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Original Article

Dopaminergic Neurotransmission Triggers Ischemia-induced Hyperactivity in Mongolian Gerbils

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It is recognized that sustained ischemia-induced hyperactivity is related to abnormalities in dopamine function. However, it is unclear that dopaminergic neurotransmission triggers such ischemia-induced hyperactivity. Therefore, the relationship between dopaminergic neurotransmission and ischemia-induced hyperactivity was investigated in an animal model using Mongolian gerbils. When haloperidol 2 mg/kg was administered i.p. 30 min after ischemia, the ischemia-induced hyperactivity at 24 h after ischemia was blocked. General behavior was similar to that of sham-operated animals. Haloperidol at doses of 0.1 and 0.2 mg/kg had no effect on locomotor activity in sham-operated animals and decreased ischemia-induced hyperactivity when the drug was administered 24 h after ischemia; these doses did not have any effect on ischemia-induced hyperactivity when the drug was administered 30 min after ischemia. On the other hand, when the animal was confined to a small, restrictive cage for the 24 h period immediately following ischemic injury, locomotor activity at 24 h after ischemia increased. Such behavior also increased in animals when they were returned to their original more permissive cages immediately after ischemia. It is conceivable that the decrease in the level of activity was not related to ischemia-induced hyperactivity. These data suggested that the inhibition of ischemia-induced hyperactivity can be induced by complete blockage of dopaminergic receptors immediately after ischemia.

Key words: ischemia, hyperactivity, dopamine, haloperidol, Mongolian gerbils

Hyperactivity induced by cerebral ischemia in Mongolian gerbils has previously been documented [1, 2, 7, 8, 12, 13, 18]. This hyperactivity, induced by cerebral ischemia, was recognized immediately after operative occlusion and continued for several days after ischemic injury [10, 11]. Many neurotransmitters are abnormally released during and immediately after ischemia; the levels of these substances tend to return to normal several hours after ischemia [6, 17]. However, ischemia-induced hyperactivity is recognized 24 h after ischemia; such hyperactivity occurs after changes in transmitters in the brain have already normalized. Therefore, it is conceivable that changes in neurotransmitter receptors during and immediately after ischemia become a trigger for longer-lasting ischemia-induced hyperactivity.

When dopamine D_2 receptor antagonists were administered 24 h after ischemia, ischemia-induced hyperactivity decreased at doses that had no effect on locomotion in sham-operated animals [1]. This finding suggests that sustained ischemia-induced hyperactivity is related to

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abnormalities in dopaminergic function. On the other hand, the cause of such ischemia-induced hyperactivity remains unclear. It also remains unclear why abnormalities in dopaminergic neurotransmission trigger hyperactivity. Therefore, in the present study, haloperidol was injected immediately after ischemic insult and the resulting hyperactivity at 24 h after ischemia was examined.

It has been reported that sensitivity to methamphetamine (MAP) can be influenced by cage size [9]. Behavioral suppression is also related to the occurrence of ischemia-induced hyperactivity. Therefore, the present experiment was performed in order to clarify whether neurotransmitter receptors or only a decrease in activity level immediately after ischemia were related to ischemia-induced hyperactivity.

Materials and Methods

Animals. Male Mongolian gerbils weighing 60–90 g were obtained from Shin Nihon Dobutsu (Saitama, Japan). All were housed in an air-conditioned room at 23 ± 1 °C. Light was provided on a 12-h light/dark cycle (7:00 am/7:00 pm). Food and water were provided *ad libitum*. All animals were thoroughly used to being handled. Each group included 5–8 animals in a cage ($22 \times 40 \times 20$ cm).

Occlusion of common carotid arteries. The Mongolian gerbils were anesthetized with ether and placed in the dorsal position. After local infiltration of xylocaine, both common carotid arteries were exposed through a ventral midline incision, and sympathetic nerves were separated, as described previously [3]. The arteries were clamped with aneurysm clips for 5 min. The clips were then removed, and the skin was sutured. Sham-operated animals were treated in the same manner, except for the absence of clip applications. The rectal temperature was maintained close to 37 °C during ischemia using a heating lamp and a heating pad.

Experimental procedure. In this experiment, locomotor activity was measured using the ANIMEX Apparatus (Muromachi Kikai Inc., Tokyo, Japan). First, animals were placed in a chamber ($22 \times 40 \times 20$ cm) on the ANIMEX Apparatus 1 day before the ischemic injury. Movements were counted for 5 min. Animals were assigned to groups such that there was no difference in the locomotor activity between the groups.

In the first experiment, 14 animals were divided into 2 groups. One group was sham operated and the other was subjected to ischemic injury.

In the second experiment, 16 animals were divided into 3 groups. The first group was sham operated and the activity of these animals was not limited. The second group was returned to the original cage immediately after ischemia, namely, the activity of these animals was not restricted. The third group was locked in a small cage ($9 \times 9 \times 17$ cm) for 24 h immediately after ischemic injury in order to limit activity. Food and water were also provided *ad libitum* to all animals.

In the third experiment, 28 animals were divided into 5 groups. Haloperidol in doses of 0.1, 0.2, and 2.0 mg/kg was administered to animals in the original cages 30 min after ischemia. The locomotor activity of these groups was measured for 5 min at 24 h after ischemia.

In the fourth experiment, 10 animals were divided into 2 groups. The locomotor activity of all animals was measured. The grouping was carried out such that there was no difference in the locomotor activity between the groups. Saline or haloperidol in a dose of 2.0 mg/kg was administered in the home cages and the locomotor activity of these animals was measured for 5 min at 24 h after injection.

Drug. Haloperidol (Takeda Pharmaceutical Co., Ltd. Osaka, Japan) at doses of 0.1, 0.2, and 2.0 mg/kg was injected i.p.

Statistical analysis. The following statistical analyses were made to assess the differences in values between groups. In the results of the ischemia-induced hyperactivity study, the differences between sham-operated and ischemia-lesioned animals were determined by *t*-test following one-way analysis of variance (ANOVA). Effects of cage scale on hyperactivity induced by cerebral ischemia were analyzed by *t*-test. In the drug administration experiment, Dunnett's test was performed to compare test animals with the control group.

Results

Changes in locomotor activity induced by cerebral ischemia in Mongolian gerbils. The increase in locomotor activity was most marked 1 day after occlusion. In the ischemic lesion group, locomotor activity 1 day after occlusion had increased significantly compared with that of the sham-operated group ($P < 0.01$ *vs.* sham operated group). This significant increase in locomotor activity in the ischemic lesion group continued for 7 days (Table. 1).

Effects of limiting locomotor activity with a small, restrictive cage for ischemia-induced hyperactivity in Mongolian gerbils. The range of behavior was limited by use of a small, restrictive cage, where animals were housed immediately after ischemic lesion for 24 h. Locomotor activity was measured for 5 min. The values of the test animals in this locomotor activity study were almost the same as that of animals kept in the original cage for 24 h after ischemia (Fig. 1).

Effects of haloperidol on ischemia-induced hyperactivity in Mongolian gerbils. Haloperidol at doses of 0.1, 0.2, and 2.0 mg/kg was administered

Table I Changes in locomotor activity induced by cerebral ischemia in Mongolian gerbils

Group	Locomotor activity (count)					
	pre-operation	1 day	3 days	7 days	14 days	28 days after occlusion
Sham operation	366.6 ± 24.2	240.5 ± 16.2	190.9 ± 12.1	243.4 ± 15.2	279.1 ± 23.1	279.5 ± 19.2
5 min ischemic	340.5 ± 24.9	629.2 ± 14.0**	466.5 ± 23.1**	400.5 ± 47.6**	355.8 ± 36.3	306.8 ± 19.3

The differences between sham-operated and ischemia-lesioned animals were determined by *t*-test following a one-way analyasis of variance (ANOVA). $^{**}P < 0.01$ *vs.* sham-operated group

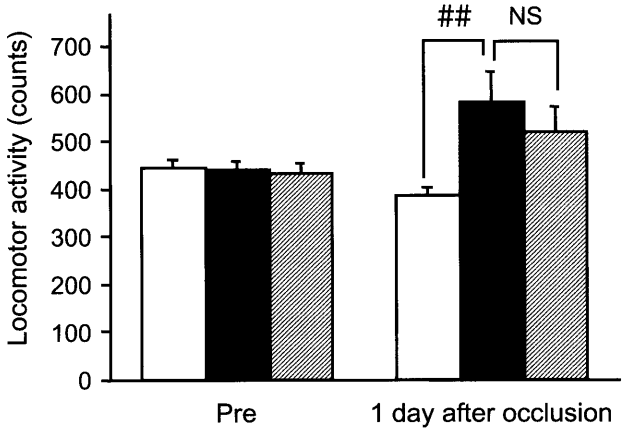

Fig. 1 Effect of constriction of movement in a small cage on ischemia-induced hyperactivity in Mongolian gerbils. □, control group; activity was not limited by cage scale, as animals were returned to their original cages (22 × 40 × 20 cm); ■, ischemic lesioned group; Group A: animals were returned to their original cages (22 × 40 × 20 cm) immediately after ischemia; ▨, ischemic lesioned group; Group B: activity of animals was limited by placement in a small cage (9 × 9 × 17 cm) immediately after ischemic injury. Results of the locomotor activity study were analyzed using Student's *t*-test to compare the sham-operated group with Group A, and Group A with Group B. ##, $P < 0.01$, sham-operated Group *vs.* Group A. NS, not significant; Group A *vs.* Group B. ##, $P < 0.01$ *vs.* sham; NS, non-significant. □, sham (n = 6); ■, control cage (n = 5); ▨, small cage (n = 5)

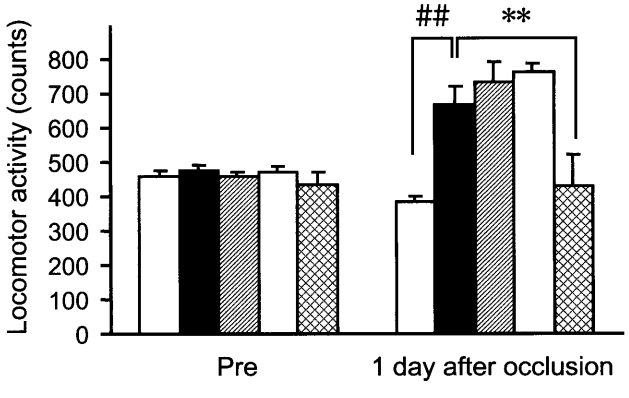

Fig. 2 Effect of haloperidol on ischemia-induced hyperactivity in Mongolian gerbils. □, sham operated group; ■, saline injected group in ischemic lesioned animals; ▨, haloperidol at a dose of 0.1 mg/kg injected into ischemic lesioned animals; ▩, haloperidol at a dose of 0.2 mg/kg injected into ischemic lesioned animals; ⊠, haloperidol at a dose of 2.0 mg/kg injected into ischemic lesioned animals. Saline and haloperidol were injected 30 min after ischemia while animals were kept in their original cages. Results of the locomotor activity study were analyzed using Student's *t*-test in order to compare the sham operated and ischemia operated-saline injected groups. ## $P < 0.01$, sham operated group *vs.* saline-injected ischemic lesioned group. The effects of haloperidol were analyzed by Dunnet's test in order to compare saline-injected group with other groups. $^{**}P < 0.01$, saline-injected group *vs.* haloperidol-injected group (dose: 2.0 mg/kg) in ischemic lesioned animals. ## $P < 0.01$ *vs.* sham, $^{**}P < 0.01$ *vs.* sham. □, sham (n = 6); ■, saline (n = 6); ▨, haloperidol 0.1 mg/kg (n = 6); ▩, haloperidol 0.2 mg/kg (n = 6); ⊠, haloperidol 2.0 mg/kg (n = 4)

30 min after ischemia and locomotor activity was measured for 5 min at 24 h after ischemia. Although hyperactivity was not influenced by the administration of haloperidol at doses of 0.1 and 0.2 mg/kg, administration of haloperidol at a dose of 2.0 mg/kg significantly reduced ischemia-induced hyperactivity ($P < 0.05$ *vs.* saline-administered group) (Fig. 2).

Effects of haloperidol on spontaneous locomotor activity 24 h after injection in Mongolian gerbils. Locomotor activity decreased 24 h after saline injection. Haloperidol at a dose of 2.0 mg/kg also decreased the value of locomotor activity 24 h after injection. However, no significant differences in locomotor activities were observed between the saline-injected and haloperidol-injected groups (Fig. 3).

Discussion

In the present experiment, ischemia-induced hyperactivity similar to that described in previous reports was observed [1, 2, 11]. It has been reported that disturbances in dopaminergic transmission are linked to cerebral ischemia [19]. Dopamine D_2 receptor antagonists dose-dependently and significantly decreased ischemia-induced hyperactivity when administered 24 h after ischemia, particularly at doses that had no effects on locomotor activity in sham-operated animals [1]. Therefore, it is assumed that dopaminergic abnormalities are present in cases of ischemia-induced hyperactivity at 24 h after ischemia.

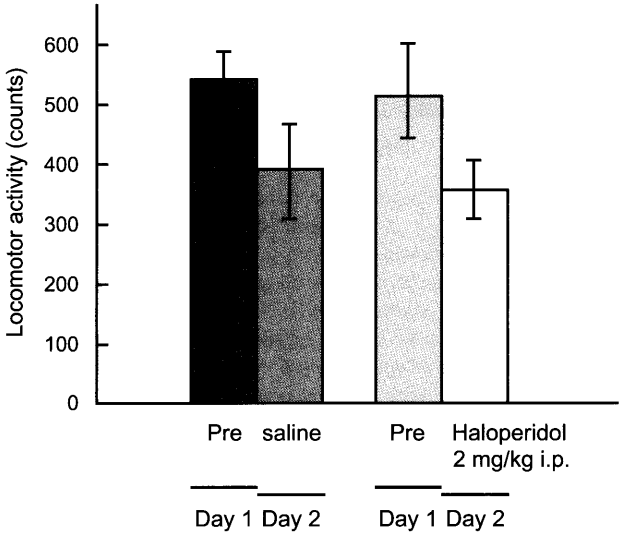

Fig. 3 Effects of haloperidol at a dose of 2.0 mg/kg on spontaneous locomotor activity observed at 24 h after injection in Mongolian gerbils. The results of the locomotor activity study were analyzed by Student's *t*-test in order to compare saline-injected and haloperidol-injected groups.

The level of dopamine was not significantly altered during and after ischemia in the cortex, hippocampus, and striatum, but the levels of its metabolites in the brain were elevated after ischemia. One such metabolite of dopamine, 3-methoxy tyramine, increased during ischemia and 5 min after ischemia [17]. Nakane *et al.* [15] reported that marked release of dopamine occurred during ischemia of bilateral carotid artery occlusion in spontaneously hypertensive rats, and calcium entry through L-type voltage-sensitive calcium channels is involved in the massive release of dopamine during cerebral ischemia. We have already reported that calcium channel blockers significantly and dose-dependently inhibited ischemia-induced hyperactivity at 24 h after ischemia; the relationship between calcium and dopaminergic function was discussed in that report [2]. Thus, it is conceivable that dopaminergic functional changes that are observable 24 h after ischemia may be linked to dopaminergic abnormalities.

In the present study, sustained ischemia-induced hyperactivity at 24 h after ischemia was significantly inhibited by the administration of haloperidol, when it was administered 30 min after ischemia at a dose of 2.0 mg/kg. On the other hand, such an inhibition was not observed at doses of 0.1 and 0.2 mg/kg; the latter doses showed no effect on locomotor activity in naive animals [1]. Furthermore, these doses inhibited ischemia-induced hyperactivity when it was administered 24 h after ischemia [1]. Therefore, a high dose of haloperidol may be needed in order to successfully inhibit dopaminergic receptor susceptibility after ischemia. In addition, this result indicates that a decrease in spontaneous locomotor activity, at least during the several hours following haloperidol injection, is related to an inhibition of hyperactivity at 24 h after ischemic injury.

It is uncertain whether or not this ischemia-induced hyperactivity is similar to observed clinical symptoms. However, the present model appears to be appropriate for clarifying psychotic disorders and abnormal behavior in situations where numerous neurotransmitters are transiently released. For example, during stressful conditions and depression, multiple transmitters are involved. In addition, tiapride, a dopamine D_2 antagonist, is effective

against abnormal behaviors and emotional disturbances after cerebral infarction. Moriguchi *et al.* [14] also reported that tiapride was effective against ischemia-induced hyperactivity, which suggests possible participation of the dopamine D_2 receptor in ischemia-induced hyperactivity.

By the way, it has been reported that sensitization to the ambulation-increasing effects of MAP was influenced by the size of the cage in which animals were placed; repeatedly, acute drug effects have been observed in such cases [9]. In the present experiment, ischemic animals were enclosed in a narrow environment for the 24-h period starting immediately after ischemia. Locomotor activity at the initiatory stage of ischemia-induced hyperactivity was inhibited. However, ischemia-induced hyperactivity appeared 24 h after ischemia in these animals. As regards these findings, behavioral suppression is not likely to be related to the occurrence of ischemia-induced hyperactivity.

On the contrary, Feeney and coworkers [7] reported a single dose of d-amphetamine given 24 h following unilateral sensory motor cortex ablation in rats; this treatment resulted in an enduring enhancement of motor recovery. Intraventiricular infusion of norepinephrine (NE) has been shown to mimic the effects of amphetamines [4, 5]. In other experiments, bilateral selective lesion of the locus coeruleus, the major source of central noradrenergic projection fibers, was found to impair behavioral recovery, in comparison with that of rats with sham locus coeruleus lesions [4, 5, 8]. With regards to these findings, it is conceivable that NE also plays an important role in ischemia-induced hyperactivity. Although haloperidol is highly selective for the D_2 receptor, it also affects serotonin and α_1 receptors more than is observed in the case of either sulpiride or pimozide [16]. Further attention should be paid to the issue of acceptor susceptibility, including noradrenergic neurons, when neurotransmitters are normalized after transient ischemia.

We have already clarified that sustained hyperactivity, which is recognized 24 h after ischemia, is related to dopaminergic mechanisms [1]. In the present experiment, it was clear that behavioral suppression was not related to the occurrence of ischemia-induced hyperactivity. The sustained hyperactivity typically observed at 24 h after ischemia was blocked by the administration of haloperidol at a high dose when it was administered 30 min after ischemia. Haloperidol has high D_2-receptor selectivity, but it also affects other neurotransmitter receptors. The mechanism of initiation of ischemia-induced hyperactivity warrants further attention.

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