Metabotropic glutamate receptor 5 (mGluR5) regulation of ethanol sedation, dependence and consumption: relationship to acamprosate actions

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Abstract
Recent studies have demonstrated that metabotropic glutamate receptor 5 (mGluR5) antagonists decrease alcohol self-administration and suggest that the anti-craving medication, acamprosate, may also act to decrease mGluR5 function. To address the role of mGluR5 in behavioural actions of ethanol and acamprosate, we compared mutant mice with deletion of the mGluR5 gene and mice treated with a mGluR5 antagonist (MPEP) or acamprosate. Lack of mGluR5 or administration of MPEP reduced the severity of alcohol-induced withdrawal (AW), increased the sedative effect of alcohol (duration of loss of righting reflex; LORR), and increased basal motor activity. The motor stimulation produced by ethanol was blocked by deletion of mGluR5, but not by injection of MPEP. Both acamprosate and MPEP increased ethanol-induced LORR and reduced AW. Importantly, the protective effects of both MPEP and acamprosate on AW were found when the drugs were injected before, but not after, injection of ethanol. This indicates that the drugs prevented development of dependence rather than merely producing an anticonvulsant action. No effects of acamprosate or MPEP on ethanol-induced LORR and AW were found in mGluR5 knockout mice, demonstrating that mGluR5 is required for these actions. mGluR5 null mutant mice showed decreased alcohol consumption in some, but not all, tests. These data show the importance of mGluR5 for several actions of alcohol and support the hypothesis that some effects of acamprosate require mGluR5 signalling.

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Introduction
Recent studies demonstrate that metabotropic glutamate receptor 5 (mGluR5) antagonists reduce alcohol self-administration as well as alcohol seeking, relapse and reward in rats and mice (Backstrom et al., 2004; Cowen et al., 2005; Hodge et al., 2006; Lominac et al., 2006). In addition, deletion of the mGluR scaffolding protein Homer2 reduced alcohol intake and blocked the development of place preference and locomotor sensitization after repeated ethanol administration (Szumlinski et al., 2005). mGluR5 signalling may also be critical in reinforcing actions of other drugs as administration of the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) resulted in a dose-dependent reduction in cocaine self-administration without affecting responding for food (Chiamulera et al., 2001) and produced attenuation of cue-induced reinstatement of cocaine (Bäckström and Hyytiä, 2006). MPEP also decreased nicotine self-administration in rats and mice (Paterson et al., 2003), and attenuated acquisition and expression of conditioned morphine reward (Popik and Wrobel, 2002). Finally, antagonism of mGluR5 function was suggested as a mechanism of action for acamprosate, one of the few FDA-approved drugs with anti-craving properties used for human alcoholics (Harris et al., 2002).

These previous studies raised several questions which we address in the present study. First, all
studies have relied on pharmacological antagonism (mainly MPEP) to infer a role for mGluR5 in alcohol consumption or reinforcement. Despite the selectivity of MPEP for mGluR5 (Gasparini et al., 1999), high doses of this drug may have additional actions involving other molecular mechanisms (Movsesyan et al., 2001; O’Leary et al., 2000). Recent evidence indicates some non-specific actions of MPEP at higher concentrations (doses) including inhibition of NMDA receptors (see Lea and Faden, 2006 for review). To extend these studies, we used null mutant mice lacking the mGluR5 protein and compared the behavioural effects of this deletion with the effects of different doses of MPEP in both wild-type and mutant mice. The latter comparison is particularly informative as it can detect actions of MPEP that are not mediated by mGluR5. We also studied behavioural actions of alcohol in the domains of dependence (acute withdrawal), sensitivity [motor stimulation, loss of righting reflex (LORR)] and alcohol consumption. We compared the traditional two-bottle choice paradigm with tests of alcohol consumption that use limited access to facilitate high alcohol consumption and high blood ethanol levels (Finn et al., 2005; Rhodes et al., 2005). Another test of voluntary ethanol intake, the four-bottle choice paradigm, has been used to promote alcohol consumption in rats (Gustafsson et al., 2007; Turyabahika-Thyen and Wolffgramm, 2006). There is little information about the pharmacological or genetic sensitivity of these tests, but we propose that they will differ in response to genetic and pharmacological manipulations. Thus, we evaluated the role of mGluR5 in several consumption paradigms. The resulting profile of the influence of mGluR5 on alcohol phenotypes using both null mutants and the mGluR5 antagonist MPEP allowed us to ask if acamprosate shared this spectrum of actions. In particular, we asked if some actions of acamprosate are lacking in null mutant mice, as would be predicted from work suggesting that acamprosate reduces the action of mGluR5 (Harris et al., 2002).

Methods

Animals

Null mGluR5/−− allele mice were created using homologous recombination as previously described (Lu et al., 1997). Breeding pairs of heterozygote mutant mice (coat color – agouti) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). The mouse colony was maintained on original mixed C57Bl/6J × 129/SvJ genetic background. To minimize possible confounding effect of genetic drift, all behavioural analyses were performed on homozygous knockout −/− and wild-type +/+ littermates generated from crosses between heterozygous animals. Mice were group-housed 4–5 to a cage based on sex and litter. Food and water were available ad libitum. The vivarium was maintained on a 12-h light–dark cycle with lights on at 07:00 hours, except experiments with ethanol intake in limited access (DID) models and effects of acamprosate on ethanol consumption. For these experiments a reversed light–dark cycle was used with lights off at 10:00 hours. The temperature and humidity of the room were controlled. All experiments were performed during the light phase of the light–dark cycle except as indicated above. Behavioural testing began when the mice were at least 2 months old. All experiments were conducted in the isolated behavioural testing rooms in the animal facility to avoid external distractions. All experiments were approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

Measurement of ethanol drinking and preference

24-h two-bottle choice

The detailed procedure has been described previously (Blednov et al., 2001). Ethanol was used in a concentration range from 3% to 12%. Male and female wild-type and mGluR5 knockout mice were used in this experiment.

24 h four-bottle choice

In the four-bottle choice paradigm, four tubes containing water, 4%, 8% and 12% of ethanol solution were available for drinking simultaneously. Tubes were weighed every day. Food was available ad libitum, and mice were weighed every 4 d. Tube positions were changed by clockwise rotation every day to control for position preferences. The quantity of ethanol consumed (g/kg body weight per 24 h) (sum of ethanol consumed from three different ethanol bottles), preference for ethanol (sum of ethanol preference of the three different ethanol bottles) and total fluid intake (g/kg body weight per 24 h) (sum of fluid consumed from four bottles) were calculated for each mouse. Only wild-type and mGluR5 null female mice were used in this test.

Acceptance during scheduled fluid access (SHAC)

An ethanol (5% solution) acceptance method using scheduled, restricted fluid access was recently found to produce high and stable ethanol intake in C57BL/6J and genetically heterogeneous mice (Finn et al., 2005).
Only wild-type and mGluR5 null female mice were used in this test.

**Limited access in the dark phase (one-bottle DID)**

Another approach for consumption of ethanol (15% solution) under conditions of limited access which achieves pharmacologically significant ethanol drinking was recently described (Rhodes et al., 2005). Only wild-type and mGluR5 null female mice have been used in this test.

**Limited access in the dark phase (two-bottle choice DID)**

This was similar to the one-bottle DID test described above except for placing two bottles containing 15% ethanol and water. The ethanol and water bottles remained in place for 3 h. After their removal mice had unlimited access to one bottle of water. The positions of bottles during the 3-h access were changed daily to avoid potential side preference. The ethanol and water bottles were weighed before placing and after removal of bottles from the experimental cage. Only wild-type and mGluR5 null female mice were used in this test.

**Motor stimulatory effect of ethanol**

Effect of ethanol on locomotor activity was measured in standard mouse cages in Opto-microvarimex (Columbus Instruments, Columbus, OH, USA) as previously described (Blednov et al., 2004). Male and female wild-type and mGluR5 knockout mice were used in this experiment.

**Response to novelty**

Locomotor activity was measured in standard mouse cages by Opto-microvarimex (Columbus Instruments). Activity was monitored by six light beams placed along the width of the cage at 2.5 cm intervals, 1.5 cm above the floor. Each experimental cage had bedding and food and was covered by a heavy plastic lid with holes for ventilation and a bottle of water. Mice were moved in their home cages to the experimental room 1 d before beginning of the experiment. Motor response to novelty was monitored on the next day every 10 min during a 3-h session that began immediately after placing the mouse into an unfamiliar experimental cage.

**Acute withdrawal severity**

Mice were scored for handling-induced convulsion (HIC) severity 30 min before and immediately before intraperitoneal (i.p.) ethanol administration. Details of the procedure have been described previously (Blednov et al., 2004; Crabbe et al., 1991). Male and female wild-type and mGluR5 knockout mice were used in this experiment.

**LORR**

Animals were injected with ethanol (3.2, 3.4, 3.6 and 3.8 g/kg i.p.) and the length of ethanol-induced LORR (sleep time) was measured as previously described (Blednov et al., 2001, 2004). Male and female wild-type and mGluR5 knockout mice were used in this experiment.

**Ethanol metabolism**

Animals were given a single dose of ethanol (3.8 g/kg i.p.) and blood samples were taken from the retro-orbital sinus at 30, 60, 120, 180 and 240 min after injection. Blood ethanol concentration (BEC) values, expressed as mg ethanol/ml blood, were determined spectrophotometrically by an enzyme assay (Lundquist, 1959). BEC values (mg/ml) were plotted vs. time (min). The ethanol clearance was calculated from the slope of a line fitted by linear regression (slope calculated as mg/dl per h).

**Considerations for selection of inbred mice and doses of MPEP**

Among inbred strains, DBA/2J mice demonstrate the strongest motor stimulation by ethanol (Dudek and Tritto, 1994) and the most severe alcohol withdrawal (Crabbe et al., 1983; Goldstein and Kakihana, 1974). Because mGluR5 knockout mice showed differences in ethanol-induced motor stimulation and severity of ethanol withdrawal, the DBA/2J mouse strain was chosen for pharmacological reproduction of behavioural phenotypes found in mutant mice.

In experiments with mice, MPEP is normally used in a range of doses of 1–30 mg/kg with i.p. administration (Brodkin et al., 2002; Li et al., 2006; Lominac et al., 2006). Although MPEP at a dose of 10 mg/kg i.p. achieved full receptor occupancy in the mouse brain 5 min post-administration, the occupancy declined steadily thereafter and returned to control levels by 2 h. Furthermore, 60 min after i.p. administration of MPEP, the ED<sub>50</sub> for receptor occupancy was 10 mg/kg (95% CI 3–40 mg/kg) (Anderson et al., 2003). Because we needed reasonably complete and relatively long-lasting inhibition of mGluR5 for our studies, we selected doses of 10 and 45 mg/kg i.p.

**Effect of acamprosate on ethanol consumption**

The effect of acamprosate was tested in two experimental paradigms—the 24-h two-bottle choice test.
and limited access in the dark phase (two-bottle choice DID). Mice (neither wild-type nor knockout) from mGluR5 colony could not be used for this purpose because of their very low level of ethanol consumption. Recently we showed that F1 hybrid FVB/NJ × C57Bl/6J female mice consume significantly more alcohol than C57Bl/6J mice (Blednov et al., 2005) in the 24-h two-bottle choice procedure. Because the effect of acamprosate on ethanol intake was tested during a relatively short (2–3 h) period of time, these hybrid mice were used in both experiments.

For the two-bottle choice paradigm, mice were given different concentrations of ethanol (3%, 6%, 9%, 12%, 15% and 18%); each concentration was presented for 4 d with daily alternation of position for ethanol and water bottles. Mice were then given 18% ethanol and water for another 2 wk to ensure stable levels of drinking. Next, all mice were injected with saline (15 min before lights off) for two consecutive days and ethanol and water were measured for the first 2 h after lights off. Mice were then divided into two groups for saline or acamprosate injections. Acamprosate (400 mg/kg i.p.) or saline were injected daily 15 min before lights off. Ethanol and water consumption were measured as described above. Data are presented as a means for 2 d (ethanol is in the right and left positions). A similar experiment with a different group of ethanol-naive mice was conducted to study the effect of acamprosate (dose of 200 mg/kg i.p.).

The two-bottle DID test was carried out as described above except 20% ethanol was used. To ensure stable levels of drinking, ethanol consumption was carried out for 18 d. At the end of this training period, the amount of ethanol consumed was 5.2 ± 0.3 g/kg per 3 h (mean for days 17–18) with high preference for ethanol (0.81 ± 0.03, mean for days 17–18). On day 19 all mice were injected with saline and divided into three groups. On days 20, 21 and 22, mice from different groups were injected with acamprosate at doses 200, 300 and 400 mg/kg i.p. All injections were made 15 min before beginning of measurement (3 h after lights off).

Drugs

MPEP (Sigma-Aldrich, St Louis, MO, USA) and acamprosate (kindly provided by Dr R. Messing) were dissolved in saline (0.9% NaCl) and injected intraperitoneally at a volume of 10 ml/kg 15 min before (or after) ethanol. All ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KT, USA) solutions for injections were prepared in saline (20% v/v) and volumes for i.p. injections were adjusted to provide appropriate doses of ethanol. Control mice received a similar volume of saline. Ethanol solutions for drinking were prepared by mixing ethanol with tap water (v/v).

Statistical analysis

Data are reported as the mean ± S.E.M. The statistics software program GraphPad Prism (Jandel Scientific, Costa Madre, CA, USA) was used throughout. To evaluate differences between groups, analysis of variance (two-way ANOVA with post-hoc Bonferroni corrections or one-way ANOVA with repeated measurement and post-hoc Dunnett’s multiple comparison test) and Student’s t test were carried out.

Results

Data are divided into three sections: ethanol actions in null mutant mice, effects of MPEP in mutant and non-mutant mice, and effects of acamprosate in mutants and non-mutants. We present data on acute alcohol dependence produced by a single injection of ethanol and measured by HIC, duration of LORR produced by ethanol, stimulation of motor activity produced by ethanol, and consumption of ethanol solutions in several different tests.

Ethanol acute withdrawal

A single dose (4 g/kg) of ethanol suppressed basal HIC in knockout as well as in wild-type mice of both sexes for about 5–6 h, followed by increased HIC (Figure 1). There were no differences between wild-type and knockout mice in basal HIC (before injection of ethanol). After injection of ethanol, knockout mice showed lower HIC scores than wild-type mice [males – effect of genotype: F(1, 330) = 7.9, p < 0.01; effect of time: F(14, 330) = 43.7, p < 0.001; females – effect of genotype: F(1, 420) = 87.8, p < 0.0001; effect of time: F(14, 420) = 56.3, p < 0.0001] (Figure 1a, b). Withdrawal severity was calculated using increases above the basal levels to give an area under the curve. There were no differences in this withdrawal measure for male mice, whereas severity of ethanol-induced withdrawal was significantly reduced in mGluR5 null female mice (Figure 1c, d).

Depressant effects of ethanol

The duration of LORR (sleep time) produced by ethanol was increased in both null allele male and female mice compared with wild-type mice [female mice – dependence on genotype: F(1, 65) = 22.9, p < 0.001; dependence on dose: F(3, 65) = 12.2, p < 0.001; male
mice – dependence on genotype: $F(1, 61) = 5.7, p < 0.05$; dependence on dose: $F(2, 61) = 23.3, p < 0.001$ (Figure 2a, b). However, post-hoc analysis revealed differences between wild-type and null mutant male mice for the effect of ethanol only at 3.8 g/kg ($p < 0.01$) whereas for female mice the differences were found at doses of 3.2 g/kg ($p < 0.05$), 3.4 g/kg ($p < 0.001$) and 3.6 g/kg ($p < 0.05$).

Motor stimulant effect of ethanol and response to novelty

$mGluR5^{+/–}$ females demonstrated higher baseline (saline injection) motor activity than wild-type females ($p < 0.05$, Student’s $t$ test) whereas the basal (saline injection) motor activity of $mGluR5^{+/–}$ males tended to be higher than wild-type ($p = 0.06$, Student’s $t$ test) (Figure 3a, c). Analysis by two-way ANOVA showed a significant genotype $\times$ dose interaction [females: $F(5, 193) = 4.2, p < 0.05$; males: $F(5, 184) = 2.9, p < 0.05$] but no effect of genotype. An effect of ethanol dose [F(5, 184) = 2.6, p < 0.05] was significant only for male mice. However, comparing the effect of ethanol on motor activity of each genotype separately by one-way ANOVA showed a strong effect of ethanol on motor activity of knockout females [F(5, 91) = 2.6, p < 0.05] and knockout males [F(5, 99) = 3.5, p < 0.01]. In contrast, an effect of ethanol on motor activity of wild-type mice was seen only for females [F(5, 102) = 4.5, p < 0.001]. To correct for baseline differences, ethanol effects were normalized by setting the activity after saline injection to 100% for each genotype and sex (Figure 3b, d). Two-way ANOVA showed a significant effect of genotype [females: F(1, 193) = 108.7, p < 0.0001; males: F(1, 184) = 34.9, p < 0.0001], dose [males: F(5, 184) = 2.4, p < 0.05] and genotype $\times$ dose interaction [females: F(5, 193) = 5.3, p < 0.05; males: F(5, 184) = 2.7, p < 0.05]. In null mice of both sexes, ethanol caused significant reduction in motor activity [females: F(5, 91) = 2.6, p < 0.05; males: F(5, 99) = 3.5, p < 0.01; one-way ANOVA]. Post-hoc analysis revealed differences between saline and ethanol at doses
for effect of genotype [females: \( F(1,522) = 22.8, \ p < 0.0001 \); males: \( F(1,486) = 43.1, \ p < 0.0001 \); and genotype \( \times \) time interaction [females: \( F(1,522) = 3.3, \ p < 0.0001 \); males: \( F(1,486) = 7.9, \ p < 0.0001 \)], than those of wild-type animals for both sexes (Figure 3e, f).

Thus, deletion of \( mGluR5 \) increases stress-related motor activity and the locomotor sedation produced by ethanol injection.

**Ethanol preference in the two-bottle choice paradigm**

In this test, mice could drink either water or an ascending series of ethanol concentrations (0, 3, 6, 9 and 12%). There were no differences in either ethanol intake, ethanol preference or total fluid intake (ethanol solution + water) between knockout and wild-type mice of either sex (Figure 4).

Two weeks after the ethanol-drinking study, the same mice were tested for saccharin (sweet) and quinine (bitter) intake and preference in an ordered-balanced experimental design. \( mGluR5^{-/-} \) knockout mice of both sexes showed similar preference for saccharin (females: 0.65 ± 0.04 and 0.78 ± 0.06; males: 0.42 ± 0.11 and 0.51 ± 0.11 for 0.033% and 0.066% saccharin respectively) compared to wild-type mice (females: 0.69 ± 0.05 and 0.79 ± 0.06; males: 0.54 ± 0.06 and 0.66 ± 0.07 for 0.033% and 0.066% saccharin respectively). No differences between \( mGluR5^{-/-} \) knockout and wild-type males were found in avoidance of quinine solutions (knockout male: 0.31 ± 0.06 and 0.27 ± 0.06; wild-type male: 0.36 ± 0.06 and 0.27 ± 0.07 for 0.03 mM and 0.06 mM quinine respectively). However, null female mice showed less avoidance for quinine solutions than wild-type females (knockout female: 0.53 ± 0.04 and 0.45 ± 0.05; wild-type female: 0.36 ± 0.07 and 0.29 ± 0.07 for 0.03 mM and 0.06 mM quinine respectively) [effect of genotype: \( F(1,58) = 8.0, \ p < 0.01 \)].

**Ethanol consumption in other tests for ethanol intake**

Because the data presented above indicate that the effects of deletion of \( mGluR5 \) were greater in female mice than in males, only females were used for three additional tests for ethanol intake. In a four-bottle choice paradigm (choice between water and three different concentrations of ethanol), \( mGluR5 \) null mice consumed less ethanol than wild-type mice [effect of genotype: \( F(1,284) = 5.1, \ p < 0.05 \)] with lower preference [effect of genotype: \( F(1,284) = 7.8, \ p < 0.01 \)] but with higher total fluid intake [effect of genotype: \( F(1,284) = 13.1, \ p < 0.001 \); effect of days: \( F(15,284) = 1.9, \ p < 0.05 \)] (Figure 5a–c).
Figure 3. mGluR5−/− mice do not show motor stimulation by ethanol (EtOH) and demonstrate a stronger motor response to a novel situation. (a) Males – motor activity (number of crossed beams) for 30-min period. (b) Males – normalized motor activity for 30-min period (activity after saline injection taken as 100%). (c) Females – motor activity (number of crossed beams) for 30-min period. (d) Females – normalized motor activity for 30-min period (activity after saline injection taken as 100%). Significant effects of individual doses of ethanol are indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, different from correspondent saline control (one-way ANOVA, post-hoc Dunnett’s multiple comparison test). Each point represents an independent group of animals. Panels (a, b): n = 25–30 per saline-treated groups of males; n = 21–23 per saline-treated groups of females; n = 10–16 per ethanol-treated groups of males; n = 12–20 per ethanol-treated groups of females. (e) Motor response (number of crossed beams) – males. (f) Motor response (number of crossed beams) – females. Panels (e, f): males, n = 14–15 per genotype; females, n = 15–16 per genotype.
A two-bottle variant of the DID model (15% ethanol vs. water) showed decreased ethanol consumption for mGluR5 null mice. Thus, wild-type mice consumed more ethanol [effect of genotype: $F(1, 189) = 32.2$, $p < 0.001$; effect of days: $F(8, 189) = 2.7$, $p < 0.01$] with higher preference [effect of genotype: $F(1, 189) = 133$, $p < 0.0001$; effect of days: $F(8, 189) = 4.1$, $p < 0.001$; genotype x days interaction: $F(8, 189) = 2.8$, $p < 0.01$] than mGluR5−/− knockout mice (Figure 5d, e). mGluR5−/− knockout mice consumed more fluid (ethanol + water) than wild-type mice [effect of genotype: $F(1, 189) = 90$, $p < 0.001$] (Figure 5f).

Limited access to a low concentration of ethanol (5%) with fluid deprivation (SHAC model) showed no differences in amount of ethanol or water consumed (Figure 6a, b).

Limited access to a high concentration of ethanol (15%) with no fluid deprivation (DID model) with one bottle of ethanol also showed no significant differences in ethanol consumption during 2 h daily access [main effect of genotype: $F(1, 39) = 2.9$, $p = 0.09$, two-way ANOVA] (Figure 6c). However, ethanol intake was gradually reduced in mGluR5 null mice during three days of drinking [$F(2, 14) = 6.5$, $p < 0.05$, one-way ANOVA] whereas in wild-type mice daily ethanol intake was gradually increased during the same period of time [$F(2, 12) = 4.1$, $p < 0.05$, one-way ANOVA].

**Ethanol metabolism**

There were no differences in metabolism of ethanol between wild-type and knockout mice. Thus, ethanol clearance expressed in mg of ethanol/dl of blood per hour was: 34.5 ± 2.7 and 33.8 ± 4 for wild-type and knockout female mice, respectively, and 26.5 ± 2.5 and 29.5 ± 1.9 for wild-type and knockout male mice, respectively.

**Pharmacological reproduction of ethanol behavioural phenotypes seen in mice lacking mGluR5**

Because deletion of mGluR5 induced stronger ethanol behavioural phenotypes in female mice than in male mice and stimulation of motor activity by ethanol is...
MPEP injected 5 h after administration of ethanol (4 g/kg) did not change the severity of ethanol-induced acute withdrawal (HIC scores and area under HIC curve) in DBA/2J mice (Figure 7a, b). In contrast,

seen most clearly in DBA/2J mice, only DBA/2J females were used to study actions of the mGluR5 antagonist (MPEP, 45 mg/kg i.p.). The basis for selection of this dose of MPEP is given in the Methods section.
injection of MPEP before injection of ethanol (4 g/kg) led to significant reduction of HIC scores [effect of treatment: $F(1, 234) = 61.7, p < 0.0001$; effect of time: $F(12, 234) = 85.9, p < 0.0001$; treatment × time interaction: $F(12, 234) = 4.2, p < 0.0001$] as well as the area under the HIC curve ($p < 0.01$, Student’s $t$ test) (Figure 7c, d).

Recent evidence indicates some non-specific actions of MPEP at higher concentrations (doses) including inhibition of NMDA receptors (see Lea and Faden, 2006 for review). To separate specific (mGluR5 blockade) and non-specific (possible direct inhibition of NMDA receptor) effects of MPEP, we studied a low dose of MPEP (10 mg/kg) on severity of ethanol-induced withdrawal and ethanol-induced LORR in mGluR5 knockout mice and their wild-type littermates. As for the higher dose (45 mg/kg), injection of MPEP (10 mg/kg injected before 4 g/kg ethanol) significantly reduced HIC scores [effect of treatment: $F(1, 210) = 13.8, p < 0.001$; effect of time: $F(14, 210) = 42.5, p < 0.001$] as well as area under the HIC curve ($p < 0.01$, Student’s $t$ test) in wild-type mice (Figure 8a, b). In contrast, the same dose of MPEP did not change ethanol-induced withdrawal in mice lacking mGluR5 (Figure 8c, d). Consistent with the experiment presented in Figure 1, the severity of withdrawal (area under the HIC curve) was significantly higher in saline-treated wild-type mice compare with saline-treated mGluR5 knockout mice ($p < 0.05$, Student’s $t$ test). No differences in severity of

Figure 7. mGluR5 antagonist reduces the severity of ethanol-induced withdrawal in DBA/2J female mice only if it was injected before ethanol. (a) Handling-induced convulsion (HIC) score – MPEP (45 mg/kg) was injected before ethanol. (b) Area under the HIC score and above the basal level – MPEP (45 mg/kg) was injected before ethanol. (c) HIC score – MPEP (45 mg/kg) was injected 5 h after ethanol. (d) Area under the HIC score and above the basal level – MPEP (45 mg/kg) was injected 5 h after ethanol ($n = 10$ for each group). ** $p < 0.01$, significant differences between control and MPEP-treated group (Student’s $t$ test).
withdrawal between saline-treated null mice and MPEP-treated wild-type mice. However, post-hoc analysis showed that only the higher dose of MPEP (45 mg/kg) (p<0.001), and not the lower dose (10 mg/kg), potentiated the duration of ethanol-induced LORR in wild-type mice (Figure 9b). Furthermore, the effect of 45 mg/kg MPEP was different not only from controls but also from a group treated with a lower dose of MPEP (p<0.001). In mGluR5 knockout mice, even the high dose of MPEP (45 mg/kg) did not produce an increase of ethanol-induced LORR (Figure 9c).

Injection of MPEP at a dose 45 mg/kg (Figure 10b) or injection of a low dose of ethanol (1.5 g/kg) (Figure 10a) increased the basal motor response of DBA/2J

Figure 8. Low dose of mGluR5 antagonist reduces the severity of ethanol-induced withdrawal in wild-type female mice but does not change the ethanol withdrawal in mGluR5−/− female mice. (a) Wild-type mice, handling-induced convulsion (HIC) score – MPEP (10 mg/kg) was injected before ethanol. (b) Wild-type mice, area under the HIC score and above the correspondent basal level – MPEP (10 mg/kg) was injected before ethanol. (c) mGluR5−/− knockout mice, HIC score – MPEP (10 mg/kg) was injected before ethanol. (d) mGluR5−/− knockout mice, area under the HIC score and above the basal level – MPEP (10 mg/kg) was injected before ethanol (wild-type mice, n=8 for each group; knockout mice, n=7 for each group). ** p<0.01, significant differences between control and MPEP-treated group (Student’s t test).
mice ($p < 0.05$ for both MPEP and ethanol effects, Student’s $t$ test). However, injection of both ethanol and MPEP appeared to produce additive effects (Figure 10c). The level of motor activity after injection of both drugs was significantly higher than the level of activity after administration of ethanol alone ($p < 0.001$, Student’s $t$ test) as well as after injection of MPEP alone ($p < 0.05$, Student’s $t$ test).
Effects of acamprosate in mGluR5 knockout and wild-type mice

Injection of acamprosate before ethanol reduced the severity of ethanol-induced withdrawal in wild-type mice \( [F(2,39)=9, p<0.001, \text{one-way ANOVA}] \). Post-hoc analysis showed that both doses of acamprosate \((200 \text{ mg/kg and } 300 \text{ mg/kg})\) significantly reduced ethanol-induced withdrawal (area under the HIC curve: \( p<0.001 \) for both doses) (Figure 11a, b). However, even the highest dose of acamprosate \((300 \text{ mg/kg})\) injected after ethanol did not change the severity of ethanol-induced withdrawal in wild-type mice (Figure 11c, d). The same dose of acamprosate \((300 \text{ mg/kg})\) did not change the ethanol-induced withdrawal in mice lacking mGluR5 (Figure 11e, f).

Acamprosate increased the ethanol-induced LORR \([F(3,44)=19.5, p<0.001, \text{one-way ANOVA}]\). However, post-hoc analysis showed that only high doses of acamprosate \((300 \text{ mg/kg, } p<0.01; \text{ 400 mg/kg, } p<0.001)\) but not a lower dose \((200 \text{ mg/kg})\) potentiated the duration of ethanol-induced LORR in wild-type mice (Figure 12a). In contrast, in mGluR5 knockout mice even the high dose of acamprosate \((400 \text{ mg/kg})\) did not increase ethanol-induced LORR (Figure 12b).

Effect of acamprosate on ethanol consumption

Hybrid FVB \( \times \) B6 F1 female mice were chosen for their high level of alcohol consumption for these experiments. Data showing the effect of daily injections of acamprosate \((400 \text{ mg/kg})\) on ethanol preference and consumption in FVB \( \times \) B6 F1 hybrid mice in the two-bottle choice paradigm are presented in Figure 13. Note that alcohol was continually available, but consumption is shown for 2 h after acamprosate injection. To attempt to correct for initial differences between two saline-treated groups of mice and facilitate presentation of the data, all parameters of ethanol and fluid intake were calculated as a percentage of the initial consumption in the saline-treated groups. Saline- and acamprosate-treated groups differed in preference for ethanol [effect of treatment: \( F(1,88)=21, p<0.001 \); effect of days: \( F(3,88)=21, p<0.01 \); treatment \( \times \) day interaction: \( F(3,88)=2.7, p<0.05 \)] as well as for amount of ethanol consumed [effect of treatment: \( F(1,88)=8.4, p<0.01 \) (Figure 13a, b)]. No differences were found for total fluid intake (Figure 13c). In contrast, injection of the lower dose of acamprosate \((200 \text{ mg/kg})\) did not affect any parameters of ethanol consumption in the two-bottle choice paradigm (data not shown).

Using a two-bottle choice paradigm with limited access to alcohol (two-bottle DID), acamprosate decreased the amount of ethanol consumed \([F(3,33)=8.8, p<0.001, \text{one-way ANOVA with repeated measures}]\) and decreased total fluid intake \([F(3,33)=6.4, p<0.01, \text{one-way ANOVA with repeated measures}]\) (Figure 14a, c). Post-hoc analyses showed significant reduction of ethanol consumption by acamprosate doses of \(300 \text{ mg/kg (} p<0.001)\) and \(400 \text{ mg/kg (} p<0.001)\) and reduction of total fluid intake at \(400 \text{ mg/kg (} p<0.001)\). There was a trend towards a reduction of ethanol preference \([F(3,33)=2.8, p=0.055, \text{one-way ANOVA with repeated measures}]\) and post-hoc analyses revealed a significant reduction of ethanol preference after injection of acamprosate at a dose of \(300 \text{ mg/kg (} p<0.05)\) (Figure 14b).

Discussion

Taken together, these results show that deletion of mGluR5 reduces the development of acute alcohol dependence, increases the sedative effects of alcohol, abolishes the motor stimulatory effect of alcohol and increases stress-related motor activity. In agreement with our data, MPEP, an antagonist of mGluR5, significantly enhanced both the sedative and hypnotic effects of ethanol in C57BL/6 mice (Sharko and Hodge, 2007). Gender differences were seen in the effect of the mutation in this study. This is very common in studies of null mutant mice (see Crabbe et al., 2006 for review). One potential problem in interpretation of results obtained with mutant mice is whether compensatory changes in expression of other genes occur as a result of deletion of the target gene (Crabbe et al., 2006; Ponomarev et al., 2006). In this context, it is important to note that three of four behavioural differences between wild-type and null mice were reproduced in DBA/2J mice after administration of MPEP. This drug had no effect in mGluR5 knockout mice, indicating that the actions we observed are probably due to blockade of mGluR5. Although, we cannot completely rule out the effects on other systems, these findings argue against a role for compensatory changes (at least for three behavioural phenotypes) and suggest a direct role for mGluR5 in alcohol actions. However, it should be noted that different doses of MPEP showed somewhat different behavioural effects in our studies. Despite the selectivity of MPEP for mGluR5 (Gasparini et al., 1999), high doses may have additional actions. For example, MPEP shows some neuroprotective activity that occurs only at concentrations that inhibit NMDA receptors and are substantially higher than that required to inhibit agonist-induced phosphoinositide

\( mGluR5, \text{ acamprosate and ethanol } 787 \)
Figure 11. Acamprosate (Acam) reduces the severity of ethanol-induced withdrawal in wild-type female mice but does not change the ethanol withdrawal in mGluR5−/− female mice. (a) Wild-type mice, HIC score – acamprosate (200 mg/kg and 300 mg/kg) was injected before ethanol. (b) Wild-type mice, area under the HIC score and above the correspondent basal level – acamprosate (200 mg/kg and 300 mg/kg) was injected before ethanol. (c) Wild-type mice, HIC score – acamprosate (300 mg/kg) was injected 5 h after ethanol. (d) Wild-type mice, area under the HIC score and above the correspondent basal level – acamprosate (300 mg/kg) was injected 4 h after ethanol. (e) mGluR5−/− knockout mice, HIC score – acamprosate (300 mg/kg) was injected before ethanol. (f) mGluR5−/− knockout mice, area under the HIC score and above the correspondent basal level – acamprosate (300 mg/kg) was injected before ethanol (n=9–14 for each group of wild-type mice; n=7–8 for each group of knockout mice). ***p < 0.01, significant differences between control and acamprosate-treated groups (one-way ANOVA, post-hoc Dunnett’s multiple comparison test).
hydrolysis (Movsesyan et al., 2001; O’Leary et al., 2000). The reduction of severity of ethanol-induced withdrawal induced by low doses of MPEP (10 mg/kg) is most likely mediated by selective inhibition of mGluR5, whereas the potentiation of alcohol sedation required higher doses of MPEP (45 mg/kg) and may involve direct blockade of NMDA receptors. This conclusion is consistent with other studies showing that NMDA antagonists increase ethanol-induced LORR (Daniell, 1990). We found that the motor-activating effects of MPEP and ethanol are at least additive (perhaps synergistic) suggesting independent mechanisms. Indeed, Kachroo et al. (2005) showed that MPEP-induced motor activation requires activation of adenosine A2A and dopamine D2 receptors but not D1 receptors, whereas activation of both dopamine D1 and D2 receptors are important for motor stimulatory effects of ethanol (Cohen et al., 1999; Lê et al., 1997; Pastor et al., 2005). Thus, the two drugs may activate multiple signalling pathways to produce a large enhancement of activity when injected together.

Other studies showed that blockade of mGluR5 by MPEP inhibited the discriminative stimulus properties of consumed ethanol (Besheer et al., 2006b), reduced ethanol-reinforced responding (Hodge et al., 2006)
and reduced the voluntary consumption of alcohol (McMillen et al., 2005). Schroeder et al. (2005) showed that MPEP may modulate both the maintenance of operant ethanol self-administration and abstinence-induced increases in ethanol intake in alcohol-prefering P rats. In mice lacking mGluR5, we found decreased alcohol consumption in one limited access test and in long-term drinking with multiple choices of ethanol concentrations (four-bottle choice test), but not in the 24-h two-bottle choice paradigm. Similar reduction of alcohol intake in a four-bottle choice paradigm was shown after administration of MPEP in rats (Backstrom et al., 2004) and mice (Lominac et al., 2006). This may demonstrate that these tests measure different aspects of alcohol intake and have distinct genetic determinants. On the other hand, alternative explanations cannot be ruled out. Thus, it is possible that the presence of high concentrations of ethanol solutions together with free choice between ethanol and water and longer duration of alcohol consumption may be required to reveal effects of deletion of mGluR5 gene on ethanol intake. Moreover, ethanol intake in rodents strongly depends on genetic background and the mixed genetic background used in this study showed low ethanol intake and preference. It is possible that a different genetic background would reveal differences between wild-type and null mice.

It should be noted, that in addition to altering ethanol consumption, mGluR5 is also important for reinforcement from other drugs of abuse. For example, mice lacking mGluR5 did not acquire intravenous cocaine self-administration (Chiamulera et al., 2001). MPEP reduced cocaine self-administration without affecting responding for food (Chiamulera et al., 2001) and decreased nicotine self-administration in both rats and mice (Paterson et al., 2003).

As mentioned above, acamprosate is one of three drugs approved by the FDA for treatment of alcoholism (Heilig and Egl, 2006). Originally proposed to be a GABA analogue, it may attenuate NMDA signalling through partial agonism at the spermidine site, or inhibit the function of mGluR5 (Harris et al., 2002; Spanagel and Zieglgansberger, 1997). Our data show similarities between the behavioural actions of acamprosate and selective reduction of mGluR5 function. Acamprosate and MPEP showed similar dose-dependent changes in behavioural effects of ethanol (reduction of severity of ethanol-induced withdrawal at low doses and potentiation of the sedative effect of ethanol at high doses) in wild-type mice and both drugs failed to produce these effects in mice lacking mGluR5. It is possible that acamprosate acts as an mGluR5 antagonist, but studies with recombinant mGluR5 receptors found no interaction with acamprosate (Reilly et al., 2008). Interestingly, both drugs reduced the severity of alcohol induced withdrawal only when given before alcohol administration, indicating that they inhibit some aspect of the development of alcohol dependence, but do not directly prevent the expression of HIC. Comparing actions of drugs on development of acute withdrawal with their ability to suppress withdrawal signs (anticonvulsant
actions) may provide a simple screening method to discover other agents useful in treating alcohol dependence.

There are several possible mechanisms by which decreased mGluR5 function could alter ethanol responses. First, ethanol has been shown to inhibit the function of mGluR5 receptors (Minami et al., 1998). If this is the main mechanism, then deletion of mGluR5 should produce actions similar to ethanol administration and also block or reduce the actions of ethanol. In general, this is not the case. Although deletion of mGluR5 increased motor activity, as do low doses of ethanol, it increased (rather than blocked) the sedative actions of ethanol. Thus, it seems that few of the alcohol behaviours can be due to inhibition of mGluR5 function. A study of the interactions of MPEP with alcohol found that the reduction in alcohol consumption requires both protein kinase C epsilon (PKCε) and phosphatidylinositol-3 kinase (Olive et al., 2005). GABA_A receptors containing α1 subunits are colocalized with mGluR5 and their ethanol sensitivity is modulated by PKCε making these receptors attractive targets for manipulation of behaviours by mGluR5 manipulation (Besheer and Hodge, 2005). Specifically, deletion of PKCε decreased ethanol consumption and increased the sedative (LORR) actions of ethanol, just as was found in our study of mGluR5 null mice (Besheer et al., 2006a; Hodge et al., 1999). However, deletion of PKCε increased the locomotor effects of ethanol, whereas we found that mGluR5 null mutants did not show any stimulatory actions of ethanol. Reduction of the discriminative stimulus actions of ethanol by MPEP appears to require cross-talk between mGluR5 and GABA_A receptors (Besheer and Hodge, 2005). Taken together, these results suggest that modulation of ethanol actions by mGluR5 is at least partially due to regulation of GABA_A receptors. NMDA receptors are also important for behavioural actions of ethanol and one role of mGluR5 is to ‘set the tone’ of NMDA receptor-mediated neurotransmission (Alagarsamy et al., 1999). In addition, GluR5-deficient mice show loss of the NMDA-receptor-mediated component of long-term potentiation (Jia et al., 1998). As noted above, NMDA antagonists increase ethanol LORR, suggesting that enhancement of the sedative actions of ethanol in mGluR5 null mice may be due to down-regulation of NMDA receptor function.

Our data show that mGluR5 are important for alcohol dependence, consumption, motor activation and sedation and support the hypothesis that some effects of acamprosate may be mediated by reduction of mGluR5 function.

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Statement of Interest

None.

References


McMillen BA, Crawford MS, Kulers CM, Williams HL (2005). Effects of a metabotropic, mglu5, glutamate...
receptor antagonist on ethanol consumption by genetic drinking rats. Alcohol and Alcoholism 40, 494–497.


