

Research report

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

Panayotis K. Thanos^{a,b,*}, Elias S. Dimitrakakis^a, Onarae Rice^a,
Andrew Gifford^a, Nora D. Volkow^a

^a Behavioral Pharmacology Lab, Department of Medicine, Brookhaven National Laboratory, Building 490,
30 Bell Avenue, Upton, NY 11973-5000, USA

^b Laboratory of Neuroimaging, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health,
Department of Health and Human Services, Park Building, Room 150, 12420 Parklawn Drive,
MSC 8115, Bethesda, MD 20892-8115, USA

Received 23 February 2005; received in revised form 6 June 2005; accepted 12 June 2005

Available online 2 September 2005

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.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Alcoholism; Gene therapy; Dependency; Ingestive behavior; Transgenic

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 opiates [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

* Corresponding author. Tel.: +1 631 344 7364; fax: +1 631 344 5311.
E-mail address: thanos@bnl.gov (P.K. Thanos).

effects within the cannabinoid system [33]. Previous studies examining the role of CB1 in addiction have shown that CB1 KO mice exhibited conditioned place preference for cocaine but not for morphine [34]; failed to self administer morphine but did self administer cocaine, D-amphetamine and nicotine [10]. In contrast, very recently it was reported that CB1 receptors play a role in the acquisition and maintenance of cocaine self-administration and that CB1 KO mice showed diminished operant responding to self-administer cocaine [50].

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/six 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-preferring (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. Results showed that 2.5 and 5 mg/kg of 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 behav-

ior, 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 Bruxells, 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, similarly 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 days, 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

by total fluid consumed \times 100. The mean intake was expressed as milliliters and grams of EtOH/kg of body weight/day.

Initially all mice were placed on a 24 h access two-bottle choice paradigm 10% (v/v) EtOH and water for 2 weeks. Next, the mice were placed on a limited 8 h access day to both liquids for 2 weeks (0830–1630 h) during the dark cycle period. This same limited access period was used throughout the remainder of the experiment. Baseline drinking was established when mice demonstrated three consecutive days of EtOH drinking within 15% of their mean. After baseline criterion drinking was achieved, mice were injected (i.p.) with 5 mg/kg of the CB1 antagonist, SR141716A (Research Triangle Institute, North Carolina). The SR141716A drug solution was prepared fresh daily with distilled water, DMSO (5%) and cremophor (5%). Each mouse was injected twice daily (08.00 and 12.00 h) for 3 days, with the SR141716A drug. EtOH drinking behavior was continually monitored for an additional 3 days (limited 8 h access/day to both liquids) before all animals were similarly treated with vehicle over 3 days (H₂O, 5% DMSO, and 5% cremophor).

2.2.2. Experiment 2: CPP

Each of the CPP boxes (MED Associates Inc.) consisted of three different chambers separated by sliding partitions. The center choice chamber was gray with a smooth PVC floor while the conditioning chambers were black with a stainless steel grid rod floor and white with a steel mesh floor. Half the animals were assigned to 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 for the experimental group of CB1 WT and KO mice (adapted from previous research [12]) and consisted of three phases as follows:

- (A) Days 1–3—Preconditioning phase. On the first 2 days, mice were handled and brought to the conditioning room to become acclimated to the test and to control for stress. On the third preconditioning day, the animals were placed in the middle chamber and allowed to explore all three of the chambers at will for a 30 min period. Data from this third day was analyzed for any unconditioned chamber preference.
- (B) Days 4–11—Conditioning phase. Half the animals were given saline in the white chamber while the other half in the black chamber on days 4, 6, 8, and 10. On days 5, 7, 9, and 11, the same animals were given ethanol (2 g/kg i.p. 20% (v/v) EtOH in saline) in the opposite chamber (for 30 min/trial).
- (C) Day 12—Test phase. Mice were placed in the middle chamber and allowed free access to all three chambers for 30 min. Each test session was recorded on videotape and analyzed.

The CPP protocol for the control group of CB1 WT and KO mice consisted of the same three 's. These animals were utilized to ascertain any innate (unconditioned) chamber preference received only saline injections during the conditioning phase (otherwise the procedure was identical to the experimental group). This group of mice was included in order to determine whether genotypes differed in unconditioned preference for the floor textures or wall colors.

2.3. Genotyping

Upon the conclusion of both experiments, the genotypes of all the mice, was verified using two methods: (a) The CB1 agonist drug, WIN 55, 212-2 and (b) PCR of tail snips:

- (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

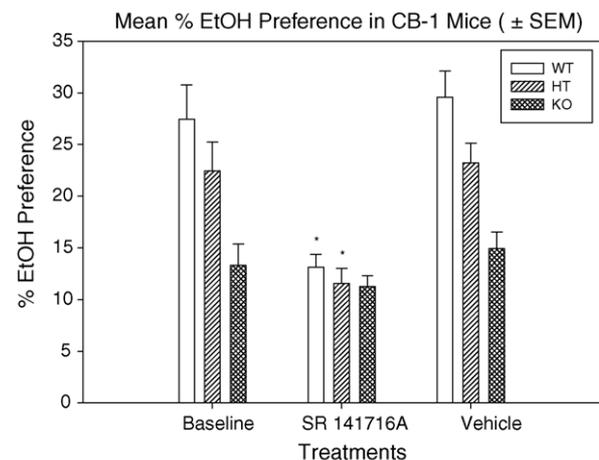
3.1. EtOH preference

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 ($F = 9.953$; 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-



* Indicates Statistically Significant Difference from Baseline and Vehicle ($p < 0.05$)

Fig. 1. Mean percent EtOH preference (\pm S.E.M.) across treatment in CB1 mice.

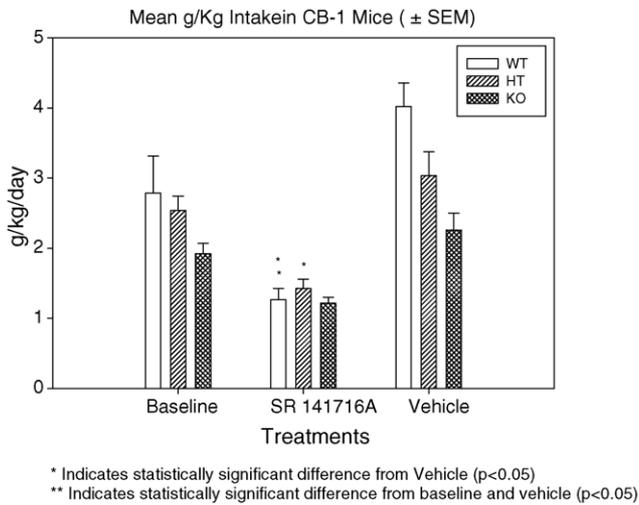


Fig. 2. Mean daily EtOH intake (g/kg/day) across treatment in CB1 mice (±S.E.M.).

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 8 h (baseline), SR141716A and vehicle drinking sessions revealed a significant difference ($F = 9.28$; $d.f. = 93.11$; $p < 0.001$; 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 ± 0.3 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; $F = 1.89$; $d.f. = 93.11$; $p = ns$). 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 intermediates 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

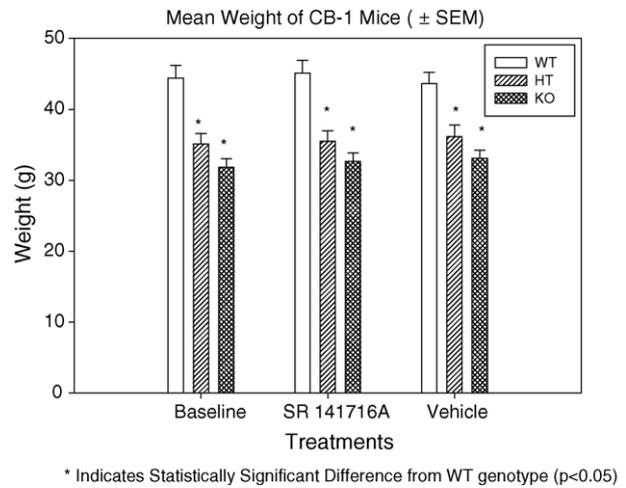


Fig. 3. Mean (±S.E.M.) weights of CB1 mice across treatment.

($F = 20.3$; $d.f. = 93.11$; $p < 0.001$; 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

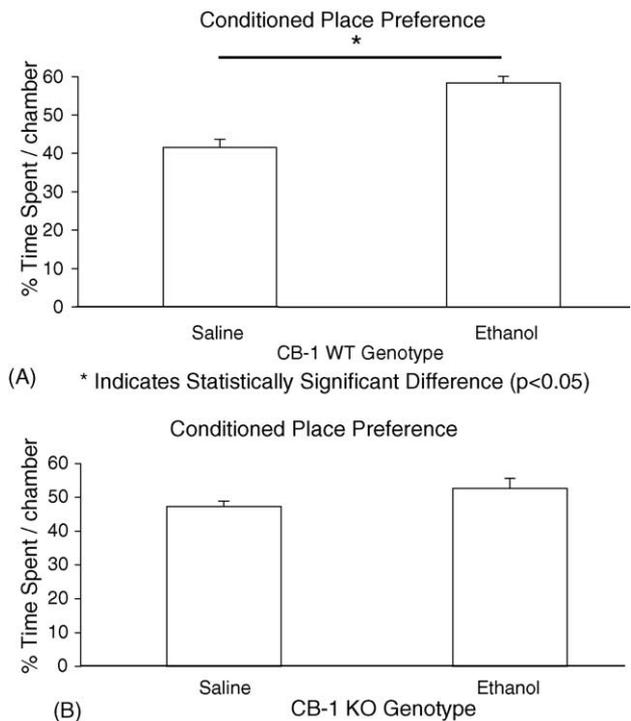


Fig. 4. (A) Conditioned place preference for the saline vs. ethanol paired chamber in CB1 WT mice. (B) Conditioned place preference for the saline vs. ethanol paired chamber in CB1 KO mice.

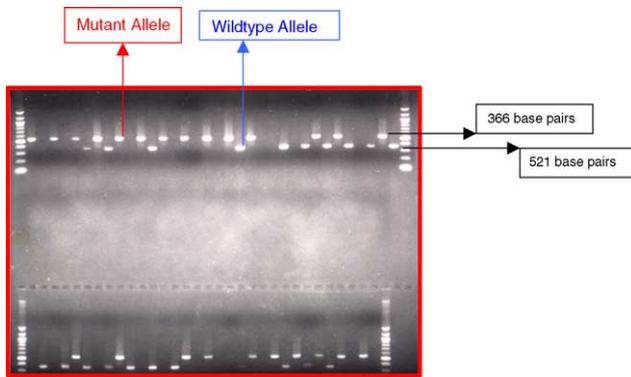


Fig. 5. Genotyping micrograph of electrophoresis agarose gel depicting CB1 wild type and mutant alleles.

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 ($F = 10.42$; d.f. = 1.32; $p < 0.05$) and genotype \times treatment interaction ($F = 10.41$; 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], D-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

or density in some way stimulated the dopaminergic reward circuit. In contrast, lack of, or diminished levels of CB1 receptors by the same token could result in a diminished level of excitation of the reward pathway. The decreased ethanol intake in CB1 KO mice may be related to several possibilities, such as a dysfunction of the dopamine system in the mesocorticolimbic reward pathway. CB1 KO mice may have decreased sensitivity to the stimulating effects of ethanol because of an alteration in dopamine signaling. This was demonstrated recently where CB1 KO mice completely lacked acute alcohol-induced dopamine release in the NAC [26]. Similarly, lack of morphine self-administration in CB1 mice was also associated with the inability of morphine to stimulate dopamine in the NAC [35].

In agreement with previous studies, we also reported that the CB1 WT mice were significantly heavier compared to the CB1 HT and CB1 KO mice [11,23]. It has been well implicated that the endocannabinoid system and the CB1 receptors were important in body weight and food intake [30,37,43,54]. While food intake was not the focus of the present study and was not measured, no significant changes in weight were observed (over the 3 days that animals were treated with the SR141716A). It is possible however, that this may have been due to the short time duration of the study. Further chronic studies would be warranted in the future to monitor effectively changes in body weight and ethanol drinking in these mice as well as the long term effects of treating these mice with SR141716A.

In summary, these results supported the notion that CB1 receptors may play a role in either mediating or facilitating the addictive properties of EtOH and EtOH-induced drug-seeking behavior. It is clear that the role of the CB1 receptor in ethanol consumption is complex and may not be unitary but may possibly involve interaction with the dopaminergic reward pathway. While the mechanism remains unclear, one possible mechanism that can be proposed is that EtOH consumption resulted in the disinhibition of excitatory projections to the VTA DA neurons and subsequent DA release in the NAc. More specifically, EtOH intake could result in the release of endocannabinoids that bind to the CB1 receptors located on cortical and hippocampal GABA neurons, resulting in decreased GABA release and consequently increased glutamate release and excitation of the VTA DA neurons. This enhanced dopaminergic drive excitation (increased DA release in the NAc) and activation of the reward circuit elicited by cannabinoids could affect alcohol drinking behavior. These findings suggested that blockade of the cannabinoid CB1 receptor should be incorporated in studying the mechanism(s) of alcohol dependency.

Recently, a general hypothesis has been proposed on the role of endocannabinoids on reward and behavior [53]. Endocannabinoid signaling could be perceived as an adaptive response to stimuli or circumstances that pose a hazard to the organism and to the brain more specifically. Such circumstances could be triggered by psychologically stressful stimuli, neurodegeneration, food and water deprivation,

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.

Acknowledgements

This work was supported by the NIAAA (AA 11034 & AA07574, AA07611) and by the U.S. Department of Energy under contract DE-AC02-98CH10886. We also thank the DOE SULI program for funding ESD. Finally, we would like to thank Dr. C. Ledent for providing the CB1 mice for this study.

References

- [1] Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P, et al. Selective inhibition of sucrose and ethanol intake by SR141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl)* 1997;132:104–6.
- [2] Baker DA, Fuchs RA, Specio SE, Khroyan TV, Neisewander JL. Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. *Synapse* 1998;30:181–93.
- [3] Basavarajappa BS, Hungund BL. Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor *N*-arachidonoylphosphatidylethanolamine in SK-N-SH cells. *J Neurochem* 1999;72:522–8.
- [4] Bedingfield JB, King DA, Holloway FA. Cocaine and caffeine: conditioned place preference, locomotor activity, and additivity. *Pharmacol Biochem Behav* 1998;61:291–6.
- [5] Chester JA, Cunningham CL. Modulation of corticosterone does not affect the acquisition or expression of ethanol-induced conditioned place preference in DBA/2J mice. *Pharmacol Biochem Behav* 1998;59:67–75.
- [6] Ciccocioppo R, Panocka I, Frolidi R, Quitadamo E, Massi M. Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. *Psychopharmacology* 1999;141:235–41.
- [7] Colombo G, Agabio R, Fa M, Guano L, Lobina C, Loche A, et al. Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. *Alcohol Alcohol* 1998;33:126–30.
- [8] Consroe P, Musty R, Rein J, Tillery W, Pertwee R. The perceived effects of smoked cannabis on patients with multiple sclerosis. *Eur Neurol* 1997;38:44–8.
- [9] Cosenza M, Gifford AN, Gatley SJ, Pyatt B, Liu Q, Makriyannis A, et al. Locomotor activity and occupancy of brain cannabinoid CB1 receptors by the antagonist/inverse agonist AM281. *Synapse* 2000;38:477–82.
- [10] Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M, et al. Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res* 2001;118:61–5.

- [11] Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 2003;112:423–31.
- [12] Cunningham CL, Howard MA, Gill SJ, Rubinstein M, Low MJ, Grandy DK. Ethanol-conditioned place preference is reduced in dopamine D2 receptor-deficient mice. *Pharmacol Biochem Behav* 2000;67:693–9.
- [13] Di Chiara G, Acquas E, Carboni E. Drug motivation and abuse: a neurobiological perspective. *Ann NY Acad Sci* 1992;654:207–19.
- [14] Fadda F, Rossetti ZL. Chronic ethanol consumption: from neuroadaptation to neurodegeneration. *Prog Neurobiol* 1998;56:385–431.
- [15] Freedland CS, Sharpe AL, Samson HH, Porrino LJ. Effects of SR141716A on ethanol and sucrose self-administration. *Alcohol Clin Exp Res* 2001;25:277–82.
- [16] Gadzicki D, Muller-Vahl K, Stuhmann M. A frequent polymorphism in the coding exon of the human cannabinoid receptor (CNR1) gene. *Mol Cell Probes* 1999;13:321–3.
- [17] Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol* 1999;370:233–40.
- [18] Gardner EL, Vorel SR. Cannabinoid transmission and reward-related events. *Neurobiol Dis* 1998;5:502–33.
- [19] Gatley SJ, Lan R, Volkow ND, Pappas N, King P, Wong CT, et al. Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB1 receptors in vivo. *J Neurochem* 1998;70:417–23.
- [20] Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur J Pharmacol* 1998;341:39–44.
- [21] Gonzalez S, Cascio MG, Fernandez-Ruiz J, Fezza F, Di Marzo V, Ramos JA. Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. *Brain Res* 2002;954:73–81.
- [22] Hoffman P. Glutamate receptors in alcohol withdrawal-induced neurotoxicity. *Metab Brain Dis* 1995;10:73–9.
- [23] Horvath TL. Endocannabinoids and the regulation of body fat: the smoke is clearing. *J Clin Invest* 2003;112:323–6.
- [24] Hungund BL, Basavarajappa BS. Are anandamide and cannabinoid receptors involved in ethanol tolerance? a review of the evidence. *Alcohol Alcohol* 2000;35:126–33.
- [25] Hungund BL, Basavarajappa BS. Distinct differences in the cannabinoid receptor binding in the brain of C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. *J Neurosci Res* 2000;60:122–8.
- [26] Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J Neurochem* 2003;84:698–704.
- [27] Khroyan TV, Baker DA, Fuchs RA, Manders N, Neisewander JL. Differential effects of 7-OH-DPAT on amphetamine-induced stereotypy and conditioned place preference. *Psychopharmacology (Berl)* 1998;139:332–41.
- [28] Kirkham TC, Williams CM. Synergistic effects of opioid and cannabinoid antagonists on food intake. *Psychopharmacology (Berl)* 2001;153:267–70.
- [29] Koob GF, Le HT, Creese I. The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. *Neurosci Lett* 1987;79:315–20.
- [30] Kunos G, Batkai S. Novel physiologic functions of endocannabinoids as revealed through the use of mutant mice. *Neurochem Res* 2001;26:1015–21.
- [31] Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 1999;283:401–4.
- [32] Lu MR, Wagner GC, Fisher H. Ethanol consumption following acute fenfluramine, fluoxetine, and dietary tryptophan. *Pharmacol Biochem Behav* 1993;44:931–7.
- [33] Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W. Self-administration of the cannabinoid receptor agonist WIN 55, 212-2 in drug-naive mice. *Neuroscience* 1998;85:327–30.
- [34] Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. *Eur J Neurosci* 2000;12:4038–46.
- [35] Mascia MS, Obinu MC, Ledent C, Parmentier M, Bohme GA, Imperato A, et al. Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB(1) receptor knockout mice. *Eur J Pharmacol* 1999;383:R1–2.
- [36] McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol* 1998;12:339–69.
- [37] McLaughlin P, Wisniecki A, Tardiff D, Liu Q, Makriyannis A, Salamone J. Cannabinoid CB1 antagonists suppress food intake in a variety of tasks in rats. *Society for neuroscience (2002 CD-ROM)* 2002;783.16.
- [38] Melis F, Stancampiano R, Imperato A, Carta G, Fadda F. Chronic ethanol consumption in rats: correlation between memory performance and hippocampal acetylcholine release in vivo. *Neuroscience* 1996;74:155–9.
- [39] Naassila M, Pierrefiche O, Ledent C, Daoust M. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology* 2004;46:243–53.
- [40] Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 1997;74:129–80.
- [41] Poncelet M, Maruani J, Calassi R, Soubrie P. Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. *Neurosci Lett* 2003;343:216–8.
- [42] Racz I, Bilkei-Gorzo A, Toth ZE, Michel K, Palkovits M, Zimmer A. A critical role for the cannabinoid CB1 receptors in alcohol dependence and stress-stimulated ethanol drinking. *J Neurosci* 2003;23:2453–8.
- [43] Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2003;284:R345–53.
- [44] Reid LD, Hunter GA, Beaman CM, Hubbell CL. Toward understanding ethanol's capacity to be reinforcing: a conditioned place preference following injections of ethanol. *Pharmacol Biochem Behav* 1985;22:483–7.
- [45] Rimondini R, Arlind C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J* 2002;16:27–35.
- [46] Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 1994;350:240–4.
- [47] Roe B, Crabtree J, Khan A. DNA isolation and sequencing. *New York: Wiley*; 1996, 1–112 pp.
- [48] Schmidt LG, Samochowiec J, Finckh U, Fiszser-Piosik E, Horodnicki J, Wendel B, et al. Association of a CB1 cannabinoid receptor gene (CNR1) polymorphism with severe alcohol dependence. *Drug Alcohol Depend* 2002;65:221–4.
- [49] Sim LJ, Hampson RE, Deadwyler SA, Childers SR. Effects of chronic treatment with delta9-tetrahydrocannabinol on cannabinoid-stimulated [35S]GTPgammaS autoradiography in rat brain. *J Neurosci* 1996;16:8057–66.
- [50] Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M, et al. Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* 2005 March 2 (Epub ahead of print).

- [51] Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science* 1997;276:2048–50.
- [52] Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* 1998;56:613–72.
- [53] van der Stelt M, Di Marzo V. The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. *Eur J Pharmacol* 2003;480:133–50.
- [54] Vickers SP, Webster LJ, Wyatt A, Dourish CT, Kennett GA. Preferential effects of the cannabinoid CB1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. *Psychopharmacology (Berl)* 2003;167:103–11.
- [55] Weiss F. Neuroadaptive changes in neurotransmitter systems mediating ethanol-induced behaviors. *NIAAA Res Monogr* 2000;34:216–313.
- [56] Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev* 1987;94:469–92.
- [57] Wu X, French ED. Effects of chronic delta9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. *Neuropharmacology* 2000;39:391–8.