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Ipek Komsuoglu Celikyurt, Hakan Kayir, Guner Ulak, Faruk B. Erden, Gokhan K. Ulusoy, Tayfun I. Uzbay

*Kocaeli University, Faculty of Medicine, Department of Pharmacology, Izmit - Turkey

Gulhane Military Medical Academy, Faculty of Medicine Department of Medical Pharmacology, Psychopharmacology Research Unit, Ankara - Turkey

*Address for correspondence

Prof. I. Tayfun Uzbay
Gulhane Military Medical Academy,
Faculty of Medicine Department of Medical Pharmacology,
Psychopharmacology Research Unit,
Etlik 06018 Ankara – TURKEY

Tel: + 90 312 304 4764
Fax: + 90 312 304 2010
E-Mail: tuzbay@gata.edu.tr
tuzbay@gmail.com
ABBREVIATION LIST

5-HT: 5-hydroxytryptamine

ANOVA: Analysis of variance

dB: Decibel

EWS: Ethanol withdrawal syndrome

Qtp: Quetiapine

Risp: Risperidone

S.E.M.: The standard error of the mean

TUBITAK: Scientific and Technological Research Council of Turkey

Zip: Ziprasidone
ABSTRACT

Comorbid substance use in schizophrenic patients is common, and substance dependence is a predictive factor for psychosis. The present study was designed to investigate the effects of risperidone, quetiapine and ziprasidone, atypical antipsychotic drugs, on ethanol withdrawal syndrome (EWS) in rats. Adult male Wistar rats were used in the study. Ethanol (7.2%, v/v) was given to rats via a liquid diet for 21 days. An isocaloric liquid diet without ethanol was given to control rats. Risperidone (1 and 2 mg/kg), quetiapine (8 and 16 mg/kg), ziprasidone (0.5 and 1 mg/kg) and vehicle were injected into rats intraperitoneally at 1.5 and 5.5 hrs of ethanol withdrawal. At the 2\textsuperscript{nd}, 4\textsuperscript{th} and 6\textsuperscript{th} hrs of ethanol withdrawal, rats were observed for 5 min, and withdrawal signs that included locomotor hyperactivity, stereotyped behavior, abnormal gait and posture, tail stiffness and agitation were recorded or rated. Following the observations at the 6\textsuperscript{th} h, the rats were tested for audiogenic seizures. All three drugs had some significant inhibitory effects on EWS-induced behavioral signs beginning at the 2\textsuperscript{nd} h of withdrawal. The drugs also significantly reduced the incidence of audiogenic seizures. Overall, risperidone and quetiapine seemed to be more effective than ziprasidone in ameliorating the withdrawal signs. Doses of the drugs used in the present study did not produce any significant changes in locomotor activities of naïve rats. Our results suggest that risperidone, quetiapine and ziprasidone had beneficial effects on EWS in rats. Thus, these drugs may be helpful for controlling withdrawal signs in ethanol-dependent patients.

Keywords: Ethanol withdrawal; Risperidone; Quetiapine; Ziprasidone; Rat(s)
1. Introduction

Epidemiologic studies and clinical assessment of schizophrenic populations indicate that there is a marked relationship between schizophrenia and addictive behaviors. Observations of the general population have recognized a high risk of alcohol dependence or substance abuse in subjects presenting with criteria for schizophrenia (Regier et al., 1990; Batel, 2000). It has also been shown that schizophrenia is four times more frequent among alcoholic than non-alcoholic subjects (Helzer and Pryzbeck, 1988). Thus, comorbid substance use in schizophrenic patients is common, and substance abuse may be acceptable as a predictive factor for psychosis (Hambrecht and Hafner, 1996).

Ethanol abuse and dependence is one of the most important worldwide public health problems. The discontinuation of chronic administration of ethanol is associated with excitatory withdrawal signs called ethanol withdrawal syndrome (EWS). EWS is the most important evidence indicating the development of physical dependence on ethanol (Jaffe, 1990). Although attenuating the severity of EWS is very important, current treatment choices are very limited except for the use of benzodiazepines. Acamprosate (a glutamate antagonist), naltrexone (an opioid antagonist) and disulfiram (an aldehyde dehydrogenase blocker) are approved for the treatment of ethanol dependence, but these medications are effective in attenuating ethanol cravings and consumption rather than treatment of EWS (Heilig and Egli, 2006). New approaches and new drug choices are necessary for treatment of EWS.

A strong body of evidence indicates that ethanol activates dopamine release from the nucleus accumbens and extended amygdala (DiChiara, 1995; Heimer et al., 1997). The action of ethanol on the mesolimbic pathway is considered to be strongly associated with susceptibility to alcoholism (Noble, 1996) and the development of craving and loss of control (Robinson and Berridge, 1993). Neurochemical findings from clinical (Roy et al., 1987; Le Marquand et al., 1994) and experimental (Murphy et al., 1987; Uzbay et al., 1998; Uzbay et
al., 2000; Uzbay, 2008) studies have also suggested that significant changes in central serotonergic neurotransmission occur during ethanol consumption and/or withdrawal. Substantial experimental evidence indicates that serotonin has a critical role in impulsivity and craving, which are frequently seen in alcoholics (Ciccocioppo, 1999), and serotonin is at least partly responsible for alcohol dependence (Myers and Martin, 1973; Schuckit, 1996). As a result, dopaminergic and serotonergic systems play an important role in the development of ethanol dependence. Thus, drugs that affect the central dopaminergic and serotonergic systems may be helpful for controlling ethanol abuse and dependence.

Atypical antipsychotic drugs are widely used in patients with schizophrenia. In contrast to classical (or typical) antipsychotics, these drugs have substantially lower risks of extrapyramidal symptoms, including tardive dyskinesia. They also modulate serotonergic and dopaminergic receptors (Schatzberg et al., 2003). Although several reports investigating the effects of new atypical antipsychotics, such as clozapine, risperidone, quetiapine and aripiprazole, on ethanol abuse and dependence have been published, many of them examined ethanol consumption, preference or craving (Drake et al., 2000; Ingman et al., 2003; Potvin et al., 2003; Martinotti et al., 2008; Vergne and Anton, 2010). These observations imply that new atypical antipsychotic drugs could be effective in the treatment of ethanol dependence, but there are limited reports investigating the effects of these drugs on EWS. In our previous studies, we tested the effects of clozapine and olanzapine, atypical antipsychotic drugs, on EWS in rats. We observed some beneficial effects of clozapine on EWS (Kayir and Uzbay, 2008). On the other hand, while olanzapine inhibited the intensity of some symptoms such as stereotyped behavior and wet dog shakes, it had some adverse effects on other signs such as abnormal gait and posture (Unsalan et al., 2008). Effects of risperidone, quetiapine and ziprasidone, relatively new and effective atypical drugs, on EWS have not been subjected to clinical or experimental studies yet.
The main objective of the present study was to investigate the effects of risperidone, quetiapine and ziprasidone on the signs of EWS in rats. Thus, the current study was focused on revealing whether these drugs are effective in attenuating ethanol withdrawal or not.

2. Materials and methods

2.1. Animals and laboratory

All procedures in this study were 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 attained, numbered 08/36 K-R on April 4, 2008. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Adult male Wistar rats (220-320 g 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 behavioral experiments involved in examining EWS were carried out in other separate and isolated laboratories, which had the same environmental conditions as the colony room.

2.2. 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 was as follows: 925-975 mL cow milk (Danone, Turkey), 25-75 mL ethanol (96.5% ethyl alcohol; Tekel, Turkish State Monopoly), 5000 IU vitamin A (Aksu Farma, Turkey) and 17 g sucrose (Uzbay and Kayaalp, 1995). This mixture supplies 1000.7 kcal/L.

At the beginning of the study, all of the rats were given the modified 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 21 days. Liquid diet was freshly prepared daily and presented at the same time of the day (09:00 h). The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as g per kg per day. Control rats (n=8) were pair fed with an isocaloric liquid diet containing sucrose as a caloric substitute for ethanol.

2.3 Drug used in the study

Risperidone (Teva Ltd. API Division, Israel), quetiapine (Sanovel, Turkey) and ziprasidone (Pfizer, Turkey) were dissolved in vehicle (0.1% acetic acid). All drugs and vehicle were injected into rats intraperitoneally at a volume of 1 mL/200 g body weight. Drug solutions were prepared freshly in the morning just before administration.

2.4 Evaluation of EWS

At the end of the exposure to the 7.2% ethanol-containing liquid diet, diet with ethanol was withdrawn and replaced with isocaloric ethanol free diet at 09:30 h. Ethanol-dependent rats were then assigned into seven groups randomly (n=8 for each group). Risperidone (1 and 2 mg/kg), quetiapine (8 and 16 mg/kg), ziprasidone (0.5 and 1 mg/kg) and vehicle were injected into the rats 30 min before ethanol withdrawal evaluation. At the 2nd, 4th and 6th hrs of ethanol withdrawal, rats were observed for 5 min, and withdrawal signs including locomotor hyperactivity, stereotyped behavior, abnormal posture and gait, tail stiffness and agitation were recorded or rated as previously described (Uzbay and Kayaalp, 1995; Uzbay et al., 1997; Kayir and Uzbay, 2008).

Locomotor activities of the rats were recorded using 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 ± S.E.M.
Grooming, sniffing, head weaving, gnawing and chewing were observed as major stereotyped behaviors during the ethanol withdrawal in the study. The total number of stereotyped behaviors for each observation period was calculated and expressed as mean ± S.E.M. Abnormal gait and posture, agitation and tail stiffness were scored 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 h of 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 liquid diet without ethanol were also evaluated for ethanol withdrawal signs parallel to ethanol-dependent groups.

All experiments were carried out during the light period. All ratings were scored by a naive observer who was blind to the treatments that the rats received.

2.5. Measurements of locomotor activity in naive control rats

The experimental drugs and vehicle were administered in seven groups of naive (not ethanol-dependent) Wistar rats. Thirty minutes after the injections, rats were put into the locomotor activity test apparatus and locomotor activities of the rats were measured for 30 min. The results of the locomotor activity tests were expressed as mean ± S.E.M.

2.6. Statistics

Changes in locomotor activity and body weight of ethanol-dependent rats as compared to ethanol non-dependent control rats were analyzed by two-way analysis of variance (ANOVA) tests (treatment x time). A two-way ANOVA (treatment x time) followed by Bonferroni or Student’s t-test was used in the evaluation of the effects of the drugs on the locomotor hyperactivity, stereotyped behaviors, latency of audiogenic seizures, abnormal gait
and posture, agitation and tail stiffness in different treatment groups. Comparison of the incidence of audiogenic seizures was completed using a chi-square test. A two-way ANOVA (group x time) was used to compare ethanol consumptions of the groups given the ethanol-containing liquid diet. The effects of risperidone, quetiapine and ziprasidone on locomotor activities of the naïve rats were also analyzed by a two-way ANOVA (group x time) followed by Bonferroni test. The level of significance was set at p<0.05.

3. Results

3.1. Rat ethanol consumption and body weight gain

Daily ethanol consumption of the rats in control and drug-treated groups ranged from 9.23±0.85 to 15.72±0.88 g/kg during the exposure to 7.2% ethanol. While there was a difference in consumption amounts between the days of the last two weeks including the 7.2% alcohol consumption, the difference between the groups was not statistically significant. The results of the two-way ANOVA on ethanol consumption data revealed significant effects for time [F(13,637)=9.694, p<0.0001] and interaction [F(78,637)=3.911, p<0.0001] effects but not a group effect [F(6,49)=2.018, p=0.081]. These data indicate that there was no significant difference in ethanol consumption between the groups. However, ethanol consumption of the rats significantly increased during exposure to the liquid diet.

Body weight changes of the ethanol-fed and control rats are presented in Table 1. Body weights of the rats increased slightly during the study in ethanol feeding groups. There was a 4.5% increase in body weight during the study in the ethanol fed groups. In control rats, an increase in body weight of approximately 17.6% was observed. This increase in body weight of the rats was statistically significant compared to the beginning of the study (Student’s t-test, p<0.05).
3.2. Behavioral changes during ethanol withdrawal

A two-way ANOVA revealed that there was a significant difference between the locomotor activities of the ethanol-dependent and non-dependent rats during the withdrawal testing [treatment effect: F(1,12)= 8.625; p=0.012]. The locomotor activity values changed during the time course of withdrawal [time effect: F(2,24)=47.627; p<0.0001], but the difference between groups was not dependent on the time course [interaction: F(2,24)=2.334; p>0.05]. Post-hoc analysis indicated significant locomotor hyperactivity in the ethanol-dependent groups at the 2nd and 4th hrs of the withdrawal testing period compared to the ethanol non-dependent vehicle groups (p<0.05) (Fig. 1). Other behavioral signs of EWS, such as stereotyped behaviors, abnormal posture and gait, tail stiffness and agitation, were apparent during the whole observation period (Fig. 2-7 A-D, dark bars).

Audiogenic seizures occurred at the 6th h of ethanol withdrawal with an incidence of 50% and latency of 12.25 ± 6.55 s in the ethanol-dependent control group (Table 2). No ethanol withdrawal signs were observed in the ethanol non-dependent rats.

3.3. Effects of risperidone on EWS

Risperidone produced significantly affected EWS-induced locomotor hyperactivity [treatment effect: F(2,20)=14.354; p<0.0001] with a significant interaction [F(4,40)=15.615; p<0.0001] with testing time point. Post-hoc analysis of the data indicated that 1 and 2 mg/kg risperidone significantly reduced ethanol withdrawal-induced locomotor hyperactivity at the 2nd h but not the 4th and 6th hrs of ethanol withdrawal (p<0.05, Bonferroni test) (Fig. 2A).

Similarly, risperidone significantly affected stereotyped behaviors observed during ethanol withdrawal [treatment effect: F(2,20)=6.138; p=0.008]. The effect of testing time and interaction between treatment and time were not significant for stereotyped behaviors [time effect: F(2,40)= 2.873; p>0.05 and interaction: F(4,40)= 1.417; p>0.05]. Post-hoc analysis of
the data indicated that 1 and 2 mg/kg risperidone significantly reduced EWS-induced stereotyped behaviors at the 2\textsuperscript{nd} h of withdrawal (p< 0.05, Bonferroni test), and this inhibitory effect on stereotyped behaviors was not observed at the 4\textsuperscript{th} and 6\textsuperscript{th} hrs of ethanol withdrawal (p\textsubscript{s}>0.05, Bonferroni test, Fig. 2B).

Risperidone significantly affected abnormal posture [F(2,20)=74.108; p<0.0001, Fig. 3A], abnormal gait [F(2,20)=154.417; p<0.0001, Fig. 3B], tail stiffness [F(2,20)=43.786; p<0.0001, Fig. 3C] and agitation [F(2,20)=43.786; p<0.0001, Fig. 3D] at the 2\textsuperscript{nd}, 4\textsuperscript{th} and 6\textsuperscript{th} hrs of ethanol withdrawal. Post-hoc analysis of data obtained from the whole observation period indicated that all doses of risperidone were significantly effective on all these signs, except the 2 mg/kg dose did not affect abnormal posture (p\textsubscript{s}<0.05, Bonferroni test).

Risperidone (1 and 2 mg/kg) also significantly reduced the incidence of audiogenic seizures appearing at the 6\textsuperscript{th} h of withdrawal (p<0.05, chi-square test) (Table 2).

3.4. Effects of quetiapine on EWS

Quetiapine significantly affected EWS-induced locomotor hyperactivity [treatment effect: F(2,21)=171.139; p<0.0001] with a significant interaction [F(4,42)=4.882; p=0.003] with testing time point. Post-hoc analysis of the data indicated that 8 and 16 mg/kg quetiapine significantly reduced EWS-induced locomotor hyperactivity at the 2\textsuperscript{nd} h of ethanol withdrawal (p<0.05, Bonferroni test, Fig. 4A).

Quetiapine significantly affected stereotyped behaviors observed during EWS [treatment effect: F(2,21)=33.140; p<0.0001, Fig. 4B]. The effect of testing time and interaction between treatment and time were not significant for stereotyped behaviors [time effect: F(2,42)=2.303; p>0.05 and interaction: F(4,42)=0.903; p>0.05]. Post-hoc analysis of the data indicated that 16 mg/kg quetiapine significantly inhibited (p\textsubscript{s}<0.05, Bonferroni test)
the stereotyped behaviors at the 2nd and 4th hrs of EWS, and this activity was not observed at the 6th h of testing (Fig. 4B).

Quetiapine also significantly affected abnormal posture $[F(2,21)=68.665; \ p<0.0001$, Fig. 5A], abnormal gait $[F(2,21)=91.263; \ p<0.0001$, Fig. 5B], tail stiffness $[F(2,21)=21.754; \ p<0.0001$, Fig. 5C] and agitation $[F(2,21)=14.646; \ p<0.0001$, Fig. 5D] at the 2nd, 4th and 6th hrs of ethanol withdrawal. Post-hoc analysis of the data obtained from the whole observation period indicated that both doses of quetiapine had significant effects on abnormal gait and posture and tail stiffness ($p_{s}<0.05$, Bonferroni test). However, the inhibitory effect of quetiapine on agitation was significant only at the 8 mg/kg dose ($p_{s}<0.05$, Bonferroni test).

Quetiapine (8 and 16 mg/kg) also significantly reduced the incidence of audiogenic seizures appearing at the 6th h of withdrawal ($p<0.05$, chi-square test) (Table 2).

3.5. Effects of ziprasidone on EWS

Ziprasidone significantly affected EWS-induced locomotor hyperactivity [treatment effect: $F(2,21)=226.464; \ p<0.0001$] with a significant interaction $[F(4,42)=2.737; \ p=0.041]$ with testing time point. Post-hoc analysis of the data indicated that 1 mg/kg ziprasidone significantly reduced EWS-induced locomotor hyperactivity at the 2nd and 6th hrs of EWS ($p<0.05$, Bonferroni test, Fig. 6A).

Ziprasidone did not significantly affect stereotyped behaviors observed during EWS [treatment effect: $F(2,21)=0.800; \ p>0.05$, Fig. 6B]. The effect of testing time and interaction between treatment and time were also not significant for stereotyped behaviors [time effect: $F(2,42)=0.155; \ p>0.05$ and interaction: $F(4,42)=1.288; \ p>0.05$].

Ziprasidone also significantly affected abnormal posture $[F(2,21)=11.972; \ p<0.0001$, Fig. 7A], abnormal gait $[F(2,21)=18.702; \ p<0.0001$, Fig. 7B], tail stiffness $[F(2,21)=9.647; \ p<0.0001$, Fig. 7C] and agitation $[F(2,21)=7.177; \ p=0.004$, Fig. 7D] during EWS. Post-hoc
analysis of the data obtained from the whole observation period indicated that ziprasidone had inhibitory effects on abnormal posture and abnormal gait only at the 6th h of EWS. Ziprasidone inhibited abnormal posture at a dose of 0.5 mg/kg but not at 1 mg/kg. However, 1 mg/kg ziprasidone affected abnormal gait (p<0.05, Bonferroni test, Fig. 7B). Tail stiffness was reversed by ziprasidone at all time points and drug doses, except at the 6th h at the 1 mg/kg dose (Fig. 7C). The inhibitory effect of ziprasidone on agitation was significant at the 2nd and 6th hrs of observation at both doses. (p<0.05, Bonferroni test).

At the dose of 1 mg/kg, ziprasidone significantly reduced the incidence of audiogenic seizures (p<0.05, chi-square test) (Table 2).

3.6. Effects of risperidone, quetiapine and ziprasidone on locomotor activity in ethanol non-dependent (naïve) rats

Risperidone (1 and 2 mg/kg) significantly affected locomotor activity of naïve (ethanol non-dependent) rats during a parallel time course with EWS experiments [treatment effect: F(2,21)=18.203; p<0.0001, Fig. 8A]. Measurement time had no effect on the results [time effect: F(2,42)=1.248; p>0.05], but the effect of risperidone on locomotor activity interacted with the measurement time [interaction: F(4,42)=3.951; p=0.008]. Post-hoc analysis indicated that 1 and 2 mg/kg risperidone significantly decreased locomotor activity of the naïve rats only at the 6th h time point (p<0.05, Bonferroni test, Fig. 8A).

Quetiapine (8 and 16 mg/kg) also significantly affected locomotor activity of naïve rats [treatment effect: F(2,21)=11.205; p<0.0001, Fig. 8B]. Measurement time independently changed the locomotor activity counts [time effect: F(2,42)=13.948; p<0.0001, interaction: F(4,42)=0.699;p> 0.05]. Post-hoc analysis indicated that the 16 mg/kg dose but not the 8 mg/kg dose of quetiapine significantly decreased locomotor activity of the naïve rats at all three time points (p<0.05, Bonferroni test, Fig. 8B).
Ziprasidone (0.5 and 1 mg/kg) significantly affected locomotor activity of naïve rats [treatment effect: F(2,21)=10.935; p<0.0001, Fig. 8C]. Measurement time also had a significant effect on the locomotor activity counts [time effect: F(2,42)=4.164; p=0.022]. A significant interaction between treatment and time was also obtained [interaction: F(4,42)=4.269; p=0.005]. Post-hoc analysis indicated that both doses (0.5 and 1 mg/kg) of ziprasidone significantly decreased locomotor activity of the naïve rats at the 2\textsuperscript{nd} h time point, and 1 mg/kg ziprasidone had a significant effect at the 6\textsuperscript{th} h time point (p\textsubscript{s}<0.05, Bonferroni test, Fig. 8C).

4. Discussion

In this study, risperidone, quetiapine and ziprasidone, relatively new atypical antipsychotic drugs, had inhibitory effects on the behavioral signs of EWS such as locomotor hyperactivity, stereotyped behavior, abnormal posture and gait, tail stiffness and agitation. Moreover, they were found to be effective on audiogenic seizures during EWS. Consistent with our previous findings (Uzbek et al., 1994; Uzbek et al., 1997; Uzbek et al., 1998; Uzbek et al., 2000a,b; Uzbek et al., 2004a; Unsalan et al., 2008), the present data demonstrated that daily ethanol consumption over 9 g/kg for 15 consecutive days produced physical dependence in rats. Majchrowicz (1975) also showed that dependence and signs of ethanol withdrawal could be produced in rats with intragastric administration of 9-15 g/kg of ethanol per day for four days. We did not measure blood ethanol levels in the present study. However, we detected high blood ethanol levels in our previous studies with the same method (Uzbek et al., 1994; Uzbek and Kayaalp, 1995; Uzbek et al., 1997; Uzbek et al., 1998; Uzbek and Erden, 2003; Uzbek et al., 2004b; Kayir and Uzbek, 2008). In addition, we observed several signs of ethanol withdrawal such as locomotor hyperactivity, stereotyped behaviors, abnormal posture and gait, tail stiffness, agitation and audiogenic seizures. Audiogenic seizure response is considered as the most reliable and easily quantifiable behavioral sign of EWS in rats.
(Majchrowicz, 1975; Trzaskowska et al., 1989; Uzbay and Kayaalp, 1995). The incidence of audiogenic seizures observed in the present study was very similar to that of our previous studies (Uzbay et al., 1994; Uzbay and Kayaalp, 1995; Uzbay et al., 1997; Uzbay et al., 2000a).

In the present study, we did not observe any body weight losses in the rats fed by liquid diet with ethanol. Even a slight body weight loss in the rats fed with an ethanol-containing liquid diet can be accepted as normal (Parale and Kulkarni, 1986; Larue-Achagiotis et al., 1990; Uzbay and Kayaalp, 1995). In fact, an increase in body weight in control rats compared to initial weights was observed at the end of the study.

Drug doses were selected according to our preliminary tests and previous studies. Because doses of the drugs higher than used in the study caused some sedative effects or slight extrapyramidal symptoms such as tremor in rats, doses up to 2, 16 and 1 mg/kg risperidone, quetiapine and ziprasidone, respectively, were used in the present study. In naïve rats, both doses of risperidone and ziprasidone and a higher dose of quetiapine (16 mg/kg) reduced locomotor activity significantly at the 2nd h of ethanol withdrawal. However, the effects of risperidone on locomotor activity disappeared at later observation periods. Even after a second injection before the 6th h of observation period, risperidone did not significantly affect locomotor activity of the naïve rats. Quetiapine (8 mg/kg) also did not significantly affect locomotor activities at all observation periods. Thus, the observed effects of these drugs on EWS seem to be specific and not dependent on other non-specific effects such as sedation and muscle relaxation. Ziprasidone exhibited some significant inhibitory effects on locomotor activities of naïve rats except at the 4th h of the observation period. Thus, some significant inhibitory effects induced by ziprasidone may be related to its sedative effects. However, because some limited beneficial effects of ziprasidone, for example inhibition of audiogenic seizures, appeared as a result of only the higher dose, we did not use lower doses of this drug.
in the present study. While risperidone and ziprasidone caused significant reductions in locomotor activities of naïve rats during the first injections and observation period, these inhibitory effects did not occur during the following observation periods, even after the second injections. It is difficult to explain this occurrence with the present data, but it may be related to acute unlike effects of the drugs in the rats followed by a tolerance development.

Both dopaminergic and serotonergic systems play a crucial role in the development of ethanol dependence (Kuriyama and Ohkuma, 1990; Uzbay, 2008), and 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). There may be potential beneficial effects of new atypical antipsychotic drugs in the treatment of the signs of ethanol withdrawal as well as blocking the craving effects of ethanol (Drake et al., 2000; Hutchison et al., 2003). In recent studies from our laboratory, we investigated and reported the effects of olanzapine (Unsalan et al., 2008) and clozapine (Kayir and Uzbay, 2008), another atypical antipsychotic drug, on EWS in ethanol-dependent rats. In the present study, we tested the effects of three additional, relatively new atypical antipsychotic agents: risperidone, quetiapine and ziprasidone, which blocks serotonin 5-HT_2 receptors as well as dopamine D_2 receptors (Stefan et al., 2002; Schatzberg et al., 2003). In our previous study, clozapine inhibited locomotor hyperactivity, stereotyped behaviors, wet dog shakes, tremors and tail stiffness during ethanol withdrawal. 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 behavior and wet dog shakes in ethanol-dependent rats by olanzapine treatment. Our results regarding the beneficial effects of risperidone and quetiapine on ethanol withdrawal-induced locomotor hyperactivity and stereotyped behaviors are in line with the results of these studies. Marked inhibitory effects of risperidone and quetiapine on locomotor
hyperactivity and stereotyped behaviors may be explained by their dopamine D₂ receptor antagonistic activity.

As previously described, combined stimulation of dopamine D₁ and D₂ receptors resulted in dose-dependent behavioral activation associated with stereotypes in rats (Longoni et al., 1987; Dall’Olio et al., 1988). Evidence also supports the hypothesis that psychostimulant-induced stereotypy is mediated through postsynaptic dopamine receptors (Feldman et al., 1997). Furthermore, it has been shown that dopamine D₂ receptor antagonists inhibit stereotyped behaviors in rats (Magnusson et al., 1986). Thus, attenuation of ethanol withdrawal-induced stereotyped behaviors by risperidone and quetiapine, which are both dopamine D₂ receptor antagonists, is not surprising.

In contrast to results obtained with olanzapine (Unsalan et al., 2008), in the present study, risperidone, quetiapine and ziprasidone did not cause any precipitation of posture and gait abnormalities in ethanol-withdrawn rats. Conversely, they significantly inhibited the intensity of abnormal posture and gait that occurred during ethanol withdrawal. Atypical antipsychotic drugs cause fewer extrapyramidal symptoms at effective doses. This may be related to their potent 5-HT₂A and relatively weak D₂ receptor blocking properties (Meltzer, 1995). Our findings regarding abnormal posture and gait are in line with this general information. Precipitation of abnormal posture and gait in ethanol-dependent rats by olanzapine treatment may be due to another property of this drug.

In the present study, we observed some biphasic profiles with risperidone and ziprasidone treatments on abnormal posture. These drugs were found to be effective on abnormal posture at their low doses, and this effect disappeared at higher doses. This situation was not observed for other behavioral signs of ethanol withdrawal. Furthermore, these drugs did not exhibit biphasic effects on locomotor activity in non-dependent rats. The biphasic
effects may be related to insufficient selectivity in the scoring method used to evaluate abnormal posture during ethanol withdrawal in the present study.

In our study, all three atypical antipsychotics were found to be effective on other behavioral signs of EWS such as tail stiffness and agitation. Agitation, together with locomotor hyperactivity, may be a reflection of anxiety occurring during ethanol withdrawal. Previous studies showed that antagonism of 5-HT\textsubscript{2} receptors, especially the 5-HT\textsubscript{2C} subtype, reduced anxiogenic behaviors during ethanol withdrawal (Lal et al., 1993; Overstreet et al., 2003; Overstreet et al., 2006). Risperidone, quetiapine and ziprasidone have antagonistic activity at the 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors. Affinity of quetiapine for 5-HT\textsubscript{2A} receptors is lower than risperidone and ziprasidone (Stefan et al., 2002; Schatzberg et al., 2003). Although literature on the role of 5-HT\textsubscript{2A} receptors in ethanol abuse and dependence is very limited, an antagonism at 5-HT\textsubscript{2A} receptors could be responsible for the beneficial effects of the drugs on EWS. These three drugs also bind to a range of non-dopaminergic and non-serotonergic targets, including glutamate, histamine, alpha-adrenergic and muscarinic receptors (Nasrallah, 2008). Interaction with other neurotransmitter systems may contribute to their beneficial effects on all of the signs of ethanol withdrawal. For instance, all three agents used in the present study have antagonistic activity at noradrenergic \(\alpha_1\) receptors, and it was shown that \(\alpha_1\)-noradrenergic receptor antagonism blocked acute withdrawal signs in ethanol-dependent rats (Walker et al., 2008).

Antipsychotic medications can lower the seizure threshold and increase the chance of seizure induction (Alldregge, 1999; Hedges et al., 2003). Thus, antipsychotic treatments during ethanol withdrawal may worsen audiogenic seizures. However, in our study, all three agents significantly reduced the incidence of audiogenic seizures. Previously, we showed that neither clozapine (Kayir and Uzbay, 2008) nor olanzapine (Unsalan et al., 2008) treatments affected the incidence and the latency of audiogenic seizures in ethanol-dependent rats. Our
previous findings showing the ineffectiveness of clozapine and olanzapine on audiogenic seizures imply that this sign of ethanol withdrawal may not be related to mechanisms modulated by clozapine and olanzapine. Our results also indicate that atypical antipsychotics are safer than classical agents during ethanol withdrawal in alcoholic patients with epilepsy.

Many reports have indicated marked reductions in the brain 5-HT levels of ethanol-withdrawn rats after seizures induced by audiogenic stimulus (Mirowski et al., 1995; Uzbay et al., 1998; Yu et al., 2000). In addition, it has been suggested that bromocriptine, a dopaminergic agonist, prevents (Trzaskowska et al., 1989) or prolongs (Uzbay et al., 1994) the latency of audiogenic seizures in ethanol-dependent rats. Thus, all of these data indicate that increased serotonergic and dopaminergic activity may be a protective response to the audiogenic seizures that occur during ethanol withdrawal in rats. Because of their dopaminergic and serotonergic antagonistic properties, the beneficial effects of risperidone, quetiapine and ziprasidone could not be explained via their interactions with serotonergic and/or dopaminergic receptor systems. Also, because H₁ receptor antagonists increased seizure susceptibility in rodents (Gerald and Richter, 1976; Sturman et al., 2001), the H₁ receptor antagonistic property of the atypical antipsychotics could not be responsible for the inhibitory effects on audiogenic seizures. However, Semenova and Ticku (1997) showed that treatment with the α₂ adrenoceptor blockers reduced the sensitivity of mice to audiogenic convulsions. The blocking effect at α₂ receptors by risperidone and quetiapine may be responsible for their beneficial effects on audiogenic seizures. The effect of ziprasidone on α₂ receptors is controversial, and it is not as clear as risperidone and quetiapine. In the present study, risperidone and quetiapine were found to be more effective than ziprasidone on audiogenic seizures. This finding may also be due to antagonistic efficacies of these drugs on α₂ receptors. Overall, in contrast to clozapine and olanzapine, marked inhibition of audiogenic seizures by risperidone, quetiapine and ziprasidone treatments is a very interesting finding.
However, we cannot currently give a definitive explanation of how these drugs inhibited the audiogenic seizures. Further studies are necessary to elucidate the mechanisms behind this phenomenon.

5. Conclusion

Our results suggest that risperidone, quetiapine and ziprasidone had significant beneficial effects on EWS in rats. Thus, these drugs may be helpful for controlling some withdrawal symptoms in ethanol-dependent patients. Moreover, we observed that risperidone and quetiapine were more effective than ziprasidone. Further research is needed to explain the differences between the activities of these three drugs.

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Figure legends

Figure 1. Changes in locomotor activity during the first 6 h of ethanol withdrawal (n=8 for each group; h=hour; * p<0.05 significantly different from control, Student’s t-test).

Figure 2. Effects of risperidone treatments on the locomotor activities (A) and stereotyped behaviors (B) of rats during ethanol withdrawal [n=8 for each group; Risp=risperidone; h=hour; Control (+), ethanol-withdrawn group treated with vehicle; * p<0.05 significantly different from Control (+), Bonferroni test].

Figure 3. Effects of risperidone treatment on abnormal posture (A), gait (B), tail stiffness (C) and agitation (D) during ethanol withdrawal [n=8 for each group; Risp=risperidone; h=hour; Control (+), ethanol-withdrawn group treated with vehicle; * p<0.05 significantly different from Control (+), Bonferroni test].

Figure 4. Effects of quetiapine treatments on the locomotor activities (A) and stereotyped behaviors (B) of rats during ethanol withdrawal [n=8 for each group; Qtp=quetiapine; h=hour; Control (+), ethanol-withdrawn group treated with vehicle; * p<0.05 significantly different from Control (+), Bonferroni test].

Figure 5. Effects of quetiapine treatment on abnormal posture (A), gait (B), tail stiffness (C) and agitation (D) during ethanol withdrawal [n=8 for each group; Qtp=quetiapine; h=hour; Control (+), ethanol-withdrawn group treated with vehicle; * p<0.05 significantly different from Control (+), Bonferroni test].

Figure 6. Effects of ziprasidone treatments on the locomotor activities (A) and stereotyped behaviors (B) of rats during ethanol withdrawal [n=8 for each group; Zip=ziprasidone; h=hour; Control (+), ethanol-withdrawn group treated with vehicle; * p<0.05 significantly different from Control (+), Bonferroni test].
Figure 7. Effects of ziprasidone treatment on abnormal posture (A), gait (B), tail stiffness (C) and agitation (D) during ethanol withdrawal [n=8 for each group; Zip=ziprasidone; h=hour; Control (+), ethanol-withdrawn group treated with vehicle; * p<0.05 significantly different from Control (+), Bonferroni test].

Figure 8. Effects of risperidone (A), quetiapine (B) and ziprasidone (C) on locomotor activity in naïve (ethanol non-dependent) rats [n=8 for each group; Risp=risperidone; Qtp=quetiapine; Zip=ziprasidone; h= hour; * p<0.05 significantly different from control, Bonferroni test].
Figure 1 (Konsuoglu Celikyurt et al.)
Figure 2 (Komsuoglu Celikyurt et al.)
Figure 3 (Komsuoglu Celikyurt et al.)
Figure 4 (Komsuoglu Celikyurt et al.)
Figure 5 (Komsuoglu Celikyurt et al.)
Figure 6 (Komsuoglu Celikyurt et al.)
Figure 7 (Komsuoglu Celikyurt et al.)
Figure 8 (Konsuoglu Celikyurt et al.)
Table 1

Changes in weight gains 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>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-) (n=8)</td>
<td>240.17 ± 6.39</td>
<td>282.50 ± 5.93*</td>
<td>+ 17.6%</td>
</tr>
<tr>
<td>Control (+) and drugs (n=56)</td>
<td>233.75 ± 9.83</td>
<td>244.37 ± 7.82</td>
<td>+ 4.5%</td>
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</tbody>
</table>

* p< 0.05, Student’s t test; Control (-), groups fed without ethanol; Control (+), groups fed with ethanol containing liquid diet.
Table 2

Effects of risperidone, quetiapine and ziprasidone on the audiogenic seizures in ethanol-dependent rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence (%)</th>
<th>Latency (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ethanol-dependent)</td>
<td>50 (4/8)*</td>
<td>12.25 ± 6.55</td>
</tr>
<tr>
<td>Risperidone (1 mg/kg)</td>
<td>0 (0/8)*</td>
<td>-</td>
</tr>
<tr>
<td>Risperidone (2 mg/kg)</td>
<td>0 (0/8)*</td>
<td>-</td>
</tr>
<tr>
<td>Quetiapine (8 mg/kg)</td>
<td>12.5 (1/8)*</td>
<td>-</td>
</tr>
<tr>
<td>Quetiapine (16 mg/kg)</td>
<td>0 (0/8)*</td>
<td>-</td>
</tr>
<tr>
<td>Ziprasidone (0.5 mg/kg)</td>
<td>25 (2/8)</td>
<td>-</td>
</tr>
<tr>
<td>Ziprasidone (1 mg/kg)</td>
<td>12.5 (1/8)*</td>
<td>-</td>
</tr>
</tbody>
</table>

n= 8 for each group; * p<0.05 significantly different from control (chi-square test); figures in the parenthesis represent the number of animals that have seizure activity after audiogenic stimulus; -: statistical analyses were not performed and data were not given in the groups having less than three rats with seizure activity.
Research Highlights

- Ethanol consumption for 21 days caused physical dependence in rats.
- Risperidone, quetiapine and ziprasidone attenuated ethanol withdrawal syndrome.
- Effective doses of drugs did not affect locomotory activity of naïve animals.
- Risperidone and quetiapine seemed to be more effective than ziprasidone.
- These drugs may be helpful for controlling of ethanol withdrawal syndrome.