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Effect of chronic acamprosate treatment on voluntary alcohol intake and β-endorphin plasma levels in rats selectively bred for high alcohol preference

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Abstract

Our previous studies have shown that repeated acamprosate administration to ethanol-naive Warsaw high preferring (WHP) rats resulted in increased plasma β-endorphin levels and at least partially prevents increases in levels of this peptide after a single administration of ethanol compared with untreated control rats. The objective of the present study, which included 45 WHP rats, was to continue the past research and investigate the effect of 10-day acamprosate treatment (200 mg/kg p.o.) on alcohol intake using a free-choice procedure and on changes in plasma β-endorphin levels while alcohol is available, and 10 days after alcohol withdrawal. Voluntary alcohol consumption increases plasma levels of β-endorphin from 440 ± 25 pg/ml to 711 ± 57 pg/ml ($p = 0.0002$). After a 10-day of alcohol withdrawal, the levels of this peptide were significantly reduced compared with levels in rats with free access to ethanol (711 ± 57 pg/ml vs. 294 ± 38 pg/ml, $p = 0.000001$) and in control naive rats (440 ± 25 pg/ml vs. 294 ± 38 pg/ml, $p = 0.044$). Chronic treatment with acamprosate increased plasma β-endorphin levels both in WHP rats with free access to ethanol (440 ± 25 pg/ml vs. 616 ± 49 pg/ml, $p = 0.008$) and in rats after ethanol withdrawal (440 ± 25 pg/ml vs. 620 ± 56 pg/ml, $p = 0.007$). In the group with free access to ethanol, there was a significant reduction in mean ethanol intake, from 6.75 ± 0.20 g/kg body weight/day to 4.68 ± 0.25 g/kg/day. Our results indicate that chronic acamprosate treatment may have beneficial effects, as it increases the β-endorphin concentration thereby compensating for β-endorphin deficiency during ethanol withdrawal. As the endogenous opioid system has an important role in the development of craving for alcohol, restoring the alcohol-induced deficits in β-endorphin levels may be an important factor to prevent craving and maintaining abstinence. We suppose that the anti-craving mechanism of acamprosate that has been reported to abolish excessive glutamate release during alcohol withdrawal may be accompanied by compensation for the β-endorphin deficiency.

Keywords: Acamprosate; Alcohol-preferring rats; β-Endorphin; Free-choice procedure; Ethanol withdrawal

Acamprosate (calcium acetyl homotaurine) is a synthetic derivative of homotaurine and has been shown to be an effective drug in the treatment of alcohol dependence. Although the precise mechanism of acamprosate action is unknown, several studies have indicated that acamprosate attenuates the hyperexcitatory effects of glutamate during alcohol withdrawal [13]. By attenuating the long-lasting glutamatergic hyperactivity, acamprosate can reduce negative craving and help maintain abstinence [13]. Some studies have also suggested that endogenous opioids, in particular endorphins, may be implicated in the development of craving for addictive drugs and alcohol [4,40,41,42,43]. Deficiencies in β-endorphin levels were observed both in abstinent alcoholic individuals and in individuals at high risk of alcoholism, as well as in alcohol-preferring animals [12,19]. Animal studies have shown that alcohol consumption stimulates the release of opioid peptides, especially β-endorphin by cells of the pituitary gland and neurons of the brain regions that are associated with the reinforcement and the reward systems [5,17,18,31,33]. The positive reinforcing properties of β-endorphin in rats injected intracerebroventriculary and measured using conditioned place preference paradigm have been reported [1]. Moreover, opioid antagonists decreased the levels of alcohol self-administration in rats [9,10,22,39], supporting a role for endogenous opioids in

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alcohol reward and reinforcing mechanisms. The ethanol-induced changes in the activity of the endogenous opioid may lead to high ethanol consumption.

In a previous study, we have shown that repeated administration of acamprosate to Warsaw high preferring (WHP) rats increased plasma β-endorphin levels [47]. A comparative clinical study by Kiefer et al. [26] has confirmed the modulating effect of acamprosate on β-endorphin release, especially in the subgroups of patients during alcohol withdrawal after a previously high alcohol intake.

It has been shown that acamprosate reduces the levels of voluntary ethanol intake in rats [6]. Many studies have confirmed the effectiveness of acamprosate on the alcohol-deprivation effect (ADE) [20,21]. It is a model of relapse in which a period of imposed ethanol deprivation results in transiently increased alcohol consumption when alcohol is again available.

The present study was designed to investigate the effectiveness of acamprosate on ethanol consumption using a model involving continuous, free access to alcohol, and monitoring the β-endorphin levels in chronic alcohol drinking WHP rats as well as in WHP rats treated with acamprosate with or without access to alcohol.

The research was performed using WHP rats, as this animal model mimics some aspects of alcohol dependence seen in human alcoholics. The WHP line fulfils most requirements of the animal model of alcoholism [15,16].

The study involved 45 female adult rats from the $F_{36,37}$ generation of the WHP animal line, each of which weighed 220–280 g and were kept under standard laboratory conditions. For consistency, we used females in the present study as in the previous studies [47,48]. It has been reported that female rodents consume more ethanol than male rodents [24]. Moreover, numerous studies have shown that ethanol administration had no effect on the estrus cycle [38,44].

Animals were housed individually in stainless steel cages equipped with two graduated drinking tubes, containing tap water or 10% (v/v) alcohol. Alcohol solution was prepared from water and a stock solution of 95% reagent-grade ethanol. Following the method of Rezvani et al. [37], rats were given free access to a solution of 10% (v/v) ethanol as a sole source of fluid for 3 days. Food was available ad libitum. This procedure allowed them to become accustomed to drinking from the tubes and to experience the taste and pharmacological properties of alcohol. For the next 21 days the rats were given a free choice between 10% alcohol and water. Alcohol intake was recorded and tubes refilled daily. The position of the alcohol and water tubes was alternated daily to control for side preference. Animals were weighed every 3 days. After the 21-day period, the rats received intragastrically 1% methylcellulose (vehicle) or acamprosate suspended in 1% methylcellulose (200 mg/kg, 2 ml/kg b.w., daily), over the course of 10 days, according to Table 1.

Sep-pak C-18 cartridges were obtained from Waters M.A., USA cat. no. WAT 020515; acetone (HPLC grade) and trifluoroacetic acid (TFA; HPLC grade) were from Baker; aprotinin (Trascolan®) was purchased in Jelfa, Poland; and acamprosate (Campral®) was from Lipha. The plasma β-endorphin radioimmunoassay kit was obtained from Phoenix Pharmaceuticals, Inc., USA.

The rats were anaesthetized with ether 24 h after the last administration of acamprosate and blood samples was collected by heart puncture. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Research Committee.

Blood samples were collected in tubes containing EDTA (1.6 mg/ml) and gently rocked several times to prevent coagulation. Samples were transferred to centrifuge tubes containing aprotinin (500 KIU/ml) and gently rocked several times to inhibit proteinase activity. The samples were then cooled in an ice-bath immediately. The plasma was separated by centrifugation at $1600 \times g$ for 15 min at 4 °C, and was then frozen and stored at −20 °C until assessment.

Plasma β-endorphin was determined after extraction by the acid–acetone method. The procedure for β-endorphin extraction used Sep-pak C-18 cartridges according to the method of Angwin and Barchas [3] that was subsequently modified by Zalewska-Kaszubska and Obzejta [46].

Before loading on Sep-Pak C-18 cartridges, plasma samples were acidified with the same volume of 1% TFA and centrifuged at 10,000 g for 20 min at 4 °C. C-18 Sep-columns were activated by passing 2 ml of acetone through them and then equilibrated twice with 2 ml of 1% TFA in distilled water. The acidified plasma solution supernatants were loaded onto the columns. The columns were washed twice with 2 ml of 1% TFA. β-endorphins were eluted with 1.5 ml of 1% TFA/acetone (25:75) and dried under vacuum. Plasma levels of β-endorphin were estimated using a radio-immunoassay kit. The intra-assay coefficient of variation was less than 9%.

All data were expressed as means ± S.E.M. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by post-hoc LSD (least significant differences) analysis. Normal distribution of data was tested using the Kolmogoroff–Smirnov test with Lillieforse correction. Differences were considered significant at $p < 0.05$.

The one-way ANOVA results showed significant main effect for changes in β-endorphin concentrations ($F_{4,40} = 10.86$, $p = 0.00005$). As shown in Fig. 1, the baseline plasma level of β-endorphin in the WHP rats was 440 ± 25 pg/ml. In rats with free access to ethanol, the levels of this peptide were significantly increased (711 ± 57 pg/ml vs. 440 ± 25 pg/ml., $p = 0.0002$). After 3 weeks of free access to ethanol followed by 10 days of alcohol withdrawal, the β-endorphin level was significantly lower than the level in the group with free access to alcohol (294 ± 38 pg/ml vs. 711 ± 57 pg/ml, $p = 0.000001$) and the control group (294 ± 38 pg/ml vs. 440 ± 25 pg/ml, $p = 0.044$). It was observed that acamprosate 200 mg/kg body weight administrated intragastrically for 10 days significantly increased β-endorphin plasma levels in the group with free access to alcohol (440 ± 25 pg/ml vs. 616 ± 49 pg/ml, $p = 0.008$) and in the abstinent rats (440 ± 25 pg/ml vs. 620 ± 56, $p = 0.007$) (Fig. 1). Baseline ethanol intake was 6.75 ± 0.20 g/kg body weight/day. As seen in Fig. 2, acamprosate significantly reduced the level of ethanol intake. The one-way ANOVA results