Chronic effect of acamprosate on amino acids during the third ethanol withdrawal period

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Abstract
The effects of chronic acamprosate (400 mg/kg/day; PO) under chronic ethanol treatment on amino acids in the hippocampus of Wistar male rats were examined during the third ethanol withdrawal period. In this study, rats were made ethanol dependent by four-week vapour inhalation. After this first cycle of chronic ethanol treatment (CET), rats underwent repeated and alternate cycles of 24h withdrawals and one week of CET. The microdialysis experiment was performed during the third ethanol withdrawal, together with the HPLC and electrochemical detection to quantify different amino acids such as aspartate, glutamate, taurine and alanine. During the first 4h of ethanol withdrawal, there were no significant changes in glutamate levels between acamprosate and non-acamprosate treated groups. During 4h and 8h 30 min glutamate levels were significantly decreased in non-acamprosate treated group by comparison to acamprosate treated group. After 10h from ethanol withdrawal glutamate levels were significantly increased in acamprosate non-treated group by comparison to acamprosate treated group. No changes were observed in other amino acids levels such as aspartate, taurine and alanine. The results of this work suggest that by acting on glutamate receptors chronic acamprosate could prevent neuroadaptation in glutamatergic system and thereby acamprosate may provide a protective mechanism against the neurotoxicity by reducing excitatory amino acids overexpression during repeated ethanol withdrawal.

Key word: Acamprosate, Glutamate, Ethanol withdrawal, Microdialysis, Hippocampus

Introduction
The ability of acamprosate to block the glutamate increase during ethanol withdrawal is an important finding for the therapeutic treatment of alcoholism and supports the glutamatergic hypothesis (Spanagel & Zieglgänsberger, 1997) for acamprosate action both in rats (Dahchour et al., 1998) and in man (Bolo et al., 1998). The latter study also suggested a central glutamatergic effect of acamprosate in the CNS. Naassila et al. (1998) showed that acamprosate would bind to a spermidine-sensitive site on the NMDA receptor and causes modulation. Moreover, acamprosate may have an excitatory or inhibitory effect on NMDA receptors depending upon the experimental conditions that are utilized (al Qatari et al., 1998) and could have a protective role against glutamate-induced excitotoxicity in ethanol withdrawal (al Qatari et al., 2001). Other study by (Rammes et al., 2001) showed that although acamprosate acts as a weak antagonist of NMDA receptors, it modulates the expression of NMDA receptor subunits in the cortex and hippocampus but not in the brainstem.

The purpose of the present study was to assess the effects of a chronic acamprosate treatment on extracellular changes in aspartate, glutamate, within the hippocampus of freely moving male rats by a microdialysis technique during the third ethanol withdrawal period.

Material and methods
2.1. Chronic ethanol treatment (CET) and surgery

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Two groups of Male Wistar rats, (350-400 g), were individually housed in plastic cages and kept in a temperature and light controlled environment (light/darkness cycle: 12 hrs light/12 hrs dark cycle), made of an isolated plastic chamber (160 x 60 x 60 cm). A mixture of alcohol and air was pulsed into the chamber via a mixing system, allowing the quantity of alcohol to be increased every 2 days during the whole experimental procedure (Le Bourhis, 1975) for four weeks. One group was orally treated with acamprosate (400 mg/kg/day), and the second group without any treatment.

During the third week the rats were removed from the chamber for surgery, which was performed under anaesthesia with chloral hydrate (400-mg/kg i. p.). Using standard stereotaxic technique a guide cannula (20 gauge stainless steel; Small Parts, Miami, FL) was implanted 1 mm above the hippocampus (A/P -4.3 mm; M/L 4.0 mm; D/V -3.0 mm), figure 1A (Paxinos & Watson, 1982) and secured to the skull with three steel screws and cranioplastic cement.

![Figure 1](image).

Figure 1. Structural similarity between acampro-sate (calcium acetylhomotaurinate) and amino acid neurotransmitters namely glutamic acid, GABA, and taurine.

2.2. Microdialysis procedure

After the fourth-week of chronic ethanol treatment, a concentric dialysis probe was inserted through the guide cannula into the hippocampus region. The dialysis membrane (0.20 mm ID, Molecular weight cut-off 18000 Da) extended 3 mm beyond the tip of the cannula, and continuously perfused with Ringer’s solution (145 mM NaCl, 4 mM KCl; 1.3 mM CaCl₂; pH 7.2) by a micro infusion pump (Infusion syringe pump 22, Harvard apparatus; USA) at 1µl/min. Microdialysis samples were collected every 20 minutes for 12h.

These experiments were approved by the Belgian governmental agency under the authorized number LA 1220028 as well as the European Communities Council Directive concerning the Use of Laboratory Animals.

2.3. Electrochemical detection

The concentration of aspartate and glutamate in each of the microdialysate samples was analyzed by HPLC with electrochemical detection and O-phthalaldehyde/ß-mercapto-ethanol (OPA/BME) precolumn derivatisation (Donzanti & Yamamoto, 1988). O-phthalaldehyde, OPA, 27 mg, was dissolved in 1 ml methanol HPLC-grade, to which 10 µl ß-mercaptoethanol (BME) was added; this solution was diluted with 9 ml of 0.1 M sodium tetraborate buffer, pH 9.3 and stored at 4°C. The working solution was prepared each day, 24 hours before use, by diluting 1 ml of the above solution in 3 ml of 0.1 M sodium tetraborate. The derivatisation procedure entail-ed mixing the dialysate (20µl) and the internal standard (10µl of homoserine 5.10⁻⁶ M) with 10 µl OPA/BME for 2 min in complete darkness, and 20µl of this mixture were injected into the HPLC system. This system consisted of a LDC Consta Metric 3200 pump delivering 1 ml/min of the mobile phase at a pressure of 5300 psi. Separation of amino acids was achieved with reversed-phase column (125 x 3 mm, ODS Hypersil 3 µm) (VDS Optilab; Germany) and detected coulometrically (ESAII, Inc., Bedford, MA) using three electrodes: a guard (500 mV), preoxidation (180 mV) and working (425 mV) electrode (Analytical cell ESA Model 5011). The mobile phase used (0.1 M Na₂HPO₄; 0.134 mM EDTA; 27% of methanol HPLC grade; 73% MilliQ H₂O; pH 6.4) was filtered through 0.2 µm cellulose nitrate filter (Gelman Sciences, Ann Arbor, MI) and degassed under vacuum, before use in the HPLC system.
The position and height of peaks of the endogenous components were compared with a standard solution containing glutamate, aspartate that was prepared in a solution of MilliQ water and HPLC grade methanol (50:50 V/V). The working solution was prepared each day by diluting the stock solution to 10-6 M in Ringer's solution and 20 µl samples of this solution were injected and quantified. All reagents used were of analytical grade from Sigma Chemical Co. (St Louis, MO). An HPLC autosampler (Model 465, Kontron Instruments, Milan, Italy) was used for sample injection and the heights and areas of the peaks were quantified by a PC Integration Pack (Kontron Instruments, Milan, Italy).

2.4. Histology and statistical analysis

Upon completion of the three cycles of alcohol intoxication and withdrawal, the rats were killed and the brain fixed with 10% formalin. Coronal sections through the extent of the cannula tracks were cut (100 µm) with a vibratome (Polaron H 1200, Biorad, Cambridge, MA) and stained with 0.5% cresyl violet. Dialysis probe placement was localized according to the atlas of Paxinos and Watson (Paxinos & Watson, 1982).

Data were analyzed by analysis of variance by the least-significant difference test of multiple comparison (Fisher's LSD Protected t-Test) to evaluate group and time differences (GB-STAT, Dynamic Microsystems, Silver Spring, MD).

Results

Effect of chronic acamprosate treatment on glutamate levels during the third ethanol withdrawal period.

Figure 2 represents glutamate levels in acamprosate treated and non-acamprosate treated rats during the third ethanol withdrawal period. Analysis of variance showed a significant differences in the time \[ F(46,845)=10.201; P>0.0001 \] and in the interaction between treatment and the time \[ F(46,845)=6.544; P>0.0001 \].

Glutamate levels were significantly decreased in non-acamprosate treated group by comparison to acamprosate treated group between 4h and 8h 30 min from ethanol withdrawal. After 10h from ethanol withdrawal glutamate levels were significantly increased in acamprosate non-treated group by comparison to acamprosate treated group.

No significant changes were observed in other amino acid levels such as aspartate (Fig. 3), taurine (Fig. 4), and alanine (Fig. 5).

![Figure 2](image-url)

**Figure 2.** Effects of an oral treatment of acamprosate on glutamate microdialysate levels as assayed during the third period of ethanol withdrawal. Data are represented as mean % of baseline values ± S.E.M. Statistical significance is represented by * \( P<0.05 \), ** \( P<0.01 \) compared the relevant control group \( n=8 \) not exposed to chronic ethanol treatment.
Figure 3. Effects of an oral treatment of acamprosate on aspartate microdialysate levels as assayed during the third period of ethanol withdrawal. Data are represented as mean % of baseline values ± S.E.M. Statistical significance is represented by * $P<0.05$, ** $P<0.01$ compared to the relevant control group (n=8) not exposed to chronic ethanol treatment.

Figure 4. Effects of an oral treatment of acamprosate on taurine microdialysate levels as assayed during the third period of ethanol withdrawal. Data are represented as mean % of baseline values ± S.E.M. Statistical significance is represented by * $P<0.05$, ** $P<0.01$ compared to the relevant control group (n=8) not exposed to chronic ethanol treatment.
Discussion

In the present study, we investigated the effects of an oral treatment of acamprosate on amino acid during the third ethanol withdrawal. As acamprosate have been already given orally to patients, it is interesting to study the effects of acamprosate given orally while chronic ethanol treatment on amino acid changes in the hippocampus during the third ethanol withdrawal.

Glutamate levels were significantly different in time, when acamprosate-treated group was compared to acamprosate non-treated group and this difference was two-phase (Fig. 1).

One potential explanation of these two-phase changes is the fact that during the first phase the blood alcohol level is still high and glutamate should be inhibited by ethanol. While the second phase the blood alcohol level is low (0.153 ± 0.020 g/l; at 12h from ethanol withdrawal) (Dahchour & De Witte, 2003) and by the consequence, rats were under ethanol withdrawal effects. The increased glutamate during the second phase could be explained as a consequence of the up-regulation of glutamate receptors particularly the NMDA receptors.

Regarding glutamate levels in acamprosate treated group, the maintained glutamate levels during all the time of the experiment could be explained as an adverse effect of acamprosate to chronic ethanol treatment on glutamate and its receptors. As such, the depressant effect of ethanol was not observed when the blood alcohol was still higher, neither glutamate increases was not seen when the blood alcohol was very low nor withdrawal effects were maximum in acampro-sate non-treated group. The NMDA receptor subunit composition and the specificity of acamprosate to act at specific NMDA receptor subunits (Spanagel et al., 1997) could explain the different results observed in different brain regions such as nucleus accumbens and hippocampus. Whether acamprosate enhances (Madamba et al., 1996 and Berton et al., 1998) or attenuates (Zeise et al., 1993 and Spanagel et al., 1996) the NMDA receptors have been largely discussed in the literatures and the discrepancy

References


