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

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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 39.7/4.5 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/V, 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 mg/2 μl, i.c.v.) and sodium nitroprusside (100 μg/2 μl, i.c.v.) suppressed epileptiform activity. Saline (2 μl) and L-arginine (300 mg/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 bicuculline (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
(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 subgroup). 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, heparin solution 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 placed over the somatomotor cortex; the common reference electrode being fixed on the pinna and the ECoG activity placed over the somatomotor cortex; the common reference electrode being fixed on the pinna and the ECoG activity placed over the somatomotor cortex; the common reference electrode being fixed on the pinna and the ECoG activity placed over the somatomotor cortex. 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

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).

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.
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 pentyleneetrazole-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^-) 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, strains 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.

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