PHARMACOLOGY AND CELL METABOLISM

Effects of Clozapine on Ethanol Withdrawal Syndrome in Rats

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Abstract — Aims: Co-morbid substance use in schizophrenic patients is common, and an important factor affects the outcome of disease. On the other hand, drug dependence is a predictive factor for psychosis. Alcohol is one of the most frequently abused psychoactive substances and may contribute psychotic symptoms in several conditions, such as withdrawal syndrome. The present study was designed to investigate the effects of clozapine on ethanol withdrawal syndrome (EWS) in rats. Methods: Adult male Wistar rats were used in the study. Ethanol (7.2%, v/v) was given to rats by a liquid diet for 14 days. An isocaloric liquid diet without containing ethanol was also given to control rats. Clozapine (2.5, 5 and 10 mg/kg) and its vehicle (0.1% acetic acid) were injected to rats subcutaneously at the 1.5th and 5.5th hours of ethanol withdrawal. At 2nd, 4th and 6th hours of ethanol withdrawal, rats were observed for 5 min and withdrawal signs that included locomotor hyperactivity, agitation, tremor, tail stiffness, stereotyped behaviour and wet dog shakes were recorded or rated. Following the observations at 6th hour, subjects were tested for audiogenic seizures. Results: Clozapine significantly and dose-dependently inhibited the EWS-induced locomotor hyperactivity, wet dog shake, stereotyped behaviour, tremor and tail stiffness. However, it did not produce any significant effect on agitation and audiogenic seizures. Doses of clozapine used in the present study did not produce any significant change on locomotor activities of naïve rats. Conclusions: Our results suggest that clozapine had some significant beneficial effects on EWS in rats. Thus, this drug may be helpful for controlling some withdrawal signs in ethanol-dependent patients.

INTRODUCTION

Epidemiologic studies in the general population and those based on the clinical assessment of schizophrenic populations have revealed a high degree of overlap between schizophrenia and addictive disorders. Surveys of the general population have identified an elevated risk of alcohol dependence or substance abuse in subjects presenting with criteria for schizophrenia (Regier et al., 1990; Batel, 2000). Helzer and Pryzbeck (1988) have also shown that schizophrenia is four times more frequent among alcoholic than non-alcoholic subjects. Thus, co-morbid substance use in schizophrenic patients is common, and an important factor affects the outcome of disease. On the other hand, drug dependence may be accepted as a predictive factor for psychosis (Hambrecht and Hafner, 1996).

Clozapine is the prototype of atypical antipsychotic drugs that are widely used in patients with resistant schizophrenia (Meltzer, 1997). Dopaminergic D1,-4 receptors are the targets for clozapine and it also modulates serotonergic 5-HT2A–C and 5-HT3, nicotinic acetylcholine α-7 nACh and α2 adrenergic receptors (Ashby and Wang, 1996; Svensson, 2003; Miyamoto et al., 2005; Singhal et al., 2007).

In animal studies, clozapine was found to have some inhibitory effects on ethanol-induced locomotor activity but could not block conditioned place preference (Thrasher et al., 1999) and ethanol drinking in alcohol-prefering AA rats (Ingman and Korpi, 2006). In contrast to these studies, Drake et al. (2000) suggested that clozapine reduces ethanol craving or consumption in human social drinkers. It has also been suggested that clozapine had highly promising results in human addicts (Potvin et al., 2003). Although these observations imply that clozapine could be effective in treatment of ethanol dependence, there is no report investigating the effects of clozapine in alcohol-dependent patients. On the other hand, ethanol withdrawal syndrome (EWS) precipitated by discontinuing chronic ethanol intake is the most important evidence indicating the presence of physical ethanol dependence either in humans or in experimental animals (Jaffe, 1990; Uzbay et al., 1994; Brust, 2004). However, effects of clozapine on ethanol withdrawal or ethanol dependence have been subjected to neither clinical nor experimental studies yet.

The main objective of the present study was to investigate the effects of clozapine, which is the prototype of atypical antipsychotics, on the signs of EWS in rats. Thus, herein the current study was focused on revealing whether clozapine is effective on ethanol withdrawal or not.

MATERIAL AND METHODS

Animals and laboratory

All procedures in this study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA). Local ethical committee approval was also taken, numbered with 05/73 on 23 October 2005. All efforts were made to minimize animal suffering to reduce the number of animals used.

Adult male Wistar rats (253–372 g weight at the beginning of the experiments) were used (n = 8 for each group). They were housed in a quiet and temperature- and humidity-controlled room (22 ± 3°C and 60 ± 5%, respectively) in which a 12-h light/dark cycle was maintained (07:00–19:00 h light). Exposure to ethanol and all behavioural experiments involved in EWS were carried out in other separate and isolated laboratories, which have the same environmental conditions with the colony room.

Chronic exposure to ethanol

For chronic ethanol exposure, the rats were housed individually and ethanol was given in the modified liquid diet as previously
described (Uzbay and Kayaalp, 1995). The rats were given a
modified liquid diet with or without ethanol ad libitum. No extra
chow or water was supplied. The composition of the
modified liquid diet with ethanol is: cow milk 925 ml (Sütas,
Bursa, Turkey), 25–75 ml ethanol (96.5% ethyl alcohol; Tekel,
Turkish State Monopoly), vitamin A 5000 IU (Aksu Farma,
Turkey) and sucrose 17 g (Uzbay and Kayaalp, 1995). This
mixture supplies 1000.7 kcal/l.

At the beginning of the study, all the rats were given the modi-
ified liquid diet without ethanol for 7 days. Then, liquid diet
with 2.4% ethanol was administered for 3 days. The ethanol
concentration was increased to 4.8% for the following 4 days
and finally to 7.2% for 14 days. Liquid diet was freshly pre-
pared daily and presented at the same time of the day (09:30h).
The weight of the rats was recorded every day, and daily ethanol
intake was measured and expressed as g per kg per day. Con-
Control rats (n = 8) were pair fed with an isocaloric liquid diet
containing sucrose as a caloric substitute to ethanol.

Drug used in the study
Clozapine (Sigma, St. Louis, MO, USA) was dissolved in 0.1%
acetic acid. Clozapine or its vehicle (0.1% acetic acid) was
injected to rats subcutaneously at a volume of 1 ml/200 g body
weight. Drug solutions were prepared freshly in the morning
just before the administration.

Determination of blood ethanol levels
Blood ethanol levels were determined in the two individual
groups of the ethanol-receiving rats (n = 7 for each group).
Blood samples were taken by intra-cardiac puncture under the
ether anaesthesia 2 h before (07:30 h) and at 6th hour of
ethanol withdrawal. Serum ethanol concentrations were
measured by using a gas chromatography-mass spectrometry
(GC-MS) autoanalyzer (Model QP5050, Schimadzu, Kyoto,
Japan).

Evaluation of EWS
At the end of the exposure to 7.2% ethanol-containing liq-
uid diet, diet with ethanol was withdrawn and replaced with
isocaloric ethanol-free diet at 09:30 h. Ethanol-dependent rats
were then assigned into four groups randomly (n = 8 for each
group). Clozapine (2.5, 5 and 10 mg/kg) and its vehicle were
injected to the rats 30 min before ethanol withdrawal evalua-
tion. At 2nd, 4th and 6th hours of ethanol withdrawal, rats were
observed for 5 min, and withdrawal signs that included loco-
motor hyperactivity, agitation, tremor, tail stiffness, stereotyped
behaviour and wet dog shakes were recorded or rated.

Locomotor activities of the rats were recorded by an open-
field locomotor activity test apparatus (Opto Varimex Minor,
Columbus, OH, USA) as a total of horizontal, vertical and
ambulatory activities and expressed as mean ± SEM.

Tremors and audiogenic seizures were assessed as incidence.
Tremor was determined after lifting rats vertically by the tail;
positive was assigned to rats showing clearly distinct forelimb
tremor (Frye et al., 1983). Grooming, sniffing, head weav-
ing, gnawing and chewing were observed as major stereotyped
behaviours during the ethanol withdrawal in the study. Total
number of stereotyped behaviours and wet dog shakes for each
observation period was calculated and expressed as mean ±
SEM. Agitation and tail stiffness were scored by using the
rating scale as previously described (Uzbay et al., 1997).

Each group received a second injection of its original drug
30 min before the 6th hour observation. After 6 h of withdrawal
testing, rats were exposed to an audiogenic stimulus (100 dB)
for 60 s in a separate and soundproof place in the laboratory. The
incidence and latency of the audiogenic seizures were recorded.

Control rats receiving no ethanol-contained liquid diet were
also evaluated for ethanol withdrawal signs as parallel to
ethanol-dependent groups.

All experiments were carried out during the light period. All
ratings were done by a naive observer, who was blind to which
treatment the rats received.

Measurements of locomotor activity in naive control rats
Clozapine (2.5–10 mg/kg) and vehicle were administered in
four groups of naive (ethanol non-dependent) Wistar rats (n =
6–7 for the groups). Thirty minutes after the injections, rats
were put into the locomotor activity test apparatus and locomo-
tor activities of the rats were measured for 15 min. The results
of the locomotor activity tests were expressed as mean ± SEM.

Statistical analysis
Changes in locomotor activity and body weight of ethanol-
dependent rats as compared to ethanol non-dependent control
rats were analysed by unpaired (between groups) Student’s
t-test. Analysis of variance (one-way ANOVA) followed by
Dunnett’s test was used in evaluation of the effects of clozap-
ine on the locomotor hyperactivity, stereotyped behaviours, wet
dog shakes, and latency and duration of audiogenic seizures.
The effects of clozapine on the intensities of agitation and tail
stiffness in different groups were analysed by the Kruskall–
Wallis test followed by the Mann–Whitney U-test. The com-
parison of the incidence of tremors and audiogenic seizures was
done by the Chi-square test. A two-way ANOVA test (group ×
time) was also used to compare ethanol consumptions of
the groups given ethanol-contained liquid diet. The level of
significance was set at P < 0.05 levels.

RESULTS

Ethanol consumption, body weight and blood ethanol levels of
the rats
Daily ethanol consumption of the rats in control and clozapine-
treated groups ranged from 9.20 ± 0.30 to 14.77 ± 0.37 g/kg
during the exposure to ethanol (7.2%) (Fig. 1). While there
was a difference in consumption amounts between the days of
the last 2 weeks including 7.2% alcohol consumption, the
difference between the two groups was not statistically sig-
nificant. The results of the two-way ANOVA test on ethanol
consumption data revealed significant effects for time \[ F(13,
364) = 43.483, P < 0.0001 \] and interaction \[ F(39, 364) =
2.134, P < 0.0001 \] effects, but not group effects \[ F(3, 28) =
2.256, P = 0.104 \]. This data indicate that there is no signif-
icant difference between the groups for ethanol consumption.
However, ethanol consumption of the rats was significantly
increased during exposure to liquid diet.

Body weight changes of the ethanol-fed and control rats are
presented in Table 1. Body weights of the rats decreased
progressively during the study in ethanol-feeding groups. When initial body weights compared to the weights at end of the study, decreases between 6.42% and 8.04% in the ethanol-fed groups were observed. Whereas in control rats, an increase in body weight of ~2.23% was also observed. There were no significant differences in body weight changes of the rats compared to beginning of the study (Student’s t-test, P > 0.05).

Serum ethanol concentrations were found as 77.71 ± 20.38 and <1 mg/dl 2 h before (07:30 h) and at 6th hour of ethanol withdrawal, respectively. There is a significant correlation between ethanol-containing liquid diet consumption and blood ethanol level of the rats, at 2 h before ethanol withdrawal \[F(1, 5) = 31.852; P = 0.002, r = 0.86\].

**Behavioural changes during ethanol withdrawal**

A significant locomotor hyperactivity was observed in the ethanol-dependent groups at the 2nd, 4th and 6th hours of the withdrawal-testing period as compared to the ethanol non-dependent vehicle groups (Student’s t-test; P values <0.05) (Fig. 2). Other behavioural signs of EWS, such as stereotyped behaviours, wet dog shakes, tail stiffness and tremors, appeared during the whole observation period (Fig. 3A–D, dark bars). However, agitation significantly appeared only at 4th hour of EWS (Fig. 3E, dark bars).

Audiogenic seizures occurred at 6th hour of ethanol withdrawal with an incidence of 63% and latency of 14.80 ± 1.38 s in ethanol-dependent control group (Table 2). No ethanol withdrawal signs were observed in the ethanol non-dependent rats.

**Effects of clozapine on EWS**

Clozapine produced some significant inhibitory effects on locomotor hyperactivity at 2nd and 6th hour of ethanol withdrawal \[F(3, 28) = 8.486; P < 0.0001 and F(3, 28) = 3.673; P = 0.024, respectively\]. Post hoc analysis of data indicated that clozapine (5 and 10 mg/kg) significantly reduced ethanol withdrawal-induced locomotor hyperactivity at 2nd hour of ethanol withdrawal. At 6th hour of ethanol withdrawal, all doses of clozapine were found to be significantly effective on locomotor hyperactivity \(P\) values <0.05, Dunnett’s test). The effect of clozapine on locomotor hyperactivity was disappeared at 4th hour of ethanol withdrawal \[F(3, 28) = 1.722; P = 0.185\] (Fig. 2).

Similarly, it produced some significant inhibitory effects on stereotyped behaviours at 2nd and 6th hours of ethanol withdrawal \[F(3, 28) = 3.613; P = 0.025 and F(3, 28) = 3.611; P = 0.025, respectively\]. Effect of clozapine on stereotyped behaviours was also disappeared at 4th hour of ethanol withdrawal \[F(3, 28) = 2.489; P = 0.081\]. Post hoc analysis of data indicated that clozapine (5 and 10 mg/kg) significantly reduced ethanol withdrawal-induced stereotyped behaviours \(P\) values <0.05, Dunnett’s test) (Fig. 3A).

Clozapine produced some significant inhibitory effects on wet dog shakes at 2nd, 4th and 6th hours of ethanol withdrawal \[F(3, 28) = 5.919, P = 0.003; F(3, 28) = 3.593, P = 0.026 and F(3, 28) = 3.344, P = 0.033, respectively\]. Post hoc analysis of data indicated that all doses of clozapine significantly reduced ethanol withdrawal-induced wet dog shakes at 2nd hour of ethanol withdrawal. In addition, at doses of 5 and 10 mg/kg, it was significantly effective at 4th hour of the withdrawal period. Clozapine (10 mg/kg) was also significantly effective on wet dog shakes at 6th hour of ethanol withdrawal \(P < 0.05\), Dunnett’s test) (Fig. 3B).

Clozapine (5 and 10 mg/kg) significantly inhibited the tremors appeared at 2nd hour of ethanol withdrawal \(P < 0.05\), Chi-square test). At dose of 5 mg/kg, it was also significantly effective \(P < 0.05\), Chi-square test) on the tremors at 6th hour of ethanol withdrawal. Any significant effect on the tremors by clozapine treatment was not observed at 4th hour of ethanol withdrawal (Fig. 3C).

Clozapine produced some significant inhibitory effects on tail stiffness at 2nd and 6th hours of ethanol withdrawal \(KW = 12.679; P = 0.005\) and \(KW = 10.686; P = 0.014\), respectively). Post hoc analysis of data obtained at 2nd hour of ethanol withdrawal indicated that all doses of clozapine were significantly effective on the tail stiffness \(P\) values <0.05, Mann–Whitney U-test). Clozapine (5 and 10 mg/kg) significantly decreased the intensity of ethanol withdrawal-induced tail stiffness at 6th hour of ethanol withdrawal \(P\) values <0.05, Mann–Whitney U-test). The effect of clozapine on tail stiffness was disappeared at 4th hour of ethanol withdrawal \(KW = 7.609; P = 0.055\) (Fig. 3D).

Clozapine did not produce any significant change on agitation scores at 2nd, 4nd and 6th hours of ethanol withdrawal \(KW = 7.011, P = 0.072; KW = 7.661, P = 0.054\) and \(KW = 2.101, P = 0.552\), respectively) (Fig. 3E). It was also ineffective on both the intensity and latency of the audiogenic seizures (Table 2).

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*Table 1. Changes in weight of the rats fed by liquid diet with or without ethanol*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Beginning of the study</th>
<th>End of the study</th>
<th>Changes during the study</th>
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<tbody>
<tr>
<td>Control (−)</td>
<td>313.63 ± 10.48</td>
<td>320.63 ± 10.37</td>
<td>+2.23%</td>
</tr>
<tr>
<td>Control (+)</td>
<td>303.00 ± 8.64</td>
<td>279.75 ± 9.48</td>
<td>−6.76%</td>
</tr>
<tr>
<td>Clozapine (2.5 mg/kg)</td>
<td>338.25 ± 11.22</td>
<td>314.75 ± 10.08</td>
<td>−6.95%</td>
</tr>
<tr>
<td>Clozapine (5 mg/kg)</td>
<td>311.63 ± 11.22</td>
<td>291.63 ± 10.08</td>
<td>−6.42%</td>
</tr>
<tr>
<td>Clozapine (10 mg/kg)</td>
<td>322.00 ± 10.84</td>
<td>296.13 ± 8.25</td>
<td>−8.04%</td>
</tr>
</tbody>
</table>

(−): ethanol non-dependent; (+): ethanol dependent.
**Effects of clozapine on locomotor activity in ethanol non-dependent (naive) rats**

Clozapine treatment (2.5, 5 and 10 mg/kg) did not cause any significant change on locomotor activity of the naive (ethanol non-dependent) rats. These doses of clozapine did not cause any withdrawal signs in these animals either (data not shown).

**DISCUSSION**

The main finding of the present study is clozapine, a prototype of atypical antipsychotic drugs, that has inhibitory effects on some signs of EWS such as locomotor hyperactivity, stereotyped behaviour, wet dog shakes, tremors and tail stiffness. On the other hand, it was found to be ineffective on the other signs of ethanol withdrawal such as agitation and audiogenic seizures. Consistent with our previous findings (Uzbay et al., 1994, 1997, 1998, 2000a, 2000b, 2004; Unsalan et al., 2008), the present data demonstrated that daily ethanol consumption of > 9 g/kg for 14 consecutive days produced physical dependence in rats. Majchrowicz (1975) also showed that the dependence and signs of ethanol withdrawal could be produced in rats with 4-day intragastric administration of 9–15 g/kg of ethanol per day. High blood ethanol level (77 mg/dl) just before ethanol withdrawal as observed in the present study also indicates an adequate ethanol consumption for the induction of EWS. Thus, we observed several signs of ethanol withdrawal such as locomotor hyperactivity, stereotyped behaviours, wet dog shakes, tremors, tail stiffness, agitation and audiogenic seizures. As we did not observe any significant change in the locomotor activity in naive group, significant effects of clozapine on EWS might not be related to other non-specific effects such as sedation, muscle relaxation or locomotor stimulation.

In the present study, we recorded some body weight losses (up to 8%) in the rats fed with liquid diet including ethanol. A slight body weight loss in the rats fed by ethanol-containing liquid diet can be accepted as usual in similar studies (Parale and Kulkarni, 1986; Larue-Achagiotis et al., 1990; Uzbay and Kayaalp, 1995). On the other hand, an increase in the body weight of control rats compared to initial weights was also observed at the end of the study.

Doses were selected according to our preliminary tests. Since doses of clozapine > 10 mg/kg caused some sedative effects in rats, doses of clozapine up to 10 mg/kg were used in the present study.

As is known, both dopaminergic and serotonergic systems play a crucial role in the development of ethanol dependence (Kuriyama and Ohkuma, 1990; Uzbay, 2008). On the other hand, recent reports indicate that there is a relationship between schizophrenia and ethanol dependence (D’Souza et al., 2006; Seeman et al., 2006; Conroy et al., 2007). It could be expected that there might be potential beneficial effects of new atypical antipsychotic drugs in the treatment of the signs of ethanol withdrawal as well as blocking craving effects of ethanol (Drake et al., 2000; Hutchison et al., 2003). In a recent study from our laboratory, we also reported that olanzapine, another atypical antipsychotic drug, had some beneficial effects on some signs of ethanol withdrawal such as stereotyped behaviours and wet dog shakes in ethanol-dependent rats (Unsalan et al., 2008). Thus, in the present study, we tested the effects of atypical antipsychotic agent clozapine that blocks serotonin 5-HT2 receptors as well as dopamine D2 receptors (Schatzberg et al., 2003). In our study, while clozapine inhibited locomotor hyperactivity, stereotyped behaviours, wet dog shakes, tremors and tail stiffness, it was ineffective on agitation and audiogenic seizures. Thrasher et al. (1999) suggested that ethanol-stimulated locomotor activity was reduced by clozapine treatment in mice. Unsalan et al. (2008) also observed inhibitory effects on stereotyped behaviour and wet dog shakes in ethanol-dependent rats by olanzapine, another atypical antipsychotic, treatment. Our
Clozapine and Ethanol Withdrawal Syndrome

Fig. 3. Effects of clozapine treatment on the signs of ethanol withdrawal syndrome \( n = 8 \) for each group; Clz. = clozapine; h = hour; \# \( P < 0.05 \) significantly different from Control (−), ∗ \( P < 0.05 \) significantly different from Control (+), Dunnett’s test for counts, Mann–Whitney U-test for score and Chi-square test for % incidence; −: ethanol non-dependent; +: ethanol dependent]. Saline or clozapine was injected 30 min before 2nd and 6th hours of observation intervals.

results involved in beneficial effects of clozapine on ethanol withdrawal-induced locomotor hyperactivity, stereotyped behaviour and wet dog shakes are in line with the results of these studies. Inhibitory effects of clozapine on wet dog shakes and stereotyped behaviours may be explained by its serotonin 5-HT\(_2\) and dopamine D\(_2\) receptor antagonistic activity, respectively.

Several reports indicated the involvement of serotonin 5-HT\(_2\) receptors in the wet dog shake behaviours and blockade of 5-HT\(_2\) receptors by antagonists inhibited wet dog shakes in rats (Yap and Taylor, 1983; Fone et al., 1991; Takao et al., 1995). 5-HT\(_2\) antagonistic activity of clozapine may cause its inhibitory effect on wet dog shakes. Inhibitory effects on stereotyped behaviour could also be explained by its dopamine D\(_2\) receptor antagonistic activity. As previously described, combined stimulation of dopamine D\(_1\) and D\(_2\) receptors resulted in

<table>
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<tr>
<th>Treatment</th>
<th>Incidence (%)</th>
<th>Latency (s)</th>
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<tr>
<td>Control (ethanol dependent)</td>
<td>63 (5/8)</td>
<td>14.80 ± 1.38</td>
</tr>
<tr>
<td>Clozapine (2.5 mg/kg)(\textsuperscript{a})</td>
<td>75 (6/8)</td>
<td>11.17 ± 2.50</td>
</tr>
<tr>
<td>Clozapine (5 mg/kg)(\textsuperscript{a})</td>
<td>38 (3/8)</td>
<td>23.33 ± 6.94</td>
</tr>
<tr>
<td>Clozapine (10 mg/kg)(\textsuperscript{a})</td>
<td>50 (4/8)</td>
<td>13.25 ± 5.41</td>
</tr>
</tbody>
</table>

\( n = 8 \) for each group; figures in the parenthesis represent the number of animals that have seizure activity after audiogenic stimulus. \( \textsuperscript{a}\)Second dose injections 30 min before 6th hour of observation period.

Table 2. Effects of clozapine on the audiogenic seizures at 6th hour of ethanol withdrawal
dose-dependent behavioural activation associated with stereotyped behaviour in rats (Longoni et al., 1987; Dall’Olio et al., 1988). Evidence also supports the hypothesis that psychostimulant stereotypy is mediated through postsynaptic dopamine receptors (Feldman et al., 1997). Furthermore, it has been shown that dopamine D2 receptor antagonists inhibit stereotyped behaviour in rats (Magnusson et al., 1986). Thus, attenuation of ethanol withdrawal-induced stereotyped behaviour by clozapine, which is a dopamine D2 receptor antagonist at the same time, is not surprising. In contrast to results obtained from olanzapine (Unsalan et al., 2008), in the present study, clozapine did not cause any precipitation of posture and gait abnormalities in ethanol-withdrawn rats. Lower affinity of clozapine for D2 receptors than olanzapine (Stefan et al., 2002) may be due to its safer effects on ethanol withdrawal. As known, dopamine D2 receptor antagonists cause some motor disturbances and locomotor inhibition in rats (Hauber and Münkler, 1997).

Furthermore, in the present study, clozapine was found to be more effective than olanzapine on the signs of ethanol withdrawal. It was effective over more signs of ethanol withdrawal than olanzapine. In addition to beneficial effects of olanzapine on stereotyped behaviours and wet dog shakes, clozapine also inhibited tremors and tail stiffness in rats. Clozapine is a multi-receptor acting antipsychotic drug. It has antagonistic activity on dopaminergic and serotonergic receptors. Clozapine has higher affinity at the D1 and D2 receptors than D2 receptor and also binds to the extra-striatal D2-like receptor, the D3 receptor. Clozapine also has antagonistic activity at the 5HT1A, 5-HT2A, 5-HT3C and 5-HT1 receptors (Meltzer, 1994; Stefan et al., 2002). It has been known that 5-HT3 receptor antagonists have anxiolytic profile in the rodents. Further evidence that 5-HT3 receptors moderate limbic dopamine function is shown by their ability to reduce both the behavioural hyperactivity and changes in limbic dopamine metabolism. Importantly, the 5-HT3 receptor antagonists are highly effective to prevent the behavioural syndrome following withdrawal from treatment with some abused substances such as diazepam, nicotine, cocaine and alcohol (Costall et al., 1990, 1993). Thus, 5-HT2 and/or 5-HT3 antagonistic properties of clozapine may also be responsible for its inhibitory effects on EWS in rats.

Clozapine decreased stereotypy, tremors and wet dog shakes at 2nd and 6th hours of ethanol withdrawal, but not at 4th hour. Clozapine has a half-life of 1.5–1.6 h in rats (Baldessarini et al., 1993). Thus disappearing the effect of drug at 4th hour may be related to its rapid metabolism. On the other hand, reappearance of the effects after second administration of clozapine, before 6th hour testing, clearly indicates that observed changes were linked to clozapine treatment.

Antipsychotic medications can lower the seizure threshold, increasing the chances of seizure induction (Allredge, 1999; Hedges et al., 2003). Some recent reports also mentioned seizures or lowered seizure threshold associated with clozapine treatment (Langosch and Trimble, 2002; White and Van Cott, 2007; Wong and Delva, 2007). Thus, we expected worsened audiogenic seizures by clozapine treatment during ethanol withdrawal. However, neither incidence nor latency of audiogenic seizures was affected by clozapine treatment in ethanol-dependent rats. Moreover, clozapine reduced the incidence and prolonged the latency of the audiogenic seizures at dose of 5 mg/kg without reaching to statistically significant levels. Discrepancy may be due to differences between the species or different nature of withdrawal-induced seizures. Rats may be more resistant to effects of clozapine on seizure threshold. Simply, our findings showing ineffectiveness of clozapine on agitation and audiogenic seizures imply that these signs of ethanol withdrawal may not be related to mechanisms modulated by clozapine.

On the other hand, clozapine is an antagonist of α2 adrenoceptors with a high affinity (Kalkman et al., 1998; Svensson, 2003). Some clinical studies also indicated that there was a relationship between ethanol withdrawal and α2 adrenoceptor modulation (Nutt et al., 1988; Fahlke et al., 1999). However, studies performed on rats showed that α2 adrenergic receptor agonists had some beneficial effects on EWS (Parale and Kulkarni, 1986; Riihioja et al., 1997). In the present study, we observed that clozapine, an α2 antagonist, significantly alleviated some ethanol withdrawal signs in most behavioural assessments, while it was ineffective in some of them. Moreover, it did not worsen any signs of ethanol withdrawal. Thus, effects of clozapine on the signs of withdrawal could not be explained by an interaction with α2 receptors.

In conclusion, our results suggest that clozapine had some significant beneficial effects on EWS in rats and this drug may be helpful for controlling of some withdrawal signs in ethanol-dependent patients. However, clozapine can cause agranulocytosis in humans (Alvir et al., 1993). Thus, this situation should be taken into consideration during the treatment.

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