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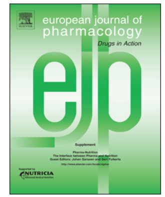

Neuropharmacology and analgesia

Acamprosate {monocalcium bis(3-acetamidopropane-1-sulfonate)} reduces ethanol-drinking behavior in rats and glutamate-induced toxicity in ethanol-exposed primary rat cortical neuronal cultures

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ABSTRACT

Acamprosate, the calcium salt of bis(3-acetamidopropane-1-sulfonate), contributes to the maintenance of abstinence in alcohol-dependent patients, but its mechanism of action in the central nervous system is unclear. Here, we report the effect of acamprosate on ethanol-drinking behavior in standard laboratory Wistar rats, including voluntary ethanol consumption and the ethanol-deprivation effect. After forced ethanol consumption arranged by the provision of only one drinking bottle containing 10% ethanol, the rats were given a choice between two drinking bottles, one containing water and the other containing 10% ethanol. In rats selected for high ethanol preference, repeated oral administration of acamprosate diminished voluntary ethanol drinking. After three months of continuous access to two bottles, rats were deprived of ethanol for three days and then presented with two bottles again. After ethanol deprivation, ethanol preference was increased, and the increase was largely abolished by acamprosate. After exposure of primary neuronal cultures of rat cerebral cortex to ethanol for four days, neurotoxicity, as measured by the extracellular leakage of lactate dehydrogenase (LDH), was induced by incubation with glutamate for 1 h followed by incubation in the absence of ethanol for 24 h. The *N*-methyl-D-aspartate receptor blocker 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5,10-imine, the metabotropic glutamate receptor subtype 5 antagonist 6-methyl-2-(phenylethynyl)pyridine and the voltage-gated calcium-channel blocker nifedipine all inhibited glutamate-induced LDH leakage from ethanol-exposed neurons. Acamprosate inhibited the glutamate-induced LDH leakage from ethanol-exposed neurons more strongly than that from intact neurons. In conclusion, acamprosate showed effective reduction of drinking behavior in rats and protected ethanol-exposed neurons by multiple blocking of glutamate signaling.

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1. Introduction

Alcoholism is a devastating chronic and progressive disorder that includes the development of ethanol tolerance, ethanol dependence, and craving and/or the manifestation of a withdrawal syndrome when ethanol is no longer available (Clapp, 2012). Acamprosate, the calcium salt of bis(3-acetamidopropane-1-sulfonate), has been marketed for the treatment of ethanol dependence as Aotal® in France (1987), Campral® in the United States (2004) and Canada (2007), and Regtect® in Japan (2013). Acamprosate promotes abstinence, and its safety has been demonstrated in numerous clinical trials worldwide (Mason, 2001).

In animal models, acamprosate decreases ethanol-intake behavior (Spanagel and Zieglgänsberger, 1997). Acamprosate also decreases the withdrawal-evoked release of the excitatory amino acid glutamate in rats, and the decrease is maintained over repeated cycles of ethanol exposure and withdrawal (Dahchour and De Witte, 2003). In addition, mutant mice with increased glutamate levels exhibit higher ethanol consumption than wild-type mice and respond better to acamprosate, providing evidence that hyperactivity of the glutamatergic system is an important neurophysiological mechanism underlying the development and maintenance of ethanol dependence (Spanagel et al., 2005). Acamprosate is thought to attenuate the hyperglutamatergic state caused by extensive ethanol exposure and repeated periods of alcohol withdrawal. However, the mechanism of action of acamprosate in the central nervous system remains unclear.

The hyperglutamatergic syndrome that occurs during ethanol withdrawal is associated not only with craving and relapse to renewed alcohol intake, but also with glutamate-induced toxicity (Kiefer and Mann, 2010). Given the widespread clinical use of acamprosate, the possible effects of this drug on neuronal survival are not only of interest in their own right, but they may also help clarify the mechanisms by which acamprosate alters neuronal

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glutamate responses. Early in vitro studies of rat neocortical cells showed that acamprosate inhibits the glutamate-induced release of lactate dehydrogenase (LDH) and entry of calcium ion (Ca^{2+}) during ethanol withdrawal (al Qatari et al., 2001). Hippocampal cell cultures obtained from the neonatal rat display neurotoxicity during ethanol withdrawal in the presence or absence of exogenously added glutamate or *N*-methyl-D-aspartate (NMDA), and this neurotoxicity is reduced by acamprosate (Mayer et al., 2002). However, the role of glutamate receptor subtypes and voltage-gated calcium channels in the inhibition by acamprosate of neurotoxicity induced by ethanol exposure in the presence or absence of exogenous glutamate is not fully understood.

In the present study, we investigated in standard laboratory Wistar rats the effect of acamprosate on voluntary ethanol consumption and increased ethanol consumption after ethanol deprivation in the two-bottle-choice drinking paradigm, in which the rats were given a choice between two drinking bottles, one containing ethanol solution and one containing water only. We also examined the protective effect of acamprosate against glutamate-induced neurotoxicity in ethanol-exposed primary neuronal cultures of rat cerebral cortex, as assessed by LDH leakage. To shed light on the mechanism of action of acamprosate, we investigated the role of glutamate receptors and voltage-gated calcium channels in glutamate-induced neurotoxicity in this neuronal model. We found evidence for the involvement of multiple glutamate signaling pathways in the ethanol-induced neurotoxicity inhibited by acamprosate.

2. Materials and methods

2.1. Animals

Male five-week-old Wistar rats (Charles River Japan, Kanagawa, Japan) were housed in individual cages in a room maintained at 20–26 °C and 35–75% relative humidity with an alternating 12-h light/dark cycle (the lights came on automatically at 8:00 a.m.). The study was conducted in compliance with the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as revised on 1 June 2006).

2.2. Preparation of acamprosate solution

Acamprosate was kindly gifted by Merck Sante (Lyon, France) and dissolved in water to give stock solutions of 40 mg/ml for experiments in vivo and 10 mM for experiments in vitro.

2.3. Voluntary ethanol consumption in rats under the two-bottle-choice drinking paradigm

A total of 80 rats were used in the experimental design shown in Fig. 1A. A 10% (v/v) solution of ethanol (henceforth termed "ethanol solution") was prepared from water and 99.5% reagent-grade ethanol (Nacalai Tesque, Kyoto, Japan). Rats were given free access to ethanol solution as the sole source of fluid for five weeks. Food was available ad libitum. After the five-week period of forced ethanol consumption, the rats were given a free choice between two drinking bottles, one containing ethanol solution and the other containing water only. The period of free choice was continued for nine weeks. The placement of the drinking bottles was reversed every day to prevent the effect of position preference. The amount of ethanol solution and water consumed were recorded every two or three days and ethanol consumption was expressed as a percentage (w/w) of total fluid consumption, a measure termed "ethanol preference". In a preliminary experiment, ethanol deprivation produced a significant increase in ethanol consumption in rats with an ethanol preference of more

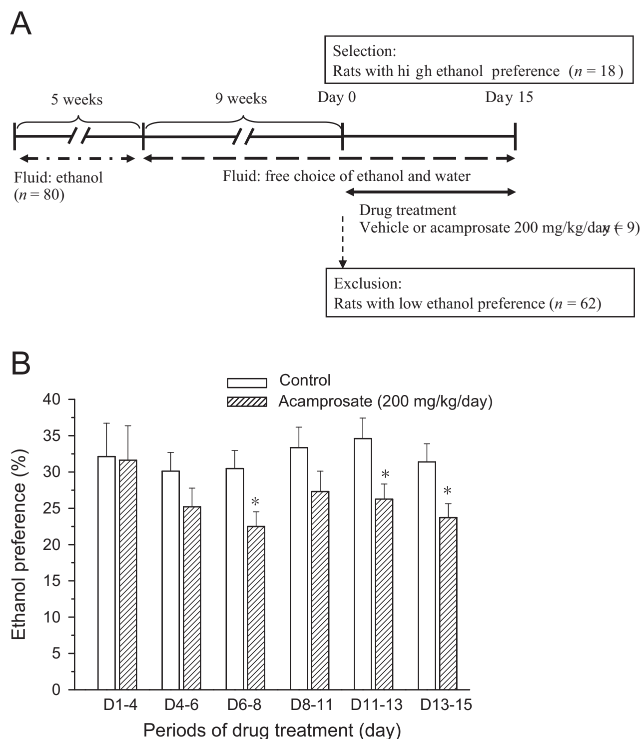

Fig. 1. Effect of acamprosate on voluntary ethanol intake in rats selected for high ethanol preference. (A) Schedule of voluntary ethanol intake in the free-choice procedure in rats before and after selection for high ethanol preference. The selected rats were given free access to ethanol solution and water and were treated orally once daily with acamprosate (200 mg/kg/day) or vehicle. (B) Effect of acamprosate on ethanol preference. Each bar represents the mean ± S.E.M. for eight rats (D6–8, D11–13 and D13–15; one rat knocked over one of the drinking-bottles, so that rat's consumption could not be measured) or nine rats (D1–4, D4–6 and D8–11) every two or three days. *$P < 0.05$ versus vehicle-treated control rats (Student's *t*-test).

than 25%, but not in rats with an ethanol preference of less than 25%. Therefore, after the nine-week period of free choice, those rats with an ethanol preference of more than 25% were selected for further analysis. These rats, 18 in number, were divided into two groups of nine by randomized block design by their ethanol preference and ethanol intake (g/kg/day) with SAS System version 8.2 (SAS Institute, Cary, NC, USA). For a period of 15 days after selection, the 18 rats continued to be presented with a free choice between ethanol solution and water. During this 15-day period, the rats in group 1 were treated orally once a day with vehicle (water) while the rats in group 2 were treated orally once a day with 200 mg/kg acamprosate, and their fluid consumption was measured and expressed as ethanol preference.

2.4. Induction of ethanol-deprivation effect in rats

A further 80 rats were used in the experimental design shown in Fig. 2A. As in the voluntary-ethanol-consumption study, a five-week period of forced ethanol consumption was followed by a nine-week period of free choice. After this, 16 rats were selected for high ethanol preference and were treated orally once a day with vehicle (control group; $n=8$) or 200 mg/kg acamprosate (treatment group; $n=8$). For the first 21 days, the rats continued to be presented with a free choice between ethanol solution and water. As a measure of basal drinking, the amount of ethanol solution and water consumed were recorded for the last two days just before the ethanol-deprivation period. On day 22, the bottle containing the ethanol solution was removed from the cage for three days. During this ethanol-deprivation period, the rats had

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