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Possible Involvement of Nitric Oxide in Quinolinic Acid-Induced Convulsion in Mice

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NAKAMURA, T. A., K. YAMADA, T. HASEGAWA AND T. NABESHIMA. *Possible involvement of nitric oxide in quinolinic acid-induced convulsion in mice.* PHARMACOL BIOCHEM BEHAV 51(2/3) 309–312, 1995.—Quinolinic acid (QA) induced clonic and tonic convulsions in mice when it was injected into the cerebral ventricle. Pretreatment with L-arginine (L-Arg), a substrate of nitric oxide (NO) synthase (NOS), and/or 5,6,7,8-tetrahydrobiopterin (THB), a cofactor of NOS, tended to potentiate QA-induced convulsion. N^G-monomethyl-L-arginine (NMMA), a competitive NOS inhibitor, diminished QA-induced convulsion. This effect of NMMA was attenuated by coadministration of L-Arg or THB. Sodium nitroprusside (SNP), which spontaneously releases NO, did not potentiate, but diminished QA-induced convulsion. These findings suggest that an endogenous NO may be involved, at least in part, in QA-induced convulsion in mice, and that an exogenous NO released from SNP may cause downregulation of *N*-methyl-D-aspartate (NMDA) receptor activity, and thereby prevent the excessive excitation of NMDA receptors and subsequent convulsion caused by QA.

Quinolinic acid *N*-Methyl-D-aspartate receptors Nitric oxide N^G-monomethyl-L-arginine Sodium nitroprusside Convulsion

ENDOGENOUS excitatory amino acids (EAA) such as glutamate and aspartate have an excitotoxic effect (26). Among EAA, quinolinic acid (QA) is one of the most potent substances and has potent neurotoxicity both in vitro (4) and in vivo (27,28). The neurotoxicity of QA occurs through the activation of the *N*-methyl-D-aspartate (NMDA) receptor complex (4,26) and is calcium-dependent (4). Activation of the NMDA receptor complex results in an increase of nitric oxide (NO) synthesis from L-arginine (L-Arg) by NO synthase (NOS) (10,16), which requires several cofactors such as NADPH, FAD, Ca^{2+}, calmodulin, and 5,6,7,8-tetrahydrobiopterin (THB) (2,11,23). NO binds to the heme moiety of guanylate cyclase and subsequently activates this enzyme (2,10).

It has been demonstrated that NMDA-induced neurotoxicity in cortical cell culture is diminished by simultaneous application of an NOS inhibitor, suggesting a role of NO in NMDA-mediated neurotoxicity (5,6). Furthermore, NMDA stimulation of cGMP levels in cortical cell culture was shown to be antagonized by inhibiting NOS (5). Agullo and Garcia (1) also demonstrated that N^G-monomethyl-L-arginine (NMMA), a competitive inhibitor of NOS (18), markedly inhibits the effect of glutamate receptor agonists on cGMP accumulation in neurons. In addition, sodium nitroprusside (SNP), which spontaneously releases NO (8) and causes a dose-dependent increase in cerebellar cGMP content (16), has been shown to produce dose-dependent cell death that parallels cGMP formation in primary cortical cultures (5).

Therefore, it was interesting to investigate the role of NO in NMDA-mediated behavior in vivo. We previously demonstrated that ICV injection of QA induces clonic and tonic convulsion and death in a dose-dependent manner, which is antagonized by 3-(RS)-2-carboxypiperazin-4-yl-propyl-1-phosphoric acid (CPP), D(−)-2-amino-7-phosphonoheptanic acid (AP-7), MK-801, and 7-chlorokynurenic acid, suggesting that QA-induced convulsion and death are mediated by NMDA receptors (24). In the present study, we investigated the role of NO in QA-induced convulsion in mice.

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METHOD

Materials

Six-week-old male mice of the ddY strain, weighing 30–35 g (Nihon SLC, Shizuoka, Japan) were used. They were given food and water ad lib and kept in a regulated environment (23 ± 1°C, 50 ± 2% humidity) with a 12 L : 12 D cycle (lights on at 0800–2000 h). QA, L-Arg, and SNP were obtained from Sigma (St. Louis, MO). THB and NMMA were obtained from Research Biochemicals, Inc. (Natick, MA) and Calbiochem Co. (LaJolla, CA), respectively.

Measurement of QA-Induced Convulsion and Drug Treatment

QA and other reagents were dissolved in phosphate-buffered saline (pH 7.4). QA was injected in a volume of 10 μl into the right side of the cerebral ventricle (ICV) 5 min after the ICV injection of test compounds. The test compounds were injected into the other side of cerebral ventricle to avoid large brain lesions in the same side by repeated injections. The ICV injection was carried out according to the method of Haley and McCormick (13). To verify the injection, a group of mice was injected ICV with india ink. The ink was diffused throughout the cerebral ventricle in > 90% of animals examined. When two or three test compounds were injected at the same time, they were mixed together at an adequate concentration and injected in a volume of 10 μl. QA was administered at a dose of 30 nmol/mouse, which induces the highest frequency of clonic and tonic convulsions but not death (24). We measured the elapsed time to develop the clonic convulsion after QA injection (onset time, s), and the duration of clonic convulsion during a 30-min observation period after QA injection (total time of clonic convulsion, s). We also measured the rate of appearance of the tonic convulsion and death as an index of the effects of the test compounds, which was calculated as follows: Rate of appearance (%) = (n animals developing convulsions or death/n animals examined) × 100.

All data are expressed as mean ± SE. Statistical significance was defined as $p < 0.05$ using Dunnett's test following one-way analysis of variance (ANOVA).

TABLE 1

EFFECTS OF L-Arg AND THB ON QA (30 nmol/BRAIN, ICV)-INDUCED TONIC CONVULSION AND DEATH

Treatment	Dose (nmol/brain) of L-Arg and THB	Tonic Convulsion (%)	Death (%)
QA + saline		25.0	0.0
QA + L-Arg	20	62.5	25.0
QA + L-Arg	50	62.5	37.5
QA + L-Arg	100	62.5	25.0
QA + THB	5	75.0	25.0
QA + L-Arg 20 + THB	5	50.0	0.0
QA + L-Arg 50 +	10	75.0	25.0
QA + L-Arg 100 +	25	62.5	25.0

L-Arg and THB were injected into the left side of cerebral ventricle. Five minutes after the injection of L-Arg and THB, QA was injected into the other side. Each value represents the rate of appearance of tonic convulsion and death caused by QA (n = 8).

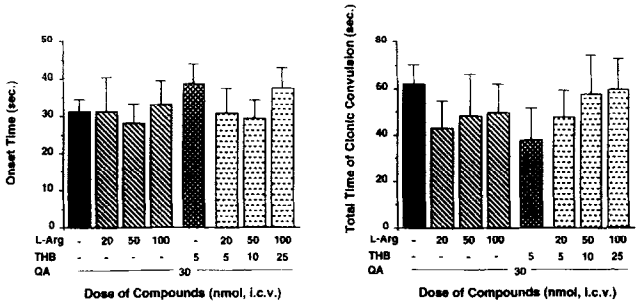

FIG. 1. Effects of L-Arg and THB on QA (30 nmol, ICV)-induced clonic convulsion. L-Arg and THB were injected into the left side of the cerebral ventricle. Five minutes after the injection of L-Arg and/or THB, QA was injected into the other side. Each value represents the mean ± SE (n = 8).

RESULTS

The ICV injection of L-Arg, THB, NMMA, or SNP alone had no effect on behavior in mice within the dose ranges examined in this study. The rate of tonic convulsion appearance and lethality caused by QA tended to increase with pretreatment with L-Arg, a substrate of NOS, and THB, a cofactor of NOS (Table 1). The onset and total time of QA-induced clonic convulsion was not affected by the pretreatment of either L-Arg, THB, or their combination (Fig. 1).

Pretreatment with NMMA (a competitive inhibitor of NOS) prolonged the onset time of QA-induced clonic convulsion. The dose-response curve was bell-shaped, and the most effective dose of NMMA was 5 nmol/mouse. The total time of the clonic convulsion was decreased by NMMA at doses of > 2.5 nmol/mouse (Fig. 2). The effects of NMMA on the onset and total time of the QA-induced clonic convulsions were completely antagonized by coadministration of L-Arg or THB (Fig. 3).

Pretreatment with SNP (a donor of NO) prolonged the onset time of QA-induced clonic convulsions, and significant differences were observed at doses of > 50 nmol/mouse (Fig. 4). The total time of the clonic convulsion was dose-depen-

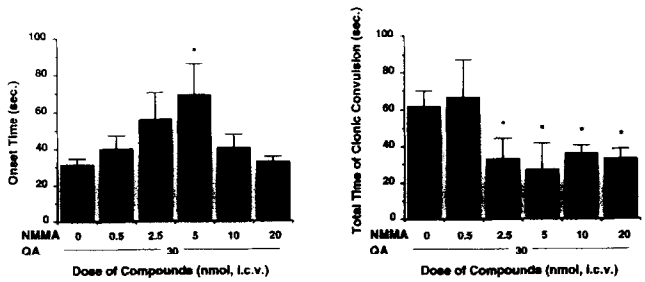

FIG. 2. Effects of NMMA on QA (30 nmol, ICV)-induced clonic convulsion. NMMA was injected into the left side of the cerebral ventricle. Five minutes after the injection of NMMA, QA was injected into the other side. Each value represents the mean ± SE (n = 8). *$p < 0.05$ vs. control.

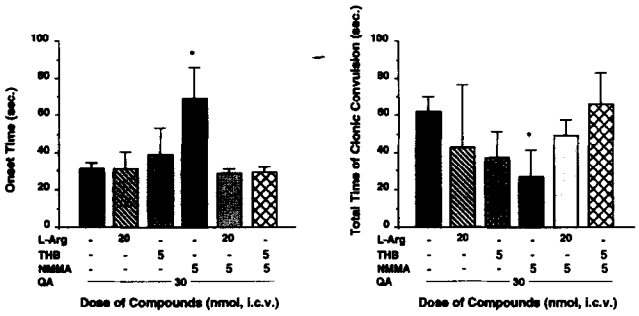

FIG. 3. Effects of coadministration of L-Arg or THB with NMMA on QA (30 nmol, ICV)-induced clonic convulsion. NMMA was coadministered with L-Arg or THB into the left side of cerebral ventricle. Five minutes after the injection of these drugs, QA was injected into the other side. Each value represents the mean ± SE (n = 8). *p < 0.05 vs. control.

dently decreased by SNP, and a significant difference was observed at the dose of 100 nmol/mouse. The rate of appearance of clonic and tonic convulsions was also dose-dependently reduced by SNP; and at the dose of 100 nmol/mouse, the tonic convulsion was completely inhibited ($p < 0.01$ by χ^2-test, data not shown).

DISCUSSION

It has been demonstrated that glutamate-induced neurotoxicity in vitro is prevented by competitive NOS inhibitors such as NMMA and L-N^G-nitroarginine (5,6). The degree of cGMP formation parallels the intensity of neuronal cell death, and NOS inhibitors inhibit glutamate-induced cGMP formation (5,6). In agreement with these experiments, we have demonstrated that NMMA inhibits QA-induced convulsion in mice and that the prevention of QA-induced convulsion produced by NMMA is attenuated by L-Arg and THB, a substrate and a cofactor of NOS, respectively. In particular, prolongation of the onset time of the QA-induced clonic convulsion produced by NMMA (5 nmol/mouse) was completely reversed to the control level. Furthermore, total time of clonic convulsion was significantly decreased by NMMA (2.5–20 nmol/mouse) compared to the control. Although we cannot explain the bimodal effects of NMMA on QA-induced convulsion (Fig. 2), our findings suggest that an endogenous NO may at least partly participate in QA-induced convulsion.

In contrast, Haberny et al. (12) demonstrated that *L*-nitroarginine potentiates QA-induced seizure and neurotoxicity in the rat hippocampus. It is also demonstrated that during status epilepticus provoked by an intra-amygdala injection of kainic acid, the severity of seizures and consequent neuronal damage increased in rats treated with L-nitroarginine (25). Because NO inhibits NMDA receptor binding in rat brain synaptic membranes (9) and blocks the excitatory responses of NMDA (20,22), the suppression of these negative feedback mechanisms exerted by NO on the NMDA receptors may be involved in potentiation by an NOS inhibitor of the neurotoxicity of excitatory amino acids such as kainic acid and QA (12,25). Regarding apparent discrepancy between our study and those by Haberny et al. (12) and Rondouin et al. (25), there may be species difference in the role of NO in the behavioral effects of QA, because we used mice as experimental animals whereas others used rats. Another possibility is that there may be site specificity in the brain for actions of QA and other test compounds, and therefore the results of ICV injections at different sides of cerebral ventricle differ from those after ICV injection at the same side (12) or intra-amygdala injection (25). Furthermore, the role of NO in the behavioral effects of QA may depend on the dose of QA used. To test this hypothesis, the dose-response effects of OA in combination with NOS inhibitors such as NMMA and N-nitro-L-arginine should be determined.

L-Arg and THB alone, or their combination, showed no effect on the onset and total time of the clonic convulsion induced by QA (Fig. 1). Although they somewhat increased the rate of appearance of the tonic convulsion and lethality, the effect reached a plateau (Table 1). One possible explanation for this result is that there may be a sufficient amount of endogenous L-Arg and THB to transmit the stimulation produced by QA. Therefore, an excessive amount of L-Arg and THB administration may have little effect on QA-induced convulsions. If this is the case, treatments that decrease the levels of endogenous L-Arg and THB in the brain could diminish QA-induced convulsions.

Organic nitrates, such as nitroglycerin, isosorbide dinitrate, and SNP, release NO and then activate soluble guanylate cyclase, which results in an increase of intracellular cGMP level (14,19). Dawson et al. (5) showed that SNP produces dose-dependent cell death that parallels cGMP formation, whereas others indicated that it inhibits NMDA receptor-mediated neurotoxicity (17), calcium influx (15), and depolarization of granule cells (7). The latter studies suggest that the effects of SNP do not appear to be due to the release of NO and activation of guanylate cyclase, but are determined by the ferrocyanide portion of the SNP molecule (7,15,17). In the present study, we observed that SNP dose-dependently reduced QA-induced convulsion. One possible explanation of the present results is that the ferrocyanide portion of the SNP molecule, but not NO released from SNP, may partly participate in the inhibitory effects of SNP on QA-induced convulsion. Furthermore, it has been shown that NO-producing agents such as SNP block both NMDA-induced currents and the associated increase in intracellular Ca^{2+} in cultured neurons, and that these effects are due to downregulation of NMDA receptor activity by reaction of NO with thiol groups of the receptor's redox modulatory site (20,22). Taking these previous findings into consideration, we believe that NO released from SNP may cause downregulation of NMDA recep-

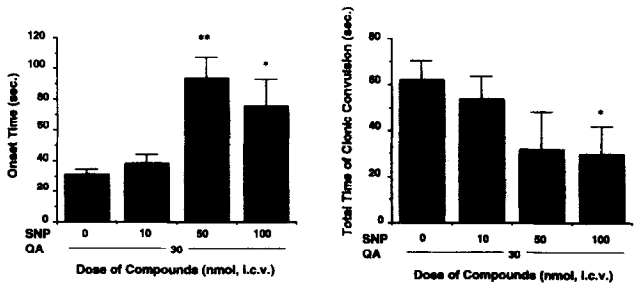

FIG. 4. Effects of SNP on QA (30 nmol, ICV)-induced clonic convulsion. SNP was injected into the left side of the cerebral ventricle. Five minutes after the injection of SNP, QA was injected into the other side. Each value represents the mean ± SE (n = 8). *p < 0.05; **p < 0.01 vs. control.

tor activity and thereby prevent the excessive excitation of NMDA receptors and subsequent convulsion caused by QA. Because NO is an important factor in the regulation of cerebral blood flow (CBF) (3,21), we cannot exclude the possibility that the effects of NMMA and SNP on QA-induced convulsion may result from the changes in CBF regulation.

In summary, our findings suggest that NO may be involved, at least in part, in QA-induced convulsions in mice. SNP protects against the QA-induced convulsion, which is probably due to the downregulation of the NMDA receptors caused by NO released from SNP.

ACKNOWLEDGEMENTS

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