Jpn. J. Pharmacol. **86**, 297 – 301 (2001)

Effects of L-Arginine on Penicillin-Induced Epileptiform Activity in Rats

Cafer Marangoz and Faruk Bağirici*

Department of Physiology, Faculty of Medicine, University of Ondokuz Mayis, 55139 Samsun, Turkey

Received December 19, 2000 Accepted April 9, 2001

ABSTRACT—It has been suggested that nitric oxide (NO) is involved in the pathophysiology of epilepsy. Data are, however controversial because it is not clear whether NO has pro- or anticonvulsant effects. The aim of this study was to investigate the effects of NO on penicillin G-induced epileptiform activity. The left cerebral cortex was exposed by craniotomy in urethane-anesthetized Wistar rats. The epileptic activity was produced by intraperitoneal injection of penicillin G (3 million U/kg, i.p.). The ECoG (electrocorticogram) activity was displayed on a four-channel recorder. At 39.7 ± 5.4 min after penicillin administration, large amplitude sharp waves appeared in the ECoG. Mean spike frequency and mean spike amplitude were calculated as 29.5 ± 3.2/min and 865 ± 91 μV, respectively, at the 55th min. 7-Nitroindazole (60 mg/kg, i.p.) injection 30 min before penicillin G administration significantly reduced the latency of epileptiform activity. Intracerebroventricular administration of L-arginine (300 μg/2 μl, i.c.v.) and sodium nitroprusside (100 μg /2 μl, i.c.v.) suppressed epileptiform activity. Saline (2 μl) and D-arginine (300 μg/2 μl, i.c.v.) administration into the cerebral ventricle were completely ineffective on epileptiform activity (P<0.01). These findings suggest that NO may be an endogenous antiepileptic substance.

Keywords: Nitric oxide, L-Arginine, Sodium nitroprusside, 7-Nitroindazole, Epileptiform activity, Penicillin

Nitric oxide (NO), which is thought to be a secondary messenger, a modulatory agent and a retrograde transmitter in the central nervous system (CNS), is a free radical gas that is not stored in vesicles and has no special protein receptor. NO is synthesized where it is needed, especially in postsynaptic cells from its precursor L-arginine by the action of the NO synthase (NOS) and exerts its physiological actions after reaching the target cells via rapid diffusion (1, 2). NO, a putative neurotransmitter in the CNS, enhances neurotransmitter release and has been implicated in the regulation at synaptic functions (3).

There are a number of different types of studies utilizing different experimental epilepsy models to reveal the association between NO and epilepsy. Although some studies suggest that NO may act as an endogenous proconvulsant substance (4 – 6), others reported that this small inorganic molecule may be anticonvulsant (7 – 9). To our knowledge, there is no study concerning the effects of NO donors L-arginine and nNOS (neuronal NOS) inhibitor 7-nitroindazole (7-NI) on the penicillin model of experimental epilepsy induced by penicillin administration among these studies.

Penicillin has structural resemblances to the GABA antagonist bicucculine (10). Due to abolished GABA inhibition after penicillin administration, excitatory effects of glutamate begins to dominate. Glutamate leads to an increase in intracellular calcium concentration through its actions on NMDA receptors. This increase in calcium then stimulates the NOS enzyme that catalyzes the formation of NO from L-arginine (11). Recently, it has been demonstrated that the L-type calcium channel blocker nicardipine suppressed epileptiform activity (12). It is reported that epileptiform spikes appeared 45 ± 3.7 min after systemic administration of 2.5 – 5 million U/kg penicillin G (13). In the present study, the effects of NO precursor L-arginine, NO donor sodium nitroprusside (SNP) and an nNOS inhibitor 7-NI on penicillin-induced experimental epilepsy were investigated via electrophysiological methods in Wistar rats.

MATERIALS AND METHODS

Experiments were performed on sixty adult male albino Wistar rats, weighing 200 – 230 g. Animals were divided into two main groups as peripherally and centrally treated groups. The peripherally treated group was divided into two subgroups as control (penicillin G, 3 million U/kg, i.p.) and 7-NI (60 mg/kg, i.p.) groups, while the centrally treated group was divided into four subgroups: saline

*Corresponding author. FAX: +90-362-457-6093
E-mail: fbagirici@yahoo.com

(2 μl, i.c.v.), D-arginine (300 μg/2 μl, i.c.v.), L-arginine (300 μg/2 μl, i.c.v.) and SNP (100 μg/2 μl, i.c.v.) groups (10 animals for each subgroups). Urethane (1.25 g/kg, i.p.) was used as anesthetic agent. The right femoral artery was tied off and used to monitor blood pressure in order to assess the general conditions of the animals. The right femoral vein was cannulated. When the blood pressure decreased, rheomacrodex was given by drop infusion. The left cerebral cortex was exposed by craniotomy. Four different corners of the scalp were each stitched by surgical threads and stretched in order to form a liquid vaseline pool (37°C). The head of the animal was immobilized in the stereotaxic head holder (Harvard Instruments, South Natick, MA, USA). Body temperature was maintained between 36.5°C and 37.5°C with a heating pad (Harvard Homoeothermic Blanket). Ag-AgCl ball electrodes were placed over the somatomotor cortex; the common reference electrode being fixed on the pinna and the ECoG activity was displayed on a four-channel recorder (Model 79 F Polygraph; Grass Inst., Quincy, MA, USA).

The epileptic activity was produced by injection of penicillin G (3 million U/kg, i.p.). Control recordings were obtained before and after penicillin G injection in the first group. Animals in the second group were injected with 7-NI (60 mg/kg, i.p.) 30 min before penicillin G (3 million U /kg, i.p.) administration and the latency of spikes was also determined in this group. Animals in the other four groups first received penicillin G (3 million U/kg, i.p.) and after the complete formation of epileptiform activity, the third, fourth, fifth and sixth groups received saline (2 μl), D-arginine (300 μg/2 μl, i.c.v.), L-arginine (300 μg/2 μl, i.c.v.) and SNP (100 μg/2 μl, i.c.v.), respectively, into cerebral ventricles via a microinjector (Hamilton Co., Reno, NV, USA).

ECoG recordings lasted 4 – 5 h for each animal. Frequencies determined by hand according to the sweep speed of the polygraph paper and the amplitude values measured via a digital compass and expressed as the mean ± S.D. Statistical analyses were done by using SPSS (Statistical Package for Social Sciences) version 10.0. Analyses of variance and Post hoc Tukey Honestly Significant Difference tests were used to compare the latencies in the first and the second groups (peripherally treated groups). Student's paired *t*-test was used in comparing the frequency and amplitude before and after the injection time of substance in centrally treated groups.

Urethane, D-arginine, L-arginine and SNP were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and they were dissolved in water. 7-NI was also obtained from Sigma, and dissolved in peanut oil. All of the solutions were prepared in coloured bottles and used immediately. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

RESULTS

Control bioelectrical brain activities were recorded before the administration of drugs, and it has been confirmed that no animal has spontaneous epilepsy (Fig. 1A). Intraperitoneally penicillin G (3 million U/kg, i.p.) administration induced epileptiform activity characterized by bilateral spikes 39.7 ± 5.4 min after injection (Fig. 1B). ECoG activity reached its maximum level 53.6 ± 6.2 min after penicillin administration and lasted for 4 – 5 h. Mean spike frequency and mean spike amplitude were calculated as 29.5 ± 3.2/min and 865 ± 91 μV, respectively, at 55 min after penicillin administration (Fig. 1C).

Peripheral administration of 7-NI

Rats in the second group first received 7-NI (60 mg/kg, i.p.) 30 min before penicillin G (3 million U/kg, i.p.) administration. Spike-wave activity appeared at 13.6 ± 4.3 min (Fig. 2B) and reached its maximum levels at 24.6 ± 5.2 min following the penicillin injection. Mean spike frequency and mean spike amplitude were calculated as 31.2 ± 3.7/min and 795 ± 91 μV, respectively. 7-NI significantly shortened the latency of the onset of epileptiform activity (P<0.01). L-Arginine (300 μg/2 μl, i.c.v.) administration into the cerebral ventricle to 7-NI-treated animals was ineffective on spike-wave activity (Fig. 2C), while 7-NI (100 μg/2 μl, i.c.v.) injection raised the frequency from 31.2 ± 3.7/min to 38.2 ± 3.9/min for ten minutes in the same animals (P<0.05, Fig. 2D).

Central administration of the drugs

Saline (2 μl, i.c.v.), D-arginine (300 μg/2 μl, i.c.v.), L-arginine (300 μg/2 μl, i.c.v.) and SNP (100 μg/2 μl, i.c.v.) were administered into cerebral ventricles of the animals

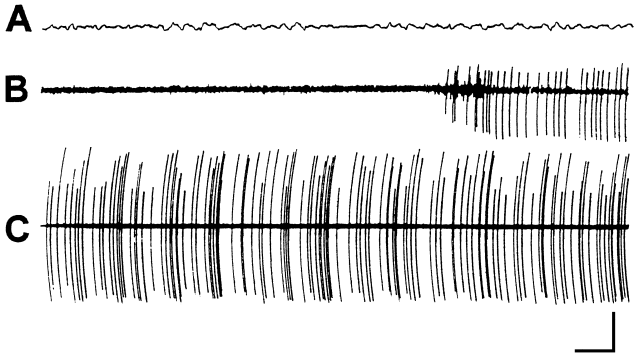

Fig. 1. Penicillin-induced epileptiform activity (3 million U/kg, i.p.). A: Control ECoG, B: 35 – 40 min after penicillin G administration, C: 50 – 55 min after penicillin G administration. Vertical bar: 300 μV, horizontal bar: 1 s for A and 20 s for B and C.

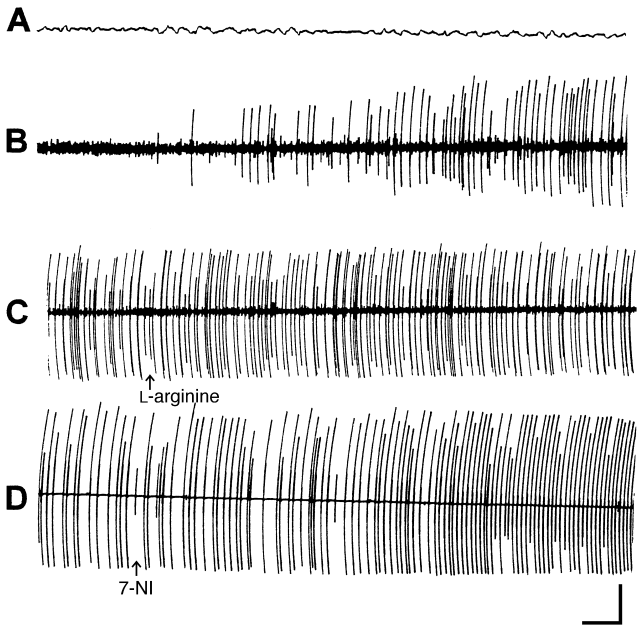

Fig. 2. Epileptiform activity in rats, which were pre-treated 7-NI (60 mg/kg, i.p.). 30 min before penicillin G administration (3 million U /kg, i.p.). Arrows show the injection time of the drugs. A: Control ECoG, B: 10 – 15 min after penicillin G administration, C: Administration of L-arginine (300 μg/2 μl, i.c.v.) into 7-NI pre-treated animals, D: Administration of 7-NI (100 μg/2 μl, i.c.v.) into 7-NI pre-treated animals. Vertical bar: 300 μV; horizontal bar: 1 s for A and 20 s for B, C and D.

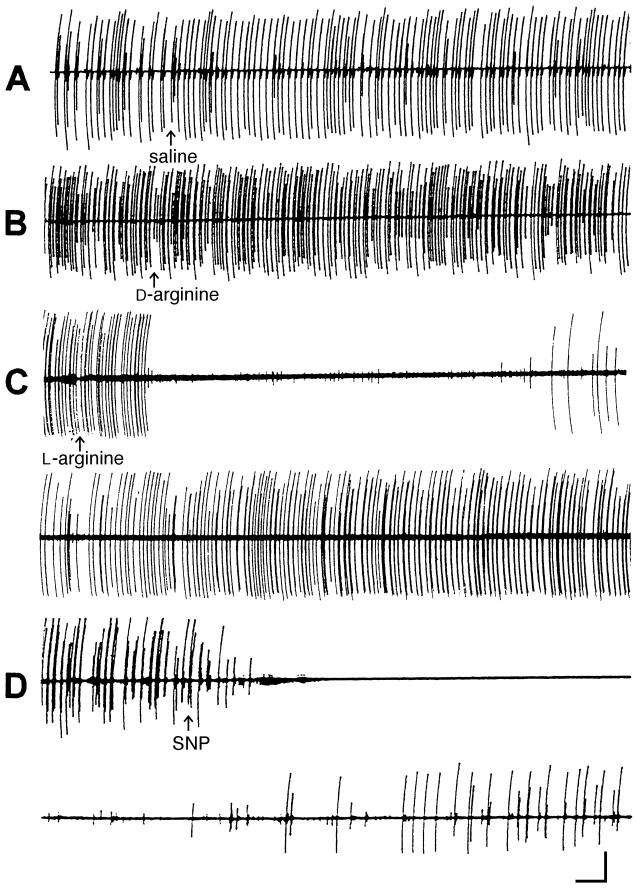

Fig. 3. Effects of saline, D-arginine, L-arginine and SNP on penicillin-induced epileptiform activity. Arrows show the injection time of the drugs. A: Administration of saline (2 μl, i.c.v.), B: Administration of D-arginine (300 μg/2 μl, i.c.v.), C: Administration of L-arginine (300 μg/2 μl, i.c.v.) (continued below), D: Administration of SNP (100 μg/2 μl, i.c.v.) (continued below). Vertical bar: 300 μV, horizontal bar: 20 s.

in other four groups via a Hamilton microinjector, respectively. Saline and D-arginine have no effect on spike frequency and amplitude (Fig. 3: A and B). However, L-arginine suppressed epileptiform activity for 5.6 ± 1.7 min (Fig. 3C) and SNP suppressed the spike activity significantly for 6.3 ± 1.9 min (Fig. 3D) ($P<0.01$).

DISCUSSION

These results suggest that systemic administration of 7-NI shortens the latency of the onset of epileptiform activity ($P<0.01$) and intracerebraventricular administration of L-arginine was ineffective in these 7-NI-pretreated animals. The results also show that saline and D-arginine administration into the cerebral ventricle were completely ineffective on epileptiform activity ($P>0.05$, Fig. 3: A and B), while L-arginine and SNP inhibited spike activity significantly ($P<0.01$, Fig. 3: C and D). Our findings agree with findings reported in several other studies, in which anticonvulsant effects of NO were also shown (14 – 16). In a different set of experiments, we found that central and peripheral administration of L-NAME (N^{ω}-nitro L-arginine methyl ester), a NOS inhibitor, has proconvulsant effects on the penicillin model of epilepsy in rats (17).

According to recent data, L-arginine may exert either an anticonvulsant or proconvulsant effect in different experimental epilepsy models. Results of earlier studies suggested that L-arginine (300 μg, i.c.v.) potentiates NMDA-induced seizures in rats and this effect reversed by L-NAME (4). The discrepancy in the effects of L-arginine on the seizure activity may be due to differences in the epilepsy models and the other experimental conditions.

Subsequently, it was demonstrated that L-arginine, at a dose of 300 μg (i.c.v.), was anticonvulsant against sound-induced seizures in DBA/2 mice and genetically epilepsy-prone rats (18).

In a recent work, in accordance with our results, it was observed that L-arginine has an anticonvulsive effect against the pentylenetetrazole-induced seizure in mice and the anti-seizure effect of L-arginine thought to be mediated by NO production from L-arginine (19).

The role of NO as a signalling molecule in the CNS is still obscure and the conflicting proconvulsant or anti-

convulsant roles of NO have long been a subject of debate. NO may be a pro- or anticonvulsant molecule depending on the model of experimental epilepsy. Results of this study and of other studies made in our laboratory showed that NO may be an endogenous anticonvulsant substance in the penicillin-induced experimental epilepsy (7). Although this study alone does not directly reveal the mechanism of the anticonvulsant effects of NO, regarding the previous studies on this subject, anticonvulsant effects of NO can be explained by the mechanisms summarized below.

Glutamate is an excitatory neurotransmitter in the brain. It also plays a critical role in epileptogenesis (20). NO is considered as a retrograde messenger involved in glutamatergic neurotransmission in the CNS (21). NO inhibits the epileptiform activity probably by blocking the NMDA receptors (22, 23). The inhibitory effects of NO on epileptiform activity may occur via at least three different mechanisms:

1. Competitive inhibition on NMDA receptors: As already known, NMDA receptor activation causes NO production and NO exerts a negative feedback inhibition on NMDA receptors in normal physiological conditions (22, 24). L-Arginine may also stimulate the production of NO and hence block the NMDA receptors.

2. NO may exert its neuroprotective and antiseizure effects by interacting with the redox regulator domain of the NMDA receptor. Although the reaction between the reduced form of NO (NO^-) and superoxide ion ($O_2^{\cdot-}$) results in the formation of peroxynitrite, which may lead to cell death, the oxidized form of NO (NO^+) reacts with the thiol groups in the redox regulator domain of NMDA receptor and exerts neuroprotective effects by this action (25, 26).

3. Results from in vitro studies suggest that the guanine nucleotides (cGMP) may cause NMDA-receptor inhibition by interacting with the recognition domain in a competitive fashion (16, 24, 25). These findings may explain the anticonvulsant effects of NO.

Results of the studies investigating the relation between NO and convulsions are contradictory. It has been shown that the central or peripheral administration of NOS inhibitors may exert opposite effects (27) and such effects have also been observed between different doses of NOS inhibitors (16, 28). It has been suggested that the additional factors including the differences in the model of experimental epilepsy, species, strands and ages of the animals used in the experiments (8, 27 – 29) may also alter the effects of NO on convulsions.

In conclusion, results obtained from the present study suggest that NO may be an endogenous anticonvulsant substance at least under these experimental conditions and further investigations need to reveal the fundamental mechanisms of NO in epilepsy.

Acknowledgment

This work was supported by The Research Fund of Ondokuz Mayis University.

REFERENCES

1 Garthwaite J: Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci **14**, 60 – 67 (1991)
2 Moncada S, Palmer RMJ and Higgs EA: Nitric oxide, physiology, pathology and pharmacology. Pharmacol Rev **43**, 109 – 142 (1991)
3 Murayama T and Nomura Y: The actions of NO in the central nervous system and in thymocytes. Jpn J Pharmacol **76**, 129 – 139 (1998)
4 Mollace V, Bagetta G and Nistico G: Evidence that L-arginine possesses proconvulsant effects mediated through nitric oxide. Neuroreport **2**, 269 – 272 (1991)
5 De Sarro G, Di Paola ED, De Sarro A and Vidal MJ: L-Arginine potentiates excitatory amino acid-induced seizures elicited in the deep prepiriform cortex. Eur J Pharmacol **230**, 151 – 158 (1993)
6 Masukawa T, Nakanishi K and Natsuki R: Role of nitric oxide in the convulsions following the coadministration of enoxacin with fenbufen in mice. Jpn J Pharmacol **76**, 425 – 429 (1998)
7 Marangoz C, Ayyıldız M and Ağar E: Evidence that sodium nitroprusside possesses anticonvulsant effects mediated through nitric oxide. Neuroreport **5**, 2454 – 2456 (1994)
8 de Vasconcelos AP, Gizard F, Marescaux C and Nehlig A: Role of nitric oxide in pentylenetetrazole-induced seizures: age-dependent effects in the immature rat. Epilepsia **41**, 4363 – 4371 (2000)
9 Jayakumar AR, Sujatha R and Paul V: Involvement of nitric oxide and nitric oxide synthase activity in anticonvulsive action. Brain Res Bull **48**, 387 – 394 (1999)
10 Marangoz C and Karatoy M: An experimental study on the inhibitory systems on the penicillin-induced epileptic focus. Atatürk Univ J Sci **1**, 112 – 122 (1982)
11 Kirkby RD, Forbes RA and Subramaniam S: Modification of kainate-induced behavioural and electrographic seizures following inhibition of nitric oxide in mice. Epilepsy Res **24**, 79 – 90 (1996)
12 Bagirici F, Gokce FM and Marangoz C: Depressive effect of nicardipine on penicillin-induced epileptiform activity in rats. Neurosci Res Commun **24**, 149 – 154 (1999)
13 Chen RC, Huang VH and How SW: Systemic penicillin as an experimental model of epilepsy. Exp Neurol **92**, 533 – 540 (1986)
14 Maggio R, Fumagalli F, Donati E, Barbier P, Racagni G, Corsini GV and Riva M: Inhibition of nitric oxide synthase dramatically potentiates seizures induced by kainic acid and pilocarpine in rats. Brain Res **679**, 184 – 187 (1995)
15 Buisson A, Lakhmeche N, Verrecchia C, Plotkine M and Boulu RG: Nitric oxide: an endogenous anticonvulsant substance. Neuroreport **4**, 444 – 446 (1993)
16 Rundfeldt C, Koch R, Richter A, Mevissen M, Gerecke U and Loscher W: Dose-dependent anticonvulsant and proconvulsant effects of nitric oxide synthase inhibitors on seizure threshold in a cortical stimulation model in rats. Eur J Pharmacol **274**, 73 – 81 (1995)
17 Bağırıcı F and Marangoz C: Proconvulsant effects of central

and peripheral administration of L-NAME on penicillin-induced epilepsy in rats. Neurosci Res Commun **28**, 107 – 114 (2001)

18 Smith SE, Man CM, Yip PK, Tang E, Chapman AG and Meldrum BS: Anticonvulsant effect of 7-nitroindazole in rodents with reflex-epilepsy may result from L-arginine accumulation or a reduction in nitric oxide or L-citrulline formation. Br J Pharmacol **119**, 165 – 173 (1996)

19 Tsuda M, Shimizu N, Yajima Y, Suzuki T and Misawa M: Role of nitric oxide in the hypersusceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice. Eur J Pharmacol **344**, 27 – 30 (1998)

20 Chen CH, Chen AC and Liu HJ: Involvement of nitric oxide and *N*-methyl-D-aspartate in acute hypoxic altitude convulsion in mice. Aviat Space Environ Med **68**, 296 – 299 (1997)

21 Kennedy MB: The biochemistry of synaptic regulation in the central nervous system. Annu Rev Biochem **63**, 571 – 600 (1994)

22 Ferraro G, Montalbano ME and La Grutta V: Nitric oxide and glutamate interaction in the control of cortical and hippocampal excitability. Epilepsia **40**, 7830 – 7836 (1999)

23 Manzoni O, Prezeau L, Marin P, Deshager S, Bockaert J and Fagni L: Nitric oxide-induced blockade of NMDA receptors. Neuron **8**, 653 – 662 (1992)

24 Manzoni O, Prezeau L, Desagher S, Sahuquent A, Sladeczek F, Bockaert J and Fagni L: Sodium nitroprusside blocks NMDA receptors via formation of ferrocyanide ions. Neuroreport **3**, 77 – 80 (1992)

25 Lei SZ, Pan ZH, Aggarwal SK, Chen HSV, Hartman J, Sucher NJ and Lipton SA: Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron **8**, 1087 – 1099 (1992)

26 Wing DA and Mitvhell JB: Chemical biology of nitric oxide: Insight into regulatory, cytotoxic, and cytoprotective mechanism of nitric oxide. Free Radic Biol Med **25**, 434 – 456 (1998)

27 Alexander CB, Ellmore TM, Kokate TG and Kirkby RD: Further studies on anti- and proconvulsant effects of inhibitors of nitric oxide synthase in rodents. Eur J Pharmacol **26344**, 115 – 125 (1998)

28 Del-Bel EA, Oliveira PR, Oliveira JA, Mishra PK, Jobe PC and Garcia-Cairasco N: Anticonvulsant and proconvulsant roles of nitric oxide in experimental epilepsy models. Braz J Med Biol Res **30**, 8971 – 8979 (1997)

29 Pereira de Vasconcelos A, Marescaux C and Nehlig A: Age-dependent regulation of seizure activity by nitric oxide in the developing rat. Brain Res Dev Brain Res **15107**, 2315 – 2319 (1998)