Effect of acute alcohol injection on plasma beta-endorphin levels in Warsaw high-preferring rats treated with acamprosate and naltrexone

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Abstract:

Naltrexone and acamprosate are the most effective drugs in reducing alcohol consumption, operating through different pharmacological mechanisms. Some studies have demonstrated that naltrexone and acamprosate in combination have significantly greater effectiveness in alcohol therapy than either agent used alone. We have previously found that beta-endorphin plasma levels are increased after repeated treatment with either of these drugs.

In this study, we examined the effect of a single administration of ethanol on the beta-endorphin levels in rats treated with both naltrexone and acamprosate. We used Warsaw High Preferring (WHP) rats and treated them for 10 days with naltrexone (2 mg/kg, *ip*) and acamprosate (200 mg/kg, *po*) or with each of these drugs separately. The control group was treated with saline and 1% methylcellulose. One hour before blood collection, the rats were injected with a single dose of ethanol or saline. We observed the increases in beta-endorphin levels after a single administration of ethanol to untreated rats compared with a single administration of saline. The same increase was observed after a single administration of ethanol to rats treated with naltrexone or naltrexone and acamprosate. However, a single injection of ethanol to rats treated only with acamprosate resulted in smaller increases in plasma beta-endorphin content. As the endogenous opioid system has an important role in the development of craving for alcohol, restoring the alcohol-induced deficits of beta-endorphin in the reward system may be an important factor contributing to preventing craving and relapse to drinking. Therefore, we suggest that the similar changes in the activity of beta-endorphin following therapy with either naltrexone alone or combined naltrexone plus acamprosate, may explain why the combined drug therapy is not more effective in treating alcoholism than naltrexone alone. The present findings support the results from some randomized clinical trials demonstrating that the efficacy of acamprosate plus naltrexone in the treatment of alcoholism was not significantly different from the efficacy of the treatment with naltrexone alone.

Key words:
beta-endorphin, naltrexone, acamprosate, ethanol, alcohol-preferring rats

Introduction

Monotherapies have shown only modest efficacy for the treatment of alcohol abuse and alcohol dependence. Some studies have shown that the combined administration of medicines with different mechanisms of action is more effective in treating alcoholism than administration of these drugs alone [21, 26, 30]. Such a combination of medicines with different mechanisms of action is likely to increase efficacy of therapy as well as allow to use lower doses of each medication, improve tolerance and reduce adverse effects. Several studies have shown that combination therapy with naltrexone and acamprosate, the most effective drugs in the treatment of alcohol dependence, have significantly greater effectiveness in alcoholism therapy than either drug used alone [13].

These two drugs reduce alcohol consumption *via* different pharmacological mechanisms. Naltrexone is an antagonist of the opioid receptors, which are known to mediate some of the rewarding effects of alcohol, and its action is thought to reduce the reinforcing effect or behavioral response to alcohol [22]. Acamprosate, which is the second most-effective agent for the treatment of alcoholism, is a structural analog of gamma-aminobutiric acid and affects both GABAergic and N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission [25]. Acamprosate is known to normalize the dysregulation of NMDA-mediated glutamatergic excitation that occurs after alcohol withdrawal and the early phases of abstinence [3]. Some randomized, placebo-controlled clinical trials have shown that both acamprosate and naltrexone used separately help maintain abstinence; however, neither monotherapy is fully effective [23, 28, 29]. Clinical observations have indicated that both naltrexone and acamprosate reduce alcohol craving [6, 24, 35]. It is thought that naltrexone is likely to reduce alcohol craving and relapse to heavy drinking through modulation of the mesolimbic dopamine activity [31]. Several studies suggest that activation of the central dopamine reward system may be associated with activation of beta-endorphin in the mesolimbic pathway [7, 12, 17].

It is well known that ethanol consumption increases the release of opioid peptides, especially beta-endorphin [37], which interacts with brain structures that are closely associated with the reward and positive reinforcement centers of the brain [18]. Some studies have suggested that endorphins may be involved in the development of craving for addictive drugs [16, 32, 33]. In alcoholics, reduced plasma beta-endorphin levels have been observed [1, 20] and may be one of the most important factors responsible for difficulties in maintenance of abstinence.

Our previous studies have shown that repeated treatment with acamprosate or naltrexone monotherapies increase the plasma beta-endorphin levels in Warsaw High-Preferring alcohol rats (WHP) [38, 39]. The WHP rat is a model that mimics some aspects of alcohol dependence seen in human alcoholics and fulfils most criteria for an animal model of alcoholism. WHP rats voluntarily drink ethanol in amounts exceeding 6 g/kg daily [27] to give blood ethanol concentrations of 0.045 g/dl. WHP rats also develop visible signs of physical dependence such as piloerection, tremor and muscle rigidity during chronic free-choice drinking [8, 9].

We have previously found that changes in the release of beta-endorphin peptide after a single administration of ethanol differ between rats treated with acamprosate and those treated with naltrexone [38, 39]. In naltrexone-treated WHP rats, a single dose of ethanol did not affect the increased plasma beta-endorphin level; however, in acamprosate-treated rats the plasma beta-endorphin levels were significantly lower compared to those observed in untreated rats injected with ethanol.

The aim of this study was to compare the response of pituitary beta-endorphin to acute alcohol administration in animals treated chronically with acamprosate and naltrexone in combination as well as in the rats treated with either naltrexone or acamprosate (monotherapy).

Materials and Methods

Animals

The experiments were performed on female adult rats from the F_{36} generation of the WHP line; each rat weighed 220–290 g and was kept under standard laboratory conditions. Thirty rats were involved in the experiment, and were divided into five groups with six animals in each group. One group (six animals) re-

ceived 10-day treatment with naltrexone administered intraperitoneally (*ip*) (2 mg/kg; 0.2 ml/100 g, daily) and acamprosate administered intragastrically (*ig*) (200 mg/kg; 0.2 ml/100 g, daily). The second group of animals received saline (*ip*) and acamprosate (*ig*), and the third group received 1% methylcellulose (*ig*) and naltrexone (*ip*) Two control groups were treated with saline *ip* (0.2 ml/100g), and with 1% methylcellulose *ig* (0.2 ml/100 g). Prior to the collection of the blood samples, rats of one of the two control groups were injected *ip* with saline, while the remaining 24 WHP rats were injected *ip* 2g with ethanol/kg (20% w/v).

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.

Materials

Sep-pak C18 cartridges were obtained from Waters (M.A., USA cat. No. WAT 020515); Acetone (HPLC grade) and trifluoroacetic acid (HPLC grade) were from Baker. Aprotinin (Trascolan) was purchased from Jelfa, Poland; Naltrexone hydrochloride was from Sigma; Acamprosate (Campral) was from Lipha. Ether was purchased from POCh, Poland. The plasma beta-endorphin radioimmunoassay kit was obtained from Phoenix Pharmaceuticals, Inc., USA.

Blood sampling procedure

Twenty-four hours after the last administration of naltrexone and acamprosate or saline and 1% methylcellulose, the rats were anesthetized with ether and blood samples were collected by heart puncture. The rats were injected *ip* with ethanol (20% w/v; 2 g/kg /10 ml) or an equal volume of saline 1 h before blood collection.

Blood samples were collected into tubes containing EDTA (1.6 mg/ml) and gently rocked several times to prevent coagulation. The samples were then 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. The plasma was separated by centrifugation

at $1,600 \times g$ for 15 min at 4°C. The plasma was frozen and stored at –20°C until assessment.

Solid phase extraction of peptides from plasma

Plasma beta-endorphin levels were determined after extraction by the acid-acetone method. The procedure for beta-endorphin extraction involved Sep-pak C 18 cartridges in accordance with the method of Angwin and Barchas [2] that was subsequently modified by Zalewska-Kaszubska and Obzejta [36].

Before loading onto Sep-Pak C-18 cartridges, samples of plasma were acidified with the same volume of 1% trifluoroacetic acid (TFA) and centrifuged at $10,000 \times g$ for 20 min at 4°C. C-18 Sep-columns were activated by passing 2 ml of acetone and subsequently equilibrated twice with 2 ml of 1% TFA in distilled water. The supernatant of the acidified plasma solution was then loaded onto the columns. The columns were washed twice with 2 ml of 1% TFA. Beta-endorphins were eluted with 1.5 ml of 1% TFA/acetone (25:75) and dried under vacuum conditions.

Plasma levels of beta-endorphin were estimated by radioimmunoassay, using a kit supplied by Phoenix Pharmaceuticals, Inc. USA.

Statistical analysis

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

Results

The one-way ANOVA demonstrated a significant main effect [$F_{(4, 25)} = 11.81$, $p < 0.05$]. As shown in Figure 1, acute administration of alcohol (2 g/kg) to WHP rats treated for 10 days with saline and 1% methylcellulose (control group of rats) induced a significant increase in the plasma beta-endorphin levels, from 292 ± 46 pg/ml to 668 ± 69 pg/ml. Rats *ip* treated with naltrexone alone (2 mg/kg) for 10 days presented a similar increase in plasma beta-endorphin
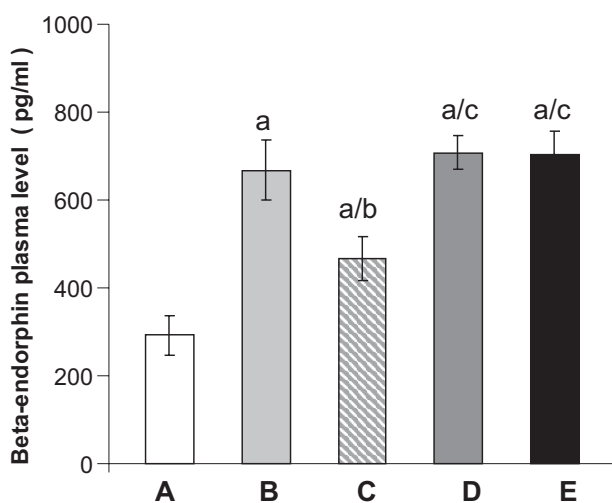
Fig. 1. Effects of a single injection of ethanol (2 g/kg) on beta-endorphin plasma level in rats treated with naltrexone (2 mg/kg) and acamprosate (200 mg/kg). Experimental groups: **A** – control group treated with saline and 1% methylcellulose for 10 days and injected with saline before blood collection; **B** – group treated with saline and 1% methylcellulose for 10 days ; **C** – group treated with acamprosate and saline for 10 days; **D** – group treated with naltrexone and 1% methylcellulose for 10 days; **E** – group treated both with naltrexone and acamprosate for 10 days. All animals in groups **B**,**C**,**D** and **E** were injected with ethanol 60 min before blood collection. Values are expressed as the mean ± SEM in each group of six rats. [a] $p < 0.05$ in comparison to control group (**A**) injected with 0.9% NaCl before blood collection; [b] $p < 0.05$ in comparison to untreated group (**B**) injected with ethanol before blood collection [c] $p < 0.05$ in comparison to group (**C**) treated with acamprosate and injected with ethanol before blood collection

levels in response to the *ip* injection of 2 g of ethanol per kg as that observed in control rats (708 ± 39 pg/ml). However, rats treated with acamprosate for 10 days presented a lower increase in plasma beta-endorphin levels in response to the *ip* injection of 2 g of ethanol per kg than either control or naltrexone-treated groups of rats (468 ± 50 pg/ml). Rats treated for 10 days with both drugs in combination presented an increase in plasma beta-endorphin levels in response to the acute administration of 2 g of ethanol per kg, that was similar to the increase observed in the control and naltrexone alone groups of rats (702 ± 56 pg/ml).

Discussion

The present study was designed to determine the effect of combined application of naltrexone and acamprosate on the changes in the plasma beta-endorphin levels in response to a single administration of ethanol to WHP rats. Our previous studies showed that chronic treatment of rats with any one of these two drugs resulted in elevated plasma beta-endorphin levels [38, 39]. The increased levels of plasma beta-endorphin were also observed in human alcoholics treated with acamprosate [14]. On the basis of published reports [32, 33] and our previous studies with acamprosate [38] and naltrexone [39], we then proposed that an increase in the beta-endorphin levels following chronic treatment with each of these drugs might be an important factor in the prevention of craving and relapse in alcohol-dependent individuals. Deficits of beta-endorphin have been observed in both abstinent alcoholics and individuals with a family history of alcoholism [5], as well as in alcohol-preferring animals [38]. It has also been observed that in the mutant mice with beta-endorphin deficiency self-administration of ethanol increased [10].

As incidental alcohol consumption often leads to relapse to alcohol abuse, we examined the effects of a single alcohol injection on the plasma beta-endorphin levels in WHP rats treated for 10 days with both naltrexone and acamprosate, the effects on the corresponding untreated control rats as well as on the rats treated with either naltrexone or acamprosate (monotherapy). After a single administration of ethanol to control rats we observed that the level of beta-endorphin increased. Similar increase was observed after a single administration of ethanol to rats treated with naltrexone or naltrexone and acamprosate in combination. However, similar injection of ethanol to rats treated with acamprosate alone resulted in a smaller increase in plasma beta-endorphin levels. We established in our previous study that plasma beta-endorphin level in WHP rats increased after chronic treatment with naltrexone [39] and acamprosate [38] and was unaffected by a single injection of ethanol. Rats treated with acamprosate alone showed a lower increase in plasma beta-endorphin levels in comparison to ethanol-injected group [38]. In these rats, chronic acamprosate treatment significantly limited the increase in this peptide after a single ethanol administration in comparison to control rats [38]. We suppose that an increase in beta-endorphin concentration to a level observed in response to a single ethanol administration may help maintain abstinence. In acamprosate-treated rats, the plasma beta-endorphin level is smaller than in control rats after acute alcohol injection and probably efficacy of this drug is limited.

Chronic naltrexone treatment increases the plasma beta-endorphin concentrations to similar levels as those observed after a single ethanol administration in control rats and this may explain the better effectiveness of this drug. These results are in agreement with those of meta-analyses that indicate that acamprosate may be more effective in maintaining complete abstinence, whereas naltrexone is better in reducing the number of days of heavy alcohol consumption [4, 19]. Feeney et al. [11] have compared the efficacy of combined acamprosate and naltrexone treatment with cognitive behavioral therapy in maintaining alcohol abstinence, with the efficacy of either monotherapy. Although they observed that combined acamprosate and naltrexone therapy produced the greatest improvement, the efficacy of the acamprosate plus naltrexone treatment was not significantly different from the efficacy of the naltrexone alone. In addition, results from pooled analysis of seven European trials including 1,485 alcohol-dependent patients showed only moderate effects with acamprosate alone. However, some evidence indicates that the combination of acamprosate with naltrexone leads to substantially better outcomes [34].

We suggest that the better effectiveness of the naltrexone therapy is due, at least in part, to the long-term blockade of the opioid receptors by naltrexone which leads to a compensatory increase in the beta-endorphin concentration ameliorating its deficiency in the mesolimbic reward system. In addition, naltrexone, which works by blocking the opioid receptors, may prevent some of the reinforcing effects of alcohol consumption. If an increase in beta-endorphin concentration to a level observed in response to a single ethanol administration helps maintain abstinence, it is more difficult to maintain abstinence after acamprosate because the acamprosate-induced increase in beta-endorphin concentration is smaller in comparison to that induced by ethanol administration in the control group of rats, and one dose of ethanol does not induce a significant increase in the beta-endorphin levels in the acamprosate-treated rats. This situation may lead to craving for alcohol and a relapse.

The results of the present study show that the response of the pituitary beta-endorphin to an acute ethanol application by rats treated with acamprosate in combination with naltrexone is similar to that by rats treated with naltrexone alone as well as to control ethanol injected rats. However, in comparison to control ethanol-treated group following an acute ethanol application, the plasma beta-endorphin levels decreased in rats treated with acamprosate alone. The present study contributes to our understanding of why naltrexone alone has shown better efficacy in clinical trials than acamprosate alone and why therapy with acamprosate and naltrexone combined is not associated with improved outcomes over the naltrexone alone therapy. However, since many factors are involved in alcohol dependence, the combined treatment with acamprosate and naltrexone may have additional benefits not associated with the opioid system. Furthermore, Kiefer and Wiedemann [15] have suggested that some alcohol-dependent patients respond selectively to naltrexone (reward-craving drinkers) or acamprosate (withdrawal-relief-craving drinkers).

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