Physiol. Res. 49: 379-385, 2000

Effects of L-arginine on Prevention and Treatment of Lithium-Pilocarpine-Induced Status Epilepticus

B. NOYAN, G. GÜLEÇ

Department of Physiology, Faculty of Medicine, Uludağ University, Bursa, Turkey

Received May 18, 1999
Accepted October 19, 1999

Summary

The effects of various doses of L-arginine, a nitric oxide substrate, on lithium-pilocarpine-induced seizures were studied in rats. Rats were implanted with chronic, stainless steel screw electrodes epidurally for electrocortical recordings. A control group received 3 mEq/kg LiCl (i.p.) and 24 h later 45 mg/kg pilocarpine HCl (i.p.). Two different experimental procedures were followed: (1) L-arginine was applied in doses of 100 mg/kg, 300 mg/kg or 500 mg/kg (i.p.), 30 min before pilocarpine injection; (2) 300 mg/kg, 500 mg/kg or 1000 mg/kg (i.p.) L-arginine was injected either 5 min or 30 min after the onset of status epilepticus (SE). L-arginine (300 mg/kg) injected 30 min before pilocarpine significantly reduced the percentage of SE, but did not change the latency to SE or 24-hour survival. These parameters were not significantly affected by the 100 mg/kg or 500 mg/kg dose of L-arginine. On the other hand, no dose of L-arginine that was applied after SE had begun, had any significant influence on the seizures. We concluded that L-arginine may prevent seizure activity in some but not all doses, and does not have any effect on the ongoing seizure activity.

Key words
Nitric oxide • L-arginine • Status epilepticus • Rat

Introduction

Temporal lobe epilepsy (TLE) is a common neurological disorder and owing to its drug-resistant nature, a large number of studies have been focused on the pathophysiological mechanisms underlying this disease. Several experimental models that mimic the drug-resistant TLE (Ben-Ari 1985, Sutula *et al.* 1989, Przewlocka *et al.* 1994) have been suggested. Of these, high-dose pilocarpine and lithium-pilocarpine models deserve attention concerning the similarity to human TLE from the view of neuropathological damage (Honchar *et al.* 1983, Turski *et al.* 1983, 1986, 1989, Mello *et al.* 1993, Cavalheiro *et al.* 1996), continuity of spontaneous recurrent seizures (Cavalheiro *et al.* 1991, 1996, Mello *et al.* 1993), and resistance to various anticonvulsants (Morrisett *et al.* 1987, Turski *et al.* 1987). In addition, the wide margin of safety between seizures and mortality is an advantage of this model (Turski *et al.* 1984, 1989).

Nitric oxide (NO) is a highly reactive and unstable free radical, which diffuses easily through the cell membrane (Garthwaite 1991, Dawson and Synder 1994, Boda and Szente 1996). It contributes to intercellular signal transduction in many tissues. In the central nervous system (CNS), it acts as a neuronal retrograde messenger (Moncada *et al.* 1989, Garthwaite 1991, De Sarro *et al.* 1993). NO is an endogenous activator of soluble guanylate cyclase, which synthetizes

Fax+4202 24920590
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

cGMP (Garthwaite *et al.* 1988, 1989, Moncada *et al.* 1991). It activates guanylate cyclase by binding to the iron of the heme, which is located at the active site of the enzyme and by changing its conformation (Bredt and Snyder 1994).

In the CNS, NO is formed from L-arginine, by calcium/calmodulin-dependent constitutive NO synthase, which is mainly activated by the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (Moncada *et al.* 1989, Moncada *et al.* 1991, Dawson and Snyder 1994, Boda and Szente 1996). In living tissues, extracellular calcium is essential for the secretion of NO from NMDA-stimulated neurons (Garthwaite *et al.* 1988). NMDA receptor activation causes an influx of a large amount of calcium into the cell through receptor-associated ion channels; calcium binds to calmodulin and activates NO synthase (Mayer and Miller 1990, Akaike *et al.* 1994).

The role of NO in epileptic seizures has been investigated in several experimental models, but the results are contradictory. In this study, the role of a NO substrate, L-arginine, in the pathogenesis or treatment of seizures was investigated in the lithium-pilocarpine model of TLE.

Methods

Male Wistar albino rats (Uludağ University, Experimental Animals Breeding and Research Center), weighing 240-340 g were kept in a temperature-controlled room (18-22 °C) and had free access to food and water.

Rats were anesthetized with 40 mg/kg (i.v.) Thiopental Sodium (Abott) and implanted chronically with four stainless steel screw electrodes (0.8 mm in diameter) epidurally to record the electrocorticogram (ECoG). The electrodes were implanted bilaterally over fronto-parietal cortices (±2 mm behind the bregma and ±2 mm lateral to the midline) and an additional electrode on the nasal bone served as the reference electrode. All electrodes were fixed to the skull with dental cement. After surgery, rats were placed in separate cages and were allowed to recover for seven days before the experiments.

Two sets of experiments were performed in order to observe the effects of various doses of L-arginine applied before or after status epilepticus (SE) initiation.

Experiment 1: Control group of rats (n=15) that were pretreated with 3 mEq/kg LiCl (i.p.) received 45 mg/kg (i.p.) pilocarpine HCl 24 h later. These rats were given vehicle 30 min before pilocarpine. L-arginine was injected in three different doses (100 mg/kg, 300 mg/kg or 500 mg/kg, i.p., 15 rats in each group) 30 min before the pilocarpine injection.

Experiment 2: SE was induced by systemic administration of pilocarpine (45 mg/kg, i.p.) to lithium-pretreated rats (3 mEq/kg, i.p., 24 h before pilocarpine). Those rats that had displayed SE received 300 mg/kg, 500 mg/kg or 1000 mg/kg (i.p.) L-arginine (15 rats in each group), 5 min or 30 min following SE initiation. Control rats in this experiment received the vehicle either 5 or 30 min after SE.

All drugs were purchased from Sigma Chemical Co. (St Louis.), LiCl was dissolved in distilled water and pilocarpine and L-arginine were dissolved in saline in a volume of 0.2 ml.

In both groups, ECoG recordings were obtained before and after the administration of each drug and the behavioral effects of these drugs were also observed. The behavioral and electrocortical changes were detected for 3 h after pilocarpine injection. The effects of various doses of L-arginine on lithium-pilocarpine-induced seizures were assessed on the basis of the SE incidence percentage, latency to SE and 24-hour survival. The effects of L-arginine on the ongoing seizures were determined according to the behavioral or electrocortical changes observed due to drug administration. In addition, the 24-hour survival was compared to the control group.

Statistical analysis of the results were performed using Fischer's Exact Test for comparing the percentage of SE and the 24-hour survival, and analysis of variance (ANOVA) to compare the latency to SE between the groups.

Results

In the control group, LiCl did not produce any behavioral or electrocortical changes. After pilocarpine injection, in addition to peripheral cholinergic stimulation signs such as piloerection, salivation, and diuresis, tremor and limbic automatism such as scratching and chewing were observed. Subsequently, some rats exhibited head nodding, rearing and forelimb clonus. Twelve rats (80 %) entered SE with an average latency of 30.41±2.07 min, which was characterized both behaviorally and electrocortically. SE continued unabated for hours. The average 24-hour survival in this group was 86.66 % (Table 1).

Experiment 1: In the groups receiving L-arginine, no dose of L-arginine caused any behavioral and electrocortical changes before pilocarpine injection (Fig. 1). The effects of 100 mg/kg, 300 mg/kg and 500 mg/kg L-arginine on the observed parameters were compared with the control group in Table 1. L-arginine (300 mg/kg) significantly decreased the percentage of SE with respect to the control group ($p<0.05$). On the other hand, it had no significant effect on the latency of SE and 24-hour survival. 100 mg/kg and 500 mg/kg doses of L-arginine did not produce any significant effects in any of the above mentioned parameters.

Table 1. Comparison of the percentage of SE, latency to SE and 24-hour survival of the rats that received L-arginine 30 min before pilocarpine with the control group

	SE (%)	Latency to SE (min) ± S.E.M	24-h survival (%)
Control group	80,00	30.41±2.07	86.66
L-arginine 100 mg/kg	86.66	36.16±6.97	100
L-arginine 300 mg/kg	33.33*	30.40±1.63	100
L-arginine 500 mg/kg	73.33	32.20±4.69	100

$p<0.05$ with respect to the control group.

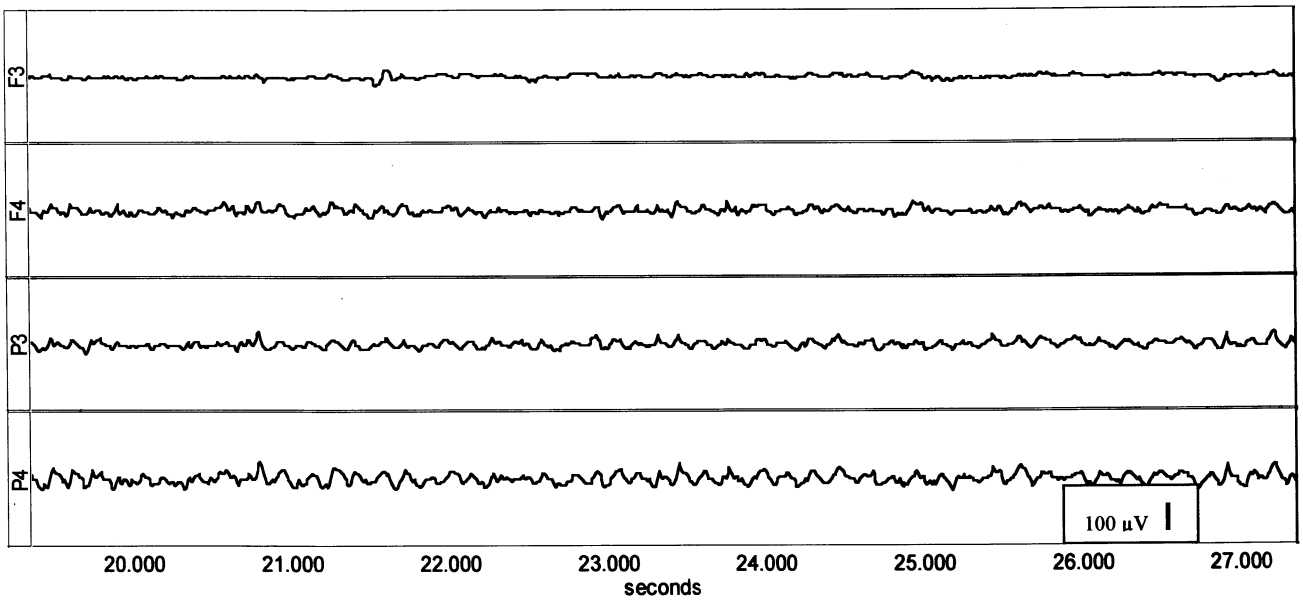

Fig. 1. *ECoG recording of a rat that had received L-arginine (300 mg/kg, i.p.) 10 min before. There is no abnormal activity before pilocarpine was injected.*

Experiment 2: When L-arginine was applied either 5 or 30 min after SE initiation, the electrocortical recordings revealed no marked changes in the seizure activity in any of the dose groups (Figs. 2 and 3). On the other hand, although slightly, the intensity of the generalized convulsions in the former group seemed to decrease regardless of the dose of L-arginine, while in the latter group no obvious behavioral changes were detected for any dose of the drug. The 24-hour survival in all groups also did not differ from those of the vehicle-treated rats ($p>0.05$) (Table 2).

Table 2. Comparison of the 24-hour survival rates of the rats that received L-arginine, 5 min or 30 min after the initiation of SE, with the vehicle-treated rats that were injected at the same time

	Injection time	24-hour survival (%)
Vehicle-treated group	Fifth min.	75.00
	Thirtieth min	85.71
L-arginine 300 mg/kg	Fifth min.	62.50
	Thirtieth min	71.42
L-arginine 500 mg/kg	Fifth min.	87.50
	Thirtieth min	100
L-arginine 1000 mg/kg	Fifth min.	75.00
	Thirtieth min	71.42

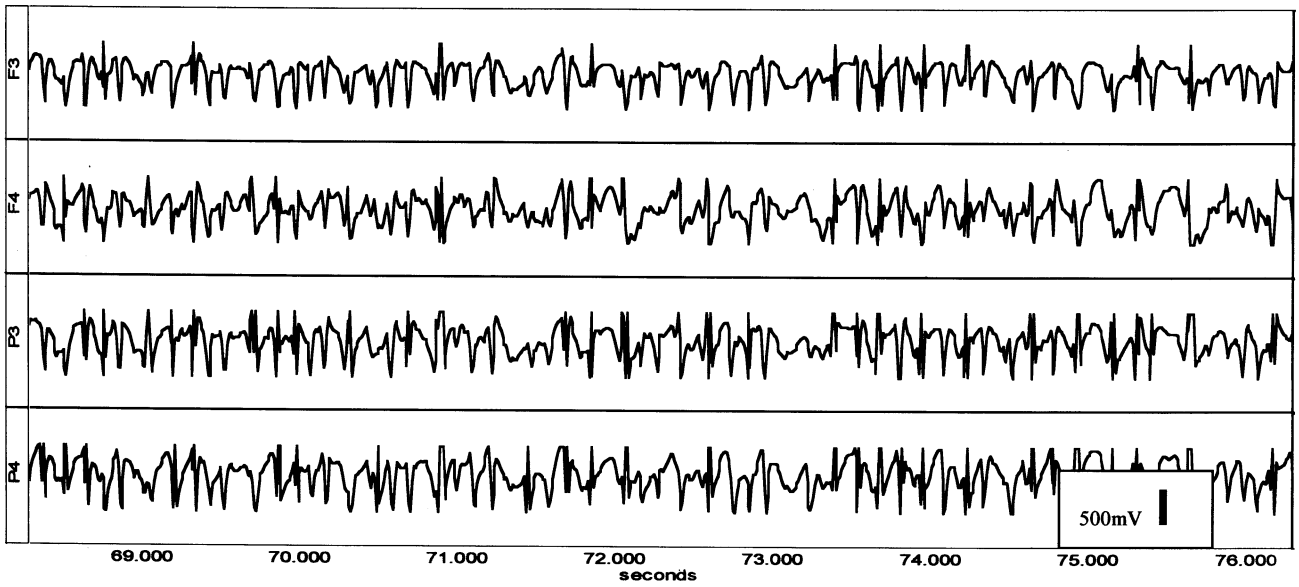

Fig. 2. *ECoG tracings recorded 20 min after onset of SE, induced by administration of 45 mg/kg (i.p.) pilocarpine to lithium-pretreated rats (3 mEq/kg, i.p. 24 hours before pilocarpine). Note the continuous, high amplitude rapid spiking that corresponds to generalized tonic-clonic seizures.*

Discussion

The results of the studies investigating the role of NO in the pathogenesis of epilepsy are quite contradictory. In one study, the effect of L-arginine on the seizures induced by microinjection of NMDA and kainic acid (KA) into the deep prepyriform cortex (DPC) was investigated (De Sarro *et al.* 1993). The authors reported that a microinjection of 5-10 nmol L-arginine into the DPC 10 min before NMDA or KA significantly potentiated the behavioral and electrocortical seizures in a dose-dependent manner. In the same study, it was shown

that a microinjection of sodium nitroprusside (SNP), which spontaneously released NO into the DPC, produced behavioral and electrographic signs of epileptic seizures. On the other hand, administration of methylene blue, a soluble guanylate cyclase inhibitor, 15 min before SNP significantly prevented seizures while 2.5 nmol SNP facilitated NMDA- or KA-induced seizures. These authors suggested that NO is synthesized from L-arginine

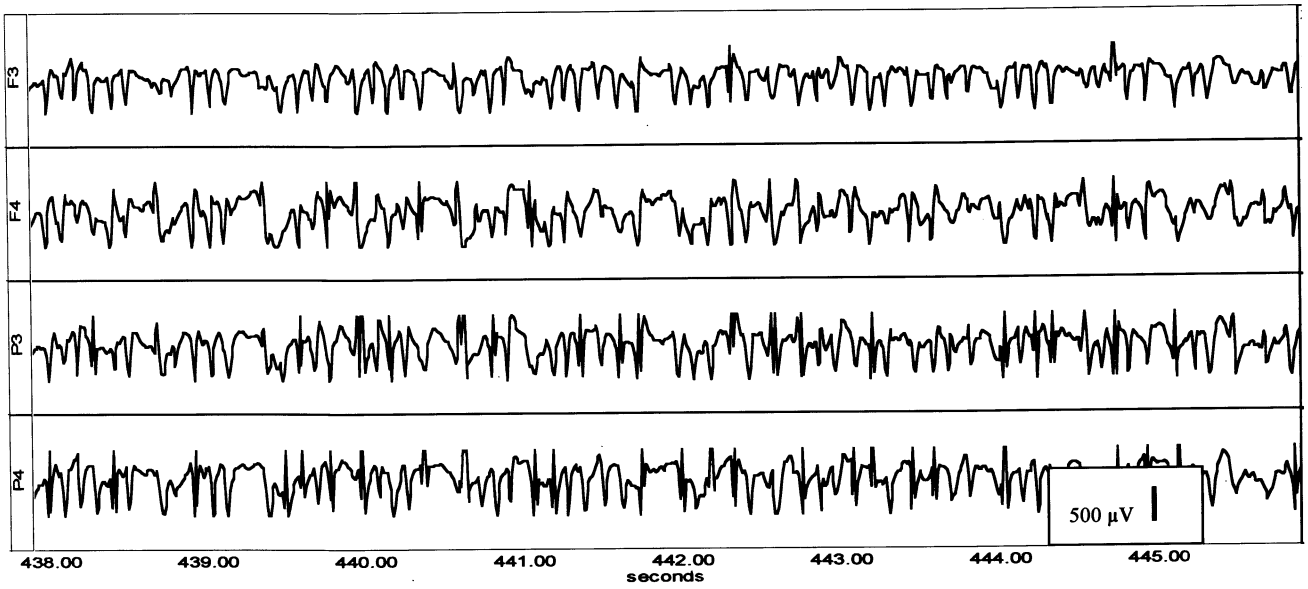

Fig. 3. *ECoG of the same rat as in Fig. 2, 10 min after administration of 500 mg/kg L-arginine on the 30th minute of SE. There is no marked change in the frequency or amplitude of the spikes.*

in the DPC due to excitatory amino acid receptor activation and that NO plays a role in the development of seizure activity.

In another study, it was shown that pretreatment with 300 µg i.c.v. L-arginine before a subconvulsive dose of i.c.v. NMDA induced behavioral and electrocortical seizures and that these seizures were prevented by L-NAME (Mollace *et al.* 1991). It was concluded that L-arginine exerted a proconvulsant activity by increasing NO synthesis. On the contrary, in mice with KA-induced seizures, 150-600 mg/kg L-arginine (i.p.) dose-dependently increased the dose of KA needed to induce clonic convulsions in 50 % of the mice (Przegalinski *et al.* 1994). Other investigators showed that sodium nitroprusside significantly prevents the penicillin-induced seizures (Marangoz *et al.* 1994) and that this effect is blocked by methylene blue and by a NO scavenger, hemoglobin. The authors have concluded that NO may be an endogenous anticonvulsant agent.

The ineffective treatment of status epilepticus with the conventional drugs poses a major problem and attempts to use several novel agents for this purpose have appeared, although hitherto only experimentally. One of the major points seems to depend on the stage of SE, since it has been pointed out that, as the SE progresses, the therapeutic effect of diazepam decreases (Walton and Treiman 1988). The authors concluded "the longer the duration of SE, the more difficult it is to control" and suggested that the EEG pattern at the time of treatment was important for the success of the therapy. In another study, it was shown that conventional anticonvulsants such as diazepam, phenytoin, carbamazepine, phenobarbital and sodium valproate, do not affect seizure activity in lithium-pilocarpine model of epilepsy when administered 60 min after pilocarpine, approximately 35 min after initiation of SE (Morrisett *et al.* 1987).

A noncompetitive NMDA antagonist MK-801 decreased the electrical and behavioral seizure activity gradually and enhanced the survival rate, when applied after 10 or 40 min of SE (Ormandy *et al.* 1989). To our knowledge, the ability of L-arginine to terminate ongoing SE has not yet been tested. Our results have revealed that administration of systemic L-arginine in doses of 300-1000 mg/kg does not affect the electrical or behavioral seizure activity induced by lithium-pilocarpine, regardless of the SE stage. Only a minor decrease in behavioral seizures was observed in rats that had received each dose of the drug 5 min after the initiation of SE, but the electrocortical findings did not change and the survival rate was not improved at all.

In this study, 300 mg/kg L-arginine significantly reduced the percentage of SE, but did not affect the

latency to SE and 24-hour survival. On the other hand, 100 mg/kg and 500 mg/kg L-arginine had no significant effect on any of these parameters. Furthermore, our attempt to terminate the ongoing SE by using different doses of L-arginine was unsuccessful. As a result, we conclude that L-arginine may prevent seizure activity in definite doses, but does not affect seizures once SE has developed. This conflicting evidence indicates the fact that more information is needed about the consequences of SE and the role of NO at each SE stage.

Acknowledgements

Preliminary communications (Gulec et al. 1999, Noyan et al. 1999) were presented at the 2nd FEPS Congress, Prague, June 30 – July 4, 1999.

References

AKAIKE A, TAMURA Y, TERADA K, NAKATA N: Regulation by neuroprotective factors of NMDA receptor mediated nitric oxide synthesis in the brain and retina. *Prog Brain Res* **103**: 391-403, 1994.

BEN-ARI Y: Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14**: 375-403, 1985.

BODA B, SZENTE M: Nitric oxide synthase inhibitor facilitates focal seizures induced by aminopyridine in rat. *Neurosci Lett* **209**: 37-40, 1996.

BREDT DS, SNYDER SH: Nitric oxide: a physiologic messenger molecule. *Am Rev Biochem* **63**: 175-195, 1994.

CAVALHEIRO EA, LEITE JP, BORTOLOTTO ZA, TURSKI WA, IKONMIDOU C, TURSKI L: Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. *Epilepsia* **32**: 778-782, 1991.

CAVALHEIRO EA, SANTOS NF, PRIEL, MR: The pilocarpine model of epilepsy in mice. *Epilepsia* **37**: 1015-1019, 1996.

DAWSON TM, SNYDER SH: Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J Neurosci* **14**: 5147-5159, 1994.

DE SARRO G, DI PAOLA ED, DE SARRO A, VIDAL MJ: L-arginine potentiates excitatory amino acid-induced seizures elicited in the deep prepiriform cortex. *Eur J Pharmacol* **230**: 151-158, 1993.

GARTHWAITE J: Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci* **14**: 60-67, 1991.

GARTHWAITE J, CHARLES SL, CHESS-WILLIAMS R: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **336**: 385-388, 1988.

GARTHWAITE J, GARTHWAITE G, PALMER RMJ, MONCADA S: NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *Eur J Pharmacol* **172**: 413-416, 1989.

GÜLEÇ G, NOYAN B, ÖZLÜK K: The effect of various doses of L-arginineon lithium-pilocarpine-induced seizures in rat. *Physiol Res* **48** (Suppl 1): S76, 1999.

HONCHAR MP, OLNEY JW, SHERMAN WR: Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. *Science* **220**: 323-325, 1983.

MARANGOZ C, AYYILDIZ M, AĞAR E: Evidence that sodium nitroprusside possesses anticonvulsant effects mediated through nitric oxide. *Neuroreport* **5**: 2454-2456, 1994.

MAYER ML, MILLER RJ: Excitatory amino acid receptors, second messengers and regulation of intracellular Ca^{2+} in mammalian neurons. *Trends Pharmacol Sci* **11**: 254-260, 1990.

MELLO LEAM, CAVALHEIRO EA, TAN AM, KUPFER WR, PRETORIUS JK, BABB TL, FINCH DM: Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia* **34**: 985-995, 1993.

MOLLACE V, BAGETTA G, NISTICO G: Evidence that L-arginine possesses proconvulsant effects mediated through nitric oxide. *Neuroreport* **2**: 269-272, 1991.

MONCADA S, PALMER RMJ, HIGGS EA: Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem Pharmacol* **38**: 1709-1715, 1989.

MONCADA S, PALMER RMJ, HIGGS EA: Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* **43**: 109-142, 1991.

MORRISETT RA, JOPE RS, SNEAD OC: Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. *Exp Neurol* **97**: 193-200, 1987.

NOYAN B, GÜLEÇ G, ÖZLÜK K: Vulnerability of two different strains of rats to lithium-pilocarpine-induced seizures. Phys Res 48 (Suppl 1): S102, 1999.

ORMANDY GC, JOPE RS, SNEAD OC: Anticonvulsant actions of MK-801 on the lithium-pilocarpine model of status epilepticus in rats. *Exp Neurol* **106**: 172-180, 1989.

PRZEGALINSKI E, BARAN L, SIWANOWICZ J: The role of nitric oxide in the kainate-induced seizures in mice. *Neurosci Lett* **170**: 74-76, 1994.

PRZEWLOCKA B, MACHELSKA AH, LASON W: Kappa opioid receptor agonists inhibit the pilocarpine-induced seizures and toxicity in the mouse. *Eur Neuropsychopharmacol* **4**: 527-533, 1994.

SUTULA T, CASCINO G, CAVAZOS J, PARADA I, RAMIREZ L: Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol* **26**: 321-330, 1989.

TURSKI WA, CZUCZWAR SJ, KLEINROK Z, TURSKI L: Cholinomimetics produce seizures and brain damage in rats. *Experientia* **39**: 1408-1411, 1983.

TURSKI WA, CAVALHEIRO EA, BORTOLOTTO ZR, MELLO LM, SCHWARTZ M, TURSKI L: Seizures produced by pilocarpine in mice: a behavioral, electroencephalographic and morphological analysis. *Brain Res* **321**: 237-253, 1984.

TURSKI L, CAVALHEIRO EA, SIEKLUCKA-DZIUBA M, IKONOMIDOU-TURSKI C, CZUCZWAR SJ, TURSKI WA: Seizures produced by pilocarpine: neuropathological sequelae and activity of glutamate decarboxylase in the rat forebrain. *Brain Res* **398**: 37-48, 1986.

TURSKI WA, CAVALHEIRO EA, COIMBRA C, BERZAGH MP, IKONOMIDOU-TURSKI C, TURSKI L: Only certain antiepileptic drugs prevent seizures induced by pilocarpine. *Brain Res Rev* **12**: 281-305, 1987.

TURSKI L, IKONOMIDOU C, TURSKI WA, BORTOLOTTO ZA, CAVALHEIRO EA: Cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. *Synapse* **3**: 154-171, 1989.

WALTON NY, TREIMAN DM: Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp Neurol* **101**: 267-275, 1988.

Reprint requests

Dr. Güldal Güleç, Uludağ University, Faculty of Medicine, Department of Physiology, 16384, Görükle, Bursa, Turkey, E-mail : guldal@uludag.edu.tr