behavioral effects of nicotine, and warrant further investigation.

Ethanol rewarding effects were also modified in FAAHKO. These mutants exhibit more preference for ethanol intake [34,15], although another study found this enhancement only in female FAAHKO [35]. These inconsistencies may be due to differences in the genetic background. Pharmacological studies showing that URB597 increases ethanol intake in mice [34,15] confirm that enhancing the anandamide levels facilitates ethanol reward. In terms of ethanol withdrawal, FAAHKO display less intense handling-induced convulsions than control mice following withdrawal from chronic ethanol [15], although no differences were observed in an acute withdrawal paradigm [34].

The effects of increasing anandamide levels on opioid and cocaine addictive properties have only been investigated in pharmacological studies. Thus, pharmacological enhancement of anandamide levels reduces morphine withdrawal [36,37•] and reinstatement of cocaine seeking behavior [38], suggesting that this pharmacological intervention could prevent cocaine craving. Interestingly, URB597, in contrast to anandamide [39] is not self-administered by monkeys, and does not reinstate drug craving in this animal species [40].

Studies on MAGL genetically modified mice

MAGL genetic disruption produces profound changes on the functional activity of the endocannabinoid system, which represents a potential limitation for the use of these genetic tools for pharmacological studies. Indeed, both constitutive MAGL disruption and chronic administration of the MAGL inhibitor JZL184 produce a sustained enhancement of 2-AG levels that leads to cross-tolerance to cannabinoid agonists, desensitization of brain CB_1R, and impaired endocannabinoid-dependent

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synaptic plasticity in mice [31]. This result is in contrast with the absence of adaptive changes on CB_1R function after FAAH disruption [5], and reveals a different role of anandamide and 2-AG in the functional activity of the endocannabinoid system. The use of pharmacological and genetic approaches has confirmed this differential role of anandamide and 2-AG in several behavioral responses [41].

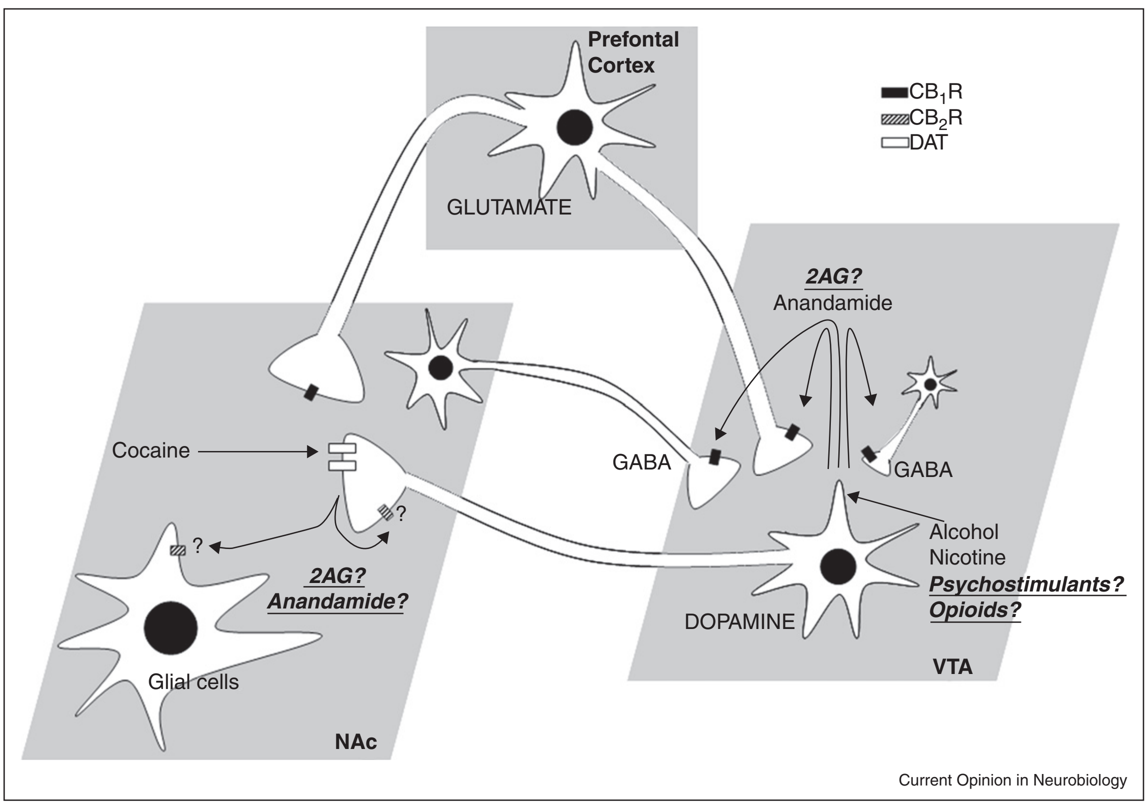
Considering these adaptive changes in the MAGL knockouts, the study of the role of 2-AG in drug addition has only been possible by using pharmacological tools [42]. The administration of JZL184 attenuated THC [31] and morphine abstinence [37•], revealing the potential interest of enhancing 2-AG tone to attenuate these withdrawal manifestations. Although 2-AG is self-administered by monkeys [43] in a similar way to anandamide [39], the possible reinforcing effects of MAGL inhibitors have not been reported yet.

A new genetic approach consisting in the overexpression of MAGL in forebrain neurons has been reported [44••]. These mutants show a significant decrease in forebrain 2-AG levels without compensatory changes in other endocannabinoid components, and have been used to evaluate the role of 2-AG in metabolic control. These transgenic mice now represent excellent tools to investigate the role of 2-AG in drug addiction.

Conclusions

The generation of genetically modified mice with selective mutations in specific components of the endocannabinoid system has provided important advances to identify the contribution of this system in drug addiction. However, several relevant issues have not been yet clarified mainly due to the limitations of the available experimental tools (Figure 1). The crucial role of CB_1R in drug addiction has been identified by using constitutive knockout mice. The recent generation of conditional

Figure 1



Mechanisms involved in the modulation of drug rewarding effects by the endocannabinoid system and remaining open questions based on recent findings using genetically modified mice. The enhancement of anandamide tone facilitates nicotine and alcohol reward as shown in FAAHKO. Nicotine and alcohol could increase dopaminergic neuron firing rates and induce anandamide release in the ventral tegmental area (VTA). Anandamide acts as retrograde messenger on presynaptic CB_1R and inhibits both GABAergic and glutamatergic inputs to VTA dopaminergic neurons. The possible effects of the genetic FAAH deletion in psychostimulant and opioid rewarding properties remain to be elucidated. The role of 2-AG in drug reward by using MAGL genetically modified mice is still unknown. On the other hand, the activation of CB_2R in the NAc decreases cocaine reward in wild-type, but not in CB_2KO. Cocaine enhances dopamine levels in the NAc directly blocking the dopamine transporter (DAT) located on dopaminergic axon terminals. CB_2R localized on astrocytes or microglia could decrease dopamine levels by regulating the release of inflammatory cytokines from these cells. Alternatively, the activation of CB_2R possibly located on dopaminergic terminals could also inhibit dopamine release in the NAc [46].

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knockouts lacking CB_1R in GABAergic interneurons or glutamatergic principal neurons [26••] will now allow to clarify the precise CB_1R circuits involved in drug addiction. A crucial limitation for the study of CB_2R localization and function is the lack of reliable antibodies. Recent studies have revealed that CB_2R in the CNS participate in drug reward. However, numerous questions remain open with regards to the possible mechanisms involved in these responses, particularly if they are mediated by CB_2R expressed in neurons or glial cells. The generation of novel conditional mutants with selective CB_2R deletion in these particular cells will be essential to further advance in this topic.

The finding that anandamide tone modulates the behavioral responses to drugs of abuse has provided an interesting target with potential therapeutic relevance. The attenuation of drug withdrawal manifestations by enhancing 2-AG tone also suggests its potential therapeutic interest. Recent studies have clearly shown a different physiological role of anandamide and 2-AG. Considering the localization of the enzymes involved in endocannabinoid synthesis and degradation [45], and the differential responses of enhancing anandamide and 2-AG tone on memory and anxiety [41], we can speculate that anandamide will have a predominant role in processing cannabinoid receptor activity mostly at GABAergic terminals. Instead, 2-AG processing could be mainly involved in cannabinoid signaling in glutamatergic neurons. Each of these conditions could lead to differential responses on the modulation of drug rewarding properties. Constitutive MAGL disruption produces profound changes on the endocannabinoid system, limiting the use of these mutants. The recent generation of transgenic mice overexpressing MAGL in forebrain neurons without producing these adaptive changes [44••] has opened for the first time the possibility to use genetic tools to evaluate the role of 2-AG in drug addiction.

The new genetic tools recently generated will allow elucidating most of the relevant questions that are still unanswered about the role of the endocannabinoid system in drug addiction, which can open novel therapeutic approaches for this brain disease.

Conflict of interest

All authors report no biomedical financial interest or potential conflict of interest.

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