The effect of alpha-tocopherol in the acute ethanol intake and its withdrawal on penicillin-induced epilepsy

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The present study was designed to investigate the influence of acute ethanol intake and its withdrawal on the anticonvulsant effect of alpha-tocopherol in penicillin-induced epileptiform activity. Ethanol-treated rats received a daily dose of 3 g/kg or 9.0 g/kg of 30% ethanol solution for 3 days. Thirty minutes after penicillin injection (500 units, i.c.), the most effective dose of alpha-tocopherol (500 mg/kg) was administered intramuscularly (i.m.). Acute administration of ethanol, in a dose of 3 g/kg, did not change either frequency or amplitude of penicillin-induced epileptiform activity, while dose of 9 g/kg ethanol significantly decreased the mean frequency of penicillin-induced epileptiform ECoG activity in the ethanol-treated group. Ethanol (9 g/kg) withdrawal also caused an increase in the amplitude of epileptiform ECoG activity in the withdrawal group. The results suggest that acute administration of high dose ethanol (9 g/kg) and alpha-tocopherol have some limited anticonvulsive effects in penicillin-induced epileptiform activity in rats.

Key words: acute, ECoG, epileptiform activity, ethanol, penicillin, alpha-tocopherol

INTRODUCTION

Ethanol is one of the most commonly used psychoactive drugs, which affects hormone- and neurotransmitter-activated signal transduction in ways that lead to short-term changes in cellular functions and long-term changes in gene expression (Faingold et al. 1998). The rapidity of effects of ethanol is due to its complete solubility in water, which results in its rapid absorption by blood and distribution throughout the highly vascularized brain (Charness 1993). Acute ethanol administration has been reported to alter excitability of neurons and to induce changes in the electrophysiology and behavioral effects of excitatory amino acid-mediated neurotransmission (Faingold et al. 1998). Previous studies examined effects of ethanol on neurons in anesthetized animals or brain slices, and reported variable effects on nerve firing, excitatory or inhibitory transmission, reducing glutamate-mediated excitation and increasing GABA-, glycine-, and adenosine-mediated inhibition (Lovinger et al. 1989, Ziskind-Conhaim et al. 2003). In addition to ethanol opposite actions on excitatory and inhibitory neurotransmitter receptors, its dual effects on epileptic seizure have also been documented (Guerrero-Figueroa et al. 1970, Mello et al. 1990, Kim et al. 1995, Fischer 2005). Acute ethanol administration revealed an anticonvulsant effect upon seizures in the amygdala and hippocampal kindling model of rats (Freeman 1978, Mello et al. 1990, Kim et al. 1995). Ethanol (2 g/kg, i.p) offered protection against NMDA, kainic acid and picrotoxin-induced convulsions and least effective against kainic acid (Kulkarni et al. 1990). It was suggested that the anticonvulsant actions of ethanol may be attributed to its ability to antagonize NMDA-mediated excitatory responses and facilitate the GABAergic transmission (Kulkarni et al. 1990). Sharma and coauthors (1991) also reported a significant protection for acute ethanol administration (0.5–2 g/kg, i.p) against NMDA-induced convulsions. Prenatal exposure to ethanol did not have long-term effects on the susceptibility to convulsions or on the anticonvulsant effect of ethanol in adult male rats in the kindling model (Kim et al. 1994). However, tolerance to anticonvulsant effect of ethanol was found greatest
in the ethanol-treated rats before convulsive stimulation (Kim et al. 1995). Moreover, acute administration of ethanol produces pronounced antiepileptogenic and anticonvulsant effects, whereas repeated administration of high doses with longer withdrawal periods leads to proconvulsant actions (Fischer and Kittner 1998). It has been suggested that the relation between ethanol intake and seizures is dose-dependent (Ng et al. 1988, Kim et al. 1994). However, several studies showed that small to modest ethanol intake does not change seizure frequency (Hoppener et al. 1983, Mattson et al. 1990).

Intake of ethanol affects the activities of antioxidant enzymes such as superoxide dismutase, catalase and GSH-Px in the brain (Somani and Husain 1997). Ethanol can induce superoxide anion or hydrogen peroxide formation in animals (Somani and Husain 1997). Alpha tocopherol, as a classical antioxidant, plays a major role in protecting brain against both epileptic discharges (Levy et al. 1992, Ayyildiz et al. 2006) and ethanol-induced toxicity (Nadiger et al. 1988, Agar et al. 2000). Animal models of epilepsy for the discovery or development of antiepileptic drugs have played a crucial role. However, it is most likely that no single animal model could be useful for all types of epilepsy. Topical and systemic administration of penicillin G is an experimental model commonly used to produce epileptic foci and interictal activity, both in the motor cortex (Collins 1978, Stankiewicz et al. 1995, Stankiewicz and Gralewicz 1996, Ayyildiz et al. 2006) and the amygdala (Fernandez-Guardiola et al. 1995, Gonzalez-Trujano et al. 2006). Penicillin-induced epileptic activity begins focally and resembles focal interictal spikes recorded in the human cortex (Fisher 1989). But then it spreads and causes generalized epilepsy. In this regard, it resembles the grand-mal epilepsy (Sagratella et al. 1985). Administration of ethanol by different routes and doses modified convulsive activity in kainic acid (Kulkarni et al. 1990), and amygdala-kindled (Mello et al. 1990) models of epilepsy. However, the data concerning the effects of acute ethanol and its withdrawal on penicillin-induced epilepsy under ECoG monitoring are still not sufficiently reported in the currently available literature. In the present study, therefore, the effects of acute ethanol intake and its withdrawal, at the doses of 3 and 9 g/kg, on epileptiform activity were investigated in the penicillin-induced epilepsy in rats. We have also analyzed the influence of acute ethanol intake and its withdrawal on the protective activity of α-tocopherol (vitamin E) in the penicillin-induced epileptiform activity.

**METHODS**

**Subjects**

Male Wistar rats (n=84), weighing 225–255 g were used in these experiments. Rats were housed individually on a 12:12-h light:dark cycle (lights on at 07.00 AM), at a temperature of 21 ± 2.8ºC and 50% humidity. The animal use protocol was approved by the institutional committee of Ondokuz Mayis University. Furthermore, animal care was accordance with governmental and institutional guidelines. Animals were assigned to the following experiments and groups: intracortical (i.c.) delivery of (1) 2.5 μl artificial cerebrospinal fluid [aCSF containing (mM): NaCl, 124; KCl, 5; KH₂PO₄, 1.2; CaCl₂, 2.4; MgSO₄, 1.3; NaHCO₃, 26; glucose, 10; HEPES, 10; pH 7.4 when saturated with 95% O₂ and 5% CO₂] (i.c.); (2) 500 units penicillin (2.5 μl, i.c.); (3) 500 mg/kg α-tocopherol (i.m.); (4) penicillin pretreated + 500 mg/kg α-tocopherol (i.m.); (5) ethanol-treated (3 g/kg, per day, for 3 days, intragastrically) + penicillin; (6) ethanol-treated (3 g/kg, per day, for 3 days, intragastrically) + penicillin + 500 mg/kg α-tocopherol (i.m.); (7) ethanol withdrawal (3 g/kg, per day, for 3 days, intragastrically) + penicillin; (8) ethanol withdrawal (3 g/kg, per day, for 3 days, intragastrically) + penicillin + 500 mg/kg α-tocopherol (i.m.). (9) ethanol-treated (9 g/kg, per day, for 3 days, intragastrically) + penicillin; (10) ethanol-treated (9 g/kg, per day, for 3 days, intragastrically) + penicillin + 500 mg/kg α-tocopherol (i.m.); (11) ethanol withdrawal (9 g/kg, per day, for 3 days, intragastrically) + penicillin; (12) ethanol withdrawal (9 g/kg, per day, for 3 days, intragastrically) + penicillin + 500 mg/kg α-tocopherol (i.m.). Each animal group was composed of seven rats.

**Induction of epileptiform activity**

The animals were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic frame (Harvard Stereotaxic Instrument). Rectal temperature was maintained between 36.5 and 37.0ºC using a feedback controlled heating system (Harvard Apparatus Limited). A polyethylene cannula was introduced into the right femoral artery to monitor blood pressure, which was kept above 100 mmHg during the experiments (mean 115 ± 8 mmHg). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.
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Fig. 1. The baseline ECoG activities (A). Intracortical injection of penicillin (500 units) induces an epileptiform ECoG activity characterized by bilateral spikes and spike-wave complexes (B). Ethanol intake (3 g/kg per day for 3 days, intragastrically) does not significantly change the mean frequency and amplitude of epileptiform activity (C). The mean frequency and amplitude of epileptiform activity are not changed in the ethanol (3 g/kg) withdrawal group (D). Ethanol intake (9 g/kg per day for 3 days, intragastrically) significantly decreases the mean frequency of epileptiform activity without changing amplitude compared with penicillin injected group (E). The mean frequency and amplitude of epileptiform activity is increased in the withdrawal (9 g/kg) group compared with penicillin-injected group (F). There is also no change in the mean of frequency and amplitude in aCSF injected rats (G). α-Tocopherol (Vitamin E), in a dose of 500 mg/kg, significantly decreases the frequency of epileptiform activity in the penicillin injected group (500 units, i.c.) (H). The mean frequency of epileptiform activity is decreased in the ethanol (3 g/kg) + α-tocopherol group compared with penicillin-injected and ethanol-treated groups (I). The mean frequency of epileptiform activity is decreased in the withdrawal (3 g/kg) + α-tocopherol group compared with penicillin-injected and withdrawal group (J). The mean frequency of epileptiform activity is decreased in the ethanol (9 g/kg) + α-tocopherol group compared with penicillin-injected and ethanol-treated groups (K). The mean frequency and amplitude of epileptiform activity are decreased in the ethanol (9 g/kg) withdrawal + α-tocopherol group compared with withdrawal and penicillin-injected groups (L). Representative ECoGs are presented in the 80 minutes from α-tocopherol or in the 110 minutes from penicillin administration (Bar 1000 μV, 30s; Bar 1000 μV, 10 s).
The left cerebral cortex was exposed by craniotomy. The epileptic focus was produced by 500 units penicillin G potassium injection (2 mm posterior to bregma and 3 mm lateral to sagittal sutures, 1 mm beneath the brain surface by a Hamilton microsyringe type 701N; infusion rate 0.5 μl/min).

**Drug and drug administration**

Ethanol was prepared 30% v/v in 0.9% physiological saline. Ethanol-treated and withdrawal rats received a daily dose of 3.0 or 9.0 g/kg ethanol solution via an oesophageal probe for 3 days. At the end of this period, ethanol administration was stopped and all rats were anesthetized for induction of epileptiform activity 3 h and 28 h after the last administration of ethanol in the ethanol-treated and withdrawal groups, respectively. Ethanol intake was administered at approximately the same time (between 9.00–10.00 AM) during the whole procedure. Blood ethanol concentrations were measured 90 min after ethanol administration in different groups (n=46).

D α-tocopherol acetate (Aksu Farma Chemical Co.) was used in the experiments. Precautions were taken to minimize exposure to the light. A dose of 500 mg/kg α-tocopherol was administered (i.m.) 30 min after penicillin (i.c.) application (Ayyildiz et al. 2006).

**ECoG recordings**

Two Ag–AgCl ball electrodes were placed over the left somatomotor cortex (electrode coordinates: first electrode; 2 mm lateral to sagittal suture and 1 mm anterior to bregma; second electrode; 2 mm lateral to sagittal suture, 5 mm posterior to bregma). The common reference electrode was fixed on the pinna. The ECoG activity was continuously monitored on a four channel recorder (PowerLab, 4/SP). All recordings were stored on a computer. The frequency and amplitude of epileptic activity were analyzed off line.

**Statistical analysis**

All statistical procedures were performed using SPSS statistical software package (version: 12.0). Statistical analysis was divided into two parts. In the first section, six groups, including penicillin, penicillin pretreated + α-tocopherol and 3 g/kg ethanol groups, were compared. In the second section, the combination of six groups, including penicillin, penicillin pretreated + α-tocopherol and 9 g/kg ethanol groups, were compared. The differences between the groups were analyzed with the one-way ANOVA. Significant differences were further evaluated using Tukey’s post hoc test. Data are expressed as the means ± SEM. Statistical significance was set at \( P<0.05 \).

**RESULTS**

The effects of acute ethanol intake and its withdrawal on penicillin-induced epileptiform activity

Baseline activities of each animal were recorded before the administration of intracortical penicillin (Fig. 1A). Intracortical injection of penicillin (500 units) induced an epileptiform ECoG activity characterized by bilateral spikes and spike-wave complexes (Fig. 1B). This ECoG activity began within 3–5 min after penicillin application and lasted for 3–5 h. It reached a constant level as to frequency and amplitude in the 30 min.

The mean spike frequency and amplitude of ECoG activity were 31.3 ± 3, 880 ± 128; 25.6 ± 1, 1025 ± 101 and 26.0 ± 3 spike/min, 940 ± 178 μV in the penicillin injected, ethanol-treated (3 g/kg), and ethanol (3 g/kg) withdrawal groups in the 120 min from penicillin injection (i.c.), respectively (Fig. 1B,C,D). The mean frequency and amplitude of ECoG activity did not significantly change in the ethanol-administered (3 g/kg, for 3 days), ethanol (3 g/kg, for 3 days) withdrawal group during experiments compared with penicillin-injected group (Fig. 2).

The mean spike frequency and amplitude of ECoG activity were 31.3 ± 3, 880 ± 128; 21.2 ± 1, 800 ± 151 and 41.6 ± 2 spike/min, 1920 ± 390 μV in the penicillin injected, ethanol-treated (9 g/kg), and ethanol (9 g/kg) withdrawal groups in the 120 min from penicillin injection (i.c.), respectively (Fig. 1B,E,F). The mean frequency of ECoG activity was decreased in the 110 min from penicillin injection in the ethanol-treated (9 g/kg) group compared with penicillin-injected group (Fig. 3).

The mean spike frequency and amplitude of ECoG activity were 31.3 ± 3, 880 ± 128; 21.2 ± 1, 800 ± 151 and 41.6 ± 2 spike/min, 1920 ± 390 μV in the penicillin injected, ethanol-treated (9 g/kg), and ethanol (9 g/kg) withdrawal groups in the 120 min from penicillin injection (i.c.), respectively (Fig. 1B,E,F). The mean frequency of ECoG activity was decreased in the 110 min from penicillin injection in the ethanol-treated (9 g/kg) group compared with penicillin-injected group (\( F_{3,36}=16.48, P<0.001, \) post hoc \( P<0.05 \), Fig. 3). There was no significant difference in the mean amplitude of ECoG activity during experiments between ethanol-treated (9 g/kg) and penicillin injected groups. The mean spike frequency of ECoG activity was significantly increased between 50 min and 120 min from penicillin injection in
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Blood ethanol concentrations (BEC) were measured 90 min after ethanol administration in different groups (n=46). Blood ethanol concentrations were 0, 238.4, 245.7, 241.5, 228.4 mg/dl, in the control, ethanol-treated (3 g/kg), ethanol-treated (3 g/kg) + α-tocopherol, ethanol (3 g/kg) withdrawal, ethanol (3 g/kg) withdrawal + α-tocopherol groups, respectively. The mean spike amplitude of ECoG activity was significantly higher in the withdrawal (9 g/kg) group than penicillin-treated group. There was also no change in the mean of frequency and amplitude in aCSF injected animals (Fig. 1G).

Fig. 2. The influence of ethanol (3 g/kg) intake and its withdrawal on the anticonvulsant effect of α-tocopherol in the mean frequency of penicillin–induced epileptiform activity in rats. Ethanol (3 g/kg per day for 3 days, intragastrically) did not affect the mean frequency of epileptiform activity. α-Tocopherol (vitamin E) , in a dose of 500 mg/kg, significantly decreases the mean of frequency of epileptiform activity in all groups. The significant effects appeared in the 60, 90 min from α-tocopherol injection in the penicillin injected and ethanol-treated (3 g/kg) groups, respectively. ▲ P<0.05, + P<0.01, ★ P<0.001.

The mean spike frequency in the ethanol treated (3 g /kg) groups
of BEC was 238.5 mg/dl in the ethanol-treated (3 g/kg) groups. Blood ethanol concentrations were 0, 294.2, 297.6, 299.4, 295.4 mg/dl, in the control, ethanol-treated, (9 g/kg) ethanol-treated (9 g/kg) + α-tocopherol, ethanol (9 g/kg) withdrawal, ethanol (9 g/kg) withdrawal + α-tocopherol groups, respectively. The mean of BEC was 296.6 mg/dl in the ethanol-treated (9 g/kg) groups. There was a significant difference between

Fig. 3. The influence of ethanol (9 g/kg) intake and its withdrawal on the anticonvulsant effect of α-tocopherol in the mean frequency of penicillin–induced epileptiform activity in rats. Ethanol (9 g/kg per day for 3 days, intragastrically) decreases the mean frequency of epileptiform activity in the 110 minutes from penicillin injection in the ethanol-treated group. The mean frequency of epileptiform activity is increased between 50 and 120 minutes from penicillin injection in the withdrawal (9 g/kg) group compared with penicillin-injected group. α-Tocopherol (vitamin E), in a dose of 500 mg/kg, significantly decreases the mean of frequency of epileptiform activity in all groups. The significant effects appeared in the 40, 60 and 120 minutes from α-tocopherol injection in the ethanol-treated, penicillin-injected and ethanol withdrawal groups, respectively. ▲ P<0.05, ★ P<0.01, ⋆ P<0.001.
3 g/kg ethanol-treated and 9 g/kg ethanol-treated groups ($P<0.001$).

The influence of ethanol intake and its withdrawal on anticonvulsant effect of $\alpha$-tocopherol in the penicillin-induced epileptiform activity

$\alpha$-Tocopherol, in a dose of 500 mg/kg, was administered 30 minutes after penicillin injection. The mean spike frequency and amplitude of ECoG activity were $10.6 \pm 2$, $790 \pm 244$; $11.5 \pm 3$, $716 \pm 132$ and $13.5 \pm 3$ spike/min, $916 \pm 456$ $\mu$V in the penicillin + $\alpha$-tocopherol, ethanol-treated (3 g/kg) + $\alpha$-tocopherol, and ethanol (3 g/kg) withdrawal + $\alpha$-tocopherol groups in the 110 min from $\alpha$-tocopherol injection (i.m.), respectively (Fig. 1H, I, J). The mean frequency of ECoG activity was decreased in the penicillin + $\alpha$-tocopherol group, ethanol-treated (3 g/kg) + $\alpha$-tocopherol, and ethanol (3 g/kg) withdrawal + $\alpha$-tocopherol groups compared with penicillin-treated group (Fig. 2). The significant effects appeared in the 60, 90 min penicillin + $\alpha$-tocopherol and ethanol-treated (3 g/kg) + $\alpha$-tocopherol groups, respectively ($F_{5,36}=9.29, P<0.001$, post hoc $P<0.05$; $F_{5,36}=16.92, P<0.001$, post hoc $P<0.001$, Fig. 2). The mean amplitude of epileptiform ECoG activity did not change after $\alpha$-tocopherol administration in all groups.

The mean spike frequency and amplitude of ECoG activity were $6.7 \pm 1$, $575 \pm 246$ and $17.6 \pm 2$ spike/min, $620 \pm 124$ $\mu$V ethanol-treated (9 g/kg) + $\alpha$-tocopherol, and ethanol (9 g/kg) withdrawal + $\alpha$-tocopherol groups in the 110 min from $\alpha$-tocopherol injection (i.m.), respectively (Fig. 1K, L). The mean frequency of epileptiform activity was decreased in the ethanol treated (9 g/kg) + $\alpha$-tocopherol and withdrawal (9 g/kg) + $\alpha$-tocopherol groups compared with penicillin group (Fig. 3). The significant effects appeared in the 40 and 120 min from $\alpha$-tocopherol injection in the ethanol treated (9 g/kg) + $\alpha$-tocopherol and withdrawal (9 g/kg) + $\alpha$-tocopherol groups, respectively ($F_{5,36}=9.04, P<0.001$, post hoc $P<0.05$; $F_{5,36}=24.93, P<0.001$, post hoc $P<0.05$, Fig. 3). $\alpha$-Tocopherol was not more effective to decrease the mean frequency of epileptiform activity in the ethanol + $\alpha$-tocopherol group than $\alpha$-tocopherol administered alone in all groups (Figs 2, 3).

Intramuscular injection of $\alpha$-tocopherol (500 mg/kg) did not cause any change in the frequency or amplitude of ECoG activity with respect to control base line in non-penicillin injected animals.

DISCUSSION

Acute ethanol administration is well known to have deleterious effects on the central nervous system (Kim et al. 2006). Animal studies have demonstrated that the acute effect of ethanol is generally inhibitory but that this can occur by increased inhibition or decreased excitation (Ziskind-Conhaim et al. 2003). Anticonvulsant or proconvulsant effects were also seen after acute ethanol administration on the epileptic seizures (Freeman 1978, Kim et al. 1995, Fischer and Kittner 1998). We provide further evidence for the effects of acute ethanol, at the dose of 9 g/kg, intake and its withdrawal on epileptiform activity in the penicillin-induced epilepsy in the rats. In addition, we have analyzed the influence of $\alpha$-tocopherol on the epileptiform activity in the penicillin model of epilepsy after acute administration of ethanol and its withdrawal in the rats.

Acute ethanol (2 g/kg, i.p.) has been already used to investigate the effects on the different experimental models of epilepsy (Kulkarni et al. 1990, Sharma et al. 1991). Moreover, we used chronically the same doses (3 and 9 g/kg) of ethanol in the penicillin-induced epilepsy models in previous studies (Kozan et al. 2006, 2007a). We also found that the short term (3 h) ethanol (3 g/kg) administration did not affect the total number of cells in the hippocampus (Kozan et al. 2007b). Therefore, in the present study, the effects of acute ethanol intake and its withdrawal, at the doses of 3 and 9 g/kg, on epileptiform activity were investigated in the penicillin-induced epilepsy in rats. The administration of ethanol (3 g/kg, for 3 days) or its withdrawal did not significantly change either the frequency or amplitude of penicillin-induced epileptiform activity in this study. This result confirms and extends previous observations by Hoppenr and coauthors (1983) who reported that the frequency of seizure activity and the level of EEG epileptiform activity did not change significantly in the patients receiving alcohol compared with the control individuals. Even though, McQuarrie and Finigl (1958) did not find changes of the mean seizure threshold during the administration of ethanol (2.0 g/kg, for 14 days) in the low-frequency electro-shock seizure threshold test in mice. On the other hand, it was found that the administration of ethanol (0.5–1.5 g/kg) 15 min prior to each pentylenetetrazol (PTZ)-stimulation dose-dependently inhibited the
progressive seizure development compared with the controls in the PTZ-kindling model (Fischer and Kittner 1998). In our previous study, the low dose of ethanol intake (3 g/kg, for 15 days) and its withdrawal also did not change the frequency and amplitude of epileptiform activity in the penicillin model of epilepsy (Kozan et al. 2006). However, the high dose of ethanol (9 g/kg, for 3 days) intake decreased the mean frequency of epileptiform ECoG activity in the 110 min from penicillin injection without changing amplitude in the present study. In contrast, Workman and colleagues (1998) reported that the high dose ethanol (6 g/kg, orally) lowered the level of electroconvulsive seizure threshold as well as the PTZ-induced seizure threshold after 8 h from a single ethanol administration. It was also suggested that occasional consumption of large quantities of alcohol may exacerbate the seizures in patients with epilepsy (Mattson et al. 1990). On the other hand, other investigations reported that acute ethanol intake has beneficial effects in progressive myoclonus epilepsy (Genton and Guerrini 1999, Jain et al. 1996). Fischer (2005) reported that the acute administration of ethanol increased the electroconvulsive threshold in two models of generalized tonic-clonic and complex partial seizures, further suggesting an anticonvulsant role for ethanol. In addition, intraperitoneal injection of vanillyl alcohol prior to ferric chloride administration significantly inhibited wet dog shakes and lipid peroxide levels in the bilateral cortex, implying both anticonvulsive and suppressive effects on the seizures for vanillyl alcohol (Hsieh et al. 2000). It is important to note that the differences in experimental design, including the differences among epilepsy models may be attributed for these contradictory results.

In this study, the mean spike amplitude of ECoG activity was significantly higher during recording, and the mean spike frequency of ECoG activity was increased between period of 50 min and 120 min in the withdrawal (9 g/kg) group compared with penicillin-treated group. The hyperexcitability in ethanol exposed rats during the acute phase of ethanol withdrawal is consistent with previous studies (Veatch and Becker 2002, Slawecki et al. 2006). The percent of EEG recording containing ‘brief spindle episodes’ activity significantly increased in time-dependent manner following withdrawal from chronic ethanol exposure (Veatch and Becker 2002). Slawecki and colleagues (2006) reported that high frequency power in the paras-
of α-tocopherol (500 mg/kg) was used to provide a maximal anticonvulsant effect in the penicillin-induced epileptiform activity (Ayyildiz et al. 2006, 2007). Therefore, the influence of acute treatment with ethanol, at the doses of 3 and 9 g/kg, intake and its withdrawal on the effects of α-tocopherol (500 mg/kg) was investigated in penicillin-induced epileptiform activity in rats. Alpha-tocopherol significantly decreased the mean frequency of epileptiform activity in all α-tocopherol administered groups compared with penicillin-induced group in the present study. There was no significant difference in the mean frequency of epileptiform activity in the ethanol treated groups compared with the penicillin-injected group. α-Tocopherol also decreased the amplitude of epileptiform activity in the withdrawal (9 g/kg) + α-tocopherol group compared with withdrawal group (9 g/kg). Therefore, we may conclude that acute ethanol intake or its withdrawal did not influence the anticonvulsant effects of α-tocopherol in the penicillin injected groups. In contrast, it was reported that acute co-medication of ethanol with valproate and carbamazepine (MES threshold test) or carbamazepine (hippocampal afterdischarge model) enhanced the effectiveness of the tested antiepileptics in both models (Fischer 2005). Fischer (2005) also noted that the total plasma levels of valproate and carbamazepine did not differ after acute ethanol administration. The blood plasma concentration of ethanol was also not changed by acute co-medication of the two antiepileptics (Fischer 2005). In addition Kleinrok and coworkers (1993) demonstrated that acute ethanol administration (2 g/kg, i.p.) increased the effectiveness of valproate and phenobarbital. This additive effect of ethanol was not seen after chronic administration of ethanol and its withdrawal (Kleinrok et al. 1993). However, chronic ethanol intake (9 g/kg, for 15 days) enhanced anticonvulsant effects of α-tocopherol in our previous study (Kozan et al. 2007a). There are several lines of circumstantial evidence suggesting that α-tocopherol has an anti-seizure activity in various models of experimental epilepsy, including the ferrous chloride model (Levy et al. 1990), the hyperbaric oxygen model (Jerrett et al. 1973), kindling, and PTZ models (Rauca et al. 2004). The results of present study show that α-tocopherol decreased the frequency of penicillin-induced epileptiform activity in the rat. α-Tocopherol significantly decreased the mean frequency of epileptiform activity in all α-tocopherol administered groups. These effects confirm results of Ribeiro and others (2005), who showed that PTZ- and MMA induced convulsions, TBARS production, and total protein carbonylation were attenuated by α-tocopherol in a dose-dependent manner. In addition, α-tocopherol might have a role in protection against epilepsy and ethanol-induced toxicity as an active oxygen free radical scavenger (Levy et al. 1992). Furthermore, Kotegawa and coworkers (1993) suggest that α-tocopherol an endogenous compound, mainly acts as chain-breaking antioxidant and protects cell membranes against oxidative damage by regulating reactive oxygen species (ROS) production and maintaining oxidative phosphorylation in mitochondria, thus accelerating restitution of high-energy phosphates.

There is extensive behavioral, electrophysiological and neurochemical evidence that the central nervous system effects of ethanol involve its effects on the action of gamma-amino-N-butyric acid (GABA) (Nishio and Narahashi 1990, Faingold et al. 1998). Acute administration of ethanol has been reported to induce changes in the electrophysiology and behavioral effects of excitatory amino acid-mediated neurotransmission, which are consistent with an important role of NMDA receptors in ethanol intoxication (Faingold et al. 1998). On the other hand, an insufficient GABAergic (GABA) inhibition was suggested as one of the reasons for central hyperexcitability in epilepsy (Corda et al. 1991, Meldrum 1995). Ethanol, at the high concentration (≥50 mM) can inhibit NMDA-activated ion current at the NMDA subtype of excitatory glutamate receptors (Lovingier et al. 1989, Randall et al. 1996), while it potentiates GABAergic-mediated Cl⁻ influx (Mehta and Ticku 1988, Sapp and Yeh 1998) in the investigated brain regions. Thus, ethanol suppresses release of glutamate, and increases in glycine and GABA (Ziskind-Conhaim et al. 2003, Zhu and Lovingier 2006). These reduced excitatory (glutamate) and an increased inhibitory (GABA) neurotransmissions may be one possible explanation of anticonvulsant effects of high dose (9 g/kg) of acute ethanol administration in the present study.

CONCLUSIONS

The present study demonstrated for the first time that acute administration of low dose ethanol (3 g/kg) does not have either anticonvulsive or proconvulsive effect, while acute administration of high dose ethanol
(9 g/kg) has anticonvulsive effect on penicillin-induced epileptiform activity in rat. α-Tocopherol, at the dose of 500 mg/kg, decreased the mean frequency of epileptiform activity in all groups, suggesting the anticonvulsive action of α-tocopherol might result from its neuroprotective actions. Ethanol (9 g/kg) withdrawal caused an increase in both the frequency and amplitude of epileptiform activity in the withdrawal group compared with other groups. However, the mechanisms underlying the influence of ethanol intake and its withdrawal on the effects of α-tocopherol in penicillin-induced epileptiform activity merit further investigation.

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