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Acamprosate Modulates Alcohol-Induced Hippocampal NMDA Receptors and Brain Microsomal Ca^{2+}-ATPase but Induces Oxidative Stress in Rat

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Abstract We investigated the effects of acamprosate on alcohol-induced oxidative toxicity, microsomal membrane Ca^{2+}-ATPase (MMCA) activity and *N*-methyl-D-aspartate receptor (NMDAR) subunits in rat brain. Forty male rats were equally divided into four groups. The first group was used as control, and the second group received ethanol. Acamprosate and acamprosate plus ethanol each day were administered to rats constituting the third and fourth groups for 21 days, respectively. Brain cortical and hippocampal samples were taken from the four groups after 21 days. Brain cortical lipid peroxidation (LP) levels and MMCA activity were higher in the alcohol group than in control, although glutathione peroxidase (GSH-Px), vitamin C, vitamin E and β-carotene values were lower in the alcohol group than in control. LP levels were further increased in the acamprosate and alcohol + acamprosate groups compared with the alcohol group. GSH-Px, vitamin A, vitamin C, vitamin E and β-carotene in the acamprosate and alcohol + acamprosate groups were further decreased compared with the alcohol group. Hippocampal NMDAR 2A and 2B subunit concentrations were lower in the alcohol group than in control, although they were increased by acamprosate and alcohol + acamprosate. Brain cortical MMCA activity was higher in the acamprosate group than in the alcohol-treated rats, although its activity was lower in the alcohol + acamprosate group than in the acamprosate group. Brain cortical reduced glutathione levels were not found to be statistically different in any of the groups. Oxidative stress has been proposed to explain the biological side effects of experimental alcohol intake. Acamprosate and alcohol-induced oxidative stress decreased brain antioxidant vitamins in the alcoholic rats.

Keywords Oxidative stress · Alcohol addiction · Hippocampus · NMDA receptor · Brain · Acamprosate

Abbreviations

CAT	Catalase
GABA	γ-Aminobutyric acid
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
LP	Lipid peroxidation
MDA	Malondialdehyde
MMCA	Microsomal membrane Ca^{2+}-ATPase
NR2A	anti-glutamate receptor NMDAR2A′
NR2B	anti-glutamate receptor NMDAR2B
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SOD	Superoxide dismutase

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Introduction

Oxidative stress is defined as an imbalance between higher cellular levels and reactive oxygen species (ROS), e.g., superoxide and hydroxyl radicals and cellular antioxidant defense (Halliwell 2006; Kovacic and Somanathan 2008). Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism, i.e., mitochondrial oxidation and phagocytosis. In order to scavenge ROS, various defense systems exist in the brain. Glutathione peroxidase (GSH-Px), a selenium-containing enzyme, is responsible for the reduction of hydro- and organic peroxides in the presence of reduced glutathione (GSH) (Whanger 2001). GSH is a hydroxyl radical and singlet oxygen scavenger which participates in a wide range of cellular functions (Whanger 2001). Vitamin E (α-tocopherol) is the most important antioxidant in the lipid phase of cells (Zingg and Azzi 2004). Vitamin C (ascorbic acid), as well as being a free radical scavenger, also transforms vitamin E to its active form (Frei et al. 1989). Vitamin A (retinol) serves as a prohormone for retinoids and is involved with signal transduction at cytoplasmic and membrane sites (Halliwell 2006).

If ROS are not controlled by the enzymatic and non-enzymatic antioxidants, they can cause oxidative injury, i.e., peroxidation of cell membrane phospholipids, proteins (receptors and enzymes) and DNA. The brain is extremely susceptible to oxidative damage induced by these ROS because it generates very high levels of ROS due to its very high aerobic metabolism and blood perfusion and relatively poor enzymatic antioxidant defense (Halliwell 2006). The brain contains polyunsaturated fatty acids (PUFAs) which can readily be peroxidized (Whanger 2001). Lipid peroxidation (LP) causes injury to cells and intracellular membranes and may lead to cell destruction and subsequently cell death (Kovacic and Somanathan 2008). Brains are protected by antioxidants from peroxidative damage (Whanger 2001). Recently, several studies have examined the role of oxidative stress in alcohol-induced brain toxicity, possibly via formation of free radicals (Shirpoor et al. 2008; Antonio and Druse 2008). It has been demonstrated that cytochrome P-450 is present in brain regions and may be an important source of ethanol-induced oxidative stress (Somani et al. 1996).

Two main pathways for transient changes in cytosolic free Ca^{2+} that are most important in cell signaling are the plasma membrane Ca^{2+}-permeant channels and the Ca^{2+}-releasing channels in the membrane of intracellular organelles, such as the endoplasmic reticulum (Bejarano et al. 2009). Among the Ca^{2+}-permeant ion channels in neuronal plasma membrane are the *N*-methyl-D-aspartate receptors (NMDARs), and this has been the focus of many studies related to the inhibitory effects of ethanol on receptor function (Littleton 1995; Chen et al. 1997). The molecular structure of NMDARs in the hippocampus consists of the NMDAR1 and NMDAR2 subunit proteins (Littleton 1995). Selective effects on the expression of the subunits of neuronal NMDAR complexes have been observed after chronic treatment of experimental animals and cells in culture with ethanol (Berton et al. 1998; Bachteler et al. 2005). The mechanisms underlying both the neuronal hyperexcitability and susceptibility to seizures observed in alcoholics during alcohol withdrawal are not completely understood. A reduction in the inhibitory effects of γ-aminobutyric acid (GABA) by downregulation of $GABA_A$ receptors, an increase in the effects of glutamate by upregulation of NMDA receptors and an increase in the activity of voltage-gated Ca^{2+} channels by upregulation of Ca^{2+}-channel expression could all be contributing factors (Littleton 1995). There are also interactions between Ca^{2+} influx, GABA and ROS. For example, the onset of oxygen-induced convulsions in epileptic patients and animals is correlated with a decrease in cerebral content of neurotransmitter GABA because of inhibition of glutamate decarboxylase by the ROS (reviewed in Nazıroğlu 2009). It has been shown that acamprosate, which has a similar chemical structure to that of GABA, increases GABA by blocking presynaptic $GABA_B$ autoreceptors (Berton et al. 1998; Allgaier et al. 2000). Hence, we chose oxidative stress and NMDAR conditions because the exact mechanism by which acamprosate alters NMDARs, microsomal (vesicle-like artifacts formed from the endoplasmic reticulum) membrane Ca^{2+}-ATPase (MMCA) and oxidative stress values are uncertain.

We aimed to evaluate whether there would be effects of acamprosate on oxidative stress, enzymatic antioxidants and MMCA activity in micosomes of rat brain cortex.

Materials and Methods

Animals

Forty male Wistar albino rats weighing 135 ± 10 g were used for the experimental procedures. Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments unless otherwise noted. The temperature was maintained at 22 ± 2°C. A 12/12 h light/dark cycle was maintained, unless otherwise noted. The experimental protocol of the study was approved by a local ethical committee of the Medical Faculty of Suleyman Demirel University (SDU) by protocol number 2009-13. Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the SDU.

Experimental Design

Acamprosate was dissolved in physiological saline (0.9%, w/v). Forty male rats were randomly divided into four equal groups as follows:

Group I was a control group ($n = 10$), and placebo (physiological saline) was intragastrically given to this group for 21 days.
Group II was an ethanol-treated group, and rats received 10 mg/kg daily ethanol intragastrically for 21 days.
Group III was an acamprosate group, and rats received 200 mg/kg daily of acamprosate intragastrically for 21 days.
Group IV was an ethanol + acamprosate group, and rats were given 200 mg/kg daily of acamprosate with 10 mg/kg daily of ethanol intragastrically for 21 days.

After 24 h from the administration of acamprosate, the animals were killed under anesthesia and brain cortical and hippocampal samples were taken.

Anesthesia and Preparation of Brain Samples

Rats were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) administered i.p. before being killed and cortical brain samples being removed. The brain was processed as follows: The cortex was dissected out after the brain was split in the mid-sagittal plane. The cortex was dissected from the total brain as described in our previous study (Eren et al. 2007).

Cortical brain tissues were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (−33°C) until processing (maximum 10 h). After weighing, half of the cortical samples were placed on ice, cut into small pieces using scissors and homogenized (2 min at 5,000 rpm) in five volumes (1:5, w/v) of ice-cold Tris–HCl buffer (50 mM, pH 7.4), using a glass Teflon homogenizer (Caliskan Cam Teknik, Ankara, Turkey).

All preparation procedures were performed on ice. The homogenate was used for determination of LP and antioxidant levels. The remaining cortical samples were used for Ca^{2+}-ATPase assay and isolation of microsomes by ultracentrifugation.

After addition of butylhydroxytoluol (4 µl/ml), brain homogenate and microsomal samples were used for immediate determination of LP levels and enzyme activities. Antioxidant vitamin analyses were performed within 3 months.

Isolation of Brain Microsomes

Tissues were cleaned, minced and then homogenized in six volumes of freshly prepared buffer A containing 0.3 mol/l sucrose, 10 mmol/l HEPES-HCl buffer (pH 7.4) and 2 mmol/l dithiothreitol. The material was homogenized with the glass Teflon homogenizer. The homogenate was centrifuged (MS 80; Sanyo, San Diego, CA) at 85,000*g* rotor (Sorvall; Teknolab, Ankara, Turkey) for 75 min. The supernatant was discarded and the pellet was resuspended in the original volume of buffer A containing 0.6 mol/l KCl using four strokes of the pestle and centrifuged again at 85,000*g* for 75 min. The pellet was resuspended in the original volume of buffer A. After centrifugation at 85,000*g* for 75 min, the pellet was suspended in buffer A using four strokes of the pestle to produce a protein concentration of 2–7 mg/ml. The complete procedure took 10–12 h for eight samples. Samples were frozen and stored at −30°C until assayed. The isolation procedure was carried out at +4°C (Nazıroğlu et al. 2009).

Measurement of Microsomal Ca^{2+}-ATPase Activity

Ca^{2+}-ATPase activity was measured spectrophotometrically by the method of Niggli et al. (1981). Assay medium contained 120 mmol/l KCl, 60 mmol/l HEPES buffer (pH 7, at 37°C), 1 mmol/l $MgCl_2$, 0.5 mmol/l K_2-ATP, 0.2 mmol/l NADH, 0.5 mmol/PEPA, 1 IU/l pyruvate kinase, 1 IU/l LDH and 500 mmol/l EGTA. After preincubation of the assay medium (total volume of 1 ml) for 4 min at 37°C, 50 mg of the microsomal homogenate were added to the medium. After 2 min, the reaction was started by addition of $CaCl_2$ (600 mmol/l). ATPase activity as oxidation of NADH was followed by continuously measuring the absorbance at 340 nm.

Based on the extinction coefficient for NADH, $\varepsilon = 6.2 \times 10^6\ M^{-1}$, the amount of NADH oxidized is equivalent to the hydrolyzed amount of ATP. Values were expressed as international units per milligram of protein.

LP Level Determinations

LP levels in the brain homogenate were measured with the thiobarbituric acid reaction by the method of Placer et al. (1966). The values of LP in the brain were expressed as micromoles per gram of protein. Although the method is not specific for LP, measurement of the thiobarbituric acid reaction is an easy and reliable method, which is used as an indicator of LP and ROS activity in biological samples.

Brain Cortical GSH, GSH-Px and Protein Assay

The GSH content of the brain homogenate was measured at 412 nm using the method of Sedlak and Lindsay (1968) as described in our own studies (Eren et al. 2007). GSH-Px activities of the brain homogenate were measured spectrophotometrically at 37°C and 412 nm according to the

method of Lawrence and Burk (1976). The protein content in the brain cortex and microsomes was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Analyses for Brain Cortical Vitamins A, C and E and β-Carotene

Vitamins A (retinol) and E (α-tocopherol) were determined in the brain samples by a modification of the methods described by Desai (1984) and Suzuki and Katoh (1990). Brain samples (250 µg) were saponified by addition of 0.3 ml KOH (60% w/v in water) and 2 ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70°C for 30 min. After cooling the samples on ice, 2 ml of water and 1 ml of *n*-hexane were added and mixed with the samples and then rested for 10 min to allow phase separation. An aliquot of 0.5 ml of *n*-hexane extract was taken, and vitamin A concentrations were measured at 325 nm. Then, reactants were added and the absorbance value of hexane was measured in a spectrophotometer at 535 nm. Calibration was performed using standard solutions of all-*trans*-retinol and α-tocopherol in hexane.

The concentrations of β-carotene in the brain samples were determined according to the method of Suzuki and Katoh (1990). Two milliliters of hexane were mixed with 250 µg brain samples. The concentration of β-carotene in hexane was measured at 453 nm in a spectrophotometer.

Ascorbic acid in brain cortical samples was quantified according to the method of Jagota and Dani (1982). Absorbance of the samples was measured spectrophotometrically at 760 nm.

Hippocampal Antiglutamate Receptor NMDAR2A′ and Antiglutamate Receptor NMDAR2B Analyses by Western Blot

Hippocampal samples were homogenated in ice-cold buffer (50 mM Tris–HCl [pH 7.5]), and an aliquot was taken for protein determination. Equal amounts of protein for each sample (20 µg of protein per lane) were separated by sodium deocyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE). Images of immunoblots were analyzed with a computerized image analysis system (Uviphoto MWV.99; Ultra-Violet Products, Cambridge, UK) (Dilek et al. 2010).

Statistical Analysis

All results were expressed as means ± SD. Significance in three groups was first checked by ANOVA Kruskal–Wallis test. Then, significant values in three groups were assessed with an unpaired Mann–Whitney *U*-test. Data were analyzed using the SPSS statistical program (version 9.05 software; SPSS, Inc., Chicago, IL). $P < 0.05$ was regarded as significant.

Results

Lipid Peroxidation

The mean brain cortical and microsomal LP levels in the four groups are shown in Table 1. The results showed that LP levels in the cortex of the alcohol ($P < 0.05$), acamprosate ($P < 0.01$) and alcohol + acamprosate ($P < 0.001$) groups were significantly higher than those in control rats. Cortical and microsomal LP levels in the acamprosate ($P < 0.05$ and $P < 0.01$) and alcohol + acamprosate ($P < 0.01$ and $P < 0.001$) groups were higher than those in alcohol-treated animals.

GSH-Px Activities and GSH Levels

The mean brain cortical and microsomal GSH-Px activities and GSH levels in the four groups are shown in Table 1. The results showed that the cortical and microsomal GSH-Px activities were significantly ($P < 0.05$) lower in the

Table 1 Effects of acamprosate and alcohol on brain cortical GSH-Px activity and levels of GSH, LP, β-carotene and vitamins A, C and E in rats (mean ± SD)

Parameters	Control ($n = 10$)	Alcohol ($n = 10$)	Acamprosate ($n = 10$)	Alcohol + acamprosate ($n = 10$)
LP (µmol/g protein)	883 ± 169	1.047 ± 247[a]	1.336 ± 204[b,d]	1.772 ± 349[c,e,g]
GSH (µmol/g protein)	23.65 ± 2.82	22.91 ± 2.48	22.68 ± 1.64	24.01 ± 2.30
GSH-Px (IU/g protein)	27.95 ± 4.92	23.95 ± 3.11[a]	20.47 ± 3.11[a]	19.81 ± 4.14[a]
Vitamin A (nmol/g tissue)	7.35 ± 0.16	6.99 ± 0.17	6.51 ± 0.28[a]	5.20 ± 0.81[b,d]
Vitamin C (µmol/g tissue)	0.95 ± 0.14	0.81 ± 0.08[a]	0.74 ± 0.06[b]	0.67 ± 0.06[c,e]
Vitamin E (µmol/g tissue)	11.51 ± 0.98	6.83 ± 0.79[b]	4.29 ± 0.83[c,d]	1.50 ± 0.36[c,f,h]
β-carotene (µmol/g tissue)	0.96 ± 0.19	0.72 ± 0.24[a]	0.39 ± 0.09[f]	0.23 ± 0.09[c,f,h]

[a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. control; [d] $P < 0.05$, [e] $P < 0.01$ and [f] $P < 0.01$ vs. alcohol group; [g] $P < 0.05$ and [h] $P < 0.01$ vs. acamprosate group

alcohol, acamprosate and alcohol + acamprosate groups than in the control. Hence, GSH-Px activity of cortical and microsomal fractions was decreased by the doses of alcohol and acamprosate. However, the cortical microsomal GSH levels did not change significantly in the four groups as a result of the treatments.

Antioxidant Vitamin Concentrations

The mean cortical concentrations of β-carotene and vitamins A, C and E in the four groups are shown in Table 1. The cortical vitamin C ($P < 0.05$), vitamin E ($P < 0.01$) and β-carotene ($P < 0.05$) concentrations were significantly lower in the alcohol group compared with the control. Cortical vitamin A ($P < 0.05$), vitamin C ($P < 0.01$), vitamin E ($P < 0.001$) and β-carotene ($P < 0.001$) concentrations were significantly higher in the acamprosate and alcohol + acamprosate groups than in the alcohol group.

MMCA Activity Asssay

The mean MMCA activities in the four groups are shown in Figure 1. MMCA activities were significantly ($P < 0.05$) lower in the alcohol group ($P < 0.05$) than in the control, although MMCA activities in the acamprosate group were significantly ($P < 0.05$) higher than those in the alcohol-treated groups. However, MMCA activities were significantly ($P < 0.05$) lower in the alcohol + acamprosate ($P < 0.05$) group than in animals treated with acamprosate alone, although they were significantly ($P < 0.05$) higher in the alcohol + acamprosate group than in the group treated with alcohol alone.

Western blot analyses of antiglutamate receptor NMDAR2A′ (NR2A) and antiglutamate receptor NMDAR2B (NR2B) are shown in Figures 2 and 3. The density of the protein band in the control group was used as the reference 100% level, and data from other groups were calculated as percentages of the control value. The percentages of NR2A and NR2B were significantly ($P < 0.05$) lower in the alcohol group than in the control, although their percentages were increased by both acamprosate ($P < 0.01$) and alcohol + acamprosate ($P < 0.001$) treatments.

Discussion

We found that brain cortical LP values were increased by experimental ethanol exposure, whereas vitamin C, vitamin E, β-carotene, MMCA and hippocampus NR2B subunit concentrations decreased. Hence, the experimental alcohol model in the animals is characterized by increased LP and decreased antioxidant vitamins, MMCA and hippocampal NR2A and -2B subunit concentrations. To our knowledge we are the first to report that administration of acamprosate caused further decrease of brain cortical vitamin A, vitamin C, vitamin E and β-carotene concentrations, whereas hippocampal NR2A and -2B subunit concentrations and MMCA activities were increased by the acamprosate treatment.

The current study indicated that alcohol administration produced a significant increase in LP levels of the cortex. Our results are in accordance with previous reports of LP increases in brain, hippocampal and cerebellar granule cells (Somani et al. 1996; Siler-Marsiglio et al. 2005; Shirpoor et al. 2008; Antonio and Druse 2008). On the other hand, the current study is the first report of brain microsomal LP in acamprosate-administered rats. Alcohol may also trigger a variety of biochemical process, including activation of membrane phospholipases, proteases and nucleases (Lieber 2000). Marked alterations in membrane phospholipid metabolism resulted in the oxidation of mitochondrial and membrane lipids, damaging membranes and leading to the formation of highly reactive oxidative components (Siler-Marsiglio et al. 2005). Free radical generation by the ethanol-induced CYP2E1 also plays a key role in oxidative stress. Inhibitors of this enzyme have great promise and are presently being evaluated clinically.

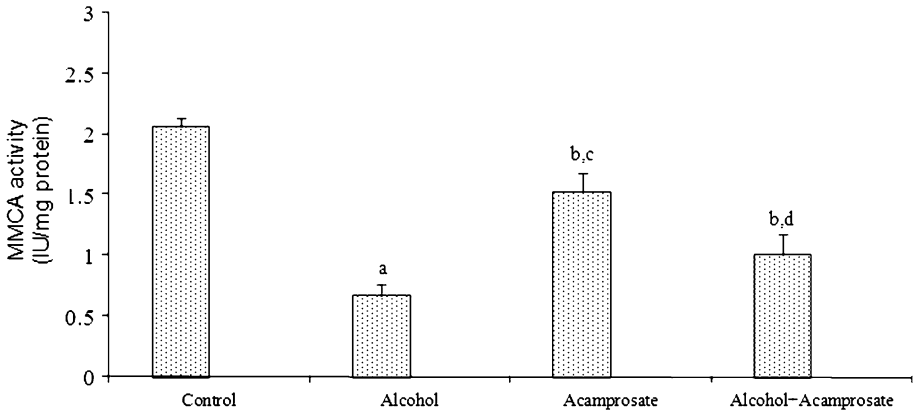

Fig. 1 Effects of alcohol and acamprosate on MMCA activity in rats (mean ± SD). [a] $P < 0.001$, [b] $P < 0.05$ vs. control; [c] $P < 0.01$ and [d] $P < 0.05$ vs. alcohol group

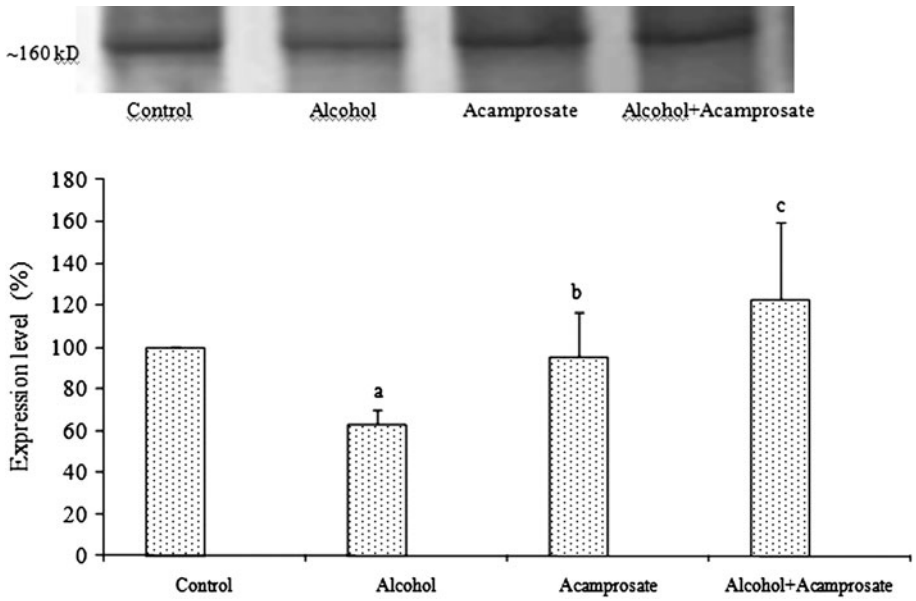

Fig. 2 Effects of alcohol and acamprosate on hippocampal NR2A subunit concentrations in rats ($n = 10$, mean ± SD). The density of the protein band in the control group was accepted as 100%, and data from other groups were calculated as percentages of the control value (mean ± SD). [a] $P < 0.001$ vs. control; [b] $P < 0.05$ and [c] $P < 0.01$ vs. alcohol group

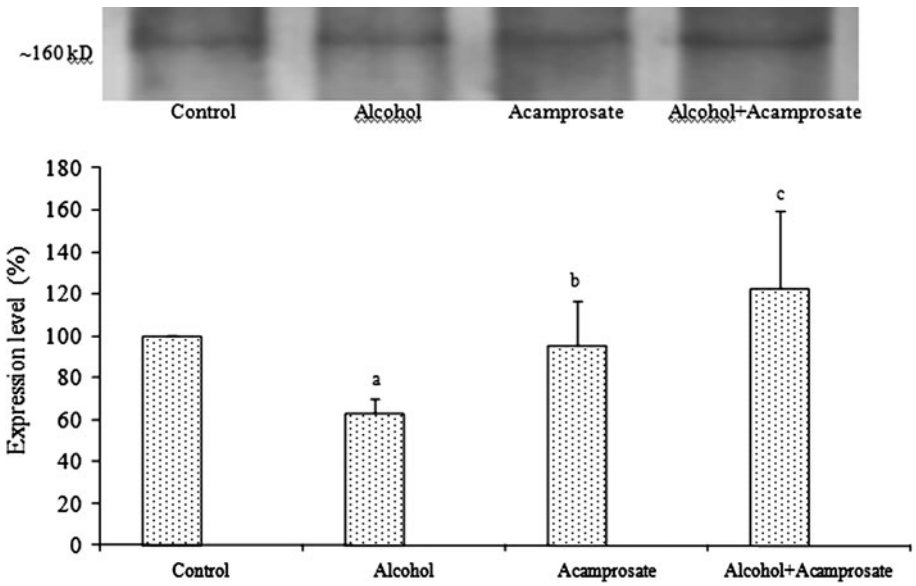

Fig. 3 Effects of alcohol and acamprosate on hippocampal NR2B subunit concentrations in rats ($n = 10$, mean ± SD). The density of the protein band in the control group was accepted as 100%, and data from other groups were calculated as percentages of the control value (mean ± SD). [a] $P < 0.001$ vs. control, [b] $P < 0.05$ and [c] $P < 0.01$ vs. alcohol group

The system is particularly interesting because of its innocuity (Lieber 2000). Hence, involvement of LP as malondialdehyde in alcohol administration can be attributed to the activation of membrane phospholipases and CYP2E1. In the current study, vitamin C, vitamin E and β-carotene concentrations in the cortex were decreased by alcohol intake. Additionally, we found that alcohol dramatically suppressed cortical GSH-Px activity. If the antioxidant vitamins decrease, superoxide radical production may increase and finally lead to oxidative stress and LP (Halliwell 2006; Kovacic and Somanathan 2008). This reduces the capacity of the brain cell to rid itself of excess LP levels in the cytoplasm and mitochondria because of decreasing GSH and GSH-Px values (Reinke 2002).

Acamprosate has been introduced for treating alcohol craving and reducing relapses in weaned alcoholics. In the current study we observed oxidant effects of acamprosate in the rat brain. Acamprosate may exert its action through the mitochondrial oxidative stress system rather than by the glutamatergic or GABAergic system. For example, taurine has an antioxidant role (Oliveira et al. 2010), and acamprosate strongly inhibits the binding of taurine to taurine receptors but has little effect on the binding of glutamate to glutamate receptors or muscimol to $GABA_A$ receptors (Wu et al. 2001). In addition, acamprosate was found to be neurotoxic, at least in neuronal cultures, triggering neuronal damage at 1 mM (Wu et al. 2001). The underlying mechanism of acamprosate-induced neuronal injury

appears to be its action in increasing the intracellular calcium ion level ($[Ca^{2+}]_i$). In accordance with the reports, we observed in the current study that the dose of acamprosate induced oxidant effects due to its neurotoxic effect.

ROS can be inactivated by antioxidant vitamins (Halliwell 2006). Vitamin E (α-tocopherol) is the most important antioxidant in the lipid phase of cells. Vitamin E acts to protect cells against the effects of free radicals, which are potentially damaging by-products of the body's metabolism (Zingg and Azzi 2004). Therefore, low antioxidant levels and high content of PUFAs results in limited antioxidant defense in brain. Vitamin E concentrations in the brain cortex were decreased in the acamprosate group. The decreased concentration of the antioxidant vitamins at the dose of acamprosate tested in our study could be due to its stimulation as a result of the increased production of free radicals.

MMCA activity and NMDA2A and -B subunit concentrations were increased by the acamprosate administration. It has also been suggested that acamprosate counteracts increases in $[Ca^{2+}]_i$ mediated by NMDA receptors and voltage-gated calcium channel activation in neurons (Allgaier et al. 2000). The alterations in MMCA activity and NMDA2A and -B subunit concentrations by alcohol, which results in the generation of ROS, could therefore be attenuated by acamprosate, which would also explain the potent action exhibited by moderate doses of this drug.

A number of reports have implicated NMDA receptor-mediated neurotransmission in alcohol's toxic effect on regulation of hippocampal spine density. Alcohol consumption causes a decrease in NMDA receptor binding and expression of the NR1 and NR2B receptor subunits in the rat hippocampus (Xu and Woodward 2006; Pickering et al. 2007). Similarly, we observed in the current study that NR2B concentrations were lower in the alcohol group than in control. Our results support those of other recent studies (Xu and Woodward 2006; Pickering et al. 2007). It was reported that acamprosate impaired both NMDA and voltage-gated calcium channels in mesencephalic neurons (Allgaier et al. 2000). Binding studies with [^{3}H]dizocilpine performed on rat whole-brain membranes suggest that acamprosate allosterically reduces NMDA receptor function by interacting with a specific spermidine-sensitive site at least at high levels of receptor activation (Naassila et al. 1998). Acamprosate reduced also the binding of [^{3}H]dizocilpine to rat neocortical membranes obtained from naive and ethanol-treated rats (Al Qatari et al. 1998). Similarly, acamprosate in the current study modulated hippocampal NMDA subunits in rats.

In conclusion, the brain results obtained in the current study on alcohol consumption and acamprosate administration are consistent with a generalized antioxidant abnormality in rats. However, acamprosate has a protective effect on hippocampal NMDA 2A and -2B subunits and brain MMCA activity in rat. The drug should be supplemented with antioxidants because of the additional oxidant effect of acamprosate.

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