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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

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