# Effects of acute acamprosate and homotaurine on ethanol intake and ethanol-stimulated mesolimbic dopamine release

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## Abstract

The purpose of the present study was to determine the acute effects of the anticraving compound acamprosate (calcium acetylhomotaurinate) and the closely related compound homotaurine on ethanol intake and ethanol-stimulated dopamine release in the nucleus accumbens. Male rats were treated with acamprosate (200 or 400 mg/kg intraperitoneally, i.p.) or homotaurine (10, 50, or 100 mg/kg i.p.) 15 min prior to access to 10% ethanol and water for 1 h in a two-bottle choice restricted access paradigm. A separate group of rats was implanted with microdialysis probes in the nucleus accumbens and given an acute injection of ethanol (1.5 g/kg i.p.) that was preceded by saline, acamprosate, or homotaurine. Acamprosate and homotaurine dose-dependently reduced ethanol intake and preference. These compounds also delayed or suppressed ethanol-stimulated increases in nucleus accumbens dopamine release, suggesting that acamprosate and homotaurine may reduce ethanol intake by interfering with the ability of ethanol to activate the mesolimbic dopamine reward system. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Acamprosate (calcium acetylhomotaurinate) is effective in reducing relapse to ethanol consumption during abstinence in both animals and humans (see Wilde and Wagstaff, 1997; Kranzler, 2000; Myrick et al., 2001 for reviews). Although relapse is a critical and defining characteristic of alcoholism, few studies have examined the ability of acamprosate to modulate acute daily ethanol consumption in nondependent subjects. In addition, very few studies have examined the ability of compounds structurally related to acamprosate, such as homotaurine, to modulate ethanol intake.

The precise neurobiological mechanisms by which acamprosate exerts its effects on ethanol consumption are largely unknown, most likely due to the elusive neuropharmacological mechanisms of action of this drug (see Littleton, 1995; Spanagel and Zieglgänsberger, 1997). The rewarding and positive reinforcing properties of ethanol are largely believed to be mediated, at least in part, by the mesolimbic dopamine system (Koob et al., 1998; Spanagel and Weiss, 1999), consisting of dopaminergic neurons in the ventral tegmental area of the midbrain that project primarily to the nucleus accumbens in the basal forebrain. Numerous studies have shown that ethanol activates this pathway to increase extracellular levels of dopamine in the nucleus accumbens (reviewed in Koob et al., 1998; Spanagel and Weiss, 1999). While no studies to date have proven clearly that these increases in extracellular dopamine levels in the nucleus accumbens contribute directly to the reinforcing properties of ethanol and subsequent self-administration behavior, further evidence supporting a role for mesolimbic dopamine in ethanol reinforcement has come from studies demonstrating that suppression of acute ethanol consumption by the opioid antagonist naltrexone is accompanied by a reduction in ethanol-stimulated dopamine release in the nucleus accumbens (Benjamin et al., 1993; Gonzales and Weiss, 1998). The current study was conducted to determine if the anticraving compound acamprosate, as well as its non-

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acetylated analogue homotaurine, exhibit inhibitory influences on acute ethanol intake, as well as ethanol-stimulated dopamine release in the nucleus accumbens.

## 2. Materials and methods

### 2.1. Animals

Male Long Evans rats (250–400 g, Harlan, Madison, WI) were housed individually under a 12:12 light–dark cycle with lights on at 0600 h. All experiments were performed during the light portion of the light–dark cycle and were performed in accordance with approved institutional protocols and National Institutes of Health guidelines.

### 2.2. Drugs

Acamprosate (calcium acetylhomotaurinate, Estech Pharmaceuticals, Seoul, Korea) and homotaurine (3-amino-1-propanesulfonic acid, sodium salt, Sigma, St. Louis, MO) were dissolved in physiological saline, pH adjusted to 6–8 when necessary, and administered intraperitoneally (i.p.) in a volume of 1 ml/kg. Acamprosate content and purity was verified by independent elemental analysis. Ethanol (95% v/v) was diluted to 20% v/v in saline and administered i.p.

### 2.3. Ethanol consumption procedures

All ethanol consumption experiments were performed on rats individually housed in standard Plexiglas cages maintained at 25 °C in a ventilated cage rack system (BioZone, Fort Mill, SC). To induce voluntary ethanol consumption, rats were water-deprived for 24 h and subsequently given concurrent access to a solution containing 10% v/v ethanol + 10% w/v sucrose and a separate water bottle for 1 h/day for 4 days. The sucrose concentration in the ethanol-containing solution was then gradually decreased every 4 days to 5%, 2%, and finally 0%. For the remainder of the experiments, rats had concurrent access to the 10% ethanol solution and water for 1 h/day at approximately 1500–1600 h while having access to food ad libitum.

Parameters measured daily were body weight (g), ethanol intake (g/kg), water intake (ml), and ethanol preference (ml ethanol/total ml consumed). Fluid intake was measured to the nearest 0.5 ml.

For drug studies, rats were injected i.p. 15 min prior to the commencement of the 1-h access session. Only two injections were given per week, with at least 2 days of baseline consumption observed between each injection. Drugs and doses were administered in random order.

### 2.4. Microdialysis procedures

For microdialysis studies, a separate group of animals was anesthetized with 2% halothane vaporized in a 1:1 mixture of $O_2$ and $N_2O$, and stereotaxically implanted with guide cannulae (CMA/11, CMA/Microdialysis, North Chelmsford, MA) aimed at the medial shell region of the nucleus accumbens (coordinates +1.7 mm anterior and +0.6 mm lateral to bregma, and −6.0 mm ventral to skull surface, according to the atlas of Paxinos and Watson, 1997). Cannulae were secured with skull screws and dental cement. The wound was treated with 2% bacitracin and 2% xylocaine topical ointments, and sutured closed with 3−0 vicryl sutures. Animals were allowed to recover in home microdialysis cages (Instech Laboratories, Plymouth Meeting, PA) for at least 5 days prior to dialysis probe implantation.

Following recovery from surgical procedures, animals were lightly reanesthetized as described above and implanted with microdialysis probes with 2-mm cuprophane membranes (6 kDa cutoff, 240 μm OD, CMA/11) to a final depth of −8.0 mm from the skull surface. Probes were continuously perfused with artificial cerebrospinal fluid (aCSF), containing 125 mM NaCl, 2.5 mM KCl, 0.5 mM $NaH_2PO_4 \cdot H_2O$, 5 mM $Na_2HPO_4$, 1 mM $MgCl_2 \cdot 6H_2O$, 1.2 mM $CaCl_2 \cdot 2H_2O$, and 5 mM D-glucose, pH = 7.3−7.5. Probes were attached to dual channel liquid swivels (Instech Laboratories) with FEP tubing (0.005 in. ID, CMA/Microdialysis) for freely moving microdialysis procedures. Animals were allowed to recover from probe implantation overnight prior to pharmacological experiments. On the next day, the aCSF flow rate was set at 2.5 μl/min, and following a 1-h reequilibration period, microdialysis samples were collected into polypropylene microcentrifuge tubes in a refrigerated microsampler (SciPro) at 15-min intervals. Perchloric acid (0.1 M final concentration) was also added to the collection tubes to prevent the oxidation of dopamine. Following collection, samples were immediately placed on dry ice and later frozen at −70 °C until analysis. All drug studies were performed within 48 h of probe implantation. Animals had access to food and water ad libitum throughout all the microdialysis procedures.

Following microdialysis procedures, animals were deeply anesthetized with Nembutal (150 mg/kg i.p.) and perfused transcardially with 100 ml of 0.9% NaCl followed by 250 ml of Streck Tissue Fixative (Streck Laboratories, La Vista, NE). Brains were then removed and placed in the same fixative for at least 48 h at 4 °C. Coronal brain sections (30 μm in thickness) were cut on a cryostat (Leica, Deerfield, IL), placed onto gelatin-coated slides, and coverslipped. Probe placement was verified under light microscopy and data from animals with probe placements outside of the nucleus accumbens were discarded.

### 2.5. Analysis of dialysate dopamine content

Dopamine levels in microdialysis samples were analyzed by standard high performance liquid chromatography (HPLC) methods. Briefly, microdialysis samples (20 μl in volume) were injected via a refrigerated autosampler (ESA,

North Chelmsford, MA, USA) onto a reversed-phase $C_{18}$ 3-μm particle column (HR-80, ESA) at a flow rate of 1.0 ml/min. Mobile phase consisted of 75 mM sodium acetate, 1.5 mM sodium dodecylsulfate, 100 μl/l triethylamine, 25 μM EDTA, 12.5% acetonitrile, 12.5% methanol, pH = 5.6. All HPLC chemicals were from Sigma or Fisher Scientific (Santa Clara, CA). Oxidation potentials were detected using an ESA Model 5011 analytical cell and Coulochem II electrochemical detector (pre-column guard cell +350 mV, reduction electrode −100 mV, and oxidation electrode +280 mV). Dopamine content of each dialysis sample was quantified by comparing computer-integrated chromatogram peaks from samples with those of 1 and 5 pg/μl dopamine standards using two-point calibration curves injected every 20 samples.

### 2.6. Data analysis

All data are presented as mean ± S.E.M. Baseline values for ethanol intake (g/kg), water intake (ml), and ethanol preference (ml ethanol/total ml consumed × 100) were determined by averaging these parameters over the first eight daily 1-h sessions following the removal of 2% sucrose from 10% ethanol solution. Data from the pharmacological challenges were compared with these baseline values using a one-way repeated measures analysis of variance (ANOVA) followed by a Neumann–Keuls post-hoc test, or when normality and equal variance tests failed, a one-way ANOVA on ranks test (SigmaStat, SPSS Science, Chicago, IL). For microdialysis studies, dialysate dopamine levels (pg/μl) in each 15-min sample were transformed to percentage of basal dopamine release, assigning a value of 100% to the average dopamine level in the four baseline samples collected prior to drug administration. Microdialysis data were analyzed using a two-way ANOVA, or when normality and equal variance tests failed, individual postinjection data points were compared with baseline values using a paired $t$-test or Wilcoxon Signed Rank test (SigmaStat).

## 3. Results

### 3.1. Effects of acute acamprosate and homotaurine on ethanol intake

Baseline ethanol intake was 1.38 ± 0.02 g/kg/h, baseline water intake was 12.36 ± 0.12 ml/h, and baseline ethanol preference was 29.31 ± 0.64%. Mean body weight of the animals prior to the initiation of 1-h daily sessions was 363 ± 2 g, and following the final 1-h session was 433 ± 7 g. As seen in Fig. 1A, saline and homotaurine 10 mg/kg had no effect on the ethanol intake, while homotaurine significantly reduced ethanol intake by 51% at 50 mg/kg ($F(1,25) = 123.23$, $P < 0.001$) and 57% at 100 mg/kg ($\chi^2 = 9.31$, $P < 0.01$). Acamprosate 200 mg/kg was ineffective in reducing ethanol intake, while acamprosate
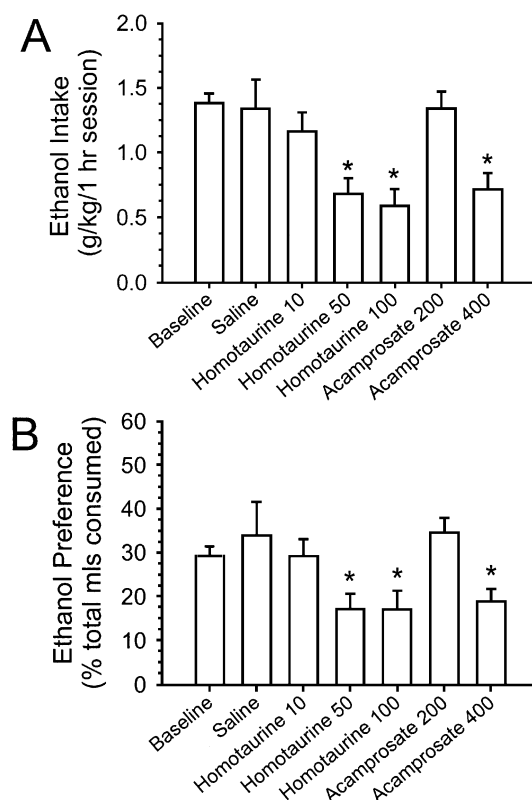


Fig. 1. Effects of acute administration of homotaurine and acamprosate on ethanol intake (A) and ethanol preference (B) in a limited access two-bottle choice paradigm in rats. Treatment and dose (in mg/kg i.p.) are given on the x-axis. For all data, $n = 13$ except for acamprosate 400 mg/kg, where $n = 10$. * $P < 0.05$ vs. baseline. Data are expressed as mean ± S.E.M.

400 mg/kg significantly reduced ethanol intake by 48% ($F(1,20) = 25.58$, $P < 0.01$). The only pharmacological treatment that produced a significant effect on water intake was homotaurine 50 mg/kg, which increased water intake by 13% ($F(1,25) = 5.20$, $P < 0.05$) (not shown). As seen in Fig. 1B, homotaurine was able to significantly reduce ethanol preference by 41% at both 50 mg/kg ($F(1,25) = 24.44$, $P < 0.001$) and 100 mg/kg ($\chi^2 = 9.31$, $P < 0.01$) doses, and acamprosate 400 mg/kg reduced ethanol preference by 35% ($F(1,20) = 6.13$, $P < 0.05$).

### 3.2. Effect of acamprosate and homotaurine on ethanol-stimulated mesolimbic dopamine release

Basal levels of dialysate dopamine were 1.0 ± 0.1 pg/μl. Because normality and equal variance tests on microdialysis data failed, individual postinjection time points were compared to baseline using a paired $t$-test or Wilcoxon Signed Rank test. As seen in Fig. 2A, a 1.5-g/kg dose of ethanol that was preceded by saline significantly increased dialysate dopamine levels by 66 ± 12% at 30 min postinjection ($W = 35.00$, $P < 0.05$), by 70 ± 8% at 45 min postinjection ($t(9) = 3.25$, $P < 0.05$), and by 29 ± 5% at 60 min postinjection ($t(9) = 2.28$, $P \leq 0.05$) which declined to basal levels thereafter. When preceded by 200-mg/kg acampro-
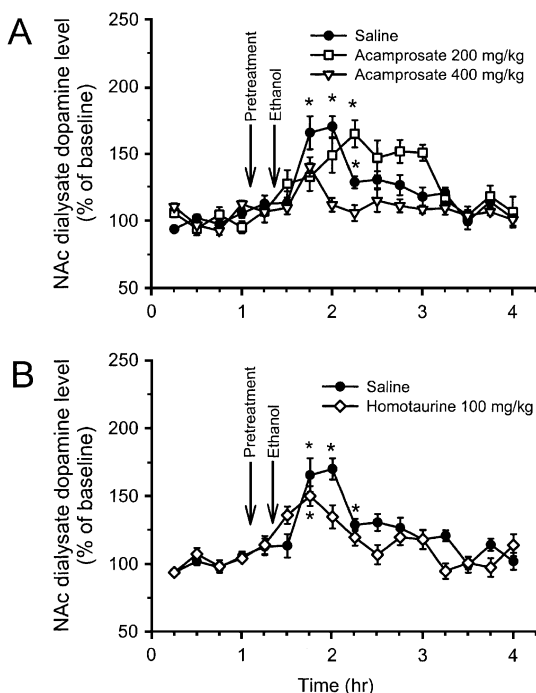
Fig. 2. (A) Effects of acute pretreatment with acamprosate 200 mg/kg (□, $n = 7$), acamprosate 400 mg/kg (▽, $n = 6$), or saline (●, $n = 9$) on increases in extracellular dopamine levels in the nucleus accumbens elicited by ethanol in freely moving rats. (B) Effects of acute pretreatment with homotaurine 100 mg/kg (◇, $n = 8$) or saline (●, $n = 9$) on increases in extracellular dopamine levels in the nucleus accumbens elicited by ethanol. Samples were collected every 15 min. Drug pretreatment was given at the first arrow and ethanol treatment was given at the second arrow. Data from the saline-pretreated animals are the same in (A) as in (B) and have been separated to facilitate the visual representation of the data. Data are expressed as mean ± S.E.M. *$P < 0.05$ vs. baseline.

sate, a $65 \pm 10\%$ peak increase in dopamine levels was observed following the ethanol ($t(7) = 2.85$, $P < 0.05$) but not until 60-min postinjection; dopamine levels declined to the baseline thereafter. Pretreatment with 400-mg/kg acamprosate blocked ethanol-stimulated increase in dialysate dopamine levels (Fig. 2A).

As seen in Fig. 2B, a 1.5-g/kg dose of ethanol that was preceded by 100-mg/kg homotaurine significantly increased dialysate dopamine levels by $50 \pm 7\%$ only at 30-min postinjection ($t(8) = 2.64$, $P < 0.05$) which declined to basal levels thereafter.

## 4. Discussion

In the present study, we investigated the ability of acamprosate and homotaurine to reduce acute ethanol intake in a limited access two-bottle choice paradigm. The restricted access paradigm was chosen since it (1) produces a greater ethanol intake than continuous (i.e., 24 h) access paradigms (Marcucella et al., 1984) and (2) has been used previously for assessing acute drug effects, including those of acamprosate, on ethanol consumption (Stromberg et al., 2001a).

In addition, this model is suitable for examining the effects of compounds with short elimination half-lives in animals, such as acamprosate (see below).

Acute administration of acamprosate suppressed ethanol intake only at 400 mg/kg and was ineffective at lower doses, consistent with previous reports (Le Magnen et al., 1987; Heyser et al., 1998; Stromberg et al., 2001a). However, homotaurine was able to suppress ethanol intake to similar degrees at doses that were much lower than that of acamprosate (50 and 100 vs. 400 mg/kg). Thus, homotaurine may be more potent than its acetylated form in suppressing acute ethanol consumption. As neither compound suppressed water intake, these data suggest that these intake-reducing effects are selective for ethanol. Further studies addressing the ability of non-acetylated homotaurine to suppress ethanol intake in other paradigms (i.e., operant self-administration and relapse models) are needed. While a previous study demonstrated no effect on repeated homotaurine administration on daily ethanol intake (Boismare et al., 1984), these negative results may be due to the lower dose used (0.26 mmol/kg or approximately 40 mg/kg), or the fact that only animals with high spontaneous ethanol preference ($> 60\%$) were used (less than 25% of the total number of rats tested).

The ability of acamprosate to reduce daily ethanol intake has been reported to increase with repeated treatments (Czachowski et al., 2001; Heyser et al., 1998), suggesting that acamprosate may not readily enter the brain after an acute injection. However, physicochemical and pharmacokinetic studies have indeed demonstrated that acamprosate indeed crosses the blood–brain barrier via a carrier transport mechanism (Chabenat et al., 1988; Durbin et al., 1995). Our findings that acute administration of acamprosate suppresses the ethanol-stimulated mesolimbic dopamine release (see below) also argue for the penetration of this compound into the brain. Similarly, homotaurine was also able to suppress ethanol-stimulated mesolimbic dopamine release, and other studies have demonstrated depressant effects in the central nervous system after acute administration at similar doses (Fariello et al., 1982; Rouhani et al., 1998; Ruiz de Valderas et al., 1991), providing further support that the effects observed in the present study are centrally mediated.

The high doses of acamprosate needed to suppress ethanol consumption in rodents may be related to the pharmacokinetics of this compound. In humans, repeated oral administration of acamprosate at 2 g/day results in steady state plasma levels of approximately 370–650 μg/l or 1 μM, and acamprosate has an elimination half-life of approximately 30 h (Saivin et al., 1998). In animals, however, the elimination half-life of this compound is approximately 30 min, whereas plasma levels of the drug are much higher than those seen in humans (Chabenat et al., 1987, 1988). Thus, high doses of this drug may be necessary to obtain relevant concentrations in the brain following an acute injection.

Using microdialysis, we also demonstrated that both acamprosate and homotaurine alter the ability of an i.p. dose of ethanol to elevate extracellular levels of dopamine in

the nucleus accumbens. In order to compare these results with those obtained in the two-bottle choice procedures, a technical issue regarding the routes of ethanol administration in these separate experiments must first be addressed. In the two-bottle choice procedures, animals consumed an average of 1.4 g/kg ethanol per 1-h session, while the animals used in the microdialysis received an i.p. injection of 1.5 g/kg ethanol. Although these experiments incorporated two different routes of ethanol administration, other microdialysis studies have demonstrated that extracellular levels of ethanol in brain are similar following the i.p. administration vs. oral self-administration of ethanol (Ferraro et al., 1990, 1991). Thus, similar brain levels of ethanol obtained in the current experiments would also be predicted.

Animals that were pretreated with saline demonstrated significant ethanol-stimulated increases in extracellular levels of dopamine in the nucleus accumbens, peaking 30–60 min following the injection and subsequently declining to basal levels. When animals were pretreated with the lower (200 mg/kg) dose of acamprosate, the time point at which the initial peak in ethanol-stimulated dopamine release observed was delayed by 30 min. Pretreatment with the higher (400 mg/kg) dose of acamprosate suppressed ethanol-stimulated increases in extracellular dopamine levels. Animals that were pretreated with homotaurine (100 mg/kg) demonstrated an initial peak in ethanol-stimulated increase in extracellular dopamine that was similar to that of the saline-pretreated animals; however, the duration of ethanol-stimulated increases in the extracellular dopamine was decreased (i.e., 45 min in saline pretreated animals vs. 15 min in homotaurine pretreated animals). Reductions in ethanol-stimulated mesolimbic dopamine release have been observed for other compounds that also suppress the ethanol consumption, such as naltrexone (Benjamin et al., 1993; Gonzales and Weiss, 1998) and γ-vinyl-γ-aminobutyric acid (γ-vinyl-GABA) (Gerasimov et al., 1999; Stromberg et al., 2001b). Thus, the ability of acamprosate and homotaurine to suppress ethanol consumption may be a result of their inhibitory actions on the mesolimbic dopamine reward system. However, as observed in the present study, the degree to which a pharmacological compound suppresses ethanol intake and ethanol-stimulated mesolimbic dopamine release may not be directly correlated. For example, while acamprosate (400 mg/kg) and homotaurine (100 mg/kg) were able to suppress ethanol intake to similar degrees, animals pretreated with this dose of homotaurine still demonstrated a significant increase (albeit shortened in duration) in ethanol-stimulated dopamine release, whereas animals pretreated with 400 mg/kg acamprosate did not demonstrate a significant increase in ethanol-stimulated dopamine release. Further investigations into the correlation between the degree of pharmacological reduction in ethanol intake and alteration in ethanol-stimulated mesolimbic dopamine release are needed to clarify this issue.

The precise mechanism(s) by which acamprosate and homotaurine suppress ethanol consumption and ethanol-stimulated mesolimbic dopamine system activity remain unclear. Homotaurine exerts agonist activity at the GABA type A (GABA_A) receptors (Allan and Harris, 1986; Andrews and Johnston, 1979; Okamoto and Sakai, 1981), and previous studies by our group have shown that the stimulation of GABA_A receptors within the nucleus accumbens suppresses ethanol self-administration (Hodge et al., 1995), whereas GABA_A receptor stimulation in the ventral tegmental area is relatively ineffective in reducing ethanol intake (Hodge et al., 1996). Rather, GABA_A receptor blockade in the ventral tegmental area appears to reduce ethanol intake (Nowak et al., 1998). Mesolimbic dopamine release is also under the inhibitory control of local GABA_A receptors in the nucleus accumbens (Yan, 1999). Thus, homotaurine may act directly in the NAc to suppress ethanol intake and ethanol-stimulated mesolimbic dopamine release. Local infusion studies are warranted to confirm this hypothesis. However, as the GABA_A systems in ventral tegmental area and other limbic regions have also been implicated in the control of mesolimbic dopamine release (Ikemoto et al., 1997; Westerink et al., 1996; Xi and Stein, 1998) and/or ethanol self-administration (Hyytia and Koob, 1995; Roberts et al., 1996), contributions of these regions to the neurochemical and behavioral effects of homotaurine observed in the present study cannot be ruled out at this point.

Despite its structural similarity to homotaurine, acamprosate does not appear to enhance the GABA_A receptor function (Berton et al., 1998; Zeise et al., 1993). Acamprosate has been shown to inhibit GABA_B receptor function in nucleus accumbens neurons (Berton et al., 1998). However, it is unlikely that acamprosate suppresses the ethanol-stimulated mesolimbic dopamine release via antagonism of GABA_B receptors in this circuitry, as it has been demonstrated that stimulation, rather than antagonism, of mesolimbic GABA_B receptors suppresses basal and drug-stimulated dopamine release in the nucleus accumbens (Kalivas et al., 1990; Westerink et al., 1996; Xi and Stein, 1998, 1999).

Recent studies have indicated a possible interaction of acamprosate with *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission in various brain regions (Allgaier et al., 2000; Al Qatari et al., 1998; Berton et al., 1998; Madamba et al., 1996; Naassila et al., 1998; Popp and Lovinger, 2000; Rammes et al., 2001; Zeise et al., 1990, 1993). However, not all of these studies concur on whether acamprosate enhances or inhibits NMDA receptor function, which is likely attributable to the different subunit composition of NMDA receptors across the various brain regions tested. Acamprosate has also recently been shown to inhibit neuronal voltage-gated calcium channel function (Allgaier et al., 2000). Given the extensive literature on regulation of mesolimbic dopamine system activity by NMDA receptors (Kalivas, 1993; Kretschmer, 1999; Westerink et al., 1996) and voltage-gated calcium channels (Cooper and White, 2000; Horne and Kemp, 1991; Rossetti et al., 1999), it is highly likely that acamprosate mediates its effects on ethanol-stimulated mesolimbic dopamine release via one

or both of these mechanism of action. Local drug administration studies are warranted to investigate this hypothesis and dissect the region(s) of the mesolimbic reward circuitry that are under the inhibitory control of acamprosate.

In conclusion, we have demonstrated that acamprosate and its non-acetylated analogue homotaurine dose-dependently suppress both acute ethanol consumption and ethanol-stimulated increases in extracellular dopamine levels in the nucleus accumbens. We speculate that the ability of these compounds to suppress ethanol intake may be via their ability to modulate ethanol's stimulatory actions on the mesolimbic reward circuitry. Further investigations into the precise neuroanatomical and neurochemical mechanisms by which homotaurine and acamprosate exert their effects will ultimately shed light on the neurobiological mechanisms controlling ethanol self-administration behavior.

## Acknowledgements

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