Cannabinoid CB₁ receptors control conditioned drug seeking

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Recent developments have implicated cannabinoid CB₁ receptors as a novel target for a new class of therapeutic agents used to treat drug addiction. CB₁ receptors are expressed in the motivational circuitry of the brain and modulate drug seeking. Blockade of the CB₁ receptor is particularly effective in reducing cue-induced reinstatement of drug seeking, an animal analogue of cue-induced relapse in human addicts. These relapse-preventing properties are observed with different classes of abused drug (i.e. psychostimulants, opiates, nicotine and alcohol). In addition, recent evidence indicates a more general role of CB₁ receptors in reward-related memories, which is consistent with the proposed role of endocannabinoids in memory-related plasticity. Relapse-preventing actions and inhibitory effects on weight gain were confirmed recently in clinical trials with the CB₁ antagonist rimonabant. Collectively, these clinical and preclinical studies suggest that antagonists of CB₁ receptors offer a novel approach in the treatment of addictive behaviours.

Drug addiction and relapse

Drug addiction is characterized by long-lasting motivational disturbances such as compulsive drug seeking and episodes of intense drug craving. The neurobiological mechanisms that underlie the persistence of such behaviour remain poorly understood. Recent advances using animal models of relapse have shown that drug seeking can be triggered by drug-associated (conditioned) cues, stressors (e.g. mild footshocks, food restriction and yohimbine) and re-exposure to the previously experienced drug [1–3], which are all events that are known to trigger drug craving and relapse in human addicts [4–6]. Therefore, stimulus-induced reinstatement of extinguished self-administration behaviour in laboratory animals (Box 1) is widely accepted as an animal model that represents defining characteristics of relapse behaviour in humans [7]. In this review, recent evidence suggesting a role for the cannabinoid system in the modulation of drug-seeking behaviour, as assessed in such models, will be provided and the possible underlying mechanisms will be discussed.

Cannabinoid-mediated modulation of relapse behaviour

The first indication of a modulatory role for cannabinoids in drug seeking came from the observation that the potent cannabinoid receptor agonist HU210 (see Chemical names) was able to reinitiate cocaine seeking following long-term (3 weeks) extinction of intravenous cocaine self-administration in rats (Figure 1a) [8]. This action was completely reversed by the selective cannabinoid CB₁ receptor antagonist SR141716A, which suggested a role for these receptors in cocaine relapse. Indeed, blockade of CB₁ receptors attenuated drug seeking evoked by cocaine-associated cues (Figure 2) and by an intravenous priming injection of cocaine [8]. Similar effects were shown in animals with a history of heroin self-administration: cannabinoid receptor agonists reinstated previously extinguished heroin seeking, whereas SR141716A attenuated heroin seeking provoked by a priming injection of heroin [9,10] or by heroin-associated cues [9].

Recent experiments with methamphetamine, alcohol and nicotine [11,12] in reinstatement models have shown that blockade of CB₁ receptors has a general role in the suppression of cue-induced relapse to drug seeking that is independent of the type of abused drug (Figure 2).

The first clinical trial with SR141716A [Acomplia™ (rimonabant)] appears to confirm the anti-relapse properties of CB₁ receptor antagonists because rates of stopping smoking were doubled in smokers who were motivated to quit and who received SR141716A compared with those who received placebo (http://en.sanofi-aventis.com/press/ppc_23312.asp?ComponentID=23312andSourcePageID=23126#1).

Neuronal mechanisms that underlie cannabinoid-mediated modulation of drug seeking

The mechanisms that underlie the modulatory role of cannabinoids on drug seeking remain to be elucidated. High levels of CB₁ receptors [13] are present in brain regions that are thought to have a key role in relapse-like behaviour and conditioning processes in laboratory animals; these regions include the prefrontal cortex, amygdala, nucleus accumbens, striatum and hippocampus [14–16]. Importantly, recent brain-imaging studies in humans revealed a similar circuitry involved in cue-elicited craving in drug addicts [17–19]. In these brain regions, CB₁ receptor activation modulates the release of a variety of neurotransmitters, including dopamine (DA), GABA and glutamate, all of which have
been implicated in the reinstatement of drug seeking (Figure 3) [7,14,20,21].

A central role for the DA-containing ventral tegmental area (VTA) and its main projection target, the nucleus accumbens, in the incentive–motivational effects of addictive drugs is well established [22,23]. Indeed, the priming effects of opiates on heroin seeking and those of psychostimulants on cocaine seeking can be mimicked by activation of mesolimbic DA-containing neurons. Thus, infusion of morphine into the VTA or of amphetamine...
directly into the nucleus accumbens reinstates lever pressing for heroin or cocaine in rats [21]. CB₁ receptors are present in high numbers on axon terminals of GABA-containing interneurons throughout the CNS. Activation of these receptors inhibits GABA release [24,25], whereas GABA in turn inhibits the activity of DA-containing projection neurons. Indeed, activation of CB₁ receptors at the level of the VTA has been shown to enhance mesolimbic DA release [26]. Therefore, the effects of cannabinoid receptor agonists on heroin and cocaine seeking might result from an enhancement of mesolimbic DA release.

In view of the evidence for a DA–cannabinoid interaction in the striatum [27], activation of endocannabinoid systems at postsynaptic sites from the DA synapse in the striatum might also contribute to drug seeking. Accordingly, DA (via activation of DA D₂-like receptors) can trigger the release of endocannabinoids in the striatum [27]. In this respect, there is general agreement that the central effects of the endocannabinoids anandamide and 2-arachidonloylglycerol are mediated by CB₁ receptors [28,29]. However, it remains to be examined whether the relapse-preventing properties of SR141716A are due to: (i) its action as a competitive antagonist at CB₁ receptors that are tonically activated by released endocannabinoids; or (ii) its action as an inverse agonist at constitutively active CB₁ receptors [30].

Other candidate neuroanatomical substrates are the basolateral amygdala and the medial prefrontal cortex.
Evidence for an important role of the basolateral amygdala in cue-induced reinstatement of both heroin [31] and cocaine [32] seeking has been provided. Interestingly, the circuitry for associative plasticity in the amygdala involves endocannabinoid signaling [33].

Blockade of CB1 receptors does not affect cocaine seeking that is reinstated by exposure to mild footshock stress [8]. This seems to be consistent with several reports suggesting a pharmacological dissociation between stress-induced relapse and cue- or drug-induced relapse [1]. For example, stress-induced reinstatement of heroin seeking is relatively insensitive to systemic application of DA receptor antagonists [1], whereas, as indicated earlier, the DA system is crucially involved in drug-induced reinstatement [20,21]. For example, the DA D1-like receptor antagonist SCH23390, the D2-like receptor antagonist raclopride and the nonselective DA receptor antagonist flupenthixol attenuate heroin-induced reinstatement, whereas only chronic blockade of DA receptors by the mixed DA receptor antagonist flupenthixol attenuates footshock-induced relapse [2]. This might relate to the findings that heroin priming induces a greater release of DA in the nucleus accumbens and a much larger increase in locomotor activity than does footshock under the conditions of the reinstatement experiments [2].

By contrast, the brain corticotrophin-releasing factor (CRF) and noradrenaline systems appear to contribute to stress-induced reinstatement of heroin and cocaine seeking, but not drug- or drug cue-induced reinstatement of heroin and cocaine seeking [1]. This pharmacological dissociation between stress- and drug- or cue-induced relapse is in agreement with anatomical disconnection studies in which local infusion of the GABA receptor agonists muscimol and baclofen were used to inactivate brain regions. These studies revealed that stress-induced, but not cue- or drug-induced, reinstatement of cocaine seeking requires the activation of the central extended amygdala [37].

From a treatment perspective this might imply that, similar to other chronic diseases, it is reasonable to expect that the treatment of drug craving and relapse will involve the use of more than one drug.

**Dissociation between primary reinforcing and secondary reinforcing effects**

SR141716A appears to be particularly effective in relapse conditions where drug seeking is triggered by renewed exposure to drug-associated stimuli or a priming dose of the drug itself. This attenuation of the so-called secondary reinforcing effects of drugs of abuse appears to be independent of cannabinoid-mediated modulation of the primary reinforcing properties of these drugs. There is general agreement that the primary reinforcing effect of cocaine, as measured in the intravenous self-administration procedure, is not altered by blockade or absence of CB1 receptors [8,38,39]. The selective effects of CB1 receptor antagonists on cue-induced relapse to cocaine seeking [i.e. on secondary reinforcement: responding for cocaine-associated stimuli in the absence of cocaine itself (Box 1)], but not on cocaine self-administration behavior is consistent with evidence suggesting that dissociable neuronal mechanisms underlie these behaviors [32,40]. For example, although D1-like receptor agonists are self-administered by laboratory animals, these compounds do not induce reinstatement of cocaine seeking and even attenuate reinstatement induced by cocaine-priming injections [20,41]. In addition, neuroanatomical studies indicate that although the basolateral amygdala is involved in the mediation of the secondary reinforcing effects of conditioned cocaine cues, the integrity of the nucleus accumbens appears to be essential for responding for cocaine itself [15,32].

With respect to the role of CB1 receptors in the primary reinforcing effects of drugs of abuse other than cocaine, a more diverse picture emerges. Heroin self-administration is minimally affected by SR141716A when responding is reinforced under a continuous reinforcement schedule [9,42,43] (i.e. in conditions where little effort is needed to obtain the drug). However, CB1 receptor blockade becomes more effective when a motivational component is added to this behaviour (i.e. when the response requirement

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**Figure 3. Pathways involved in cue- and drug-induced relapse.** The most important neuroanatomical sites and neuronal pathways involved in cue- and drug-induced reinstatement of drug seeking are shown. This overview is based on a variety of studies using (temporary) lesion methods, pharmacological activation or electrical stimulation techniques. The anatomical sites where CB1 receptors exert their modulatory action on drug-seeking behavior are still unknown. All of the depicted brain areas have high levels of cannabinoid receptors and many functional interactions have been reported between CB1 receptors and the DA, GABA and glutamate systems shown here. For example, CB1 receptor activation enhances the release of DA from ventral tegmental area (VTA)-originating neurons by disinhibition of GABA-containing interneurons in this area. At the level of the nucleus accumbens (NAc), the release of glutamate from neurons originating in several cortical and subcortical areas that are known to be involved in relapse is modulated by CB1 receptors. The basolateral amygdala and hippocampus have an important role in mediating discrete and contextual cue-induced relapse, and CB1 receptors in these areas are involved in memory-related plasticity. Pharmacological intervention studies using local application of CB1 receptor agonists and antagonists are needed to identify the CB1 receptors responsible for the attenuation of drug seeking. Abbreviation: PFC, prefrontal cortex.
to obtain a heroin infusion is high or progressively increases) [9,42].

Close relationships between the cannabinoid system and alcohol are well established [44,45]. Blockade of CB1 receptors reduce alcohol drinking under a variety of conditions, although part of this effect can be attributed to a more general suppression of food and fluid intake [45,46].

The exact role of CB1 receptors in nicotine reinforcement remains to be resolved. SR141716A decreases nicotine self-administration in rats [47]. Environmental stimuli associated with nicotine appear to be particularly important in the maintenance of nicotine self-administration behaviour [48,49]. Therefore, this decrease might be the result of an attenuation of the secondary reinforcing properties of nicotine as is evident from our reinstatement experiments [11] and from a recent report by Cohen and co-workers [49]. Experiments with CB1 receptor knockout mice, however, indicate that CB1 receptors are not required to acquire nicotine self-administration behaviour [38].

Collectively, the effects of CB1 receptor blockade on drug self-administration (primary reinforcement) appear to be drug specific and/or depend on the motivational state of the animal. These observations argue against the possibility that cannabinoid receptor antagonists affect the ability of an animal to perform an operant response as a result of, for example, motor disturbances or other side-effects. By contrast, suppression of secondary reinforcing effects of drugs of abuse appears to be a more general and highly reproducible result of CB1 receptor blockade.

Cannabinoids and memory-related plasticity

Because retrieval of memories associated, through Pavlovian conditioning, with self-administered drugs appear to be crucial to the persistence of drug addiction, it is relevant to note that CB1 receptors and endocannabinoids are abundant in memory-related brain areas, and modulate memory [33,50,51]. For example, the endocannabinoid system controls the extinction of aversive memories [52]. Cannabinoids appear to modulate memory by changing synaptic plasticity. Endocannabinoids function as retrograde signaling messengers and mediate long-term depression or potentiation of synaptic transmission in several addiction and memory-related brain areas, including the nucleus accumbens, prefrontal cortex, amygdala and hippocampus [51,53]. Recently, even a single exposure to Δ9-tetrahydrocannabinol (Δ9-THC), the active constituent of marihuana, produced a long-lasting (days) blockade of endocannabinoid-mediated retrograde signaling in the nucleus accumbens and hippocampus [54]. Therefore, interference with the endocannabinoid system could change the impact of reward-related memories. In this context, it is worth noting that heroin seeking was enhanced for several days following a single priming injection of a cannabinoid receptor agonist (Figure 1b) [10], which might be attributed to a long-lasting disruption of the extinction process. Interestingly, non-CB1 receptor-mediated effects of cannabinoids on extinction of a previously established cocaine place preference were reported recently [55].

Specifity of the effects of CB1 receptor blockade

This more general role of endocannabinoids in memory-related processes such as extinction, consolidation and retrieval might not only form an alternative explanation for the anti-craving properties of SR141716A, but might also imply that the actions of cannabinoids on these processes are not limited to drug reinforcers. It is evident that the nucleus accumbens and its associated circuitry are also involved in a variety of non-drug related behaviors. In fact, the nucleus accumbens subserves behaviors linked to reward and motivation per se, including natural rewarding behaviors such as feeding, drinking and sex [56]. Using a similar reinstatement paradigm as for heroin, cocaine and alcohol seeking, cue-elicited sucrose seeking following long-term extinction was also attenuated by SR141716A [11]. It is well established that CB1 receptors have an important role in food intake through leptin-regulated endocannabinoids at the level of the hypothalamus [57]. These observations, together with those showing that SR141716 reduced sensitivity to rewarding electrical brain stimulation [58] and blocked the acquisition of food-induced place preferences [59], suggests that the anorectic actions of CB1 receptor antagonists are, at least in part, mediated by a suppression of the salience or incentive value of food-associated stimuli. The recent finding that Δ9-THC reinstated both alcohol and sucrose seeking is consistent with this idea. [60]. This general role of cannabinoids on conditioned reinforcement implies that these modulatory actions are probably not the result of drug-induced changes in the endocannabinoid system, although such changes have been reported [61].

From a treatment perspective, the generality of the effects of cannabinoids on motivational processes, might lead to undesirable side-effects (e.g. sex drive) during long-term treatment with cannabinoid receptor antagonists. Such adverse effects, however, were not observed in a recently published clinical trial with rimonabant in overweight and obese patients after treatment for 1 year with the highest (20 mg) dose [62].

Concluding remarks

The preclinical studies performed to date provide a solid framework for an important role of CB1 receptors in the neuronal mechanisms that underlie relapse to drug seeking and, in particular, conditioned responding to drugs and drug-associated stimuli. The relapse-preventing properties of SR141716A (rimonabant) were confirmed recently in a first clinical trial in smokers. In addition, a number of drugs have been tested in clinical trials to date for their possible anti-craving properties, such as WIN552122, a compound that has been shown to reduce cue-elicited drug seeking in animal models [63].

Chemical names

HU210: (8аR)-trans-3-{1,1-dimethyl-heptyl}-6а,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol
SCH23390: (±)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
SR141716A: N-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyld-4-methylpyrazole-3-carboxamide
WIN552122: (R)-{+}[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphtalenylmethanone mesylate
1-year treatment with rimonabant was associated with clinically meaningful weight loss. [62]. The aforementioned preclinical observations indicate that both actions could, at least in part, be the result of a common mechanism (i.e. reducing the impact of both drug- and food-associated stimuli). Finally, it appears that the extinction-reinstatement model shows good predictive validity regarding the clinical efficacy of compounds used for the treatment of drug abuse and eating disorders.

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