Published in final edited form as:

Alcohol Clin Exp Res. 2008 February ; 32(2): 188–196. doi:10.1111/j.1530-0277.2007.00569.x.

Effects of Acamprosate on Neuronal Receptors and Ion Channels Expressed in *Xenopus* Oocytes

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Abstract

Background—Acamprosate (calcium acetylhomotaurinate) has proven to be a moderately effective pharmacological adjunct for the treatment of alcoholism. However, the central nervous system mechanism by which acamprosate reduces alcohol relapse remains unclear. Here we survey a number of metabotropic receptors, ligand-gated ion channels, and voltage-gated ion channels, to determine if acamprosate has actions at these sites in the central nervous system.

Methods—*Xenopus* oocytes were injected with cDNAs or cRNAs encoding metabotropic glutamate receptors 1 and 5, M1 muscarinic receptors, glycine $\alpha 1$ homomeric and $\alpha 1\beta 1$ heteromeric receptors, γ -aminobutyric acid A (GABA_A $\alpha 4\beta 3\delta$, $\alpha 4\beta 3\gamma 2s$, and $\alpha 1\beta 2\gamma 2s$) receptors, vanilloid receptor 1, and various combinations of α and β subunits of voltage-gated Na⁺ channels. Electrophysiological responses were measured using two-electrode voltage clamp parameters after activation with agonists or voltage steps (for the voltage-gated channels). Acamprosate (0.1 to 100 μ M) was pre-applied for 1 minute, followed by co-application with agonist. Acamprosate was also applied with ethanol to determine if it altered ethanol responses at some of these receptors and channels.

Results—None of the receptors or ion channels responded to acamprosate alone. Acamprosate also failed to alter the activation of receptors or channels by agonists or after activation of voltage-gated channels. There was no effect of acamprosate on ethanol responses at GABA_A $\alpha 1\beta 2\gamma 2s$ receptors or Na⁺ channels.

Conclusions—Acamprosate does not significantly modulate the function of these receptors and ion channels at clinically relevant concentrations. Thus, the clinical effectiveness of acamprosate in the treatment of alcoholism is not likely due to direct effects on these receptors or ion channels.

Keywords

Acamprosate; Ethanol; Ligand-Gated Ion Channels; Voltage Gated Ion Channels; Metabotropic Receptors

Relapse into drug taking after a period of sobriety is a major problem in the treatment of addiction and in the treatment of alcoholism especially (Stalcup et al., 2006). Efforts have focused on the development of pharmacological agents that have the potential to curb the drug craving which precedes and usually precipitates a relapse (Heilig and Egli, 2006). Acamprosate (calcium acetylhomotaurine) was approved first in France in 1989 for the treatment of alcohol dependence and later in the United States in 2002. Over 1.4 million

alcohol-dependent patients world-wide have been treated with acamprosate, which shows moderate efficacy at reducing relapse (Mason, 2001). However, attempts at determining the site of action of acamprosate in the central nervous system have produced equivocal results (De Witte et al., 2005; Littleton and Zieglansberger, 2003).

Mouse models of alcoholism have shown clear effects of acamprosate on behavioral responses to alcohol. Mutant mice that have increased brain glutamatergic tone drink large quantities of alcohol, and acamprosate administration reduces both the excessive alcohol consumption and high glutamate tone (Spanagel et al., 2005). In addition to reducing alcohol consumption, acamprosate inhibits the acute activating effects of alcohol and locomotor sensitization in wild-type mice (Kotlinska et al., 2006). Alcohol locomotor sensitization is also reduced by acamprosate to a greater degree in a high alcohol-preferring mouse line compared to a low alcohol-preferring mouse line (Chester et al., 2001). Acamprosate appears to alter conditioned responses to alcohol. For example, acamprosate inhibits the development of conditioned place preference to alcohol (McGeehan and Olive, 2003) and conditioned abstinence behavior from repeated alcohol exposure, a measure of alcohol negative reinforcement (Cole et al., 2000).

Most studies aimed at identifying a mechanism of action of acamprosate have focused on the glutamatergic system (De Witte et al., 2005). However, the majority of these studies are difficult to interpret because the concentrations of acamprosate tested were at least 10 times greater than that seen in humans during treatment (Johnson et al., 2003; Mason et al., 2002). For example, in 2 independent clinical trials, healthy patients were administered 1 to 3 g of acamprosate orally once a day for 6 days, and the peak plasma concentration of acamprosate achieved was approximately 1 μ M (Johnson et al., 2003; Mason et al., 2002). Early studies suggested that acamprosate at high concentrations (1 mM) inhibits glutamate receptor activated responses in vitro and in vivo (Zeise et al., 1993), and 300 μ M acamprosate enhanced *N*-methyl-D-aspartate (NMDA) receptor function in hippocampal and accumbens brain slices (Berton et al., 1998; Madamba et al., 1996). Furthermore, additional studies on NMDA receptors concluded that acamprosate may interact with the polyamine site as a partial agonist (Naassila et al., 1998; Popp and Lovinger, 2000; al Qatari et al., 2001), while another study suggested that acamprosate may have weak antagonistic properties at NMDA receptors (Rammes et al., 2001). Finally, one study suggested that acamprosate may inhibit metabotropic glutamate receptors (mGluR), in particular mGluR5 (Harris et al., 2002). None of these studies detected direct actions of clinically relevant (e.g., 1 to 3 μ M) concentrations of acamprosate on receptor or channel function.

Other neurotransmitter systems have been less studied with respect to the site of action of acamprosate. It is plausible that acamprosate may interact with inhibitory neurotransmitter systems, such as GABA and glycine because of the similarity in structure between acamprosate and these neurotransmitters (Olive, 2002). However, there is no direct evidence that acamprosate modulates the function of GABA_A or glycine receptors. Finally, voltage-gated channels could also represent a target for the action of acamprosate in the central nervous system because of their important role in cellular excitability.

In this study, we tested the effects of acamprosate on several metabotropic receptors including, mGluR1, mGluR5 and M1 muscarinic receptors. We also determined whether acamprosate has any effects on ligand-gated and voltage-gated channels including: GABA_A, glycine, vanilloid-1 receptors (VR1) and voltage-gated Na⁺ channels (Nav). We chose these candidate receptors and channels in part because with the exception of mGluR1, all of them are affected by ethanol in vitro. In addition, we tested the ability of acamprosate to alter ethanol responses on some of these receptors. We found no effects of acamprosate on these receptors or ion channels when recombinantly expressed in *Xenopus* oocytes.

MATERIALS AND METHODS

Materials

Chemicals (glutamate, acetylcholine, capsaicin, GABA, glycine) were purchased from Sigma Aldrich (St Louis, MO). Calcium acamprosate was provided by Dr. R. Messing (Gallo Center, San Francisco, CA). Stock solutions of acamprosate were dissolved in distilled H₂O and then diluted in modified Barth's solution (MBS), Frog Ringer's solution, or ND-96 depending on the receptor tested.

cDNA Constructs and Preparation of cRNA

Expression plasmids containing full length mGluR1 and mGluR5 (S890G) were mutated to the wild-type receptor and these were provided by Dr. R. Gereau (Salk Institute, La Jolla, CA). M1 muscarinic receptor expression plasmid was obtained from Dr. T. Bonner (NIMH, Bethesda, MD). The expression plasmid for VR1 was obtained from Dr. D. Julius (University of California, San Francisco, CA). γ -aminobutyric acid type A (GABA_A) receptor $\alpha 1$, $\beta 2$, and $\gamma 2s$ subunit clones were obtained from Dr. P. Whiting (Merck Sharpe Dome, Hertfordshire, UK). γ -aminobutyric acid type A receptor cDNAs were provided by Dr. R. W. Olsen (University of California, Los Angeles, CA, rat $\alpha 4$ and δ), Dr. L. Mahan provided rat $\beta 3$, and Dr. M. H. Akabas provided rat $\gamma 2s$ (Yeshiva University, Bronx, New York). Glycine $\alpha 1$ was obtained from Dr. P. Schoffield (Prince of Wales Medical Research Institute, Randwick, Australia) and $\beta 1$ expression plasmids were obtained from Dr. H. Betz (Max-Planck-Institute for Brain Research, Frankfurt, Germany). Voltage-gated sodium channel α Nav 1.2 clone was a gift from Dr. W. Catterall (University of Washington, Seattle, WA). The cDNAs encoding α Nav 1.4 and $\beta 1$ subunit were obtained from Dr. A. George (Vanderbilt University, Nashville, TN). The cDNA encoding α Nav 1.6 clone was obtained from Dr. A. Goldin (University of California, Irvine, CA). cRNA was prepared for oocyte injection for mGluR1, mGluR5, M1, $\alpha 4\beta 3\delta$ and $\alpha 4\beta 3\gamma 2s$ containing GABA_A receptor, VR1 receptor and sodium channel subunits. In vitro transcription of cRNA was carried out according to the instructions of the mMessage kit (Ambion, Austin, TX). cDNA was used for expression of glycine receptor subunits and $\alpha 1\beta 2\gamma 2s$ containing GABA_A receptors.

Site-Directed Mutagenesis

A mutant mGluR5 clone (S890G) was mutated back to the wild-type sequence by changing glycine at position 890 to serine. Mutagenesis was carried out according to the manufacturer's instructions of the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) using commercially made mutagenic primers (Operon, Alameda, CA). The wild-type cDNA was confirmed by automated fluorescent DNA sequencing.

Xenopus Oocyte Preparation

Extraction of ovarian tissue from *Xenopus laevis* frogs was in accordance with the National Institutes of Health guide for the care and use of laboratory animals. Tissue was placed in MBS containing 88 NaCl, 1 KCl, 10 HEPES, 0.82 MgSO₄, 2.4 NaHCO₃, 0.91 CaCl₂, and 0.33 mM Ca(NO₃)₂, and adjusted to pH 7.5. Following manual isolation of *Xenopus laevis* oocytes with forceps, oocytes were treated with collagenase type 1A solution containing 0.5 mg/ml collagenase, 83 NaCl, 2 KCl, 1 MgCl₂, and 5 mM HEPES, adjusted to pH 7.5, for 10 minutes. Oocytes were injected with wild-type receptors and ion channel cRNA or cDNA using a microdispenser (Drummond Scientific, Broomwall, PA). Injected oocytes were singly stored in incubation media at 13°C. Incubation media is composed of MBS supplemented with 10 mg/l streptomycin, 10,000 units/l penicillin, 50 mg/l gentamicin, 90 mg/l theophylline, and 220 mg/l sodium pyruvate (Sigma Chemical Co., St Louis, MO).

Xenopus Oocyte Electrophysiology

Electrophysiological measurements were made in oocytes 1 to 10 days after injection depending on the receptor expressed. Oocytes were placed in a chamber (approximately 100 μ l volume) and perfused (2.0 ml/min) with MBS using a peristaltic pump (Cole-Parmer Instruments Co., Chicago, IL) through 18-gauge polyethylene tubing (Becton Dickinson, Sparks, MD). Oocytes were impaled in the animal pole with 2 glass electrodes filled with 3 M KCl and clamped at -70 mV using an OC725C oocyte clamp (Warner Instruments, Hamden, CT). Currents were continuously plotted on a chart recorder (Cole-Parmer Instrument Co.). In each experiment predrug baseline agonist responses were measured after application of agonist for 20 to 30 seconds. After a 15 to 20 minutes washout, acamprosate was pre-applied for 1 minute to allow equilibration in the bath and to measure any direct effects on receptor function. Following this 1 minute pre-application period, acamprosate was co-applied with agonist for 20 to 30 seconds, followed by a 15 to 20 minutes washout. A postdrug baseline agonist response was measured again after application of the agonist for 20 to 30 seconds. For experiments with GABA_A $\alpha 1\beta 2\gamma 2s$ and glycine $\alpha 1$ and $\alpha 1\beta$ receptors, we tested the effects of acamprosate on agonist responses at the effective concentrations (EC_{5-10}) concentration. The EC_{5-10} concentration was determined for each oocyte by first applying a maximal concentration (1 mM) of agonist. This protocol is based on previous studies indicating that enhancement and inhibition of receptor function can be detected at this concentration of agonist (Mihic et al., 1997). The details of the protocol used for GABA_A receptors containing $\alpha 4\beta 3\delta$ and $\alpha 4\beta 3\gamma 2s$ receptors were published (Borghese et al., 2006). Briefly, we used an EC_{20} concentration of GABA determined from a 1 mM GABA concentration. Details of the protocol used for mGluR1/5 and M1 muscarinic receptors were published (Minami et al., 1998; Sanna et al., 1994). For these metabotropic receptors, we chose a low concentration of agonist (100 μ M) which corresponds to approximately EC_{10} to avoid receptor desensitization.

For experiments with Nav channels, electrophysiological procedures were as previously described (Shiraishi and Harris, 2004). Nav α subunit cRNA was co-injected with $\beta 1$ cRNA at a ratio of 1:10 (total 22 ng) into *Xenopus* oocytes and electrophysiological recording was measured 2 to 6 days after injection. Oocytes were perfused with Frog Ringer's solution containing 115 NaCl, 2.5 KCl, 1.8 CaCl₂, and 10 mMHEPES (pH7.5 adjusted with NaOH) at a rate of 2 ml/min at room temperature. Recording electrodes (<0.5 M Ω) filled with 3 M KCl were inserted into the oocyte. A Warner oocyte clamp OC 725C (Warner Instruments) was used to voltage-clamp, and pulses were applied and data were acquired using pClamp software (Axon Instruments, Foster City, CA). The amplitude of expressed Na⁺ currents was typically 2 to 15 μ A. These currents were completely blocked by external applications of tetrodotoxin (200 nM). We had reported that alcohol and anesthetics inhibit sodium channels and the inhibition was greater when the number of channels in the inactivated state was increased (Shiraishi and Harris, 2004). Because one-half of the channels were in the inactivated state at a holding potential of approximately -60 mV, the currents were elicited by a 50 milliseconds depolarizing pulse to -20 mV from holding potential, causing half-maximal current ($V_{1/2}$) (around -60 mV) every 10 second. In addition, we tested the effects of acamprosate at voltages that elicited maximal currents (V_{max}) (around -90 mV). Finally, current-voltage (I/V) plots were constructed from several voltage potentials in the presence and absence of acamprosate. For construction of I/V plots, currents were elicited by 50 milliseconds depolarizing pulses from -80 to 60 mV in 10 mV increments from a holding potential of -90 mV. Acamprosate was applied for 1 minute to allow a complete change of solution in the bath.

Effects of Acamprosate on Ethanol Action

The effect of acamprosate on ethanol potentiation of GABA_A $\alpha 1\beta 2\gamma 2$ s containing receptors was determined by co-applying ethanol (200 mM) with acamprosate (1, 10, 100 μ M) and EC₅₋₁₀ GABA. The percent ethanol potentiation of the GABA control response in the presence and absence of acamprosate was calculated and used for statistical analysis. The effect of acamprosate on ethanol inhibition of Na⁺ channel current was determined by co-applying ethanol (200 mM) with acamprosate (1, 10, 100 μ M) from the V_{1/2} holding potential. The percent ethanol inhibition of the voltage control response in the presence and absence of acamprosate was calculated and used for statistical analysis.

Statistics

In each oocyte, predrug and postdrug agonist responses were used as a baseline to determine the effect of acamprosate on receptor function. These values are expressed as a percent of the agonist control or voltage control for the sodium channels in all figures and used for statistical analysis with the one-way repeated measures ANOVA and Dunnett's post hoc test. Significant effects were set at $p < 0.05$. Because there was no current produced by application of acamprosate alone these data could not be analyzed.

RESULTS

Metabotropic Receptors

Several metabotropic receptors are sensitive to physiologically relevant concentrations of ethanol in vitro, including mGluR5 (Minami et al., 1998) and M1 muscarinic receptors (Sanna et al., 1994). Group I metabotropic glutamate receptors (mGluR1/5) and M1 muscarinic receptors are G-protein coupled receptors that activate phospholipase C, ultimately resulting in increased intracellular calcium. This produces a calcium-activated chloride conductance which is used as a measure of receptor activation (Sanna et al., 1994).

Metabotropic Glutamate Receptors—Previous studies suggested that acamprosate may act by inhibiting the metabotropic glutamate type 5 receptor (Harris et al., 2002). In oocytes injected with mGluR5 cRNA, acamprosate (1 or 100 μ M) did not affect receptor function directly as observed during the 1 minute pre-application of acamprosate. In addition, acamprosate did not alter the glutamate response during co-application with glutamate. These data are shown in Fig. 1A. Summary data for the effect of acamprosate on agonist responses are shown in Fig. 1B expressed as a percent of the glutamate control responses. One-way repeated measures ANOVA confirmed the observation that acamprosate did not alter mGluR5 function [$F_{(2,14)} = 1.6, p > 0.1$].

Acamprosate was tested on recombinant mGluR1 receptors, which are similar in structure and second messenger coupling as mGluR5 receptors. Representative current responses after application of 1 or 100 μ M acamprosate are shown in Fig. 2A. Acamprosate did not alter mGluR1 function when applied in the absence of glutamate nor did it alter the agonist response. Figure 2B summarizes data from mGluR1-injected oocytes after application of 0.1 to 100 μ M acamprosate co-applied with agonist. There was no significant effect of acamprosate on the glutamate responses [$F_{(5,31)} = 2.2, p > 0.05$].

Muscarinic Receptors—Representative current tracings from oocytes injected with M1 receptors are shown in Fig. 3A. Acamprosate did not affect the receptor in the presence or absence of agonist. The percent of the acetylcholine response after acamprosate (0.1 to 10 μ M) is shown in Fig. 3B. There was no significant effect of acamprosate on M1 receptors [$F_{(3,24)} = 0.02, p > 0.1$].

Ligand-Gated Ion Channels

Vanilloid-1 Receptors—Ethanol was previously shown to elicit and potentiate nociceptor responses via VR1 (Trevisani et al., 2002). Capsaicin (3 μ M), which elicited an EC₅₀ response was co-applied with 3 concentrations of acamprosate (1, 10, and 100 μ M) in turn (Fig. 4A). None of these concentrations of acamprosate affected the capsaicin response (Fig. 4B) when compared to the control capsaicin response with the one-way repeated measures ANOVA [$F_{(3,19)} = 3.1, p > 0.05$] nor did acamprosate affect receptor function in the absence of agonist.

Glycine Receptors—Glycine receptors are one of the major inhibitory neurotransmitter receptors in the central nervous system and are potentiated by low concentrations of ethanol (Mihic et al., 1997). Glycine $\alpha 1$ homomeric receptors were expressed in oocytes and current responses were measured before, during and after acamprosate (1 and 10 μ M). Acamprosate did not alter channel function during the pre-application period (Figs 5A, 5C, 5E and 5G). Figure 5A shows representative current tracings for glycine $\alpha 1$ receptors activated by glycine. Summary data are shown in Fig. 5B expressed as a percent of the control glycine response. There was no significant effect of acamprosate on glycine $\alpha 1$ receptor function [$F_{(2,23)} = 2.8, p > 0.05$] (Fig. 5B). We also tested the effects of acamprosate on $\alpha 1\beta$ heteromeric glycine receptors. Current tracings are shown in Fig. 5C. Summary data expressed as a percent of the glycine response are shown in Fig. 5D and statistical analysis indicated no significant effect [$F_{(2,23)} = 3.0, p > 0.05$].

Taurine is a partial agonist at glycine receptors and we wondered if acamprosate might affect taurine responses because it is a taurine analog. Acamprosate (1 and 10 μ M) was tested on glycine $\alpha 1$ receptors before, during and after application of taurine as shown in the tracing of Fig. 5E. Acamprosate had no significant effect on the taurine response [$F_{(7,23)} = 1.9, p > 0.1$] as shown in the summary graph in Fig. 5F. Similarly, acamprosate did not affect taurine responses at $\alpha 1\beta$ heteromeric glycine receptors (Fig. 5G). Summary data shown in Fig. 5H indicated no significant effect of acamprosate [$F_{(5,17)} = 1.2, p > 0.1$].

GABA_A Receptors— γ -aminobutyric acid receptors mediate fast synaptic inhibition in the central nervous system and are a major site of action of ethanol (Mihic et al., 1997). We tested acamprosate (1, 10, and 100 μ M) on a number of different GABA_A receptor subunit combinations. Acamprosate had no effect on receptor function when applied alone during the pre-application period (Figs 6A, 6C, 6E). Figure 6A shows a representative current tracing for GABA_A $\alpha 4\beta 3\delta$ receptors. There was no significant effect of acamprosate detected as shown in the summary data of Fig. 6B [$F_{(3,23)} = 3.5, p > 0.1$]. Acamprosate did not alter $\alpha 4\beta 3\gamma 2s$ receptor function (Fig. 6C). Summary data shown in Fig. 6D confirmed this observation [$F_{(3,22)} = 0.92, p > 0.05$]. Finally, acamprosate had no effect on $\alpha 1\beta 2\gamma 2s$ containing receptors (Fig. 6E), which was confirmed by statistical analysis of the summary data in Fig. 6F [$F_{(3,33)} = 1.7, p > 0.1$].

Voltage-Gated Channels

Na⁺ Channels—Voltage-gated Na⁺ channels play a major role in neuronal excitability and are inhibited by ethanol in vitro (Shiraishi and Harris, 2004). Nav α 1.2, 1.4 or 1.6 were co-expressed individually with the Na⁺ channel $\beta 1$ subunit. Acamprosate did not alter channel function during the pre-application period. A representative current tracing at V_{max} and V_{1/2} is shown in Fig. 7A for data obtained before, during and after acamprosate application for an oocyte expressing Nav 1.2 and $\beta 1$ subunits. Summary data are shown in Fig. 7B for each Na⁺ channel α subunit with the $\beta 1$ subunit. There was no significant effect of acamprosate (1, 10 and 100 μ M) on Na⁺ channel function. Representative current traces during voltage steps of Nav 1.2 co-expressed with the $\beta 1$ subunit are shown in Fig. 7C with and without

acamprosate. The current-voltage relationships are plotted in Fig. 7D. Acamprosate did not alter the Na⁺ channel current at any of these voltage potentials.

Effect of Acamprosate on Ethanol Action

Another hypothesis we considered was that acamprosate alters ethanol responses at some of the receptors and channels we tested above. We tested the ability of acamprosate to alter the ethanol responses at GABA_A and Na⁺ channels. Acamprosate did not alter the ethanol potentiation of $\alpha 1\beta 2\gamma 2$ s containing GABA_A receptors as shown in Fig. 8A [$F_{(3,31)} = 2.8$, $p = 0.06$], although there appeared to be a trend for a reduction in the ethanol response. We also tested the effect of acamprosate on the ethanol inhibition of sodium channels (Fig. 8B). There was no effect of acamprosate on the ethanol inhibition of Nav1.2, 1.4 or 1.6 channels.

DISCUSSION

The mechanism of action of acamprosate has remained elusive since it was first discovered to reduce alcohol drinking in rats (Boismare et al., 1984) and in detoxified alcoholics (Lhuintre et al., 1985). In this study, we tested the effects of clinically relevant concentrations of acamprosate (1 μ M), as well as higher concentrations (≥ 10 μ M), on recombinant metabotropic receptors, ligand-gated ion channels, and voltage-gated ion channels expressed in *Xenopus* oocytes. As mentioned previously, the typical plasma concentration of acamprosate achieved in patients is approximately 1 μ M (Johnson et al., 2003; Mason et al., 2002). We found that acamprosate does not appear to significantly modulate the function of any of these receptors or ion channels tested here in vitro at concentrations observed during treatment. Also, acamprosate does not affect ethanol responses at GABA_A receptors or Na⁺ channels.

A previous study implicated mGluRs as sites of action of acamprosate (Harris et al., 2002). This study found that 10 μ M acamprosate prevented the binding of trans-(1S,3R)-1-amino-1,3-cyclopentanedicarboxylic acid (*trans*-ACPD), an mGluR1 and mGluR5 agonist, in rat brain homogenates, suggesting that acamprosate directly interacts with group I mGluRs. In addition, the neurotoxic effects of *trans*-ACPD on hippocampal cultures were reduced by a mGluR5 non-competitive antagonist (SIB-1893) and acamprosate, while NMDA induced neurotoxicity was not altered by application of these drugs (Harris et al., 2002). In contrast, we found no significant effect of acamprosate on either mGluR1 or mGluR5 function in vitro. It is important to note that the concentrations of acamprosate used in the present study included the range of concentrations achieved during treatment in humans (e.g., 1 μ M), as well as much higher concentrations (100 μ M) (Johnson et al., 2003; Mason et al., 2002). We found no effects of acamprosate at either low (≤ 1 μ M) or high concentrations (≥ 10 μ M) on mGluR1 and mGluR5. From our data, we conclude that clinically relevant concentrations of acamprosate have little to no effect on the electrophysiological responses of mGluR1 and mGluR5 expressed in *Xenopus* oocytes. The discrepancies between our study and the one by Harris et al., 2002 could be because of differences in the responses measured (e.g., receptor binding vs. electrophysiology) and/or differences in the preparations used (e.g., rat brain vs. *Xenopus* oocytes).

To further explore potential sites of action of acamprosate, we surveyed a number of other receptors and ion channels that are expressed in the nervous system and are affected by physiological concentrations of ethanol. These included: VR1 receptors, GABA_A and glycine receptors, M1 muscarinic receptors, and Nav channels. We did not test NMDA receptors because previous studies using the same methodology showed a very small inhibitory effect of high concentrations of acamprosate (Rammes et al., 2001). Acamprosate was without effect on all of the receptors and ion channels tested here at concentrations seen in patients during treatment, as well as at higher concentrations. Our findings are consistent

with other studies showing that acamprosate has only little to no effect on brain receptors and voltage-gated channels (e.g., calcium channels) when tested using a different methodology. (Littleton and Zieglansberger, 2003). It is possible that the moderate effectiveness of acamprosate in the treatment of alcoholism may be because of some neuroadaptive compensation after chronic treatment, which was suggested in a previous study of NMDA receptors (Rammes et al., 2001). Specifically, Rammes et al., 2001 found that chronic treatment with acamprosate in rats altered brain abundance of specific NMDA receptor subunits. In addition, the possibility still exists that acamprosate produces its clinical effects by acting on some intracellular protein or enzyme. Global brain gene expression analysis after acamprosate treatment may help clarify this possibility.

Two large multi-center clinical trials of acamprosate for the treatment of alcoholism have been published in the United States and Australia (Anton et al., 2006; Morley et al., 2006). Both of these studies concluded that acamprosate had no beneficial effect in reducing drinking in the populations tested. However, smaller trials in Europe showed that acamprosate is effective in reducing relapse (Soyka and Chick, 2003). One of the major differences in the two recent American and Australian acamprosate trials and those conducted in Europe is the degree to which the subjects showed clinically defined dependence. It has been suggested that acamprosate efficacy is greater in those individuals having a higher degree of dependence, such as those individuals that require medical detoxification before acamprosate administration (Dahchour et al., 2000; al Qatari et al., 2001).

In summary, our data shows that acamprosate has little to no effect on electrophysiological properties of a number of receptors and ion channels expressed in the nervous system. Acamprosate also had no significant effect on ethanol responses at GABA_A receptors or Na⁺ channels. The effectiveness of acamprosate in the treatment of alcoholism cannot be attributed to a direct action at these sites.

Acknowledgments

This research funded by the National Institute of Alcohol Abuse & Alcoholism grant AA06399-25 and an endowment from M. June and J. Virgil Waggoner. We thank Dr. Junichi Ogata for help with the sodium channel experiments and Kathy Carter and Marni Martinez for technical assistance.

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Fig. 1.

Acamprosate does not modulate metabotropic glutamate receptor (mGluR5) function. *Xenopus* oocytes were injected with (mGluR5) cRNA, and calcium-activated chloride currents were measured after application of glutamate and co-application with acamprosate. **(A)** Representative current tracings after application of 100 μ M glutamate or acamprosate (1 and 100 μ M) are shown. **(B)** Summary data showing mean $\pm$ SEM of the percent of the glutamate response in 5–6 oocytes per bar.

Fig. 2.

Acamprosate does not modulate metabotropic glutamate receptor (mGluR1) function. *Xenopus* oocytes were injected with mGluR1 cRNA, and calcium-activated chloride currents were measured after application of glutamate and co-application with acamprosate. **(A)** Representative current tracings after application of 100 μ M glutamate or acamprosate (1 and 100 μ M) are shown. **(B)** Summary data showing mean $\pm$ SEM of the percent of the glutamate response from 5 to 7 oocytes per bar.

Fig. 3.

Acamprosate does not modulate muscarinic receptor (M1) function. *Xenopus* oocytes were injected with M1 cRNA, and calcium-activated chloride currents were measured after application of acetylcholine and co-application with acamprosate. **(A)** Representative current tracings after application of 100 μ M acetylcholine or acamprosate (1 and 10 μ M) are shown. **(B)** Summary data showing mean $\pm$ SEM of the percent of the acetylcholine response from 4 to 6 oocytes per bar.

Fig. 4.

Acamprosate does not modulate vanilloid receptor function. (A) Representative current tracings after application of 3 μ M capsaicin alone or with acamprosate (1, 10 and 100 μ M). (B) Summary data showing the mean $\pm$ SEM of the percent of the capsaicin response from 5 oocytes.

Fig. 5.

Acamprosate does not modulate glycine receptor function. **(A)** Glycine $\alpha 1$ receptor current tracing during application of acamprosate and co-application with EC₅₋₁₀ glycine. **(B)** Summary data from **A** showing mean $\pm$ SEM of the percent of the glycine response from 8 oocytes per bar. **(C)** Glycine $\alpha 1\beta$ receptor current tracing during application of acamprosate and co-application with EC₅₋₁₀ glycine. **(D)** Summary data from **C** showing mean $\pm$ SEM of the percent of the glycine response in 8 oocytes per bar. **(E)** Glycine $\alpha 1$ receptor current tracing during application of acamprosate and co-application with EC₅₋₁₀ taurine. **(F)** Summary data from **E** showing mean $\pm$ SEM of the percent of the taurine response in 8 oocytes per bar. **(G)** Glycine $\alpha 1\beta$ receptor current tracing during application of acamprosate and co-application with EC₅₋₁₀ taurine. **(H)** Summary data from **G** showing mean $\pm$ SEM of the percent of the taurine response in 6 oocytes per bar.

Fig. 6.

Acamprosate does not modulate γ -aminobutyric acid type A receptor (GABA_A) function. **(A)** GABA_A ($\alpha 4\beta 3\delta$) receptor current tracing during application of acamprosate and co-application with EC_{20} GABA. **(B)** Summary data from **A** showing mean $\pm$ SEM of the percent of the GABA response in 6 oocytes per bar. **(C)** GABA_A ($\alpha 4\beta 3\gamma 2s$) receptor current tracing during application of acamprosate and co-application with EC_{20} GABA. **(D)** Summary data from **C** showing mean $\pm$ SEM of the percent of the GABA response in 6 oocytes per bar. **(E)** GABA_A ($\alpha 1\beta 2\gamma 2s$) receptor current tracing during application of acamprosate and co-application with EC_{5-10} GABA. **(F)** Summary data from **E** showing mean $\pm$ SEM of the percent of the GABA response in 7 to 10 oocytes per bar.

Fig. 7. Acamprosate does not modulate voltage-gated Na^+ channel (Nav) function. **(A)** Representative current tracing at V_{max} (-90 mV) and $V_{1/2}$ before, during and after application of acamprosate ($1 \mu\text{M}$) is shown in an oocyte expressing Nav 1.2 and $\beta 1$ subunits. **(B)** Summary data showing the mean $\pm$ SEM of the percent of the control responses in 4–5 oocytes per bar. **(C)** Na^+ channel current tracings in the presence and absence (control) of acamprosate elicited by depolarizing steps from -80 to 60 mV from a holding potential of -90 mV **(D)** Current-voltage plots in the presence and absence of acamprosate.

Fig. 8.

Acamprosate does not alter ethanol responses at γ -aminobutyric acid type A [(GABA_A) $\alpha 1\beta 2\gamma 2s$] receptors or voltage-gated Na⁺ channels (Nav). **(A)** Ethanol potentiation of GABA_A receptors expressed as a percent of the GABA response in the presence and absence of acamprosate. Bars represent the mean $\pm$ SEM from 8 oocytes per bar. **(B)** Ethanol inhibition of Nav expressed as a percent of the control response in the presence and absence of acamprosate. Bars represent the mean $\pm$ SEM from 7 oocytes per bar.