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SEROTONERGIC ANTI-DEPRESSANTS AND ETHANOL WITHDRAWAL SYNDROME: A REVIEW

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Abstract — Aim: To review laboratory findings on the effects of anti-depressant agents that interact with the serotonergic system on signs of ethanol withdrawal syndrome in rats. **Method:** Adult Wistar rats received a modified liquid diet to produce ethanol dependence. Signs of ethanol withdrawal, locomotor hyperactivity, stereotyped behaviour, tremor, wet dog shakes, agitation, and audiogenic seizures, were evaluated for the first 6 h of ethanol withdrawal. The effects of the anti-depressants fluoxetine, venlafaxine, escitalopram, tianeptine, and extract of *Hypericum perforatum* (St. John's wort) (HPE) were examined. **Results:** Some beneficial effects of fluoxetine, tianeptine, HPE, escitalopram and venlafaxine on ethanol withdrawal signs were observed, ranked as follows: fluoxetine = tianeptine > HPE > escitalopram > venlafaxine. **Conclusions:** Tianeptine and fluoxetine seem to be potent pharmacologically active agents on ethanol withdrawal syndrome in rats. Thus, these anti-depressants may be useful in treatment of ethanol withdrawal syndrome in patients with alcoholism. In addition to serotonergic effects, interactions with nitrergic, glutamatergic, and adenosinergic systems may also provide a significant contribution to the beneficial effects of these drugs on ethanol withdrawal syndrome.

INTRODUCTION

Ethanol abuse and dependence remain among the most common substance abuse problems worldwide. The discontinuation of chronic administration of ethanol is associated with excitatory withdrawal signs called ethanol withdrawal syndrome. Ethanol withdrawal syndrome is the most important evidence, which indicates the development of a physical dependence to ethanol (O'Brien, 1996). Although the signs of ethanol withdrawal syndrome in humans (Thompson, 1978) and rodents (Majchrowicz, 1975; Uzbay and Kayaalp, 1995; Uzbay *et al*., 1997) have been well described, the mechanisms underlying physical dependence to ethanol and ethanol withdrawal syndrome are poorly understood. Among the numerous neurotransmitter systems implicated in the pharmacological effects of ethanol, the serotonergic system has received particular attention. Serotonergic system has been shown to play an important role in the regulation of ethanol intake, preference, and dependence via central mechanisms (Roy *et al*., 1987; Rezvani *et al*., 1990; Ferreria and Soares-DaSilva, 1991; McBride *et al*., 1991; Sellers *et al*., 1992; Wallis *et al*., 1993; LeMarquand *et al*., 1994; Uzbay *et al*., 1998, 2000).

Depression is an important psychiatric disorder that affects individuals' quality of life and social relations directly. Depression is characterized by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness, and amotivation, as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances, and loss of appetite. When the symptoms are very severe, major depression is considered. The prevalence of major depression is approximately 9% both in the United States and Europe (Fichter *et al*., 1996; Lepine *et al*., 1997). A decrease in serotonergic activity is associated with depression. In experimental studies, a decrease in brain serotonergic activity due to social isolation is known for decades (Garattini *et al*., 1967). Specifically, rodents show hyperactive and aggressive behaviour during long-term social isolation, which can be blocked with anti-depressant treatment (Garzon and del Rio, 1981). These social isolation forms based on serotonin deficiency are used as experimental depression model in rodents (Leonard, 1998). On the other hand, selective serotonin re-uptake inhibitors (SSRIs) and some post-synaptic receptor agonists, which increase serotonergic activity in the synaptic space, are used widely and effectively to treat depression (Cowen, 1998; Vaswani *et al*., 2003).

Alcoholism and depression are often associated in psychiatric patients. Many alcoholic patients have symptoms of depression (Weissman and Myers, 1980; Miguel-Hidalgo and Rajkowska, 2003). A positive association between high ethanol intake and a depression-like status has been suggested also in genetically selected ethanol-preferring AA rats (Kiianmaa *et al*., 1991) and in fawn-hooded rats (Overstreet *et al*., 1992). Previous studies from our laboratory also indicated a significant decrease in striatal serotonin levels of rats during early ethanol withdrawal (Uzbay *et al*., 1998) and chronic ethanol consumption (Uzbay *et al*., 2000). These observations imply that there might be a correlation between decreased serotonergic activity and ethanol dependence. Thus, drugs that increase serotonergic activity in synapses could be useful in treatment of ethanol withdrawal or dependence. Furthermore, some anti-depressant drugs are in general use for patients with ethanol dependence. They are mainly indicated in the treatment of ethanol withdrawal and combined psychiatric disorders (Miller, 1995; Favre *et al*., 1997; Myrick *et al*., 2001). Although effects of some serotonergic anti-depressants on ethanol intake and/or ethanol abuse have been investigated in several studies, data relating to the action of anti-depressant drugs during ethanol withdrawal period are very limited.

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An ethanol-dependent rat model was developed in our Psychopharmacology Research Unit by a modified liquid diet technique in 1995 (Uzbay and Kayaalp, 1995). Numerous experimental studies were, and are being performed in our laboratory to understand the mechanism and etiology of alcoholism. Some of these were involved in the effects of anti-depressant drugs on ethanol withdrawal syndrome in rats. In this review, it was aimed to analyse the results obtained from ethanol-dependent rat models treated with various anti-depressant drugs that interact with the serotonergic system via different mechanisms. A relationship between the drug effects and the signs of ethanol withdrawal has also been evaluated and discussed.

ETHANOL-DEPENDENT RAT MODEL BY THE MODIFIED LIQUID DIET

Subjects and laboratory

Procedure in all studies was in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA). Adult Wistar rats (182–339 g in weight at the beginning of the experiments) were subjects. They were housed in a quiet and temperature- and humidity-controlled room ($22 \pm 3^{\circ}$C and $65 \pm 5\%$, respectively), in which a 12-h light/dark cycle was maintained (07:00–19:00 light).

Chronic ethanol administration to rats

For chronic ethanol administration, the rats were housed individually and ethanol was administered in the modified liquid diet as previously described (Uzbay and Kayaalp, 1995). The rats received 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: cow milk 925 ml (Mis Süt, Turkey), 25–75 ml ethanol (96.5% ethyl alcohol; Tekel, Turkish State Monopoly), vitamin A 5000 IU (Akpa İlaç Sanayi, Turkey) and sucrose 17 g (Uzbay and Kayaalp, 1995). This mixture supplied 1000.7 kcal/l.

At the beginning of the study, 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 another 21 days. Liquid diet was prepared on a daily basis and presented at the same time each day. The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as g/kg/day. Control rats were pair fed with an isocaloric liquid diet containing of sucrose as a caloric substitute to ethanol.

Drugs used and dose regimens in the studies

Fluoxetine (2.5–10 mg/kg, Sigma Chemical—USA), an SSRI, venlafaxine (5–40 mg/kg, White Company, İstanbul—Turkey), a serotonin/noradrenalin re-uptake inhibitory agent, escitalopram (2.5–10 mg/kg, Lundbeck—Denmark), a bounding agent at the primary site of pre-synaptic serotonin transporter, tianeptine (5–20 mg/kg, Servier Laboratory—France), a serotonin uptake stimulatory agent, were injected in rats intraperitoneally. Extract of *Hypericum perforatum* (HPE) was prepared by using aerial parts of St John's wort at Anadolu University, Department of Pharmacognosy as previously described (Ozturk *et al*., 1996) and injected in rats intraperitoneally, as well.

Doses of the anti-depressants were selected from our preliminary experiments and previous studies. Since higher doses of anti-depressants used in our studies caused sedation and impairment of motor co-ordination, higher doses were not tested.

Evaluation of ethanol withdrawal syndrome

At the end of 7.2% ethanol-containing liquid diet administration, ethanol was withdrawn from the daily diet. Ethanol-dependent rats were then assigned to several groups ($N = 8–10$ for each group). Anti-depressants and saline were injected in the rats 30 min before ethanol withdrawal testing. The rats were then observed for 5 min at the 2nd, 4th and 6th h of the withdrawal period. At each observation time, the rats were assessed simultaneously for the following behavioural conditions: agitation, tremor, stereotyped behaviour, wet dog shakes and audiogenic seizures as previously described (Uzbay and Kayaalp, 1995; Uzbay *et al*., 1997).

Ethanol consumption, weight changes and blood ethanol levels

Daily ethanol consumption of the rats in the control and anti-depressant treated groups ranged from 10 to 17 g/kg during exposure to ethanol (7.2%).

No significant weight loss was observed in any of the studies, as body weights of the rats increased progressively during the study.

Blood ethanol levels were found higher than 150 mg/dl in ethanol feeding groups.

EFFECTS OF ANTI-DEPRESSANTS ON ETHANOL WITHDRAWAL SYNDROME

Fluoxetine

Fluoxetine is an SSRI that exhibits anti-depressant activity in experimental models (Detke *et al*., 1995; Contreras *et al*., 2001) and clinical trials (Stokes and Holtz, 1997; Vaswani *et al*., 2003). Fluoxetine increases serotonergic transmission in synaptic cleft (Stahl, 1996). Studies suggest that SSRIs, such as zimelidine, citalopram, and fluoxetine, may reduce ethanol consumption, and that is not thought to be an anti-depressant effect (Miller, 1995). Limited clinical studies indicated that fluoxetine reduces the extent of anxiety and depression during ethanol withdrawal (Romeo *et al*., 2000) and at its anti-depressant doses; it is able to prevent relapses in patients with alcoholism (Janiri *et al*., 1996).

A detailed study reported the effects of fluoxetine on several signs of ethanol withdrawal in rats (Uzbay *et al*., 2004). In this study, fluoxetine inhibited withdrawal-induced locomotor hyperactivity and attenuated the severity or incidence of the signs of ethanol withdrawal, such as agitation, increased

Table 1. Acute effects of ip administration of fluoxetine on the signs of ethanol withdrawal syndrome in rats

Doses and observation intervals	Ethanol withdrawal signs					
	LH	Agitation	Stereotype	Tremors	WDS	AS
2nd h of EWS						
2.5 mg/kg	0	+	0	+	+	−
5.0 mg/kg	+	+	0	+	+	−
10.0 mg/kg	0	+	+	+	+	−
4th h of EWS						
2.5 mg/kg	0	0	0	+	+	−
5.0 mg/kg	0	0	0	+	0	−
10.0 mg/kg	0	0	0	0	+	−
6th h of EWS[a]						
2.5 mg/kg	0	0	0	+	0	+
5.0 mg/kg	+	+	0	+	0	+
10.0 mg/kg	0	+	0	+	0	+

(Uzbay *et al*., 2004); ip, Intraperitoeal; EWS, Ethanol withdrawal syndrome; LH, Locomotor hyperactivity; WDS, Wet dog shake; AS, Incidence of audiogenic seizure; −, Not evaluated; 0, Ineffective; +, Statistically significant attenuation.

[a] All doses were repeated 30 min before the 6th h of evaluation.

stereotyped behaviour, wet dog shakes, and tremors, dose-dependently. It also reduced markedly the incidence of audiogenic seizures (Table 1). Preventive effects of fluoxetine were seen particularly on agitation, wet dog shakes, tremors, and audiogenic seizures. Effective doses of fluoxetine did not cause any significant change in locomotor activities of the naïve (not ethanol-dependent) rats. Moreover, the inhibitory effects of fluoxetine on the signs of ethanol withdrawal were specific and not related to other non-specific effects, such as sedation and muscle relaxation. These observations clearly showed that fluoxetine is a pharmacologically active agent on mechanisms involved in development of physical dependence on ethanol in rats, and it may have a potential therapeutic effect in the treatment of ethanol-type dependence.

Escitalopram

Escitalopram is an active enantiomer of citalopram, which is an SSRI. It has a proven efficacy in the treatment of major depression, like other SSRIs. It has been shown in non-clinical and clinical experiments that it has greater efficacy than equivalent doses of citalopram (Auquier *et al*., 2003; Lepola *et al*., 2003; Sánchez *et al*., 2004). Unlike classical SSRIs, it is bound at the primary site of pre-synaptic serotonin transporter (SERT) with a very high affinity, and it has higher serotonergic activity than the classical SSRIs (Sánchez *et al*., 2004).

In the light of information above, it could be expected that escitalopram is more effective than the classical SSRIs, such as fluoxetine on ethanol withdrawal syndrome. Thus, the effects of escitalopram on ethanol dependence or ethanol withdrawal have been evaluated in our laboratory (Saglam *et al*., 2006). In contrast to our expectations, in this study, escitalopram was found to be less effective when compared to fluoxetine. While fluoxetine had additional preventive effects on locomotor hyperactivity, agitation, and audiogenic seizures (Uzbay *et al*., 2004), escitalopram was not effective on these signs of ethanol withdrawal. Its beneficial effects on ethanol withdrawal syndrome were found to be limited. It only produced a significant attenuation on tremors (Table 2). Although it produced some significant decrease on stereotyped behaviours and wet dog shakes, these effects were limited and not dose-dependent (Saglam *et al*., 2006). Thus, results of this study suggest that escitalopram has some limited beneficial effects on ethanol withdrawal syndrome in rats. However, it does not have superiority over fluoxetine for treatment of ethanol withdrawal syndrome in rats.

Venlafaxine

Venlafaxine is a bicyclic phenylethylamine derivative which inhibits pre-synaptic re-uptake of serotonin, noradrenaline, and, to a lesser extent, dopamine (Holliday and Benfield, 1995). Thus, it increases serotonergic and noradrenergic transmission in synaptic cleft (Stahl, 1996). Venlafaxine exhibits an anti-depressant activity in experimental models and clinical trials (Mitchel and Fletcher, 1993; Holliday and Benfield, 1995; Dierick, 1997). Anxiety is also a sign of withdrawal of the drugs that were abused, and produced physical dependence, such as ethanol in humans (Schuckit, 2000; De Witte *et al*., 2003) and rats (Gatch *et al*., 2000). Additionally, several clinical reports have suggested that venlafaxine has beneficial effects in some kind of anxiety disorders (Gelenberg *et al*., 2000; Ninan, 2000; Gorman, 2003).

Evidence also showed that venlafaxine strongly attenuated morphine withdrawal in rats (Lu *et al*., 2001). However, studies assessing the effect of venlafaxine on ethanol withdrawal syndrome or ethanol dependence were limited. Therefore, the first detailed study investigating the effects of venlafaxine effects on ethanol withdrawal syndrome were performed in our laboratory.

In this study, no prominent effect on locomotor hyperactivity, agitation, stereotyped behaviour and wet dog shake

Table 2. Acute effects of ip administration of escitalopram on the signs of ethanol withdrawal syndrome in rats

Doses and observation intervals	Ethanol withdrawal signs					
	LH	Agitation	Stereotype	Tremors	WDS	AS
2nd h of EWS						
2.5 mg/kg	0	0	0	0	+	−
5.0 mg/kg	0	0	0	+	+	−
10.0 mg/kg	0	0	0	+	0	−
4th h of EWS						
2.5 mg/kg	0	0	0	0	0	−
5.0 mg/kg	0	0	0	0	0	−
10.0 mg/kg	0	0	0	0	0	−
6th h of EWS[a]						
2.5 mg/kg	0	0	0	0	+	0
5.0 mg/kg	0	0	+	0	0	0
10.0 mg/kg	0	0	0	0	0	0

(Saglam *et al*., 2006); ip, Intraperitoneal; EWS, Ethanol withdrawal syndrome; LH, Locomotor hyperactivity; WDS, Wet dog shake; AS, Incidence of audiogenic seizure; −, Not evaluated; 0, Ineffective; +, Statistically significant attenuation.
[a] All doses were repeated 30 min before the 6th h of evaluation.

Table 3. Acute effects of ip administration of venlafaxine on the signs of ethanol withdrawal syndrome in rats

Doses and observation intervals	Ethanol withdrawal signs					
	LH	Agitation	Stereotype	Tremors	WDS	AS
2nd h of EWS						
5.0 mg/kg	0	0	0	−	0	−
10.0 mg/kg	0	0	0	−	0	−
20.0 mg/kg	0	0	0	−	0	−
40.0 mg/kg	0	0	0	−	0	−
4th h of EWS						
5.0 mg/kg	0	0	0	−	0	−
10.0 mg/kg	0	0	0	−	0	−
20.0 mg/kg	0	0	0	−	0	−
40.0 mg/kg	0	0	0	−	0	−
6th h of EWS[a]						
5.0 mg/kg	0	0	0	−	0	0
10.0 mg/kg	0	0	+	−	0	0
20.0 mg/kg	0	0	0	−	0	+[$]
40.0 mg/kg	0	0	0	−	0	0

(Saglam *et al*., 2004) ip, Intraperitoneal; EWS, Ethanol withdrawal syndrome; LH, Locomotor hyperactivity; WDS, Wet dog shake; AS, Incidence of audiogenic seizure; ($, significant prolonged latency); −, Not evaluated; 0, Ineffective; +, Statistically significant attenuation.
[a] All doses were repeated 30 min before the 6th h of evaluation.

by acute venlafaxine treatment was observed. However, venlafaxine had some limited preventive effects on the audiogenic seizures. It significantly prolonged the latency of audiogenic seizures at the dose of 20 mg/kg and reduced the incidence of the seizures without reaching a statistically significant level at the dose of 40 mg/kg (Saglam *et al*., 2004) (Table 3). As a result, venlafaxine did not seem to be an agent as effective as fluoxetine to control ethanol withdrawal syndrome.

Tianeptine

Tianeptine is a tricyclic drug that exhibits anti-depressant activity in experimental models (Curzon and Datla, 1993) and clinical trials (Guelfi, 1992; Saiz-Ruiz *et al*., 1998). The neurochemical properties of tianeptine vary from those of other tricyclic and non-tricyclic anti-depressants. It is a unique type of anti-depressant that produces its effect by enhancing, rather than inhibiting, serotonin re-uptake (Mennini *et al*., 1987).

Table 4. Acute effects of ip administration of tianeptine on the signs of ethanol withdrawal syndrome in rats

Doses and observation intervals	Ethanol withdrawal signs					
	LH	Agitation	Stereotype	Tremors	WDS	AS
2nd h of EWS						
5.0 mg/kg	0	0	0	0	+	−
10.0 mg/kg	0	0	0	+	+	−
20.0 mg/kg	+	0	+	+	+	−
4th h of EWS						
5.0 mg/kg	0	0	0	0	0	−
10.0 mg/kg	0	0	0	+	0	−
20.0 mg/kg	0	+	+	+	+	−
6th h of EWS[a]						
5.0 mg/kg	0	0	0	+	0	0
10.0 mg/kg	0	+	0	+	+	+
20.0 mg/kg	0	+	+	+	+	+[$]

(Uzbay *et al*., 2006) ip, Intraperitoneal; EWS, Ethanol withdrawal syndrome; LH, Locomotor hyperactivity; WDS, Wet dog shake; AS, Incidence of audiogenic seizure; ($, significant prolonged latency); −, Not evaluated; 0, Ineffective; +, Statistically significant attenuation.
[a] All doses were repeated 30 min before the 6th h of evaluation.

Limited clinical studies indicated that tianeptine has beneficial effects for patients with alcoholism. Malka *et al*. (1992) showed that long-term tianeptine treatment results in marked and consistent improvement in depression and anxiety scores after alcohol withdrawal. In addition, Favre *et al*. (1997) suggested that tianeptine prevents alcoholic relapses in patients.

In experimental studies, Daoust *et al*. (1992) showed that tianeptine decreases ethanol intake of male Wistar rats without causing any significant change on either their food intake or body weight. File *et al*. (1993) also suggested that tianeptine is able to reverse the anxiogenic effects of ethanol withdrawal in the social interaction test in rats. However, ethanol withdrawal consists of more than anxiety. Other symptoms such as locomotor hyperactivity, agitation, increased stereotyped behaviour and wet dog shakes, tremors, and audiogenic seizures also appear during ethanol withdrawal in rodents. In a recent study, Uzbay *et al*. (2006) reported results from a detailed study investigating the effects of both acute and chronic tianeptine treatment on ethanol withdrawal syndrome in rats. Both acute and chronic administration of tianeptine attenuated severity of ethanol withdrawal syndrome dose-dependently. However, acute tianeptine treatment was found to be more effective than chronic treatment. While acute tianeptine treatment was effective on all the signs of ethanol withdrawal (locomotor hyperactivity, agitation, increased stereotyped behaviour, wet dog shakes, tremors, and audiogenic seizures) (Table 4), chronic treatment was ineffective on locomotor hyperactivity and agitation. In addition, chronic tianeptine treatment did not produce any significant effect on ethanol intake of the rats. Results of this study indicated that tianeptine may be a potent and pharmacologically active agent on ethanol withdrawal syndrome in rats. It may be useful in treatment of ethanol dependence as well as depression in patients with history of ethanol abuse.

Extract of Hypericum perforatum (HPE, St. John's wort)

HPE has been usually called St John's wort, and commonly used in folk medicine of several European countries. Several preclinical studies indicate that extract of the common plant HPE may be useful for treatment of disorders, especially depression, originating from the central nervous system. Thus, the anti-depressant-like effect of HPE has been reported in rodents (Butterweck *et al*., 1997; Ozturk, 1997; De Vry *et al*., 1999). Several meta-analyses and overviews of randomized clinical trials also consistently show that HPE displays a clear anti-depressant action and it has been used for the treatment of mild to moderate depression (Linde *et al*., 1996; Melchart, 1996; Volz, 1997; Kasper and Dienel, 2002). HPE has some serotonergic properties reducing 5-HT re-uptake and inhibiting monoamine oxidase (MAO) activity (Neary and Bu, 1990; Perovic and Muller, 1995; Cott, 1997; Bennett *et al*., 1998; Greeson *et al*., 2001) like other anti-depressant drugs.

Some experimental studies have been reported involving the effects of HPE on ethanol abuse and dependence. It was suggested that HPE inhibits ethanol intake and preference in several strains of ethanol-preferring rats (De Vry *et al*., 1999; Rezvani *et al*., 1999; Perfumi *et al*., 1999, 2001, 2002) and mice (Wright *et al*., 2003). In a recent report, Perfumi *et al*. (2005) showed that HPE significantly reduced ethanol self-administration, while it did not modify saccharin self-administration. They also observed that HPE abolished the increased ethanol intake following ethanol deprivation. Thus, these results suggested that HPE might be a useful agent in the treatment of ethanol abuse and dependence.

Although the effects of HPE on ethanol preference and intake have been investigated in detailed studies, only one study investigating the effects of HPE on ethanol withdrawal syndrome was reported (Coskun *et al*., 2006). In this study, HPE blocked both locomotor hyperactivity and stereotyped behaviours especially at 2nd and 6th h of ethanol withdrawal. In addition, it attenuated the incidence of tremor in

Table 5. Acute effects of ip administration of extract of *Hypericum perforatum* on the signs of ethanol withdrawal syndrome in rats

Doses and observation intervals	Ethanol withdrawal signs					
	LH	Agitation	Stereotype	Tremors	WDS	AS
2nd h of EWS						
25.0 mg/kg	+	−	+	0	−	−
50.0 mg/kg	+	−	+	0	−	−
100.0 mg/kg	+	−	+	0	−	−
200.00 mg/kg	+	−	+	0	−	−
4th h of EWS						
25.0 mg/kg	0	−	0	0	−	−
50.0 mg/kg	0	−	0	+	−	−
100.0 mg/kg	+	−	+	+	−	−
200.00 mg/kg	0	−	0	0	−	−
6th h of EWS[a]						
25.0 mg/kg	+	−	+	0	−	+[$]
50.0 mg/kg	+	−	+	0	−	+[$]
100.0 mg/kg	+	−	+	0	−	+
200.00 mg/kg	+	−	+	0	−	0

(Coskun *et al*., 2006) ip, Intraperitoneal; EWS, Ethanol withdrawal syndrome; LH, Locomotor hyperactivity; WDS, Wet dog shake; AS, Incidence of audiogenic seizure; HPE, Extract of *hypericum perforatum* [Yield of HPE were 27.4% (w/v), doses were expressed as dried extract (mg/kg) body weight]; ($, significant prolonged latency); −, Not evaluated; 0, Ineffective; +, Statistically significant attenuation.
[a] All doses were repeated 30 min before the 6th h of evaluation.

ethanol-dependent rats at 4th h of ethanol withdrawal. HPE (100 mg/kg) produced a significant attenuation in the incidence of the audiogenic seizures appearing in 6th h of ethanol withdrawal. Latency of the audiogenic seizures was also prolonged significantly by HPE (25 and 50 mg/kg) treatment (Table 5). These results imply that HPE may be useful in the treatment of ethanol withdrawal syndrome.

DISCUSSION AND CONCLUSION

Effects of each anti-depressant on the signs of ethanol withdrawal during observation terms are shown in Tables 1–5. Their comparative effects were also summarized in Table 6. As shown in the Tables, treatment of tianeptine, fluoxetine, HPE, escitalopram, and venlafaxine have some beneficial effects on the signs of ethanol withdrawal in rats. Effectiveness of the anti-depressants was as follows: fluoxetine = tianeptine > HPE > escitalopram > venlafaxine. Since any significant changes on the open field locomotor activities in naïve groups were not observed, the beneficial effects of the anti-depressants on ethanol withdrawal syndrome could not be due to other non-specific effects, such as sedation or muscle relaxation. As shown in Tables 1–5, actions of the drugs are either lost or weakened at the 4t h-h-withdrawal. This may be related to elimination of single dose of tested drugs. Thus, second injections were repeated before 6th-h-observations.

Neurochemical findings from clinical (Roy *et al*., 1987; LeMarquand *et al*., 1994) and experimental (Murphy *et al*., 1987; Uzbay *et al*., 1998, 2000) studies suggested significant changes in central serotonergic neurotransmission in ethanol dependence. On the other hand, we hypothesized that there might be a significant association between decreased serotonergic activity and ethanol dependence (Uzbay *et al*., 1998, 2000). Our findings indicated some beneficial effects on withdrawal signs treated by fluoxetine, escitalopram, and HPE to support this hypothesis.

As venlafaxine inhibits the re-uptake of serotonin more than noradrenaline, and even more than dopamine in synaptic cleft (Muth *et al*., 1986; Holliday and Benfield, 1995), serotonergic property of this drug may also be responsible for its prolonging effects of latency of audiogenic seizures. Ineffectiveness of venlafaxine on other signs of withdrawal may be due to its stimulative effects on noradrenalin re-uptake. Thus, ethanol withdrawal syndrome is especially characterized by the signs of overactivity of the sympathetic nervous system (Linnoila *et al*., 1987; De Witte *et al*., 2003). Inhibition of noradrenaline re-uptake, besides serotonin, by venlafaxine and increased noradrenergic activity in synaptic cleft might mask or prevent its beneficial effects on locomotor hyperactivity, agitation, stereotyped behaviour, and wet dog shakes. Some increase in agitation scores by venlafaxine treatment (Saglam *et al*., 2004) also supports this idea. Thus, signs such as agitation and hyperreflexia, during ethanol withdrawal are related to increased noradrenergic activity (Linnoila *et al*., 1987).

The beneficial effects of HPE might also be related to serotonergic mechanisms. HPE has some serotonergic properties, reducing serotonin re-uptake and inhibiting MAO activity (Neary and Bu, 1990; Perovic and Muller, 1995; Calapai *et al*., 1999) like other anti-depressant drugs.

Unlike the classical SSRI anti-depressants, escitalopram is bound at the primary site of SERT with a very high affinity. In the central nervous system, the concentration of active serotonin in the synaptic cleft is regulated by

Table 6. Comparative effects of anti-depressants on the signs of ethanol withdrawal syndrome in rats

	Ethanol withdrawal signs					
Drugs	LH	Agitation	Stereotype	Tremors	WDS	AS
Fluoxetine	↓↓	↓↓	↓	↓↓↓	↓↓↓	↓↓↓
Tianeptine	↓	↓↓	↓↓	↓↓↓	↓↓	↓↓↓
HPE	↓↓↓	−	↓↓↓	↓	−	↓↓
Escitalopram	0	0	↓	↓↓	↓↓	0
Venlafaxine	0	0	0	0	0	↓

LH, Locomotor hyperactivity; WDS, Wet dog shake; AS, Audiogenic seizure; HPE, Extract of *Hypericum perforatum*; −, Not evaluated; 0, Ineffective; ↓, Mild inhibitory effect; ↓↓, Moderate inhibitory effect; ↓↓↓, High inhibitory effect.

SERT (Tatsumi *et al*., 1997; Sanchez *et al*., 2004). SERT is also responsible for termination or modulation of the action of serotonin released from the pre-synaptic neuron. Thus, escitalopram has higher serotonergic activity than the classical SSRIs (Lepola *et al*., 2003; Sánchez *et al*., 2004) and it could be expected that escitalopram is more effective than the classical SSRIs, such as fluoxetine on ethanol withdrawal syndrome. In contrast to our expectations, Saglam *et al*. (2006) found that escitalopram is less effective compared to fluoxetine. While fluoxetine had additional protective effects on locomotor hyperactivity, agitation, and audiogenic seizures (Uzbay *et al*., 2004), escitalopram was not effective on these signs of ethanol withdrawal. These findings imply that more selective serotonergic activity does not mean more effectiveness on ethanol withdrawal syndrome. Further studies are needed to clarify the lower effectiveness of escitalopram on ethanol withdrawal syndrome.

On the other hand, additional effects of fluoxetine on nitric oxide (NO) may contribute to its stronger activity on ethanol withdrawal. Several studies have shown that NO synthase (NOS) inhibitors cause a prominent attenuation in the signs of ethanol withdrawal syndrome in rats (Uzbay and Oglesby, 2001). Wegener *et al*. (2003) suggested that local administration of serotonergic anti-depressants, such as fluoxetine, tianeptine, paroxetine, citalopram, and imipramine, significantly decrease hippocampal NOS activity in rat brain. In addition, previous studies indicated that fluoxetine has some NOS inhibitory effects in humans (Yaron *et al*., 1999) and rats (Luo and Tan, 2001).

Different from SSRIs and other anti-depressants, tianeptine was shown to enhance serotonin uptake selectively in rat brain synaptosomes (Mennini *et al*., 1987). Thus, this drug can be described as a serotonin re-uptake enhancer, an atypical anti-depressant. However, in a recent study from our laboratory, it was found that tianeptine and fluoxetine, but not venlafaxine, have similar discriminative stimulus properties in rats (Alici *et al*., 2006). Clinical anti-depressant efficacy of tianeptine has also been found to be similar to that of SSRIs (Lŏo *et al*., 1999; Waintraub *et al*., 2000) and other tricyclic-depressants (Guelfi, 1992; Staner and Mendlewicz, 1993).

Recent studies indicate that anti-depressant effects of this drug may be attributable to non-serotonergic mechanisms, including its capacity to buffer excitatory amino acid receptors against stress (Kole *et al*., 2002). Tianeptine reverses the adverse effects of stress on brain morphology and synaptic plasticity by reducing excessive accumulation of intracellular calcium, which results from stress-induced excitatory amino acid activation (McEwen and Magarinos, 2001). It also prevents stress-induced increase in glutamate transporter mRNA levels in rat hippocampus (Reagan *et al*., 2004). On the other hand, many studies have shown a clear role of excitatory amino acid stimulation in the development of ethanol dependence (Rossetti and Carboni, 1995; Tsai *et al*., 1995; Hardy *et al*., 1999). In addition, blockade of NMDA receptors markedly reduces ethanol withdrawal signs in rodents (Morriset *et al*., 1990; Liljequist, 1991; Thomas *et al*., 1997). Furthermore, adenosine A_1 agonistic agents have also inhibitory effect on ethanol withdrawal syndrome in rats (Concas *et al*., 1996; Kaplan *et al*., 1999) and it has been shown that tianeptine has anti-convulsant activity via adenosine A_1 receptor stimulation (Uzbay *et al*., 2007). In addition, similar to fluoxetine, tianeptine also decreases hippocampal NOS activity in rats (Wegener *et al*., 2003). Glutamatergic, nitrergic, and adenosinergic mechanisms may be responsible for the prominent beneficial effects of tianeptine on ethanol withdrawal syndrome.

In the light of the results gained by five anti-depressants agents, it can be concluded that fluoxetine and tianeptine are potent and pharmacologically active agents on ethanol withdrawal syndrome. They may be useful in treatment of ethanol dependence as well as depression in patients with history of ethanol abuse. HPE, escitalopram, and venlafaxine also have limited beneficial effects on some signs of withdrawal. In addition, anti-depressants did not cause any deteriorating effect on any of the signs of ethanol withdrawal syndrome. It implies that anti-depressants could also be used safely in patients suffering from alcoholism.

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