ELSEVIER

European Journal of Pharmacology 394 (2000) 221–231

ejp

www.elsevier.nl/locate/ejphar

Interaction of acamprosate with ethanol and spermine on NMDA receptors in primary cultured neurons

R. Lisa Popp *, David M. Lovinger

Department of Molecular Physiology and Biophysics, Vanderbilt School of Medicine, Nashville, TN 37232, USA

Received 29 February 2000; accepted 3 March 2000

Abstract

The *N*-methyl-D-aspartate (NMDA) receptor has been implicated as a putative sight of action for acamprosate, a novel drug that reduces craving for alcohol. The purpose of this study was to assess the effect of acamprosate on the function of native NMDA receptors expressed in primary cultured striatal and cerebellar granule cells, as well as ethanol inhibition and spermine modulation of these receptors, using whole-cell patch-clamp electrophysiological techniques. Under all circumstances, acamprosate (0.1–300 μM) did not alter NMDA- or glutamate-induced currents. Acamprosate did not alter the inhibitory effects of ethanol (10–100 mM) on receptor function. In a subpopulation of striatal neurons, acamprosate did reverse the potentiating effects of spermine. These findings indicate that although acamprosate may modify polyamine modulation of the NMDA receptor, acamprosate alone does not alter receptor function nor does it modify ethanol inhibition of this receptor expressed in primary cultured striatal and cerebellar granule neurons.

Keywords: Acamprosate; Ethanol; Spermine; NMDA receptor; Primary cultured neuron; Patch-clamp, whole-cell

1. Introduction

Acamprosate (calcium-acetyl homotaurinate) has been shown to decrease the propensity to consume alcohol in both humans and rodents who are physically dependent upon the drug. Acamprosate significantly reduces alcohol consumption in rodents that have become dependent on ethanol due to chronic exposure (Le Magnen et al., 1987; Gewiss et al., 1991; Spanagel et al., 1996a; Holter et al., 1997), but acamprosate has less of an effect on alcohol consumption in alcohol naive rats (Le Magnen et al., 1987). During the withdrawal phase of chronic ethanol exposure, acamprosate has been reported to decrease hyperlocomotion (Spanagel et al., 1996b) and spontaneous activity (Gewiss et al., 1991) but not hypothermia (Spanagel et al., 1996b). This decrease in motor activity correlates with an observed decrease in ethanol withdrawal-induced elevation of c-*fos* mRNA, an index of neuronal hyperexcitability in rats (Putzke et al., 1996). In microdialysis studies acamprosate blocks the elevated glutamate release in certain brain areas during ethanol withdrawal in rats (Dahchour et al., 1998). However, in all of the abovementioned animal studies, acamprosate did not alter alcohol brain or blood levels, nor did acamprosate substitute for ethanol in an ethanol discrimination test (Grant and Woolverton, 1989; Spanagel et al., 1996c).

Double-blind placebo-controlled randomized clinical studies have found acamprosate to successfully reduce measures of relapse in detoxified alcoholics in a dose-dependent manner (Lhuintre et al., 1990; Paille et al., 1995; Sass et al., 1996; Whitworth et al., 1996; Pelc et al., 1997; Poldrugo, 1997). Acamprosate at the doses administered in these studies (1.3–2.0 g per day) produced minor side effects and is, in and of itself, not addictive. In the absence of ethanol, acamprosate appears to have little effect on behavior or cognition (Schneider et al., 1998). Although this drug is currently being used to maintain abstinence in recovering alcoholics, the mechanism by which acamprosate prevents relapse remains unknown.

Because the molecular structure of acamprosate is similar to several endogenous amino acids, excitatory amino acid receptors are a putative sight of action (Durbin et al.,

* Corresponding author. Department of Pharmacology and Toxicology, College of Pharmacy, Austin, TX 78712-A1915 USA. Tel.: +1-512-471-1018; fax: +1-512-475-6088.

E-mail address: lisa.popp@mail.utexas.edu (R.L. Popp).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00195-3

1996). Evidence using electrophysiological techniques implicates the *N*-methyl-D-aspartate (NMDA) receptor, an excitatory amino acid receptor, in acamprosate's actions (Zeise et al., 1993; Madamba et al., 1996; Berton et al., 1998). In vivo extracellular recordings indicate that acamprosate inhibits glutamate-induced firing of rat neocortical cells without a reduction in spontaneous activity (Zeise et al., 1993). In addition, Zeise et al. (1993) also reported a decrease in the excitatory response elicited by various glutamatergic agonists obtained from in vitro intracellular recordings of cortical neurons. These data suggest that acamprosate inhibits post-synaptic glutamatergic neurotransmission. However, acamprosate has also been shown to significantly increase excitatory post-synaptic potentials in rat hippocampal CA1 neurons (Madamba et al., 1996) and in the nucleus accumbens (Berton et al., 1998) and this effect is specific for the NMDA receptor. Contrary to these results, direct assessment of receptor function using whole-cell patch-clamp techniques has shown that 10^{-9} M acamprosate decreases NMDA-induced currents in human embryonic kidney (HEK) 293 cells transiently expressing NMDA NR1 NR2A receptors (Spanagel et al., 1997).

A possible explanation for the different effects of acamprosate on NMDA receptors has been that acamprosate modulates NMDA receptor function in a manner similar to polyamines. This hypothesis is based primarily on data obtained from radio-ligand binding studies indicating that acamprosate binds to a spermidine-sensitive site on the NMDA receptor (Al Qatari et al., 1998; Naassila et al., 1998). It is well documented that polyamines have potentiating as well as inhibitory effects on NMDA receptor function depending upon receptor subunit composition and experimental conditions used (for review see Williams, 1997). Therefore, since there is evidence that acamprosate both enhances (Madamba et al., 1996; Berton et al., 1998) as well as attenuates (Zeise et al., 1993; Spanagel et al., 1997) NMDA receptor function, acamprosate may modulate NMDA receptor function through the polyamine site on the NMDA receptor. However, it must be noted that effects of acamprosate have not included direct agonist activation of NMDA receptors expressed in isolated neurons. Thus, it is not yet clear if acamprosate has direct effects on the receptor.

The purpose of this study was to directly assess the effect of acamprosate on NMDA receptor function using whole-cell patch-clamp electrophysiological techniques as well as to explore possible interactions of acamprosate with ethanol and spermine on NMDA receptor function. Our studies indicate that micromolar concentrations of acamprosate do not significantly alter NMDA receptor function nor do these concentrations effect the inhibitory actions of ethanol on this receptor in either primary cultured striatal or cerebellar granule cells. Acamprosate did not enhance spermine potentiation of NMDA-induced currents. However, in some striatal neurons, acamprosate did reverse the potentiating effects of spermine.

2. Methods

2.1. Preparation and maintenance of striatal and cerebellar granule cell cultures

All drugs were purchased from Sigma, St. Louis, MO unless otherwise noted; all serum was purchased from Gibco/BRL, Gaithersburg, MD. Striatal cultures were prepared using striatal tissue from e15–e16 Sprague–Dawley embryonic rats. These methods have been described in detail elsewhere (Popp et al., 1998) and were modified for these experiments in that cells were initially seeded at 1×10^6 cells per 35 mm dish. Cells were maintained in a 37°C incubator with an atmosphere of 90% O_2/10% CO_2. Six through fourteen-day old neurons were used for the electrophysiological studies. After 7 days in vitro, 0.5 ml of minimum essential medium (Gibco/BRL) containing 5% horse serum and 2 mM L-glutamine was added to each dish.

Cerebellar granule cells cultures were prepared using cerebellar tissue from 6 to 8 day-old Sprague-Dawley rats. These methods have been previously published (Popp et al., 1999). Six through twenty-eight days in vitro neurons were used for the electrophysiological studies. Every 7 days in vitro, existing medium was supplemented with 0.5 ml of fresh feeding medium [minimum essential medium containing 5% fetal bovine serum, 2 mM L-glutamine, 25 mM KCl, penicillin/streptomycin (100 U/100 μg/ml), and a fluorodeoxyuridine/uridine, 35 μm/15 μm mixture]. Cells were maintained at 37°C in a humidified atmosphere of 95% O_2/5% CO_2. This method of nourishment minimizes excitotoxic cell death due to the introduction of fresh medium (Popp et al., 1999).

2.2. Preparation, maintenance and transfection of HEK 293 cells

HEK 293 cells were purchased from the American Tissue Type Culture collection (Bethesda, MD). Cells were grown in feeding medium consisting of minimum essential medium, 10% fetal bovine serum, 1% L-glutamine, 1% penicillin/streptomycin (stock concentration: 5000 U penicillin/5000 μg streptomycin/ml) (Gibco/BRL) and maintained in an incubator with an atmosphere of 95% O_2/5% CO_2. Transient expression of functional receptors containing NMDA NR1-1a and NR2B subunits was accomplished using the calcium phosphate method (Chen and Okayama, 1987). This procedure was modified for volume and includes the addition of 1 mM 2-amino-5-phosphonovalerate (RBI, Natick, ME) and has been previously published (Popp et al., 1998). Transfected cells were initially incubated at 37°C in a 97% O_2/3% CO_2 atmosphere for approximately 24 h. Cells were washed with Dulbecco's phosphate-buffered saline. Fresh feeding medium was then added to the dish, and the cells were returned to the 5% CO_2 incubator. Cells were used

for electrophysiological experiments 40 to 48 h post transfection.

2.3. Whole-cell patch-clamp recordings

Culture dishes were placed on the stage of an inverted microscope (NIKON, Garden City, NY) and superfused at 1–2 ml/min with external medium (150 mM NaCl, 2.5 mM CaCl, 10 mM HEPES, 10 mM glucose [J.T. Baker, Phillipsburg, NJ] and 200 nM tetrodotoxin, pH adjusted to 7.4 with NaOH and osmolality adjusted to 333–336 mmol/kg with sucrose [J.T. Baker]). During experiments in which glutamate was the agonist, 20 μM 6-Nitro-7-sulphamoylbenzo[f]-quinoxaline-2,3-dione (NBQX) (Tocris Cookson, St. Louis, MO) was included in the external solution. All recordings were performed at room temperature using the axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, CA). For neuronal recordings, the solution in the patch pipette contained: 100 mM *N*-methyl-D-glucamine, 100 mM $MeSO_3$, 40 mM CsF, 10 mM HEPES, 1 mM $MgCl_2$, 5 mM QX-314 (RBI) and 5 mM EGTA, pH adjusted to 7.4 with CsOH and osmolality adjusted to 314–317 with sucrose. Patch pipettes had a tip resistance of 4.0–5.0 MΩ and recordings were made with series resistance of < 10 MΩ for striatal neurons and 7–5 MΩ for CGC. The patch pipette solution used for recording from the HEK 293 cells contained: 140 mM CsCl, 2 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, pH and osmolality were the same as previously described. The electrode resistance was 2–3 MΩ and the series resistance was < 11 MΩ when recording from HEK 293 cells. Recordings were conducted at -25 mV in experiments involving spermine. This holding potential diminishes the voltage-dependent polyamine channel block and maximizes the potentiating effects of polyamines on NMDA-induced currents (Williams, 1994). For all other drug applications, the holding potential was -60 mV.

2.4. Drug application

All drugs were dissolved in the external medium and delivered by gravity from solution-containing reservoirs placed above the preparation, gated by plastic stopcocks and connected to a linear array of microcapillary tubes (0.32 mm inner diameter). This system allowed for rapid solution perfusion of the neuron being studied and rapid solution exchange ($\sim$ 150 ms). Spermine and acamprosate (Groupe Lipha, Lyon, France) were made from stock solutions originally dissolved in dH_2O that had been stored at -20°C. Final concentrations were 100 μM for spermine and 0.1, 1, 10, 100 and 300 μM acamprosate for the dose response experiments and 10 μM for all other experiments unless otherwise noted. Ethanol (AAper Alcohol and Chemical, Shelbyville, KY) concentrations were 10, 50 and 100 mM. Each drug was applied simultaneously with the NMDA receptor agonists: 100 μM NMDA and 10 μM glycine or 10 μM L-glutamate and 10 μM glycine. Current was low pass filtered at 1 kHz using a 3-pole Bessel filter. Signals were digitized and current traces measured using pClamp 6.0 software (Axon Instruments). In all experiments, peak amplitudes were compared between agonists alone and agonists combined with the respective drugs. These values were the mean peak amplitude of 2–3 agonist plus drug applications normalized to the mean peak agonist-induced current amplitudes (2–3) obtained before and after drug application. Steady-state current values were obtained by measuring the difference between two cursors placed at time points immediately before and four to six s after drug application. The exact time point was consistent for all recordings within a given cell. Peak current values were taken at the time peak amplitude was observed. The mean value of steady-state and peak currents obtained from several applications of ethanol or acamprosate or the two drugs combined was normalized to the mean values obtained from several agonist-induced currents just prior to and following experimental drug application.

2.5. Statistics

All averaged data values are expressed as mean ± S.E.M. We used a Repeated Measures design and thus employed the One Sample *t*-test (Statview II, Abacus Concepts, Berkley, CA) to compare the percent change from control values attributable to acamprosate. This statistical analysis was done for both peak and steady-state current values and was employed in the experiments designed to study the effect of acamprosate on NMDA- and glutamate-induced currents of NMDA receptors expressed in striatal and cerebellar granule cells.

Steady-state/peak (SS/Pk) current ratios were determined using the mean current values determined for each cell and summed within each given experiment. One-way Analysis of Variance (ANOVA) was used to determine statistical differences between SS/Pk values obtained from control conditions (no acamprosate present) and conditions in which acamprosate was present.

Differences in ethanol inhibition of NMDA-induced current attributable to acamprosate were analyzed using a two-way ANOVA. The experimental design was a completely randomized factorial design with two-treatment levels, acamprosate and ethanol concentration. The variable acamprosate consisted of the presence or absence of acamprosate and there were three ethanol concentration groups: 10, 50 and 100 mM.

For the experiments involving 300 μM acamprosate, the paired *t*-test was used to compare the effect of acamprosate on ethanol inhibition within the same cell. This statistical analysis was also used to analyze the effect of acamprosate on ethanol inhibition of glutamate-induced currents and each concentration of ethanol was analyzed separately.

The Dunn's Multiple Comparison test was used to assess if acamprosate concentration affected spermine

Table 1
Effect of Acamprosate on NMDA-induced currents. Acamprosate (0.1–100 μM) had no significant effect on 100 μM NMDA-induced peak (Pk) and steady-state (SS) currents in primary cultured cerebellar granule cells and striatal neurons. Percent of control (no acamprosate) values + S.E.M. *t*-values were determined using a Repeated Measures design; One Sample *t*-test

Acamprosate (μM)	SS				Pk			
	0.1	1	10	100	0.1	1	10	100
Striatal cells								
% of control	117 ± 10	115 ± 8	104 ± 11	118 ± 17	94 ± 4	100 ± 4	106 ± 4	100 ± 4
t	1.7	1.9	0.4	1.1	1.7	0.02	1.4	0.9
P	0.1	0.1	0.7	0.3	0.1	0.98	0.2	0.9
N	10	8	7	11	10	8	7	11
Cerebellar granule cells								
% of control			97 ± 6	100 ± 5			96 ± 3	97 ± 2
t			0.5	0.05			2.5	1.9
P			0.6	0.96			2.5	0.8
N			14	13			14	13

modulation of NMDA receptor function in primary cultured striatal neurons. Further analysis on the possible interaction of acamprosate and spermine on NMDA receptor function was performed using a one-way ANOVA. Variables were acamprosate alone, spermine alone and acamprosate and spermine combined. The Multiple *t*-test (*t*) was the post-hoc test used to identify individual group differences. The criterion for statistical significance was $P \leq 0.05$.

3. Results

3.1. Acamprosate does not alter NMDA-induced currents

We have previously reported that the NMDA NR2 receptor subunit composition differs between primary cultured striatal (Popp et al., 1998) and cerebellar granule cells (Popp et al., 1999). Both Western blot analysis and pharmacological manipulation indicate that the NR2B is the primary NR2 NMDA receptor subunit expressed in primary cultured striatal cells whereas the NR2A subunit predominates in primary cultured cerebellar granule cells. We used these two in vitro systems to study the effect of acamprosate on NMDA-induced currents as well as acamprosate's interactions with ethanol on NMDA receptor function.

In primary cultured striatal cells, acamprosate concentrations of 0.1, 1, 10 and 100 μM had no effect on the peak amplitude of NMDA-induced currents. Although mean steady-state values for NMDA-induced currents were higher than control values in the presence of acamprosate these increases were not statistically significant. Similar results were observed in cerebellar granule cells maintained for 6 to 28 days in vitro in that neither 10 nor 100 μM acamprosate altered NMDA-induced steady-state or peak currents. Acamprosate also did not alter SS/Pk values, a measurement of receptor desensitization. The lack of effect on SS/Pk values was not surprising given that acamprosate did not alter steady-state nor peak current values. In striatal neurons, SS/Pk values in the presence of 100 μm acamprosate were 0.25 ± 0.02 compared to control values, of 0.22 ± 0.03, $F(1,20) = 0.59$; 0.24 ± 0.04 for 10 μM acamprosate compared to control values of 0.22 ± 0.05, $F(1,12) = 0.07$; 0.25 ± 0.01 for 1 μM acamprosate compared to control values of 0.22 ± 0.02, $F(1,14) = 1.07$ and 0.27 ± 0.04 for 100 nM acamprosate compared to control values of 0.22 ± 0.03, $F(1,18) = 1.49$. Acamprosate (100 and 10 μM) did not alter SS/Pk values in cerebellar granule cells: $F(1,24) = 0.02$ and $F(1,26) = 0$, respectively. The data from these experiments are summa-

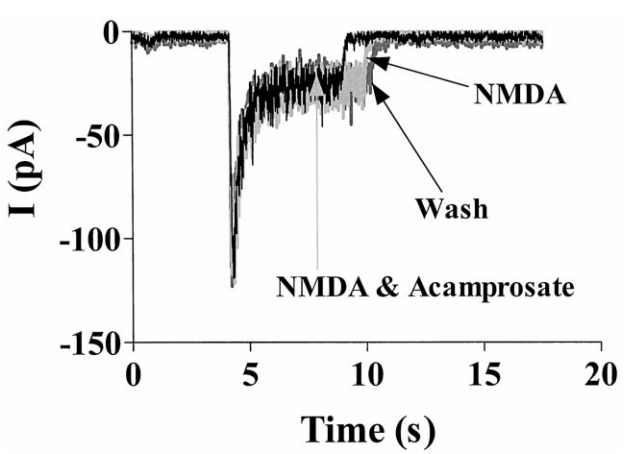

Fig. 1. Effect of acamprosate on NMDA-induced currents. The NMDA receptor agonists used in this experiment were 100 μM NMDA with 10 μM glycine and are indicated as NMDA and Wash. Acamprosate (10 μM) did not alter agonist-induced steady-state or peak current amplitudes. Traces were taken from a 7 days in vitro striatal neuron. The holding potential in these electrophysiological experiments was −60 mV.

rized in Table 1. Application of acamprosate at concentrations from 10–300 μM in the absence of any NMDA receptor agonist did not produce any ion current in either striatal or cerebellar granule cells (data not shown). Representative current traces depicting the lack of acamprosate's effect on NMDA-induced currents from receptors expressed in a primary cultured striatal cell are shown in Fig. 1.

3.2. Acamprosate does not alter ethanol inhibition of NMDA- or glutamate-induced currents

These experiments were designed to determine the affect of acamprosate on the NMDA receptor at pharmacotherapeuticaly relevant concentrations (10^{-6} M) (Zeise et al., 1993; Al Qatari et al., 1998). Therefore, 10-μM acamprosate was used to study the effect of acamprosate on ethanol inhibition of NMDA-induced currents unless otherwise noted. The effect of acamprosate on ethanol (10, 50 and 100 mM) inhibition of 100 μM NMDA and 10 μM glycine-induced steady-state and peak currents expressed in primary cultured striatal neurons (7 to 14 days in vitro) was assessed. Acamprosate did not significantly alter ethanol inhibition of NMDA-induced steady-state current ($F(1,42) = 0.05$) and this was observed at all concentrations of ethanol tested (interaction $F(2,42) = 0.05$). Acamprosate did not alter inhibition of NMDA-induced peak current ($F(2,42) = 0$) at any ethanol concentration examined ($F(2,42) = 1.15$). Inhibition of NMDA-induced current increased significantly with an increase in ethanol concentration for steady-state currents ($F(2,42) = 7.79$; $P \leq 0.0013$) and for peak currents ($F(2,42) = 32.9$; $P \leq 0.0001$). Acamprosate had no effect on SS/Pk current values: $F(1,12) = 0.5$; $F(1,14) = 3.06$ and $F(1,14) = 0.02$ for 10, 50 and 100 mM ethanol, respectively. Data from these analyses are summarized in Fig. 2A. Fig. 2B contains

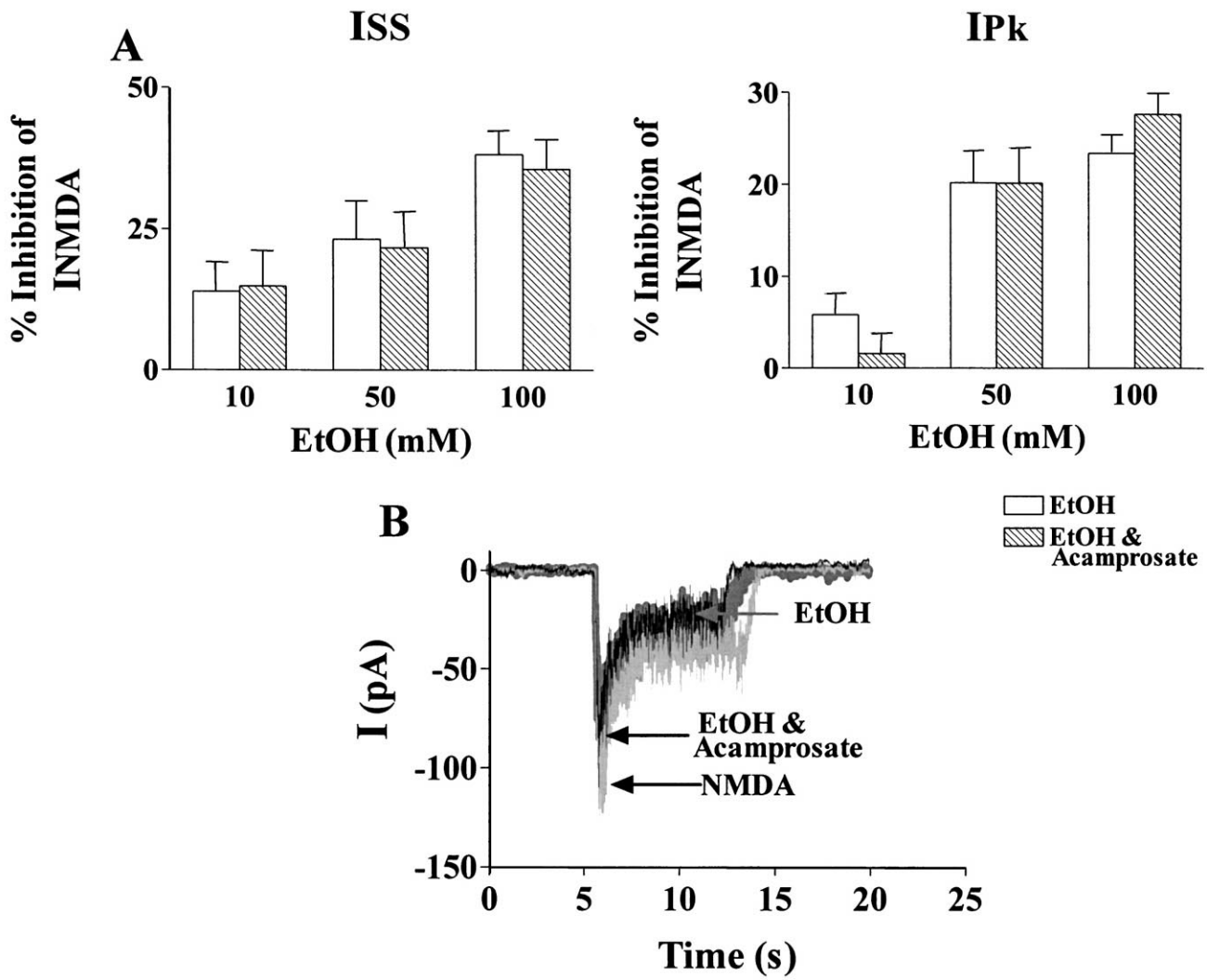

Fig. 2. (A) Summary of the effect of 10 μM acamprosate on the inhibition of NMDA-induced steady-state and peak currents produced by three concentrations of ethanol; $N = 8$ cells for each ethanol concentration. (B) Representative current traces taken from an 11-day in vitro striatal neuron depicting the lack of effect of 10 μM acamprosate on inhibition of NMDA-induced current by 100 mM ethanol.

Table 2
Effect of acamprosate on glutamate-induced peak and steady-state currents. Acamprosate (10 μM) effect on current induced by 10 μM glutamate and 10 μM glycine in primary cultured cerebellar granule cells and striatal neurons. Values are percent of control (no acamprosate) values ± S.E.M. *t* values were determined using a Repeated Measures design; One Sample *t*-test

	Steady-state	Peak
Striatal cells		
% of control	101.2 ± 3.5%	107 ± 5.4%
t	0.4	0.23
P	0.7	0.23
N	4	4
Cerebellar Granule Cells		
% of control	88 ± 9.0%	100.4 ± 2.6%
t	1.5	0.13
P	0.2	0.9
N	5	5

representative traces depicting equal inhibition produced by ethanol and by ethanol in the presence of 10 μM acamprosate.

It has been reported that a concentration of 300 μM acamprosate is required to significantly enhance NMDA-mediated synaptic neurotransmission in nucleus accumbens neurons (Berton et al., 1998). Experiments designed to assess the effect of a higher concentration of acamprosate (300 μM) revealed no change in ethanol (10 and 100 mM) inhibition of NMDA-induced currents in both striatal cells and cerebellar granule cells. In striatal cells, the percent inhibition by 100 mM ethanol was 38.35 ± 8.3 for steady-state current and 28.34 ± 3 for peak current, and inhibition by 100 mM ethanol in the presence of 300 μM acamprosate was 41.6 ± 4.3 for steady-state current and 29.15 ± 4.2 for peak current ($N = 4$). Paired *t*-test values were $t(3) = 0.2$ and $t(3) = 0.4$ for steady-state and peak currents, respectively. Additionally, 300 μM acamprosate did not significantly alter 10 mM ethanol effects of NMDA-induced steady-state current. For NMDA-induced steady-state current ethanol produced a 12.23 ± 11.8% increase; $t(3) = 0.2$ and ethanol in presence of acamprosate produced a 1.6 ± 16.5% inhibition ($t(3) = 0.8$. For NMDA-induced peak currents ethanol (10 mM) resulted in a 1.6 ± 4.8% inhibition and ethanol and acamprosate resulted in 1.87 ± 4.5% inhibition ($t(3) = 0.2$. $N = 4$ for both ethanol concentrations. Once again there was no effect of acamprosate on SS/Pk values under these experimental conditions: $F(1,6) = 0.6$ and $F(1,6) = 0.14$ for 100 and 10 mM ethanol, respectively. Results were similar for experiments conducted in cerebellar granule cells in that 300 μM acamprosate did not effect ethanol's actions on NMDA-induced currents nor SS/Pk values (data not shown).

Glutamate is believed to be the endogenous NMDA receptor agonist. Thus, we performed additional experiments examining ethanol–acamprosate interactions using glutamate as the receptor agonist instead of NMDA. When glutamate was the agonist, 10 μM acamprosate did not alter glutamate-induced peak or steady-state currents in primary cultured striatal or cerebellar granule cells (Table 2). Ethanol mediated inhibition of steady-state and peak glutamate-induced currents was unaffected by 10 μM acamprosate. In cerebellar granule cells (Fig. 3A), 100 mM ethanol inhibition of steady-state current in the presence and absence of acamprosate was 28.4 ± 3.8 and 35.6 ± 3.6; $t(14) = 1.56$, respectively and inhibition of peak current was 29.38 ± 4.0 for ethanol alone and 33.53 ± 3.9 for ethanol in the presence of acamprosate; $t(14) = 1.67$. Additionally, acamprosate did not significantly alter the inhibition of steady-state glutamate-induced currents by 10 mM ethanol: 7.5 ± 3.0% inhibition for ethanol alone compared to 8.13 ± 3.6% inhibition for ethanol and acamprosate combined, $t(14) = 0.15$, nor for inhibition of glutamate-induced peak currents (15.23 ± 3.4 and 12.23 ± 3.0 for 10 mM ethanol and ethanol plus acamprosate, respectively; $t(14) = 1.1$. $N = 15$ for both concentrations of ethanol. Similar results were observed in primary cultured striatal neurons (Fig. 3B) For receptors expressed in both types of primary cultured neurons, acamprosate did not alter SS/Pk values at all concentrations of ethanol tested when glutamate was the agonist (data not shown).

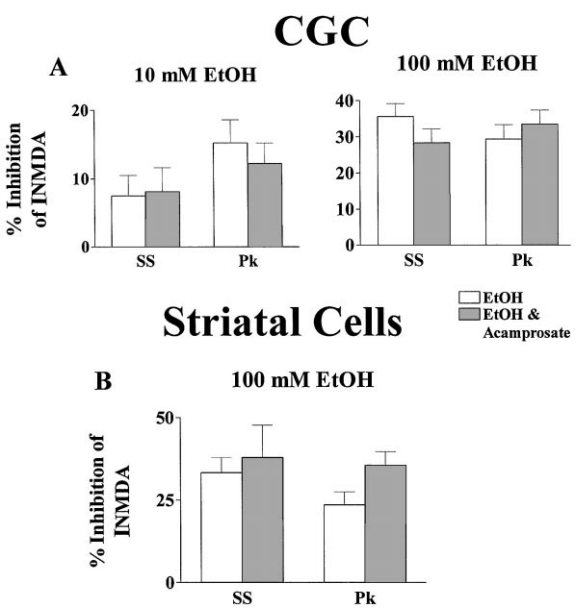

Fig. 3. (A) The effect of acamprosate on glutamate-induced currents in cerebellar granule cells. Acamprosate (10 μM) failed to significantly alter ethanol inhibition (10 or 100 mM) of glutamate-induced (10 μM glutamate plus 10 μM glycine) peak or steady-state currents in primary cultured cerebellar granule cells 7 to 21 days in vitro. $N = 15$ for both concentrations of ethanol. (B) Acamprosate also failed to alter 100 mM ethanol inhibition of glutamate-induced peak and steady-state currents mediated by NMDA receptors expressed in primary cultured striatal cells 7 to 14 days in vitro. Paired *t*-values were $t = 0.7$, $df = 7$ and $t = 2.26$, $df = 7$ for steady-state and peak currents, respectively, $N = 8$.

3.3. Acamprosate interaction with spermine

We have previously shown that the NMDA receptors in primary cultured striatal cells 7 to 21 days in vitro exhibit NMDA-induced currents that have a wide range of responses to spermine, being either potentiated, inhibited or unaffected by 100 μm spermine (Popp et al., 1998). It has been speculated that the conflicting results concerning acamprosate's actions on NMDA-mediated synaptic transmission may result from acamprosate acting at the NMDA receptor polyamine site. Although we had not observed a potentiating nor inhibiting effect of acamprosate on NMDA-induced currents, acamprosate could interact with the polyamine site on the NMDA receptor so as to alter polyamine modulation of NMDA receptor function.

Two concentrations of acamprosate (10 and 100 μM) were used to study the possible interaction with spermine on NMDA receptor function of receptors expressed in primary cultured striatal cells. As in our previous experiment (Popp et al., 1998) we observed three populations of neurons: one in which 100 μM spermine potentiated NMDA-induced steady-state current (> 10% above control

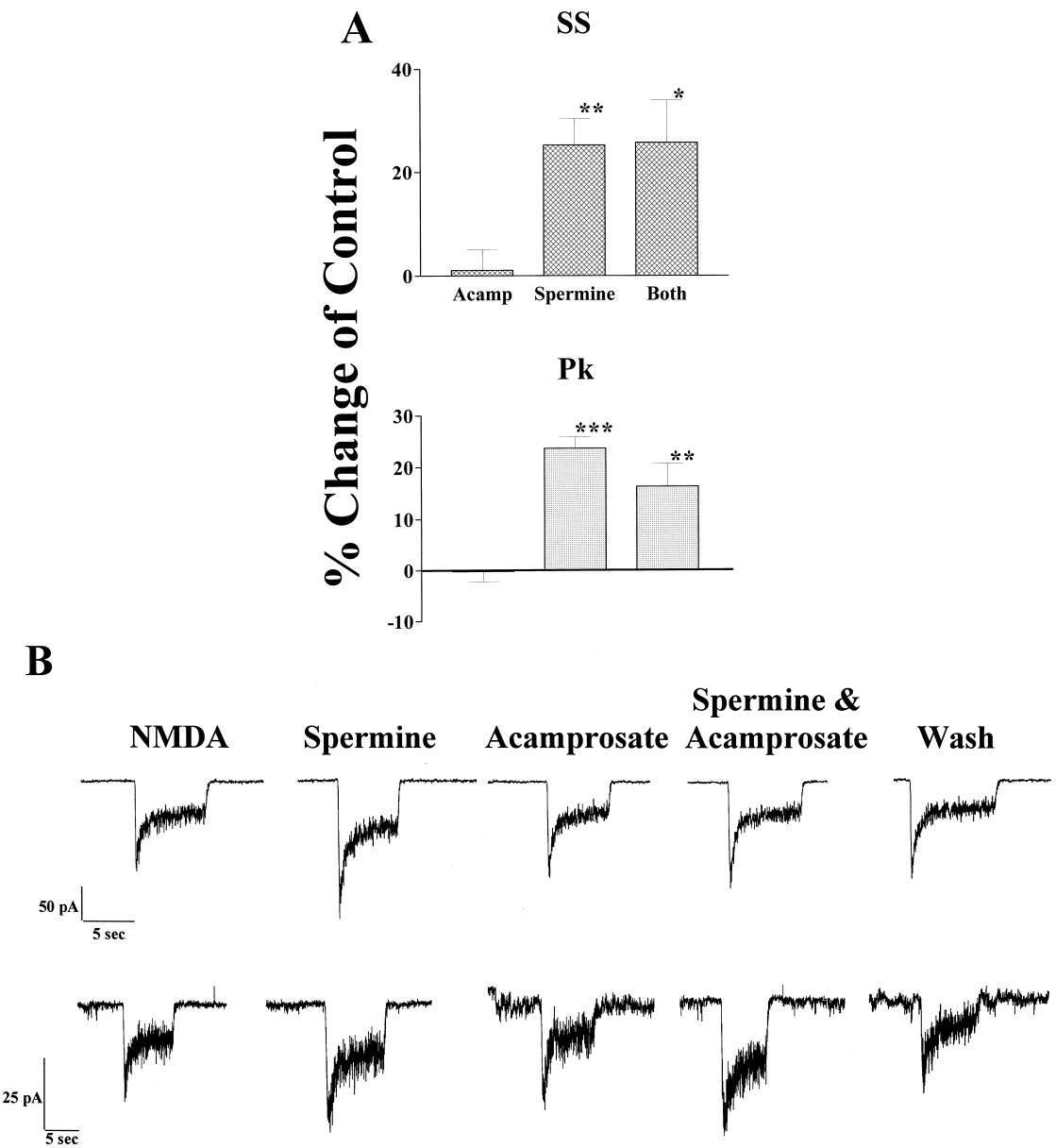

Fig. 4. (A) Acamprosate interaction with spermine in primary cultured striatal neurons. Acamprosate (10 μM) did not significantly alter 100 μM spermine potentiation of NMDA-induced currents of NMDA receptors contained in primary cultured striatal cells. For both peak ($N = 15$) and steady-state ($N = 12$) current, spermine and spermine plus acamprosate significantly enhanced NMDA-induced currents when compared to NMDA and acamprosate alone at the $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ or $^{***}P \leq 0.001$ levels. (B) Representative current traces illustrating that, in some cells, 10 μM acamprosate reversed the potentiating affects of spermine (as shown in the top set of current traces), whereas in other cells, acamprosate did not affect spermine potentiation of NMDA-induced current (bottom set of current traces). Holding potential was −25 mV; traces were taken from 7 to 9 days in vitro striatal neurons.

value, $N = 13$); a population in which NMDA-induced steady-state current was inhibited ($< 90\%$ of control value, $N = 3$) and a population of neurons in which NMDA-induced steady-state current was unaffected by 100 μM spermine ($N = 7$).

Since our previous results indicated that acamprosate did not affect NMDA-induced currents at any concentration, we performed the Dunn's Multiple Comparison test to compare the effect of 10 and 100 μM acamprosate alone and acamprosate plus spermine on NMDA-induced current. Once again, acamprosate did not significantly alter NMDA-induced steady-state ($t(10) = 0.5$) or peak current ($t(13) = 1.02$). Nor did acamprosate significantly effect spermine potentiation at either concentration of acamprosate for NMDA-induced steady-state ($t(10) = 1.62$) or peak current ($t(13) = 0.9$). Therefore, the data from the two concentrations of acamprosate were combined and a one-way ANOVA was performed. The major effect revealed by this analysis was a spermine enhancement of NMDA receptor-mediated current regardless of the presence or absence of acamprosate. The ANOVA analysis revealed that NMDA-induced current was significantly altered as a function of the different drug combinations: peak current, ($F(2,46) = 20.8$; $P \leq 0.001$) and steady-state current ($F(2,35) = 6.75$; $P = 0.003$). These analyses contained only data from cells in which peak or steady-state currents were potentiated ($\geq 10\%$ control values) by spermine. The Multiple *t*-test was employed to identify individual group differences. Both peak and steady-state NMDA-induced currents were significantly enhanced under both the spermine and spermine plus acamprosate conditions relative to the response to NMDA in the presence of acamprosate alone. For steady-state current: $t_{(acamp,sperm)}(33) = 2.95$; $P \leq 0.05$ and $t_{(acamp,both)}(33) = 3.02$; $P \leq 0.01$) and for peak current: $t_{(acamp,sperm)}(42) = 5.66$; $P \leq 0.001$; $t_{(acamp,both)}(42) = 3.89$; $P \leq 0.01$, where acamp is acamprosate alone, sperm is spermine alone and both is spermine coapplied with acamprosate. Potentiation of NMDA-induced current produced by spermine was not significantly different when spermine was co-applied with acamprosate: $t(33) = 0.06$ for steady-state current and $t(42) = 1.77$ for peak current. These data are summarized in Fig. 4A. It has not been previously noted that spermine alters the desensitized state of the NMDA receptor and results from experiments presented throughout this paper indicate that acamprosate does not alter SS/Pk values. Results from experiments assessing the effect of spermine, acamprosate or spermine and acamprosate on SS/Pk values are in agreement with these findings. This was true for experiments using 100 μM acamprosate ($F(2,30) = 0.19$) or 10 μM acamprosate ($F(2,24) = 1.6$).

Although acamprosate had no statistically significant effect on spermine potentiation of NMDA-induced currents, we observed a very small population of neurons (16%) in which acamprosate completely reversed the potentiating effects of 100 μM spermine. This phenomenon was seen exclusively for 10 μM acamprosate and not for 100 μM acamprosate. Fig. 4B contains a representative current trace from a striatal cell in which acamprosate did not reverse spermine potentiation (top panel) and a current trace from a cell in which acamprosate did reverse spermine potentiation (bottom panel).

As previously reported (Popp et al., 1998), 100 μM spermine potentiated NMDA-induced peak and steady-state currents by $21.2 \pm 8.4\%$ and by $15.9 \pm 11\%$, respectively in HEK 293 cells expressing NMDA NR1-1a and NR2B subunits. Acamprosate (10 μM) did not alter NMDA-induced current nor did it alter the potentiating effects of 100 μM spermine. The percent change was -3.4 ± 4.4 and 14.6 ± 7.6 for NMDA-induced peak currents in the presence of: 10 μM acamprosate and spermine plus acamprosate, respectively. The percent change of NMDA-induced steady-state current was: acamprosate, -4.9 ± 3.8 and 13.6 ± 12.5 for spermine and acamprosate ($N = 5$).

4. Discussion

The purpose of this study was multifaceted. We wanted to assess the effect of acamprosate on the function of native NMDA receptors expressed in primary cultured neurons using whole-cell patch-clamp electrophysiological techniques. The two in vitro systems used in our experiments differ in their NMDA receptor NR2 subunit composition (Popp et al., 1998, 1999). This enabled us to assess if the NR2 subunit composition was related to acamprosate's action on NMDA receptor function. Acamprosate has been shown to successfully decrease the propensity to drink alcohol in both animals and humans made dependent on the drug. Therefore, another purpose of this study was to assess the interaction of acamprosate and ethanol on NMDA receptor function. Lastly, since an acamprosate binding site has been identified in neuronal preparations which implicates the polyamine site on the NMDA receptor (Al Qatari et al., 1998; Naassila et al., 1998) we wanted to examine the effect of acamprosate on polyamine modulation of the NMDA receptor. We observed no direct modulation by acamprosate of NMDA- or glutamate-induced steady-state or peak currents for NMDA receptors expressed in primary cultured striatal cells or cerebellar granule cells. Furthermore, acamprosate concentrations up to 300 μM did not alter ethanol inhibition of NMDA steady-state or peak currents. As previously reported (Popp et al., 1998) we did observe three effects of the polyamine spermine on NMDA-induced currents. In instances when spermine potentiated NMDA-induced currents, in a small number of cells, 10 μM acamprosate completely negated the potentiating effects of spermine. In the majority of cells, acamprosate did not alter spermine's actions.

It has been reported that acamprosate (100–1000 μM) inhibits (Zeise et al., 1993) or enhances excitatory postsynaptic potentials (Madamba et al., 1996; Berton et al.,

1998). These concentrations are higher than clinically relevant concentrations which are approximately in the 10^{-6} M range (Zeise et al., 1993; Al Qatari et al., 1998). However, high drug concentrations are sometimes required to observe effects in brain slice experiments. It has also been reported that acamprosate, at 10^{-9} M concentrations, inhibits the function NMDA receptors with an NR1 NR2A subunit composition expressed in HEK 293 (Spanagel et al., 1997). Our findings differ from the previous results (Zeise et al., 1993; Madamba et al., 1996; Spanagel et al., 1997; Berton et al., 1998) in that we did not observe any direct effect of acamprosate on NMDA receptor function in the two neuronal culture systems studied. This could be attributable to the lower acamprosate concentrations used in this study compared to those used in previous studies (Zeise et al., 1993; Madamba et al., 1996). However, 100 μM acamprosate did not have any effect on NMDA receptor function in our experiments, a concentration which did produce effects in two of the three studies previously cited (Zeise et al., 1993; Madamba et al., 1996). Furthermore, we also observed no effect of 300 μM acamprosate on NMDA-induced currents. Thus, the different results in these studies cannot be explained solely on the basis of acamprosate concentration differences.

Explanations for the difference in results previously reported have been proposed (Madamba et al., 1996). One possible explanation, which could also explain the different results observed in the current study, is that the effect of acamprosate may vary between brain regions. It is also possible that the type of preparation influences the effect of acamprosate in some way that is as yet poorly understood. The study by Zeise et al. (1993) was conducted in vivo while the experiments in the Madamba et al. (1996) and Berton et al. (1998) studies were performed using a hippocampal and nucleus accumbens slice preparation, respectively. Neurons grown in primary culture were used in our experiments. The development of neurons in culture does not always parallel that which occurs in vivo (Popp et al., 1999). Furthermore, in the Zeise study, the various synaptic components of the post synaptic potentials were not isolated pharmacologically as in the two subsequent studies (Madamba et al., 1996; Berton et al., 1998). Thus, substantiating the argument used by Madamba et al. (1996) that the resultant overall decreases seen in excitatory post synaptic potentials by Zeise et al. (1993) could have masked the increase in NMDA-mediated excitatory post synaptic potentials.

Although Madamba et al. (1996) were able to isolate and identify the action of acamprosate as affecting an NMDA-mediated post synaptic potential, it is impossible to unequivocally state that acamprosate is directly altering NMDA receptor function in a brain slice system. Neurons in brain slices contain numerous synaptic connections, and it is also possible that the drug may work by releasing a neurotransmitter. By directly monitoring receptor function in individual neurons grown in culture, we can largely avoid this potential problem. In both culture preparations, we observed no change in either NMDA-induced steady-state or peak currents attributable to acamprosate 0.1–300 μM. These results were obtained through examination of native receptors which we have previously identified to contain the NMDA NR2B (striatal cells) or the NR2A2B or NR2A (cerebellar granule cells) subunits. These results differ from those reported from Spanagel et al. (1997) who reported 20–30% inhibition of NMDA-induced currents mediated by NMDA receptors expressed in HEK 293 cells (Lovinger and Zieglgänsberger, 1996; Spanagel et al., 1997). However, this discrepancy may be due to differences between native receptors and receptors expressed in heterologous systems.

To assess if acamprosate modulated the inhibitory affects of ethanol on NMDA-induced currents, three concentrations of ethanol were used: a low concentration (10 mM), moderately high (50 mM) and a high concentration of ethanol (100 mM). Neither a physiologically relevant concentration of acamprosate (10 μM) nor a high concentration (300 μM) significantly altered ethanol inhibition of NMDA-induced peak or steady-state current. This lack of effect was observed at all concentrations of ethanol studied and in both striatal neurons and in cerebellar granule cells. These observations indicate that acamprosate does not produce its anti-craving effects by preventing EtOH effects on the NMDA receptor.

Since glutamate is the endogenous ligand mediating NMDA receptor function we wanted to determine if acamprosate might alter ethanol inhibition when the receptor was activated by glutamate. As seen with NMDA, 10 μM acamprosate did not alter glutamate-induced steady-state or peak currents from NMDA receptors expressed in either striatal or cerebellar granule cells. Nor did acamprosate alter ethanol inhibition of glutamate-induced currents. A lack of preferential action of acamprosate on NMDA receptor activation by different agonists was also reported in previous studies (Zeise et al., 1993). Thus, the lack of acamprosate action in cultured neurons is not related to the species of agonist used to activate receptors.

Acamprosate appeared to have inhibitory (Zeise et al., 1993) as well as potentiating (Madamba et al., 1996; Berton et al., 1998) effects on excitatory amino acid transmission. Therefore it was proposed that acamprosate might be working at the polyamine site on the NMDA receptor because polyamines can inhibit or potentiate NMDA receptor function via different allosteric sites (for review see Williams, 1997). Binding studies supported this postulate. Binding of [^{3}H]acamprosate to rat brain membranes has been observed with a K_D of 120 μM and a B_{max} of 450 pmol/mg of protein (Naassila et al., 1998). This study revealed that although there exists abundant acamprosate binding sites within the central nervous system, only a ''subgroup'' of binding sites are also spermidine sensitive. Naassila et al. (1998) reported that spermidine inhibited acamprosate binding with an IC_{50} of 13.32

$\pm$ 1.1 μM and acamprosate could displace [^{14}C] spermidine with an IC_{50} of 645 μM. These investigators further characterized the acamprosate binding site and attempted to identify its relationship with the NMDA receptor. Several of their results suggested that the interaction between acamprosate and spermidine is allosteric in nature. They also provided evidence for a direct effect of acamprosate on NMDA receptor function, which is influenced allosterically by its interactions with other ligands. Their data also suggest that acamprosate modulation of NMDA receptor function depends upon the availability and concentration of spermidine.

As in the Naassila et al. (1998) studies, a ''subset'' (16%) of neurons examined in our experiments exhibited an allosteric interaction between acamprosate and spermine on the NMDA receptor. In this group of neurons, the interaction is such that the net effect of acamprosate is to reduce or eliminate the potentiating effects of polyamines on NMDA-induced currents. However, unlike the results observed by Naassila et al., we observed a subset of spermine sensitive neurons, which were acamprosate insensitive. At this time, we do not have an explanation for the difference in results. However, the acamprosate-polyamine interaction suggested by our data and that of Naassila et al. (1998) are likely to be physiologically relevant since under physiological conditions (i.e. in the presence of Mg^{2+}), the inhibitory effects of polyamines are probably negligible (Williams, 1997) and the potentiating polyamine effects predominate. On the basis of previously published immunohistochemical and pharmacological data (Popp et al., 1998) we know that the predominant NMDA NR2 subunit present in our cultured striatal neurons is the NR2B co-assembled with NR1 subunits that contain and lack the N-terminal cassette. Therefore, under saturating glycine conditions and a holding potential of -25 mV, we would expect to see spermine potentiation in some neurons and spermine inhibition of NMDA-induced currents in others. If acamprosate was replicating spermine's actions directly, it would be expected that NMDA-induced currents that are potentiated by spermine should also be potentiated by acamprosate. We did not observe this result. In the absence of spermine, acamprosate did not alter NMDA-induced currents from control values. Furthermore, cells that contained NMDA receptors that were potentiated by spermine were not further potentiated by acamprosate. In fact, NMDA-induced current values did not differ significantly between the spermine and the spermine with acamprosate values. However, in a small population of cells, a low concentration of acamprosate (10 μM) completely negated the potentiating effects of spermine on NMDA-induced current. A similar result was reported by Naassila et al. (1998) in that at low acamprosate concentrations (<1 μM), the interaction between spermidine and acamprosate appeared competitive with the net result being an inhibition of spermidine potentiation as seen by a decrease in [^{3}H] dizocilpine binding.

How acamprosate attenuation of polyamine potentiation of NMDA receptor function might play a role in preventing relapse to alcohol consumption remains unknown. It has been reported that acamprosate has no effect on animals that have not been previously exposed to ethanol (Le Magnen et al., 1987). Chronic ethanol consumption causes many physiological changes, some of which may be involved in acamprosate's actions. Some of these changes may be the site of acamprosate's action, which under normal conditions are unaffected by the drug. It has been reported that the NMDA NR2B subunit increases following chronic ethanol exposure (Follesa and Ticku, 1996). The mRNA of the NR1-1a-type subunits increases relative to the NR1-1b-type subunit following chronic ethanol exposure and remains elevated up to forty-eight h after withdrawal (Hardy et al., 1999). Chronic ethanol exposure results in an increase in polyamines and in ornithine decarboxylase (the rate-limiting enzyme for polyamine biosynthesis) in the brains of rats made dependent on the drug (Davidson and Wilce, 1998). A positive correlation was found to exist between these increases in ornithine decarboxylase activity/polyamine levels and the severity of withdrawal behavior in these rats. These findings suggest that chronic EtOH exposure may produce a pattern of changes that favors acamprosate-polyamine interactions at the NMDA receptor since subunits that confer polyamine potentiation are increased while levels of polyamines increase.

On the basis of these reports and our current observations we hypothesize that acamprosate may minimize or negate effects due to physiological changes produced by chronic ethanol exposure. Specifically, acamprosate may negate the potentiating effects of increased concentrations of endogenous polyamines on upregulated NR2B and NR1-1a subunits incorporated into some NMDA receptors. Binding studies support this hypothesis in that chronic exposure to ethanol and/or acamprosate produce changes that favor inhibitory effects of acamprosate on NMDA receptor function (Al Qatari et al., 1998). Future studies, which focus on the effect of acamprosate under chronic ethanol conditions, may elucidate a central nervous system site of action for this drug and this site may involve allosteric modulation of polyamine's actions on the NMDA receptor.

Acknowledgements

This investiation was supported by AA08986 and AA05458. We thank Groupe Lipha (Lyon, France) for kindly providing the acamprosate.

References

Al Qatari, M., Bouchenafa, O., Littleton, J., 1998. Mechanisms of action of acamprosate: Part II. Ethanol dependence modifies effects of

acamprosate on NMDA receptor binding in membranes from rat cerebral cortex. Alcohol. Clin. Exp. Res. 22, 810–814.

Berton, F., Francesconi, W.G., Madamba, S.G., Zieglgänsberger, W., Siggins, G.R., 1998. Acamprosate enhances *N*-methyl-D-aspartate receptor-mediated neurotransmission but inhibits presynaptic $GABA_B$ receptors in nucleus accumbens neurons. Alcohol. Clin. Exp. Res. 22, 183–191.

Chen, C., Okayama, H., 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. 7, 2745–2752.

Dahchour, A., De Witte, P., Bolo, N., Nedelec, J.-F., Muzet, M., Durbin, P., Macher, J.-P., 1998. Central effects of acamprosate: Part 1. Acamprosate blocks the glutamate increase in the nucleus accumbens microdialysate in ethanol withdrawn rats. Psy. Res. 82, 107–114.

Davidson, M., Wilce, P., 1998. Chronic ethanol treatment leads to increased ornithine decarboxylase activity: implications for a role of polyamines in ethanol dependence and withdrawal. Alcohol. Clin. Exp. Res. 22, 1205–1211.

Durbin, P., Hulot, T., Chabac, S., 1996. Pharmacodynamics and pharmacokinetics of acamprosate: an overview. In: Soyka, M. (Ed.), Acamprosate in Relapse Prevention of Alcoholism. Springer-Verlag, Heidelberg, Germany, p. 47.

Follesa, P., Ticku, M.K., 1996. Chronic ethanol-mediated up-regulation of the *N*-methyl-D-aspartate receptor polypeptide subunits in mouse cortical neurons in culture. J. Biol. Chem. 271, 13297–13299.

Gewiss, M., Heidbreder, C., Opsomer, L., Durbin, P., De Witte, P., 1991. Acamprosate and diazepam differentially modulate alcohol-induced behavioral and cortical alterations in rats following chronic inhalation of ethanol vapour. Alcohol Alcohol. 26, 129–137.

Grant, K.A., Woolverton, W.L., 1989. Reinforcing and discriminative stimulus effects of Ca-acetyl homotaurine in animals. Pharmacol. Biochem. Behav. 32, 607–611.

Hardy, P.A., Chen, W., Wilce, P.A., 1999. Chronic ethanol exposure and withdrawal influence NMDA receptor subunit and splice variant mRNA expression in the rat cerebral cortex. Brain Res. 819, 33–39.

Holter, S.M., Landgraf, R., Zieglgänsberger, W., Spanagel, R., 1997. Time course of acamprosate action on operant ethanol self-administration after ethanol deprivation. Alcohol. Clin. Exp. Res. 21, 862–868.

Le Magnen, J., Tran, G., Durlach, J., Martin, C., 1987. Dose-dependent suppression of the high alcohol intake of chronically intoxicated rats by Ca-acetyl homotaurinate. Alcohol 4, 97–102.

Lhuintre, J.P., Moore, N., Tran, G., Steru, L., Langrenon, S., Daoust, M., Parot, P., Ladures, P., Libert, C., Boismare, F., Hillemand, B., 1990. Acamprosate appears to decrease alcohol intake in weaned alcoholics. Alcohol Alcohol. 25, 613–622.

Lovinger, D.M., Zieglgänsberger, W., 1996. Interactions between ethanol and agents that act on the NMDA-type glutamate receptor. Alcohol. Clin. Exp. Res. Suppl. 20, 187A–191A.

Madamba, S.G., Schweitzer, P., Zieglgänsberger, W., Siggins, G.R., 1996. Acamprosate (calcium acetylhomotaurinate) enhances the *N*-methyl-D-aspartate component of excitatory neurotransmission in rat hippocampal CA1 neurons in vitro. Alcohol. Clin. Exp. Res. 20, 651–658.

Naassila, M., Hammoumi, S., Legrand, E., Durbin, P., Daoust, M., 1998. Mechanism of action of acamprosate: Part I. Characterization of spermidine-sensitive acamprosate binding site in rat brain. Alcohol. Clin. Exp. Res. 22, 802–809.

Paille, F.M., Guelfi, J.D., Perkins, A.C., Royer, R.J., Steru, L., Parot, P., 1995. Double-blind randomized multicentre trial of acamprosate in maintaining abstinence from alcohol. Alcohol Alcohol. 30, 239–247.

Pelc, I., Verbanck, P., Le Bon, O., Gavrilovic, M., Lion, K., Lehert, P., 1997. Efficacy and safety of acamprosate in the treatment of detoxified alcohol-dependent patients. A 90-day placebo-controlled dose-finding study. Br. J. Psychol. 171, 73–77.

Poldrugo, F., 1997. Acamprosate treatment in a long-term community-based alcohol rehabilitation programme. Addiction 92, 1537–1546.

Popp, R.L., Lickteig, R., Browning, M.D., Lovinger, D.M., 1998. Ethanol sensitivity and subunit composition of NMDA receptors in cultured striatal neurons. Neuropharmacology 37, 45–56.

Popp, R.L., Lickteig, R., Lovinger, D.M., 1999. Factors that enhance EtOH inhibition of *N*-methyl-D-aspartate receptors in cerebellar granule cell neurons. J. Exp. Ther. Pharmacol. 289, 1564–1574.

Putzke, J., Spanagel, R., Tolle, T.R., Zieglgänsberger, W., 1996. The anti-craving drug acamprosate reduces c-*fos* expression in rats undergoing ethanol withdrawal. Eur. J. Pharmacol. 317, 39–48.

Sass, H., Soyka, M., Mann, K., Zieglgänsberger, W., 1996. Relapse prevention by acamprosate: results from a placebo-controlled study on alcohol dependence. Arch. Gen. Psychiatry 53, 673–680.

Schneider, U., Wohlfahrt, K., Schulze-Bonhage, A., Haacker, T., Caspary, M., Zedler, M., Emrich, H.M., 1998. Lack of psychotomimetic or impairing effects on psychomotor performance of acamprosate. Pharmacopsychiatry 31, 110–113.

Spanagel, R., Holter, S.M., Allingham, K., Landgraf, R., Zieglgänsberger, W., 1996a. Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat. Eur. J. Pharmacol. 305, 39–44.

Spanagel, R., Putzke, J., Stefferl, A., Schobitz, B., Zieglgänsberger, W., 1996b. Acamprosate and alcohol: II. Effects on alcohol withdrawal in the rat. Eur. J. Pharmacol. 305, 45–50.

Spanagel, R., Zieglgänsberger, W., Hundt, W., 1996c. Acamprosate and alcohol: III. Effects on alcohol discrimination in the rat. Eur. J. Pharmacol. 305, 51–56.

Spanagel, R., Holter, S., Putzke, J., Pestel, E., Wetzel, C., Zieglgänsberger, W., 1997. Behavioral, molecular and electrophysiological characterization of the action of acamprosate. Alcohol. Clin. Exp. Res. Suppl. 21, 637.

Whitworth, A.B., Fischer, F., Lesch, O.M., Nimmerrichter, A., Oberbauer, H., Platz, T., Potgeiter, A., Walter, H., Fleischhacker, W.W., 1996. Comparison of acamprosate and placebo in long-term treatment of alcohol dependence. Lancet 347, 1438–1442.

Williams, K., 1994. Mechanisms influencing stimulatory effects of spermine at recombinant *N*-methyl-D-aspartate receptors. Mol. Pharmacol. 46, 161–168.

Williams, K., 1997. Modulation and block of ion channels: a new biology of polyamines. Cell Signal 9, 1–13.

Zeise, M.L., Kasparov, S., Capogna, M., Zieglgänsberger, W., 1993. Acamprosate (calciumacetylhomotaurinate) decreases postsynaptic potentials in the rat neocortex: possible involvement of excitatory amino acid receptors. Eur. J. Pharmacol. 231, 47–52.