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Cannabidiol inhibits sucrose self-administration by CB1 and CB2 receptor mechanisms in rodents

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Abstract

A growing number of studies suggest therapeutic applications of cannabidiol (CBD), a recently U.S. Food and Drug Administration (FDA)–approved medication for epilepsy, in treatment of many other neuropsychological disorders. However, pharmacological action and the mechanisms by which CBD exerts its effects are not fully understood. Here, we examined the effects of CBD on oral sucrose self-administration in rodents and explored the receptor mechanisms underlying CBD-induced behavioral effects using pharmacological and transgenic approaches. Systemic administration of CBD (10, 20, and 40 mg/kg, ip) produced a dose-dependent reduction in sucrose self-administration in rats and in wild-type (WT) and $CB1^{-/-}$ mice but not in $CB2^{-/-}$ mice. CBD appeared to be more efficacious in $CB1^{-/-}$ mice than in WT mice. Similarly, pretreatment with AM251, a CB1R antagonist, potentiated, while AM630, a selective CB2R antagonist, blocked CBD-induced reduction in sucrose self-administration, suggesting the involvement of CB1 and CB2 receptors. Furthermore, systemic administration of JWH133, a selective CB2R agonist, also produced a dose-dependent reduction in sucrose self-administration in WT and $CB1^{-/-}$ mice, but not in $CB2^{-/-}$ mice. Pretreatment with AM251 enhanced, while AM630 blocked JWH133-induced reduction in sucrose self-administration in WT mice, suggesting that CBD inhibits sucrose self-administration likely by CB1 receptor antagonism and CB2 receptor agonism. Taken together, the present findings suggest that CBD may have therapeutic potential in reducing binge eating and the development of obesity.

KEYWORDS

cannabidiol, cannabinoid, CB1 receptor, CB2 receptor, feeding behavior, sucrose self-administration

INTRODUCTION

It is well known that excessive food intake and binge eating contribute to the national epidemic of obesity. The endocannabinoid system has been implicated in numerous aspects of eating-related behaviors and disorders.[1,2] Previous studies have demonstrated that CB1R agonists can induce overeating,[3] while CB1R antagonists suppress food intake and weight gain in rodents,[4,5] suggesting potential use of CB1R antagonists in treatment of body overweight and obesity. However, the related clinical trials have been terminated worldwide because of the depressive and anxiogenic properties of CB1R antagonists.[6-8]

In recent years, much attention has been given to cannabidiol (CBD), the second major component extracted from the *Cannabis sativa* plant.[9] Unlike Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component in cannabis, CBD is devoid of psychotropic effects.[10] Recent research suggests that CBD may have a wide range

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of medical applications in treatment of epilepsy,[11,12] anxiety,[13,14] schizophrenia,[15,16] neurodegenerative disorders,[17,18] and even cancer.[19] A significant progress in cannabinoid research has been made such as CBD was approved by the US Food and Drug Administration (FDA) in June 2018 for treatment of epilepsy. Furthermore, it has been reported that CBD may have therapeutic potential in controlling food intake and preventing obesity. A number of studies indicate that CBD can inhibit food consumption[20-22] and responding for food or sweetened water in rats and monkeys.[23,24] In contrast, a lack of CBD effects on food-related behaviors in rats or mice has also been reported.[25-27] Thus, more research is required to determine the role of CBD in controlling body weight and obesity. Given recent findings that CBD has the ability to reduce alcohol[28], cocaine,[29] or methamphetamine[30] self-administration, in the present study, we systemically examined the pharmacological action of CBD on oral sucrose self-administration and explored the receptor mechanisms through which CBD alters sucrose taking.

Sucrose is a natural energy source and reward (sweet) substance that provides higher reward valence even than cocaine.[31] The consumption of highly sweetened diets is now highly prevalent in developed countries and is thought to contribute to the current obesity epidemic.[32] Therefore, oral sucrose self-administration is a commonly used animal model to study feeding, binge-eating, and food-taking disorders. CBD has been shown to have multiple acting targets including CB1 and CB2 receptors,[33] GPR55 receptor,[34] 5-HT_{1A} receptor,[35] GPR3, GPR6, and GPR12,[36] μ and δ opioid receptors,[37] vallinoid receptor 1 (TRPV1),[38] and peroxisome proliferator–activated receptor γ (PPAR γ).[39] However, more recent studies suggest that CBD is a potent allosteric modulator of CB1 and CB2 receptors with nanomolar binding affinity.[40-43] These new findings suggest that CB1 and CB2 receptors could be important targets in the pharmacological action produced by CBD. To test this hypothesis, we used pharmacological and transgenic approaches to explore a possible involvement of CB1 and CB2 receptors in CBD-induced behavioral effects.

METHODS

Animals

Adult male Long Evans rats and mice were used in this study. Male wild-type (WT) and $CB1^{-/-}$ mice with C57BL/6J genetic backgrounds were bred at the National Institute on Drug Abuse (NIDA) from $CB1^{+/-}$ breeding pairs that were generously donated by Dr Andrea Zimmer. $CB2^{-/-}$ mice with C57BL/6J genetic backgrounds were bred from $CB2^{+/-}$ breeding pairs that were generously donated by Dr George Kunos (National Institute on Alcohol Abuse and Alcoholism). All animals were matched for age (8-14 wk) and weight (rats, 250-350 g; mice, 25-35 g). They were housed in a climate-controlled animal colony room on a reversed light-dark cycle (lights on at 7:00 PM; lights off at 7:00 AM) with free access to food and water. One week prior to the start of experiments, animals received daily rations of food in order to maintain their weights at 85% of free feeding values. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the US National Research Council and were approved by the Animal Care and Use Committee of the NIDA of the US National Institutes of Health (NIH).

Drugs

CBD was generously provided by the NIDA Drug Supply Program and was dissolved in the 5% cremophor. Sucrose (Sigma-Aldrich) was dissolved in 0.9% physiological saline to achieve 5% concentration that was delivered in a volume of 0.1 or 0.02 mL onto a food trough JWH133, AM251, and AM630 were purchased from Tocris Bioscience. JWH133 was dissolved in soya oil-based Tocrisolve-100 (Tocris Bioscience, USA). AM251 and AM630 were dissolved in 5% cremophor and were administered intraperitoneally (ip).

Procedure

Operant conditioning chambers

Sucrose self-administration experiments were conducted in operant conditioning chambers (Med Associates, USA), each placed in a ventilated, sound-attenuating cubicle. Each operant chamber was equipped with two levers located 2.5 cm above the floor, a cue light, a speaker located 5 cm above the active lever, and a food trough onto which liquid sucrose was delivered upon a lever press.

Oral sucrose self-administration

Procedures for oral sucrose self-administration in rats and mice were the same as we reported previously.[44,45] Briefly, animals were trained to self-administer sucrose under a fixed ratio 1 (FR1) schedule of reinforcement during daily 3-hour sessions. Responding on an active lever activated the syringe pump causing the delivery of 5% liquid sucrose onto a liquid food receptacle (0.1 mL per delivery in rats and 0.02 mL per delivery in mice) and the presentation of the light/tone cue above the active lever. Responses on an inactive lever were counted but had no consequences. During the 4.2-second infusion period, additional responses on the active lever were recorded but did not lead to additional infusions. To prevent satiation of sucrose reward, we set a maximal number of 100 sucrose deliveries during each 3-hour session. Animals were tested with different compounds once stable sucrose self-administration was achieved, defined as (i) at least 20 sucrose rewards earned per 3-hour session, (ii) less than 20% variability in daily sucrose intakes across two consecutive sessions, and (iii) an active/inactive lever press ratio exceeding 2:1. All animals met these criteria before being tested with one of the compounds.

Experiment 1. Effects of CBD on sucrose self-administration in rats

We first assessed the effects of CBD on sucrose self-administration in rats under an FR5 schedule of reinforcement. Animals (n = 12) were

first trained to self-administer sucrose under FR1 schedule of reinforcement and then on an FR5 schedule of reinforcement. After stable self-administration was achieved for at least three consecutive days, rats were injected with one of the CBD doses (vehicle, 20 or 40 mg/kg, ip) 30 minutes prior to a self-administration session. On following days, rats were restabilized and later retested with a different dose of CBD. The order of CBD doses that the animals were tested with was counterbalanced. Only the animals (n = 9) received all three doses of CBD treatment were included in the data analysis.

Experiment 2. Effects of CBD on sucrose self-administration in WT, CB1$^{-/-}$, and CB2$^{-/-}$ mice

To ensure that our results are reproducible across different species and to determine the potential involvement of CB1 and/or CB2R mechanisms in CBD action, we then assessed the effects of CBD on sucrose self-administration in WT (n = 12), CB1$^{-/-}$ (n = 6), and CB2$^{-/-}$ (n = 7) mice. The experimental procedures for sucrose self-administration in mice were the same as those in Experiment 1, except the FR1 schedule of reinforcement was used throughout the experiment. The drug treatment was the same as described above. Only the animals (nine WT, six CB1-KO, and six CB2-KO) received all three doses of CBD treatment were included in the data analysis.

Experiment 3. The effects of CBD treatment on sucrose self-administration in the presence of CB1 or CB2 receptor antagonism

To confirm the findings observed in the above transgenic mice, we further assessed the effects of CB1 or CB2R antagonists on CBD-induced reduction in sucrose self-administration. After stable responding was achieved, WT mice were divided into four groups—vehicle (5% cremophor) group (n = 12), vehicle + CBD (20 mg/kg) group (n = 12), AM251 (3 mg/kg) + CBD (20 mg/kg) group (n = 12), and AM630 (3 mg/kg) + CBD (20 mg/kg) group (n = 8). Thirty minutes prior to the test session, mice were injected according to the assigned treatment and later were allowed to self-administer sucrose under an FR1 schedule of reinforcement, under the same condition as described above. We then observed the effects of vehicle (Tocrisolve-100; n = 12), AM251 (3 mg/kg; n = 11), or AM630 (3 mg/kg; n = 9) alone on oral sucrose self-administration under an FR1 schedule of reinforcement in WT mice.

Experiment 4. The effect of CBD treatment on sucrose self-administration under a PR schedule of reinforcement in WT mice

To determine whether CBD treatment alters motivation for sucrose self-administration, WT mice were trained to self-administration sucrose first under the FR1 and then progressive ratio (PR) schedules of reinforcement. Under a PR schedule, requirement of lever presses for a single sucrose delivery was progressively raised within each session according to the following PR series—1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, and 603—so that the animal reached a breakpoint.[46,47] Final ratio (or breakpoint) was defined as the number of lever presses completed for the last sucrose delivery before a 1-hour period during which no sucrose reward was obtained. Animals were allowed to continue daily sessions of sucrose self-administration until day-to-day variability in final ratio fell within 1 to 2 ratio increments for three consecutive days. On the testing day, 30 minutes prior to a test session, mice were injected with one of the CBD doses (0, 20, or 40 mg/kg) and then allowed to press the lever for sucrose under the PR schedule of reinforcement. The order of drug injections was counterbalanced.

Experiment 5. The effects of JWH133 on sucrose self-administration in mice

In this experiment, we further explored whether the selective CB2R agonist, JWH133, alters sucrose-self administration in WT (n = 11), CB1$^{-/-}$ (n = 6), and CB2$^{-/-}$ (n = 6) mice in a way similar to CBD. The self-administration procedures were the same as in Experiment 2. After stable responding was achieved for at least three consecutive days, each animal was injected with one of the JWH133 doses (vehicle, 10 or 20 mg/kg, ip) 30 minutes prior to self-administration test session. Each animal received three doses of drug injections 3 to 5 days apart.

Experiment 6. The effects of JWH133 on sucrose self-administration in the presence of CB1 or CB2 antagonism

Similarly as in Experiment 3, we used pharmacological approaches to observe whether pretreatment with CB1 or CB2R antagonist can block JWH133-induced reduction in sucrose self-administration. Again, the experimental procedures were the same as in Experiment 3. Additional four groups of WT mice—vehicle (Tocrisolve-100; n = 12), vehicle (Tocrisolve-100) + JWH133 (20 mg/kg; n = 11), AM251 (3 mg/kg) + JWH133 (20 mg/kg; n = 9), and AM630 (3 mg/kg) + JWH133 (20 mg/kg; n = 8)—were used to assess the effects of AM251 or AM630 pretreatment on JWH133 action in sucrose self-administration.

Data analyses

All data were expressed as mean ± SEM. In Experiments 1 and 2, percentage of baseline sucrose intake for each animal was calculated by dividing the number of sucrose deliveries on the test day by the average number of sucrose deliveries earned in the last two baseline days (before the test day).

One-way ANOVAs for repeated measures (RMs) over drug dose were used for statistical analysis followed with post hoc Bonferroni tests with correction for multiple group comparisons. Statistical significance was defined as $P < 0.05$.

RESULTS

CBD inhibits sucrose self-administration in rats

Figure 1A shows the acquisition of responding for sucrose that reached the asymptote after five sessions of training. Figure 1B shows

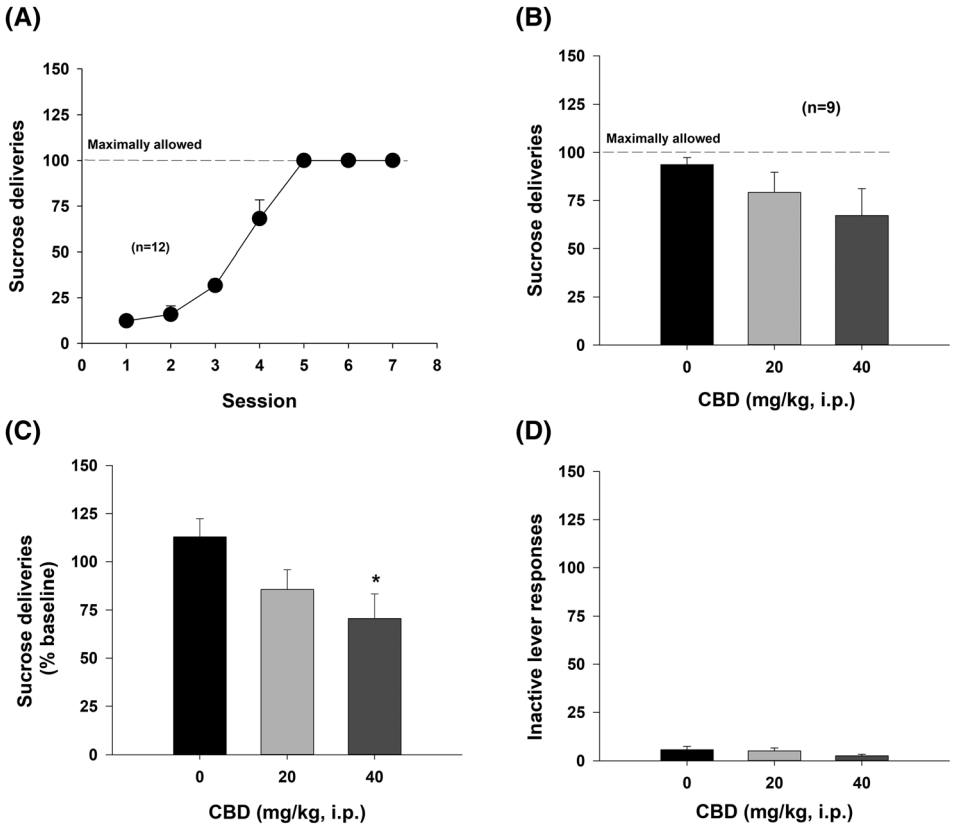

FIGURE 1 The effects of cannabidiol (CBD) on sucrose self-administration under an FR5 schedule of reinforcement in rats. A, Mean (±SEM) number of sucrose deliveries during the training. B, Mean (±SEM) number of sucrose deliveries in the absence or presence of the CBD treatment. C, Percent baseline of sucrose deliveries. D, Mean (±SEM) number of inactive lever presses. *$P < 0.05$, compared with vehicle (0 mg/kg) control group

that systemic administration of CBD produced a trend but not a significant reduction in the total number of sucrose self-administration in rats (one-way RM ANOVA, $F_{2,16} = 3.316$, $P = 0.062$). However, when we analyzed percent baseline of sucrose intake, we found that CBD treatment produced a significant and dose-dependent reduction in sucrose self-administration (Figure 1C, $F_{2,16} = 4.159$, $P = 0.035$). Post hoc Bonferroni tests revealed that the 40 mg/kg, but not 20 mg/kg, dose of CBD significantly reduced sucrose intake ($P < 0.05$). Figure 1D shows inactive lever responding, indicating that CBD had no effect on inactive lever presses ($F_{2,16} = 1.542$, $P = 0.224$), suggesting no significant sedative effects under the CBD treatment.

CBD inhibits oral sucrose self-administration in mice

Figure 2A shows the acquisition of responding for sucrose during the training where CB1-KO mice took longer to stabilize and reach the asymptote. Figure 2B shows that systemic administration of CBD produced a significant and dose-dependent reduction in sucrose self-administration in WT (one-way RM ANOVA, $F_{2,16} = 3.938$, $P = 0.04$) and CB1$^{-/-}$ ($F_{2,10} = 2.97$, $P = 0.01$) mice but not in CB2$^{-/-}$ ($F_{2,10} = 0.892$, $P = 0.44$) mice. Figure 2C shows that CBD treatment significantly inhibited percent baseline of sucrose intake in WT (one-way RM ANOVA, $F_{2,16} = 3.91$, $P = 0.041$) and CB1$^{-/-}$ ($F_{2,10} = 7.37$, $P = 0.01$) mice but not in CB2$^{-/-}$ ($F_{2,10} = 1.546$, $P = 0.26$) mice. Post hoc Bonferroni tests for multiple group comparisons revealed that CBD, at 20 mg/kg, significantly lowered sucrose intake in WT and CB1$^{-/-}$ mice ($P < 0.05$). Figure 2D shows that systemic administration of CBD did not alter inactive lever presses in any strain of mice.

Blockade of CB2, not CB1, receptors blocks CBD action

Figure 3A (left panel) shows that CBD, at 20 mg/kg, significantly inhibited sucrose self-administration in the absence of AM251 (a CB1R antagonist) or AM630 (a CB2R antagonist). This effect was enhanced in the presence of AM251 pretreatment but blocked by AM630 pretreatment. A one-way ANOVA revealed a significant treatment effect ($F_{3,44} = 20.388$, $P < 0.001$, Figure 3A). Post hoc Bonferroni tests for multiple group comparisons indicated that CBD-induced reduction in self-administration and AM251-induced enhancement of CBD action were statistically significant. Figure 3A (right panel) shows neither AM251 nor AM630 alone altered sucrose intake in WT mice (one-way ANOVA, $F_{2,29} = 0.617$, $P = 0.574$). Figure 3B shows that CBD treatment significantly lowered breakpoint for sucrose self-administration in WT mice (one-way ANOVA, $F_{2,16} = 9.55$, $P = 0.001$). Post hoc Bonferroni tests

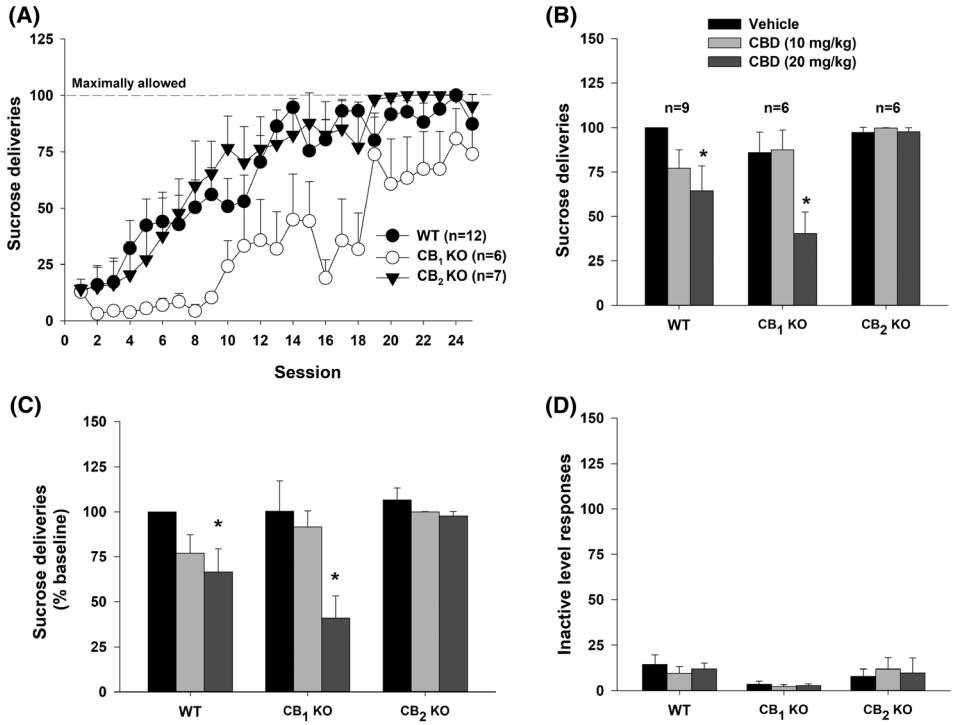

FIGURE 2 The effects of cannabidiol (CBD) on sucrose self-administration under an FR1 schedule of reinforcement in wild-type (WT), $CB1^{-/-}$, and $CB2^{-/-}$ mice. A, Mean (±SEM) number of sucrose deliveries during the training. B, Mean (±SEM) number of sucrose deliveries in the absence or presence of the CBD treatment. C, Percent baseline of sucrose intake. D, Mean (±SEM) number of inactive lever presses. $^{*}P < 0.05$, compared with vehicle control group in each genotype of mice

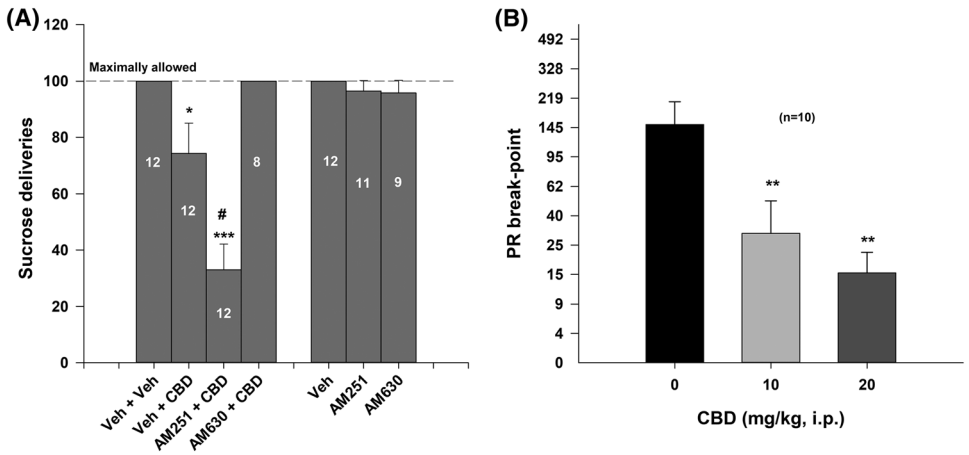

FIGURE 3 The effects of cannabidiol (CBD) on sucrose self-administration in wild-type (WT) mice in the presence of AM251 (a CB1R antagonist, 3 mg/kg) or AM630 (3 mg/kg, a CB2R antagonist) or under PR schedule of reinforcement. A, Mean (±SEM) number of sucrose deliveries under an FR1 schedule of reinforcement. B, PR breakpoint in sucrose self-administration in the presence or absence of CBD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with (Veh + Veh) group (A) or in vehicle (0 mg/kg) control group. $^{\#}P < 0.05$, compared with (Veh + CBD) group

revealed that reductions produced by 10 and 20 mg/kg doses of CBD were statistically significant as compared with the vehicle control group ($P < 0.001$).

JWH133 inhibits sucrose self-administration in mice

On the basis of our findings that a CB2R mechanism underlies the pharmacological action of CBD, we assessed whether systemic administration of JWH133, a selective CB2R agonist, can produce a similar reduction in sucrose self-administration. Figure 4A shows that JWH133, at 10 and 20 mg/kg doses, significantly inhibited sucrose self-administration in WT ($F_{2,20} = 16.0$, $P < 0.001$, one-way RM ANOVA) and $CB1^{-/-}$ ($F_{2,10} = 8.184$, $P < 0.01$) mice but not in $CB2^{-/-}$ ($F_{2,10} = 0.455$, $P = 0.647$) mice. Post hoc Bonferroni tests indicated that reductions observed in WT or $CB1^{-/-}$ mice were statistically significant after 10 or 20 mg/kg CBD treatment ($P < 0.05$).

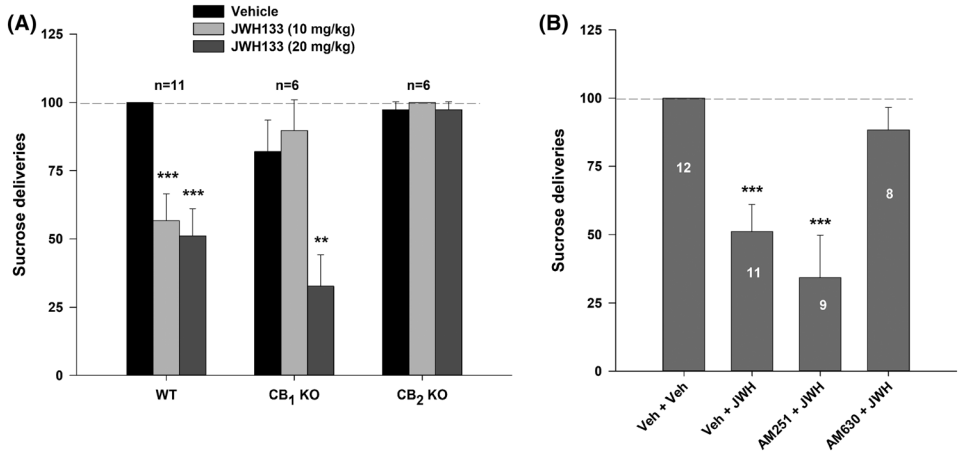

FIGURE 4 The effects of JWH133, a selective CB2R agonist, on sucrose self-administration in mice. A, Treatment with JWH133 (10 and 20 mg/kg) dose-dependently inhibited sucrose self-administration in wild-type (WT) and CB1$^{-/-}$ mice but not in CB2$^{-/-}$ mice. B, Pretreatment with AM630 (3 mg/kg, ip), but not AM251 (3 mg/kg, ip), blocked JWH133-induced reduction in sucrose self-administration. $^{**}P < 0.01$, $^{**}P < 0.001$, compared with vehicle (A) or (Veh + Veh) (B) control group

AM630 blocks JWH133 action in sucrose self-administration

Figure 4B shows the effects of pretreatment with AM251 or AM630 on CBD action, indicating that AM251 (3 mg/kg, ip) failed, but AM630 blocked CBD-induced reduction in sucrose self-administration. A one-way ANOVA revealed a significant treatment effect ($F_{3,36} = 12.00$, $P < 0.001$). Post hoc Bonferroni tests revealed that CBD-induced reduction was statistically significant after vehicle or AM251 pretreatment ($P < 0.05$).

DISCUSSION

The goals of this study were to investigate whether CBD can reduce sucrose intake in rodents and to explore the receptor mechanisms underlying CBD action. The major findings include the following: (1) Systemic administration of CBD produced a significant and dose-dependent reduction in sucrose-self administration in rats and mice under FR1, FR5, and PR schedules of reinforcement, suggesting a reduction in sucrose reward. (2) Pharmacological blockade or genetic deletion of CB1Rs failed to block CBD-induced reduction in sucrose self-administration. However, blockade of CB1Rs by AM251 produced an effect similar to CBD, while pretreatment with AM251 produced an enhancement in CBD action, suggesting possible involvement of CB1R antagonism in CBD action. (3) Pharmacological blockade or genetic deletion of CB2Rs blocked the pharmacological action of CBD in sucrose self-administration, suggesting a CB2R mechanism involvement. And finally, (4) stimulation of CB2Rs by JWH133 produced a reduction in sucrose self-administration in a way similar to CBD. Taken together, these findings suggest that CBD-induced reduction in sucrose self-administration may be mediated by inhibition of CB1Rs and stimulation of CB2Rs.

CBD inhibits oral sucrose intake

Cannabis contains more than 100 chemical components that share a similar chemical cannabinoid structure. CBD was isolated from cannabis in 1940[48] and then identified as a nonpsychotropic cannabinoid in 1970.[9] Since then, a growing body of research has shown that CBD could be a useful and promising medication for the treatment of epilepsy, substance abuse and dependence, schizophrenia, pain, anxiety, depression, sleep disorders, and Parkinson disease.[49,50] In June 2018, the U.S. FDA approved Epidiolex (CBD) for treatment of seizures associated with two rare and severe forms of epilepsy.

An important finding in the present study is that CBD is also effective in attenuation of sucrose intake and motivation for sucrose, as assessed by reductions in sucrose self-administration and breakpoints for sucrose reward. CBD appears more potent and effective in mice than in rats since higher doses of CBD are required in rats to produce a significant reduction in sucrose self-administration. This may be related to the different pharmacokinetic profiles of CBD in rats versus mice. The present finding is consistent with previous reports that CBD is effective and useful at controlling food intake and body weight and in preventing the development of obesity.[20-22,51] It is also consistent with previous reports that CBD may have a therapeutic value to treat substance use disorders and obesity as it has the ability to reduce rewarding effects of alcohol[28], cocaine, and methamphetamine.[29,30,52]

Although CBD failed to alter heroin self-administration, it reduced cue-induced reinstatement of drug seeking[53] and facilitated the extinction of psychostimulant-induced conditioned place preference (CPP).[54] Studies with human subjects revealed that CBD reduced cigarette consumption in smokers[55] and counteracted euphoric as well as negative effects of Δ^9-THC in cannabis users.[56-58] Interestingly, CBD itself lacks rewarding properties as it failed to induce CPP, withdrawal symptoms or altered motor behavior.[10] It also failed to affect brain

stimulation reward in rodents.[52] These findings suggest that CBD has no abuse potential by itself.[59]

Role of CB1 receptor in CBD action

However, a challenge in CBD research is that the molecular targets underlying CBD's action are ambiguous. On the basis of the structural similarity to Δ^9-THC, it was initially believed that similar receptor mechanisms underlie CBD's action. However, experimental evidence suggests that CBD has a very low affinity (K_i = 10μM~30μM) and shows little agonist activity (EC_{50} > 10μM) at the CB1 and CB2Rs, as compared with Δ^9-THC (K_i = 3nM~80nM for CB1/CB2Rs),[33] suggesting non-CB1 and non-CB2 mechanism involvement. Alternatively, a growing body of literature indicates that CBD has higher affinity at many other molecular targets, including GPR55, TRPV1, PPAR γ, opioid, and serotonin receptors (5-HT_{1A}).[60,61] However, a majority of the above findings derives from in vitro cell lines, and currently, there is a lack of convincing evidence indicating that any of these targets directly contribute to therapeutic effects produced by CBD in vivo or in human clinical trials.

The second important finding in the present study is that CBD-induced reduction in sucrose self-administration may be related to CB1R inactivation. This is based on the findings that pharmacological blockade of CB1Rs by AM251 produced a reduction in sucrose self-administration in a way similar to CBD, and pretreatment with AM251 (30 min prior to CBD) produced an augmented reduction in sucrose self-administration. This finding is consistent with previous observation of food intake and body weight in rats fed with a high-fat or high-sugar diet.[51] In addition, growing evidence demonstrates that CBD may act as allosteric CB1R antagonist (see discussion below). These findings suggest that AM251 and CBD may act on CB1Rs, producing an additive or synergistic effect although their exact binding sites on CB1Rs are unclear. We note that genetic deletion of CB1Rs failed to alter CBD action. This should not be used to exclude CB1R involvement since in the absence of CB1Rs, CBD may act on other targets (such as CB2Rs, discussed below) producing pharmacological effects. Overall, the hypothesis about the role of CB1Rs in CBD action is consistent with previous reports that Δ^9-THC and the endocannabinoid anandamide increase food consumption by stimulation of CB1Rs.[62-65] Accordingly, blockade of CB1Rs produces an inhibitory effect on food-taking behavior.

The molecular mechanisms through which CBD modulates CB1R function are unclear. As stated above, CBD has very low affinity at the orthosteric binding site of CB1Rs,[33,38] suggesting that CBD may not alter endocannabinoid binding to the orthosteric site of the CB1Rs. However, more recent studies suggest that CBD is a potent noncompetitive negative allosteric modulator (or antagonist) of CB1Rs.[42,43,66,67] CBD reduces both G protein–dependent signaling and β-arrestin 2 recruitment using Δ^9-THC and 2-AG as orthosteric probes at CB1Rs. These findings suggest that CBD-induced reduction in sucrose self-administration may be mediated by functional inhibition of CB1Rs at its allosteric binding site.

We note that in addition to binding to CB1Rs, CBD and AM251 also have nanomolar range binding affinities at other targets, such as GPR55 receptors[34] and μ-opioid receptors.[37,68] AM251 has been shown to exert "off-target" effects in vitro and in $CB1^{-/-}$ mice.[68] Thus, it is conceivable that the robust reduction in sucrose intake observed with the combined treatment of AM251 and CBD might involve GPR55 and μ-opioid receptor sites. In fact, others have shown that opioid receptors play an important role in food-related behaviors.[28]

Role of CB2 receptor in CBD action

Perhaps the most important finding in the present study is that a CB2R mechanism may also underlie CBD action in sucrose self-administration. This is based on the several lines of evidence. First, deletion of CB2Rs abolished CBD action; second, pretreatment with AM630, a selective CB2R antagonist, prevented CBD action; third, JWH133, a selective CB2R agonist, produced a similar effect as CBD; and fourth, pretreatment with AM630 blocked JWH133 action on sucrose self-administration. These findings are consistent with previous reports indicating that a CB2R-dependent mechanism is closely associated with CBD-induced reduction in food intake, body weight, and obesity[21,69,70] and with CBD-produced neuroprotection.[50,71]

The molecular mechanisms through which CBD modulates CB2R function are still not fully understood. As stated above, CBD has low affinity for either CB1 or CB2 orthosteric binding site, and therefore, diverse noncannabinoid molecular targets have been proposed. Using [^{35}S]-GTPγS binding assays, Pertwee and coworkers provided the first evidence for CBD to antagonize the effects of CP-55,940 at hCB_2 and to display *CB2R inverse agonism*.[33,43] A decade later, CBD has been proposed as a potential allosteric ligand of CB2Rs with a high affinity at the allosteric binding site of CB2Rs even at nanomolar concentrations and the ability to significantly decrease affinity (K_D) of the orthosteric agonist (red CM-157).[40,72] In similar conditions, CBD by itself did not significantly affect CB2R-coupled cAMP and ERK1/2 signaling but blocked the action of the selective CB2R agonist JWH133 in a dose-dependent manner at nanomolar concentrations. Furthermore, a very recent paper reported that CBD is a partial CB2R agonist in the absence of orthosteric ligands.[66] Taken together, these data suggest that CBD acts as an *allosteric CB2R inverse agonist* and functionally antagonizes action produced by orthosteric CB2R agonists in a noncompetitive manner.

It is unknown how CBD inhibits sucrose self-administration via CB2Rs at neural circuit level. We and others have reported that CB2Rs are expressed in the brain and are functionally involved in drug reward and addiction.[73,74] CB2Rs have been identified on the cell bodies of dopaminergic (DA) neurons in the ventral tegmental area (VTA)[45,75,76] as well as on the terminals of these neurons in the nucleus accumbens (NAc),[77,78] two brain regions critical for reward and addiction.[79] We previously reported that stimulation of VTA or

NAc CB2Rs inhibits neuronal firing of DA neurons and decreases DA release in the NAc.[75,76] Stimulation of CB2Rs by JWH133 inhibits cocaine self-administration, cocaine-induced locomotor hyperactivity and CPP, and electrical brain stimulation reward.[80-82] Correspondingly, deletion of CB2Rs leads to enhanced CPP or locomotor response to cocaine or ethanol in mice,[82-85] while overexpression of CB2Rs decreases cocaine sensitization and cocaine self-administration.[77] These findings suggest that CBD-induced reduction in sucrose self-administration may be mediated by a CB2-dependent mechanism in the mesolimbic DA reward system.

Given that stimulation of CB2Rs by JWH133 (an orthosteric CB2R agonist) produces an inhibitory effect on VTA DA neuronal activity and NAc DA release),[75,76,82] one may expect that CBD, an allosteric *inverse agonist* of CB2Rs, would produce an *increase* in VTA DA neuronal activity and NAc DA release. However, by directly measuring extracellular DA level in the NAc using in vivo brain microdialysis, we found that CBD alone produced a 20% reduction (nonsignificant) in extracellular NAc DA, and pretreatment with CBD significantly reduced cocaine-enhanced DA,[86] suggesting that a DA-dependent mechanism may underlie CBD action. We cannot use these data to support or against whether CBD is an allosteric CB2R inverse agonist since CBD has multiple acting targets, and the observed DA effect could be a final net effect of multiple actions after CBD administration. In addition, the effects of CBD on NAc DA are conflicting. For example, microinjections of CBD into a lateral ventricle (icv) or lateral hypothalamus produced significant increases in the extracellular DA in the NAc.[87,88] However, intra-NAc microinjection of CBD produced an inhibitory effect on VTA DA neuronal activity by itself[89] and attenuated amphetamine-induced locomotor sensitization and VTA DA neuronal sensitization.[89,90] Although the authors proposed that 5-HT_{1A} mechanisms may underlie intra-NAc CBD-induced reduction in NAc DA,[90,91] there is no direct evidence to support it. Furthermore, CBD has been reported to inhibit synaptic uptakes of DA in vitro synaptosome preparations.[92] Clearly, more studies are required to determine whether and how CBD alters the mesolimbic DA system activity.

In addition to the above DA mechanism, CBD was reported to inhibit the degradation of the endocannabinoid anandamide by fatty acid amide hydrolase[93] and inhibit the cellular uptake of anandamide.[38] Given that anandamide a weak partial CB2R agonist, it is unsure whether such an enhanced anandamide-CB2R mechanism is involved in CBD action observed in the present study. In contrast, the endocannabinoid 2-arachidonoylglycerol (2-AG) is a full CB1 and CB2 receptor agonist.[94] If CBD similarly elevates brain 2-AG levels, it may well explain the present finding. More studies are required to test this hypothesis.

Lastly, sucrose intake may also alter neuronal activity and hormonal release in the hypothalamus and mesolimbic regions, leading to the development of preference for palatable sweetened food and the alterations in metabolism and body weight.[95,96] Thus, it is conceivable that CBD, in addition to reducing sucrose reward acutely, may also reverse hypothalamic and mesolimbic dysfunctions, leading to persistent preference for healthy diet.

In summary, the present findings suggest that CBD may have certain therapeutic potential in controlling binge eating and treating obesity. Our findings are of particular importance given prevalence of obesity in the United States and successful pharmacotherapies to treat obesity are yet to be developed. In the series of experiments, we have demonstrated that CBD and JWH133 have the ability to reduce food reward and motivation to seek sweetened food. Our data pave the way toward better understanding the role of CB1 and CB2 receptors in food reward and promoting CBD as potential therapeutics for the treatment and prevention of obesity.

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AUTHORS CONTRIBUTION

Z-XX designed the experiments. G-HB and YH performed the experiments. G-HB and EG analyzed data and prepared the figures. EG and Z-XX wrote the manuscript.

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