The Role of Nitrergic System in Lidocaine-Induced Convulsion in the Mouse

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ABSTRACT—The effects of *N*-nitro-L-arginine-methyl ester (L-NAME) a nitric oxide (NO) synthase inhibitor and L-arginine, a NO precursor, were investigated on lidocaine-induced convulsions. In the first experiment, four groups of mice received physiological saline (0.9%), L-arginine (300 mg/kg, i.p.), L-NAME (100 mg/kg, i.p.) and diazepam (2 mg/kg), respectively. Thirty minutes after these injections, all mice received lidocaine (50 mg/kg, i.p.). In the second experiment, four groups of mice received similar treatment in the first experiment, and 30 min after these injections, all mice received a higher dose of lidocaine (80 mg/kg). L-NAME (100 mg/kg, i.p.) and diazepam (2 mg/kg) significantly decreased the incidence of lidocaine (50 mg/kg)-induced convulsions. In contrast, the L-arginine treatment increased the incidence of lidocaine (80 mg/kg, i.p.)-induced convulsions significantly. These results may suggest that NO is a proconvulsant mediator in lidocaine-induced convulsions.

Keywords: Lidocaine, Convulsions, *N*-nitro-L-arginine-methyl ester, L-arginine

The neuropharmacologic signs of toxicity from local anesthetics are excitation of the central nervous system (CNS) and generalized tonic clonic convulsions. Several neuromediators and mechanisms may be involved in lidocaine-induced convulsions. It is generally agreed that local anesthetics may induce convulsions by depressing inhibitory neurons, thereby allowing facilitation of excitatory neuronal activity (1–3). Nitric oxide (NO), a putative neurotransmitter in the CNS, enhances neurotransmitter release and has been implicated in the regulation at synaptic functions (4). Although much experimental evidence has indicated that brain NO plays a role in various models of convulsions, the role of NO in such convulsions is still unclear and has not been investigated widely. The aim of this study was to investigate the effects of NO on lidocaine-induced convulsions.

MATERIAL AND METHODS

Animals

Male Albino mice weighing 25–40 g ($n = 242$) were used in all experiments. Animals were housed in groups of eight in a temperature controlled room ($20 \pm 2^\circ\text{C}$) with a 12-h light 12-h dark cycle (lights on at 07.00 a.m.). The

animals had a period of adaptation for three days and allowed free access to food and water prior to the experiments. All experiments were performed in the afternoon because of the possible effects of circadian rhythms on experiments. Each animal was used once. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

Data analyses

The incidence of convulsions were obtained from the appearance of clonic convulsions for each group. The statistical analysis of significance of the difference in incidence of convulsions was made by the chi square test.

Drugs

N-nitro-L-arginine-methyl ester (L-NAME, Sigma), L-arginine (Sigma) and lidocaine (Sigma) were dissolved in physiological saline (0.9%). The commercial preparation of diazepam was diluted in 5% propylene glycol aqueous solution. In the first experiments, three groups of mice received physiological saline (0.9%), L-arginine (300 mg/kg), L-NAME (100 mg/kg) and diazepam (2 mg/kg), respectively. Thirty minutes after these injections, all mice received lidocaine (50 mg/kg). In the second set of experiments, the remaining groups of mice received similar treatment as in the first experiment, and 30 min after the injections, all mice received lidocaine (80 mg/kg). All drugs

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were administered intraperitoneally at doses of 0.01 ml /10 g body weight and equivalent volumes of vehicle were injected in control groups. Groups of 22 to 36 mice were used for each treatment. L-NAME, L-arginine or vehicle were administered at 30 min before the lidocaine treatment.

RESULTS

Lidocaine produced ataxia, loss of righting reflex and clonic convulsions 5 min after the injection. The effects of L-arginine (300 mg/kg), L-NAME (100 mg/kg, i.p.) and diazepam (2 mg/kg, i.p.) on the incidence of lidocaine-induced convulsions are shown in Table 1. In the L-NAME-treated mice, the incidence of lidocaine (50 mg/kg, i.p.)-induced convulsions were decreased. Conversely the incidence of lidocaine (80 mg/kg, i.p.)-induced convulsion were increased significantly by L-arginine treatment.

DISCUSSION

There are some contradictory reports about the role of the nitric system in convulsions. Marangoz and Agar (5) have suggested that NO may be an endogenous anticonvulsant substance in the penicillin model of experimental epilepsy. In the same way, Jayakumar et al. (6) have found that L-arginine, a precursor of NO, inhibited picrotoxin-induced convulsions, suggesting that NO may play a role as an endogenous anticonvulsant substance. In contrast to these reports, Mülsch et al. (7) demonstrated an increase in NO formation in kainate-treated mice, but a reduction in both NO production and kainate-induced convulsions in mice pretreated with nitric oxide synthase (NOS) inhibitor. They suggested that NO has a proconvulsant role in kainate-induced convulsions. Kyrkby et al. (8) have suggested that NO may be an endogenous anticonvulsant in kainate-induced convulsions.

Contradictory results about the role of NO in convulsions

may be explained by the different experimental convulsion models and convulsant agents.

Del-Bel et al. (9) have examined the role of NO in convulsion using different agents and models and suggested that the effects of NOS inhibitors vary depending on the seizure model. For this reason, we investigated the function of NO using a different model in this study. In the present study, the incidence of lidocaine-induced convulsions in mice decreased significantly when L-NAME was administered prior to lidocaine, whereas L-arginine increased the convulsions induced by lidocaine. Glutamate is an excitatory neurotransmitter in the brain. It also plays a critical role in epileptogenesis. Glutamate antagonists are potent anticonvulsants in a wide variety of animal models (10 – 12).

There are some evidence that local anesthetics may induce convulsions by activating glutamatergic receptors (10 – 13). Both *N*-methyl-D-aspartic acid (NMDA) and non-NMDA receptor systems have been shown to be involved in local anesthetic-induced convulsions (13). It has been reported that CGS 19755, a competitive NMDA-receptor antagonist, increased the lidocaine-induced seizure threshold (11). In the CNS, NO is considered as a retrograde messenger involved in glutamatergic neurotransmission (14). NO is produced by NOS from L-arginine and NOS is activated by glutamate via NMDA receptor (15). Mülsch et al. (7) have reported that the epileptogenic activity of kainate, a glutamatergic agonist, is likely to be based on a NO response in the amygdale/temporal cortex. Acute increase in NO formation may also be responsible for other neuroexcitator-induced seizures.

Recent reports indicate that NOS inhibitors have both anticonvulsant (16 – 19) and proconvulsant actions (6, 20 – 22). NOS inhibitors have been reported to exert an anticonvulsant effect on NMDA and kainate-induced convulsions (7). In the present study, L-NAME, a NOS inhibitor, significantly reduced lidocaine-induced convulsions. In the

Table 1. The effects of L-arginine, L-NAME and diazepam on the incidence of lidocaine-induced convulsions in mouse

Drugs	Dose mg/kg	Lidocaine mg/kg	n	Numbers of animals with convulsions	% of animals with convulsions
Control	0	50	22	10	45
	0	80	32	27	86
L-Arginine	300	50	36	14	41
	300	80	32	32	100*
L-NAME	100	50	28	3	11*
	100	80	30	18	60
Diazepam	2	50	32	2	6*
	2	80	30	2	7*

* $P < 0.05$, significantly different from control.

light of the data presented in this study, it is possible to suggest that the modulation of glutamatergic activity by lidocaine may result in enhanced NO signaling. Thus, NO may affect lidocaine-induced convulsion by altering glutamatergic activity (23). According to the results that L-arginine was ineffective on convulsions induced by low doses of lidocaine and that L-NAME was ineffective on convulsions caused by high doses of lidocaine suggest that the glutamatergic system may not be sufficiently modulated in these groups.

We have demonstrated that the nitric system may have an excitatory effect on lidocaine-induced convulsions, and NO may play an important role as an endogenous convulsant substance in mice.

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