Nitric Oxide Synthase Inhibitors Induce Motor Abnormality in Mice

III. 4. Nitric Oxide Synthase Inhibitors Induce Motor Abnormality in Mice

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Introduction

Nitric oxide (NO) is an intracellular and short-lasting messenger molecule that is synthesized from L-arginine in several tissues by a reaction catalyzed by NO synthase (NOS)[1,2]. In the central nervous system (CNS), NO has been proposed to be implicated in many physiological, pathological and biochemical conditions, such as learning and memory[3,4]. NOS has been also localized in the neuronal cells in specific regions, with highest expression in neurons of the cerebellum and olfactory bulb of rat brains, by immunohistochemical studies[5]. Therefore, these observations are of interest with regard to the role of NO in the regulation of neuronal function.

Several studies suggest that N^G-nitro-L-arginine, a non-selective NOS inhibitor, can cause catalepsy in experimental animals[6-8]. It is known that NOS-positive cells are found in the striatum[9] and antagonism of NO formation has been shown to decrease dopamine release in this structure[10]. However, a recent study indicates that NOS inhibitors can cause a marked increase of dopamine content in the rat striatum[11]. Thus conflicting results regarding the possible role of NO are apparent in dopaminergic transmission.

It is known that catalepsy has been defined as a failure to correct an extremely imposed posture. Therefore, it has been widely used to evaluate motor effects of various drugs, particularly those related to the extrapyramidal system[12]. Previous studies also reported that pole test may be of value in the screening of anti-parkinsonian agents[13]. In the present study, therefore, we investigated possible motor effects of N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective NOS inhibitor, and 7-nitroindazole (7-NI), a neuronal NOS (nNOS) inhibitor, using both catalepsy and pole tests in mice. We also examined the dopamine and its metabolite contents in each drug-treated mouse.

Material and Methods

Male ddy mice, 5 weeks old (25-32g) were used in this study. The animals were housed in air-conditioned rooms at room temperature of 22±1°C and under a 12-hr light-dark cycle with standard food and tap water available at libitum.

7-NI (Research Biochemicals Int.), L-NAME hydrochloride (Research Biochemicals Int.) and L-arginine (Sigma) were used in this study. 7-NI (40, 80 and 160 mg/kg) was suspended in peanut oil and was given subcutaneously (sc) in mice. L-NAME was dissolved in saline and was given sc in mice. L-Arginine was dissolved in saline and was injected intraperitoneally (ip) in mice.

Catalepsy test

To measure cataleptic symptoms such as akinesia and rigidity, bar-test catalepsy was evaluated by placing both forepaws of the mouse over a horizontal bar (diameter: 0.2 cm), elevated 15 cm from floor. The time during which the animals maintained this position was recorded up. In preliminary study, the test was performed at immediately before (0 hr) and 1, 2, 4 and 24 hr after the treatment of each drug. For the evaluation of drugs, the test was performed at immediately before (0 hr) and 2 and 4 hr after drug treatment. Each group contained 7 mice. All values were expressed as means±S.E. and statistical significance was evaluated using an analysis of variance (ANOVA) followed by Williams multiple range test.

Pole test

To measure the degree of bradykinesia, a typical symptom of parkinsonism, pole test was performed according to the method of Ogawa et al.[13] with minor modifications[14]. The mouse was placed head upward on the top of a rough-surfaced pole (8 mm in diameter and 50 cm in height) which was wrapped doubly with gauze to prevent slipping: the time until it turned completely downward (Tturn) and the time until it climbed down to the floor (TLA) were examined. In preliminary study, the test was performed at immediately before (0 hr) and 1, 2, 4 and 24 hr after the treatment of each drug. For the evaluation of drugs, the test was performed at immediately before (0 hr) and 2 and 4 hr after drug treatment. Each group contained 7 mice. All values were expressed as means±S.E. and statistical significance was evaluated using an analysis of variance (ANOVA) followed by Williams multiple range test.

Measurement of dopamine and its metabolites

The mice were killed by cervical dislocation at 4 hr after the treatment of each drug. After decapitation, brains were quickly removed and the two striata were rapidly dissected out freehand on an ice-cold glass Petri dish. Samples were immediately weighted, then frozen and stored at –80°C until assay. The dissection procedure was performed in less than 2 min. Striata were sonicated ice-cold 0.2M perchloric acid containing 100 ng/ml isoproterenol as internal standard. Homogenates were centrifuged at 3000 rpm for 20 min at 4°C. The

supernatant was filtered (pore size 0.45μm, Millipore filter) and a 30-μl aliquot of the supernatant was used for determination of the dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and isoproterenol by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD) (Eicom, Japan). The mobile phase consisted of 0.1M sodium citrate-0.1M sodium acetate solution (pH 3.5) including 1.064 M octane sulfonic acid and 0.013 mM Na_2EDTA and 15% (v/v) methanol. The recoveries of dopamine, DOPAC, HVA and isoproterenol through the present procedures were >93%. Levels of dopamine, DOPAC and HVA were calculated from the comparison of simple peak area with internal standard peak region and are expressed as μg/g tissue weight. Each group contained 4 mice. All values were expressed as means±S.E. and statistical significance was evaluated using an analysis of variance (ANOVA) followed by Williams multiple range test.

Results

Effect of L-NAME treatment on motor activity

L-NAME caused a significant and dose-dependent cataleptic effect in the range of 40-160 mg/kg as shown in Fig. 1. The cataleptic effect developed gradually from 1 hr after L-NAME injection and lasted more than 4 hr. After 24 hr, L-NAME-induced cataleptic effect was not observed in mice. In pole test, L-NAME also produced a prolongation of Tturn and TLA in a dose-dependent manner as shown in Fig. 2. These motor deficits were especially noted in higher dose of 160 mg/kg and developed gradually from 1 hr after L-NAME treatment and lasting more than 4 hr. After 24 hr, however, this effect was not evident in mice.

Effect of 7-NI treatment on motor activity

7-NI caused a significant and dose-dependent cataleptic effect in the range of 40-160 mg/kg as shown in Fig. 3. The cataleptic effect developed gradually from 1 hr after 7-NI injection and lasted more than 4 hr. After 24 hr, 7-NI-induced cataleptic effect was not observed in mice. In pole test, 7-NI also produced a prolongation of Tturn and TLA in a dose-dependent manner as shown in Fig. 4. These motor deficits were especially noted in higher dose of 160 mg/kg and developed gradually from 1 hr after 7-NI treatment and lasting more than 4 hr. After 24 hr, however, this effect was not significance in mice.

Effect of each drug on the content of dopamine and its metabolites in the striatum

Four hours after L-NAME (160 mg/kg) treatment, no significant change in dopamine, DOPAC and HVA content were observed in the striatum. Therefore, the change for dopamine turnover was not found. On the other hand, 7-NI (160 mg/kg) caused a significant increase in the striatal dopamine content 4 hr after the treatment. In contrast, no

significant change in DOPAC and HVA content was observed. For dopamine turnover, 7-NI produced a significant reduction in the striatum (Fig. 5).

Effect of L-arginine on L-NAME- or 7-NI-induced catalepsy

L-NAME or 7-NI increased catalepsy compared with the effect of vehicle. The effects were especially noticed at 2 and 4 hr after each drug treatment. The cataleptic action of L-NAME or 7-NI was significantly antagonized by the treatment with L-arginine (Fig. 6).

Discussion

In this study, L-NAME and 7-NI also caused a significant and dose-dependent cataleptic effect in the range of 40-160 mg/kg. In pole test, both drugs produced a significant prolongation of Tturn and TLA in a dose-dependent manner. The motor deficit in 7-NI-treated mice was more pronounced than that in L-NAME-treated animals. These findings strongly indicate that NOS inhibitors can induce motor deficiency in mice.

It is possible that NOS inhibitors decrease locomotor activity by interfering with striatal dopamine. For example, it is known that antagonism of NO formation attenuates dopamine release in the striatum[10,15] and inhibits the increased locomotor activity found after dopamine agonist treatment[16,17]. In the present study, L-NAME showed no significant change in dopamine, DOPAC and HVA content in the striatum. On the other hand, 7-NI caused a significant increase in the striatal dopamine content, whereas this drug showed no significant change in DOPAC and HVA content in the striatum. That is why 7-NI produced a significant decrease in the dopamine turnover. Thus, these results suggest that the mechanism in the motor deficit caused by NOS inhibitors is different from that in the motor deficit induced by the decrease of dopaminergic neurotransmission in the striatum, such as haloperidol. Recent studies demonstrate that 7-NI has a MAO inhibitory-like property besides its action on NOS[11,18]. Therefore, it is suggested that NOS inhibitor can cause a significant increase in dopamine content in the striatum. These observations are, at least in part, consistent with our present findings.

To further precise the mechanism of NOS inhibitors, we studied the effect of L-arginine on motor deficit induced by NOS inhibitors. In the present study, the cataleptic action of L-NAME or 7-NI was significantly antagonized by the treatment with L-arginine, suggesting that inhibition of NO formation may be implicated. These findings suggest that NO also plays a key role in control of motor behavior. In the present study, however, it is difficult to infer at the moment whether antagonism of the peripheral or the central enzyme is involved. From our findings that the motor deficit induced by 7-NI was more pronounced than that caused by L-NAME, it is conceivable that the inhibition of nNOS may induce motor deficit in mice.

NO has complex interactions with NMDA-mediated neurotransmission. For example, NO can mediate the NMDA-induced increase in cyclic GMP, but inhibit NMDA-

induced increase in intracellular Ca^{2+} and NOS activity, and antagonize the NMDA receptors[19-22]. It has been also suggested that NO can regulate excitatory amino acid release[23]. In the present study, therefore, the action of NOS inhibitors against NMDA-mediated neurotransmission may explain the motor deficit induced by these inhibitors. However, further studies are necessary to clarify the precise mechanisms for our findings.

In conclusion, our results show that NOS inhibitors can induce motor deficit in mice. Furthermore, we speculate that nNOS may play some role in control of motor behavior.

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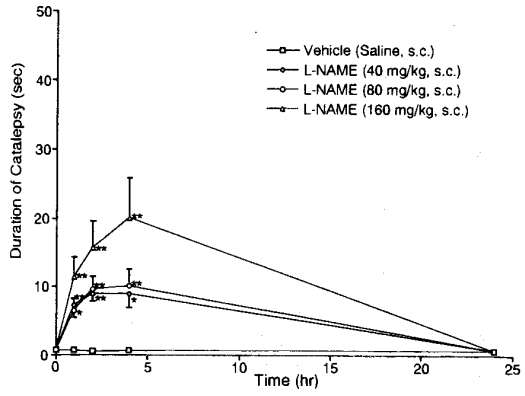

Fig. 1. Time course of the catalepsy induced by L-NAME in mice. Points show the means±S.E. of 7 mice. *p<0.05, **p<0.01 vs. the corresponding value in vehicle group (Williams multiple range test).

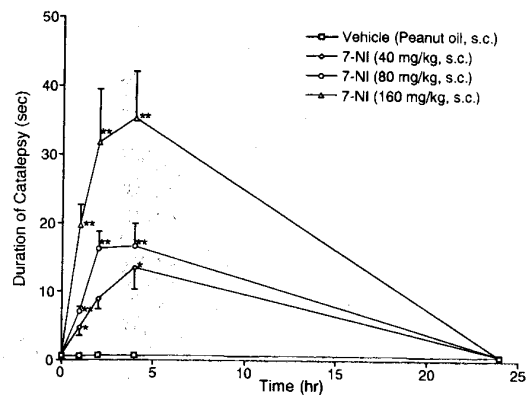

Fig. 3. Time course of the catalepsy induced by 7-nitroindazole (7-NI) in mice. Points show the means±S.E. of 7 mice. *p<0.05, **p<0.01 vs. the corresponding value in vehicle group (Williams multiple range test).

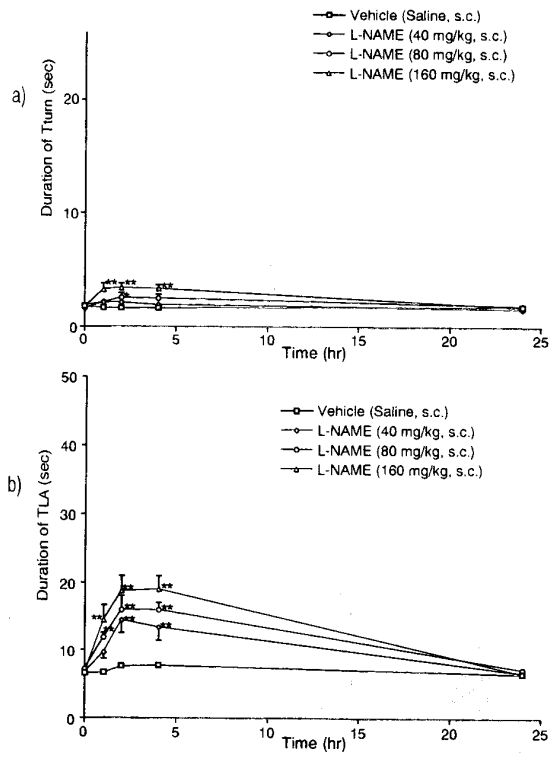

Fig. 2. Time course of the values of the Tturn and TLA in L-NAME-treated mice. Points show the means±S.E. of 7 mice. *p<0.05, **p<0.01 vs. the corresponding value in vehicle group (Williams multiple range test). a) Tturn; b) TLA.

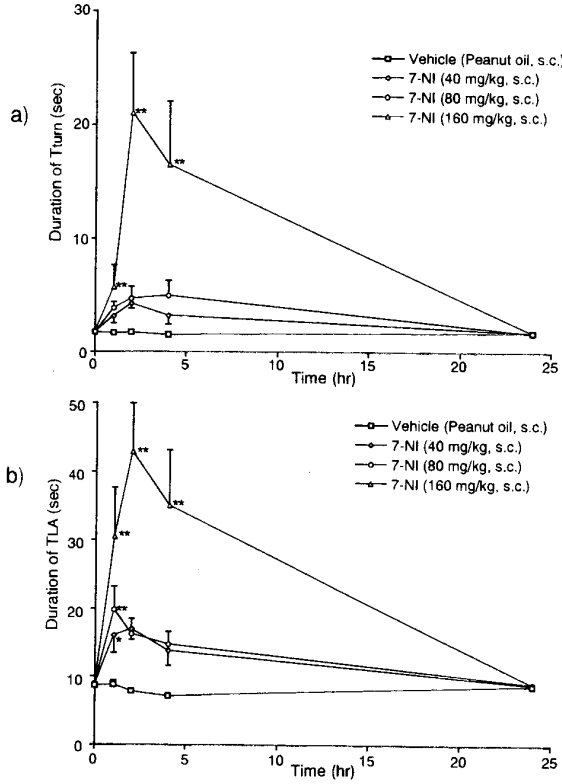

Fig. 4. Time course of the values of the Tturn and TLA in 7-NI -treated mice. Points show the means±S.E. of 7 mice. *p<0.05, **p<0.01 vs. the corresponding value in vehicle group (Williams multiple range test). a) Tturn; b)

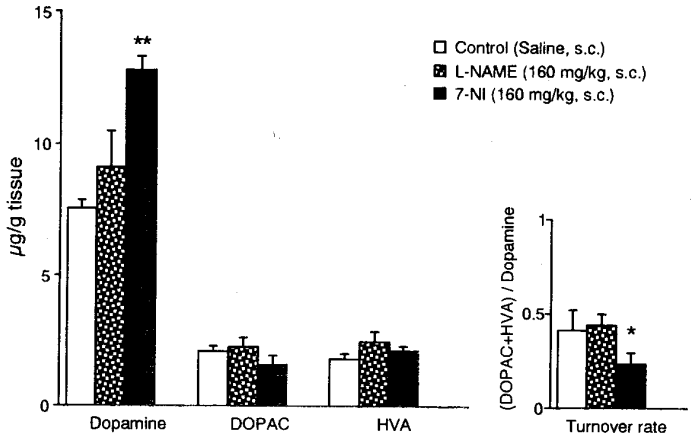

Fig. 5. Effects of L-NAME and 7-NI on the content of dopamine, DOPAC and HVA and turnover (DOPAC+HVA/Dopamine) in mice. Points show the means±S.E. of 4 mice. *p<0.05, **p<0.01 vs. control group (Williams multiple range test). The measurement of dopamine, DOPAC and HVA was examined 4 hr after each drug treatment

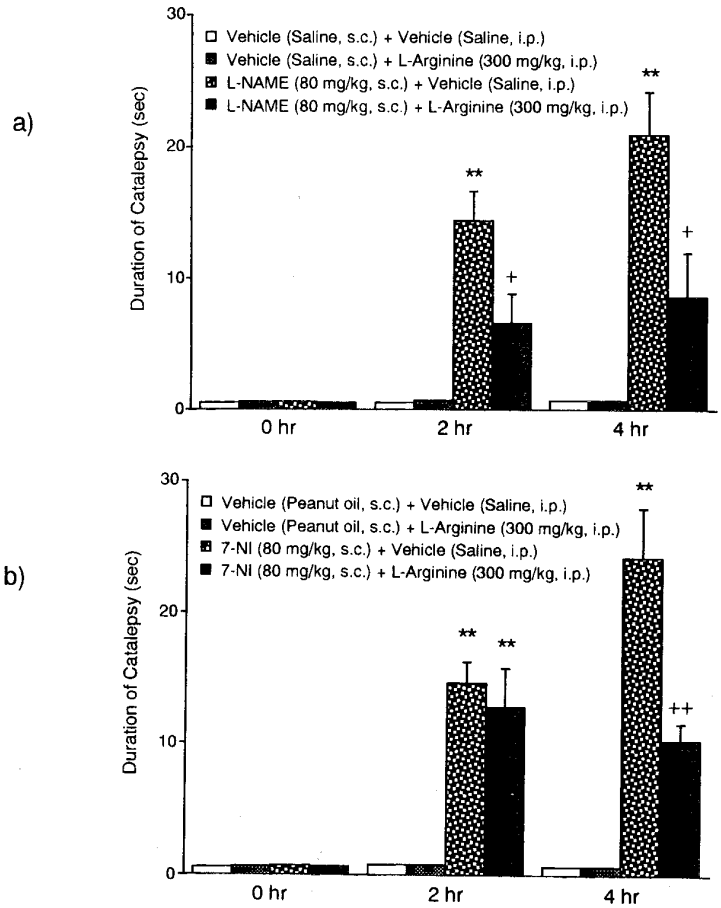

Fig. 6. Antagonitic effects of L-arginine on L-NAME or 7-NI-induced catalepsy in mice. *p<0.05, **p<0.01 vs. the corresponding value in vehicle group (Williams multiple range test). +p<0.05, ++p<0.01 vs. L-NAME + Vehicle group or 7-NI + Vehicle group (Student's t-test). a) L-NAME; b) 7-NI