Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors

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Abstract

Cannabinoids are postulated to play a role in modulating the reinforcing effects of abused drugs, including alcohol. Experiment 1 examined alcohol self-administration in cannabinoid CB1 receptor knockout (KO), heterozygous (HT) and wild type (WT) mice in a two-bottle choice paradigm. Mice were trained in a limited 8 h access/day to 10% (v/v) EtOH (EtOH) versus water. After baseline drinking levels (% EtOH preference and total EtOH intake (g/kg)), results indicated that the CB1 knockout mice displayed significantly lower baseline EtOH consumption compared to wild type mice. Subsequently, treatment with SR141716A (5 mg/kg) significantly attenuated EtOH intake in the WT and HT mice but had little effect on the knockout mice.

Experiment 2 examined the CB1 WT and CB1 KO strains in a conditioned place preference (CPP) procedure between saline and 2 g/kg EtOH. The CB1 WT mice spent significantly more time in the EtOH-paired versus saline-paired chambers, whereas no significant preference was observed in the CB1 KO mice. Finally, we observed that CB1 KO mice were significantly lighter than WT and HT and that SR141716A did not significantly alter body weight. These results demonstrate that the cannabinoid CB1 receptor is an essential component of the molecular pathways underlying the reinforcing effects of alcohol. Thus, medications targeting the CB1 receptors may be beneficial for the treatment of alcoholism.

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1. Introduction

EtOH abuse and addiction is a complex social and psychiatric problem that involves a variety of neurotransmitter systems, such as dopamine (DA) [13,29,55,56], GABA [24], glutamate [22], serotonin [32], acetylcholine [38] and the cannabinoid system [25]. EtOH abuse also involves a variety of receptors, second messenger systems and genes [14].

The cannabinoid system is not only involved in the effects of marijuana [19], but may be involved in the reinforcing properties of major abused drugs, such as EtOH and opioids [18] as well as other psychiatric or neurological diseases as supported by various genetic studies [8,16,48]. The cannabinoid CB1 receptor in particular has been widely examined and reported to have an interesting though complex role in addiction.

CB1 receptor activation increases the activity of DA neurons in the ventral tegmental area, thereby increasing DA release in the NAc [20,51] which may have an effect in modulating the brains reward circuitry. Prior research has reported that the CB1 receptor appears to be the site of reinforcing
The human CB1 receptor gene (CNR1) was recently analyzed in alcoholics versus non-alcoholic controls [48]. The observed frequency of the A allele was 31.2% for controls and 42.1% for alcoholics. These results suggested that that homozygous genotype CNR1 1359A/A confers vulnerability to alcoholism [48]. Chronic alcohol exposure caused a decrease in anandamide in the midbrain [21], while it increased anandamide content in the limbic forebrain [3,21].

These results suggested the involvement of endocannabinoid transmission in the reward circuit activated by alcohol [21].

Several animal studies have indicated that the cannabinoid system can influence the rewarding effects of EtOH. This is based on several observations: lower alcohol consumption in C57BL/6 mice [1]; a reduction in EtOH intake by SR141716A [7,41] and ethanol sensitivity [39] and withdrawal symptoms completely absent in cannabinoid CB1 receptor-deficient mice [39,42].

SR141716A has been shown to be a very selective ligand for the CB1 receptor and a potent antagonist [46]. SR141716A has been shown to block the actions of the cannabinoids in several behaviors [40], such as suppressing alcohol intake [28,41]. Sardinian alcohol-prefering (SP) rats, selectively bred for high EtOH preference and consumption were used to study the efficacy of SR141716A, in reducing voluntary EtOH intake [7]. Rats were given limited access to food and (10%) EtOH (4 h/day) and were treated with acute i.p. administration of 2.5 and 5 mg/kg SR141716A decreased EtOH intake while 10 mg/kg reduced both food and EtOH intake [7]. In another study, C57BL/6 in a two-bottle choice procedure were given free access to water and (10%) EtOH [1], when treated with SR141716A (0.3–3 mg/kg), significantly reduced EtOH consumption. More recently, Long Evans rats trained on a fixed ratio operant response protocol to self-administer EtOH were treated with SR141716A (0.3–3 mg/kg, i.p.), produced dose-related decreases the number of lever presses and EtOH intake [15].

Based on the previous studies with SR141716A, we hypothesized that wild type homozygous, heterozygous and CB1 receptor deficient mice would display differences in their alcohol-drinking behavior. In the present study, we examined the role of the CB1 receptor using CB1 transgenic mice, in a two-bottle choice paradigm, to measure EtOH preference, and total EtOH consumption. We thus hypothesized that CB1 homozygous, heterozygous and CB1 deficient mice would display differences in their alcohol-drinking behavior, and that treatment with SR141716A would modulate that behavior. Furthermore, the present study examined CB1 transgenic mice in a conditioned place preference (CPP) paradigm for ethanol. In this procedure, the animals tendency to approach or avoid environmental cues previously paired with the drug [2,4,27,52]. Several studies have demonstrated that as with other drugs of abuse, animals display CPP to EtOH [5,6,12,44,49]. Therefore, another objective of the present study was to determine if the CB1 receptor is important in Pavlovian conditioning to the EtOH-paired environmental cues. We hypothesized that CB1-deficient mice would not show EtOH-paired CPP as compared to their wild type littermates.

2. Materials and methods

2.1. Animals

2.1.1. Experiment 1: two-bottle choice EtOH drinking

Adult male \(N=29\) CB1 transgenic mice (CD1 strain, obtained from C. Ledent in Universite libre de Brussels, Belgium) [31] were individually housed in a 12/12 h reverse light/dark cycle, as well as a temperature and humidity controlled room. Details on the generation of these animals has been previously described [31]. Briefly, using the 129/Sv mouse genome library, the CB1 gene was cloned and the single coding exon was mapped and sequenced. Using R1 cells and aggregation with CD1 eight-cell stage embryos homologous recombination was performed [31]. Heterozygous mice were bred for five generations on a CD1 background before generating the CB1 WT and KO mice used in this study.

Specifically, the study consisted of three groups of animals: (a) CB1 homozygous (+/+)(\(n=12\)), (b) heterozygous (+/−)(\(n=7\)) and (c) the CB1 knockout (−/−) mice (\(n=10\)). All studies were conducted in accordance with the guidelines established by the National Institutes of Health in The Guide For Care and Use of Laboratory Animals.

2.1.2. Experiment 2: conditioned place preference

Adult male \(N=38\) CB1 transgenic mice (CD1 strain, obtained individually housed in a 12/12 h reverse light/dark cycle, as well as a temperature and humidity controlled room were used for this experiment. More specifically, 20 CB1 homozygous (WT) and 18 CB1 deficient (KO) mice were used.

2.2. Procedures

2.2.1. Experiment 1: two-bottle choice EtOH drinking

We utilized the standard two-bottle choice protocol, which is a widely used model that captures aspects of voluntary alcohol consumption in humans [36]. One bottle contained water and the other bottle ethanol. Volumetric consumption data was recorded from both drinking bottles every 3 days at the same time (1630). Each home cage contained two 25 ml Pyrex glass bottles, capped with rubber stoppers fitted with stainless steel tips. Each of those, the bottles were emptied cleaned and refilled to 25 ml. Every 3 days, the bottles were switched to eliminate a position preference. All animals were given unrestricted food access (Purina rodent lab diet). Drinking preference was assessed as the amount of EtOH consumed divided...
Days 1–3—Preconditioning phase. On the first 2 days, mice for the experimental group (10 CB1 WT and 9 CB1 KO) and the other half of the animals were assigned to the control group. The CPP protocol consisted of three phases as follows: (A) Behavioral response to WIN 55, 212: Animals were administered 1 mg/kg WIN 55, 212 via a tail vein and the presence or absence of a classic CB1-induced catalepsy in the wild type and heterozygous versus the knockouts, respectively, was verified by visual inspection [9].

(b) Animals were genotyped using the tail snip method [47]. DNA was collected for all the mice, amplified using PCR and loaded into an agarose gel for electrophoresis.

3. Results

3.1. EtOH preference

The CB1 WT mice showed the highest EtOH preference on average when compared to the CB1 HT and the CB1 KO. A one way repeated measures ANOVA on percent ethanol preference across all three strains of mice during baseline, SR141716A and vehicle drinking sessions revealed a significant difference (F=9.95; d.f. =93.11; p<0.001; Fig. 1).

All pairwise multiple comparison procedures (using the Holm–Sidak method) revealed several significant differences (p<0.05; Fig. 1). Specifically, SR141716A (12.9 ± 1.19%) significantly attenuated % EtOH preference compared to baseline (26.7 ± 3.3%) and vehicle (30.3 ± 2.5%) for the CB1 WT mice. Similarly, SR141716A (10.1 ± 0.8%) reduced EtOH preference compared to baseline (24.6 ± 3.2%) and vehicle (23.8 ± 1.9%) for the CB1 HT mice. In contrast, SR141716A had no significant effect on EtOH preference in CB1 KO mice. In addition, similar pairwise multiple comparisons were found to be significantly different (p<0.05) between WT (26.7 ± 3.3%) and KO (14.5 ± 2.2%) mice in baseline and vehicle (30.3 ± 2.5% and 14.9 ± 1.6% respectively) drinking.

3.2. EtOH intake

In addition to measuring EtOH preference over water, the data was also analyzed by measuring total EtOH consump-

![Graph](image-url)
tion (per day; Fig. 2). This indicated a similar trend. The highest level of EtOH intake was observed in CB1 WT mice followed by HT and KO mice. A one way repeated measures ANOVA on percent ethanol intake across all three strains of mice during 24 and 9 h (baseline), SR141716A and vehicle drinking sessions revealed a significant difference ($F = 9.28; \text{d.f.} = 93.11; p < 0.001$; Fig. 2).

Pairwise multiple comparison procedures (using the Holm–Sidak method) revealed several significant differences ($p < 0.05$; Fig. 2). Specifically, SR141716A (1.3 ± 0.1 g/kg) significantly attenuated EtOH intake compared to baseline (2.9 ± 0.5 g/kg) and vehicle (4.0 ± 0.5 g/kg) for the CB1 WT mice. Similarly, SR141716A (1.2 ± 0.1 g/kg) reduced EtOH intake compared to vehicle (3.1 ± 0.3 g/kg) for the CB1 HT mice. In contrast, SR141716A had no significant effect on EtOH intake in CB1 KO mice (Fig. 2). Likewise, pairwise multiple comparisons were found to be significantly different ($p < 0.05$) between WT (4.0 ± 0.3 g/kg) and KO (2.2 ± 0.2 g/kg) mice in vehicle drinking (Fig. 2).

Finally, no significant effect of SR141716A was found on water intake across treatment in any of the groups of mice (one way repeated measures ANOVA, $F = 1.89; \text{d.f.} = 93.11; p = \text{ns}$). Specifically the mean (±S.E.M.) water intake during vehicle versus SR141716A treatment across genotype was as follows: 15.86 ± 1.37 and 14.32 ± 0.62 for WT, 13.80 ± 0.62 and 14.8 ± 0.63 for HT, and 14.4 ± 0.59 and 14.12 ± 0.99 for KO.

### 3.3. Weight

The CB1 WT mice (45.0 ± 5.8 g) were significantly heavier than the CB1 KO (32.2 ± 3.5 g) and the CB1 HT (35.2 ± 3.9 g) mice had intermediate weights between those of the WT and the KO mice (Fig. 3). One way repeated measure ANOVA of the weights of the three strains of CB1 mice throughout the experiment revealed a significant difference ($F = 20.3; \text{d.f.} = 93.11; p < 0.001$; Fig. 3). Pairwise multiple comparisons (Holm–Sidak method) revealed no significant differences across treatment (baseline, SR141716A, vehicle), but there were significant differences in weight across strains for each treatment ($p < 0.05$). In all cases, CB1 mice showed significantly greater weight than HT and KO mice.

### 3.4. CPP

EtOH CPP is illustrated in Fig. 4 for each strain as a percentage of total time spent per chamber. The WT (Fig. 4A) and KO (Fig. 4B) mice showed significant CPP for ethanol compared to baseline (saline).
mice demonstrated a significantly greater time spent in the EtOH-paired chamber as compared to the saline-paired chamber. The mean time (seconds) spent in the EtOH-paired chamber was 405.12 ± 28.98 s, while the mean time spent in the saline was 288.6 ± 22.98 s. However, the CB1 KO mice showed less of a difference in mean time spent between the EtOH-paired chamber (321.06 ± 35.58 s) and the saline (288.54 ± 18.42 s). Using a two-way ANOVA for genotype and treatment, we found a significant genotype (\(F = 10.42; \text{d.f.} = 1.32; \ p < 0.05\)) and genotype × treatment interaction (\(F = 10.41; \text{d.f.} = 1.32; \ p < 0.05\)) effects. Pairwise multiple comparisons (Holm–Sidak method) revealed significant greater time spent in the ethanol-paired chamber compared to vehicle for the D2 WT mice (\(t = 3.002; \ p < 0.05\)). In contrast, similar pairwise comparisons did not reveal significant differences in time spent/chamber for the D2 KO mice (\(t = 1.483; \ p = ns\)).

As expected, the saline treated control group of mice did not exhibit a significant preference. A paired t-test comparison revealed no statistical difference in the mean time spent in the white chamber with the steel mesh floor (339.66 ± 20.22 and 318.28 ± 18.98, respectively) for the CB1 WT and KO mice as compared to the mean time spent in the black chamber with a stainless steel grid rod floor (371.76 ± 22.68 and 364.22 ± 19.35, respectively, \(p > 0.05\)). These results revealed that there was no significant place preference for either of the two chambers in both the CB1 WT and KO mice.

3.5. Genotyping

In electrophoresis, the highest allele marking is the mutant allele while the allele on the bottom is the WT allele; because the CB1 KO has an amplification of about 366 base pairs while the WT have an amplification of 521 base pairs (see Fig. 5).

4. Discussion

Dopaminergic transmission has been shown to be preferentially increased by natural rewards (food, water, sex) under normal and deprivation conditions, as well as in response to novel stimuli. While no CB1 receptor mRNA or protein has been shown in dopamine neurons in the VTA, increased dopamine release and firing of dopaminergic neurons have been found after systemic administration of CB1 agonists, or blocked by CB1 antagonists [20,51,57]. This suggested that the endocannabinoid system could exert a trans-synaptic control over dopamine neurons in the reward center of the brain.

The present study supported the hypothesis that the CB1 receptors play a critical role in mediating the reward and pleasure properties of alcohol, contributing to alcohol dependency and abuse. These findings were consistent with earlier studies [20,33,51], that reported the involvement of the CB1 in the addictive drug abuse. It has been reported that the CB1 receptors were not involved in the reinforcing properties of certain drugs of abuse, such as morphine [10,31], but were for others such as to cocaine [50], \(\alpha\)-amphetamine and nicotine [10]. The present findings were in agreement with several studies that have demonstrated a relationship between the CB1 receptors and ethanol abuse [1,7,17,25,45].

CB1 HT and KO mice showed 18% and 51%, respectively lower baseline EtOH preference when compared to CB1 WT mice. SR141716A (5 mg/kg) treatment showed the strongest effect on the CB1 WT and HT mice, and significantly attenuated their EtOH preference and intake by almost half. In contrast SR141716A did not significantly lower ethanol preference or intake in the KO mice.

The CPP data were also very important, and suggested that the CB1 receptors played an important role in alcohol abuse. These results indicated that when given a choice of environments, the CB1 WT mice preferred the EtOH-paired chamber to the saline or vehicle-paired chamber. Specifically, WT mice spent about 40% more time in the EtOH-paired chamber versus the saline-paired. In contrast, the CB1 deficient mice did not show a preference between the two chambers spending approximately equal time in each. The CPP results illustrated that the CB1 receptor were involved in the saliency of the environmental cues associated with stimuli, such as EtOH and overall in the EtOH-induced drug seeking behavior.

In agreement with the data recently reported [26,41], we observed that voluntary alcohol consumption was appreciably lower in CB1 KO mice compared to CB1 WT. In addition, we observed that SR141716A, attenuated EtOH consumption in CB1 WT mice, and this was in accord with a previous report [41]. This effect on EtOH intake in the CB1 transgenic mice by the CB1 antagonist SR141716A, was in agreement with previous reports in rats [7] and C57BL/6 mice [1]. The present data supported the idea that endocannabinoid tone played an important role in the regulation of ingestive behaviors. Moreover, the present study also demonstrated that the CB1 HT mice displayed EtOH preference and intake at intermediate levels between the CB1 WT and KO mice. This could suggest that the CB1 genotype quantitatively influenced EtOH consumption and played a modulatory role in reward. The presence of CB1 receptors at an elevated level...
Endocannabinoid signaling could then compensate the behavior and alcohol and drug abuse and fear. The inhibitory actions of endocannabinoids could then compensate the behavior and the abnormal neurochemistry associated with these conditions, such as modify dopaminergic signaling characteristic of alcohol abuse. Much work however remains to fully understand the mechanism(s) of the endocannabinoid—dopamine relationship in alcohol abuse, so as to assess this and other hypotheses. Future studies will continue to examine the complexity of the endocannabinoid relationship with dopaminergic signaling and, hence behavior under normal and alcohol abuse conditions.

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