Drug Addiction

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Abstract Many drugs of abuse, including cannabinoids, opioids, alcohol and nicotine, can alter the levels of endocannabinoids in the brain. Recent studies show that release of endocannabinoids in the ventral tegmental area can modulate the reward-related effects of dopamine and might therefore be an important neurobiological mechanism underlying drug addiction. There is strong evidence that the endocannabinoid system is involved in drug-seeking behavior (especially behavior that is reinforced by drug-related cues), as well as in the mechanisms that underlie relapse to drug use. The cannabinoid CB_1 antagonist/inverse agonist rimonabant

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has been shown to reduce the behavioral effects of stimuli associated with drugs of abuse, including nicotine, alcohol, cocaine, and marijuana. Thus, the endocannabinoid system represents a promising target for development of new treatments for drug addiction.

Keywords Drug addiction • Cannabinoids • Endocannabinoids • Self-administration • Relapse • Reward • THC

Abbreviations

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
VTA	ventral tegmental area
DAT	dopamine transporter
THC	delta-9-tetrahydrocannabinol
FAAH	fatty acid amide hydrolase

1 Introduction

1.1 Drug Addiction

The abuse of drugs and alcohol is a major problem worldwide, costing 250 billion dollars annually due to premature deaths, healthcare expenditures, reduction of productivity, lost earnings and drug-related crime in the United States alone (estimated by US National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism). Drug addiction is considered to be a chronic, relapsing disorder characterized by compulsive drug-seeking, by continued use despite serious negative socioeconomic and health consequences, and by loss of control over drug use (Cami and Farre 2003). The World Health Organization and the American Psychiatric Association use the term "substance dependence" rather than "drug addiction." Both terms are used interchangeably in the literature, but the latter term is less likely to be confused with physical dependence and emphasizes the behavioral component of the process. According to the DSM-IV (American Psychiatric Association 1994), three or more of the following must be present in order to diagnose substance dependence: (a) symptoms of tolerance, (b) symptoms of withdrawal, (c) large amounts of drug taken, (d) unsuccessful attempts or desire to control use, (e) considerable time spent obtaining the substance, (f) reduction of social and occupational activities due to abuse, (g) continued use of a substance despite physical or psychological problems.

Repeated drug use arises from the drug's neurochemical actions that produce positive reinforcing effects, progressively leading to neurobiological changes in the brain reward circuits and behaviors characteristic of addiction: tolerance, sensitization, dependence, withdrawal and craving (Kreek et al. 2002). The transition from casual drug use to drug addiction might also involve an additional source of reinforcement, such as the reduction of a negative emotional state during acute abstinence (Koob et al. 1998). The combination of positive (e.g., euphoria) and negative (e.g., alleviation of dysphoria or withdrawal symptoms) reinforcement may provide a powerful motivational force for compulsive drug taking. Associated neurobiological changes and behavioral abnormalities and deficits in cognitive function may persist for months or years after discontinuation of drug use (Cami and Farre 2003).

1.2 Endocannabinoid System in Brain Reward Circuitry

The initial events that lead to drug addiction involve acute effects at the specific sites of action of the abused drug. These sites of action (e.g., G-protein coupled receptors and ligand-gated ion channels) typically activate neural circuits associated with positive reinforcement/reward, particularly the mesocorticolimbic dopaminergic system. This system, originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens, olfactory tubercle, frontal cortex, and amygdala (Wise 2004), interacts with glutamatergic projections from the cerebral cortex, hippocampus, and amygdala, and thus regulates responses to natural reinforcers such as food, drink, social interactions or sex (Kauer 2004). The mesocorticolimbic dopaminergic system is part of a brain reward circuit that has long been thought to play a major role in mediating the reinforcing/rewarding effects of drugs of abuse (Di Chiara et al. 1999; Koob 1992; Wise and Bozarth 1987). Abused drugs (like opioids, cannabinoids, psychostimulants, alcohol, nicotine, sedative-hypnotics, anxiolytics, and anesthetics) directly or indirectly elevate extracellular levels of dopamine in the shell of the nucleus accumbens (Brodie et al. 1999; Chen et al. 1990; Masuzawa et al. 2003; Pontieri et al. 1995, 1996; Spyraki and Fibiger 1988; van der Laan et al. 1992).

In the striatum, cannabinoid CB_1 receptors are localized presynaptically in GABAergic and glutamatergic nerve terminals, but also postsynaptically in the dendritic shafts and spines of both enkephalinergic and dynorphinergic GABAergic efferent neurons (Fusco et al. 2004; Hohmann and Herkenham 2000; Kofalvi et al. 2005; Pickel et al. 2004, 2006). When these cells are depolarized, endocannabinoids can be released in the nucleus accumbens (Robbe et al. 2001) and VTA (Melis et al. 2004; Riegel and Lupica 2004), where they modulate the excitatory (glutamatergic) and inhibitory (GABAergic) inputs that control dopaminergic neurons of the mesocorticolimbic pathway by acting as retrograde messengers on CB_1 receptors. Endocannabinoids are also involved in synaptic plasticity in the mesolimbic system – please see the chapter, "Endocannabinoid Signaling in Neural Plasticity."

The dopaminergic system has a well-established role in the reinforcing effects of drugs of abuse. It has become increasingly clear that the endocannabinoid system can modulate dopaminergic reward circuits, which suggests that endocannabinoids also play a major role in the mechanisms underlying drug addiction.

1.3 Release of Endocannabinoids by Abused Drugs

The endocannabinoid system can modulate the primary rewarding effects of non-cannabinoid drugs of abuse, and this ability appears to depend on endocannabinoid release in the VTA (Lupica and Riegel 2005). This hypothesis is consistent with evidence that repeated non-contingent drug administration alters levels of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG).

Analysis of AEA and 2-AG levels in brains of animals treated chronically with cocaine, nicotine, or ethanol showed that chronic cocaine administration produced a modest but significant decrease in the content of 2-AG in the limbic forebrain (Gonzalez et al. 2002). In contrast, chronic ethanol and nicotine exposure produced an increase in AEA content in this area. Chronic ethanol administration caused a decrease in the contents of both AEA and 2-AG in the midbrain. Chronic nicotine exposure increased both AEA and 2-AG in the brainstem and decreased their content in the hippocampus, striatum and cerebral cortex. It appears that the most consistent finding with these drugs of abuse is that chronic administration led to an elevation in endocannabinoid levels in the limbic system. This observation is consistent with the notion that endocannabinoids enhance the reinforcing effects of addictive drugs by increasing dopamine release via the inhibition of GABA release in the limbic system. Chronic administration of Δ^9-tetrahydrocannabinol (THC) itself decreases the levels of AEA and 2-AG in the striatum (Di Marzo et al. 2000). Chronic morphine administration decreases 2-AG levels in the striatum without altering AEA levels (Gonzalez et al. 2003; Vigano et al. 2003). Acute morphine administration, on the other hand, increased AEA levels and decreased 2-AG levels in the striatum (Vigano et al. 2004). Thus, the selection of the time point for endocannabinoid analysis is critical for determination of the nature of alterations in endocannabinoid levels.

It should be noted that, in the studies just described, endocannabinoid levels were measured in postmortem rat brain tissue, usually at a single time-point after chronic administration of the drugs. Therefore, it is not clear whether these findings reflect sustained changes in the brain endocannabinoid levels, or acute alterations of endocannabinoid formation. Another potential problem with these results is that endocannabinoid levels in brain tissue are affected by rapid postmortem increases in endocannabinoid formation (Bazinet et al. 2005; Patel et al. 2005). In addition, these studies have used non-contingent drug administration, which can produce neurochemical, proteomic, and genomic effects that differ substantially from those induced by free-choice self-administration (Jacobs et al. 2003). Thus, further

research is required to determine whether endocannabinoid levels would be affected in the same way if the drugs were self-administered.

In vivo microdialysis techniques offer an effective means of studying levels of neurotransmitters during drug self-administration. However, microdialysis is difficult to perform with AEA and 2-AG due to their highly lipophilic nature and instability. The first study in which endocannabinoid levels were measured by microdialysis showed that local intrastriatal administration of the dopamine D_2 agonist quinpirole elevated levels of AEA, but not 2-AG (Giuffrida et al. 1999). Caille and colleagues (Caille et al. 2007) were the first to measure changes in endocannabinoid levels during self-administration of a drug of abuse. They found that self-administration of cocaine did not alter either AEA or 2-AG levels in the nucleus accumbens, but self-administration of heroin increased AEA and decreased 2-AG levels, and self-administration of ethanol increased 2-AG without altering AEA levels. These data provide in vivo evidence for an endocannabinoid involvement in the motivational effects of ethanol and heroin but not cocaine.

An exciting new analytical method, combining online in vivo brain microdialysis with solid-phase extraction–liquid chromatography–tandem mass spectrometry, allows real-time detection of trace amounts of endocannabinoids in the extracellular fluid. This technique has been used to show that the CB_1 receptor antagonist/inverse agonist rimonabant increased, whereas the CB_1 receptor agonist WIN 55,212-2 decreased, AEA release in the rat hypothalamus (Bequet et al. 2007). Interestingly, the same treatments induced opposite changes in 2-AG release. The same study also shows that inhibition of fatty acid amide hydrolase (FAAH), the primary enzyme responsible for AEA degradation, induced an increase in outflow of AEA, but not 2-AG. In this study, FAAH was inhibited by systemic administration of URB597, a selective FAAH inhibitor now entering clinical trial.

1.4 Endocannabinoids in Drug-Seeking and Relapse

Relapse to drug use, even after a long period of forced or voluntary withdrawal and detoxification, is one of the defining features of addiction, and perhaps the most important impediment to effective treatment (American Psychiatric Association 1994; O'Brien 2001). Reinstatement of drug-seeking behavior in laboratory animals is an experimental procedure that is used to model relapse. In this model, animals are initially trained to self-administer drugs intravenously by making an operant response (e.g., pressing a lever). Subsequently, the drug-reinforced behavior is extinguished by replacing the self-administered drug solution with saline or by disconnecting the infusion pump. After extinction of the drug-reinforced behavior, reinstatement of drug-seeking behavior can be tested by one of several procedures that mirror the triggers that induce relapse in humans. These include non-contingent injection of a drug (drug-induced reinstatement), presentation of visual or auditory stimuli that had previously signaled availability or delivery of the drug (cue-induced

reinstatement), or exposure to a brief period of intermittent foot-shock in the self-administration chamber (stress-induced reinstatement).

The first evidence for involvement of the endocannabinoid system in reinstatement of extinguished drug-seeking behavior was provided by De Vries and colleagues (De Vries et al. 2001). They found that the synthetic CB_1 receptor agonist HU210 could reinstate cocaine-seeking behavior. The CB_1 antagonist/inverse agonist rimonabant blocked this effect and also reduced cocaine- and cue-induced reinstatement, but not stress-induced reinstatement. Another CB_1 antagonist/inverse agonist, AM251, was later found to block cocaine-induced reinstatement of drug-seeking behavior (Xi et al. 2006). Rimonabant has since been found to reduce reinstatement induced by heroin, methamphetamine, nicotine, WIN 55, 212-2 and ethanol, and to attenuate or block cue-induced reinstatement of the seeking of cocaine, nicotine, heroin, methamphetamine, and alcohol in rodents (De Vries and Schoffelmeer 2005; Fattore et al. 2007). Rimonabant blocks both THC-induced and cue-induced reinstatement of THC-seeking behavior in non-human primates (Justinova et al. 2008b). At present, studies investigating a potential ability of CB_1 receptor blockade to alter reinstatement have been described as indicating that rimonabant is unable to affect stress-induced relapse to cocaine or ethanol seeking (Fattore et al. 2007). Although stress-induced relapse has received less attention than drug- and cue-induced relapse, the evidence accumulated to date indicates that endocannabinoid signaling might not be involved in stress-induced reinstatement.

Early clinical trials examining the effectiveness of rimonabant as an aid in smoking cessation and obesity treatment were very promising. Rimonabant has been approved and marketed as an anti-obesity treatment, but not an anti-smoking treatment, in the European Union and in a number of other countries. However, in 2006 the American Food and Drug Administration (FDA) declined to approve rimonabant for smoking cessation and required further studies before final approval for weight management. This was due to concerns over possible depression-like side effects. It has been suggested that CB_1 neutral antagonists may be devoid of the side effects produced by CB_1 antagonists/inverse agonists, and neutral antagonists are now being tested in animal studies (Salamone et al. 2007; Sink et al. 2008). Thus, while manipulations of the endocannabinoid system show promise for the treatment of addiction, there is not yet a specific clinically tested compound that has been shown to be both effective and safe.

2 Cannabinoids

Cannabinoids, usually abused by humans in the form of marijuana, have become the most frequently abused illicit class of drugs in the United States. There is ample evidence that most of the centrally mediated effects of cannabinoids occur through the endocannabinoid system. The effects of marijuana in humans are quite complex and highly variable depending on the dose of the drug, environment and expectations of the user. The subjective effects may include excitement and dissociation of

ideas, quickening of mental associations, heightened perception, distortion of the sense of time, irresistible impulses and illusions accompanied by decrease of psychomotor activity (Dewey 1986). In addition to its euphorigenic properties (Haney et al. 1997), marijuana can produce anxiety, analgesia, hypothermia, increased appetite, anti-emetic effects, vasorelaxation, and alterations in cognition and memory (Hollister 1986). A controlled study in healthy cannabis users showed that the intoxicant effects are clearly mediated by CB_1 receptors (Huestis et al. 2001). In rodents, administration of cannabis or its major psychoactive ingredient, THC, produces a characteristic combination of four signs, analgesia, hypoactivity, catalepsy and hypothermia, associated with anxiogenesis, memory changes and cardiovascular changes (Chaperon and Thiebot 1999). The tetrad of behavioral and physiological assays (motor activity, ring catalepsy, body temperature and analgesia tests) was developed to assess in vivo activity of cannabinoid analogs in mice (Martin et al. 1991). In monkeys, disruption of behavior and static ataxia have also been observed (Branch et al. 1980).

Although cannabis dependence is often considered to be a less serious problem than dependence on other drugs, the number of people seeking treatment for cannabis use in the US is higher than the number seeking treatment for cocaine use (CEWG 2007; Substance Abuse and Mental Health Services Administration 2007). Cannabis produces clear subjective motivational responses in humans, leading to drug-seeking and drug-taking behavior (Maldonado 2002). Many different animal models are used to elucidate the consequences of chronic exposure to cannabinoids and to predict their abuse liability. Tolerance and withdrawal syndromes provide only a partial correlate of their addictive properties. The reinforcement-related motivational properties of drugs, including cannabinoids, can be evaluated using several different behavioral models: drug self-administration, conditioned-place preference/aversion and drug-discrimination paradigms.

2.1 Self-Administration of Cannabinoids

2.1.1 Drug Self-Administration Paradigm

Drug self-administration behavior is one of the most direct and productive approaches for studying the reinforcing effects of psychoactive drugs, which are critical in determining their abuse potential (Johanson and Balster 1978). Even in a non-dependent state, animals and humans will readily self-administer drugs of abuse. Allowing limited access to drugs provides a reliable model for their acute reinforcing effects and a means for exploring the neuropharmacological mechanisms involved in these effects (Koob and Weiss 1990). Reliable and persistent self-administration behavior has now been demonstrated in laboratory animals for almost all drugs abused by humans, including psychostimulants, opiates, ethanol, nicotine (Collins et al. 1984; Goldberg et al. 1981; Koob and Weiss 1990; Yokel 1987; Young and Herling 1986), and recently marijuana (THC) (Justinova et al. 2003, 2008b; Tanda et al. 2000).

During the intravenous self-administration procedure, animals are allowed to self-administer a drug by making an operant response, such as pressing a lever or inserting their nose into a hole (a "nose-poking" response), which activates a pump to intravenously deliver the drug. Subjects are prepared with intravenous catheters. Primates often wear a vest to protect the catheter. In many studies, a certain number of responses is required for each injection, in a procedure known as a fixed-ratio schedule. The behavioral measures in drug self-administration studies include the rate of responding and the number of drug injections delivered. Although there are many variations of the self-administration paradigm, usually the reinforcing efficacy of a tested drug is compared to a standard drug of known abuse potential from a similar pharmacological class and also to the drug's vehicle in the same subject (Bergman and Johanson 1985; Tanda et al. 2000; Young and Woods 1981). These studies are often performed in rhesus (*Macaca mulatta*) or squirrel (*Saimiri sciureus*) monkeys, which have learned to self-administer a drug, for example cocaine, under a schedule requiring a certain number of responses to obtain each injection (e.g., ten-response, fixed-ratio schedule of drug injection) (Goldberg et al. 1971). The drugs being tested are then substituted for the training drug and evaluated for their ability to maintain a level of responding resulting in their frequent injection. It is important to mention that the functional state of the brain reward circuits in naïve versus experienced drug self-administering animals is different, and neurobiological adaptations might predispose to or limit subsequent self-administration (Young et al. 1981).

To study the relative reinforcing efficacy of drugs and compare the effects of pharmacological treatments, progressive-ratio schedules of drug self-administration are often used, in which the number of responses required for each injection increases progressively within a session until the drug-seeking response ceases (Arnold and Roberts 1997). Progressive-ratio schedules allow an estimation of the maximal effort an individual will put forth under a specified set of conditions to obtain a particular reinforcement. The behavioral measure obtained is the maximal number of responses an animal will perform for a single drug injection, often called the "break-point," which is taken as a measure of the motivational strength of the reinforcing event and predicts rewarding efficacy of drugs (Hodos 1961). Many different and more complicated schedules of reinforcement also exist and are used to focus on various aspects of addiction. One of these variations, the second order schedule, is discussed in detail in Sect. 2.1.3.

2.1.2 Fixed-Ratio Schedule

THC and Synthetic Cannabinoids

During the last three decades, many attempts to demonstrate intravenous self-administration of THC or of synthetic CB_1 receptor agonists by experimental animals were relatively unsuccessful (for review see Justinova et al. 2005a; Tanda and Goldberg 2003). In none of these studies were THC or synthetic cannabinoids clearly shown to maintain self-administration behavior that was

persistent, dose-related and susceptible to vehicle extinction and subsequent reinstatement. Only a few studies reported self-administration of THC at levels higher than vehicle controls. In one of these studies (Kaymakcalan 1973), two monkeys out of six acquired THC self-administration behavior, but only after withdrawal from forced automatic intravenous injections of THC, when overt signs of physical dependence occurred.

Although THC has not been found to maintain persistent intravenous self-administration in mice or rats, it has been reported to be self-administered intracerebroventricularly (Braida et al. 2004) and into the VTA and the shell of the nucleus accumbens (Zangen et al. 2006) in rats. There have also been several reports of intravenous and intracerebroventricular self-administration of synthetic CB_1 receptor agonists in rodents: WIN 55, 212-2 (Ledent et al. 1999; Martellotta et al. 1998), CP55940 (Braida et al. 2001b; Navarro et al. 2001) and HU210 (Navarro et al. 2001) in mice and WIN 55, 212-2 in rats (Fattore et al. 2001; Spano et al. 2004). However, the experimental procedures employed in some of these studies limit the scope and generality of the findings. For example, the studies with mice (Ledent et al. 1999; Martellotta et al. 1998; Navarro et al. 2001) employed 1-day experimental tests during which mice were restrained for acute intravenous administration through the tail vein. This procedure provides little information about acquisition, extinction and relapse to self-administration behavior. In contrast, the study by Fattore and colleagues (Fattore et al. 2001) utilized unrestrained, freely moving rats as subjects that were allowed to self-administer WIN 55, 212-2 over repeated daily sessions. Spano and colleagues (Spano et al. 2004) used the same model to provide the first evidence of drug-induced reinstatement of cannabinoid-seeking behavior.

It is important to note that chronic diet restriction (rats were maintained at 80% of their normal body weight) was a necessary condition in the study by Fattore and colleagues (Fattore et al. 2001), since rats on an unrestricted diet did not acquire cannabinoid self-administration behavior. Diet restriction has been repeatedly shown to increase a wide variety of appetitive behaviors, including self-administration of drugs from each of the major classes of abused drugs (Carroll and Meisch 1984). Thus, the need for food restriction may simply indicate that cannabinoid agonists are only weakly reinforcing in rats, or that they may have aversive effects that can counteract their reinforcing effects. In another series of studies, food was not only restricted in the rats, but delivered during the THC self-administration sessions (Takahashi and Singer 1979, 1980). In those studies, THC self-administration behavior above placebo levels was found in diet-restricted rats (maintained at 80% of normal body weight), under conditions where a food pellet was automatically delivered once every minute. However, this self-administration behavior immediately decreased to placebo levels when food restriction was discontinued, suggesting that this was probably a schedule-induced adjunctive behavior, rather than a case of drug reinforcement, per se.

Robust, dose-related, intravenous self-administration of THC by animals was first demonstrated under a fixed-ratio schedule in squirrel monkeys (Tanda et al. 2000). This study utilized monkeys with cocaine self-administration experience that were not food-deprived and had access to THC only after at least 1 week of saline extinction. The dose range of THC in this study (1–8 g kg^{-1} per injection)

was lower than that previously used in THC self-administration studies and comparable to that received from smoking a marijuana cigarette (Agurell et al. 1986; Tanda et al. 2000). Under these conditions, monkeys readily acquired THC self-administration behavior. Once acquired, self-administration behavior could be rapidly extinguished by substituting vehicle for THC or by administering the CB_1 antagonist/inverse agonist, rimonabant, suggesting that the behavior was mediated by CB_1 receptors. The opioid-receptor antagonist naltrexone can also partially reduce THC self-administration (Justinova et al. 2004).

Although earlier attempts to obtain THC self-administration behavior in monkeys that had prior experience with cocaine self-administration were unsuccessful – even when THC was directly substituted for cocaine with no intervening vehicle extinction (Harris et al. 1974) – the fact that the monkeys in the study by Tanda and colleagues (Tanda et al. 2000) had prior experience with cocaine raised the possibility that cocaine might induce persistent neurobiological adaptations that subsequently predispose animals to self-administer THC (Maldonado 2002). However, such adaptations are clearly not a necessary condition, since further experiments established that drug-naïve squirrel monkeys readily acquired THC self-administration behavior (Justinova et al. 2003).

The ability of THC to maintain drug-taking behavior in monkeys without a history of exposure to other drugs shows that this drug possesses reinforcing properties of its own that are not dependent on prior self-administration of other drugs. These findings support the previous conclusion that, under certain experimental conditions, THC has a pronounced abuse liability that is comparable to that of other drugs of abuse (Justinova et al. 2005a). Self-administration of THC by squirrel monkeys provides the most reliable animal model for human marijuana abuse available to date. This animal model now makes it possible to study the relative abuse liability of other natural and synthetic cannabinoids and to preclinically assess new therapeutic strategies for the treatment or prevention of marijuana abuse in humans.

AEA and Methanandamide

Building on the procedures that were successfully used to obtain THC self-administration in squirrel monkeys, it was shown that the endocannabinoid AEA is also self-administered by squirrel monkeys, with or without previous exposure to other drugs (Justinova et al. 2005b). This study also showed that methanandamide, a longer-lasting synthetic analog of AEA, can serve as an effective reinforcer of drug-taking behavior when self-administered intravenously by squirrel monkeys. The reinforcing effects of both AEA and methanandamide in squirrel monkeys appear to be mediated by CB_1 receptors, because pre-session treatment with the CB_1 antagonist/inverse agonist rimonabant dramatically decreased self-administration behavior for both cannabinoids. The fact that the endocannabinoid AEA is self-administered is consistent with the hypothesis that the release of endogenous cannabinoids is involved in brain reward processes and that activation of CB_1 receptors by AEA is part of the signaling of natural rewarding events (Solinas

et al. 2007d, 2008). As discussed below, intravenous self-administration of AEA by squirrel monkeys provides a procedure for studying the potential abuse liability of medications that activate the endogenous cannabinoid system by interfering with inactivation of endocannabinoids and for investigating mechanisms involved in the reinforcing effects of endocannabinoids.

Fatty Acid Amide Hydrolase (FAAH) Inhibitors and AEA Transport Inhibitors

URB597

Interest in the development of medications that enhance endocannabinoid signaling in the brain without inducing the psychotropic side effects associated with systemic administration of direct acting CB_1 receptor agonists (like THC) led our laboratory to study the selective FAAH inhibitor URB597, focusing on evaluating its abuse liability and measuring its effects on endocannabinoid levels in the brain. We found (Justinova et al. 2007, 2008a) that URB597 suppresses FAAH activity and increases AEA levels in regions of the squirrel monkey brain that participate in motivational, cognitive and emotional functions. This effect is accompanied by a marked decrease in the levels of 2-AG, which would presumably have major effects on endocannabinoid signaling in the brain. This was surprising, because URB597 does not affect activity of the 2-AG-metabolizing enzyme; it may be due to enhanced levels of AEA causing a compensatory up-regulation in 2-AG mobilization.

We further observed that, over a broad range of experimental conditions, URB597 does not display overt reinforcing properties in monkeys. Indeed, the drug did not have reinforcing effects (i.e., was not self-administered more than vehicle) even when its cumulative intake exceeded by several fold a fully effective dose for FAAH inhibition. Furthermore, neither previous cocaine nor THC exposure predisposed monkeys to self-administer URB597. Indeed, even monkeys that had previously self-administered AEA at very high rates failed to self-administer the FAAH inhibitor. Finally, URB597 did not alter the reinforcing effects of THC or cocaine, and did not reinstate extinguished drug-seeking behavior in monkeys that had previously self-administered THC or cocaine. These results indicate that the potentiation of endogenous AEA-mediated transmission produced by URB597 is insufficient per se to produce reinforcing effects. Our findings further imply that FAAH inhibitors such as URB597 – which have demonstrated analgesic, anxiolytic, antidepressant and antihypertensive properties in rodents (Gobbi et al. 2005; Jayamanne et al. 2006; Kathuria et al. 2003) – may be used in humans without anticipated risk of inducing abuse or provoking relapse to drug use in abstinent individuals.

AM404

Another mechanism by which brain levels of AEA can be increased is by inhibition of AEA transport into neurons. The most studied drug of this class is AM404,

which has been found to exert anxiolytic effects (Bortolato et al. 2006), serve as a reinforcer of intravenous drug-taking behavior in squirrel monkeys, and reinstate extinguished drug-seeking behavior (Justinova and Goldberg 2004). These findings are consistent with the fact that AM404 produces conditioned place preference, an indication of rewarding properties, in rats housed under enriched conditions (Bortolato et al. 2006). On the other hand, place preference does not develop with URB597 under the same conditions (Gobbi et al. 2005). Although both AM404 and URB597 do not have THC-like discriminative or neurochemical effects in rodents (Gobbi et al. 2005; Solinas et al. 2006a, 2007c), only AM404 has motivational effects in rodents and primates that suggest the potential for abuse.

2.1.3 Second-Order Schedule and Drug Seeking

The second-order schedule of drug self-administration (Goldberg et al. 1975; Schindler et al. 2002) has been strongly advocated as an animal model that can be used to focus on drug-seeking behavior, as opposed to drug taking (Arroyo et al. 1998; Everitt and Robbins 2000). This drug-seeking schedule incorporates drug-related stimuli that model the environmental cues that maintain drug seeking and induce drug craving and relapse in humans. Unlike fixed-ratio schedules of drug self-administration, which can only be used to evaluate the ability of a treatment to block the effects of the abused drug after it has been self-administered, second-order schedules can be used to evaluate treatments that target drug seeking, per se, before the abused drug is received. This is important because treatments that reduce drug seeking might provide an especially effective means of achieving and maintaining drug abstinence. In addition to studying the effects of treatments on drug seeking, the second-order schedule can also be used to study relapse induced by drug-related cues, as well as relapse caused by re-exposure to the abused drug or exposure to other drugs. The study by Justinova and colleagues (Justinova et al. 2008b), described in detail below, took advantage of all of these features of a second-order schedule to study the effects of treatments on the maintenance of and relapse to THC seeking.

In the first study utilizing a second-order schedule of THC self-administration (Justinova et al. 2008b), squirrel monkeys' lever-pressing responses intermittently produced brief presentations of a visual stimulus (a colored light). This drug-seeking response produced only the stimulus until the end of the 30-min session, when the last response of the session produced both the stimulus and intravenous delivery of THC. Monkeys' THC-seeking behavior occurred at a high rate even though the drug was not delivered until the end of the session. This behavior depended on both the delivery of THC and the response-contingent presentations of the drug-paired stimulus. That is, when the brief light stimulus was not presented during the session, THC seeking decreased abruptly and continued to occur at a low rate even when THC was still delivered paired with the stimulus at the end of each session. When both the stimulus and THC delivery were discontinued,

responding ceased, but it was immediately reinstated when stimulus presentations were reinstituted. Thus, like re-exposure to the drug, re-exposure to THC-associated stimuli (cues) was a highly effective trigger for relapse following a period of abstinence.

When the THC-seeking procedure was used to evaluate the effects of potential therapeutic treatments, it was found that the CB_1 antagonist/inverse agonist, rimonabant, was highly effective in reducing the drug-seeking response. Importantly, treatment with rimonabant produced an immediate decrease in THC seeking, indicating that rimonabant blocked the ability of the stimulus to maintain THC seeking. This finding is consistent with a number of studies showing that rimonabant can reduce the behavioral effects of stimuli associated with other drugs of abuse, including nicotine, alcohol, cocaine and heroin (Cohen et al. 2005; De Vries and Schoffelmeer 2005; Fattore et al. 2007; Le Foll and Goldberg 2005; Maldonado et al. 2006), as well as the effects of similar cues under second-order food-seeking procedures (Evenden and Ko 2007; Thornton-Jones et al. 2005). Thus, this effect of rimonabant on responding maintained by drug-paired cues appears to be a general effect, unlike its ability to reduce drug-taking behavior, which seems to be limited to specific drugs (De Vries and Schoffelmeer 2005). This suggests that the ability to block both drug seeking (behavior reinforced by drug-related cues) and drug taking (behavior reinforced directly by the drug) might make rimonabant and similar drugs especially useful for treating cannabinoid use disorders.

In contrast with rimonabant, treatment with the opioid antagonist, naltrexone, had a more limited effect under the second-order schedule. In another study by Justinova and colleagues (Justinova et al. 2004), naltrexone produced a partial reduction in THC taking under a fixed-ratio schedule over most of a 5-day course of treatment. However, under the second-order schedule, naltrexone only decreased THC seeking during the first 2 days of treatment. These results might suggest that, like rimonabant, naltrexone can alter both THC seeking and THC taking, but that naltrexone only partially blocks the reinforcing effects of THC. This finding is consistent with the many studies showing functional interactions between the cannabinoid and opioid systems, but it appears that an opioid antagonist alone might not provide significant protection against drug seeking induced by THC-related environmental cues.

During reinstatement testing with the second-order schedule, it was also found that THC seeking was reinstated when the monkeys were passively exposed to THC, AEA, methanandamide, or the AEA transport inhibitor AM404. Also consistent with evidence for functional links between the cannabinoid and opioid systems (see Sect. 3 for more details), passive exposure to morphine reinstated THC seeking. Although it has been shown that passive cannabinoid exposure can reinstate cocaine seeking in rats (De Vries et al. 2001; Xi et al. 2006), cocaine did not reinstate THC seeking in the second-order study. This finding is consistent with those of Spano and colleagues (Spano et al. 2004), who found that the cannabinoid agonist, WIN 55, 212-2, or heroin, reinstated seeking of WIN 55, 212-2 in rats, but cocaine did not.

Rimonabant and naltrexone were tested to determine whether they could block the reinstating effects of passive exposure to THC or morphine. The cannabinoid

antagonist/inverse agonist only blocked the effects of the cannabinoid agonist, and the opioid antagonist only blocked the effects of the opioid agonist. These findings contrast with those of Spano and colleagues (Spano et al. 2004) that rimonabant and the opioid antagonist, naloxone, were both capable of preventing WIN 55, 212-2-induced as well as heroin-induced reinstatement of WIN 55, 212-2 seeking in rats. This discrepancy could be due to differences between rats and monkeys, or due to differences between THC and WIN 55, 212-2, which show different profiles of non-cannabinoid receptor binding.

2.2 Conditioned Place Preference and Aversion with Cannabinoids

2.2.1 THC and Synthetic Cannabinoids

An alternative way to assess the rewarding effects of cannabinoids in experimental animals is to study cannabinoid-induced conditioned place preference. Although methodological details differ among laboratories, a typical place-conditioning experiment involves differentially pairing a distinct set of environmental (contextual) cues with the effects of a drug. This occurs in a training chamber with two compartments. During the conditioning procedure, the animal receives the drug in one compartment and receives vehicle in the other. These pairings are repeated several times over a number of days. Following conditioning, a choice test is conducted in which a door is opened between the two compartments, and the animal is allowed unrestricted access to both contexts in the absence of the drug. An increase in time spent in the drug-paired context relative to a control value is taken as evidence that the drug has rewarding effects. On the other hand, a decrease in time spent in the drug-paired context is taken as evidence that the drug has aversive effects.

Unfortunately, the results of conditioned place preference studies with cannabinoid agonists have ranged from positive place preference to no effect to place aversion. THC, as well as synthetic cannabinoid agonists like CP55940 (McGregor et al. 1996), WIN 55, 212-2 (Chaperon et al. 1998) and HU210 (Cheer et al. 2000), can induce conditioned place aversion in rats (Hutcheson et al. 1998; Mallet and Beninger 1998; Parker and Gillies 1995; Sanudo-Pena et al. 1997) and mice (Valjent and Maldonado 2000). THC-induced conditioned place preferences have been reported within limited dose ranges and under restricted experimental conditions in rats and in mice (Braida et al. 2004; Ghozland et al. 2002; Le Foll et al. 2006; Lepore et al. 1995; Valjent and Maldonado 2000). CP55940-induced conditioned place preference have been reported in rats (Braida et al. 2001a). Interestingly, THC microinjections into the VTA or the shell of the nucleus accumbens can produce conditioned place preference in rats (Zangen et al. 2006). Because of the unresolved inconsistencies in this area of research, it is difficult to draw general conclusions on whether cannabinoids have rewarding or aversive effects in this paradigm.

One explanation for this inconsistency might be that THC's rewarding effects in place-conditioning procedures are often masked or reversed by its aversive effects. Differences are reported to exist in the rewarding and aversive effects of cannabinoids in rats and mice in a measure of anxiety. Cannabinoid agonists produced predominantly anxiolytic effects in mice, but predominantly anxiogenic effects in rats (Haller et al. 2007). There also seem to be different mechanisms involved in the THC-induced conditioned place preference compared to aversion. It was found that aversions in mice depend on kappa-opioid receptors (Cheng et al. 2004; Ghozland et al. 2002) and endogenous dynorphin (Zimmer et al. 2001), while preference depends on mu-opioid receptors (Ghozland et al. 2002).

Given the difficulty in obtaining cannabinoid self-administration in rodents, place-preference procedures will likely remain a valuable alternative for studying the abuse-related effects of cannabinoid agonists, despite the fact that results have so far been inconsistent. One approach that does not appear to have been attempted is to use cannabinoid-induced place preference to study reinstatement, as has been done with morphine and other drugs (Parker and Mcdonald 2000).

2.2.2 AEA

There are only two studies to date that evaluated rewarding or aversive effects of AEA in a place-conditioning procedure. First, Mallet and colleagues (Mallet and Beninger 1998) compared effects of THC and AEA. Rats in this study received injections of the potent, but non-selective, FAAH inhibitor phenylmethylsulfonyl fluoride (PMSF) prior to AEA injections in order to prolong its half-life. The study showed that THC, but not AEA, induced significant conditioned place aversion. Second, Scherma and colleagues (Scherma et al. 2008a) found that AEA alone had no effects on place conditioning, but it induced conditioned place aversion when its metabolism was inhibited by the selective FAAH inhibitor URB597, which by itself does not produce conditioned place preference or aversion (Gobbi et al. 2005; Kathuria et al. 2003). The latter study by Scherma and colleagues used intravenous catheters for AEA delivery, while in the former study AEA was injected intraperitoneally. It is possible that, when injected intraperitoneally, AEA availability was not sufficient to produce effects in the place preference procedure because of hepatic first-passage metabolism, which does not favor rapid entry of AEA into the brain.

2.3 Discriminative-Stimulus Effects of Cannabinoids

Drug discrimination is a powerful behavioral assay for discerning similarities and differences among drugs active in the central nervous system (CNS). The subjective and perceptible CNS effects of a compound can be evaluated in this paradigm by training subjects to respond differently when these effects are present versus when

they are absent. During drug-discrimination training, the interoceptive effects of a training drug (e.g., THC) are established as a cue for performing a specific operant response (e.g., lever pressing reinforced by food). One of the widely used protocols is the two-lever choice drug-discrimination procedure. Pressing one lever is reinforced during sessions when the training drug has been injected, and pressing on a second lever is reinforced during sessions when vehicle has been injected. Lever choice during test sessions can be used as an indication of whether a novel drug has effects similar to the training drug, or whether a potential therapeutic alters the effects of the training drug (Solinas et al. 2006b). The range of effects measured by drug discrimination is wider than those of direct measures of reward and reinforcement and can include aversive, anxiogenic or anxiolytic effects (Colpaert 1999).

Discriminative-stimulus effects of CB_1 agonists (like THC) in animals show a high degree of pharmacological specificity. Generally, only CB_1 agonists produce discriminative-stimulus effects similar to THC, and only CB_1 antagonists block them (Jarbe et al. 2001; Solinas et al. 2004, 2007c; Wiley et al. 1995a, b). Among non-cannabinoid drugs, only pentobarbital and diazepam have been found to produce partial generalization to a cannabinoid cue (Barrett et al. 1995; Mokler et al. 1986; Wiley and Martin 1999). The effect of diazepam was not blocked by the CB_1 antagonist/inverse agonist rimonabant, suggesting that this effect is mediated by an interaction with the GABAergic system (Wiley and Martin 1999).

Several studies have investigated whether endogenous cannabinoid ligands produce THC-like discriminative stimulus effects when they are systemically administered. AEA does not generally produce THC-like responding in monkeys and rats in drug-discrimination studies or does so only at very high doses that also dramatically depress rates of responding (Burkey and Nation 1997; Jarbe et al. 2001; Wiley et al. 1997, 1998). However, metabolically stable, synthetic analogs of AEA, methanandamide, O1812 and AM1346, did induce THC-like responding (Alici and Appel 2004; Burkey and Nation 1997; Jarbe et al. 2006; Wiley et al. 2004). Thus, AEA's fast metabolic inactivation is likely responsible for its observed weak THC-like discriminative-stimulus effects.

When metabolic inactivation of AEA via FAAH was blocked by the FAAH inhibitor URB597, AEA produced dose-related THC-like discriminative-stimulus effects (Solinas et al. 2007c). URB597 alone did not produce any THC-like effects, even at doses several times higher than those that potentiated the effects of AEA (Gobbi et al. 2005). Another compound interfering with AEA inactivation, AM404, which is thought to inhibit the transport of AEA into neurons, produced no THC-like effects itself, but also did not potentiate the THC-like effects of AEA (Solinas et al. 2007c). These different effects of FAAH blockade and blockade of AEA transport on THC-like discriminative effects of AEA suggest that membrane transport is not the main mechanism for AEA inactivation in the brain regions mediating the discriminative-stimulus effects of THC. Interestingly, nicotine was shown to produce THC-like discriminative effects after FAAH inhibition with URB597 (Solinas et al. 2007b), which implicates nicotine-induced increases in

the release of endocannabinoids in another effect observed in the study, the ability of nicotine to potentiate the discriminative effects of THC.

2.4 Tolerance, Physical Dependence and Behavioral Sensitization

2.4.1 Tolerance

The chronic administration of natural or synthetic cannabinoid agonists induces tolerance to most of their pharmacological effects in numerous animal species (Abood and Martin 1992). Tolerance has been shown to develop to the effects of cannabinoids involving antinociception, decreased locomotion, hypothermia and catalepsy, and neuroendocrine effects (Martin 2005), but studies of tolerance to the effects of THC on learning and memory in rats have been contradictory (Delatte et al. 2002; Nava et al. 2001). The development of cannabinoid tolerance is rapid, and a marked decrease of the acute response can sometimes be observed after only the second administration of a cannabinoid agonist (Abood and Martin 1992; Hutcheson et al. 1998). It has been reported that the total number of CB_1-binding sites significantly decreases in several brain areas, including the striatum, cortex, limbic system and cerebellum, during chronic administration of cannabinoids (Rodriguez de Fonseca et al. 1994; Rubino et al. 2000b, c). Also, there are other cellular adaptations observed in some brain regions which play an important role in the induction of synaptic plasticity due to cannabinoid chronic exposure, such as increased activation of the cAMP pathway (Rubino et al. 2000b) and adaptations in the ERK cascade (Rubino et al. 2004, 2005). Together, the downregulation of CB_1 receptors along with the changes in these second messenger systems seems to be responsible for the development of cannabinoid tolerance.

Furthermore, there seems to be a relationship between the status of the CB_1 receptors and the levels of endocannabinoids. In rats tolerant to THC, there are alterations in endocannabinoid content in various brain regions (Martin 2005). Specifically, AEA concentrations were increased in the limbic forebrain and decreased in the striatum, midbrain and diencephalon of THC-tolerant rats (Gonzalez et al. 2004). 2-AG concentrations increased in the cerebellum, brainstem and hippocampus, whereas they decreased only in the striatum. It appears that the most consistent findings with a number of centrally acting drugs of abuse is that chronic administration leads to an elevation in endocannabinoid levels in the limbic system (see Sect. 1.3). This observation is consistent with the notion that endocannabinoids enhance the reinforcing effects of addictive drugs by increasing dopamine release via the inhibition of GABA release in the limbic system (Martin 2005).

Several studies have revealed that cross-tolerance develops for four of the main behavioral/physiological effects of different exogenous CB_1 agonists (analgesia, hypoactivity, catalepsy and hypothermia) (Pertwee et al. 1993). However, there is not always cross-tolerance between AEA and other cannabinoids. For example,

THC and AEA did not show cross-tolerance to hypothermic effects (Pertwee et al. 1993), but did show cross-tolerance to antinociceptive effects (Welch 1997). Cross-tolerance between opioid and cannabinoid compounds has also been revealed. Morphine and THC elicit cross-tolerance to antinociceptive and hypothermic effects in mice (Thorat and Bhargava 1994). On the other hand, AEA-tolerant mice were not cross-tolerant to opioids (Welch 1997). Results such as these probably indicate that tolerance to some effects of AEA involves cannabinoid mechanisms, but tolerance to other effects of AEA does not.

2.4.2 Physical Dependence

Abstinence from cannabis use by chronic users does not produce signs of withdrawal as pronounced as those seen in opioid, ethanol, or barbiturate users. Nonetheless, withdrawal from THC has been reported to induce withdrawal symptoms in both humans (including craving for the drug, decreased appetite, sleep disturbances, anger and aggression (Haney et al. 1999a, b)) and animals (Aceto et al. 1996; Taylor and Fennessy 1982; Verberne et al. 1981). It is likely that the severity of these withdrawal symptoms when use is discontinued is limited by the slow release of THC from its depot in fat tissues, where it is stored due to its highly lipophilic nature. This hypothesis is consistent with the fact that administration of the CB_1 antagonist/inverse agonist rimonabant generally precipitates a pronounced withdrawal syndrome in animals that have been chronically treated with cannabinoids (Aceto et al. 1995; Costa et al. 2000; Hutcheson et al. 1998). There are conflicting reports on the ability of rimonabant to precipitate withdrawal signs in rats chronically treated with AEA, which has a short duration of action (Aceto et al. 1998; Costa et al. 2000).

The behavioral signs of CB_1 antagonist-precipitated cannabinoid withdrawal in rodents include increased grooming, wet-dog shakes, a hunched-back posture, piloerection, body tremors, paw tremors and ptosis. The CB_1 antagonist/inverse agonist rimonabant failed to precipitate behavioral manifestations of abstinence in CB_1 knockout mice given long-term treatment with THC (Ledent et al. 1999), indicating further that somatic signs of abstinence are CB_1-receptor mediated. Microinjection of rimonabant into the cerebellum induced severe manifestations of abstinence in mice dependent on WIN 55, 212-2 (Castane et al. 2004). When the CB_1 antagonist/inverse agonist was administered into the hippocampus and the amygdala, a moderate but significant withdrawal syndrome was also observed. However, no signs of withdrawal were induced when rimonabant was microinjected into the striatum. The cerebellum, and to a lesser extent the hippocampus and the amygdala, participates in the behavioral expression of cannabinoid withdrawal (Castane et al. 2004).

Neurochemical adaptive changes have also been demonstrated during antagonist-precipitated cannabinoid withdrawal in rats and mice, including activation of corticotropin releasing factor (Rodriguez de Fonseca et al. 1997), pronounced increases in the activity of the cAMP pathway in the cerebellum (Hutcheson et al.

1998), and decreases in dopamine transmission in the shell of the nucleus accumbens (Tanda et al. 1999). Some of these signs also occur during withdrawal from other drugs of abuse, such as alcohol (Rossetti et al. 1991), cocaine (Richter et al. 1995) and morphine (Acquas et al. 1991).

Spontaneous cannabinoid withdrawal produced significant time-related alterations in gene transcription (Oliva et al. 2003), such as decreased tyrosine hydroxylase mRNA levels in the ventral tegmental area and increased levels in substantia nigra; increased proenkephalin gene expression in caudate-putamen, nucleus accumbens, olfactory tubercle and piriform cortex; and increased pro-opiomelanocortin gene expression in the arcuate nucleus of the hypothalamus. These alterations induced by spontaneous cannabinoid withdrawal could play a role in the altered vulnerability to other drugs of abuse, as well as in schizoaffective disorders, observed in cannabis users.

2.4.3 Behavioral Sensitization

Behavioral sensitization, an increased response to the drug after repeated exposure, is another adaptive neurobiological alteration that occurs after repeated exposure to drugs. The ability to produce this phenomenon is shared by many drugs abused by humans (e.g., opioids, psychostimulants, nicotine and phencyclidine) and has been proposed to play a role in addiction (Robinson and Berridge 1993, 2001), particularly in drug-seeking behavior persisting long after discontinuation of drug use (De Vries et al. 1998). Repeated exposure to cannabinoid agonists can induce behavioral sensitization (Cadoni et al. 2001; Pontieri et al. 2001b), which is typically observed as an increase in behavioral activity in response to a drug challenge given weeks after the last training injection. However, a recent study (Varvel et al. 2007) was not able to replicate THC-induced behavioral sensitization in rodents under various protocols. Cross-sensitization may occur between cannabinoid agonists and other drugs abused by humans, including heroin (Pontieri et al. 2001a), morphine (Cadoni et al. 2001) and amphetamine (Lamarque et al. 2001).

The adaptive neurobiological changes underlying cannabinoid-induced behavioral sensitization are only beginning to be understood. Altered CB_1 receptor functionality in the striatum and cerebellum of sensitized rats has been observed, as well as lost responsiveness to cannabinoids by the cAMP pathway in the cerebellum (Rubino et al. 2003). In another study (Cadoni et al. 2008), rats pre-exposed to THC showed behavioral sensitization associated with a reduced stimulation of dopamine transmission in the nucleus accumbens shell and an increased stimulation in the nucleus accumbens core in response to THC challenge. Animals pre-treated with morphine showed behavioral sensitization and differential changes in the dopamine response to a THC challenge, with a decreased response in the shell and an increased response in the core. This suggests that THC-induced behavioral sensitization is associated with changes in the responsiveness of dopamine transmission in the nucleus accumbens subdivisions that are similar to those observed with sensitization induced by other drugs of abuse.

3 Opioids

The existence of functional, bidirectional interactions between the endogenous cannabinoid and opioid systems has been demonstrated in numerous studies. Both systems participate in the common circuits involved in the addictive properties of different drugs of abuse. Mu-opioid and CB_1-cannabinoid receptors are both expressed in brain areas involved in reward processes where they share common signaling cascades (Fattore et al. 2005; Maldonado and Rodriguez de Fonseca 2002). The endocannabinoid system is crucial not only for opioid-induced rewarding effects and relapse, but also in the development of physical dependence during chronic opioid administration.

Cross-dependence has been reported between opioid and cannabinoid compounds. In morphine- or methadone-dependent rodents, the opioid antagonist naloxone precipitated a withdrawal syndrome, which was attenuated by THC or AEA (Hine et al. 1975; Lichtman et al. 2001; Vela et al. 1995). Similarly, morphine decreased withdrawal signs in THC-dependent mice undergoing rimonabant-precipitated withdrawal (Lichtman et al. 2001). Furthermore, rimonabant induced behavioral alterations usually associated with opioid withdrawal when given to morphine-dependent rats, and naloxone induced an opioid withdrawal syndrome when given to animals made cannabinoid-dependent by repeated administration of the potent cannabinoid agonist HU210 (Navarro et al. 1998). However, long-term treatment with rimonabant reduced the intensity of naloxone-precipitated withdrawal in morphine-tolerant animals (Rubino et al. 2000a). In CB_1 knockout mice, the severity of naloxone-precipitated morphine withdrawal was robustly attenuated (Ledent et al. 1999). Reciprocally, the expression of cannabinoid withdrawal was decreased in pre-proenkephalin knockout mice compared to wild-type (Valverde et al. 2000). In contrast, rimonabant-precipitated withdrawal in THC-dependent mice was not affected by deletion of mu, kappa, or delta opioid receptors (Ghozland et al. 2002). Another study (Castane et al. 2003) suggested that cooperative actions of both mu and delta receptors were required for the expression of THC dependence.

Studies of rewarding effects of opioids confirm involvement of the endocannabinoid system. In CB_1 knockout mice, morphine did not induce intravenous self-administration (Cossu et al. 2001), but place-conditioning studies show that morphine-induced place preference may or may not develop in these mice dependent on the conditioning paradigm used (Martin et al. 2000; Rice et al. 2002). Rimonabant reduced opioid self-administration and blocked development of heroin-induced conditioned place preference in rodents (De Vries et al. 2003; Navarro et al. 2001). The effects of CB_1 antagonist/inverse agonists like rimonabant appear to be relatively weak when the effort required to obtain heroin is low (fixed-ratio 1 schedules), but become more pronounced when the effort is high (progressive-ratio schedules) (De Vries et al. 2003; Solinas et al. 2003). Furthermore, CB_1 agonists increased the motivation to self-administer heroin under a progressive-ratio schedule (Solinas et al. 2005). On the other hand, opioid antagonists can block

cannabinoid-induced place preference or cannabinoid self-administration in rodents and primates (Braida et al. 2001b; Justinova et al. 2004). Deletion of mu-opioid receptors in mice abolished THC place preference, and deletion of kappa-opioid receptors abolished THC place aversion, while unmasking THC place preference (Ghozland et al. 2002). This suggests an opposing activity of mu- and kappa-opioid receptors in modulating reward pathways.

The role of the endocannabinoid system in relapse to opioid use has also been established. Blockade of CB_1 receptors can prevent heroin-induced reinstatement of heroin-seeking behavior after a long period of extinction, and CB_1 agonists can reinstate heroin-seeking behavior in rats (De Vries et al. 2003; Fattore et al. 2003; Solinas et al. 2003). Rimonabant can also block cue-induced heroin seeking in rats (De Vries et al. 2003). On the other hand, heroin reinstated cannabinoid-seeking behavior after a long period of abstinence, and this effect was blocked by rimonabant (Spano et al. 2004). In the same study, naloxone blocked heroin-induced cannabinoid-seeking behavior, which further supports the existence of bidirectional opioid–cannabinoid interactions in the central mechanisms underlying relapse. However, in squirrel monkeys, morphine-induced reinstatement of THC seeking under a second-order schedule was not blocked by rimonabant, and THC-induced reinstatement was not blocked by naltrexone (details in Sect. 2.1.3).

Both opioids' and cannabinoids' rewarding effects are related to their facilitatory effects on mesolimbic dopamine transmission. Heroin or morphine-induced activation of dopamine transmission in the nucleus accumbens does not appear to be mediated by CB_1 receptors, because rimonabant does not block this effect (Caille and Parsons 2003; Tanda et al. 1997) and CB_1 knockout mice show normal accumbal morphine-induced dopamine elevations (Mascia et al. 1999). Naloxone, on the other hand, prevented the cannabinoid-induced dopamine elevations in the same area (Tanda et al. 1997).

4 Alcohol

The endogenous cannabinoid system is involved in both the rewarding effects of alcohol and in relapse to alcohol abuse (Vengeliene et al. 2008). The endocannabinoid system seems to participate in alcohol's rewarding properties by modulating its effects on activation of mesolimbic dopamine transmission. Pharmacological blockade of CB_1 receptors blocks dopamine-releasing effects of alcohol, and alcohol did not increase extracellular levels of dopamine in the nucleus accumbens of CB_1 knockout mice (Cohen et al. 2002; Hungund et al. 2003). Alcohol acutely inhibits endocannabinoid transmission (Ferrer et al. 2007), which in turn leads to above normal endocannabinoid transmission in reward-related brain areas during chronic alcohol administration, as was revealed by the downregulation of CB_1 receptors and by increased levels of AEA and 2-AG (Hungund and Basavarajappa 2004).

Pharmacological manipulations of the CB_1 receptors showed that, generally, CB_1 agonists increase (Colombo et al. 2002) and CB_1 antagonist/inverse agonists decrease rodents' oral alcohol consumption in self-administration studies (Arnone et al. 1997; Cippitelli et al. 2005). Although CB_1 receptor blockade can cause suppression of fluid and food intake (McGregor and Gallate 2004), CB_1 antagonist/inverse agonists were still found to decrease alcohol's rewarding effects when this confounding factor was controlled in place-conditioning procedures by giving alcohol intraperitoneally to bypass the oral route of administration (Gessa et al. 2005; Lallemand and De Witte 2006). Moreover, genetic manipulations of the CB_1 receptor confirmed that rewarding effects of ethanol require CB_1 receptor activation, since knockout mice consumed less alcohol in most studies (Crabbe et al. 2006) and did not develop place preference for an alcohol-paired environment (Thanos et al. 2005).

Exposure to the CB_1 agonists WIN 55, 212-2 or THC promotes relapse to alcohol use in abstinent rats (Lopez-Moreno et al. 2004; McGregor et al. 2005), and the CB_1 antagonist/inverse agonist rimonabant blocks cue-induced relapse to ethanol seeking (Cippitelli et al. 2005). The latter study also showed that in a strain of rats bred for its ethanol preference (alcohol-preferring Marchigian Sardinian – msP rats), there is increased CB_1 receptor mRNA expression in brain areas relevant for the processing of reward and reward-associated behaviors. This suggests that altered function of the CB_1 receptor system may be linked to genetic vulnerability to alcohol misuse. In fact, it has recently been reported (Zuo et al. 2007) in a large case-controlled sample that the human CB_1 receptor, which is encoded by the CNR1 gene, may play a role in the development of alcoholism.

There is also a question of whether increased AEA levels in the brain contribute to sustained high levels of alcohol drinking or facilitate relapse to alcohol seeking. Studies in rodents have yielded a spectrum of results so far. One study showed that chronic alcohol-induced increases in extracellular AEA were due to inhibition of AEA transport, but not FAAH, in cerebellar granular neurons of mice (Basavarajappa et al. 2003). Blockade of AEA transport by AM404 in Wistar rats reduced alcohol self-administration, but did not affect the relapse induced by contextual cues associated with ethanol (Cippitelli et al. 2007). Genetic ablation of FAAH in mice resulted in increased alcohol preference and intake (Blednov et al. 2007). Pharmacological inhibition of FAAH by URB597 produced increased alcohol intake in wild-type mice (Blednov et al. 2007), but had no effect on alcohol intake in Wistar or msP rats (Cippitelli et al. 2008). In the latter study, URB597, like AM404, did not affect relapse to alcohol seeking induced by either cues or stress. The lack of effect of AM404 and URB597 on relapse to alcohol seeking suggests the absence of a primary role of AEA in the regulation of alcohol-ingestive behaviors in the rat.

5 Nicotine

The endocannabinoid system is critically involved in the addictive effects of nicotine. Preclinical evidence clearly implicates CB_1 receptors in nicotine addiction, which has led to clinical trials indicating that CB_1 receptor antagonists

(rimonabant) could be useful as therapeutic agents for smoking cessation (Fernandez and Allison 2004). Rimonabant was shown to block nicotine-induced conditioned place preference, nicotine self-administration, cue-induced reinstatement of nicotine seeking, as well as nicotine-induced dopamine release in the nucleus accumbens shell in rats (Cohen et al. 2002; Cohen et al. 2005; De Vries and Schoffelmeer 2005; Le Foll and Goldberg 2004). CB_1 knockout mice did not develop nicotine-induced place preference, but they self-administered nicotine similarly to the wild-type (Castane et al. 2002; Cossu et al. 2001; Merritt et al. 2008). Genetic deletion or pharmacological inhibition of FAAH by URB597 enhanced the expression of nicotine-induced place preference in mice (Merritt et al. 2008). In contrast, in rats pharmacological inhibition of FAAH by URB597 markedly inhibited the development of nicotine-induced place preference, reduced nicotine-induced reinstatement of drug seeking and reduced nicotine-induced dopamine elevations in the nucleus accumbens shell (Scherma et al. 2008b). Also in rats, inhibition of FAAH by URB597 prevented nicotine-induced activation of dopaminergic neurons in the VTA (Pistis et al. 2008). These results point to drugs that inhibit FAAH as potentially useful agents in the treatment of tobacco dependence in humans.

Interactions between nicotine and the endocannabinoid system may underlie the widespread practice of cannabis and tobacco co-administration in humans. For example, in place conditioning procedures, sub-threshold doses of nicotine and THC produced place preference when given in combination (Valjent et al. 2002). Also, nicotine potentiates the discriminative-stimulus effects of low doses of THC, and this effect is mediated in part by the release of AEA (Solinas et al. 2007b). It was further shown that systemic administration of the 7-nicotinic acetylcholine receptor (nACh) antagonist methyllycaconitine significantly reduced not only the discriminative effects of THC and WIN 55, 212-2 and the self-administration of WIN 55, 212-2, but also the ability of THC to increase dopamine levels in the nucleus accumbens shell (Solinas et al. 2007a). These findings suggest that drugs that block 7-nACh receptors can counteract the addictive properties of THC and may be potentially useful agents in the treatment of cannabis abuse in humans.

6 Psychostimulants

The mechanism of action of psychostimulants differs from that of other drugs of abuse. Psychostimulants enhance the activity of dopaminergic neurons by directly acting on the reuptake of monoamines binding to one or multiple monoamine transporters (Rothman and Baumann 2003). There are two primary mechanisms by which psychostimulants affect the dopamine transporter (DAT), but the end result is to inhibit the elimination of dopamine from the synapse and therefore increase the quantity and half-life of synaptic and extrasynaptic dopamine levels (Kalivas 2007). Psychostimulants can be separated into “uptake blockers” (cocaine and methylphenidate) and “releasers” (amphetamines) based on the mechanism of their acute effect on neurotransmitter flux through the DAT. Cocaine binds to DAT,

but is not transported into the presynaptic terminal as surrogate dopamine. Amphetamines also bind to DAT, but also translocate into the cell in place of dopamine and enter the dopamine synaptic vesicles. This causes a large buildup of dopamine in the cytosol and reversal of the direction of DAT to release dopamine into the extracellular space. The general separation of drugs into these two classes helps to functionally distinguish the pharmacological profiles of some of the most commonly used psychostimulants. For example, uptake blockers cause little or no persistent dopamine deficits, whereas releasers can cause persistent deficits in monoaminergic neurons (Riddle et al. 2005).

6.1 Cocaine and Methylphenidate

6.1.1 Cocaine

Results of many preclinical studies indicate that CB_1 receptors are not involved in the primary reinforcing effects of cocaine. For example, the ability to self-administer cocaine was unaffected in CB_1 knockout mice, as was development of cocaine-induced place preference (Cossu et al. 2001; Martin et al. 2000). Blockade of CB_1 receptors by rimonabant did not interfere with cocaine self-administration in mice, rats or monkeys (De Vries et al. 2001; Lesscher et al. 2005; Tanda et al. 2000). However, there are also contrasting reports, such as the demonstration that rimonabant can affect acquisition of cocaine-induced conditioned place preference (Chaperon et al. 1998). Another report (Soria et al. 2005) showed reduced acquisition of cocaine self-administration in CB_1 knockout mice and that the maximal effort to obtain cocaine (break-point under a progressive-ratio schedule) was also significantly reduced in CB_1 knockout mice or after CB_1-receptor blockade in wild-type mice. In the same study, acute cocaine administration induced a similar enhancement in extracellular levels of dopamine in the nucleus accumbens of both CB_1 knockout and wild-type mice. This impairment in cocaine self-administration indicates decreased motivation for cocaine-seeking behavior, suggesting a role for CB_1 receptors in consolidation of the cocaine addictive process, but not in its acute effects on mesolimbic dopaminergic transmission (Maldonado et al. 2006).

The endocannabinoid system does appear to be capable of influencing the reinstatement of extinguished cocaine self-administration behavior, since CB_1 agonists can induce reinstatement of cocaine seeking (De Vries et al. 2001; Spano et al. 2004) (see Sect. 1.4 for details). Cocaine, on the other hand, does not reinstate extinguished cannabinoid-seeking behavior (Justinova et al. 2008b; Spano et al. 2004). Recent evidence shows that acute cocaine administration could alter synaptic plasticity in the brain reward system (i.e., nucleus accumbens) by abolishing a retrograde long-term depression (LTD) mediated by endocannabinoids (Fourgeaud et al. 2004). Behavioral sensitization to cocaine is accompanied by a decrease in excitatory drive to the nucleus accumbens (Thomas et al. 2001) and a reduction of basal extracellular glutamate in the nucleus accumbens (Pierce et al.

1996). Thus the abolition of endocannabinoid-mediated LTD in the nucleus accumbens of cocaine-exposed animals might serve as a compensatory mechanism to counterbalance the general decrease in glutamatergic activity measured in response to cocaine (Fourgeaud et al. 2004). Although the endocannabinoid system does not appear to participate in the primary reinforcing effects of cocaine, it is important for maintaining cocaine-seeking behavior, probably by modulating synaptic processes induced by cocaine (Maldonado et al. 2006).

6.1.2 Methylphenidate

Brain dopaminergic and noradrenergic systems play an important role in impulsive behavior, which is manifested at pathological levels in attention-deficit/hyperactivity disorder (ADHD), for which methylphenidate shows therapeutic efficacy. Impulsivity also plays a crucial role in drug addiction, and prolonged drug intake produces disturbances in inhibition of behavior that might contribute to the compulsivity associated with addiction (Jentsch and Taylor 1999). This hypothesis, that drug addiction and impulsivity are strongly interrelated, has been supported by several recent studies in both humans and laboratory animals demonstrating that elevated impulsivity might predispose individuals to initiate or maintain drug seeking and taking (Pattij and Vanderschuren 2008).

The endocannabinoid system, and particularly CB_1 receptors, has been implicated in higher cognitive functions including attention. In healthy volunteers, marijuana and THC have been demonstrated to increase the occurrence of risk-taking behavior in the laboratory and induce impulsive action in a stop signal task, but not delay aversion (McDonald et al. 2003; Ramaekers et al. 2006), which suggests a role for the cannabinoid system in impulsivity. A recent study provided evidence for a differential involvement of the endocannabinoid system in independent measures of impulsivity, as the CB_1 antagonist/inverse agonist rimonabant primarily affected inhibitory control, and did not affect either impulsive choice nor response inhibition, whereas the CB_1 agonist WIN 55, 212-2 only slightly affected response inhibition (Pattij et al. 2007).

6.2 Amphetamine, Methamphetamine and 3,4-Methylendioxymethamphetamine (MDMA)

Dopamine–endocannabinoid interactions have been suggested to be important for the development of amphetamine-induced behavioral sensitization. AEA and 2-AG are differentially modulated by dopamine, via activation of D_1 and D_2 receptors (Patel et al. 2003), which play a significant role in the induction and expression of amphetamine sensitization. Repeated exposure to THC can induce behavioral sensitization not only to cannabinoids, but also to psychostimulants, including

amphetamine (Gorriti et al. 1999; Lamarque et al. 2001). In line with this finding is the report that CB_1 knockout mice failed to sensitize to the locomotor stimulant effects of amphetamine (Thiemann et al. 2008). Furthermore, amphetamine-sensitized wild-type animals in that study had decreased levels of AEA and 2-AG in the ventral striatum (which contains the nucleus accumbens). It seems that amphetamine, which directly increases dopamine activity, can trigger a compensatory reduction in cannabinoid levels, most likely via *trans*-synaptic mechanisms within mesolimbic circuitry (van der Stelt and Di Marzo 2003)). However, amphetamine also releases endocannabinoids in rat amygdala, producing LTD by a dopamine-independent mechanism mediated by CB_1 receptors (Huang et al. 2003), and these endocannabinoids participate in the synaptic plasticity produced by amphetamine in mesocorticolimbic structures (Wolf et al. 2004).

Studies investigating involvement of the endocannabinoid system in the reinforcing effects of amphetamines show conflicting results. Amphetamine is self-administered in CB_1 knockout mice (Cossu et al. 2001). On the other hand, the CB_1 antagonist/inverse agonist AM251 decreased and AEA and methanandamide increased methamphetamine self-administration under a fixed-ratio schedule in rats (Vinklerova et al. 2002). Rimonabant was also shown to block methamphetamine- and cue-induced reinstatement of methamphetamine-seeking behavior in rats (Anggadiredja et al. 2004). Studies with MDMA showed contradictory effects as well. Blockade of CB_1 receptors antagonized MDMA-induced place preference (Braida et al. 2005), but increased intracerebroventricular self-administration of MDMA (Braida et al. 2004). The increase in operant responding induced by rimonabant indicates a decreased motivation to self-administer amphetamine and its derivatives, suggesting that the endocannabinoid system influences the mechanisms regulating MDMA's reinforcing effects (Sala and Braida 2005).

It is important to note that, as with alcohol, marijuana, and heroin, a human genetic variant of the cannabinoid CB_1 receptor gene *CNR1* has been associated with susceptibility to cocaine and amphetamine dependence (Ballon et al. 2006; Comings et al. 1997; Zhang et al. 2004).

7 Endocannabinoid System and Treatment of Drug Addiction

As can be seen by the large number of studies in this area in recent years, the role of the endocannabinoid system in drug abuse and addiction is the focus of intense activity. This interest is generated for several important reasons. Endocannabinoids appear to modulate the direct reinforcing effects of many drugs, the ability of these drugs to induce relapse, and perhaps most interestingly, the ability of drug-related cues to induce relapse. The abuse of cannabis itself is a widespread phenomenon, and large numbers of people seek treatment for cannabis dependence each year. Cannabinoid antagonists represent a unique approach to the treatment of substance abuse (including obesity and addiction to both licit and illicit drugs). Along with replacement therapy (e.g., methadone, nicotine replacement), aversion therapy

(e.g., Antabuse), and antagonist or mixed agonist therapies that are specific for opioid addiction (e.g., naltrexone and buprenorphine, respectively), manipulations of the endocannabinoid system offer one of the very few kinds of pharmacotherapeutic treatments that have shown promise for treating addiction. Among these treatments, cannabinoid-based therapies may be the only ones with the potential to target addiction and relapse, per se, as opposed to targeting the abuse of a single substance. Unfortunately, the recent rejection of the CB_1 antagonist/inverse agonist rimonabant as an aid to smoking cessation by the FDA indicates that the search for a cannabinoid-related treatment for addiction is just beginning. Recently developed neutral antagonists that in animals appear to lack the unwanted side effects of CB_1 antagonist/inverse agonists such as rimonabant (details in Sect. 4), as well as drugs such as FAAH inhibitors that alter endocannabinoid signaling, are two examples of potentially useful approaches to cannabinoid-related treatment of addiction. As our understanding of the endocannabinoid system rapidly increases, it is hoped that the promise of safe and effective therapies based on this system will soon be realized.

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