Brief Communications

Cannabidiol, a Nonpsychotropic Component of Cannabis, Inhibits Cue-Induced Heroin Seeking and Normalizes Discrete Mesolimbic Neuronal Disturbances

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There remains debate regarding the impact of cannabis on neuropsychiatric disorders. Here, we examined the effects of cannabidiol (CBD), a nonpsychotomimetic constituent of cannabis, on heroin self-administration and drug-seeking behavior using an experimental rat model. CBD (5–20 mg/kg) did not alter stable intake of heroin self-administration, extinction behavior, or drug seeking induced by a heroin prime injection. Instead, it specifically attenuated heroin-seeking behavior reinstated by exposure to a conditioned stimulus cue. CBD had a protracted effect with significance evident after 24 h and even 2 weeks after administration. The behavioral effects were paralleled by neurobiological alterations in the glutamatergic and endocannabinoid systems. Discrete disturbances of AMPA GluR1 and cannabinoid type-1 receptor expression observed in the nucleus accumbens associated with stimulus cue-induced heroin seeking were paralleled by neurobiological alterations in the glutamatergic and endocannabinoid systems. Discrete disturbances of AMPA GluR1 and cannabinoid type-1 receptor expression observed in the nucleus accumbens associated with stimulus cue-induced heroin seeking were normalized by CBD treatment. The findings highlight the unique contributions of distinct cannabis constituents to addiction vulnerability and suggest that CBD may be a potential treatment for heroin craving and relapse.

Introduction

There continues to be major controversy as to whether cannabis should be legalized given some of its medicinal benefits, which are countered by the negative impact of cannabis on physical and mental health. Early cannabis use is associated with the development of psychotic disorders (Andreasson et al., 1987; Arseneault et al., 2002; Hall and Degenhardt, 2008) and with drug-seeking behavior, which characterizes the chronic, relapsing disorder rats could directly control their drug intake and drug-seeking vulnerability using a drug self-administration model such that antipsychotic and anxiolytic properties (Crippa et al., 2004; Zuardi et al., 2006).

In this study, we evaluated CBD effects in relation to addiction vulnerability using a drug self-administration model such that rats could directly control their drug intake and drug-seeking behavior, which characterizes the chronic, relapsing disorder of drug dependence. We focused on the potential influence of CBD on heroin-related behaviors given the strong neurobiological interactions between the cannabinoid and opioid systems (Rodriguez et al., 2001; Schoffelmeer et al., 2006). CBD was studied during different behavioral phases—maintenance, extinction, and relapse. Various factors can induce drug relapse and we specifically examined the impact of drug-associated environmental cue and heroin prime that are well documented to promote drug-seeking behavior and reinstate drug intake in experimental animal models (See, 2002; Shaham et al., 2003) and to induce drug craving in humans (Childress et al., 1993; Sinha et al., 2000). Neurobiological correlates to the behavioral effects were also evaluated in the striatum, a region critical for reward, goal-directed behavior, and habit formation (Everitt and Robbins, 2005).

Materials and Methods

Animals. Male Long–Evans rats, weighing 230–250 g at the beginning of the experiment, were obtained from Taconic. They were housed in a humidity- and temperature-controlled environment on a reversed 12 h light/dark cycle (lights off at 9:00 A.M.) with ad libitum access to food and water. Rats were allowed to acclimate in their new environment and were handled daily for 1 week before the start of the experiment. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the local Animal Care and Use Committee.

Intravenous heroin self-administration. The self-administration procedure was performed as described previously (Ellgren et al., 2007; Spano et al., 2007). Briefly, catheters (CamCath) were implanted into the right jugular vein under isoflurane anesthesia (2.4–2.7%; Baxter). Following 1 week of recovery from surgery, self-administration training began during the dark phase of the light/dark cycle in operant equipment which was fitted with infrared beams to measure locomotor behavior (MED Associates). Animals were allowed 3 h daily access to heroin (30 μg/kg/infu-
sion, diacetylmorphine–HCl (obtained from NIDA Drug Supply) under a fixed ratio-1 reinforcement schedule in which one active lever press resulted in a single drug infusion (85 μl over 5 s) and activation of a white conditioned stimulus light situated above the active lever. During training, animals were food restricted (20 g/d) and subsequently given ad libitum access to food after stable heroin intake behavior was achieved. Stable self-administration behavior was defined as at least 10 responses on the active lever and at least a 2:1 ratio in active/inactive lever presses for three consecutive sessions, with <15% variation. After stable heroin intake behavior was achieved, the animals were divided into different groups balanced for the number of active lever presses to subsequently evaluate the effects of CBD (5, 20 mg/kg, i.p.) dissolved in 3% Tween 80; NIDA Drug Supply) or vehicle (3% Tween 80) on heroin self-administration behavior.

In addition to the effects of CBD on heroin intake, the impact on drug-seeking behavior was also evaluated. After the drug maintenance phase, animals were kept drug-free in their home cage for 2 weeks. At the end of the drug withdrawal period, drug-seeking behavior was studied during reinstatement sessions initiated by exposure to the conditioned stimulus light cue or a heroin prime injection (0.25 mg/kg, i.p.). Heroin intake behavior was also evaluated. After the drug maintenance phase, animals were food restricted (20 g/d) and subsequently given ad libitum access to food after stable heroin intake behavior was achieved. Stable self-administration behavior was defined as at least 10 responses on the active lever and at least a 2:1 ratio in active/inactive lever presses for three consecutive sessions, with <15% variation. After stable heroin intake behavior was achieved, the animals were divided into different groups balanced for the number of active lever presses to subsequently evaluate the effects of CBD (5, 20 mg/kg, i.p.) dissolved in 3% Tween 80; NIDA Drug Supply) or vehicle (3% Tween 80) on heroin self-administration behavior.

CBD effects were also evaluated in regard to extinction of heroin self-administration behavior. The extinction sessions were conducted at the end of the maintenance phase of heroin self-administration, which lasted ~2 weeks. The testing conditions were the same as during training, except that presses on the previously active lever was replaced by saline infusion and there was no activation of the cue light.

Overall, 155 rats were trained to self-administer heroin in this study and 18 were excluded because of loss of catheter patency, poor health, or failure to acquire heroin self-administration.

Postmortem brain studies. To test neurobiological systems associated with CBD’s effects on cue-induced heroin-seeking behavior, we studied the striatum in postmortem brain samples 1 h after the drug-seeking session in which rats were given vehicle or CBD. A group of saline animals were also included that were processed through the same behavioral protocol; the rats did not show active saline self-administration behavior. In addition to the effects of CBD on heroin intake, the impact on drug-seeking behavior was also evaluated. After the drug withdrawal period, drug-seeking behavior was studied during reinstatement sessions initiated by exposure to the conditioned stimulus light cue or a heroin prime injection (0.25 mg/kg, i.p.). Heroin intake behavior was also evaluated. After the drug maintenance phase, animals were kept drug-free in their home cage for 2 weeks. At the end of the drug withdrawal period, drug-seeking behavior was studied during reinstatement sessions initiated by exposure to the conditioned stimulus light cue or a heroin prime injection (0.25 mg/kg, i.p.). Heroin intake behavior was also evaluated. After the drug maintenance phase, animals were kept drug-free in their home cage for 2 weeks. At the end of the drug withdrawal period, drug-seeking behavior was studied during reinstatement sessions initiated by exposure to the conditioned stimulus light cue or a heroin prime injection (0.25 mg/kg, i.p.). Heroin intake behavior was also evaluated. After the drug maintenance phase, animals were kept drug-free in their home cage for 2 weeks. At the end of the drug withdrawal period, drug-seeking behavior was studied during reinstatement sessions initiated by exposure to the conditioned stimulus light cue or a heroin prime injection (0.25 mg/kg, i.p.). Heroin intake behavior was also evaluated. After the drug maintenance phase, animals were kept drug-free in their home cage for 2 weeks. At the end of the drug withdrawal period, drug-seeking behavior was studied during reinstatement sessions initiated by exposure to the conditioned stimulus light cue or a heroin prime injection (0.25 mg/kg, i.p.).

Comparison with CBD. NIA. CBD (5–20 mg/kg, i.p.) did not affect the number of lever presses (b) or locomotor activity (c) during maintenance of heroin self-administration. Data represent mean ± SEM; n = 7–9/group.

Figure 1. CBD effects on heroin self-administration. a. Rats readily maintained stable self-administered heroin from approximately the sixth training session. b. CBD (5–20 mg/kg, i.p.) did not affect the number of lever presses (b) or locomotor activity (c) during maintenance of heroin self-administration. Data represent mean ± SEM; n = 7–9/group.

Results

CBD specifically affects heroin-seeking behavior induced by conditioned cue

Experiment 1

Rats quickly learned to self-administer heroin such that a significantly higher number of responses were observed on the active lever at the fourth training session (Fig. 1a) (p < 0.001) with stable heroin self-administered behavior acquired after the sixth session (lever presses × training session interaction; F(15, 135) = 15.57, p < 0.001). After acquisition of stable self-administration behavior, animals were divided into subgroups with equal heroin intake behavior to evaluate the effects of CBD or vehicle treatment. Initial CBD pilot studies indicated a greater protracted effect of the drug (data not shown), and thus the effects of CBD
administration were examined after 30 min and 24 h time periods. The maintenance of heroin intake behavior was not affected following CBD administration (5 and 20 mg/kg, i.p.) at either time point (Fig. 1b). CBD also failed to alter locomotor activity (Fig. 1c), which was simultaneously monitored during the self-administration session.

To assess the potential impact of CBD on heroin-seeking behavior, rats were re-exposed to the heroin self-administration chamber and stimulus light cue 14 d following drug abstinence. During the drug-seeking session, no drug reinforcement was obtained upon lever pressing. Vehicle-treated animals showed robust lever pressing during the drug-seeking session, however, this response was inhibited by a single CBD injection administered 24 h (Fig. 2a) (5 mg/kg, p < 0.05), but not 30 min (data not shown), before the session. The impact of CBD on drug-seeking behavior was very specific, affecting only active lever presses following the light cue exposure (Fig. 2a) (p < 0.01). Various time periods were evaluated in another set of animals after CBD injections (5 mg/kg daily over 3 d) in the reinstatement sessions. Intriguingly, there remained a significant decrease in active, but not inactive, lever presses for heroin seeking monitored even 2 weeks after the last CBD treatment (Fig. 2b) (p < 0.001).

To determine the effects of CBD on other conditions known to induce relapse, additional sets of animals were tested during a drug-seeking session that was initiated by a heroin prime injection (0.25 mg/kg, i.p.). CBD, both acute and 24 h after injection, had no effect on either active or inactive lever presses following the heroin prime (data not shown).

Experiment 2
The impact of CBD on extinction of heroin self-administration was also examined in which the context of the self-administration chamber, but not the cue light, was extinguished. CBD injections given 24 h before the initial extinction session and throughout the extinction training did not alter the rate of decline in the active lever-pressing or alter the inactive lever press responses compared with vehicle control animals (Fig. 3a). After a 2 week abstinence period, re-exposure to the cue light reinstated lever pressing in vehicle animals, but this was attenuated in animals administered CBD 24 h before the relapse session (Fig. 3b) (p < 0.01). Animals carried through the extinction paradigm were also exposed to a drug-seeking session that was initiated by a heroin prime injection (0.25 mg/kg, i.p.). CBD, both acute (data not shown) and 24 h after injection, had no effect on either active or inactive lever presses (Fig. 3c) induced by the heroin prime.

Altogether, these results suggest that CBD has a protracted neurobiological effect to counter long-lasting neuroadaptations that specifically govern conditioned cue-induced drug-seeking behavior and relapse.

**CBD effects on mesolimbic CB1 and GluR1 receptors**

CBD has been reported to be an inverse agonist at CB1Rs (Thomas et al., 2007) and an agonist at the transient receptor potential V1 (TRPV1) and TRPV2 (Qin et al., 2008). We examined the striatum, a region critical for reward, goal-directed behavior, and habit formation (Everitt and Robbins, 2005), in animals following the drug-seeking session. TRP proteins and mRNA levels were extremely low in the forebrain, though they were detected in the dorsal root ganglion (data not shown). CB1R mRNA expression was significantly increased in the ventral striatum (NAc), the core subdivision in heroin rats that received vehicle treatment before drug seeking (Fig. 4a) (p < 0.05). The heroin–CBD group showed decreased CB1R mRNA expression in the NAc core and shell subdivisions compared with heroin–vehicle animals even 2 weeks after the last CBD injection (Fig. 4a) (p < 0.05 and p < 0.01). Moreover, this CBD effect was also evident in the most medial division of the dorsal striatum (p < 0.05), which is innervated by limbic cortices, but no CBD effects were evident in the dorsolateral division that receives primarily sensorimotor cortical input (supplemental Table 1, available at www.jneurosci.org as supplemental material). Similar to alterations of the CB1R transcript, CB1R protein levels tended to be increased in the NAc of heroin–vehicle animals (Fig. 4b,c) (significant in the lateral NAc shell; p < 0.05), which were decreased both 24 h and 2 weeks after CBD treatment [medial (p < 0.05) and lateral (p < 0.01) NAc shell]. These findings suggest that CBD’s effects on CB1R expression have a mesolimbic specificity in the striatum. Interestingly, CBD administered in heroin-naïve animals acutely reduced CB1R expression in the NAc but not in the dorsal striatum; no significant alterations were observed with repeated CBD exposure (supplemental Table 2, available at www.jneurosci.org as supplemental material).

Drug-seeking behavior and relapse have been strongly linked to dysregulation of glutamate (Kalivas and Volkow, 2005; LaLumiere and Kalivas, 2008). We studied mRNA and protein levels of several markers relevant to glutamatergic function;
markers related to opioid transmission were also evaluated given the relevance to heroin. Most showed only heroin-associated effects with no alterations induced by CBD. For example, mRNA levels of mGluR5, abundantly expressed in medium spiny striatal efferent neurons and intricately linked to endocannabinoid-mediated synaptic plasticity (Katona and Freund, 2008), were strongly downregulated to the same extent in the heroin–vehicle and heroin–CBD groups (Fig. 4d) \(p < 0.001\). In contrast, AMPA CB1R receptors, which are highly implicated in drug-seeking behavior (Conrad et al., 2008), were significantly altered only in the heroin–CBD animals; GluR2/3 were not robustly expressed in the striatum and CDB administration on its own did not alter expression levels of any of the glutamatergic markers studied (data not shown). As shown in Figure 4, e and f, heroin–vehicle animals, with strong cue-induced relapse behavior, had marked reduction of AMPA GluR1 protein expression in the NAc core \(p < 0.001\), medial shell \(p < 0.01\), and lateral shell \(p < 0.05\), with no significant effect in the dorsal striatum (supplemental Table 1, available at www.jneurosci.org as supplemental material). However, 24 h after CBD, GluR1 protein expression was significantly normalized in the NAc core \(p < 0.001\) and medial shell \(p < 0.05\). A similar pattern was observed even 2 weeks following the last repeated CBD treatment, but the effect was most evident in the NAc core (Fig. 4e) \(p < 0.01\).

**Discussion**

The current study has revealed unique properties of the phyto-cannabinoid CBD and underscores the contrasting characteristics of the main constituents of cannabis in relation to addiction vulnerability. Compared with the documented effects of THC to enhance heroin self-administration (Solinas et al., 2004; Ellgren et al., 2007), the present data demonstrated that CBD specifically inhibited reinstatement of cue-induced heroin seeking. The specificity of CBD to cue-induced reinstatement was also emphasized by the observation that the compound still inhibited drug relapse behavior in animals extinguished to the environmental context (self-administration chamber) previously associated with heroin. The results are striking given the very selective and protracted effects of CBD. Although CBD significantly altered drug-seeking behavior promoted by conditioned cue, it failed to influence drug seeking initiated by a heroin prime. Whether CBD induces some perceptual alterations that compromise cue- but not priming-induced reinstatement of drug seeking remains to be determined. The apparent diminished impact of CBD in the presence of heroin was also evident during the drug maintenance phase, in which CBD did not modify stable heroin intake behavior. Thus CBD does not appear to interfere with the reinforcing effects of heroin at least on a FR-1 schedule. Interestingly, the ability of CBD to reduce heroin-seeking behavior at least 2 weeks after exposure was nevertheless still observed when CBD had been administered during the active phase of heroin self-administration. These findings emphasize that CBD retains its effects to modulate neural mechanisms relevant to cue-induced drug relapse vulnerability even in the presence of heroin.

The observation that CBD’s effects on cue-induced drug-seeking behavior was apparent 24 h and 2 weeks, but not after 30 min, following administration may suggest delayed pharmacological actions of the drug. However, it is important to note that behavioral effects have been observed immediately after administration of CBD at the dose range currently studied on, for example, its anxiolytic properties (Guimarães et al., 1994; Moreira et al., 2006). Moreover, acute administration of CBD has been shown to enhance the extinction of cocaine- and amphetamine-induced conditioned place preference without affecting learning or retrieval (Parker et al., 2004). There was also no evidence in our study that CBD affected extinction learning. CBD did not alter the extinction of heroin seeking. The potential influence of CBD
on psychostimulant-seeking behavior needs to be examined with an operant self-administration procedure to determine the specificity of CBD to different classes of drugs and different relapse models.

The protracted behavioral effects of CBD, in addition to its specific influence on heroin-seeking behavior, strongly implied a long-term impact on synaptic plasticity, the pathology of which is hypothesized to underlie compulsive disorders such as drug addiction. The endocannabinoid and glutamatergic systems have been tightly linked to synaptic plasticity (Kauer and Malenka, 2007). In addition to its high potency at the CB1R (Thomas et al., 2007), CBD has also been reported to alter the hydrolysis of the endocannabinoid anandamide (Bisogno et al., 2001). The reduction of CB1R expression in the NAc when CBD was administered alone was short-term though its impact was enduring in heroin-seeking animals. Attenuation of the elevated expression CB1R mRNA and protein levels in the NAc by CBD in heroin rats, which paralleled the behavioral alterations, is consistent with the observation that inhibition of the CB1R inhibits cue-induced drug-seeking behavior (De Vries et al., 2003). Various lines of evidence have clearly documented the critical role of AMPA GluR1 in drug-seeking behavior though most studies have focused on psychostimulant drugs (Anderson et al., 2008; Conrad et al., 2008). Of the few investigations that have evaluated opiates, the expression of AMPA receptors in prefrontal cortical synaptic membranes was reported to be reduced in heroin-abstinent animals after re-exposure to heroin cues (Van den Oever et al., 2008) and glutamate arising from the prefrontal cortex was increased in the NAc core (LaLumiere and Kalivas, 2008). The specific cellular localization of the GluR1 was not examined in the current study, which limits interpretations as to the dynamic cellular distribution of AMPA receptors relevant to drug-seeking behavior. Moreover, it is important to note that a similar alteration of glutamate levels was reported in the NAc core with both cue and heroin prime (LaLumiere and Kalivas, 2008). Thus, other mechanisms than NAc core glutamatergic disturbances most likely underlie CBD’s apparent ability to alter cue- but not priming-induced reinstatement of drug seeking. Nevertheless, the observation that GluR1 disturbances in the NAc associated with heroin seeking were absent in those administered CBD is intriguing. Most studies have focused on the NAc core in relation to glutamatergic involvement in drug-seeking behavior, but CBD’s effects on GluR1 were also apparent in the NAc shell, though to a weaker extent than the core. Further studies are required to determine the specific contribution of the NAc subregions as well as other brain regions implicated in cue-induced reinstatement to the actions of CBD. Although additional studies are needed to fully elucidate the molecular mechanisms of CBD in regard to its direct and indirect effects on heroin-seeking behavior, the mesolimbic specificity and protracted effects of CBD on CB1R and GluR1 is interesting given the role of the limbic system in goal-directed behavior.

Of the over 1 million opiate-dependent subjects today, only less than a quarter of such individuals receive treatment, which have traditionally targeted μ-opioid receptors. Although such treatment strategies including methadone have improved substance abuse outcome, they do not effectively block opiate craving in all patients (Walter et al., 2008) and thus are still associated with high rates of relapse and ultimately the continued cycle of opioid abuse. The fact that drug craving is generally triggered by exposure to conditioned cues makes the current results particularly fascinating. Moreover, the observation that CBD did not cause gross motor impairment as evident by a lack of effect on inactive lever presses or on locomotor behavior is consistent with the weak side effects that have been reported with this compound in humans (Consroe et al., 1991; Tomida et al., 2006). In addition, CBD lacks hedonic properties on its own (Parker et al., 2004). Overall, the observations of this study suggest the potential for CBD as a treatment strategy given its specificity to attenuate cue-induced drug-seeking behavior, preferential impact on mesolimbic neuronal populations, and enduring neural actions. Clearly, greater attention needs be given to the potential role of CBD in the treatment of addiction and other mental health disorders.

References


