EFFECT OF NALTREXONE ADMINISTRATION ON SHORT-TERM MEMORY IN CHRONICALLY ETHANOL-TREATED OUTBRED RATS

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Abstract — Aims: The purpose of this study was to evaluate the effect of naltrexone treatment for 21 consecutive days on short-term memory in ethanol-prefering and non-prefering outbred rats. Methods: Ethanol preferring, non-prefering and control Wistar rats were treated with naltrexone (0.1 mg/kg intraperitoneally (i.p.)) for 21 consecutive days. Short-term memory was assessed by using an olfactory social recognition test. Results: A single administration of naltrexone (0.1 mg/kg i.p.) to non-ethanol-treated animals facilitated social memory, whereas the drug did not affect short-term memory in either group of chronically ethanol-treated rats. Multiple naltrexone treatment also lowered alcohol intake in ethanol-prefering rats. Conclusion: Naltrexone–ethanol interaction does not seem to produce any negative effect on the short-term memory in outbred rats.

INTRODUCTION

In the past decade there has been increasing interest in the use of pharmacotherapy to improve the effectiveness of alcoholism treatment (Kranzler, 2000; Anton, 2001; O’Leary et al., 2001; Rosenberg et al., 2002). One of the most effective drugs for the treatment of alcohol dependence seems to be naltrexone, a non-selective opioid antagonist, which decreases the likelihood of relapse into heavy drinking, most likely through a reduction of craving and/or the reinforcing effects of ethanol, although the mechanisms underlying its efficacy have not yet been satisfactorily explained (Johnson and Ait-Daoud, 2000; Sinclair, 2001).

It is generally accepted that the opioid system can contribute to the rewarding effects of ethanol (Koob and Le Moal, 2000); however, the role of opioids and ethanol interaction in the learning and memory processes is far from well understood (Heyne et al., 2000).

There are few reports concerning naltrexone effects on memory in humans, and the published data often led to inconclusive results. For example, naltrexone alone showed an impairment on verbal memory tasks (Chaves et al., 1988) or reduced the attentional efficiency and sensitivity in word recognition tasks (Mason et al., 2002), although subjects receiving the drug performed better on test of recognition memory when the state of arousal was heightened (Katzen-Perez et al., 2001). Concerning the naltrexone and ethanol interaction, it is known that this drug augments the sedative, and reduces the positive, reinforcing effects of ethanol without affecting psychomotor performance in humans (Swift et al., 1994). As follows from a study on human volunteers, naltrexone did not eliminate the negative effect of ethanol, but also did not have a significant effect on memory task performance (Rush and Ali, 1999).

One of the tests used for research on memory and learning processes in experimental animals is the social memory test (Thor and Holloway, 1982; Dantzer et al., 1987; Popik and van Ree, 1998). It can be assumed that this memory takes the form of short-term memory, strictly related to sensory memory (Dantzer et al., 1987). It is known that an acute intraperitoneal ethanol administration in low doses (0.5 and 1.0 g/kg i.p.) improves social memory in rats (Prediger and Takahashi, 2003). Similarly, in our earlier study, we found that animals subjected to long-term alcohol effects and disturbed circadian cycle differed in their performance of social memory tasks (Mikolajczak et al., 2001). Moreover, since it is known from new data that the opioid system has an important role in the acquisition and consolidation olfactory memory in rat pups (Roth and Sullivan, 2003), therefore choosing the social memory test for studying a naltrexone and ethanol interaction seems to be appropriate.

As the data on the effects of naltrexone on learning and memory processes are ambiguous and available results of ethanol and naltrexone interaction were obtained after a single administration of these compounds, this study was undertaken to establish the effect of multiple administrations of this drug on the short-term memory assessed by the social memory test in outbred ethanol ‘preferring’ and ‘non-prefering’ rats.

MATERIALS AND METHODS

Animals

The experiments were performed on male Wistar rats housed individually in their home cages [standard plastic cages 35 (l) × 13 (h) cm with stainless steel covers], kept on a reversed 12 h/12 h dark/light cycle (lights on: 19.00–07.00 hours) under constant ambient conditions (20 ± 2 °C; relative humidity, 65%). The rats were given free access to standard laboratory diet [pellets, Labofood B (LSM); Feeds and Concentrates Production Plant, Poland] and had tap water.
freely available in their home cages. The animals weighed 179.0 ± 1.5 g (n = 106) at the beginning of the experiments. Juvenile (~30-day-old) male Wistar rats kept in standard plastic cages with seven animals in each were used as social stimulus. Animals (n = 75) were forced to drink only ethanol solution [12% (w/w) from 95% stock ethanol; Polmos, Poland] for 2 months (~9 g/kg/day). During the next 4 weeks the animals were presented with a free choice paradigm between tap water and ethanol. This procedure led to a distinction between two groups of ethanol-treated animals: (1) rats with a mean intake of ethanol exceeding 50% of their total fluid intake (>4 g/kg/day), i.e. ‘preferring’ (PRF; n = 25); and (2) rats for whom alcohol solution constituted less than 50% of total fluid intake, i.e. ‘non-preferring’ (NPF; n = 31). Additionally, for comparative purposes, throughout the whole period of chronic ethanol treatment, an ethanol-naive control group of animals (CR) received only tap water (n = 29).

The volumes of ethanol intake were converted to a value in g/kg/day and expressed as a mean ± SEM for the group during the last week of drug treatment. Similarly, the total fluid intake (sum of intakes of water and ethanol solution) was also expressed as ml/kg/day for the groups during the last week of drug administration. Preference (as %) was calculated as the amount of ethanol consumed (ml/kg/day) / total fluid intake (ml/kg/day) × 100. The body weight of animals after the drug treatment period was measured.

Social memory test
A social memory test design based on olfactory recognition, which allowed measurement of the short-term memory conditions with a short-term recognition procedure, was used (Thor and Holloway, 1982; Sawyer et al., 1984; Dantzer et al., 1987; Griffin and Taylor, 1995). Briefly, the adult test rat was presented to a juvenile (~30 days old) male rat (social stimulus) for 5 min, and its social-investigatory behaviour (defined as being proximally oriented to the juvenile rat or as having a direct contact with the other rat by sniffing, following, grooming or generally inspecting any body surface of the juvenile) was measured with a hand-held cumulative timer to the nearest 0.1 s (T1-first encounter). Next, after 30 min the same procedure was repeated with the same juvenile rat (known juvenile rat) (T2-second encounter). Between the two successive exposures, the juveniles were housed individually. For evaluation of non-specific effects, an unknown new juvenile was exposed to the rat during ‘second exposure’ (T2). As in our other reports, the test values were expressed as a ratio (ratio of investigation duration) of the time spent on investigation during T2 divided by T1 (Mikolajczak et al., 2001, 2002). All investigations were conducted in the cages of adult rats during the dark phase (between 09.00 and 15.00 hours) in a dimly illuminated, sound-proof room.

All adult rats were preliminarily tested (before introducing a drug treatment to adult rats) during the four consecutive days preceding the experiment (pre-test; data not shown) using the procedure described by others (Popik and van Ree, 1998). Generally, each adult rat was exposed to various juvenile rats, and only animals that reliably investigated the juvenile rats during pre-test without displaying aggressive or sexual behaviour were used in the experimental part of this study.

Dose-dependent effect of naltrexone on social memory
The first step of the experiment was the assessment of the dose-dependent effect of naltrexone hydrochloride (Sigma-Aldrich) on social memory. Adult rats (ethanol-naive) were treated with a single dose of naltrexone (0.1, 1.0, 3.0 and 10.0 mg/kg i.p.) or 0.9% saline (vehicle) 30 min before T2. The experiment was performed using five separate groups of adult rats (n = 7 in each group) and 28 juveniles.

Effect of naltrexone on social memory in chronically ethanol-treated rats
After 4 weeks of voluntary ethanol intake, groups of rats (PRF + naltrexone, n = 7; NPF + naltrexone, n = 7; CR + naltrexone, n = 7) were treated with naltrexone (0.1 mg/kg i.p. dissolved in 0.9 % saline) for 21 consecutive days. This dose of naltrexone was chosen according to above-mentioned acute experiment. The control groups were given the corresponding volume of saline (PRF + saline, n = 7; NPF + saline, n = 7; CR + saline, n = 7). On day 21 of the experiment, the social memory (T1) was assessed and the last dose of naltrexone or saline was administered 30 min before T2. On the next day (day 22), the same procedure was repeated for all rats, but the known juvenile rat was replaced by an unknown juvenile rat during the second encounter (measuring non-specific effect).

Locomotor activity
On day 23 of naltrexone treatment, locomotor activity 30 min after naltrexone or saline administration was evaluated using a licensed activity meter (PAN), which recorded their activity with electromechanical counters (Mikolajczak et al., 2001, 2002). The data obtained were expressed as signals corresponding to spontaneous movements for 5 min.

Statistical analyses
All values were expressed as means ± SEM (n equals the number of rats included in each analysis). The statistical comparison of results was carried out using one-way analysis of variance (ANOVA), followed by a Duncan post hoc test, to analyse the total fluid intake, ethanol intake and locomotor activity data. Kruskall–Wallis nonparametric ANOVA followed by a Mann–Whitney test and Friedman non-parametric ANOVA were applied for memory task data analysis.

RESULTS
Single administrations of naltrexone in doses of 0.1; 1.0; 3.0 and 10.0 mg/kg, i.p., to control (non-ethanol-treated) animals significantly affect social memory (ANOVA Kruskal–Wallis: H(4,30) = 12.2; P < 0.02), with the results being strongly statistically significant for 0.1 mg/kg, when compared with the control group, which was administered saline (P < 0.02) (Fig. 1). After administration of naltrexone in doses 1.0 and 3.0 mg/kg, the differences were not strongly statistically significant (P < 0.06 and P < 0.1, respectively). No statistically significant difference compared with the control group was observed after the highest dose of naltrexone (P > 0.1). Joint analysis of ratio of investigation duration for the first (known juvenile) and the second (unknown juvenile) day, revealed that the values differed significantly (ANOVA
specificity by comparison of ratio of investigation duration for known
animals. (ANOVA: \(F(3,24) = 7.11; \ P < 0.01\) or \(F(3,24) = 6.12; \ P < 0.01\), respectively) (Table 1). Thus, PRF + saline rats drank more ethanol than did NPF + saline rats (\(P < 0.01\)), and a similar pattern was observed with regard to preference. The naltrexone-receiving PRF rats (PRF + naltrexone) drank less alcohol and exhibited less preference for ethanol, compared with the control rats receiving saline (\(P < 0.05\)) (Table 1), whereas the administration of naltrexone to NPF rats (NPF + naltrexone) did not result in any statistically significant differences in alcohol intake or preference compared with the saline group.

Analysis of the overall changes in total fluid intake by the animals during naltrexone administration did not reveal statistically significant differences (ANOVA: \(F(5,36) = 1.88; \ P > 0.1\)). Although naltrexone rats drank less, the differences were not statistically significant, compared with the saline rats (Table 1). Similarly, naltrexone and alcohol drinking did not significantly change the animals’ body weight (ANOVA: \(F(5,36) = 0.22; \ P > 0.1\) (Table 1).

Multiple administration of naltrexone and saline to the rats drinking ethanol on a prolonged basis (PRF and NPF animals) and to CR animals yielded mean test values that characterized social memory and differed in a statistically significant degree from the first day of the test (ANOVA Kruskal–Wallis: \(H(5,36) = 12.4; \ P < 0.03\) (Fig. 2). No general variability between the obtained mean values was observed on the second day of the test (ANOVA Kruskal–Wallis: \(H(5,36) = 7.73; \ P > 0.1\). However, mean ratio of investigation duration values for the first and the second day of social memory testing differed to a statistically significant degree (ANOVA Friedman: \(H(1,37) = 21.4; \ P < 0.001\), which confirms the specificity of the results obtained in our experiment. The observed variability as expressed through the values of the Kruskal–Wallis test for the first day of social memory testing was due to the differences in task performance by NPF animals, yet the differences between NPF + saline and CR + saline animals were only weakly significant (\(P < 0.06\) (Fig. 2). Further analysis of the detailed data for the first testing day has revealed that naltrexone did not affect social memory in any of the tested animals as compared with the saline-treated animals (\(P > 0.1\)).

Spontaneous activity measurements showed that there was no significant variability in the mean activity level of the animals (ANOVA: \(F(5,37) = 1.39; \ P > 0.1\) (Fig. 3). Further analysis has revealed that the level of spontaneous activity of naltrexone-receiving rats did not differ significantly from that of the other animals in any of the tested groups (\(P > 0.1\)). Minor differences in the level of spontaneous activity were noticed only in the NPF group (NPF + saline vs CR + saline; \(P < 0.06\)).

**DISCUSSION**

Two groups of rats (PRF and NPF) differing in voluntary ethanol intake were used in this study and the observed differences in alcohol drinking were in line with our previous report (Mikolajczak et al., 2001). The ethanol drinking pattern

![Fig. 1. Dose-dependent effect of a single naltrexone administration on social memory expressed as a ‘ratio of investigation duration’ in rats.](image-url)

**Table 1. Parameters of alcohol intake after multiple naltrexone treatment in ethanol-preferring (PRF), ethanol-non-preferring (NPF) and control (CR) rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Total fluid intake (ml/kg/day)</th>
<th>Ethanol intake (g/kg/day)</th>
<th>Preference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR + saline</td>
<td>528 ± 25</td>
<td>89.4 ± 2.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CR + naltrexone</td>
<td>508 ± 15</td>
<td>77.1 ± 4.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NPF + saline</td>
<td>535 ± 32</td>
<td>92.8 ± 5.4</td>
<td>2.6 ± 0.9</td>
<td>24 ± 8</td>
</tr>
<tr>
<td>NPF + naltrexone</td>
<td>519 ± 14</td>
<td>84.9 ± 7.4</td>
<td>2.0 ± 0.5</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>PRF + saline</td>
<td>517 ± 17</td>
<td>92.8 ± 5.1</td>
<td>7.5 ± 1.4*</td>
<td>67 ± 13*</td>
</tr>
<tr>
<td>PRF + naltrexone</td>
<td>512 ± 19</td>
<td>80.6 ± 2.4</td>
<td>4.0 ± 1.8†</td>
<td>41 ± 7†</td>
</tr>
<tr>
<td>ANOVA: (F(5,36) = )</td>
<td>0.22; (P &gt; 0.1)</td>
<td>1.88; (P &gt; 0.1)</td>
<td>7.11; (P &lt; 0.01)</td>
<td>6.12; (P &lt; 0.01)</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± SEM for seven animals in each group. *ANOVA for \(F(3,24)\). **Statistically significant difference vs. NPF + saline, \(P < 0.01\). (Duncan post hoc test). †,‡,§ Statistically significant difference versus PRF + saline, \(P < 0.01\) or 0.05 (respectively); Duncan post hoc test.
observed in our PRF group (>4.0 g/kg/day and >50% preference) is in agreement with the suggestion, that level drinking may be considered as ‘preference’ (Colombo, 1997).

Naltrexone treatment lowered alcohol intake in PRF animals, but not that of NPF rats, thus confirming the results of other studies, which found that naltrexone, in the dose used in our study, is capable of reducing alcohol intake in experimental animals (Franck et al., 1998; Spanagel and Holter, 2000; Stromberg et al., 2001). However, the nature of naltrexone-induced lowering of ethanol intake in PRF rats is far from understood (Froehlich et al., 1998; Hyyttia et al., 1999; Parkes and Sinclair, 2000).

Naltrexone is known to not only suppress alcohol drinking in PRF animals, but also to affect overall fluid intake in chronically ethanol-treated animals. In our study, naltrexone did not affect total fluid intake in all investigated rats. This is consistent with the opinion that the inhibitory effect of naltrexone on fluid intake exists only at high naltrexone doses, in contrast with the reported effect of a 0.1 mg/kg dose (Holter and Spanagel, 1999).

In the social memory test used in this study, the second exposure of the young rat was done on the same day and after 30 min from the first exposure. Such a test protocol allows effective evaluation of the factors which have a negative impact on short-term memory (Thor and Holloway, 1982; Dantzer et al., 1987; Perio et al., 1989; Popik and van Ree, 1998; Prediger and Takahashi, 2003). Moreover, using the social recognition paradigm is an effective procedure which can help to exclude the effects of drugs appearing as ‘false positive’ in other tests aimed at measuring memory.

Acute naltrexone administration in the lowest dose (0.1 mg/kg i.p.) produced a significant facilitation in the social memory of control rats. However, at higher doses (1.0–10.0 mg/kg i.p.) a tendency to diminished effects was found. The acute effect of opioid agonists and antagonists on memory processes is not clear. Considering the effects of opioid antagonists on social memory it was found that acute naltrexone, the other non-specific opioid antagonist, per se (1.0 mg/kg i.p.) did not affect this kind of memory in rats (Prediger and Takahashi, 2003). It has been observed that a single administration of naltrexone in the dose used in our study did not significantly affect the results of the passive avoidance test, whereas at double this dose, the effect depended on the strain of the tested mice, with both negative and positive effects on memory consolidation being observed (Castellano et al., 1999). It is known that the results of the passive avoidance test, especially in young animals, depend on the opioid receptor subtype being involved (Freeman and Young, 2000) and a role for endomorphins 1 and 2 in the impairment of short-term memory mediated through µ receptors is proposed (Ukai et al., 2000, 2001). It should also be stressed that, despite naltrexone not being a non-selective antagonist at opioid receptors, it is postulated that it blocks predominantly µ receptors when used in lower doses, as in our study (Tempel et al., 1985; Millan et al., 1988; Morris et al., 1988). Hence, it may be speculated that naltrexone in the dose of 0.1 mg/kg shows its activity on social memory by blocking the µ receptors, whereas at higher doses the other opioid receptor subtypes are involved. However, it should be stressed that the mechanism of the positive effect of acute naltrexone on short-term memory observed in this study expressed by the social recognition paradigm is unknown, therefore more studies, especially at the molecular level, are needed.

On the basis of the assessment of rats’ social memory after a single naltrexone administration, in further investigations the 0.1 mg/kg, i.p., dose of the drug was considered appropriate.
Chronic ethanol intake affected social memory in investigated rats, however, the effects were not so strong. In this study we observed that short-term memory showed a weak impairment in NPF rats, whereas in PRF animals, a statistically insignificant tendency to perform worse was found. As NPF showed a weak higher locomotor activity when compared with CR or PRF animals, an eventual sedative component of action of ethanol in NPF rats can be excluded in this study. As in our previous studies, short-term memory improvement could be observed in the same test both in PRF and NPF animals (Mikolajczak et al., 2001). It should, however, be noted that the cited results were those of PRF and NPF animals with disrupted diurnal cycles. Therefore, it can be said that the observed differences in this study between PRF and NPF are in agreement with opinions that sometimes NPF-like animals exhibit rather higher sensitivity towards prolonged ethanol intoxication, whereas PRF-like rats tolerate many of the behavioural patterns of ethanol better, which may be related to their genetic predisposition (Hammoumi et al., 1997; Möller et al., 1997; Chester et al., 2003).

Multiple administration of naltrexone to the PRF, NPF and CR rats has revealed that the drug did not affect social memory in any of the tested animals. It should also be emphasized that the results of social memory tests were not made worse as a result of the sedative activity of naltrexone, as there was no significant naltrexone-induced reduction in spontaneous activity level in any of the tested groups of animals. It is known that acute co-administration of ethanol and nalozone (1.0 mg/kg i.p.) reverses the memory-enhancing effect of ethanol in rats, whereas nalozone exerts no effects when administered alone (Prediger and Takahashi, 2003). However, it should be stressed that the cited results of ethanol–naloxone interaction were obtained when the same juvenile was re-exposed at 120 min, whereas in this study a 30-min interexposure interval was used. Due to the fact that the 30-min interval is generally used to demonstrate possible ‘anamnesic’ effects subsequent to a given drug administration, whereas the 120-min interval is rather selected as a temporal window suitable for testing memory-enhancing treatments (Danzter et al., 1987; Prediger and Takahashi, 2003), it cannot be excluded that chronic ethanol intake and the different test procedure are responsible for the observed effects of naltrexone in comparison to the results of Prediger and Takahashi (2003).

In conclusion, it seems justified to state that multiple administration of naltrexone does not disrupt short-term memory in chronically ethanol-treated rats. Although the results of experimental studies cannot be readily paralleled with clinical practice, it may be postulated that the proposed use of naltrexone as an agent to treat alcohol addiction does not seem to be related to any negative effects on the short-term memory.

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REFERENCES


