Cannabinoids as Therapeutics
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Cannabinoids and drugs of abuse

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Introduction

Derivatives of *Cannabis sativa*, such as marijuana and hashish, are the most widely consumed illicit drug: almost half of all 18-year olds in the USA and in most European countries admit to having tried it at least once, and 10% of that age group are regular users. There have been many subjective accounts of the cannabis 'high'. A typical 'high' is preceded initially by a transient stage of tingling sensations felt in the body and head accompanied by a feeling of dizziness or light-headedness. The 'high' is a complex experience, characterized by a quickening of mental association and a sharpened sense of humor, sometimes described as a state of "fatuous euphoria". As reported by Atha and Bianchard [1] in a survey of 1333 young British cannabis users the most common benefit reported were relaxation and relief from stress, insight/personal development and euphoria, but 21% of the users also described some adverse effects, including impaired memory, paranoia and amotivation/laziness. As with other intoxicant drugs, little is known about the brain mechanisms that underlie the cannabis high. The intoxicant effects are clearly mediated via CB_1 receptors. In a well-controlled study in 63 healthy cannabis users [2] who received either a CB_1 receptor antagonist (Rimonabant) or placebo and smoked either a Δ^9-tetrahydrocannabinol (Δ^9-THC)-containing or placebo marijuana cigarette, Rimonabant blocked the acute psychological effects of the active cigarettes. Moreover, self ratings of cannabis intoxication correlated most markedly with increased blood flow in the right frontal region as demonstrated using positron emission tomography (PET) to measure changes in cerebral blood flow.

The potential ability of cannabis derivatives to produce dependence in humans is still a controversial issue. Earlier clinical literature (for reviews see [3–5]) suggested that tolerance also occurs after repeated administration of Δ^9-THC in humans, although many of these studies were poorly controlled. But for many years cannabis was not considered to be a drug of addiction. Withdrawal of the drug did not lead to any obvious physical withdrawal syndrome either in people or in animals, and animals failed to self-administer the drug, a behavior usually associated with drugs of addiction.

Attitudes have changed markedly in recent years. According to the Diagnostic and Statistical manual of Mental Disorders (DSM IV) [5a] criteria

(American Psychiatric Association, 1994) for 'substance dependence' and 'substance abuse', surprisingly a high proportion of regular cannabis users appear to fall into these categories. Recent studies [6] indicated that almost one-third of regular cannabis users fell within the definition of 'substance abuse' or 'substance dependence'. Moreover, carefully controlled studies have also shown that a reliable and clinically significant withdrawal syndrome does occur in human cannabis users when the drug is withdrawn. The symptoms include craving for cannabis, decreased appetite, sleep difficulty and weight loss, and may sometimes be accompanied by anger, aggression, increased irritability, restlessness and strange dreams [7].

The existence of abuse liability of cannabinoids in animals is much more clearly observable. Processes involved in substance abuse are neurobiologically and behaviorally complex. Tolerance and withdrawal syndrome represent adaptive responses to the prolonged exposure of neurons to drugs, but the main factor common to all drugs of abuse is their ability to induce drug-seeking behavior, which is due to the positive reinforcing effects of the drugs. Several behavioral models have been used to evaluate tolerance and withdrawal, as well as the rewarding effects of cannabinoids, which will be briefly summarized here together their proposed molecular basis.

Tolerance

Chronic administration of natural or synthetic cannabinoid agonists in different animal species induces tolerance to most of their pharmacological effects (see [8, 9] for review). Although some papers have reported that pharmacokinetic events take place during the development of cannabinoid tolerance [10, 11], there is general agreement that this phenomenon is pharmacodynamic in nature. The best-known events that occur after development of cannabinoid tolerance are receptor downregulation and uncoupling from the G protein system, which ends in receptor desensitization (see [8] for review). Besides these alterations, other cellular adaptations are present in the brain of cannabinoid-tolerant rats, such as modulation of effector proteins. Specifically, it has been shown that increased activation of the cAMP pathway (i.e. cAMP accumulation and protein kinase A activity) [12–15], together with adaptations in the extracellular-signal-regulated kinase (ERK) cascade [16], were observed in some cerebral regions of chronic Δ^9-THC-treated animals. An elegant demonstration of the involvement of the Ras/ERK pathway in development of cannabinoid tolerance comes from studies in Ras-GRF1-knockout mice [16], a useful model where cannabinoid-induced ERK activation is lost. These animals did not develop tolerance to Δ^9-THC's analgesic and hypolocomotor effects, suggesting that the ERK cascade could play a pivotal role in the induction of synaptic plasticity due to chronic cannabinoid exposure. Finally, recent work reported that the pyrazolopyrimidine (PP1), the Src family tyrosine kinase inhibitor, reversed Δ^9-THC-induced tolerance, supporting a role for Src

tyrosine kinase in phosphorylation events in Δ^9-THC-tolerant mice [15]. Taken together, these recent data seem to indicate an outstanding role in cannabinoid tolerance for some protein kinases (protein kinase A, ERK, Src tyrosine kinase), suggesting that cannabinoid tolerance could be depicted as activity-dependent synaptic plasticity. Whether and how these kinases could contribute to CB_1 receptor downregulation or desensitization remains to be determined. In line with this view, large-scale analysis of gene-expression changes during acute and chronic exposure to Δ^9-THC in rat hippocampus [17] revealed that the altered genes were predominantly associated with membrane repair and synaptic structures, indicating that they are involved in transcription or proteosomal processes, possibly reflecting a change in neuronal capacity to deal with the ubiquitous consequences of chronic cannabinoid receptor activation over long time periods.

Finally, it cannot be ruled out that prolonged activation of cannabinoid receptors also leads to decreased endocannabinoid content and signalling in the striatum and to increased anandamide formation in the limbic forebrain [18], areas involved in the tonic control of movements and in reinforcement processes.

Physical dependence

Although the presence of spontaneous withdrawal after chronic cannabinoid treatment is also controversial in animals, there are no doubts that administration of the CB_1-selective antagonist SR-141716A precipitates a pronounced withdrawal syndrome in animals that have been chronically treated with cannabinoids (see [8, 9] for review). Biochemical indicies of adaptive changes have been demonstrated during cannabinoid withdrawal and they include compensatory changes in the cAMP pathway in the cerebellum [12–14, 19], which appears to be a key area in the modulation of somatic expression of cannabinoid abstinence syndrome. These findings directly demonstrated that, in analogy with other addictive drugs, the activation of the cAMP pathway is a crucial phenomenon at the onset of Δ^9-THC-withdrawing behaviors. Interestingly, a key structure in controlling this process could be the cerebellum, a region not previously associated with drug abuse, and whose participation in cognitive networks is actually a most exciting field of investigation. Moreover, activation of corticotropin-releasing factor [20] and a decrease in mesolimbic dopamine transmission [21, 22] have also been observed in withdrawn rats, strengthening the evidence that cannabinoids share with other drugs of abuse those neurochemical properties that are regarded as the biological substrate of drug addiction.

Behavioral sensitization

Behavioral sensitization represents another adaptive neurobiological alteration that occurs after repeated exposure to drugs and plays a role in drug

addiction, particularly in drug-seeking behavior that persists long after the discontinuation of drug use [23]. Rats repeatedly treated with Δ^9-THC for several days (3/5 days) and then challenged with Δ^9-THC 2/3 weeks after the last Δ^9-THC injection show a greater behavioral activation than rats repeatedly treated with vehicle [24, 25]. The molecular underpinnings of this phenomenon are still not well understood, but they involve altered CB_1 receptor functionality in the striatum and cerebellum of sensitized rats [26]. Moreover, in the cerebellum the cAMP pathway and the ERK cascade seem to lose their responsiveness to cannabinoids ([26] and T. Rubino et al., unpublished results). Preliminary data obtained in our laboratory indicate differential responsiveness of specific transcription factors in selected brain areas (striatum, prefrontal cortex and hippocampus) of pre-exposed rats, supporting the working hypothesis that relapse can be viewed as a certain kind of memory (addiction memory) since the brain obviously remembers the prior administration of the drug and induces craving.

Drug discrimination

Drug discrimination is a behavioral procedure based on the ability of a drug to induce a specific set of interoceptive stimulus conditions perceived by the animals that might be predictive of the subjective reports of perceptions/feelings induced by the same drug in humans. As a result, studies of the subjective effects of new drugs in both humans and animals have been relatively good predictors of either or not a drug will be abused. Since animals do not easily self-administer cannabinoids, the drug-discrimination procedure has long been the primary animal model available for evaluating the potential abuse liability of cannabinoids [27]. Cannabinoid drugs show a pharmacological specificity in this behavioral procedure. Thus, in animals trained to discriminate injections of Δ^9-THC from injections of saline, only drugs that possess the ability to activate CB_1 cannabinoid receptors fully generalize to the Δ^9-THC training stimulus (see [9] for review). Moreover, the discriminative stimulus effects of Δ^9-THC and other synthetic CB_1 agonists can be completely blocked by pre-treatment with the selective CB_1 receptor antagonist SR-141716A [28], further demonstrating that the cannabinoid discrimination is mediated by CB_1 receptors [29, 30]. In contrast, anandamide and stable analogs of this endocannabinoid do not fully substitute for Δ^9-THC in monkeys and rats [31–33], or has done so only at doses that severely decrease food-mantained responding [32]. The fast reuptake and rapid metabolism of anandamide by the fatty acid amide hydrolase enzyme is a likely explanation for why anandamide, which is a partial agonist of CB_1 receptors, just like Δ^9-THC, usually fails to produce Δ^9-THC-like discriminative-stimulus effects. Anandamide has been shown to have cannabinoid-like discriminative stimulus effects under some situations. Recently Jarbe et al. [33] demonstrated that methanadamide was successfully used as a training stimulus in rats,

and Δ^9-THC produced complete generalization. Anandamide was able to produce generalization to the methanandamide but not to the Δ^9-THC training stimulus that could be related to the different affinities of Δ^9-THC and methanadamide for CB_1 receptors, resulting in a discriminative stimulus for methanadamide with an intensity and a quality closer to the anandamide stimulus as compared to the Δ^9-THC stimulus. It could be also the case that anandamide and methanadamide but not Δ^9-THC possess affinity for a subpopulation of receptors other than CB_1. Unfortunately the ability of SR-141716A to block the generalization to anandamide was not tested. Among non-cannabinoid drugs, only the benzodiazepine diazepam has been found to produce partial generalization to cannabinoid training stimulus that was SR-141716A-insensitive, suggesting that this effect is mediated by an interaction through the GABAergic system [34].

Self-administration

Drug self-administration behavior has been one of the most direct and productive approaches for studying the rewarding properties of abused drugs. Using this methodology, it has been possible to study neuropharmacological mechanisms involved in such behaviors and preclinically evaluate therapeutic strategies for treatment of drug abuse. Since 1970, all attempts to obtain a robust procedure for Δ^9-THC self-administration have failed and this has been fundamental to claims of a differential status for cannabinoids with respect to major abused drugs. Within the last few years, however, reinforcing effects of some synthetic CB_1 cannabinoid agonists have been reported using intravenous self-administration procedures in rats and mice [35–37], although the experimental procedures employed in each of these studies limit the generality of the findings. Persistent intravenous self-administration of Δ^9-THC itself was first demonstrated in squirrel monkeys by Tanda et al. [38]. However, monkeys in this study had a history of cocaine self-administration, raising the possibility that persistent neurobiological adaptations might subsequently predispose animals to self-administer Δ^9-THC. This problem was successfully overcome by Justinova et al. [39], who demonstrated that Δ^9-THC-self-administration behavior was initiated and subsequently maintained at very high rates in monkeys with no history of exposure to other drugs, showing that this drug possesses reinforcing properties of its own that are not dependent on prior self-administration of other drugs. Thus self-administration of Δ^9-THC by squirrel monkeys provides a reliable animal model of human marijuana abuse, suitable for studying the relative abuse liability of other natural and synthetic cannabinoids and for developing new therapeutic strategies for the treatment or prevention of marijuana abuse in human.

Conditioned place preference

The conditioned place preference procedure is a classical procedure that provides an indication of drug-related reward/aversion effects in animals. Previous studies into the reinforcing properties of cannabinoids have produced conflicting evidence with respect to the generation of place preference. Some studies have shown that Δ^9-THC can produce place preference [40, 41], whereas others reported place aversion [12, 42–46] or no effect [41]. The discrepancies in results have been interpreted as being due to differences in apparatus, experimental design and the subjects used. Positive place preferences, when found, are usually highly dose-dependent, often occurring at only a single dose either in mice or in rats using Δ^9-THC as well as synthetic cannabinoid compounds [40, 41, 47, 48]. Indeed, place preference was obtained with a low dose of Δ^9-THC in mice (1 mg/kg) when they received a previous priming Δ^9-THC exposure in the home cage before the conditioning sessions [41]. Place aversion properties are often produced by Δ^9-THC and synthetic cannabinoids either in rats or in mice using similar dose ranges and standard place preference procedures [40, 43–46]. These apparently conflicting results could be explained by the possible dysphoric/anxiogenic consequences of the first cannabinoid exposure that could mask the development of positive place preference [41]. Discrepant results are also present for the CB_1 receptor antagonist SR-141716A: while some papers reported a positive place preference in rats [44, 49] some others failed to demonstrate either place preference or place aversion [45, 47]. These opposite results do not allow us to precisely indicate a role for endocannabinoid tone as a physiological system to suppress reward or to induce aversion.

Neurochemical correlates of cannabinoid rewarding properties

The mesolimbic dopaminergic system is part of a brain reward circuit that has been long thought to play a major role in mediating reinforcing/rewarding effects of drugs of abuse [50]. Many drugs abused by humans share the common property of selectively increasing dopamine release in the nucleus accumbens, the major terminal area of the mesolimbic dopamine system, but this has been a matter of debate with regard to Δ^9-THC and other cannabinoids. It is now well-accepted that cannabinoids are able to increase dopamine levels in the shell compared with the core of the nucleus accumbens, likely through an opioid receptor-mediated mechanism or a direct activation of dopaminergic neurotransmission in the nucleus accumbens (see [9] for review). Moreover, cannabinoids might exert part of their reinforcing effects through the endogenous opioid system [51, 52]. For example, Δ^9-THC-induced conditioned place preference is suppressed in μ-opioid receptor-knockout mice [48] and Δ^9-THC-induced self-administration can be blocked by μ-opioid receptor antagonists [36, 37]. The neurochemical mechanism of the interaction between

the endocannabinoid and opioid systems has not been elucidated, but might involve cannabinoid-induced synthesis and release of endogenous opioids or converging signal transduction pathways if the receptors are co-expressed [52].

Cannabinoid system and drug addiction

Animal models of drug reward provide evidence that endogenous cannabinoids play a role in determining the rewarding effects not only of cannabis but also of other psychoactive drugs, such as ethanol, cocaine, morphine, nicotine and amphetamine. Plenty of published works report the involvement of cannabinoid processes in positive reinforcement activated by both natural rewards and drugs of abuse. For example, in CB_1-knockout mice nicotine was not able to induce place preference as it does in wild-type mice [53], and administration of SR-141716 in the rat decreased nicotine self-administration [54]. These results suggest that activation of the endogenous cannabinoid system may participate in the motivational effect of nicotine; thus SR-141716 may be effective as an aid for smoking cessation.

Results on morphine-conditioned place preference in CB_1-knockout mice had controversial effect: in one study [55] morphine induced conditioned place preference in wild-type mice but failed to produce any response in knockout mice, indicating the inability of morphine to induce rewarding effects in the absence of CB_1 cannabinoid receptors. In a more recent work [56] CB_1 receptor-knockout mice developed a strong place preference to morphine, similar to that in wild-type Swiss-Webster mice, thus not supporting a contribution of the brain cannabinoid system to morphine reward. A possible explanation for this discrepancy could rely in the slightly more intensive conditioning paradigm and differences in the nature of conditioning chambers used for the experiment in the last paper. However, self-administration studies support the idea that the CB_1 cannabinoid receptor is essential for the modulation of morphine's rewarding effects. Cossu et al. [57] found that morphine did not induce intravenous self-administration in mutant CB_1 receptor-knockout mice, whereas it was significantly self-administered by the corresponding wild-type mice. Approaches involving the CB_1 antagonist SR-141716 gave more compelling results. Recently it was shown that SR-141716A pretreatment dose-dependently reduced operant heroin self-administration by male Wistar rats under a fixed-ratio schedule of reinforcement, and significantly lowered the breaking point of responding for heroin under a progressive-ratio schedule of reinforcement [58]. In the same line Solinas et al. [59] reported that SR-141716A markedly decreased heroin self-administration under the progressive-ratio schedule. In contrast, SR-141716A had no effect on heroin self-administration under the fixed-ratio schedule at heroin doses of 50 or 100 μg/kg per injection, but produced small decreases in self-administration at lower doses (25 and 12.5 μg/kg per injection). These data demonstrate that the cannabinoid CB_1 receptor antagonist SR-141716 produces a clear attenuation, but not a com-

plete blockade, of the reinforcing effects of heroin, suggesting a facilitatory modulation of opioid reinforcement by endogenous cannabinoid activity that is unmasked by CB_1 receptor blockade. All these lines of evidence provide support for the potential efficacy of cannabinoid CB_1 antagonists in the prevention and treatment of opioid addiction.

Evidence for endocannabinoid involvement in the rewarding effects of ethanol also exists (see [60] for review). Here we only cite the latest papers. Voluntary ethanol intake was significantly lower in $CB_1^{-/-}$ *versus* $CB_1^{+/+}$ young male mice [61–63]. Moreover, administration of the cannabinoid CB_1 receptor antagonist SR-141716 significantly reduced ethanol intake in CB_1 wild-type (+/+) mice [61] and rats [54]. The role of endocannabinoids and CB_1 receptor in alcohol-drinking behavior is now unequivocal; thus SR-141716 may be effective in reduction of alcohol consumption. Surprisingly the combination of the synthetic cannabinoid agonist CP-55,940 with MDMA (methylenedioxymethamphetamine; ecstasy) in rat reduced the number of drug-associated lever pressings compared to the single drugs [64] and pre-treatment with SR141716A significantly increased MDMA self administration. At first glance these data seem to suggest that the endocannabinoid system might have negative effects rather than the positive ones shown in the above cited studies. The nature of this interaction remains unclear due to the lack of studies on dopamine levels in mesolimbic structures that could add further insight on the neurochemical correlates of MDMA's reinforcing properties.

Finally, particularly relevant seems to be the role of endocannabinoid tone in relapse to drugs of abuse. This aspect of drug addiction assumes a striking interest in the human context. In fact, detoxification from drug addiction has been a medical problem for as long as drugs have been abused, due to relapse occurring even after prolonged drug-free periods. Several reinstatement models are currently available to investigate major factors contributing to relapse and have been used to study the involvement of the cannabinoid system. In recent work Fattore et al. [65] reported that the CB_1 receptor antagonist SR-141716A prevented heroin-induced reinstatement of heroin-seeking behavior but did not show any effect *per se*, suggesting that CB_1 receptor blockage alters the reinforcing consequences of heroin administration. Moreover, in animals with a history of heroin self-administration, cannabinoid primings elicit relapse to heroin-seeking behavior following an extended drug-free period. Similar results were also obtained by De Vries et al. [66]: the potent cannabinoid agonist HU-210 reinstated heroin seeking, whereas SR-141716A attenuated both heroin-primed and cue-induced heroin seeking following a 3-week extinction period. The same group [67] found very similar results also in animals withdrawn from cocaine self-administration: HU-210 provoked relapse to drug seeking after a prolonged withdrawal period, while blockade of CB_1 receptor attenuated the relapse induced by re-exposure to cocaine-associated cues or cocaine itself. The CB_1 cannabinoid antagonist was also used on alcohol-deprivation effects (i.e. the temporary increase in alcohol intake after a period of alcohol withdrawal) in Sardinian alcohol-preferring

(sP) rats [68]. As expected, alcohol-deprived rats virtually doubled voluntary alcohol intake during the first hour of re-access. Acute administration of SR-141716 completely abolished the alcohol-deprivation effect. These results suggest that the cannabinoid CB_1 receptor is part of the neural substrate mediating the alcohol-deprivation effect and that SR-141716 may possess anti-relapse properties.

Taken together, these results seem to indicate that SR-141716 could specifically counteract reward-related behaviors, whatever the specific factors involved in the action of each reinforcer, and that cannabinoid CB_1 receptors could be crucially involved in the neurobiological events evoked by appetitive reinforcers. However this does not necessarily mean that a permanent endogenous cannabinoid tone exists to ensure the organism a basal hedonic level. Thus it can be postulated that cannabinoid-related processes are elicited and maintained by pleasant reinforcers. This suggests that the activation of reward system could be under the permissive control of some complex CB_1-related cannabinoid processes which are required for the perception of the incentive value of positive reinforcements. Is the recent finding that Rimonabant reduces food intake in obesity and tobacco consumption in more than 500 adults underlying the relevance of therapeutic modulation of the endocannabinoid system?

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