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Effect of acute administration of ethanol on beta-endorphin plasma level in ethanol preferring and non-preferring rats chronically treated with naltrexone

Jadwiga Zalewska-Kaszubska [a,*], Dorota Gorska [a], Wanda Dyr [b], Elzbieta Czarnecka [a]

[a] Department of Pharmacodynamics, Medical University of Lodz, Muszynskiego 1, PL 90-151 Lodz, Poland
[b] Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland

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Abstract

An ample support can be found in professional literature for the hypothesis that the endogenous opioid system plays an important role in developing a craving for alcohol. It is well established that people with a genetic deficit of beta-endorphin are particularly susceptible to alcoholism. In our study, we looked into the beta-endorphin plasma level of animals with high- and low-risk of alcohol dependency after repeated treatment with naltrexone, the opioid antagonist known to be effective in the treatment of alcoholism. We used the Warsaw High Preferring (WHP) and Warsaw Low Preferring (WLP) rats and treated them for 10 days with naltrexone in a dose of 2 mg/kg i.p. One hour before blood collection the rats were injected with a single dose of ethanol. A prolonged naltrexone treatment or a single application of ethanol resulted in the increase of the beta-endorphin plasma level. In the WLP rats repeated naltrexone treatment prevents the ethanol-induced increase in beta-endorphin plasma level. In the WHP rats the level of this peptide was similar to it while they were undergoing the naltrexone treatment or had received a single alcohol injection. This finding supports the proposition that the endogenous opioid system plays an important role in developing a craving for alcohol. It is likely that effectiveness of naltrexone in reducing craving for alcohol results from the attenuation of the rewarding properties of ethanol and restoring the beta-endorphin deficit in reward system.

Keywords: Beta-endorphin; Naltrexone; Ethanol; Alcohol preferring rats

1. Introduction

Naltrexone, a nonselective opioid antagonist, is considered to be one of the most effective drugs used in the treatment of alcohol dependence. It is well accepted that naltrexone reduces the risk of heavy drinking among abstinent alcoholics (Kranzler et al., 2003). Some suggest that naltrexone may be even better suited for patients who continue drinking alcohol at the beginning of their treatment as compared to abstainers (Killeen et al., 2004). Pharmacological studies indicate that naltrexone as well as other opioid receptor antagonists significantly decreases alcohol intake (Cichelli and Lewis, 2002; Coonfield et al., 2002; O'Malley et al., 2002; Stromberg et al., 2002; Mhatre and Holloway, 2003; Stromberg, 2004). According to Ferraro et al. (2002), naltrexone's effectiveness results from the fact that it modifies the taste of alcohol to become more aversive. However, it is more likely that the efficacy of naltrexone results not from the alteration of taste but from its influence on the opioid system. Numerous reports support the hypothesis that the endogenous opioid system plays an important role in alcohol acceptance or lack thereof. It is well known that ethanol consumption increases the release of opioid peptides, especially beta-endorphin (Zalewska-Kaszubska and Czarnecka, 2005), which interacts with brain structures closely involved in the reward and positive reinforcement system (Koob et al., 1998). The after-alcohol-consumption release of beta-endorphin has been documented both in animals (Olive et al., 2001) and humans (Dai et al., 2002). A number of studies suggest that activation of beta-endorphin in the mesolimbic pathway may be

* Corresponding author.
E-mail address: jzalewska@pharm.am.lodz.pl (J. Zalewska-Kaszubska).

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associated with the activation of the central dopamine reward system (Koob et al., 1994; Froehlich, 1996; Di Chiara, 1997). Coonfield et al. (2004) showed that the effect of acute naltrexone treatment might be different in ethanol preferring rat lines. In our earlier studies, we observed that there was a different release of beta-endorphin peptide in rats selectively bred for preference as opposed to those bred for non-preference for alcohol, and the difference was striking after a single injection of ethanol (Zalewska-Kaszubska et al., 2005). The alcohol preferring rats, even after a single ethanol application, developed a higher beta-endorphin plasma concentration. Genetically determined, increased responsiveness of the opioid system to alcohol may contribute to a predisposition for alcoholism.

The reported difference in response to alcohol, depending on the genetic susceptibility, have encouraged us to study the effects of naltrexone treatment on beta-endorphin levels in rats with a low or high preference to alcohol. Thus, the aim of the present study was to investigate whether, after a repeated treatment with naltrexone, a single application of alcohol causes changes in the beta-endorphin plasma level in rat lines selectively bred for preference (Warsaw High Preferring–WHP) as opposed to those bred for non-preference (Warsaw Low Preferring–WLP) of alcohol. The WHP line fulfills a majority criteria for an animal model of alcoholism: they voluntary drank ethanol in the amount of 4–8 g/kg daily and achieved an ethanol concentration in blood of 0.045 g/dl. They also developed visible signs of physical dependence (Dyr and Kostowski, 2000, 2004). The WHP rats were less receptive to the sedative effect of ethanol in comparison to the WLP rats. Also, neurochemical studies proved that in the WHP rats, striatal concentrations serotonin and dopamine as well as their metabolites were lower in comparison with the WLP rats (Dyr et al., 1998).

Using the two types of rats, our goal is to investigate what changes in the beta-endorphin plasma levels occur in the treated subject after an incidental alcohol intake during the naltrexone treatment.

2. Materials and methods

2.1. Animals

The experiments were carried out on female adult rats from the $F_{29–31}$ generation of the WHP and WLP rat lines, weighting 320–400 g and kept under standard laboratory conditions. Forty-eight rats divided into 8 groups with 6 animals in every group participated in the experiment. Four groups consisted of WHP rats (24 animals) and 4 groups of WLP rats (24 animals). Two groups of the WHP rats (12 animals) and two groups of WLP rats (12 animals) received naltrexone intraperitoneally (2 mg/kg; 0.2 ml/100 g body weight, daily). Before a blood collection, 6 rats from the WHP group and 6 rats from WLP group were injected with ethanol while the remaining 6 WLP and 6 WHP rats were injected with saline in the same volume. The other 24 rats (12 WHPs and 12 WLPs) had been treated for 10 days only with saline in the same volume and before their blood was collected received an injection of ethanol or saline in the same way as naltrexone-treated rats.

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.

2.2. Blood sample procedure

24 hours after the last administration of naltrexone or saline, the rats were anaesthetized with ether and their blood samples were collected by heart puncture. The rats were injected with ethanol (20% w/v; 2 g/kg/10 ml) or saline in the same volume 1 h before blood collection.

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

2.3. Materials

Sep-pak C 18 cartridges were obtained from Waters MA, USA cat. No. WAT 020515; Acetone (HPLC grade) and trifluoroacetic acid (HPLC grade) were from Baker. Aprotinin (Trascolan®) was purchased in Jelfa, Poland; Naltrexone hydrochloride was from Sigma. Ether was purchased in POCh, Poland. The plasma beta-endorphin radioimmunoassay kit was obtained from Phoenix Pharmaceuticals, Inc., USA.

2.4. Solid phase extraction of peptides from plasma

Plasma beta-endorphin was determined after extraction by the acid–acetone method. The procedure for beta-endorphin extraction was based on the use of Sep-pak C 18 cartridges in accordance with the method by Angwin and Barchas (1982), modified by Zalewska-Kaszubska and Obzejta (2004).

Before loading on Sep-Pak C-18 cartridges, plasma in 2 ml volume was acidified in the same volume of 1% trifluoroacetic acid (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 and subsequently equilibrated twice with 2 ml of 1% TFA in distilled water. The supernatant of acidified plasma solution was 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.

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

2.5. Statistical analysis

All data were expressed as mean ± S.E.M. Statistical analysis was performed using three-way analysis of variance 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 $p<0.05$.

3. Results

As shown in Fig. 1 acute administration of alcohol (2 g/kg body weight) to rats treated for 10 days with saline caused the statistically significant increase of beta-endorphin plasma level both in low- and high-preferring rats. The main effect for alcohol was ($F_{1,40}=6.04$, $p<0.05$). After a single application of ethanol, the beta-endorphin plasma level increased from 292±69 pg/ml to 615±92 pg/ml and from 478±58 to 740±79 pg/ml in WHP and WLP rats, respectively. There was not a statistically significant main effect for strain and for naltrexone treatment. In three-way analysis of variance, three two-way interaction effects was observed. There was statistically significant interaction effects for strain × naltrexone treatment ($F_{1,40}=12.89$, $p<0.05$); strain × alcohol treatment ($F_{1,40}=7.32$, $p<0.05$) and naltrexone × ethanol treatment ($F_{1,40}=14.07$, $p<0.05$). There was also a statistically significant three-way interaction effect for all of factors: strain × naltrexone treatments × ethanol treatment ($F_{1,40}=4.22$, $p<0.05$). Naltrexone administrated peritoneal for 10 days in dose 2 mg/kg body weight caused statistically significant increase of beta-endorphin plasma level, both in WHP (600±61 pg/ml) as in WLP rats (641±35 pg/ml). The 10-day treatment with naltrexone prevented the increase of beta-endorphin level after a single application of ethanol in WLP rats (356±75 pg/ml); however, the plasma peptide level was 763±61 pg/ml in WHP rats. Post hoc LSD test has shown significant differences between WHP and WLP rats (Fig. 1).

4. Discussion

A lot of research has proven that naltrexone is more effective than placebo in reducing relapse rates and reducing the craving for alcohol (Davidson et al., 1999; Chick et al., 2000; Morris et al., 2001; Kranzler et al., 2003; Killeen et al., 2004).

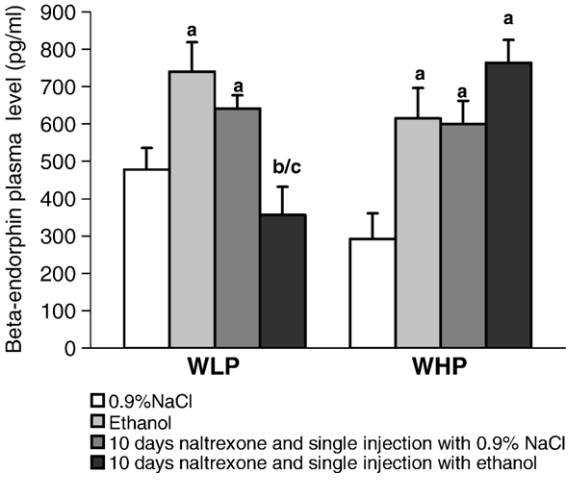

Fig. 1. Effect of single injection of ethanol (2 g/kg) on β-endorphin plasma level in low (WLP) and high preferring rats (WHP) after 10 days administration of naltrexone. Values are expressed as mean±S.E.M. in each group of 6 rats. (a) $P<0.05$ in comparison to group injected with 0.9% NaCl. (b) $P<0.05$ in comparison to group treated with ethanol. (c) $P<0.05$ in comparison to group treated with naltrexone.

In our study, we administered a 10-day naltrexone treatment to adult females of WLP and WHP rats in order to observe how the treatment affects the level of beta-endorphin plasma in their bodies. As in our previous study, we again observed that even a single injection with ethanol increases the level of this peptide. An increase in the beta-endorphin level results in the stimulation of dopaminergic pathways in the brain (Di Chiara, 1997). Beta-endorphin can stimulate a dopamine release through two mechanisms: (1) directly by interacting with opioid receptors on the dopamine producing neurons in nucleus accumbens or (2) indirectly in the ventral tegmental area by interfering with opioid receptors located on the GABA neurons (inhibition of GABA leads to increased dopamine production and release in nucleus accumbens). This excessive release of dopamine is related to positively reinforcing effect of alcohol and is supposed to lead to the development of the alcohol dependence (Heinz, 2002; Bowirrat and Oscar-Berman, 2005).

By denying access to the opioid receptors, one observes that the alcohol preferring rats decrease their alcohol intake and a reduction in the increase of opioid activity induced by ethanol also occurs (Cowen and Lawrence, 2001), thus reducing at least partly the reinforcing properties of alcohol. A large body of research has proven that long-lasting blockade of opioid receptors induces supersensitivity of opioid receptors (Lesscher et al., 2003; Gullapalli and Ramarao, 2002; Lee and Yoburn, 2000). In our study, we have observed that repeated treatment by naltrexone leads to the increase in the beta-endorphin level both in the WHP and WLP rats. The increase in the level of this peptide was similar in both lines of rats. Kosten et al. (1986) also reports an increase in the beta-endorphin level in opioid-dependent humans who had been treated with naltrexone for extended periods of time. However, Ernst et al. (1993), examining the influence of naltrexone in autistic children, did not find any specific influence on the beta-endorphin plasma level.

We have observed that repeated naltrexone treatment prevented the increase in beta-endorphin plasma level after a single application of ethanol to WLP rats but not to WHP rats. In our earlier study with acamprosate, we demonstrated that repeated acamprosate treatment also prevented the increase in beta-endorphin level after a single injection of ethanol only in the WLP rats (Zalewska-Kaszubska et al., 2005). In contrast to our other studies, we have not observed differences between both strains in the basic levels of beta-endorphin. In the WHP rats treated with naltrexone, the level of this peptide after a single injection with ethanol was similar to it had been while they were undergoing the naltrexone treatment or had received a single alcohol injection. Our observations are in agreement with the study by Na and Lee (2002). They observed a higher increase in beta-endorphin concentration in social drinker volunteers after alcohol consumption preceded by naltrexone treatment as compared to a placebo receiving group (Na and Lee, 2002). Taking under consideration the increased responsiveness of the opioid system, it is possible to understand high efficacy of naltrexone. One of the explanations is that naltrexone, by blocking the opioid receptors, might reduce the

reward after the alcohol drinking. It is probably why people taking naltrexone while consuming alcohol do not experience its reinforcing effects, and so, the amount of alcohol consumed and the craving for alcohol decline. However, naltrexone helps decrease the craving for alcohol but does not treat addiction. A meta-analysis of randomized controlled trials is leading to the conclusion that there is strong evidence that naltrexone significantly reduces alcohol relapses to heavy drinking, the frequency of alcohol consumption and alcohol craving (Streeton and Whelan, 2001; Srisurapanont and Jarusuraisin, 2005). Probably in alcohol-dependent humans and in humans with the inclination for dependence as well as in rats preferring alcohol, a long-term blockade of the opioid receptors during naltrexone treatment is able to cause the compensatory increase in the beta-endorphin plasma level and equalizes its deficit. It is possible that in this way the disturbed balance in the mesolimbic reward system is restored. However, a genetic susceptibility to alcoholism is a complicating factor and we suppose that the administration of naltrexone may have different effects on the opioid system on different individuals. Oslin et al. (2003) reported that functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Subjects with a mutation A118G (Asn40Asp) variant receptor who were treated with naltrexone had significantly lower rates of relapse and a longer time to return to heavy drinking (Oslin et al., 2003). This variant of mu-receptor has shown to be increased allele frequencies in alcohol-dependent subjects (Rommelspacher et al., 2001; Zalewska-Kaszubska and Czarnecka, 2005).

The high naltrexone effectiveness in the treatment of alcoholism might result from both its blocking effect on the opioid system of positive reinforcement and partly from increasing the beta-endorphin concentration. The relationship between the mechanism underlying the differential sensitivity of alcohol preferring and non-preferring rat lines to the effects of repeated naltrexone treatment and ethanol-induced beta-endorphin response remains to be determined and requires more extensive investigations.

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