INTRODUCTION

The endocannabinoid system consists not only of G-protein-coupled cannabinoid receptors but also other components such as enzymes [i.e. fatty acid amidohydrolase (FAAH)] and endogenous ligands. There are two types of cannabinoid receptors, CB₁ and CB₂. The CB₁ cannabinoid (CB₁) receptor is predominantly expressed in brain at relatively high levels in hippocampus, cerebellum and spinal cord and thus, often referred to as the brain cannabinoid receptor. CB₁ receptors are also expressed at low levels in peripheral tissues including spleen, testis and leucocytes (Herkenham et al., 1991; Bouaboula et al., 1993; Lévénès et al., 1998; Navarro et al., 1998). The CB₂ receptor is referred to as the peripheral cannabinoid receptor because it mainly shows peripheral expression in immune cells (Munro et al., 1993; Facetti et al., 1995). To date, there are two endogenous ligands of CB₁ receptors, namely anandamide and 2-arachidonylglycerol, which mimic the pharmacological action of Δ⁹-tetrahydrocannabinol, the active compound of marijuana and other synthetic agonists (Devane et al., 1992; Mechoulam and Fride, 1995; Mechoulam et al., 1995; Stella et al., 1997). There is growing evidence for the implication of endogenous cannabinoids in biological functions, including the control of appetite and food intake (Di Marzo et al., 2001), the modulation of some of the pharmacological effects of ethanol (Basavarajappa et al., 1998, 2000; Basavarajappa and Hungund, 1999a,b, 2002; Hungund and Basavarajappa, 2000a,b; Hungund et al., 2002) and drinking behaviour (Arnone et al., 1997; Colombo et al., 1999; Gallate et al., 1999; Gallate and McGregor, 1999; Rodríguez de Fonseca et al., 1999; Freedland et al., 2001; Colombo et al., 2002; Hungund et al., 2002).

Chronic ethanol administration has a dual effect on the cannabinoid receptor, increasing the level of both endogenous cannabinoid agonists, anandamide and 2-arachidonylglycerol, while downregulating the CB₁ receptor number and function, thereby suggesting a role for the endocannabinoid system in the neurobiological effects of ethanol (Basavarajappa et al., 1998b, 2000; Basavarajappa and Hungund, 1999a,b). Nonetheless, ethanol did not produce any effects on CB₁ receptor binding and mRNA levels in rats (Gonzalez et al., 2002). However, a recent study by Ortiz et al. (2004) showed that forced consumption of high quantity of ethanol for a long period significantly decreased the gene expression of the CB₁ receptors in the caudate-putamen, the ventromedial nucleus of hypothalamus and both CA1 and CA2 fields of the hippocampus. The last finding is in accordance with Basavarajappa et al. (1998) and Basavarajappa and Hungund (1999a). The result obtained by Gonzalez et al. (2002) could be due to differences in the quantity of ethanol and the duration of ethanol administration.

CP-55,940, a CB₁ receptor agonist, promoted alcohol craving in rats (Gallate et al., 1999), as well as voluntary ethanol intake in Sardinian alcohol-prefering (sp) rats (Colombo et al., 2002). WIN-55,212-2, another CB₁ receptor agonist, also promoted voluntary ethanol intake in sp rats (Colombo et al., 2002).

Numerous studies have shown that the CB₁ receptor agonist SR 141716 reduces ethanol intake (Arnone et al., 1997; Colombo et al., 1998; Rodríguez de Fonseca et al., 1999; Freedland et al., 2001) and ethanol craving (Gallate and McGregor, 1999) in different rat strains. In addition,
SR 141716 suppressed the ethanol deprivation effects (i.e. the temporary increase in ethanol intake after a period of ethanol withdrawal) in sP rats (Serra et al., 2002). All these results suggest that the blocking of CB₁ receptor decreases the consumption of ethanol. Nonetheless, it is also important to mention that in our previous study in Wistar rats, we showed that the cannabinoid receptor antagonist SR 141716 profoundly altered ethanol preference in chronically pulmonary alcoholised rats depending on the dose and time of administration. Doses of 3 or 10 mg/kg/day, administered during chronic pulmonary alcoholisation enhanced ethanol preference whereas its administration during the ethanol withdrawal stage after alcoholisation induced a decrease in ethanol preference (Lallemand and De Witte, 2001). We have also shown that the action of SR 141716 was dependent on a number of factors, including the duration of ethanol intoxication as well as the number of ethanol re-exposures and ethanol withdrawals (Lallemand et al., 2004).

All these previous studies used antagonists and agonists of CB₁ receptors. In certain circumstances, some antagonists have side-effects, which could alter/modify their actions. For example, SR-141716, a CB₁ receptor antagonist, can show agonist property (Shire et al., 1999).

An alternative to avoid these possible pharmacological side-effects is the use of null mutant mice. The development of transgenic CB₁ knockout mice has provided the opportunity to study the role of the CB₁ receptor system in the regulation of ethanol consumption (Ledent et al., 1999; Zimmer et al., 1999). CB₁−/− mice with C57 background showed decreased ethanol intake and preference. These effects were associated with a dramatic sensitivity to the hypothermic and hypolocomotor effects in response to low doses of ethanol (Naassila et al., 2004). These mice also showed an increased intensity of ethanol withdrawal-induced convulsions. Female CB₁+/− mice consumed more ethanol than male CB₁+/+ mice; in addition, this gender difference was observed in both genotypes — female CB₁−/− mice showed a decreased ethanol consumption compared with that of female CB₁+/+ mice, but did consume the same quantity of ethanol as did male CB₁+/+ mice. Hungund et al. (2003) observed similar results, although the gender difference in ethanol consumption observed between female and male CB₁−/− mice was abolished in CB₁+/+ mice. These results were also observed in the study of Poncelet et al. (2003) using CB₁−/− mice with C57BL/6 × 129/Ola F2 background.

CB₁−/− with C57BL/6J background had a higher preference for ethanol but only for a few days (Racz et al., 2003). After the cessation of chronic ethanol administration, these mice did not exhibit withdrawal symptoms. After mild intermittent foot-shock stress, alcoholized CB₁−/− mice did not consume an increased amount of ethanol as did the CB₁+/+ mice for the next 24 h. The activation of CB₁ receptors in wild-type mice will also contribute to the high ethanol preference exhibited by C57BL/6J mice (Wang et al., 2003) as SR 141716 is able to reduce ethanol drinking when administered to these mice and not in CB₁−/− mice. Young and old CB₁−/− mice with this genetic background displayed low ethanol preference. On the contrary, CB₁+/+ mice presented an age-dependent decline in ethanol preference, suggesting that the decline in ethanol preference is related to a loss of cannabinoid signalling in the limbic forebrain.

It could be hypothesized that there was an interaction of gender and expression of phenotype associated with the CB₁ gene mutation. The total fluid intake was similar between the different genotypes, although differences were evident between males and females within the same genotype. CB₁−/− male mice did not show the acute ethanol-induced increase in dopamine levels in nucleus accumbens compared with CB₁+/+ mice, which would indicate that activation of the limbic system was required for the reinforcing effects of ethanol (Hungund et al., 2003).

The purpose of our study was to investigate the effect of a low to high acute intraperitoneal ethanol injection on blood ethanol concentration (BEC), as well as the effects of non-forced ethanol administration and forced chronic pulmonary ethanol intoxication on ethanol preference by comparing CB₁−/− and CB₁+/+ mice to ascertain the precise involvement of the cannabinoid system on ethanol-related behavioural effects.

**MATERIALS AND METHODS**

Homozygous CB₁−/− male mice were compared with homozygous CB₁+/+ male wild-type mice. CB₁+/+ and CB₁−/− mice were from a C57BL/6J × 129/Ola (Harlan) F2 genetic background and generated as described previously (Robbe et al., 2002; Ravinet-Trillou et al., 2003). No backcrosses were performed. These mice were provided by Sanofi-Recherche Synthélabo (Montpellier, France). The mice were housed in clear plastic cages with steel wire fitted tops and wood chip bedding under standard conditions (normal 12 h light–dark cycles, light on at 08:00 h, constant room temperature of 25 ± 1°C) with commercial lab chow diet and tap water available ad libitum during the entire experiments.

**Acute ethanol experiments**

BEC was assayed in CB₁−/− and CB₁+/+, 30–32 g, 12-weeks-old, male mice, housed 5/cage, after an intraperitoneal injection of ethanol. The experiment was carried out in their home cages. Blood samples were collected from the retro-orbital sinus under slight ether anaesthesia where necessary, into haematocrit tubes at 20, 40 min and 1, 2, 4, 9 and 12 h, after either 1 or 3 g/kg ethanol doses (15% v/v), while an additional two samples at 14 and 16 h were also collected after the 5 g/kg dose. This procedure followed the schedule of blood drawing used by Bruguerolle and Dubus (1993), Bruguerolle et al. (1994) and Hettiarachchi et al. (2001). Blood from each haematocrit tube was transferred into microcentrifuge tubes containing sodium fluoride as an anticoagulant. The concentration of blood ethanol was assayed by an alcohol-dehydrogenase-based method (Aufrère et al., 1997).

**Chronic ethanol experiments**

*Non-forced ethanol administration experiments.* CB₁−/− and CB₁+/+, 30–32 g, 12-weeks-old, male mice, were housed 2/cage. Fluid intake (water and 10% v/v ethanol when present) was recorded every 1 or 2 days, and body weight every week.

Free-choice period. Two drinking bottles were placed in each cage, one containing tap water and the other, 10% v/v ethanol solution. The mice had continuous access to the
drinking tips of both tubes. The position of the tubes was changed every day, in order to avoid possible bias due to place preference. The ratio of the 24 h intake from the ethanol bottle versus total fluid intake was used to define preference and the absolute amount (g/kg body weight/day) of ethanol consumed was also calculated.

**Forced chronic pulmonary ethanol administration procedure.** The motility of CB\(^{-/-}\) and CB\(^{+/+}\), 30–32 g, 12 weeks old, male mice, was recorded, after 3 weeks of acclimatization, for 18 h by the MacLab system, the recordings being combined for each hourly interval. The apparatus has been described in detail previously (Lallemand and De Witte, 2001).

Forced chronic alcoholization was induced in these mice, housed in pairs of two, within a plastic chamber (120 × 60 × 60 cm) by pulmonary inhalation of a mixture of ethanol and air. The mixture was pulsed into the chamber via a mixing system that allowed the quantity of ethanol to be combined for each hourly interval. The apparatus has been described in detail previously (Lallemand and De Witte, 2001).

During these 18 h using the same apparatus described above. The motility of each mouse was recorded after 3 weeks of acclimatization. The motility was pulsed into the chamber via a mixing system that allowed the quantity of ethanol to be combined for each hourly interval. The apparatus has been described in detail previously (Lallemand and De Witte, 2001).

The motility of CB\(^{-/-}\) and CB\(^{+/+}\), 30–32 g, 12 weeks old, male mice, was recorded, after 3 weeks of acclimatization, for 18 h by the MacLab system, the recordings being combined for each hourly interval. The apparatus has been described in detail previously (Lallemand and De Witte, 2001).

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Blood from each haematocrit tube was transferred into microcentrifuge tubes containing sodium fluoride as an anticoagulant. The concentration of blood ethanol was assayed by an alcohol-dehydrogenase-based method.

Withdrawal motility and free-choice period. At the end of the forced chronic pulmonary alcoholisation period the motility and ethanol preference was studied in these two groups of mice. For the measurement of ethanol preference, the mice from each strain underwent three successive steps (Le Bourhis, 1977) on cessation of the chronic ethanol intoxication. First, full beverage deprivations, i.e. the drinking bottles were removed during the last 6 h of the chronic alcoholisation procedure and the following 18 h of the withdrawal period. The motility of each mouse was recorded during these 18 h using the same apparatus described above. Secondly, a 10% (v/v) ethanol solution was given as the sole drinking fluid during the following 24 h. Thirdly, a free-choice beverage situation [water vs 10% (v/v) ethanol solution] was presented for a period of 39 days. During this free-choice period, the fluid consumptions were recorded daily and ethanol consumption expressed as a percentage of total fluid intakes and as ethanol intake in g/kg of body weight. The positions of the drinking bottles were changed every day to avoid position preference. BECs were assayed at different time points during the free-choice period by the method described above. The weight of animals was recorded every 3 or 4 days.

In all experiments, the results are presented as mean ± standard error (SE) except where stated otherwise. In all experiments, groups were compared by two-way analysis of variance (ANOVA) (genotype; time) with repeated measures on time. Where appropriate, post hoc pair wise comparisons were analysed by the least-significant difference test of multiple comparisons (Fisher LSD protected t-test) (GB-STAT 5.3 for Windows, Dynamic Microsystems, Silver Spring, MD, USA). Criterion for significance was set at \( P < 0.05 \) for all tests.

The Belgian Governmental Agency under the authorized number LA 1220028 as well as the European Communities Council Directive concerning the Use of Laboratory Animals approved these experiments.

**Products**

Absolute ethanol, used in the free-choice paradigm and acute experiment, was obtained from Labotec (La Gleize, Belgium). Ethanol at 15% (w/v) was prepared for i.p. injection in 0.9% saline. Ethanol at 97% was obtained from Belgalco SA (Belgium). Sodium fluoride was from Sigma Aldrich, (Steinheim, Germany).

**RESULTS**

**Acute ethanol experiments**

The BECs were similar in both groups of mice after either 1 or 3 g ethanol/kg doses \([F(1,56) = 0.0005, \ P = 0.982] and \([F(1,56) = 0.1161, \ P = 0.7421]\) respectively). However, after an acute injection of 5 g/kg of ethanol, CB\(^{-/-}\) mice showed significant differences compared to the CB\(^{+/+}\) mice \([F(1,8) = 19.254, \ P = 0.0022]\), with a significantly higher BEC than the CB\(^{-/-}\) mice \([F(9,72) = 2.981, \ P = 0.0045]\) (Fig. 1).

**Chronic ethanol experiments**

**Non-forced ethanol administration experiments.** After 1 week of measurements, CB\(^{-/-}\) mice showed a significantly higher water consumption in comparison to CB\(^{+/+}\) mice \([F(1,54) = 6.8364, \ P = 0.0176]\) (data not shown). The mean water consumptions over the time of the experiment were 10.8 ± 1.24 and 13.8 ± 0.87 ml, respectively for CB\(^{+/+}\) and CB\(^{-/-}\) mice.

At the conclusion of the study, the CB\(^{-/-}\) mice showed a significantly lower mean weight in comparison to controls \([F(1,38) = 7.3466, \ P = 0.01]\). The mean weights were 32.18 ± 0.62 and 30.16 ± 0.36 g, respectively, for CB\(^{+/+}\) and CB\(^{-/-}\) mice.

**Free choice.** CB\(^{-/-}\) mice showed a significantly reduced ethanol preference (expressed as a percentage of total fluid intake) in comparison to control mice \([F(1,12) = 8.6787, \ P = 0.0122]\) (Fig. 2A). There was also a significant interaction between genotype and time \([F(8,96) = 2.1965, \ P = 0.0342]\). Nonetheless, when ethanol preference is expressed as ethanol intake in g/kg of body weight, the genotype significance disappeared totally and only the interaction remained \([F(38,418) = 3.9539, \ P < 0.0001]\) (Fig. 2B). The mean ethanol intake over the time of the experiment was 12.28 ± 0.48 and 13.12 ± 0.59 g/kg/day, respectively, for CB\(^{-/-}\) and CB\(^{+/+}\) mice.

When comparing liquid type consumptions, i.e. water and ethanol, ethanol volume consumed in mice of either genotype was not significantly different \([F(1,12) = 0.2861, \ P = 0.6025]\). On the contrary, CB\(^{-/-}\) mice consumed significantly more water than CB\(^{+/+}\) mice \([F(1,12) = 14.1872, \ P = 0.0027]\).

During the free-choice period the total consumption (water + 10% v/v ethanol) of CB\(^{-/-}\) mice was not significantly different in comparison to control mice \([F(1,26) = 3.3544, \ P = 0.0785]\) (data not shown), but there was a significant interaction between genotype and time \([F(8,208) = 4.3224, \ P < 0.0001]\). Nonetheless, the total consumption of
CB^{−/−} mice was always above that of control mice. The mean water consumptions over the time of the experiment were 9.67 ± 0.70 and 7.23 ± 0.33 ml/24 h, respectively, for CB^{−/−} and CB^{+/+} male mice.

**Forced chronic ethanol pulmonary administration experiments.** The motility of CB^{−/−} mice, prior to forced chronic ethanol pulmonary administration, was not significantly different in comparison to CB^{+/+} mice \([F(1,150) = 0.7872, P = 0.382]\). In the CB^{−/−} mice the mean BEC assayed at different time points during the forced chronic alcoholization regime were significantly different than the mean levels in the CB^{+/+} mice \([F(1,26) = 25.887, P < 0.0001]\) characterized by a significant higher BEC level at both 10 and 11 days after the commencement of forced chronic pulmonary alcoholization. \(F(6,156) = 7.931, P < 0.0001\) (Fig. 3). At 10 days, the mean BEC was 3 fold higher in the CB^{−/−} mice than in the CB^{+/+} mice, whereas at 11 days, it showed a 2 fold increase. However, on Day 13 no significant difference in mean BEC was assayed.

During the forced chronic pulmonary alcohol period, water consumption of CB^{−/−} mice was not significantly different \([F(1,18) = 1.7514, P = 0.2023]\) (data not shown). The mean water consumptions over the time of the experiment were 9.82 ± 0.5 and 8.76 ± 0.48 ml/24 h, respectively, for CB^{−/−} and CB^{+/+} mice.

CB^{−/−} mice had a significantly lower body weight than CB^{+/+} mice \([F(1,38) = 7.3466, P = 0.01]\). The mean weights over the time of the experiment were 30 ± 0.46 and 32 ± 0.62 g, respectively, for CB^{−/−} and CB^{+/+} mice.

Following forced chronic pulmonary alcoholization, similar motilities were assayed for both CB^{−/−} and control mice \(F(1,340) = 0.8442, P = 0.3704\) as they were also similar prior to the chronic pulmonary alcoholization.

Free choice. During the first 24 h period after forced chronic pulmonary alcoholisation, there were no significant differences in ethanol consumption between CB^{−/−} and CB^{+/+} mice \([F(1,11) = 0.4936, P = 0.4969]\). The mean ethanol consumptions in the alcoholized CB^{+/+} group and in the alcoholized CB^{−/−} group were, 18.69 ± 1.69 ml and 16.75 ± 1.29 ml, respectively. During the free-choice period, ethanol preference, expressed as percentage of total fluid consumption, of CB^{−/−} mice showed no significance at the genotype level when compared with CB^{+/+} mice \([F(1,11) = 2.1819, P = 0.1677]\) (Fig. 4A). There was also absence of significance when ethanol preference was expressed as ethanol intake/kg body weight \([F(1,11) = 1.6614, P = 0.2239]\) (Fig. 4B). Nonetheless, in both representations of ethanol preference, there were always significant interactions between genotype and time \([F(38,418) = 2.345, P < 0.0001\) and \(F(38,418) = 3.9539, P < 0.0001\), respectively, for percentage of total fluid consumption (Fig. 4A) and ethanol intake expressed in g/kg body weight (Fig. 4B)]. The mean ethanol intakes in all experiments were 17.96 ± 0.52 and 22.05 ± 0.69 g/kg, respectively, for CB^{+/+} and CB^{−/−} mice.

When the ethanol and water consumptions in each genotype were compared, no significant differences between the two liquids in both CB^{+/+} \([F(1,12) = 0.0356, P = 0.8534]\) and CB^{−/−} mice \([F(1,10) = 3.5586, P = 0.0886]\) were apparent. Nonetheless, there were always significant interactions between liquid type and time in both genotypes \([F(38,456) = 8.1675, P < 0.0001\) and \(F(38,380) = 6.2574, P < 0.0001\), respectively, for CB^{+/+} and CB^{−/−}]. In CB^{+/+} mice, the consumptions of water...
and ethanol were very similar (8.42 ± 0.25 and 8.93 ± 0.23 ml, respectively, for ethanol and water). In contrast, in CB1⁻/⁻ mice, the ethanol consumption was always lower than the intake of water (8.81 ± 0.27 and 11.21 ± 0.21 ml, respectively, for ethanol and water). In addition, water intake of CB1⁻/⁻ mice was higher than in the CB1⁺/+ mice.

The total liquid consumption was not significantly different in CB1⁻/⁻ and CB1⁺/+ mice \([F(1,11) = 1.3981, \ P = 0.262]\), although there was a significant interaction between genotype and time \([F(38,418) = 3.5209, \ P < 0.0001]\). The total consumption values for CB1⁻/⁻ mice were always greater or at the same level for those of the CB1⁺/+ mice.
During the free-choice paradigm, there was no significant difference between the BEC values at the genotype level \([F(1,4) = 3.092, P = 0.1535]\); the values assayed being less than 0.02 g/l in both groups of mice (data not shown). However, there was a significant interaction between genotype and time \([F(5,20) = 2.849, P = 0.0422]\), as well as for time \([F(5,20) = 20.192, P < 0.0001]\).

During the free-choice period, CB1\(^{-/-}\) mice showed a significantly lower body weight than the CB1\(^{+/+}\) mice \([F(1,18) = 9.5004, P = 0.0064]\) (data not shown). There was also a significant interaction between genotype and time \([F(10,180) = 6.851, P < 0.0001]\). The body weight of CB1\(^{-/-}\) mice at the beginning of the study was 31.2 ± 0.6 and 31.14 ± 0.67 g at its conclusion. The body weight of CB1\(^{+/+}\) mice was 32.36 ± 0.75 g at the beginning of the study and was 37.64 ± 1.39 g at the end.

**DISCUSSION**

Recently, mouse specific gene deletions have been used to investigate the role of the endocannabinoid system in alcohol research. In this study we assessed the effect of CB1 receptor null mutation on ethanol preference in both non-alcoholized and chronically alcoholized mice as well as ethanol clearance after an acute ethanol i.p. injection. The acute ethanol injection in mice lacking the CB2 receptor showed an unexpected result in that the ethanol peak concentration for the high ethanol dose, 5 g/kg, induced a significantly higher ethanol peak concentration in CB1\(^{-/-}\) mice. However, the ethanol elimination rates for the lower doses, 1 and 3 g/kg, were similar in both CB1\(^{-/-}\) and CB1\(^{+/+}\) mice. This has not been described previously in the literature for CB1\(^{-/-}\) mice. Nonetheless, the influence of the cannabinoid system on the metabolism of ethanol was reported in one study where the administration of cannabinoid receptor inhibitor SR 141716 induced no changes in ethanol metabolism in rats (Colombo et al., 1998). It is difficult to interpret these present results. As the 1 and 3 g/kg ethanol doses showed no significant change between CB1\(^{-/-}\) and CB1\(^{+/+}\), we hypothesized that, with respect to the high dose of ethanol used, the lack of CB1 cannabinoid receptors in the enteric nervous system, particularly at the level of the gastrointestinal tract of CB1\(^{-/-}\) mice, might interfere with the absorption/distribution of ethanol (Batkai et al., 2001; Pertwee, 2001). However, this lack would intervene only with a high acute dose of ethanol.

In mice with non-forced ethanol administration, ethanol preference ratio was significantly reduced in CB1\(^{-/-}\) mice, but when ethanol preference was expressed as g/kg body weight per day, no significances appeared. These results are in agreement with those obtained by Wang et al. (2003) for ethanol preference ratio. Nonetheless, other studies by Hungund et al. (2003) and Poncelet et al. (2003) observed that ethanol intake expressed in g/kg body weight/day was significantly reduced in CB1\(^{-/-}\) mice as well. This discrepancy on the preference in ethanol intake was unclear. In our study, the absence of significance in preference as expressed in g/kg body weight/day is mainly the result of a higher, but not significant, total liquid intake of the CB1\(^{-/-}\) mice.

In chronically forced alcoholized mice, the BEC in CB1\(^{-/-}\) mice peaked faster than in CB1\(^{+/+}\) mice, although the maximum values obtained were not significantly different. This result has not been observed in previous studies, although Colombo et al. (1998b) showed that the antagonism at CB1 cannabinoid receptors did not modify ethanol metabolism. In our study, the difference between CB1\(^{-/-}\) and CB1\(^{+/+}\) mice was noted only during the increase of BEC but not at the end of the chronic alcoholization period. Unlike other chronic alcoholization
procedures, our protocol of chronic alcoholization is a forced one, i.e. animals were unable to adjust the amount of ethanol ingested by themselves. Our procedure of chronic alcoholization induced other mechanisms involved in ethanol metabolism microsomal ethanol oxidizing system MeOS/cytochrome P450IIE (Lieber, 1999) and alcohol dehydrogenase (Kishimoto et al., 1995), which have not been studied to date in these knockout animals.

After forced chronic pulmonary alcoholization, the ethanol consumption in \( \text{CB}_1^{-/-} \) mice was similar to that of \( \text{CB}_1^{+/+} \) mice when access to 10% (v/v) ethanol solution was given. In contrast, when \( \text{CB}_1^{-/-} \) mice had access to both drinking bottles, i.e. free choice, their ethanol preference was significantly lower than \( \text{CB}_1^{+/+} \) mice when expressed as percentage of total consumption. This result is in agreement with our previous study in Wistar rats of the action of the CB1 cannabinoid receptor inhibitor SR 141716 (Lallemand and De Witte, 2001) and data reported recently by Hungund et al. (2003), Poncelet et al. (2003), Racz et al. (2003), Wang et al. (2003) and Naassila et al. (2004), which show that a CB1 receptor

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 4** (A) Time course of ethanol preference expressed as percentage of total fluid intake of \( \text{CB}_1^{-/-} \) and \( \text{CB}_1^{+/+} \) mice after forced chronic pulmonary alcoholization. Open squares represent the \( \text{CB}_1^{+/+} \) mice and filled squares the \( \text{CB}_1^{-/-} \) mice. Significant time points between \( \text{CB}_1^{+/+} \) mice (control) and \( \text{CB}_1^{-/-} \) mice are represented by \(*P<0.05\) and \(**P<0.01\). Results are presented as mean ± SE. (B) Time course of ethanol preference expressed as g/kg body weight/day ethanol intake of \( \text{CB}_1^{-/-} \) and \( \text{CB}_1^{+/+} \) mice after forced chronic pulmonary alcoholization. Hatched bars represent the \( \text{CB}_1^{+/+} \) mice and filled bars the \( \text{CB}_1^{-/-} \) mice. Significant time points between \( \text{CB}_1^{+/+} \) mice (control) and \( \text{CB}_1^{-/-} \) mice are represented by \(*P<0.05\) and \(**P<0.01\). Results are presented as mean ± SE.
antagonist decreases ethanol consumption in rats and mice. Nevertheless, when ethanol preference is expressed as g/kg body weight/day, CB₁⁻/⁻ mice presented significant ethanol intake time point higher than CB₁+/⁺ mice.

In both non-forced alcoholized and chronically forced alcoholized experiment, the CB₁⁻/⁻ mice showed a significantly lower weight than the CB₁+/⁺ mice. This result was in contradiction to the results from a previous study (Wang et al., 2003) where no difference was observed when the animals had free access to the food. A weight difference between CB₁⁻/⁻ and CB₁+/⁺ mice has been described between gender (Hungund et al., 2003) when there is restricted food access. In another study, CB₁⁻/⁻ mice gained less weight than CB₁+/⁺ mice when fed with high fat diet (Ravinet-Trillou et al., 2003). Conversely, these data could be interpreted as a higher weight gain by CB₁+/⁺ mice, which is in accordance with the results of Wang et al. (2003), although in our experiments the mice had full access to the food. This effect could be due to the length of the experiment and the presence of ethanol, which modulates endocannabinoid levels in neuronal cells (Gonzalez et al., 2002).

Both CB₁ genotypes showed no significant differences in their motility irrespective of whether they were chronically forced alcoholized or not. There was also no difference in motilities before and after chronic alcoholization. These results are in agreement with those observed in the study of Racz et al. (2003) where CB₁⁻/⁻ mice showed no withdrawal symptoms when compared with CB₁+/⁺ mice. In contrast, Naassila et al. (2004) reported an increased ethanol withdrawal severity in CB₁⁻/⁻ mice. This discrepancy in the results obtained in those studies may be caused by the use of different measures for alcohol withdrawal symptoms.

In conclusion, these data showed: (1) a higher BEC in CB₁⁻/⁻ mice after a high acute ethanol dose of 5 g/kg; (2) during forced chronic pulmonary alcoholization, higher BEC levels are reached at an earlier time point in CB₁⁻/⁻ mice, and (3) CB₁⁻/⁻ mice show a lower ethanol preference. These results strongly support an important role for the endocannabinoid-CB₁ receptor system in ethanol drinking behaviour as well as other actions of ethanol. Further studies of enzymes involved in the pharmacokinetics of ethanol are needed to explain the apparent differences in ethanol absorption/distribution observed in CB₁⁻/⁻ mice after high doses of ethanol.

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