ABSTRACT—Ischemia-induced hyperactivity is recognized several hours after both common carotid arteries’ occlusion for 5 min in Mongolian gerbils, and it continues for at least 7 days. The aim of this study is to investigate the possible mechanisms of this abnormal behavior. Methamphetamine (MAP) (1 and 3 mg/kg) was administered for 7 days and imipramine (IMP) (5 and 10 mg/kg) was administered for 7 or 14 days. Bilateral carotid artery was occluded for 5 min 24 h after the last administrations of these drugs. MAP, which had been administered every day for 1 week, showed marked inhibition in the ischemia-induced hyperactivity. However, IMP did not have any effect even though it had been injected every day for 2 weeks. Hippocampal CA1 neuronal changes also appeared in the MAP- and IMP-administered groups. As the dopaminergic neurotransmission is facilitated by the repeated administration of MAP, the ischemia-induced hyperactivity may be related to abnormalities in dopaminergic function. The participation of the other neurotransmitters is also discussed.

Keywords: Ischemia, Hyperactivity, Methamphetamine, Imipramine, Mongolian gerbil

The abnormal behaviors induced by some drugs are mainly caused by changes in the complicated neurotransmitters in the brain (1–3). In an experiment on transient ischemic attack using Mongolian gerbils, many transmitters are released during and immediately after transient ischemia (4). Although attention has been increasingly directed to the “delayed neuronal death in hippocampal CA1 neurons” elicited by transient ischemia, it is possible that functional deficit will occur after the carotid arteries occlusion. In fact, studies for effects of cerebral ischemia in Mongolian gerbils have documented memory impairment; i.e., functional abnormalities similar to many of those observed in hippocampal lesion studies (5–8). A good correlation has been found between hippocampal CA1 neuronal death and memory impairment in the passive avoidance task (5, 9, 10).

On the other hand, changes in locomotor activity induced by cerebral ischemia in Mongolian gerbils have also been reported (11–15). Although the delayed neuronal death in the hippocampal CA1 neuron is found 2 or 3 days after the transient ischemia, the hyperactivity can be seen from 2 or 3 h after the ischemia, reaches the peak 24 h later and continued for 7 days (15, 16). It is conceivable that the abnormal release of neurotransmitters may be a trigger of ischemia-induced hyperactivity. When dopamine (DA) D2-receptor antagonists were administered at the peak time of hyperactivity (24 h after the ischemia), they inhibited the hyperactivity at doses that had no effect on locomotor activity in sham-operated animals (16). This means that the hyperactivity, which has already occurred, may be related to the dopaminergic neurotransmission. However, it is unclear how ischemia-induced hyperactivity occurred and continued for 7 days and what is the key neurotransmitter for this long lasting behavior.

The aim of this study is to investigate the possible involvement of neurotransmission in ischemia-induced hyperactivity in Mongolian gerbils. Repeated administration of methamphetamine (MAP) can cause functional supersensitivity (17, 18). This phenomenon is at least partly due to the increasing basal extra-cellular levels of DA in the mesolimbic terminal areas when the animals are receiving daily psycho-stimulant injections (19–21). On the other hand, imipramine (IMP) inhibits the uptake of noradrena-
line and serotonin (5-HT), and it is well known that \( \beta \) - and 5-HT\(_2\) receptors show down regulation when IMP is administered repeatedly.

Therefore, in the present study, we observed the effect of repeated pretreatment with IMP or MAP on the ischemia-induced hyperactivity.

MATERIALS AND METHODS

Animals

Fifty-eight male Mongolian gerbils weighing 60 – 90 g were obtained from Shin Nihon Dobutsu (Saitama). They were housed in an air-conditioned room at 22 ± 1°C. Light was provided on a 12-h light/dark cycle with lights on at 7:00 a.m. Food and water were provided ad libitum. All animals had become thoroughly used to being handled. Animals were divided into 9 groups and each group had 5 – 6 animals.

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 the sympathetic nerves were separated, as described previously (5). 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). All animals were put in the chamber (22 × 40 × 20 cm) one by one on the ANIMEX Apparatus. The following grouping was carried out on the premise that there was no difference in the locomotor activity between the groups. All drugs were administered i.p.

Group 1: saline injection for 7 days and sham-operation was performed 24 h after the last administration of saline, Group 2: saline injection for 7 days, Group 3: MAP 1 mg /kg injection for 7 days, Group 4: MAP 3 mg/kg injection for 7 days, Group 5: IMP 5 mg/kg injection for 7 days, Group 6: IMP 10 mg/kg injection for 7 days, Group 7: saline injection for 14 days and sham-operation was performed 24 h after the last administration of saline, Group 8: saline injection for 14 days, Group 9: IMP 5 mg/kg injection once a day for 14 days, and Group 10: IMP 10 mg /kg injection for 14 days. All injections were performed once a day. The bilateral carotid artery was occluded for 5 min 24 h after the last administration of saline, MAP or IMP in Groups 2 – 6 and 8 – 10. Locomotor activities were measured 30 min after each drug-injection in Groups 1 – 6 for 5 min. In Groups 7 – 10, locomotor activities were measured 30 min after drug-injection on day 1, 3, 7 and 14 for 5 min. In addition, in each group, locomotor activities were measured for 5 min 3 and 24 h after sham or ischemic occlusion.

Histopathology of the hippocampus

Seven days after ischemia (six days after the locomotor activity test), the same animals were anesthetized with ether, and the brains were perfused with a 10% buffered formalin solution administered through the left cardiac ventricle. The hippocampal region, cut coronally into 3- to 4-mm-thick slices, was embedded in paraffin and processed using the step-section technique. The preparations were stained with hematoxylin-eosin and cresyl-violet by an investigator blind to the experimental conditions. Ischemic neuronal damage was graded on a scale of 0 – 3 as follows: 0 (−), normal neurons; 1 (+), a few neurons damaged (as few as one neuron damaged); 2 (++), many neurons damaged; and 3 (+++), the majority of neurons damaged (15).

Drugs

MAP (Dainippon Pharmaceutical Co., Ltd., Osaka) and IMP (Sigma, St. Louis, MO, USA) were used. All drugs were dissolved in physiological saline (0.9% sodium chloride). Each drug was administered i.p. in a volume of 0.1 ml per 100 g body weight.

Statistical analyses

Dunnet’s test was performed following one-way or two-way analysis of variance (ANOVA).

RESULTS

Effect of MAP on locomotor activity in Mongolian gerbils

MAP at a dose of 1 mg/kg was administered every day for 7 days, and locomotor activity was measured 30 min after each injection for 5 min. Hyperactivity and stereotyped behavior were not recognized at this dose. However, at the dose of 3 mg/kg, locomotor activity increased from day 1 to day 7 of MAP injection (Fig. 1).

Effect of chronic MAP administration on ischemia-induced hyperactivity in Mongolian gerbils

In 1 mg/kg MAP treated animals, hyperactivity was also recognized at 24 h after the ischemia. However, at the dose of 3 mg/kg, MAP significantly inhibited the hyperactivity (Fig. 2). In addition, at 3 h after the cerebral ischemia, hyperactivity was seen in both groups (Fig. 2).
Effect of IMP on locomotor activity in Mongolian gerbils

IMP at doses of 5 and 10 mg/kg was administered every day for 7 or 14 days. In each group, the locomotor activity was almost the same as that of the saline-treated group, except for day 14 (Fig. 3).

Effect of chronic IMP administration (14 days) on ischemia-induced hyperactivity in Mongolian gerbils

In all groups treated with IMP at 5 or 10 mg/kg for 14 days, hyperactivity occurred at 3 and 24 h after the occlusion (Fig. 4).

Histopathological changes in the hippocampus induced by cerebral ischemia in MAP and IMP administered Mongolian gerbils

In the sham-operated gerbils, CA1 neurons in the hippocampus were readily visible. Seven days after a 5 min ischemia, the CA1 neurons appeared to be destroyed, and severity of the neuronal damage was 3 (+++) in 100% of the 5-min occlusion group. At that time, neuronal changes also appeared in the MAP- and IMP-administered groups (Fig. 5).

DISCUSSION

Hyperactivity induced by cerebral ischemia was significantly inhibited by repeated MAP injection at a dose of 3 mg/kg, but it was not inhibited by the repeated administration of IMP.

Dopaminergic mechanisms within the nucleus accumbens and striatum play an important role in the control of locomotor activity. Intra cerebral injections and lesion studies have revealed that both the mesolimbic and nigrostriatal DA pathways and their terminal regions, particularly the nucleus accumbens and the striatum, mediate the locomotor activity. Stimulation of dopaminergic neurons in these areas causes an increase in locomotor activity, whereas decreased DA activity elicits a depression of spontaneous behavior (22). While DA seems to be the primary neurotransmitter in inducing locomotor activity, the fine articulation and prolongation of locomotion involves transmitters such as norepinephrine (NE) (22). It has been reported that NE affects DA via a pathway between the locus coeruleus and the nigrostriatal system that enhances impulse flow between the substantia nigra and the striatum (23). Furthermore, other neurotransmitters such as...
5-HT interact directly with DA neurons in an inhibitory capacity. Sufficient stimulation or inhibition could substantially alter locomotor activity in the absence of any direct experimental manipulation of DA neurons or receptors themselves (22). The central action of MAP is mediated through noradrenergic, dopaminergic and serotonergic neurons (24). It has been known that repeated exposure to psychostimulants produces behavioral sensitization and an exaggerated motor response to subsequent challenge administration of psychostimulants (17, 18, 25). Efforts to elucidate the neurochemical mechanisms involved in the development of behavioral sensitization to psychostimulants have found that this phenomenon is at least partly due to increasing basal extracellular levels of DA in the mesolimbic terminal areas when the animals are receiving daily psychostimulants injections (19–21). Wagner et al. (26) reported that high-dose-MAP treatment caused an irreversible decrease in the number of DA re-uptake sites in rat striatum. Nakayama et al. (27) also reported that the treatment regimen of gradually escalating doses of MAP induces a long-lasting decrease of DA uptake sites in rat striatum.

Furthermore, transient ischemia can lead to disturbances in the metabolism of a neurotransmitter (28). It has previously been shown that ischemia alters not only monoaminergic (29), but also other neurotransmitter metabolic pathways (30). The activity of monoamine oxidase is inhibited (31) and the uptake function of dopamine neuro-
transmission is destroyed by global ischemia in the rats (32). With respect to these findings, it is also assumed that ischemia-induced hyperactivity results from destruction of the uptake function of DA neurotransmission, which is similar to that of MAP treatment.

Arai et al. (33) reported that the MAP-induced sensitization was impaired by 5-min ischemia. They suggested that the impairment of synaptic plasticity might be induced in advance of morphological damage. Although the methods are different from our present experiment, the impairment of synaptic plasticity may be induced by ischemia in Mongolian gerbils. In the present experiment, the ischemia-induced hyperactivity was significantly inhibited by repeated treatment of 3 mg/kg MAP at 24 h after the carotid artery occlusion, but not at 3 h. This means that DA is increased by the transient ischemic attack and stimulates the post-synaptic dopamine receptor. Because the destruction of the uptake function of DA neurotransmission had already been induced in MAP pretreated animals, the basal extracellular DA level increased significantly, and the marked downregulation of post-synaptic dopamine receptor occurred at 24 h after the ischemia. In addition, the impairment of synaptic plasticity may be induced gradually during 24 h, and DA release may be reduced. Therefore, it is difficult to maintain the hyperactivity for 24 h.

Stummer et al. (34) reported that the protection by pre-

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**Fig. 5.** Neuronal damage in hippocampal CA1 neurons induced by cerebral ischemia in Mongolian gerbils. A: Saline was injected for 7 days and sham operation was performed 1 day after the last saline injection. Seven days after sham operation, neurons in the hippocampal CA1 were normal. B: MAP at 3 mg/kg was injected for 7 days and the bilateral common carotid artery was occluded for 5 min 1 day after the last MAP injection. Seven days after ischemia, neurons in hippocampal CA1 showed degeneration, destruction or disappearance of Nissl bodies. Scale bars: 40 μm.
ischemic locomotor activity may involve enhanced post-ischemic reperfusion, leading to more rapid normalization of electrical tissue conductance and thus of cell volume. In the present experiment, locomotor activity increased every day by the injection of MAP. Therefore, it is conceivable that the pre-ischemic physical activity is attributable to the early recovery of hyperactivity. Certainly, physical exercise offers protection against the consequences of ischemia; however, in the present experiment, MAP did not have any effect on hippocampal CA1 delayed neuronal death. In addition, we surmised that this behavioral change induced by cerebral ischemia does not correlate directly with hippocampal damage. This is because we found that when Mongolian gerbils were subjected to 5-min ischemia again 1 month after the initial 5-min ischemia, which had caused complete degeneration of hippocampal CA1 neurons, locomotor activity even 1 day after was again significantly increased (15).

Feeney and coworkers (35) reported a single dose of d-amphetamine given 24 h following unilateral sensorimotor cortex ablation in the rat resulted in an enduring enhancement of motor recovery. Amphetamine-facilitated recovery is hypothesized to be due to its specific effects on central NE. Intra-ventricular infusion of NE mimics the effect of amphetamine (36, 37). In other experiments, bilateral selective lesions of the locus coeruleus, the major source of central noradrenergic projection fibers, was found to impair behavioral recovery as compared to rats with sham locus coeruleus lesions (36–38). Given the hypothesis that amphetamine acts through NE, other drugs that enhance NE release or decrease its metabolism would be expected to be beneficial (39). With regard to these facts, noradrenergic neuronal changes may be related with the inhibition of ischemia-induced hyperactivity by repeated MAP administration.

It is well known that down-regulation of β-adrenergic post synaptic receptor and 5-HT, especially 5-HT2 post-synaptic receptor, are observed when tricyclic antidepressants are administered for 2 weeks (40). If NE is related to the ischemia-induced hyperactivity, it may be changed by the repeated administration of tricyclic antidepressants. In the present experiment, the traditional tricyclic antidepressant, IMP did not show any effect on ischemia-induced hyperactivity. Although the receptor-binding experiment was not carried out in the present study, β- and 5-HT2 receptor may not be related directly to the ischemia-induced hyperactivity.

Recently, we found that when haloperidol at a dose of 2 mg/kg, which did not have any effect on the gerbil behavior 24 h after drug administration, was administered i.p. 30 min after ischemia, the ischemia-induced hyperactivity at 24 h after ischemia was blocked. This inhibition was not recognized with haloperidol at a dose of 0.2 mg/kg. Therefore, these data suggested that the inhibition of ischemia-induced hyperactivity could be complete blockage of dopaminergic receptors immediately after ischemia (41).

In the present study, it is conceivable that the inhibition of ischemia-induced hyperactivity in the animal repeatedly administered MAP is mainly related to the changes in dopaminergic neuronal transmission.

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