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

Ramazan Kozan, Mustafa Ayyildiz, Mehmet Yildirim, and Erdal Agar*

Department of Physiology, Faculty of Medicine, University of Ondokuz Mayis, 55139 Samsun, Turkey,
*Email: eragar@omu.edu.tr

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 $21 \pm 2.8°C$ 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; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgSO_4$, 1.3; $NaHCO_3$, 26; glucose, 10; HEPES, 10; pH 7.4 when saturated with 95% O_2 and 5% CO_2] (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^{-1}, i.p.) and placed in a stereotaxic frame (Harvard Stereotaxic Instrument). Rectal temperature was maintained between 36.5 and 37.0°C 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.
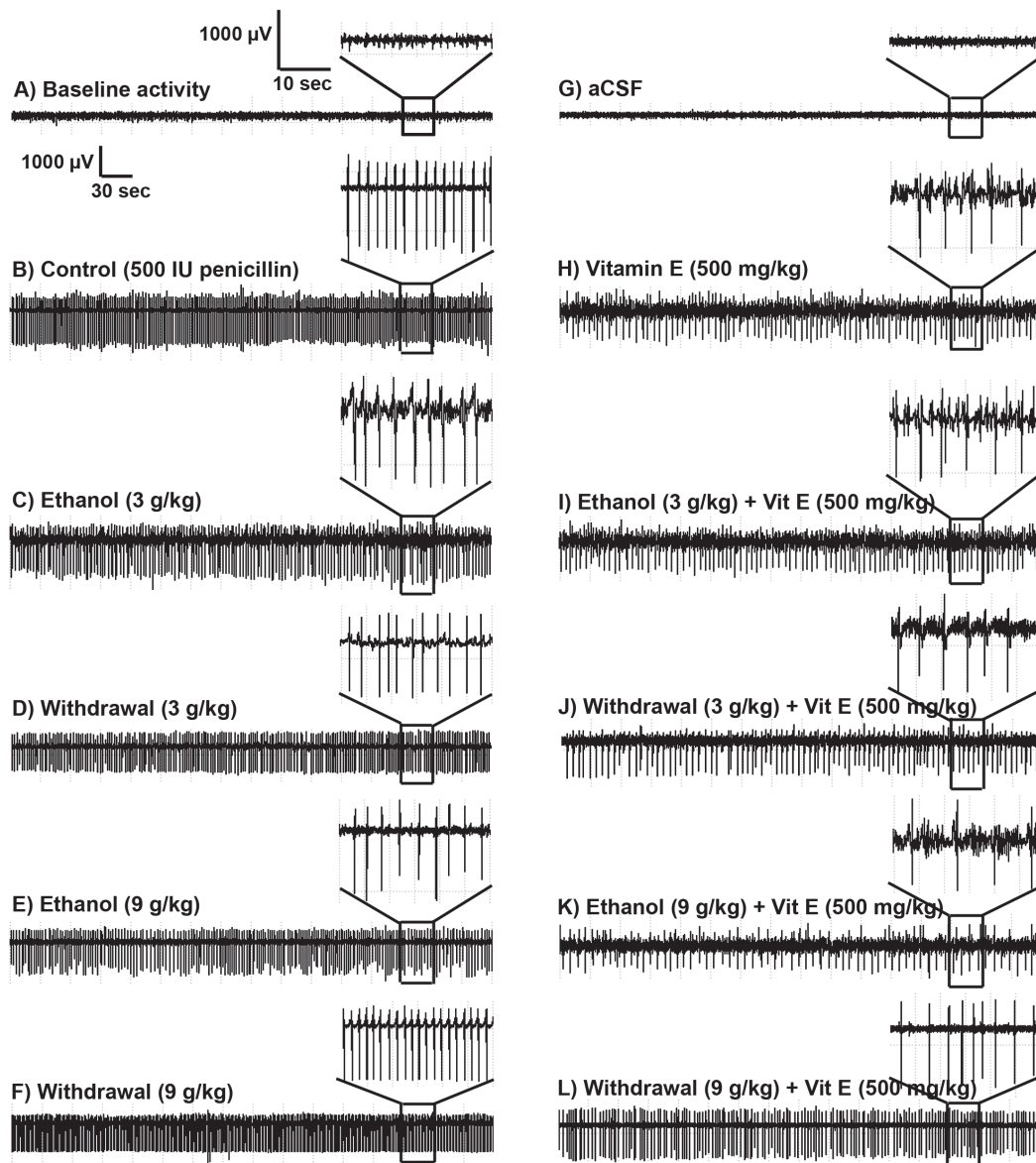
Fig. 1. The baseline ECoG activities (A). Intracortical injection of penicillin (500 units) induces an epileptiform ECoG activity characterized by bilateral spikes and spike-wave complexes (B). Ethanol intake (3 g/kg per day for 3 days, intragastrically) does not significantly change the mean frequency 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 ($F_{5,36}$=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

the withdrawal (9 g/kg) group compared with penicillin-treated group ($F_{5,36}$=24.93, $P<0.001$, *post hoc P<0.05*, Fig. 3). 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).

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
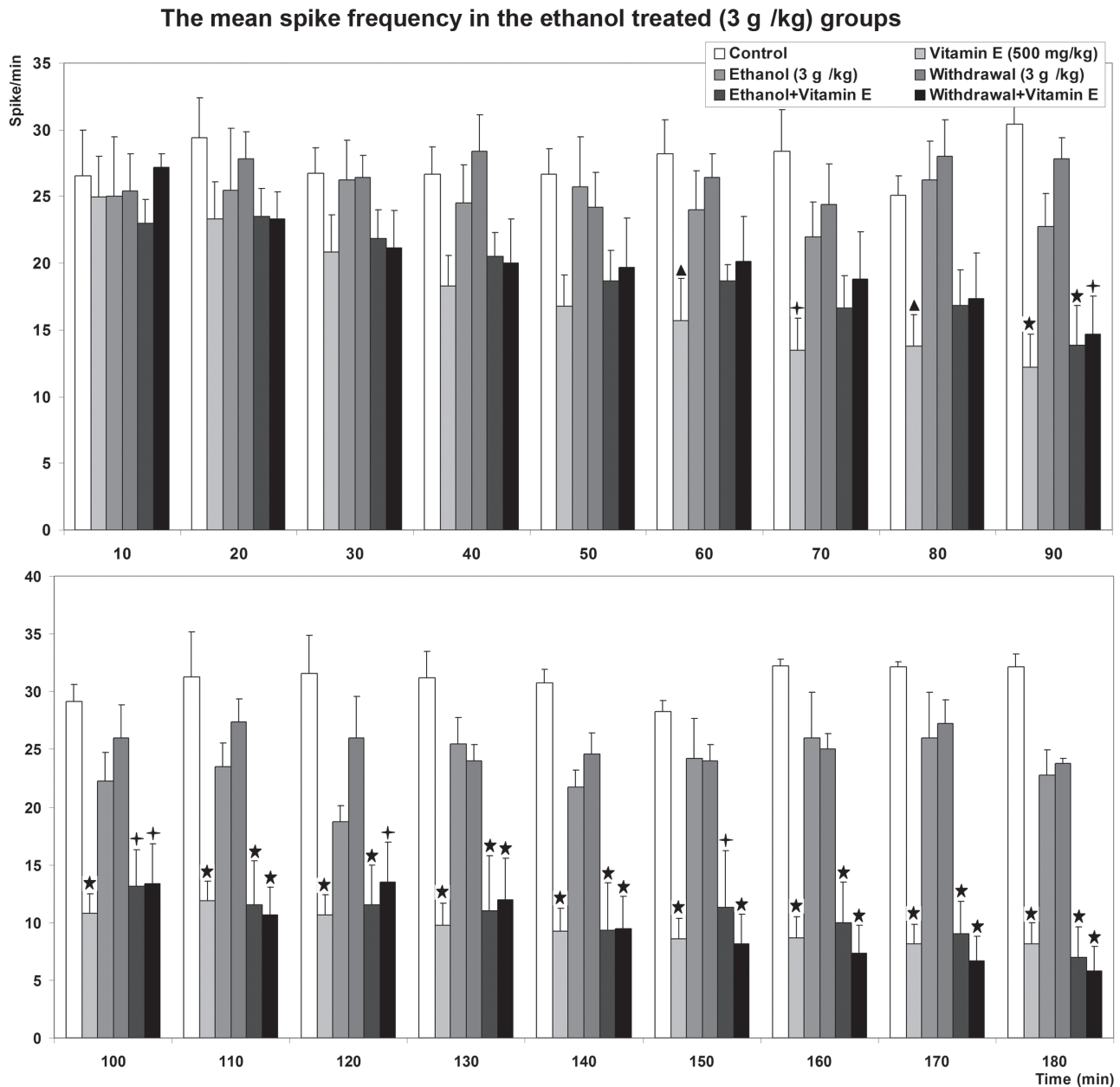

Fig. 2. The influence of ethanol (3 g/kg) intake and its withdrawal on the anticonvulsant effect of α-tocopherol in the mean frequency of penicillin–induced epileptiform activity in rats. Ethanol (3 g/kg per day for 3 days, intragastrically) did not affect the mean frequency of epileptiform activity. 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. ▲ $P<0.05$, ✦ $P<0.01$, ★$P<0.001$.

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
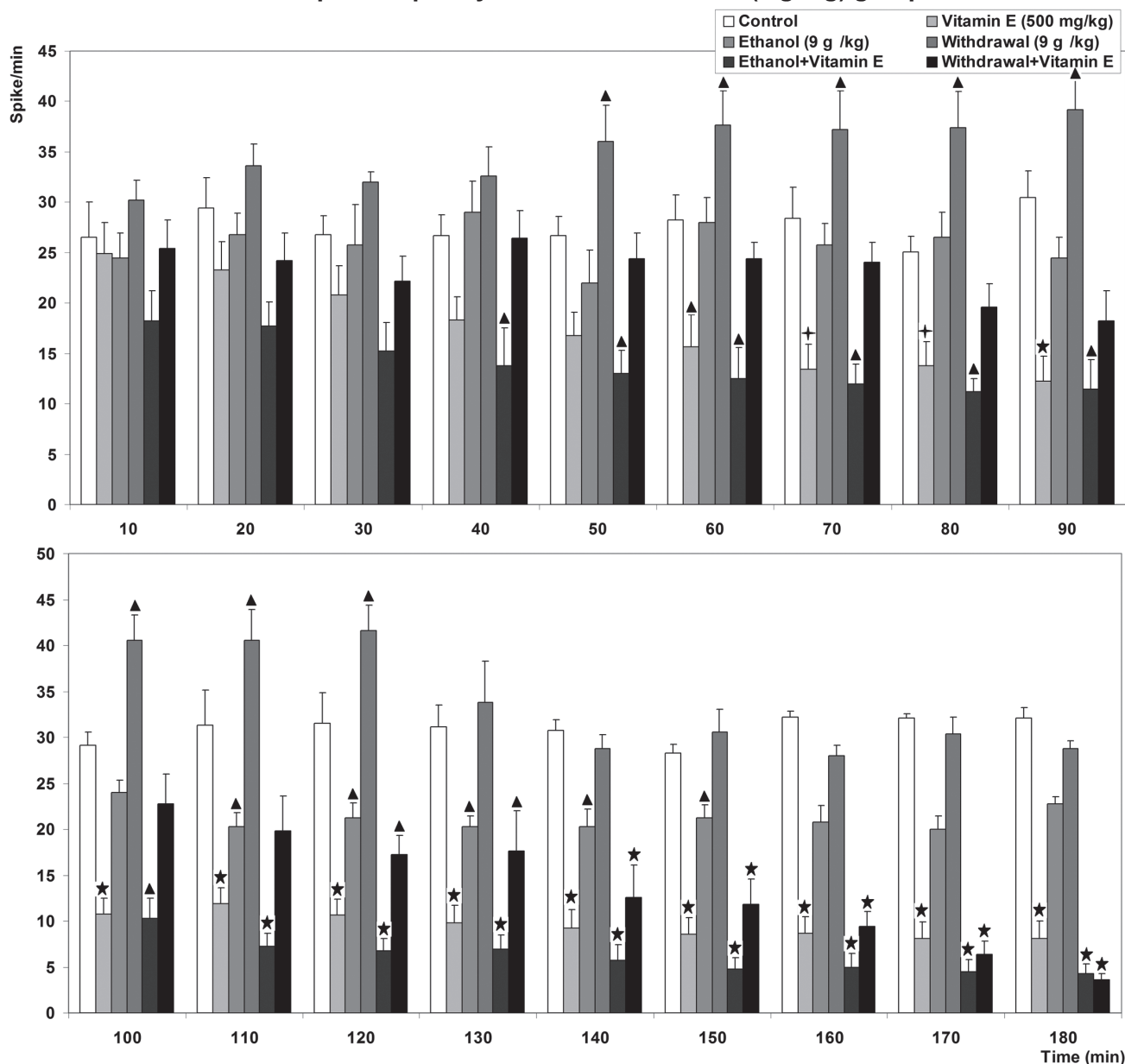

Fig. 3. The influence of ethanol (9 g/kg) intake and its withdrawal on the anticonvulsant effect of α-tocopherol in the mean frequency of penicillin–induced epileptiform activity in rats. Ethanol (9 g/kg per day for 3 days, intragastrically) decreases the mean frequency of epileptiform activity in the 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 α-tocopherol in the penicillin-induced epileptiform activity

α-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 ± 2, 790 ± 244; 11.5 ± 3, 716 ± 132 and 13.5 ± 3 spike/min, 916 ± 456 μV in the penicillin + α-tocopherol, ethanol-treated (3 g/kg) + α-tocopherol, and ethanol (3 g/kg) withdrawal + α-tocopherol groups in the 110 min from α-tocopherol injection (i.m.), respectively (Fig. 1H, I, J). The mean frequency of ECoG activity was decreased in the penicillin + α-tocopherol group, ethanol-treated (3 g/kg) + α-tocopherol, and ethanol (3 g/kg) withdrawal + α-tocopherol groups compared with penicillin-treated group (Fig. 2). The significant effects appeared in the 60, 90 min penicillin + α-tocopherol and ethanol-treated (3 g/kg) + α-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 α-tocopherol administration in all groups.

The mean spike frequency and amplitude of ECoG activity were 6.7 ± 1, 575 ± 246 and 17.6 ± 2 spike/min, 620 ± 124 μV ethanol-treated (9 g/kg) + α-tocopherol, and ethanol (9 g/kg) withdrawal + α-tocopherol groups in the 110 min from α-tocopherol injection (i.m.), respectively (Fig. 1K,L). The mean frequency of epileptiform activity was decreased in the ethanol treated (9 g/kg) + α-tocopherol and withdrawal (9 g/kg) + α-tocopherol groups compared with penicillin group (Fig. 3). The significant effects appeared in the 40 and 120 min from α-tocopherol injection in the ethanol treated (9 g/kg) + α-tocopherol and withdrawal (9 g/kg) + α-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). α-Tocopherol was not more effective to decrease the mean frequency of epileptiform activity in the ethanol + α-tocopherol group than α-tocopherol administered alone in all groups (Figs 2, 3).

Intramuscular injection of α-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 α-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 Hoppener 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 Fingl (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 electroshock 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 coauthors (1958) reported that the high dose ethanol (6 g/kg, orally) lowered the level of electroconvulsive 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 1990, 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 lipids 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 parietal cortical EEG was increased in ethanol exposed adolescents during withdrawal. However, they noted that acoustic startle response amplitude was reduced in the chronic ethanol exposed rats during the acute phase of ethanol withdrawal (Slawecki et al. 2006). Moreover, it was demonstrated that withdrawal from a single bolus dose of ethanol produces a transient but significant elevation in reward thresholds only with highest ethanol dose tested (Schulteis and Liu 2006). In the rodent models of alcohol withdrawal, animals are exposed to alcohol by intragastric intubation, inhalation, or feeding in a nutritionally complete liquid diet for periods of 2 to 21 days (Rogawski 2005). The animals exhibit sound-evoked audiogenic seizures or handling-induced convulsions during the 1- to 3-day period after cessation of alcohol intake and may also experience spontaneous generalized seizures (Rogawski 2005). It is also important to note that several lines of evidence indicate that rats were rendered physically dependent on ethanol by intragastric administration of ethanol at a dosage of 9 to 15 g/kg/day for 4 days (Majchrowicz 1975, Majchrowicz et al. 1976, Derr and Derr 1985).

In this study, we used urethane-anesthetized animals in all groups including control group to examine the effects of ethanol and the involvement of α-tocopherol in these effects. Urethane has been widely used as an anesthetic in *in vivo* experiments. Many researches noted that more than 100 studies are published each year using 'urethane anesthetized' animals (Soma 1983, Maggi and Meli 1986, Hara and Harris 2002, Bosnak et al. 2007). However, only a few epileptic models analyzed under urethane anesthesia, none of them exhibited high frequency of tonic-clonic activity (Heltovics et al. 1995). Suzuki and Smith (1988) reported that general anesthetics (ether, urethane and phenobarbital) all reduced spontaneous electroencephalograhic spikes. However, both ether and phenobarbital, at the anesthetic doses, completely abolished spontaneous electroencephalograhic spikes (Suzuki and Smith 1988). In addition, it was reported that urethane has less influence on the neuronal activity of a wide variety of central nervous system areas, including the cerebral cortex (Angel and Gratton 1982).

On the other hand, several studies showed that α-tocopherol has a neuroprotective role against epilepsy and ethanol-induced toxicity (Levy et al. 1992, Agar et al. 2000). In our previous studies, a high dose

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 after-discharge 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 ($\geq$50 mM) can inhibit NMDA-activated ion current at the NMDA subtype of excitatory glutamate receptors (Lovinger et al. 1989, Randoll et al. 1996), while it potentiates $GABA_A$-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 Lovinger 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.

ACKNOWLEDGEMENT

REFERENCES

Agar E, Demir S, Amanvermez R, Bosnak M, Ayyildiz M, Celik C (2000) The changes in lipid peroxidation and GSH levels in the cerebellum of rats induced by ethanol consumption are prevented by vitamin E. Neurosci Res Commun 27: 191–197.

Angel A, Gratton DA (1982) The effect of anaesthetic agents on cerebral cortical responses in the rat. Br J Pharmacol 76: 541–549.

Ayyildiz M, Yildirim M, Agar E (2006) The effect of vitamin E on penicillin-induced epileptiform activity in rats. Exp Brain Res 174: 109–113.

Ayyildiz M, Yildirim M, Agar E (2007) The involvement of nitric oxide in the anticonvulsant effects of α-tocopherol on penicillin-induced epileptiform activity in rats. Epilepsy Res 73: 166–172.

Bosnak M, Ayyildiz M, Yildirim M, Agar E (2007) The role of nitric oxide in the anticonvulsant effects of pyridoxine on penicillin-induced epileptiform activity in rats. Epilepsy Res 76: 49–59.

Charness ME (1993) Brain lesions in alcoholics. Alcohol Clin Exp Res 17: 2–11.

Collins RC (1978) Kindling of neuroanatomic pathways during recurrent focal penicillin seizures. Brain Res 150: 503–517.

Corda MG, Orlandi M, Lecca D, Carboni G, Frau V, Giorgi O (1991) Pentylenetetrazol-induced kindling in rats: effect of GABA function inhibitors. Pharmacol Biochem Behav 40: 329–333.

Derr RF, Derr M (1985) Suppression of an ethanol withdrawal syndrome in rats by 3-hydroxybutyrate. Life Sci 36: 763–767.

Faingold CL, N'Gouemo P, Riaz A (1998) Ethanol and neurotransmitter interactions-from molecular to integrative effects. Prog Neurobiol 55: 509–535.

Fernandez-Guardiola A, Martinez A, Fernandez-Mas R (1995) Repeated penicillin − induced amygdala epileptic focus in freely moving cats. EEG, polysomnographic (23 h recording), and brain mapping study. Epilepsy Res 22: 127–136.

Fisher RS (1989) Animal models of the epilepsies. Brain Res 14: 245–278.

Fischer W, Kittner H (1998) Influence of ethanol on the pentylenetetrazol-induced kindling in rats. J Neural Transm 105: 1129–1142.

Fischer W (2005) Influence of ethanol on the threshold for electroshock-induced seizures and electrically-evoked hippocampal afterdischarges. J Neural Transm 112: 1149–1163.

Freeman FG (1978) Effects of alcohol on the kindled seizures tresholds in rats. Pharmacol Biochem Behav 8: 641–644.

Genton P, Guerrini R (1990) Antimyoclonic effects of alcohol in progressive myoclonus epilepsy. Neurology 49: 1412–1416.

Gonzalez-Trujano ME, Tapia E, Lopez-Meraz L, Navarrete A, Reyes-Ramirez A, Martinez A (2006) Anticonvulsant effect of annona diversifolia saff. and palmitone on penicillin-induced convulsive activity: A behavioral and EEG study in rats. Epilepsia 47: 1810–1817.

Guerrero-Figueroa R, Rye MM, Gallant DM, Bishop MP (1970) Electrographic and behavioral effects of diazepam during alcohol withdrawal stage in cats. Neuropharmacology 9: 143–150.

Hara K, Harris A (2002) The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. Anesth Analg 94: 313–318.

Heltovics G, Boda B, Szente M (1995) Anticonvulsive effect of urethane on aminopyridine-induced epileptifrom activity. Neuroreport 6: 577–580.

Hoppener RJ, Kuyer A, Van der Lugt PJM (1983) Epilepsy and alcohol: the influence of social alcohol intake on seizures and treatment of epilepsy. Epilepsia 24: 459–471.

Hsieh CL, Chang CH, Chiang SY, Li TC, Tang NY, Pon CZ, Hsieh CT, Lin JG (2000) Anticonvulsive and free radical scavenging activities of vanillyl alcohol in ferric chloride-induced epileptic seizures in Sprague-Dawley rats. Life Sci 67: 1185–1195.

Jain S, Tamer SK, Hiran S (1996) Beneficial effect of alcohol in hereditary cerebellar ataxia with myoclonus (progressive myoclonic ataxia): report of two siblings. Mov Disord 11: 751–752.

Jerrett SA, JeVerson D, Mengel CE (1973) Seizure H2O2 and lipid peroxidase in brain during exposure to oxygen under high pressure. Aeroesp Med 44: 40–44.

Kim CK, Dalal S, Pinel JPJ, Weinberg J (1994) Prenatal ethanol exposure: susceptibility to convulsions and ethanol's anticonvulsant effects in amygdala-kindled rats. Alcohol Clin Exp Res 18: 1506–1514.

Kim CK, Pinel JPJ, Dalal S, Kippin TE, Kalynchuk LE, Payne GJ (1995) Contingent drug tolerance: differential tolerance to the anticonvulsant, hypothermic, and ataxic effects of ethanol. Pharmacol Biochem Behav 52: 531–539.

Kim HJ, Choi KM, Ku BM, Mun J, Joo Y, Han JY, Kim YH, Roh GS, Kang SS, Cho GJ, Choi ES (2006) Acute ethanol administration decreases GAP-43 and phosphorylated-GAP-43 in the rat hippocampus. Brain Res 1112: 16–25.

Kleinrok Z, Dziki M, Janczarek T (1993) The influence of ethanol on pentylenetetrazol-induced seizures and anticonvulsant activity of phenobarbiatal and valproate against maximal electroshoch in mice. Pol J Pharmacol 45: 361–368.

Kotegawa M, Sugiyama M, Shoji T, Haramaki N, Ogura R (1993) Effect of α-tocopherol on high energy phosphate metabolite levels in rat heart by P-NMR using a Langend of perfusion technique. J Mol Cell Cardiol 25: 1067–1074.

Kozan R, Ayyildiz M, Yildirim M, Agar E (2006) The effects of ethanol intake and withdrawal on penicillin-induced epileptiform activity in rats. Brain Res Bull 71: 111–115.

Kozan R, Ayyildiz M, Yildirim M, Agar E (2007a) The influence of ethanol intake and its withdrawal on the anticonvulsant effects of α-tocopherol in penicillin-induced epileptiform activity in rats. Neurotoxicology 28: 463–470.

Kozan R, Ayyildiz M, Bas O, Kaplan S, Agar E (2007b) Short-term ethanol administration does not change the total pyramidal neuron number of the rat hippocampus: A stereologic study. Adv Ther 24: 231–238.

Kulkarni SK, Mehta AK, Ticku MK (1990) Comparison of anticonvulsant effects of ethanol against NMDA-, kainic acid-and picrotoxin-induced convulsions in rats. Life Sci 46: 481–487.

Levy Sl, Burnham WM, Bishai A, Hwang PA (1990) An evaluation of the anticonvulsant effects of vitamin E. Epilepsy Res 6: 12–17.

Levy SL, Burnham WM, Bishai A, Hwang PA (1992) The anticonvulsant effects of vitamin E: a further evaluation. Can J Neurol Sci 19: 201–203.

Lovinger DM, White G, Weight FF (1989) Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243: 1721–1724.

Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations. Part 3. Other systems and conclusions. Experientia 42: 531–537.

Majchrowicz E (1975) Induction of physical dependence upon ethanol and the associated behavioral changes in rats. Psychopharmacologia 43: 245–254.

Majchrowicz E, Hunt WA, Piantadosi C (1976) Suppression by 1,3-butanediol of the ethanol withdrawal syndrome in rats. Science 194: 1181–1182.

Mattson RH, Fay ML, Sturman JK (1990) The effect of various patterns of alcohol use on seizures in patients with epilepsy. In: Alcohol and Seizures: Basic Mechanisms and Clinical Concepts (Porter RJ, Mattson RH, Cramer JA, Diamond I, Eds.). Davis Co., Philadelphia, PA, p. 233–240.

McQuarrie DG, Fingl E (1958) Effects of single doses and chronic administration of ethanol on experimental seizures in mice. J Pharmacol Exp Ther 124: 264–271.

Mehta AK, Ticku MK (1988) Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves gamma-aminobutyric acid-gated chloride channels. J Pharmacol Exp Ther 246: 558–564.

Meldrum BS (1995) Neurotransmission in epilepsy. Epilepsia 27 (Suppl 1): S3–S11.

Mello LEAM, Masur J, Formigoni MLOS, Kireeff W, Cavalheiro EA (1990) Amygdaloid kindling and kindled seizures in rats receiving chronic ethanol administration. Epilepsy Res 7: 95–104.

Nadiger HA, Marcus SR, Chandrakala MV (1988) Lipid peroxidation and ethanol toxicity in rat brain: effect of vitamin E deficiency and supplementation. Med Sci Res 16: 1273–1274.

Ng SKC, Hauser A, Brust JCM, Susser M (1988) Alcohol consumption and withdrawal in new-onset seizures. New Engl J Med 319: 666–673.

Nishio M, Narahashi T (1990) Ethanol enhancement of GABA-activated chloride current in rat dorsal root ganglion neurons. Brain Res 518: 283–286.

Randoll LA, Wilson WR, Weaver MS, Spuhler-Phillips K, Leslie SW (1996) N-methyl-D-aspartate-stimulated increases in intracellular calcium exhibit brain regional differences in sensitivity to inhibition by ethanol. Alcohol Clin Exp Res 20: 197–200.

Rauca C, Wiswedel I, Zerbe R, KeilhoV G, Krug M (2004) The role of superoxide dismutase and α-tocopherol in the development of seizures and kindling induced by pentylenetetrazol-influence of the radical scavenger α-phenyl-N-tert-buthyl nitrone. Brain Res 109: 203–212.

Ribeiro MCP, Avila DS de, Schneider CYM, Hermes FS, Furian AF, Oliveira MS, Rubin MA, Lehmann M, Kreiglstein J, Mello CF (2005) α-tocopherol protects against pentylenetetrazol-and methylmalonate-induced convulsions. Epilepsy Res 66: 185–194.

Rogawski MA (2005) Update on the neurobiology of alcohol withdrawal seizures. Epilepsy Curr 5: 225–230.

Sagratella S, Niglio T and Carolis AS (1985) An investigation on the mechanism of anticonvulsant action of ketamine and phencyclidine on convulsions due to cortical application of penicillin in rabbits. Pharmacol Res Commun 17: 773–786.

Sapp DW, Yeh HH (1998) Ethanol GABA$_A$ receptor interactiona: a comparison between cell lines and cerebellar Purkinje cells. J Pharmacol Exp Ther 284: 768–776.

Schulties G, Liu J (2006) Brain reward deficits accompany withdrawal (hangover) from acute ethanol in rats. Alcohol 39: 21–28.

Sharma AC, Thorat SN, Nayar U, Kulkarni SK (1991) Dizocilpine, ketamine and ethanol reverse NMDA-induced EEG changes and convulsions in rats and mice. Indian J Physiol Pharmacol 35: 111–116.

Slawecki CJ, Roth J, Gilder A (2006) Neurobehavioral profiles during the acute phase of ethanol withdrawal in adolescent and adult Sprague-Dawley rats. Behav Brain Res 170: 41–51.

Soma LR (1983) Anesthetic and analgesic considerations in the experimental animal. Ann NY Acad Sci 406: 32–47.

Somani SM, Husain K (1997) Interaction of exercise training and chronic ethanol ingestion on antioxidant system of rat brain regions. J Appl Toxicol 17: 329–336.

Stankiewicz J, Stankiewicz M, Wiaderna-Stepień D, Tomas T, Gralewicz S (1995) Effects of repeated systemic penicillin injections on nonconvulsive and convulsive epileptic seizures in the rat. Acta Neurobiol Exp (Wars) 55: 281–287.

Stankiewicz J, Gralewicz S (1996) The influence of repeated systemic penicillin injections at subconvulsive doses on spontaneous spike-wave discharges in the rat. Acta Neurobiol Exp (Wars) 56: 673–681.

Suzuki SS, Smith GK (1988) Spontaneous EEG spikes in the normal hippocampus. Effects of ether, urethane, phenobarbital, atropine, diazepam and bicuculline. Electroencephalogr Clin Neurophysiol 70: 84–95.

Veatch LM, Becker HC (2002) Electrographic and behavioral indices of ethanol withdrawal sensitization. Brain Res 946: 272–282.

Workman RL Jr, Swinyard EA, Rigby OF, Swinyard CA (1958) Correlation between anticonvulsant activity and plasma concentration of ethanol. J Am Pharmac Assoc 47: 769–772.

Zhu PJ, Lovinger DM (2006) Ethanol potentiates GABAergic synaptic transmission in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. J Neurophysiol 96: 433–441.

Ziskind-Conhaim L, Gao B, Hinckley C (2003) Ethanol dual modulatory actions ob spontaneous postsynaptic currents in spinal motoneurons. J Neurophysiol 89: 806–813.