Neuroscience Letters 490 (2011) 90–95

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Acamprosate reduces ethanol drinking behaviors and alters the metabolite profile in mice lacking ENT1

Moonnoh R. Lee[a,d,1], David J. Hinton[a,1], Jinhua Wu[a], Prasanna K. Mishra[d], John D. Port[e], Slobodan I. Macura[d], Doo-Sup Choi[a,b,c,*]

[a] Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN, USA
[b] Department of Psychiatry and Psychology, Mayo Clinic College of Medicine, Rochester, MN, USA
[c] Neurobiology of Disease Program, Mayo Clinic College of Medicine, Rochester, MN, USA
[d] Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, USA
[e] Department of Radiology, Mayo Clinic College of Medicine, Rochester, MN, USA

ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form
10 December 2010
Accepted 12 December 2010

Keywords:
Alcoholism
ENT1
Glutamate
Taurine
In vivo MRS
Acamprosate

ABSTRACT

Acamprosate is clinically used to treat alcoholism. However, the precise molecular functionality of acamprosate in the central nervous system remains unclear, although it is known to antagonize glutamate action in the brain. Since elevated glutamate signaling, especially in the nucleus accumbens (NAc), is implicated in several aspects of alcoholism, we utilized mice lacking type 1 equilibrative nucleoside transporter (ENT1), which exhibit increased glutamate levels in the NAc as well as increased ethanol drinking behaviors. We found that acamprosate significantly reduced ethanol drinking of mice lacking ENT1 (ENT1$^{-/-}$) while having no such effect in wild-type littermates. We then analyzed the basal and acamprosate-treated accumbal metabolite profiles of ENT1$^{-/-}$ and wild-type mice using *in vivo* 16.4 T proton magnetic resonance spectroscopy (MRS). Our data show that basal glutamate + glutamine (Glx), glutamate, glutamine and *N*-acetylaspartatic acid (NAA) levels are increased in the nucleus accumbens (NAc) of ENT1$^{-/-}$ compared to wild-type mice. We then found that acamprosate treatment significantly reduced Glx and glutamine levels while increasing taurine levels in the NAc of only ENT1$^{-/-}$ compared to their saline-treated group while normalizing other metabolite compared to wild-type mice. This study will be useful in the understanding of the molecular basis of acamprosate in the brain.

Acamprosate (Campral®) is a taurine homologue, which has been FDA-approved and is clinically used to treat alcoholism [34]. However, the effect of acamprosate is not uniform in all patients, with some patients responding better to this drug than others [15]. Therefore, understanding the molecular function of acamprosate and how brain metabolites are affected by its administration will help in the prediction of a successful reduction in alcohol drinking in humans [12,18].

Adenosine is one of the main inhibitory neuromodulators that regulates glutamate levels in response to alcohol [1]. An ethanol-sensitive nucleoside transporter, type 1 equilibrative nucleoside transporter (ENT1), regulates adenosine levels in response to acute ethanol treatment [16]. Human genetic association studies indicate that allelic variants of *SLC29A1* (human ENT1) are associated with an alcohol abuse phenotype in women [10]. A number of recent studies also suggest that ENT1 gene expression is inversely correlated with ethanol drinking and sensitivity to the acute intoxicating effects of ethanol in several rodent models [1,5,19,24,25]. Specifically, mice lacking ENT1 (ENT1$^{-/-}$) exhibit reduced ataxic and hypnotic effects of acute ethanol intoxication as well as increased ethanol drinking compared to wild-type littermates [4,5,17]. Based on our previous studies, these behaviors appear to be mediated by constitutively increased glutamate levels in the NAc of ENT1$^{-/-}$ mice [4,5,17]. Since chronic ethanol treatment and withdrawal are known to increase extracellular glutamate levels in several brain regions [22,23], ENT1$^{-/-}$ mice may be used as a model representing adenosine-mediated, constitutively increased glutamate signaling in alcoholism [4,5,17].

Here we report that acamprosate significantly reduces ethanol drinking in ENT1$^{-/-}$ mice. Using *in vivo* 16.4 T [^{1}H] MRS, we investigated the basal and acamprosate treated metabolite profile in the NAc of ENT1$^{-/-}$ mice. Our results indicate that acamprosate may

* Corresponding author at: Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA. Tel.: +1 507 284 5602; fax: +1 507 266 0824.
E-mail address: choids@mayo.edu (D.-S. Choi).
[1] These authors contributed equally to this study.

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.12.033

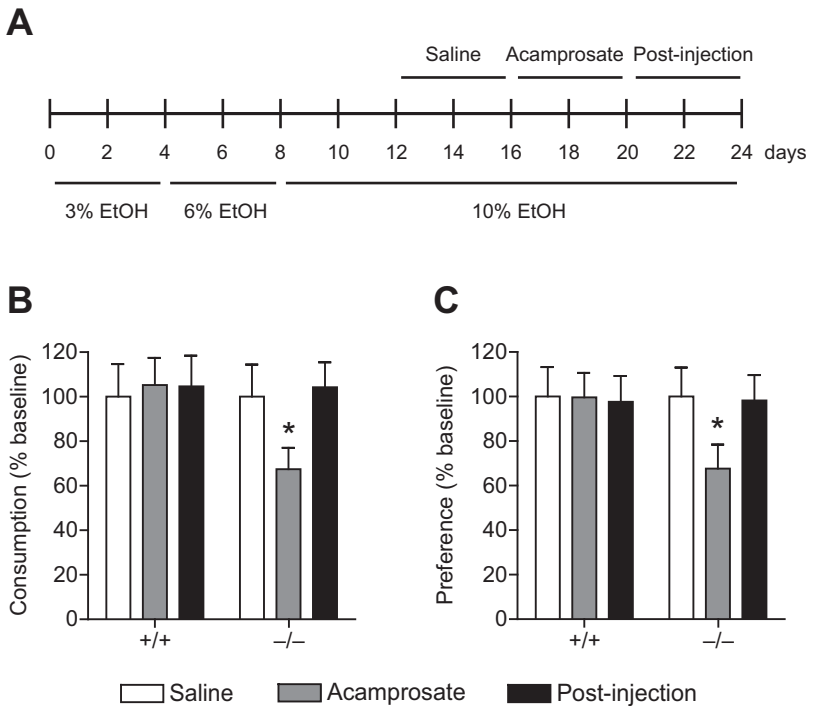

Fig. 1. Reduced ethanol consumption and preference by acamprosate administration in ENT1$^{-/-}$ mice. (A) Mice were given 12 days to acclimate to a 10% (v/v) ethanol concentration. Then mice were injected with saline and acamprosate (4 days each) during the two-bottle drinking experiment. The post-injection period refers to the 4 days following the acamprosate injection period. (B) Reduced ethanol consumption and (C) preference in ENT1$^{-/-}$ mice during the acamprosate administration period compared to the saline injection period. $n = 15$–16 mice per genotype and per treatment group. All data are expressed as mean ± SEM. *$p < 0.05$ compared to respective saline treatment groups by one-way repeated measures ANOVA.

reduce ethanol drinking by normalizing Glx levels and increasing taurine levels in the NAc of ENT1$^{-/-}$ mice.

ENT1$^{-/-}$ mice were generated as described previously [5]. F1 generation mice were generated by crossing 129X1/SvJ background heterozygous ENT1 mice with C57BL/6J wild-type mice. The C57BL/6J and 129X1/SvJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The heterozygous F1 mice were then crossed to generate F2 generation mice. Only 8–16 week old male F2 generation homozygote wild-type and ENT1$^{-/-}$ mice were used in all experiments. Animal care and handling procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committees in accordance with NIH guidelines.

The effect of acamprosate on oral alcohol self-administration and preference were examined using a modified two-bottle choice drinking experiment [13]. To examine the effect of acamprosate on ethanol consumption and preference, mice were given 200 mg/kg (*i.p.*) acamprosate (Estechpharma, Korea) every 12 h for four additional days after mice steadily consumed 10% ethanol in a two-bottle choice drinking experiment.

In vivo magnetic resonance spectroscopy (MRS) was performed on a 16.4 T (700 MHz) Bruker Avance III 700WB spectrometer with an 89 mm vertical bore (Bruker BioSpin, Billerica, MA). Mice were anesthetized in an isolated chamber using 3.5% isoflurane. For the basal metabolite level measurements, mice were injected with saline (*i.p.*) and to analyze the effect of acamprosate, mice were given a 200 mg/kg *i.p.* injection of acamprosate. Mice were immediately placed in the anesthesia chamber for 5–10 min following injections and then inserted into the probe for scanning. For both experiments, the mouse typically spent about 95 min inside the magnet (5 min preparation time and 90 min scan time). Accumbal metabolites were quantified using LCModel Version 6.2-2 software [20]. Metabolites were quantified as percent ratio to total creatine (creatine + phosphocreatine) levels as an internal control to standardize for animal and instrumental variations as well as for the total number of cells analyzed within the volume of interest (VOI). Pilot fast low-angle shot (FLASH) images were recorded for placement of an 8 μl (2 mm × 2 mm × 2 mm) accumbal VOI [28]. Then, method specific local magnet field homogenization was performed. A point-resolved spectroscopy sequence (PRESS) was used with a repetition time (TR) of 1768 ms, an echo-time (TE) of 10 ms, and a number of averages (NA) of 3072. Consistent spectral resolution was ensured by considering LCModel calculated Cramér–Rao lower bounds. LCModel quantified glutamate + glutamine (Glx) with a Cramér–Rao lower bounds of less than 10%; *N*-acetylaspartatic acid (NAA), glutamate, glutamine, glycerophosphocholine + phosphocholine (GPC + PCh), guanosine, taurine, γ-aminobutyric acid (GABA), *myo*-inositol, and phosphocreatine with a Cramér–Rao lower bounds of less than 20%; and *N*-acetylaspartylglutamic acid (NAAG), alanine and lactate with a Cramér–Rao lower bounds of less than 35% in all spectra.

All data are presented as the mean ± SEM (standard error of the mean). Data were analyzed by two-tailed *t*-test, one-way or two-way repeated measures ANOVA. Results were considered significantly different when $p < 0.05$.

We examined whether acamprosate could reduce ethanol drinking behaviors in ENT1$^{-/-}$ mice in a two-bottle drinking experiment. When analyzing the drinking behaviors of ENT1$^{-/-}$ and wild-type mice we found that acamprosate significantly reduced ethanol consumption (Fig. 1B; $n = 16$ per genotype) and preference (Fig. 1C; $n = 15$–16 per genotype) of ENT1$^{-/-}$ mice compared to respective saline injection periods in a two-bottle choice drinking experiment. As reported, ENT1$^{-/-}$ mice consume more ethanol than wild-type mice under basal conditions [5]. Thus, to examine the antidipsotrophic effect of acamprosate, we analyzed the data after normalization by baseline. For ethanol consumption, one-way repeated measures ANOVA on ENT1$^{-/-}$ mice indicated a significant effect of acamprosate treatment in ENT1$^{-/-}$ mice ($F_{2,24} = 8.395$, $p = 0.002$) but not in wild-type littermates. For ethanol preference,