Cannabinoid Tolerance and Dependence

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Abstract The use of marijuana for recreational and medicinal purposes has resulted in a large prevalence of chronic marijuana users. Consequences of chronic cannabinoid administration include profound behavioral tolerance and withdrawal symptoms upon drug cessation. A marijuana withdrawal syndrome is only recently gaining acceptance as being clinically significant. Similarly, laboratory animals exhibit both tolerance and dependence following chronic administration of cannabinoids. These animal models are being used to evaluate the high degree of plasticity that occurs at the molecular level in various brain regions following chronic cannabinoid exposure. In this review, we describe recent advances that have increased our understanding of the impact of chronic cannabinoid administration on cannabinoid receptors and their signal transduction pathways. Additionally,

we discuss several potential pharmacotherapies that have been examined to treat marijuana dependence.

Keywords Cannabinoid · Dependence · Marijuana · THC · Withdrawal · Tolerance · Rimonabant · Cannabis

1 Introduction

The worldwide use of cannabis for recreational as well as for medicinal purposes has resulted in a large population of individuals chronically using this drug. Consequently, many recent reviews have focused on the long-term consequences of chronic marijuana use, particularly as it relates to tolerance and dependence (Lichtman et al. 2002; Maldonado 2002; Maldonado and Rodriguez De Fonseca 2002; Tanda and Goldberg 2003; Martin et al. 2004). Although similar antecedents (i.e., prolonged exposure to a particular drug) lead to tolerance and withdrawal, both processes are mediated by different neurochemical and neuroanatomical substrates. The profound behavioral tolerance that occurs during repeated administration of Δ^9-tetrahydrocannabinol (THC), the primary active constituent of marijuana, or other cannabinoid agonists illustrates the high degree of plasticity that can occur within the endocannabinoid system. This plasticity can be attributed, in part, to changes that occur to the CB_1 receptor, cell signaling processes, and possibly changes in levels of endogenous cannabinoids in the CNS. In contrast, termination of chronic cannabinoids results in a variety of withdrawal symptoms in humans as well as laboratory animals. In humans, abstinence from continual marijuana use leads to delayed withdrawal symptoms manifested as physiological symptoms of decreased appetite and weight loss, as well as emotional changes, which include irritability, anxiety, restlessness, and strange dreams. Acceptance that these symptoms reflect a clinically significant withdrawal syndrome is gaining. In addition, the availability of several laboratory animal models of cannabinoid withdrawal is playing an important role in understanding cannabinoid dependence and may contribute to the development of pharmacotherapies. In this review, we will discuss published research that has been conducted on (1) characterizing cannabinoid tolerance and dependence, (2) providing insight into the mechanisms of action underlying cannabinoid tolerance and dependence, and (3) evaluating potential pharmacotherapies to treat cannabinoid withdrawal.

2 Reinforcing Effects of Cannabinoids in Animals

Despite the fact that marijuana has consistently been the most commonly used illicit drug for more than 25 years (Johnston et al. 2004), it is only relatively recently that cannabinoids have been shown to elicit rewarding effects in animal models of addiction, including drug self-administration, conditioned place-preference, and

intracranial self-stimulation paradigms. As this issue is the topic of several recent reviews (Gardner 2002; Maldonado 2002; Maldonado and Rodriguez de Fonseca 2002; Tanda and Goldberg 2003; Varvel et al. 2004), it will only be briefly discussed here.

The most compelling preclinical evidence suggesting that a drug has reinforcing properties comes from drug-self administration studies. Most early studies attempting to determine whether THC is self-administered in laboratory animals met with failure, except for studies by Takahashi and Singer showing that THC self-administration occurs in food-deprived rats when a fixed-time 1-min non-contingent food delivery schedule is operating (Takahashi and Singer 1979, 1980). However, the refinement of procedural issues, particularly the use of sufficiently low doses that lacked both motor and aversive effects, has led to several recent papers demonstrating that mice (Martellotta et al. 1998; Ledent et al. 1999), rats (Braida et al. 2001b; Fattore et al. 2001), and squirrel monkeys (Tanda et al. 2000; Justinova et al. 2003) will self-administer Δ^9-THC as well as exogenous cannabinoids (e.g., WIN 55,212-2 and CP 55,940).

In the conditioned place preference (CPP) paradigm, subjective effects of a given drug are repeatedly paired with stimuli associated with one of two experimental chambers, while the other chamber is repeatedly paired with vehicle injections. On test days, the subjects are given free access to both chambers, and the relative amount of time spent in the drug-paired chamber is taken as an indicator of the rewarding/aversive properties of that drug. As in the case of self-administration, the occurrence of preference for the chamber paired with drug is generally inversely related to drug dose: low doses lead to a place preference (Lepore et al. 1995; Valjent and Maldonado 2000; Braida et al. 2001a,b; Ghozland et al. 2002) while high doses elicit conditioned place aversions (Sanudo-Pena et al. 1997; Hutcheson et al. 1998; Zimmer et al. 2001).

The third general technique that has been used to infer the rewarding properties of drugs is the propensity of a drug to decrease the threshold for electrical self-stimulation of neural reward circuits (see Wise 1996). Substantial work demonstrating that Δ^9-THC decreases brain-stimulation reward thresholds comes from the laboratory of Gardner and colleagues (Gardner et al. 1988, 1989; Gardner and Lowinson 1991). Interestingly, the positive effects of Δ^9-THC in this paradigm are strain dependent, with the greatest efficacy in Lewis rats, followed by Sprague-Dawley rats; there is a complete lack of efficacy in Fischer rats (Lepore et al. 1996).

3 Overview of Cannabinoid Tolerance in Whole Animals

THC and a variety of cannabinoid agonists have been reliably shown to produce a constellation of pharmacological effects as evaluated in a variety of animal behavioral models, including the dog-static ataxia test, rat and monkey drug discrimination, and the tetrad test in mice (i.e., depression of spontaneous activity, antinociception, hypothermia, and catalepsy) (Dewey et al. 1972; Chaperon and Thiebot 1999; Martin 2002). These behavioral effects are well known to undergo

tolerance upon subchronic dosing of THC and potent synthetic cannabinoid agonists, such as WIN 55,212-2, CP 55,940, and HU-210. Not only do these behaviors undergo tolerance at different rates (Fan et al. 1994; De Vry et al. 2004), but also they recover at different rates following cessation of cannabinoid administration (Bass and Martin 2000). These findings suggest that the various behaviors may be subserved by distinct mechanisms for the production and maintenance of tolerance.

THC has also been shown to produce dose-dependent decreases in cerebral glucose utilization, especially in structures subserving limbic and sensory functions (Freedland et al. 2002). After subchronic dosing of THC, the rates of glucose utilization in the majority of brain structures were similar to control levels, indicating that tolerance had taken place (Whitlow et al. 2003). Remarkably, THC continued to produce acute alterations in functional activity in mesolimbic and amygdalar regions, despite repeated THC administration, suggesting the provocative possibility that behaviors subserved by these structures (e.g. anxiety, stress, reward, and memory) may continue to be affected by THC, even after chronic dosing (Whitlow et al. 2003).

Below, we will discuss the cellular changes that occur following repeated cannabinoid administration, and which may underlie behavioral tolerance. However, it is important to note that associative learning is also known to contribute to drug tolerance and dependence (Siegel and Ramos 2002). Using a Pavlovian conditioning paradigm, it was shown that repeated administration of HU-210 resulted in a more rapid development of tolerance to the decreased ambulatory behavior when the drug was administered in the testing environment than when it was given in a separate context (Hill et al. 2004).

3.1 Cellular and Molecular Changes Associated with Cannabinoid Tolerance

The profound tolerance that occurs following repeated administration of cannabinoids illustrates the high degree of plasticity that can occur in the endocannabinoid system. This plasticity can be attributed, in part, to changes that occur to the CB_1 receptor, which include sequestration into an intracellular vesicle (internalization) and either receptor degradation (downregulation) or recycling to the cell membrane (Ferguson and Caron 1998; Krupnick and Benovic 1998). Several significant changes have been demonstrated to occur to CB_1 signaling pathways. Acute stimulation of CB_1 receptors has been demonstrated to activate the pertussis toxin-sensitive G proteins (Howlett et al. 2002), the consequence of which includes inhibition of adenylyl cyclase, decreased Ca^{2+} conductance, and increased K^+ conductance. Further downstream, many intracellular kinases are activated including the mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases type 1 and 2 (ERK1/2) (Bouaboula et al. 1995; Rueda et al. 2000), protein kinase B (PKB, also, known as Akt) (Gomez Del Pulgar et al. 2000), and focal adhesion kinase (FAK) (Derkinderen et al. 1996). Repeated administration of cannabinoids leads to receptor/G protein uncoupling and desensitization. Inter-

estingly, these changes appear to vary between brain regions, which is likely to account for differences in tolerance to various cannabinoid-mediated behaviors. These processes are discussed in the following sections.

3.2 CB_1 Receptor Downregulation

Several groups have demonstrated that repeated cannabinoid administration consistently leads to a reduction in CB_1 receptor levels in brain. Specifically, repetitive treatment with THC, CP 55,940, or WIN 55,212-2 results in a decrease in CB_1 receptor binding in brain sections or membrane homogenates from whole brain (Oviedo et al. 1993; Romero et al. 1997; Breivogel et al. 1999; Rubino et al. 2000b; Sim-Selley and Martin 2002). Autoradiographic studies have allowed analysis of a large number of brain regions and have revealed that downregulation occurs in all CB_1 receptor-containing brain regions following subchronic administration of WIN 55,212-2 or THC, including cerebellum, hippocampus, caudate-putamen, globus pallidus, substantia nigra, prefrontal, cingulate and entorhinal cortices, nucleus accumbens, amygdala, hypothalamus, thalamus, and periaqueductal gray (Sim-Selley and Martin 2002). However, the magnitude of downregulation varies in different brain regions. For example, comparatively small changes are found in the basal ganglia output nuclei (globus pallidus, entopeduncular nucleus, and substantia nigra). In addition, regional differences in CB_1 receptor downregulation are not constant throughout the period of tolerance development (Romero et al. 1998b; Breivogel et al. 1999). Downregulation occurs more rapidly and with greater magnitude in the hippocampus and cerebellum compared with the basal ganglia.

Although the mechanisms that regulate CB_1 receptor synthesis, posttranslational modification, degradation, and internalization remain largely unknown, studies are beginning to address this area. In hippocampal neuronal cultures and in a Xenopus oocyte expression system, CB_1 receptor desensitization has been shown to require G protein-coupled receptor kinase (GRK) and β-arrestin (Jin et al. 1999; Kouznetsova et al. 2002). Internalization of CB_1 receptors following agonist treatment in CB_1 receptor-transfected cells (Hsieh et al. 1999) and hippocampal cultures (Coutts et al. 2001) has been shown to lead to internalization of CB_1 receptors.

Another possible explanation for decreased CB_1 receptor binding after chronic cannabinoid treatment is that receptor synthesis has been attenuated. However, studies investigating CB_1 receptor mRNA following repeated cannabinoid administration have resulted in mixed results. In one study, CB_1 receptor mRNA was decreased only in the caudate-putamen of animals treated with THC or CP 55,940 (Rubino et al. 1994). The duration of THC treatment as well as the interval between drug injection and sacrifice appear to contribute to the direction and magnitude of change in CB_1 receptor mRNA (Zhuang et al. 1998). However, the results of this study also suggested that alterations in CB_1 receptor synthesis underlie adaptations in the caudate-putamen, but not in the hippocampus and cerebellum.

3.3 CB_1 Receptor-Activated G Proteins and Effectors

Agonist-stimulated [^{35}S]GTPγS binding has been a very useful assay to investigate the consequences of receptor downregulation and desensitization. Following THC administration for 3 weeks, a high level of CB_1 receptor desensitization occurs in many brain areas of the rat, including the hippocampus, cerebellum, caudate-putamen, globus pallidus, substantia nigra, septum, and cortex (Sim et al. 1996a). The pattern of desensitization was similar to that of downregulation, with the globus pallidus and substantia nigra exhibiting the smallest magnitude of change. Repeated administration of THC, WIN 55212-2, CP 55,940 or anandamide results in CB_1 receptor desensitization (Breivogel et al. 1999; Rubino et al. 2000a,b; Sim-Selley and Martin 2002). As in the case of downregulation, desensitization develops at different rates and magnitudes in different brain regions (Breivogel et al. 1999). Desensitization in the hippocampus occurs rapidly and with a large magnitude, while desensitization in the cerebellum develops at a slightly slower rate, but to the same magnitude, as compared to the hippocampus. However, the caudate putamen and globus pallidus exhibit slower rates of development and smaller magnitudes of desensitization than found in hippocampus and cerebellum. Whereas changes in G protein mRNA have been found and could contribute to loss of CB_1 receptor-mediated G protein activity, these alterations were not accompanied by concomitant changes in protein expression (Rubino et al. 1997). Thus, it is unclear whether the loss of CB_1 receptor-mediated G protein activity is the result of CB_1 receptor downregulation, receptor–G protein uncoupling, altered G protein expression, or some combination of these adaptations.

The results of cell culture models have yielded convincing evidence demonstrating that subchronic cannabinoid administration leads to desensitization of adenylyl cyclase and ERK1/2 (Rinaldi-Carmona et al. 1998). In contrast, the results have been somewhat mixed when attempts have been made to examine this signaling pathway in whole animals. Mice treated repeatedly with CP 55,940 did not exhibit altered CB_1-mediated inhibition of adenylyl cyclase in the cerebellum (Fan et al. 1996). In contrast, several other studies have reported increases in basal, forskolin or Ca^{2+}-stimulated adenylyl cyclase activity in the cortex, striatum, and cerebellum of mice treated subchronically with THC (Hutcheson et al. 1998; Rubino et al. 2000b; Tzavara et al. 2000).

Tolerance procedures have great utility for identifying signaling events that are uniquely specific to the pharmacological effects of cannabinoids. In particular, regional brain differences in the magnitude and time-course of downregulation and desensitization appear to occur concomitantly (Sim-Selley 2003). For example, the time-courses for development and recovery of tolerance that develops to cannabinoid hypothermia, hypomotility, antinociception, and memory impairment appear to be associated with CB_1 receptor adaptation in hypothalamus, striatum, cerebellum, periaqueductal gray, spinal cord, and hippocampus.

CB_1 receptor-mediated G protein activation leads to activation of $G\alpha_i$, attenuation of adenylyl cyclase activity, decreased cyclic AMP (cAMP) synthesis, and ultimately decreased protein kinase A (PKA) activity (Howlett et al. 2002). Chronic

cannabinoid administration elevates cAMP that consequently increases PKA activity (Rubino et al. 2000b), an effect that is augmented by administration of a CB_1 receptor antagonist (Hutcheson et al. 1998; Tzavara et al. 2000). Free $\beta\gamma$-dimers also regulate other cellular signaling events, such as ion channel conductance, phospholipid metabolism, and the activity of several intracellular kinases. Cannabinoids stimulate ERK1/2 activation in cultured cell lines (Bouaboula et al. 1995; Sanchez et al. 1998) and brain (Derkinderen et al. 2003). Interestingly, in hippocampus and neuroblastoma cells, ERK1/2 activation by cannabinoids was downstream of inhibition of the cAMP/PKA pathway (Davis et al. 2003; Derkinderen et al. 2003). In astrocytoma cells, however, this activation required $G\beta\gamma$-sensitive phosphoinositide 3-kinase (PI3K), similarly to cannabinoid activation of PKB/Akt (Galve-Roperh et al. 2002). Cannabinoids also activate p38 MAPK and c-Jun N-terminal kinase (JNK) in several cell types (Liu et al. 2000; Rueda et al. 2000), though only p38 was activated in hippocampus (Derkinderen et al. 2001a). There is also evidence for cannabinoid activation of non-receptor tyrosine kinases in brain, including a neuronal FAK isoform that was activated via recruitment of the Src family kinase Fyn (Derkinderen et al. 2001b). Similarly to ERK1/2 activation, evidence suggested that these signaling events were a result of inhibition of cAMP/PKA signaling.

Plasticity of an endogenous system often occurs via phosphorylation events. It appears that the endocannabinoid system is no exception. Src tyrosine kinase and PKA are involved in tolerance to spinally mediated cannabinoid analgesia (Lee et al. 2003). Tolerance to THC was reversed with either an Src family tyrosine kinase inhibitor or a PKA inhibitor. Inhibitors of protein kinases C and G (PKC and PKG) and of PI3K were ineffective in altering tolerance to this effect, despite an earlier report that PKC activation disrupted CB_1 receptor signaling through direct phosphorylation of the receptor (Garcia et al. 1998). Underscoring the importance of PKA in the maintenance of THC tolerance, another study also reported that intracerebroventricular administration of a PKA inhibitor reversed tolerance to the antinociceptive, cataleptic, and hypomotility, but not hypothermic, effects of THC (Bass et al. 2004). However, Src tyrosine kinase may also play a role in the THC tolerance, albeit a less prominent one than PKA; its inhibition reversed tolerance to decreased locomotor activity, but failed to affect other in vivo actions of THC. In contrast, PKG and PKC inhibitors failed to affect any measures of tolerance. Collectively, these data suggest that PKA activity plays a major role in THC-induced tolerance, and that THC produces its multiple effects through different signaling pathways. If direct involvement of specific protein kinases in cannabinoid tolerance is confirmed, future studies will need to determine the targets of these protein kinases.

3.4 Changes in Endogenous Cannabinoid Levels

In addition to altering CB_1 receptors and the signal transduction pathways, repeated cannabinoid administration has been associated with differential changes of endogenous cannabinoids in a regionally dependent manner. Reliable decreases of both anandamide and 2-arachidonoyl glycerol (2-AG) were found in striatum,

while increases of only anandamide were found in limbic forebrain of THC-tolerant rats (Di Marzo et al. 2000; Gonzalez et al. 2004). Additionally, significant increases of anandamide were found in limbic forebrain following repeated THC dosing (Gonzalez et al. 2004). No significant differences of either endogenous cannabinoid were found in cerebral cortex of THC-tolerant rats (Di Marzo et al. 2000; Gonzalez et al. 2004). However, the effects of repeated THC administration on 2-AG in cerebellum, brain stem, and hippocampus are less certain. Whereas a recent study reported increased levels of 2-AG in these brain areas (Gonzalez et al. 2004), an earlier study by the same group found no differences (Di Marzo et al. 2000). Nonetheless, intriguing possibilities concerning the consequences of altered levels of endogenous cannabinoid content in specific brain regions of THC-dependent animals have been proposed. Specifically, the altered levels of endogenous cannabinoids may be part of a homeostatic mechanism and have important ramifications for motor behavior and emotional states, as well as for cannabinoid dependence (Gonzalez et al. 2004).

4
Characterization of Cannabinoid Dependence

Although chronic cannabis users have long been known to undergo withdrawal upon abrupt discontinuation of the drug (Fraser 1949; Wikler 1976), a marijuana withdrawal syndrome is not yet included in the Diagnostic and Statistical Manuel of Mental Disorders (DSM-IV 1994). Two factors may contribute to the reluctance of accepting the notion that marijuana dependence is clinically relevant. First, the likelihood of progressing from occasional drug use to daily use is considerably lower for marijuana than for other drugs of abuse such as nicotine, cocaine, or heroin (Anthony et al. 1994). However, given the fact that marijuana has consistently been the most frequently used illicit drug in the United States (Johnston et al. 2004), it should not be surprising that the estimated proportion of Americans dependent on marijuana was 4.2%, which was higher than all other illicit drugs, including those that have a greater abuse potential such as cocaine (2.7%) and heroin (0.4%) (Anthony et al. 1994). Second, the delayed onset of cannabis withdrawal symptoms due to the long half-life of THC is also likely to contribute to the lingering doubts concerning the clinical relevance of a cannabinoid withdrawal symptom. However, a growing body of research indicates that abrupt discontinuation following prolonged cannabinoid administration can lead to physical withdrawal symptoms in humans as well as in laboratory animals.

4.1
Clinical Significance of Cannabis Withdrawal

Although it has been contended that further controlled research is needed to diagnose a withdrawal syndrome in human marijuana users (Smith 2002), criteria for cannabinoid withdrawal have been proposed (Budney et al. 2003). Moreover, converging lines of evidence from retrospective, outpatient, and inpatient stud-

ies indicate a pattern of cannabis withdrawal signs. In an early inpatient study, Jones and his colleagues reported that subjects reported a variety of subjective effects upon abrupt discontinuation from chronic oral THC, which included strange dreams, decreased appetite, restlessness, irritability, sweating, chills, and nausea (Jones and Benowitz 1976; Jones et al. 1976). More recently, similar abstinence symptoms were reported by subjects following abrupt withdrawal from continued administration of either oral THC (Haney et al. 1999a) or inhalation of smoked marijuana (Haney et al. 1999b). In these studies, subjects were found to experience increased anxiety, irritability, and stomach pain, as well as exhibit decreases in food intake. The authors of these studies suggested that daily marijuana use might be sustained, in part, to alleviate or avoid withdrawal effects.

Although a cannabis withdrawal syndrome can be obtained in a controlled laboratory setting, these findings do not address whether a cannabis withdrawal syndrome represents a clinically significant malady. The results of both retrospective and outpatient studies addressing this issue support the hypothesis that a cannabis withdrawal syndrome is indeed clinically relevant. In one study, marijuana users who were identified from a population of alcohol-dependent subjects recalled a variety of symptoms from when they had previously abstained from marijuana smoking (Wiesbeck et al. 1996). These symptoms included nervousness, sleep disturbances, and changes in appetite. Despite the inherent limitations associated with retrospective self-report data in polysubstance abuse subjects, this pattern of withdrawal symptoms was similar to those described in the laboratory studies investigating cannabis and THC and distinct from those associated with other drugs. In another retrospective study, adults seeking treatment for marijuana dependence recalled similar symptoms upon their most recent period of abstinence that included craving for marijuana, irritability, nervousness, restlessness, depressed mood, increased anger, sleep difficulties, strange dreams, decreased appetite, and headaches (Budney et al. 1999). In addition, the amount of marijuana smoked per day yielded a positive correlation with withdrawal severity.

These findings reported in retrospective and laboratory studies have been corroborated in an outpatient study of regular marijuana users (Budney et al. 2001). In this experiment, subjects were instructed to smoke marijuana for 5 days, followed by a 3-day abstinence period, and then this cycle was repeated. Statistically significant withdrawal symptoms reported during each abstinence period included marijuana craving, decreased appetite, sleep difficulty, and a global withdrawal discomfort score that consisted of the other three measures as well as self-reported data of anger, depressed mood, headaches, irritability, nervousness, restlessness, and strange dreams. Additionally, the subjects lost a significant amount of weight during each abstinence phase. The fact that the withdrawal symptoms increased during abstinence from marijuana smoking, returned to baseline when smoking was reinitiated, and increased again during the second abstinence period suggests that the effects were caused by cessation of marijuana use.

In an outpatient study of marijuana users, Budney and his colleagues attempted to characterize the time course and recovery of marijuana withdrawal symptoms (Budney et al. 2003). In addition to the observations that the onset (i.e., 1–3 days) and peak effects (i.e., 2–6 days) were quite gradual, the duration of these effects

was quite prolonged (i.e., 4–14 days). The protracted nature of marijuana withdrawal may contribute to the difficulties in maintaining abstinence. Collectively, this pattern of results is consistent with the hypothesis that cannabis withdrawal symptoms contribute to continued marijuana use.

4.2 Investigation of Cannabinoid Withdrawal in Laboratory Animal Models

Two general categories of dependent measures are used to assess withdrawal in laboratory animals: (1) recording the occurrence of behavioral and physiological changes and (2) the use of operant procedures. In the former approach, subjects are observed for alterations in behavior, and these behaviors are generally either scored as quantal responses or quantified. In the latter approach, animals that had been previously trained to emit an operant response (e.g., lever pressing) for food reinforcement will exhibit decreases in response rates during withdrawal. In both cases, readministration of the drug results in response rates returning to normal. Below, we will first discuss the results of abstinence withdrawal studies conducted in laboratory animals followed by precipitated withdrawal studies.

There are two types of procedures that are used to induce withdrawal in drug-dependent organisms, abstinence withdrawal and precipitated withdrawal. Abstinence withdrawal occurs when drug administration is abruptly discontinued or reduced, following prolonged exposure to the drug. As the body metabolizes and/or excretes the agent, physiological symptoms ranging from mild rebound to severe life-threatening effects can emerge. The pharmacokinetic and pharmacodynamic characteristics of the drug, as well as the degree of dependence, influence the specific withdrawal syndrome, its intensity, and the onset of withdrawal responses. In contrast, a second procedure used to induce withdrawal is the use of a receptor antagonist that precipitates withdrawal in a drug-dependent organism. The antagonist displaces the agonist from the receptor, immediately eliciting withdrawal effects. A typical clinical example of precipitated withdrawal is the treatment of an opioid overdose with naloxone or other opioid receptor antagonist. Upon near instantaneous reversal of respiratory depression and other overdose symptoms, an opioid-dependent individual will present with opioid withdrawal effects. The precipitated withdrawal procedure has been particularly useful in investigating cannabinoid withdrawal symptoms in laboratory animals; however, there are currently no published reports in which this procedure has been used in humans. Below, we will review studies examining cannabinoid dependence in humans and laboratory animals, as well discuss potential pharmacotherapies to alleviate cannabinoid withdrawal symptoms.

4.2.1 Abstinence Withdrawal Versus Precipitated Withdrawal in Laboratory Animals

Research investigating abstinence withdrawal following repeated cannabinoid administration in laboratory animals has led to mixed results. A variety of uncon-

ditional behavioral effects including hyperirritability, tremors, and anorexia have been reported (Kaymakcalan and Deneau 1972), though other studies failed to observe abrupt withdrawal effects following subchronic THC administration to dogs (Mcmillan et al. 1971) or rats (Leite and Carlini 1974; Aceto et al. 1996). Indeed, rhesus monkeys that received chronic THC and trained to press a lever for food exhibit a marked suppression in response rates; an effect that was reversed upon readministration of drug (Beardsley et al. 1986). In addition, rats have been observed to exhibit small, but significant increases in wet-dog shakes and facial rubbing 24 h following discontinuation of continuous infusion of a medium ramping dose, but not a low or high ramping dose, of WIN 55,212-2 (Aceto et al. 2001). Similarly, cessation following repeated CP 55,940 treatment in mice led to subtle behavioral changes that included increases in motor activity and rearing, but decreases in grooming, wet-dog shakes, and rubbing behavior that were interpreted as a withdrawal syndrome (Oliva et al. 2004). The long half-life of these drugs further contributes to the difficulty in studying cannabinoid withdrawal in nonhuman animals. Other related factors that complicate the investigation of abrupt cannabinoid withdrawal in laboratory animals include species differences, strain differences, the time at which withdrawal is assessed, the particular withdrawal measures that are scored, and other methodological issues.

The development of SR 141716 and other selective CB_1 receptor antagonists resulted in powerful pharmacological tools to investigate cannabinoid pharmacology (Rinaldi-Carmona et al. 1994). In addition to its usefulness in determining whether the acute pharmacological actions of an agent are mediated through a CB_1 receptor mechanism and to infer endogenous cannabinoid tone, SR 141716 has been demonstrated to precipitate withdrawal reactions following subchronic administration of cannabinoid agonists in mice (Cook et al. 1998; Rubino et al. 1998), rats (Aceto et al. 1995; Tsou et al. 1995), and dogs (Lichtman et al. 1998). The specific withdrawal effects are species specific, and even within a species a variety of factors affects the withdrawal behavior, including strain, dosing regimen, and test conditions.

Rats exhibit a variety of somatic withdrawal signs that include wet-dog shakes, facial rubs, horizontal and vertical activity, forepaw fluttering, chewing, tongue rolling, head shakes, retropulsion, myoclonic spasms, front paw treading, and eyelid ptosis (Aceto et al. 1995; Tsou et al. 1995). Additionally, SR 141716 dose-dependently reduced food-maintained response rates of THC-tolerant rats, but had no effect in non-tolerant rats (Beardsley and Martin 2000). This disruption of response rates occurred in the absence of somatic withdrawal signs, suggesting that antagonist-precipitated disruptions of operant behavior may be a more sensitive measure of THC withdrawal than the occurrence of unconditional withdrawal behaviors. Others have reported increases in the rate-suppressing effects of SR 141716 in both naïve and THC-tolerant rats, though significantly greater suppression occurred in the tolerant rats compared to naïve animals (Freedland et al. 2003). Again, this effect occurred in the absence of profound somatic signs of withdrawal, further indicating that food-maintained responding is a sensitive measure of the effects of SR 141716-precipitated cannabinoid withdrawal.

SR 141716 was found to precipitate a constellation of withdrawal signs in THC-tolerant dogs that included excessive salivation, vomiting, diarrhea, restless behavior (e.g., circling), trembling, and decreases in social behavior (Lichtman et al. 1998). Several of these signs bore some resemblance to those reported in humans undergoing abrupt withdrawal from THC, including restlessness, nausea, and loose stools (Jones et al. 1981).

Notwithstanding the influence that species and strain differences contribute to the specific withdrawal effects that are observed, the utility of the animal models is verified by the fact that SR 141716 reliably precipitates withdrawal in animals treated repetitively with cannabinoids.

4.2.2 SR 141716 Inverse Activity

One complication in interpreting the results of experiments using SR 141716 to precipitate withdrawal in cannabinoid-dependent animals is that it has inverse agonist properties, in addition to its antagonist actions. Specifically, SR 141716 was found to decrease [^{35}S]GTPγS binding in membranes isolated from human cannabinoid CB_1 receptor-transfected CHO cells (Landsman et al. 1997; Pan et al. 1998), an effect opposite that of cannabinoid agonists (Sim et al. 1996b; Burkey et al. 1997). The observation that SR 141716 is over 7,000-fold more potent as a CB_1 receptor antagonist than as an inverse agonist indicates a high degree of a selectivity as antagonist (Sim-Selley et al. 2001). However, it is difficult to determine whether SR 141716's inverse agonist properties play a role in precipitating cannabinoid withdrawal.

Another important consideration in the design and interpretation of SR 141716-precipitated withdrawal studies is that SR 141716 given alone has been shown to produce mild withdrawal-like effects in naïve (Rodriguez De Fonseca et al. 1997) or vehicle-treated animals (Aceto et al. 1995), such as scratching of the face and body (Aceto et al. 1996; Rubino et al. 1998), head shakes (Cook et al. 1998; Lichtman et al. 2001a), and forepaw fluttering (Rubino et al. 1998). Some of these intrinsic effects of SR 141716 have been linked to other receptor systems. For example SR 141716-induced head twitches were completely blocked by a serotonergic 5-HT_{2A}/5-HT_{2C} receptor antagonist and were partially blocked by an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist as well as by a tachykinin natural killer (NK)1 antagonist (Darmani and Pandya 2000). On the other hand, because SR 141716 lacks affinity for these receptors (Rinaldi-Carmona et al. 1994), it is likely that the serotonergic 5-HT_{2A}/5-HT_{2C}, AMPA/kainate,, and tachykinin NK1 receptor antagonists acted "downstream" from the CB_1 receptor.

It remains to be determined whether SR 141716-induced behaviors are due to inverse agonist activity, blockade of endogenous cannabinoid tone, or noncannabinoid sites of action. However, these intrinsic behavioral effects of SR 141716 do not occur universally. Moreover, the magnitude of SR 141716-induced head shakes and paw tremors in naïve animals is generally significantly less than that found in

cannabinoid-dependent animals (Aceto et al. 1995, 1996; Aceto et al. 1998; Cook et al. 1998). Nonetheless, the fact that SR 141716 can produce "withdrawal-like" behavior in naïve animals underscores the importance of including an appropriate control group comprising animals that receive repeated doses of vehicle, in order to control for the intrinsic effects of SR 141716 at testing.

4.2.3
SR 141716 Precipitated Anandamide Withdrawal?

SR 141716 reliably precipitates mild to moderate withdrawal responses following subchronic dosing of a variety of cannabinoid agonists including THC, WIN 55,212-2, CP 55,940, and HU-210. However, studies evaluating the effect of this antagonist in rats treated repeatedly with anandamide have led to mixed results. SR 141716 failed to precipitate withdrawal in rats that were infused continuously with anandamide over a 4-day period (Aceto et al. 1998). On the other hand, both abstinence withdrawal and SR 141716-precipitated withdrawal were reported to occur in rats treated repeatedly with daily i.p. injections of anandamide (20 mg/kg) (Costa et al. 2000). In addition to the procedural differences between the two studies, the short half-life of anandamide (Willoughby et al. 1997) contributes to the difficulty of evaluating this endocannabinoid in the whole animal. However, the use of mice deficient in fatty acid amide hydrolase (FAAH), the primary enzyme responsible for anandamide catabolism (Cravatt et al. 2001), and selective inhibitors of this enzyme (Kathuria et al. 2003; Lichtman et al. 2004) will be of value for investigating both the dependence liability and other behavioral effects of anandamide. Interestingly, a single nucleotide polymorphism found in the human gene that encodes FAAH, which produces a variant that displays an enhanced sensitivity to proteolytic degradation, was found to be associated with both street drug use and problem drug/alcohol use (Sipe et al. 2002). This finding suggests the intriguing possibility that the FAAH-endocannabinoid system may play a regulatory role in addictive behavior.

4.3
Neuroadaptive Changes Underlying Cannabinoid Dependence

While it is clear that repeated stimulation of CB_1 receptors by cannabinoid agonists is necessary for the development of cannabinoid dependence, recent research is just beginning to shed light on the underlying cellular mechanisms of action as well as brain regions that mediate these effects. Most of the research directed toward these issues has employed an approach in which cannabinoid-dependent animals are subjected to either a precipitated withdrawal or abstinence withdrawal. The subjects are then euthanized and the biochemical and molecular indices are examined. Several measures of interest include assessing changes of the CB_1 receptor, the signal transduction pathway of this receptor, and other neurochemical systems that affect or are affected by this process. Many studies use a strategy in which

behavioral withdrawal signs are also assessed in an attempt to elucidate the relationship between cannabinoid dependence and the underlying neuroadaptations.

4.3.1 Role of the CB_1 Receptor in Cannabinoid Dependence

Repeated administration of a cannabinoid agonist generally results in decreases in CB_1 receptor density in a variety of brain regions as measured by radioligand binding (Romero et al. 1998a; Breivogel et al. 1999). At the level of the G protein, daily injections of THC for 21 days produced significant decreases of CB_1 receptor-stimulated G protein activity in various brain regions, including hippocampus, cerebellum, caudate-putamen, globus pallidus, substantia nigra, septum, and various regions of cortex. In addition to being region-dependent, this desensitization was time-dependent and appeared to be specific for CB_1 receptors and not other G protein-coupled receptors (Breivogel et al. 1999). Although these biochemical correlates are believed to play an important role in the development of tolerance, their role in dependence is less clear. CP 55,940-dependent mice during abstinence were found to undergo subtle behavioral changes that included increases in motor activity and rearing, but decreases in grooming, wet-dog shakes, and rubbing behavior, which were associated with upregulation of CB_1 gene expression in caudate-putamen, ventromedial hypothalamic nucleus, central amygdaloid nucleus, and in the CA1 field of hippocampus, but a decrease in the CA3 field of hippocampus (Oliva et al. 2004).

4.3.2 Cellular Mechanisms Underlying Cannabinoid Dependence

Recent studies have linked alterations in the cAMP second messenger cascade with cannabinoid withdrawal. SR 141716 administered to THC-dependent mice resulted in significant increases of both basal and forskolin-stimulated adenylyl cyclase activity in the cerebellum, but not in other brain regions including the cortex, hippocampus, striatum, and periaqueductal gray (Hutcheson et al. 1998). Similarly, significantly higher levels of calcium–calmodulin-stimulated adenylyl cyclase were found in the cerebellum of THC-dependent rats undergoing withdrawal than in non-dependent rats treated with SR 141716. In another well-designed study (Rubino et al. 2000c), G protein, adenylyl cyclase, and PKA activity were assessed in cerebral cortex, striatum, hippocampus, and cerebellum of rats undergoing precipitated withdrawal. Significant increases of adenylyl cyclase and PKA activity were found in the cerebellum of these animals. However, no effects were found on either receptor density or G protein activity. These findings further implicate the involvement of the cerebellum in cannabinoid dependence and suggest that changes are occurring downstream from the CB_1 receptor.

Functional evidence also suggests that the adenylyl cyclase second messenger cascade in the cerebellum may be involved in cannabinoid withdrawal. An intracerebellar infusion of the cAMP blocker Rp-8Br-cAMPs reduced several behavioral

signs of withdrawal including tremors, ataxia, mastication, front paw tremors, ptosis, piloerection, and wet-dog shakes in THC-dependent mice following SR 141716 challenge (Tzavara et al. 2000). Interestingly, Sp-8Br-cAMPs, a cAMP analog, actually induced each of these behavioral effects in vehicle-treated mice. Taken together with the biochemical data, these intriguing findings suggest that upregulation of cAMP signal transduction in the cerebellum may represent a critical biochemical event underlying precipitated withdrawal.

In addition, there is evidence accumulating that implicates the involvement of corticotropin-releasing factor (CRF) and other hormones associated with stress in cannabinoid dependence. SR 141716 significantly elevated plasma corticosterone levels in rats treated subchronically with HU-210 compared with non-dependent rats that were administered SR 141716 or dependent rats not going through precipitated withdrawal (Rodriguez De Fonseca et al. 1997). In another study, however, SR 141716 failed to significantly increase plasma levels of corticosterone in THC-dependent rats (Gonzalez et al. 2004). However, it is unclear whether this apparent failure to replicate was due to a great deal of variability associated with measuring endocrine levels or other methodological differences, such as the use of different cannabinoid agonists to induce dependence (i.e., HU-210 versus THC).

4.3.3
Brain Areas Implicated in Cannabinoid Dependence

As described above (see Sect. 4.3.2) inhibition of adenylyl cyclase in the cerebellum was found to significantly decrease the expression of cannabinoid withdrawal behaviors (Tzavara et al. 2000). Other compelling evidence supporting the notion that CB_1 receptors in the cerebellum play a predominant role in cannabinoid dependence is recent work from Valverde and colleagues. They found that intracerebral injections of SR 141716 into the cerebellum of mice treated repeatedly with WIN 55,212-2 precipitated robust withdrawal responses, which included significant increases in wet-dog shakes, body tremor, paw tremor, piloerection, mastication, genital licks, and sniffing (Castane et al. 2004). Microinjection of SR 141716 into the hippocampus and the amygdala precipitated a moderate but still significant withdrawal syndrome, suggesting the involvement of these brain regions as well. However, SR 141716 infusion into the striatum failed to elicit any significant increases in withdrawal signs. Collectively, these exciting findings strongly implicate the involvement of the cerebellum and possibly the hippocampus and amygdala in cannabinoid withdrawal (Castane et al. 2004).

The role of CRF during cannabinoid withdrawal appears to involve the amygdala. Specifically, SR 141716 challenge to cannabinoid-dependent rats led to significant concomitant increases in CRF and Fos-immunopositive cell activity in the central nucleus of the amygdala (Rodriguez De Fonseca et al. 1997). Similar alterations in amygdaloid CRF function have also been found following ethanol, cocaine, and opioid withdrawal (for review see Weiss et al. 2001). Moreover, SR 141716 challenge to HU-210-tolerant rats led to increases in mRNA CRF in the central amygdala compared to that of HU-210-tolerant subjects not administered SR 141716 (Caberlotto

et al. 2004). An interpretation of this finding is that increased gene expression in the amygdala contributes to increased CRF release and behavioral withdrawal signs during precipitated withdrawal. It should be noted that the increase in mRNA CRF levels was only a relative increase, as levels did not significantly differ from those of untreated control subjects. It was proposed that repeated HU-210 administration activated a hitherto unknown counter-regulatory process, thereby returning the system to equilibrium in the presence of the drug (Caberlotto et al. 2004). In this provocative model, withdrawal would occur when SR 141716 rapidly displaces the agonist from the receptor so as to cause the purported counter-regulatory process to go unchecked, despite the apparently normal CRF mRNA levels.

Many other brain regions are also affected by SR 141716-precipitated withdrawal. SR 141716 significantly increased CRF-mRNA levels in the paraventricular hypothalamic nucleus of THC-tolerant rats compared to a non-tolerant group (Gonzalez et al. 2004). Additionally, Fos-immunopositive activity has been reported to occur in the accumbens shell, piriform cortex, hippocampus, caudate putamen, ventral pallidum, ventral tegmental area, locus coeruleus solitary tract, and area postrema (Rodriguez De Fonseca et al. 1997). It remains to be established whether these biochemical changes represent an underlying mechanism of action for cannabinoid dependence or are merely correlated with this phenomenon.

4.4 Influences of Other Neurochemical Systems on Cannabinoid Dependence

Considerable evidence is emerging that supports an interaction between endocannabinoid and opioid systems on many physiological processes (for a review see Varvel et al. 2004). For example, an acute injection of morphine significantly reduced the magnitude of SR 141716-precipitated THC withdrawal (Lichtman et al. 2001b). Genetic alteration of the opioid system has also been found to ameliorate SR 141716-precipitated cannabinoid withdrawal. Cannabinoid withdrawal symptoms, as well as tolerance, were significantly diminished in pre-proenkephalin-deficient mice compared to the wild-type mice (Valverde et al. 2000). In contrast, dynorphin knockout mice failed to exhibit statistically significant changes in either THC tolerance or THC withdrawal (Zimmer et al. 2001). On the other hand, assessing cannabinoid withdrawal in μ-opioid receptor knockout mice has led to contradictory results. While these mice exhibited a significant attenuation of SR 141716-precipitated THC paw tremors and head shakes compared to the wild-type controls in one study (Lichtman et al. 2001b), another study found that THC withdrawal was unaffected by deletion of μ, δ, or κ receptors (Ghozland et al. 2002). Still, another study found that THC withdrawal effects were significantly decreased in mutant mice deficient of both μ- and δ-opioid receptors, suggesting the involvement of multiple opioid receptors in the expression of cannabinoid withdrawal (Castane et al. 2003). A variety of methodological factors could contribute to the apparent discrepant results among these studies, including the background strain and dosing regimen.

Studies examining acute blockade of opioid receptors in cannabinoid-tolerant animals have also reported inconsistent findings, with some finding that naloxone precipitated withdrawal (Hirschhorn and Rosecrans 1974; Kaymakcalan et al. 1977). Similarly, naloxone precipitated withdrawal in rats following repeated injections of the potent cannabinoid analog HU-210 for 15 days (Navarro et al. 1998). It should be noted that considerable toxicity occurred following chronic high doses of THC (Kaymakcalan et al. 1977), though no such toxicity was reported in the other studies. Conversely, naloxone was ineffective in precipitating withdrawal in THC-dependent monkeys (Beardsley et al. 1986), pigeons (Mcmillan et al. 1971), or mice (Lichtman et al. 2001b). Considerable methodological differences used among the studies, including the selection of agonist, species, dosing regimen, and the dependence measures, make it difficult to account for the differential effectiveness of the antagonist in precipitating withdrawal. In general, however, the cannabinoid dosing regimen employed in studies that find naloxone precipitated withdrawal tend to be greater than those in which naloxone failed to elicit any withdrawal effects. Nonetheless, naltrexone failed to precipitate any apparent withdrawal effects in marijuana smokers (Haney et al. 2003a).

It has been well established that clonidine, as well as other α_2-agonists, abrogates many of the withdrawal effects in morphine-dependent animals (Fielding et al. 1978) as well as in human opioid addicts (Gold et al. 1978). Clonidine also ameliorated SR 141716-precipitated paw tremors in THC-dependent mice independently of motor depressive or motor impairment effects (Lichtman et al. 2001a). Although clonidine may hold some promise for treating withdrawal, its hypotensive side effects (Gossop 1988) must be considered before any potential development for its use in alleviating drug withdrawal.

4.5 Pharmacotherapies for Cannabis Dependence

Despite a great need, there are currently no efficacious pharmacotherapies for the treatment of cannabinoid dependence (for a review see McRae et al. 2003). Haney and her colleagues have made important inroads into this area by evaluating several potential treatments, including two antidepressants, bupropion (Zyban) (Haney et al. 2001) and nefazodone (Serzone) (Haney et al. 2003b), the mood stabilizer divalproex, and oral THC (Haney et al. 2004) in double-blind studies (see Table 1). During abstinence from marijuana, the placebo treatment group reliably reported significant increased ratings of marijuana craving, misery, anxiety, trouble sleeping, "strange dreams," chills, irritability, and muscle pain, but decreased ratings of feeling high, content, friendly, social, mellow, and self-confident. Oral THC decreased many of these withdrawal measures such as marijuana craving, feelings of anxiety, misery, trouble sleeping, and chills, and increased the ratings of self-confidence (Haney et al. 2004). In addition, total daily caloric intake was significantly decreased during the withdrawal period in the placebo group and increased in the oral THC group.

Table 1. Potential pharmacotherapies to treat marijuana dependence

Drug/class	Positive effects for marijuana withdrawal	Potential problems	Reference
Oral THC/ cannabinoid agonist	Decreased marijuana craving and withdrawal symptoms	Potential dependence to THC	Haney et al. 2004
Divalproex/ mood stabilizer	Decreased marijuana craving	Worsened anxiety and other withdrawal indices	Haney et al. 2004
Nefazodone (Serzone)/ antidepressant	Decreased anxiety	Ineffective on most withdrawal measures; may cause liver failure	Haney et al. 2003b
Naltrexone/opioid antagonist	No apparent effect	Increased "positive" subjective effects of oral THC	Haney et al. 2003a
Bupropion (Zyban)/ antidepressant	No apparent benefit	Worsened anxiety and other withdrawal indices	Haney et al. 2001
Fluoxetine (Prozac)/ antidepressant	Reduced marijuana use in a subgroup of depressed alcoholics	Controlled studies needed to evaluate efficacy during marijuana withdrawal	Cornelius et al. 1999

The results of other potential pharmacotherapies were less encouraging than those found for THC. Divalproex decreased marijuana craving during marijuana abstinence; however, it increased ratings of anxiety, irritability, bad effect, and sleepiness during marijuana abstinence (Haney et al. 2004), thus limiting its utility. Although bupropion has been well established to be an effective treatment for nicotine withdrawal (Ferry and Johnston 2003; Richmond and Zwar 2003), it not only failed to alleviate symptoms associated with marijuana withdrawal, but also exacerbated them (Haney et al. 2001). Specifically, bupropion worsened mood during marijuana abstinence as reflected by increased self-reports of irritability, misery, restlessness, depression, and lack of motivation compared to placebo. It also worsened subjective reports of sleep quality during marijuana withdrawal compared to placebo. One concern with bupropion is that its stimulatory effects were the cause of the increased severity of withdrawal effects. Therefore, a subsequent study examined whether nefazodone, an antidepressant with sedative properties would attenuate symptoms of marijuana withdrawal. Indeed, the effects of nefazodone maintenance on marijuana were more promising than those of bupropion. Nefazodone decreased subjective ratings of anxiety and muscle pain, but failed to ameliorate the increased ratings of irritability and misery, and decreased rating of sleep quality during marijuana withdrawal (Haney et al. 2003b). In any event, the recent finding that nefazodone is associated with the risk of hepatic failure (FDA 2004) would certainly diminish enthusiasm to develop this drug to treat cannabinoid dependence.

The results of a study conducted in a subgroup of depressed alcoholic marijuana users suggest that fluoxetine (Prozac) may also have some promise for treating marijuana dependence (Cornelius et al. 1999). Specifically, this serotonin re-uptake

inhibitor significantly reduced marijuana use as well as depression and alcohol consumption in this subgroup, suggesting that daily marijuana use may result from an attempt to self-medicate for depression (see Table 1). Further controlled studies will be needed to assess the efficacy of fluoxetine in treating marijuana withdrawal symptoms.

Another study by Haney and her colleagues was designed to examine the effects of naltrexone in heavy marijuana users. The goals of this experiment were to examine whether naltrexone would (1) block marijuana's pharmacological effects, and (2) precipitate withdrawal in heavy marijuana users (Haney et al. 2003a). Naltrexone failed to elicit these actions; however, it did increase many of the "positive" subjective effects of oral THC in heavy marijuana smokers. An implication of these findings is that naltrexone may be expected to increase marijuana use.

Currently, oral THC appears to be the best candidate agent to treat marijuana dependence. Of significance, THC was also shown to ameliorate withdrawal symptoms in both monkeys and mice (Beardsley et al. 1986; Lichtman et al. 2001a). However, none of the other agents listed in Table 1 has been evaluated in preclinical models of cannabinoid withdrawal. Thus, it will be important to establish whether the various animal models of cannabinoid dependence are relevant to marijuana-dependent humans. Clearly, the availability of a viable animal model could facilitate the development of effective pharmacotherapies to treat cannabis dependence.

5 Conclusions

The availability of laboratory animal models of cannabinoid tolerance and dependence has greatly increased the ability to investigate the mechanisms underlying these processes. A substantial effort has been focused on characterizing the adaptive changes that occur to the CB_1 receptor and its signal transduction pathways in response to repeated stimulation by cannabinoid agonists. A compelling body of research has led to the wide acceptance that tolerance to the pharmacological effects of cannabinoids is strongly associated with downregulation and desensitization of this receptor. Moreover, a growing body of in vitro and in vivo evidence is beginning to establish that PKA may play an integral role in the maintenance of cannabinoid tolerance.

Cannabinoid-tolerant mice have been demonstrated to exhibit a constellation of somatic withdrawal signs as well as decreases in food-reinforced behavior upon abrupt discontinuation of drug or challenge with a CB_1 receptor antagonist. Additionally, cannabinoid withdrawal has been shown to elicit a variety of physiological responses associated with stress, such as elevations in corticotropin releasing factor and Fos-immunopositive cell activity. The preponderance of evidence from human research supports the notion that cannabinoid dependence is clinically significant and that a need for treatment is warranted. It will be important to establish whether the animal models of dependence will be of value in developing pharmacotherapies for the treatment of cannabis dependence. A multidisciplinary

approach examining molecular and cellular changes in conjunction with animal models of dependence, and clinical trials will undoubtedly further our basic understanding of cannabinoid dependence as well as develop pharmacotherapies for cannabinoid dependence disorders.

Acknowledgements.
This work was supported by NIH grants DA03672, DA005274, DA14277, and DA15197.

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