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The Effects of Ascorbic Acid on Penicillin-induced Epileptiform Activity in Rats

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Summary: *Purpose:* Epileptic seizure results from excessive discharge in a population of hyperexcitable neurons. A number of studies help to document the effects of active oxygen free radical scavengers such as α-tocopherol or ascorbic acid (vitamin C). In the present study, we examined the effects of ascorbic acid, at the six different doses, on penicillin-induced epileptiform activity.

Methods: A single microinjection of penicillin (2.5 μl, 500 units, intracortically) into the left sensorimotor cortex induced epileptiform activity within 2–5 min, progressing to full seizure activity lasting ~3–5 h. In the first set of experiments, 30 min after penicillin injection, six different doses of ascorbic acid (25, 50, 100, 200, 400, or 800 mg/kg) were administered intraperitoneally (IP). The other group of animals received the effective dose of ascorbic acid (100 mg/kg, IP) for 7 days. Ascorbic acid administration was stopped 24 h before penicillin treatment. Another group of rats received the effective dose of ascorbic acid (100 mg/kg, IP) 30 min before penicillin treatment. In the second set of experiments, the lipid peroxidation (MDA) and reduced glutathione (GSH) levels of brain were measured in the control, control + ascorbic acid, penicillin, and penicillin + ascorbic acid groups.

Results: Ascorbic acid, at the low dose (50, 100 mg/kg, 30 min after penicillin injection), decreased both the frequency and amplitude of penicillin-induced epileptiform activity in rats. Ascorbic acid, at intermediate doses (200, 400 mg/kg, 30 min after penicillin injection), decreased the frequency of epileptiform activity without changing the amplitude. Ascorbic acid, at the lowest dose (25 mg/kg) and highest dose (800 mg/kg) (30 min after penicillin injection), did not change either the frequency or amplitude of epileptiform activity. Ascorbic acid, at the low dose (100 mg/kg) was the most effective dose in changing the frequency and amplitude of penicillin-induced epileptiform activity. Pretreatment with ascorbic acid (100 mg/kg) 30 min before penicillin treatment caused a significant delay in the onset of penicillin-induced epileptiform activity. Pretreatment with ascorbic acid (100 mg/kg) for 7 days did not change the latency of epileptiform activity. The most effective dose of ascorbic acid (100 mg/kg) prevented both the decrease in GSH level and the increase in lipid peroxidation level (MDA) occurring after penicillin-induced epileptiform activity.

Conclusions: These data indicate that ascorbic acid has neuroprotective activity against penicillin-induced epileptiform electrocorticogram activity. **Key Words:** Epileptiform activity—Glutathione—Lipid peroxidation—Penicillin—Vitamin C.

Epilepsy is one of the most common neurologic disorders, characterized by unprovoked, recurring seizures that disrupt the nervous system and can cause mental and physical dysfunction (Dichter, 1994). Epileptogenic processes have been associated with imbalance between excitatory and inhibitory control systems in selective regions of the brain (Brailowsky and Garcia, 1999). Most epileptic seizures are due to discharges generated in cortical and hippocampal structures, although subcortical structures also are involved in some seizure types (Avanzini and Franceschetti, 2003). Many previous studies have demonstrated that the abnormal metabolism of trace elements, electrolytes, and antioxidants might be involved in the pathophysiology of severe mental and neurologic disorders, including epilepsy (Hamed and Abdullah, 2004). Arzimanoglou et al. (2002) noted that anticonvulsant treatment of epilepsy has always been related to neuroprotection, because it aims to reduce the duration of or totally to suppress seizures.

Membrane lipid derangements, including lipid peroxidation (LPO) and free radical production, have been reported to contribute to epileptogenesis (Frantseva et al., 2000; Patel et al., 2001). The biologic effects of free radicals are controlled in vivo by a wide range of antioxidants, such as vitamin E, vitamin C, vitamin A, and glutathione (Halliwell and Gutteridge, 1990). Ascorbic acid has many functions in the brain, and in the neuronal microenvironment, it functions as neuromodulator, as well as an antioxidant/free radical scavenger (Niki, 1991; Frei et al.,

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1992). It has been suggested that ascorbic acid has neuroprotective properties in some experimental models of excitotoxic neurologic disorders, such as Huntington disease (Rebec et al., 2003), cerebral ischemia (MacGregor et al., 2003), and seizure activity induced by various agents, such as iron (Yamamoto et al., 2002), methylmalonic acid (Fighera et al., 1999), and pentylenetetrazol (PTZ) (Oliveira et al., 2004). Yamamoto et al. (2002) demonstrated that an ascorbate synthetic derivative prevents the occurrence of ferric ion–induced epileptic discharges in a rat model of post-traumatic epilepsy. Further evidence for an anticonvulsant role for ascorbate comes from a study that reported that ascorbate, at high doses (300 mg/kg), protected against PTZ-induced convulsions, further suggesting an anticonvulsant and neuroprotective role for ascorbic acid (Oliveira et al., 2004). They also reported that ascorbate, at a low dose (30 mg/kg), did not alter PTZ-induced convulsions. Conversely, ascorbate, at an intermediate dose (100 mg/kg), potentiated the duration of convulsive episodes (Oliveira et al., 2004). The fact that ascorbate, at the low doses (50–200 mg/kg), has been reported to enhance amphetamine-induced behavior activation (Wambebe and Sokomba, 1986) and conditioned place preference (Pierce et al., 1995), and that ascorbate, at a high dose (500 mg/kg), had either no, or an opposing effect, on these behaviors (Tolbert et al., 1979; Pierce et al., 1995) constitutes further evidence for a biphasic effect of ascorbate on central nervous system functions. Therefore, in the present study, we decided to investigate the effect of ascorbic acid, at six different doses of 25, 50, 100, 200, 400, and 800 mg/kg, on the frequency and amplitude of penicillin-induced epileptiform activity in the rats. We also evaluated the levels of lipid peroxidation and reduced glutathione in the brain after seizure.

MATERIALS AND METHODS

Animals

One hundred-five male Wistar rats (OMU Animal Research Center, Samsun, Turkey) (230–270 g), maintained on a 12-h light/dark cycle, with free access to tap water and standard laboratory food, were used. All experimental protocols were conducted in accordance with governmental approval according to local guidelines for the care and use of laboratory animals. Rats were assigned to the following experiments and groups. In the first set of experiments for electrophysiological recordings: Intracortical (IC) delivery of (a) 2.5 μl artificial cerebrospinal fluid (aSCF), containing (m*M*): 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) (IC); (b) 500 units penicillin (2.5 μl, IC); (c) 100 mg/kg ascorbic acid (IP); (d) penicillin-pretreated + 25 mg/kg ascorbic acid (IP); (e) penicillin-pretreated + 50 mg/kg ascorbic acid (IP); (f) penicillin-pretreated + 100 mg/kg ascorbic acid (IP); (g) penicillin-pretreated + 200 mg/kg ascorbic acid (IP); (h) penicillin-pretreated + 400 mg/kg ascorbic acid (IP); (i) penicillin-pretreated + 800 mg/kg ascorbic acid (IP); (j) 100 mg/kg ascorbic acid (IP) (30 min before penicillin) + penicillin-pretreated; (k) 100 mg/kg ascorbic acid for 7 days (IP) + penicillin-pretreated + 100 mg/kg ascorbic acid (30 min after penicillin, IP).

In the second set of experiments for biochemical analysis: (l) 2.5 μl aCSF (IC); (m) 2.5 μl aCSF (IC) + 100 mg/kg ascorbic acid (IP); (n) 500 units penicillin (2.5 μl, IC); (o) 500 units penicillin (2.5 μl, IC) + 100 mg/kg ascorbic acid (IP). Each animal group was composed of seven rats.

Induction of epileptiform activity

The animals were anesthetized with urethane (1.25 g/kg, IP) and placed in a stereotaxic frame. Rectal temperature was maintained between 36.5 and 37.0°C by using a feedback-controlled heating system. A polyethylene cannula was introduced into the right femoral artery to monitor blood pressure, which was kept >100 mm Hg during the experiments (mean, 120 ± 5 mm Hg). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.

The left cerebral cortex was exposed by craniotomy. The epileptic focus was produced by 500 units penicillin G potassium injection (short-term experimental model of focal epilepsy; 1 mm beneath the brain surface by a Hamilton microsyringe type 701N (Aldrich, Milwaukee, WI, U.S.A.); infusion rate, 0.5 μl/min) (Wyler and Ward, 1980; Ayyildiz et al., 2006a).

Drug and drug administration

L-Ascorbic acid (Aldrich) (pH 7.0) was used in the experiments. Ascorbic acid, at doses of 25, 50, 100, 200, 400, and 800 mg/kg, were administered (IP) 30 min after penicillin (IC) application. Ascorbic acid–pretreated rats, received a daily dose of 100 mg/kg ascorbic acid (IP) for 7 days. At the end of this period, ascorbic acid administration was stopped, and 24 h after the last administration of ascorbic acid, all rats were anesthetized for induction of epileptiform activity. Ascorbic acid, in a dose of 100 mg/kg, was administered (IP) 30 min after penicillin (IC) application in this group. An additional group of animals was pretreated at 30 min with ascorbic acid (100 mg/kg, IP) before penicillin treatment. Penicillin was prepared in sterile apyrogen distilled water and administered intracortically in a volume of 2.5 μl (pH 7.6).

Electrocorticographic (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, AD Instruments, Castle Hill, Australia). All recordings were stored on a computer. The frequency and amplitude of epileptiform activity was analyzed offline.

Biochemical analysis

Animals were killed by decapitation 1 h after substance injection and the head pinned onto a dissecting dish and immersed in oxygenated aCSF at 4°C (Agar et al.,1996). The skull was opened with small iris scissors, after removing the scalp and other soft tissue. Each brain was carefully removed from the skull, and the spinal cord was severed at the spinal–medullary junction (Agar et al., 2003). The level of lipid peroxidation and GSH were measured in brain by spectrophotometric methods (Aykaç et al., 1985; Casini et al., 1986)

Measurement of lipid peroxidation

Lipid peroxidation was estimated as malondialdehyde (MDA) levels reacting with thiobarbituric acid, according to methods described by Casini et al. (1986) and expressed as nanomoles of MDA per gram wet weight. In brief, tissue samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (Heideloph Diax 900, Schwabach, Germany). After centrifugation of the homogenate at 3,000 rpm for 10 min (Hermle Z 323 K, Tuttlingen, Germany), 750 μl of supernatant was added to an equal volume of 0.67% (m/vol) thiobarbituric acid and heated at 100°C for 15 min. The absorbance of the samples was measured at 535 nm with a spectrophotometer. Lipid peroxide levels are expressed in terms of malondialdehyde equivalents by using an extinction coefficient of 1.56×10^5 mol/cm.

Measurement of glutathione

The reduced GSH levels were determined by a modified Ellman's method (Aykaç et al., 1985) and expressed as micromoles of GSH per gram wet weight. In brief, after centrifugation of homogenate at 3,000 rpm for 10 min, 0.5 ml of supernatant was added to the 2 ml of 0.3 *M* Na_2HPO_4 2 H_2O solutions. A 0.2-ml solution of dithiobis (2-nitrobenzoate) and 0.4 mg/ml 1% sodium citrate was added. The absorbance at 412 nm was measured immediately after mixing. The GSH levels were calculated by using an extinction coefficient of 13,600 mol/cm.

Statistical analysis

All statistical procedures were performed by using the SPSS (SPSS Inc., Chicago, IL, U.S.A.) statistical software package. Statistical analyses were carried out by one-way analysis of variance (ANOVA), followed by a post hoc Tamhane test to correct for multiple comparisons of treatments. The latency of epileptiform activity and the level of MDA and GSH were compared by a post hoc Bonferroni test. Data are expressed as the mean ± SEM. Statistical significance was set at $p < 0.05$.

RESULTS

Effects of ascorbic acid on epileptiform ECoG activity

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 2–5 min after penicillin application and lasted for 3–5 h. It reached a constant level as to frequency and amplitude in 30 min. The mean of spike frequency and amplitude were 28 ± 3 spike/min, 1,018 ± 181 μV, respectively. Ascorbic acid was administered 30 min after penicillin injection. The low dose of ascorbic acid (25 mg/kg) did not significantly change the frequency (21.4 ± 2.1 spike/min) of epileptiform activity (Fig. 1C, Fig. 2). Ascorbic acid, at the doses of 50, 100, 200, 400, mg/kg, significantly decreased the mean frequency of epileptiform activity to 13.6 ± 2.3, 11.1 ± 1.7, 15.7 ± 2.1, and 19.8 ± 2.9 spike/min in the 60 min after ascorbic acid injection (IP), respectively, and lasted for 2 h (Fig. 1D, E, F, G; Fig. 2). Ascorbic acid, in a dose of 800 mg/kg, did not significantly change the frequency (23.6 ± 3.4 spike/min) of epileptiform activity (Fig. 1H, Fig. 2). The best and earlier effects appeared in the group given ascorbic acid (100 mg/kg) (Fig. 2 and Fig. 3). The frequency and amplitude of epileptiform activity were 12.2 ± 1.4 spike/min, 411 ± 103 μV, and 11.8 ± 2.1 spike/min, 424 ± 141 μV after 80 min from ascorbic acid injection, in ascorbic acid (100 mg/kg, IP) supplemented groups (30 min and 7 days before penicillin injection), respectively (Fig. 1J and K). Pretreatment with ascorbic acid (100 mg/kg, IP) 30 min before penicillin treatment significantly increased the latency of epileptiform activity from 156.22 ± 12 s to 325.82 ± 82 s compared with penicillin-injected groups ($p < 0.01$) (Fig. 4). The latency of epileptiform activity did not significantly change in the group that received a daily dose of 100 mg/kg ascorbic acid (IP) for 7 days (Fig. 4). Ascorbic acid, at doses of 50 and 100 mg/kg, significantly decreased the mean amplitude of epileptiform activity to 419 ± 141, 400 ± 123 μV in 90 min after ascorbic acid injection (IP), respectively, and lasted for 90 min (Fig. 1D and E, Fig. 3). The significant effects appeared in the 60 min after ascorbic acid (50 and 100 mg/kg) injection and lasted for 90 min (Fig. 3). Ascorbic acid, at doses of 25, 200, 400, and 800 mg/kg, did not significantly change the mean amplitude of epileptiform activity during the experiments (Fig. 3). Intraperitoneal injection of ascorbic acid, in a dose of 100 mg/kg, did not cause any change in the frequency or amplitude of ECoG activity with respect to the control baseline in non–penicillin-injected animals (Fig. 1I). No change was found in the mean of frequency and amplitude in aCSF-injected animals (Fig. 1L).

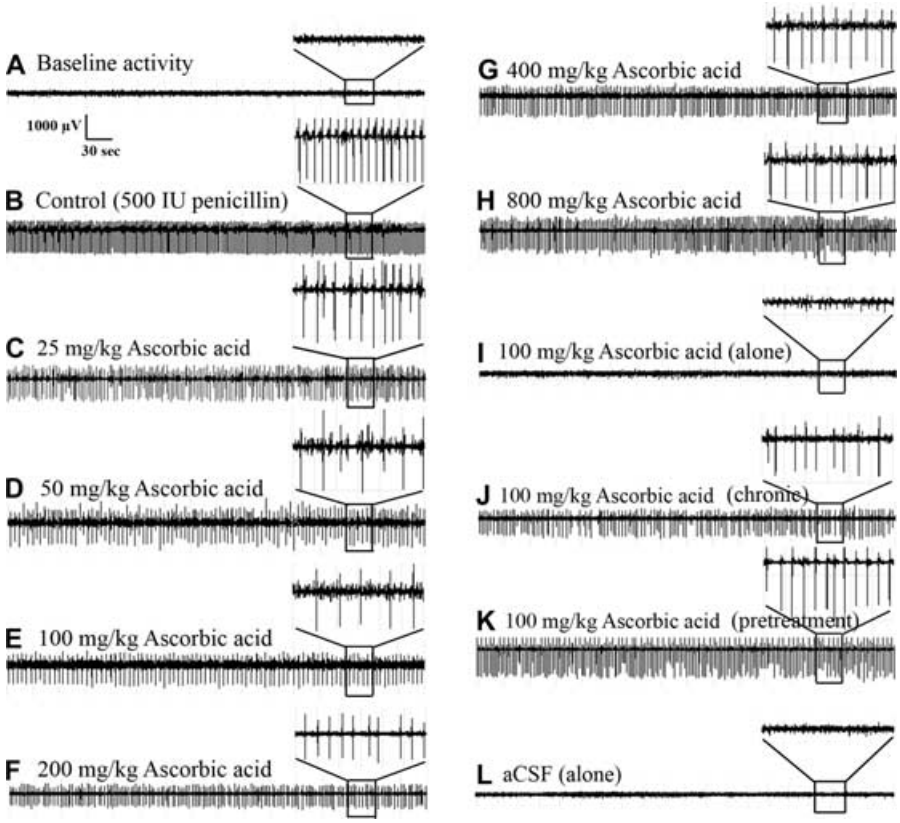


FIG. 1. A: Baseline ECoG activity before penicillin or the injection of other substances. **B:** Intracortical injection of penicillin (500 IU) induced epileptiform activity on ECoG. **C:** Ascorbic acid, in a dose of 25 mg/kg, did not change either the frequency or amplitude of penicillin-induced epileptiform activity. **D:** Ascorbic acid, in a dose of 50 mg/kg, decreased both the frequency and amplitude of penicillin-induced epileptiform activity. **E:** Ascorbic acid, in a dose of 100 mg/kg, decreased both the frequency and amplitude of penicillin-induced epileptiform activity. Ascorbic acid, in a dose of 100 mg/kg, was the most effective dose in changing the frequency and amplitude of epileptiform activity. **F:** Ascorbic acid, in a dose of 200 mg/kg, decreased the frequency of penicillin-induced epileptiform activity without changing amplitude. **G:** Ascorbic acid, in a dose of 400 mg/kg, decreased the frequency of penicillin-induced epileptiform activity without changing the amplitude. **H:** Ascorbic acid, in a dose of 800 mg/kg, did not change either the frequency or amplitude of penicillin-induced epileptiform activity. **I:** Representative ECoGs are presented 80 min after 100-mg/kg ascorbic acid administration. **J:** The frequency and amplitudes of epileptiform activity were decreased in ascorbic acid (100 mg/kg, IP) supplemented (for 7 days before penicillin injection) group. **K:** The frequency and amplitude of epileptiform activity were decreased in ascorbic acid (100 mg/kg, IP) pretreated (30 min before penicillin injection) group. **L:** Representative ECoGs are presented for the 80 min after aCSF administration.

Epileptiform activity-induced alteration in the MDA and GSH levels in rat brain: effects of ascorbic acid

The level of lipid peroxidation (MDA, in nmol/g) was found to be 25.42 ± 3.2, 68.90 ± 0.8, 21.38 ± 0.6, and 22.40 ± 0.2 in the controls, penicillin (500 units), control + ascorbic acid (100 mg/kg, IP), and penicillin + ascorbic acid (100 mg/kg, IP) groups, respectively (Figs. 4 and 5). The increase of lipid peroxidation was almost 171% in the penicillin-injected group compared with controls. Administration of ascorbic acid (100 mg/kg, IP) reversed the increased level of lipid peroxidation in the penicillin + ascorbic acid group. No significant difference was found between non–penicillin-injected groups and the penicillin + ascorbic acid–injected group (Figs. 4 and 5).

The level of GSH (in μmol/g) was found to be 7.73 ± 0.07, 1.10 ± 0.21, 7.51 ± 0.12, and 6.81 ± 0.6 in the control, penicillin (500 units), control + ascorbic acid (100 mg/kg, IP), and penicillin + ascorbic acid (100 mg/kg, IP) groups, respectively. Penicillin-induced epileptiform activity produced a 602% decrease in the brain GSH level of the penicillin-injected group compared with controls (Figs. 4 and 5). Administration of ascorbic acid (100 mg/kg, IP) prevented the decrease in GSH level in the penicillin + ascorbic acid group (Figs. 4 and 5). No significant difference was found between non–penicillin-injected groups and penicillin + ascorbic acid–injected group (Figs. 4 and 5).

DISCUSSION

Many previous studies demonstrated that the abnormal metabolism of trace elements, electrolytes, and antioxidants might be involved in the pathophysiology of severe mental and neurologic disorders including epilepsy (Hamed and Abdullah, 2004; Hamed et al., 2004). Epileptic seizures have been studied in genetic,

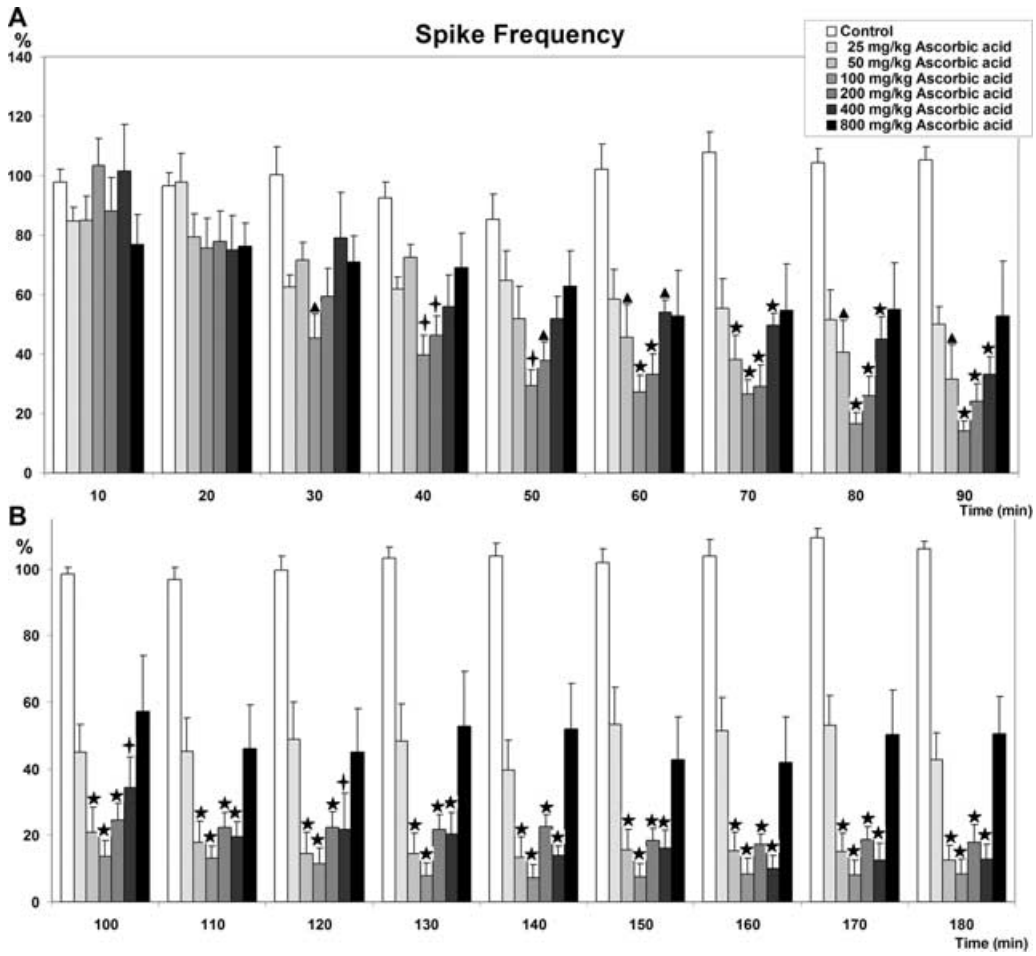


FIG. 2. A, B: The effects of ascorbic acid on the mean spike frequency of penicillin-induced epileptiform activity. Ascorbic acid, at doses of 50, 100, 200, and 400 mg/kg, significantly decreased the mean frequency of epileptiform activity. The best and earlier effects appeared in the 100-mg/kg ascorbic acid (IP)–administered group. Ascorbic acid, at doses of 25 and 800 mg/kg, did not change the mean frequency of epileptiform activity. ▲$p < 0.05$; ✚$p < 0.01$; ★$p < 0.001$.

electrophysiologic, and chemical models (MacNamara et al., 1993; Yamamoto et al., 2002; Oliveira et al., 2004; Ayyildiz et al., 2006b). A variety of animal seizure models help to document the effects of ascorbic acid and specify its action (Tolbert et al., 1979; Pierce et al., 1995; Yamamoto et al., 2002; Oliveira et al., 2004). In the present study, we provide further evidence for the effects of ascorbic acid, at the doses of 25, 50, 100, 200, 400, and 800 mg/kg (IP), on epileptiform activity in the penicillin-induced epileptiform activity in rats.

Ascorbic acid is probably the most important water-soluble antioxidant in the brain extracellular fluid, in addition to its cooperative antioxidant role in regenerating reduced α-tocopherol in membranes (Niki, 1991). Although ascorbic acid has an important antioxidant role to counter oxidative stress, ascorbic acid will also form reactive oxidants, especially in the presence of transition metals, and evidence suggests that ascorbic acid participates in pro-oxidant reactions under certain conditions (Sadrzadeh and Eaton, 1988; Prat and Turrens, 1990; Layton et al., 1998). Oliveira et al. (2004) reported that ascorbate, at a high dose (300 mg/kg), protected against PTZ-induced convulsions, further suggesting an anticonvulsant and neuroprotective role for this vitamin. They also indicated that ascorbate, at an intermediate dose (100 mg/kg), potentiated the duration of the convulsive episodes (Oliveira et al., 2004). The results of present study confirm that ascorbic acid, at doses of 200 and 400 mg/kg, decreased the frequency of penicillin-induced epileptiform activity in the rats. Furthermore, ascorbic acid, at doses of 50 and 100 mg/kg, also decreased both the frequency and amplitude of epileptiform activity. Ascorbic acid, in a dose of 100 mg/kg, was the most effective dose in decreasing the frequency and amplitude of epileptiform activity in the present study. In addition, the frequency of epileptiform activities was decreased, and the occurrence of epileptiform activities was delayed in the ascorbic acid–pretreated

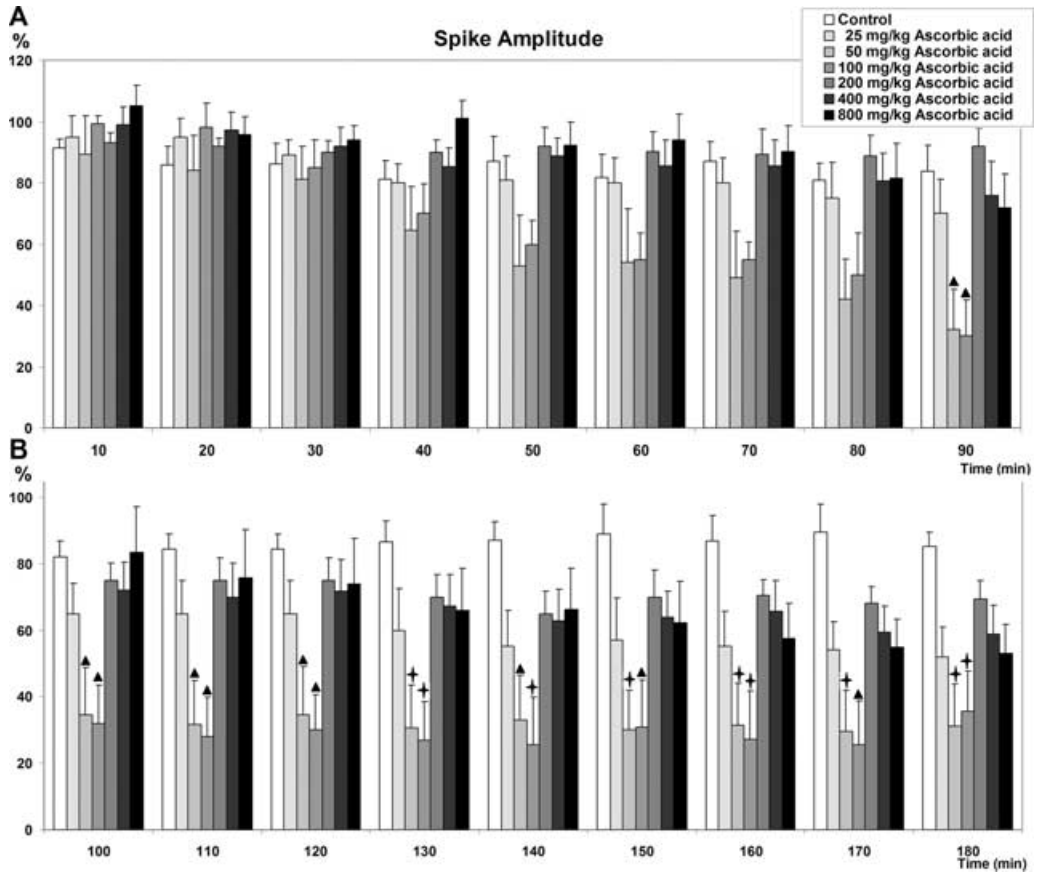


FIG. 3. A, B: The effects of ascorbic acid on the mean spike amplitude of penicillin-induced epileptiform activity. Ascorbic acid, at doses of 50 and 100 mg/kg, significantly decreased the mean amplitude of epileptiform activity. The significant effect appeared in the 90 min after ascorbic acid (50, 100 mg/kg, IP) injection. Ascorbic acid, at doses of 25, 200, 400, and 800 mg/kg, did not change the mean amplitude of epileptiform activity.▲$p < 0.05$;✚$p < 0.01$.

group (100 mg/kg, IP, 30 min before penicillin treatment), which confirms the results of Yamamoto et al. (2002), who showed that with the injection of synthetic derivative ascorbate intraperitoneally, in a dose of 200 mg/kg, 60 min before the injection of $FeCl_3$, the occurrence of epileptic discharges was prevented or delayed in a rat model of post-traumatic epilepsy. They also reported that the percentage occurrence of epileptiform activity was significantly lower in the ascorbate synthetic derivative (80 mg/kg/day for 7 days) + ferric ion–pretreated rats compared with the ferric ion–pretreated group (Yamamoto et al., 1992). Ascorbate was found significantly effective at doses of ≥30 mg/kg, with total protection against kainate at 50 mg/kg (MacGregor et al., 1996). However, ascorbic acid, in a dose of 25 mg/kg, did not change either the frequency or amplitude of epileptiform activity in the present study, which is consistent with the results of the Oliveira et al. (2004) study, concerning the effect of ascorbate, at a low dose (30 mg/kg), on PTZ-induced convulsions.

Ascorbate, at a high dose (500 mg/kg), produced either no effect or an opposing effect on these behaviors and constitutes further evidence for a biphasic effect of ascorbate on central nervous system functions (Tolbert et al., 1979; Pierce et al., 1995). Wang et al. (1992) provided evidence to support the involvement of free radical intermediates in iron–ascorbic acid reactions. Their results confirm the suggested mechanisms of both the adverse and protective effects of ascorbic acid in biologic systems (Wang et al., 1992). Moreover, it was reported that ascorbate acts as prooxidant in the presence of iron and shows excellent scavenging properties in the absence of iron (Fischer-Neilsen et al., 1992). They also suggested that no further benefit could be achieved by increasing the intake of ascorbate, while the prooxidant action will increase, and the net effect is to shift to the deleterious side (Fischer-Neilsen et al., 1992). The results of the present study confirm that ascorbic acid, at a high dose (800 mg/kg), did not significantly change either the frequency or amplitude of penicillin-induced epileptic activity in the rat. Conversely, α-tocopherol, at a high dose (500 mg/kg), another active oxygen free radical scavenger, was used to provide a maximal anticonvulsant effect in the penicillin-induced epileptiform activity in our previous study (Ayyildiz et al., 2006b).

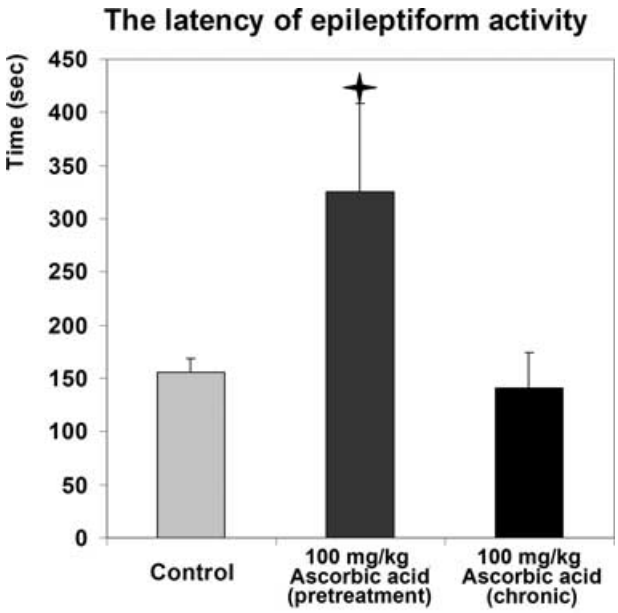


FIG. 4. The effects of ascorbic acid on the latency of penicillin-induced epileptiform activity. Pretreatment with ascorbic acid (100 mg/kg, IP) 30 min before penicillin treatment significantly increased the latency of epileptiform activity compared with penicillin-injected groups. Pretreatment with ascorbic acid (100 mg/kg, IP) for 7 days before penicillin treatment did not change the latency of epileptiform activity compared with penicillin-injected groups.✚$p < 0.01$.

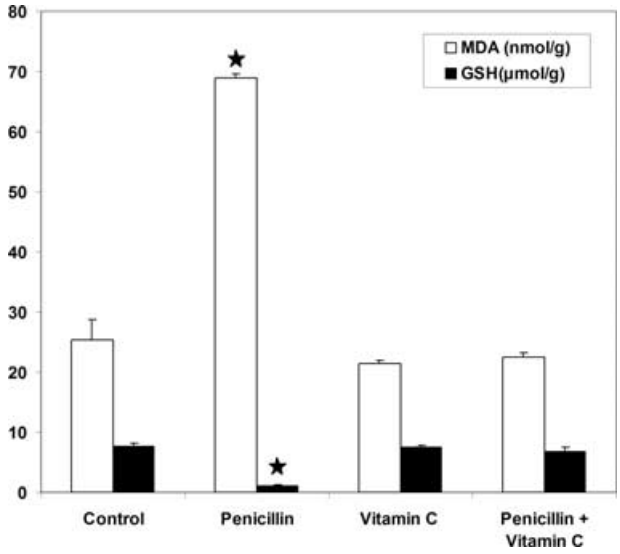


FIG. 5. The effect of ascorbic acid, in a dose of 100 mg/kg, on the level of lipid peroxidation (MDA, nmol/g) and reduced glutathione (GSH, μmol/g) in the rat brain after penicillin-induced epileptiform activity. Penicillin-induced epileptiform activity caused an increase of 171% in lipid peroxidation and a decrease of 602% in GSH level of the brain compared with controls. Administration of ascorbic acid reversed the increased lipid peroxidation and decreased GSH level in the brain of rat.★$p < 0.001$.

The results of the present study show that GSH was reduced, whereas lipid peroxidation was increased after penicillin-induced epileptiform activity. Increased lipid peroxidation of brain tissue has been reported in ferric chloride and kainate-induced epilepsy in rats (Singh and Pathak, 1990; Bruce and Baudry, 1995). Frantseva et al. (2000) also presented evidence that LPO increases during seizures in the kindling model of epilepsy, suggesting that the free radical increase during seizures occurs independent of iron salts or excitotoxins. Conversely, previous studies using various methods reported low GSH levels in the brain in different models of epilepsy (Abe et al., 2000; Freitas et al., 2005). The most effective dose of ascorbic acid (100 mg/kg) prevented both the decrease in GSH level and the increase in level of lipid peroxidation (MDA) in the penicillin-administered rats in this study, confirming the result of the Shin et al. (2005) study, which reported that treatment with ascorbate, at doses of 50 and 100 mg/kg, significantly attenuated trimethyltin (TMT)-induced seizures as well as the initial oxidative stress, impaired glutathione homeostasis, and neuronal degeneration in a dose-dependent manner in rats (Shin et al., 2005). However, Oliveira et al. (2004) reported that the effects of ascorbate are complex, and other mechanisms, unrelated to its reactive species scavenger ability, are claimed to explain the neuroprotective actions of this vitamin. Thus more research will be needed before a final conclusion about the possible mechanisms of ascorbic acid effects on penicillin-induced epileptiform ECoG activity.

Another important point to discuss is the interaction between ascorbic acid and potassium penicillin G used in this study. A study by Pfeifer and Webb (1976) documents an incompatibility between penicillin and ascorbic acid due to the function of pH. It was noted that penicillin can degrade rapidly in an acid environment (Pfeifer and Webb, 1976). However, the presence of the ascorbate ion did not alter the stability of potassium penicillin G in the two different solutions with 5% dextrose over an 8-h period (Pfeifer and Webb, 1976). Penicillin (pH 7.6) and ascorbic acid (pH 7.0) were injected separately at different times in a different manner in the present study. Therefore we may conclude that ascorbic acid injection might not represent an incompatible admixture with penicillin in this study.

In conclusion, we demonstrated that ascorbic acid, at low (50, 100 mg/kg) and intermediate doses of (200, 400 mg/kg), significantly decreased the frequency of penicillin-induced epileptiform activity. Ascorbic acid, at low doses, also decreased the amplitude of epileptiform activity. Ascorbic acid, at doses of 25 and 800 mg/kg, was not effective in changing either the frequency or amplitude of epileptiform activity. Ascorbic acid, in a dose of 100 mg/kg, was the most effective in reducing the frequency and amplitude of epileptiform activity. An increase in the level of lipid peroxidation and a severe depletion in the GSH level were seen in the brain after penicillin-induced epileptiform activity in the rats. The treatment of rats with

ascorbic acid, in a dose of 100 mg/kg, resulted in an elevation in the GSH level with a decrease in lipid peroxidation in the brain. These data clearly show that ascorbic acid partially prevented the occurrence of penicillin-induced epileptic discharges, suggesting a neuroprotective role. However, the exact mechanisms of these effects of ascorbic acid are still a crucial issue.

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