Effect of Minaprine and Other Reference Drugs on Passive Avoidance Impairment Induced by Cerebral Ischemia in Mongolian Gerbils

Yasuko KARASAWA, Hiroaki ARAKI, Shigeru OKUYAMA, Hironaka AIHARA and Susumu OTOMO
Department of Pharmacology, Research Center, Taisho Pharmaceutical Co., Ltd., Yoshino-cho, Ohmiya, Saitama 330, Japan
Accepted March 30, 1990

Abstract—We examined the characteristics of cerebral ischemia-induced behavioral deficit in the passive avoidance task and the effect of minaprine and other cytoprotective drugs on passive avoidance deficit induced by cerebral ischemia in Mongolian gerbils. Severe impairment of passive avoidance was apparent when the duration of the ischemia exceeded 2 min. Histopathological ischemic neuronal damage in CA1 neurons at 7 days after occlusion was also induced when the ischemia was over 2 min. Otherwise, although cerebral ischemia was carried out at 5 min, 2 hr, 5 hr or 24 hr after the training session, the passive avoidance deficit was produced 24 hr after the training session. When the training session was carried out 24 hr before the occlusion, minaprine, which was administered 30 min before the occlusion, led to a recovery of the response latency. Pentobarbital, diazepam and ethylapovincamine improved the passive avoidance deficit induced by 5-min bilateral carotid artery occlusion. On the other hand, the passive avoidance deficit was not ameliorated by Ca++-hopantenate, nicardipine and idebenone. The hippocampal damage at 7 days after occlusion was prevented by the drugs that ameliorated the passive avoidance deficit. The relationship between passive avoidance deficit and CA1 neuronal death in the hippocampus induced by cerebral ischemia warrants further attention.

Neurons in the CA1 region in the hippocampus of gerbils are selectively vulnerable to ischemia (1), and these neurons often show delayed development of morphologically obvious cell death after reversible ischemia (2–4). We reported that severe behavioral abnormality in the passive avoidance task was apparent when training was carried out 2 or 14 days after ischemia induced by a 5-min bilateral occlusion of the carotid arteries in Mongolian gerbils (5). Under such conditions, the amplitude of the hippocampal theta waves was decreased, and Nissl’s degradation was apparent in the CA1 neurons in the hippocampus.

Minaprine [3-(2-morpholino-ethylamino)-4-methyl-6-phenylpyridazine dihydrochloride] is a newly developed psychotropic drug effective for treating the various types of mental depression seen clinically (6–8) and multi-infarct dementia (9). The drug also shows a protective effect against cerebral anoxia and cerebral ischemia, in a variety of animal models (10). When minaprine was given 30 min before the 5-min occlusion of bilateral carotid arteries in Mongolian gerbils, there was a significant improvement in passive avoidance impairment, no decrease in the amplitude of the hippocampal theta waves, and the destruction of CA1 neurons was considerably diminished (11).

One of the objectives of the present study was to elucidate the characteristics of cerebral ischemia-induced passive avoidance deficits and the other was to compare the effect of minaprine with other cytoprotective drugs on the passive avoidance test and histopathological hippocampal changes induced
by 5-min bilateral common carotid arteries occlusion in Mongolian gerbils.

Materials and Methods

Animals: Male Mongolian gerbils, supplied by Shin Nihon Dobutsu Co., Ltd. (Saitama, Japan) and weighing 55 to 80 g, were housed in an air-conditioned room at 22±1 °C. Light was provided on a 12 hr light-dark cycle with lights off at 7:00 p.m. Food and water were provided ad libitum.

Occlusion of common carotid arteries: The gerbils were anesthetized lightly with ether and placed in the supine position. After local infiltration of xylocaine, both common carotid arteries were exposed through a ventral midline incision and carefully separated from the adjacent vein and sympathetic nerves. The arteries were then clamped with aneurysm clips. At determined times, the clips were removed and the skin was sutured. Sham-operated animals were treated in the same manner, except that clamping was not done.

Passive avoidance task: The gerbils were trained in a conventional step-down type passive avoidance apparatus, divided into the safe and grid parts. The experimental chamber (22.5×20.0×19.5 cm) was made of acrylfiber. The floor had a grid of stainless rods and a 2 mA scrambles shock generator (Muromachi

Fig. 1. Experimental schedule of passive avoidance task and cerebral ischemia. a: The training and test sessions of the passive avoidance were carried out 2 and 3 days after a 1, 2, 3, 4 or 5 min occlusion of the bilateral common carotid arteries. b: The training session of the passive avoidance test was first carried out and bilateral common carotid arteries were clamped for 5 min. The latency between the training session and cerebral ischemia was 2, 5, 24 and 24 hr in experiments A, B, C and D, respectively. The interval between occlusion and the test was 24, 24, 24 and 48 hr, respectively.
Kikai Co. Ltd., Tokyo, Japan). The safe part (20.0×9.5×3.0 cm) was made of acrylfiber and was fixed at one side of the chamber. Each animal was placed initially on the safety platform. When the gerbil stepped down onto the grid floor, it received a foot shock. Initially, the gerbils repeatedly stepped up and down, but eventually remained on the platform. This training session lasted for 300 sec. The step down latency in Mongolian gerbils did not increase with one foot shock, and this is completely different from mice or rats. However, there was a marked prolongation of latency in the passive avoidance task in Mongolian gerbils that received foot shock repeatedly within 5 min. In the test trial, the gerbil was placed on the safety platform and the response latency, i.e., the time before it stepped down to the grid floor, was measured. If the gerbil did not step down to the grid floor within 60 sec, a ceiling score of 60 sec was assigned. Two types of investigation were performed. In the first experiment, the training and test sessions of the passive avoidance were carried out 2 and 3 days after 1, 2, 3, 4 or 5 min occlusion of the bilateral common carotid arteries (Fig. 1a). In the second experiment, the training session of the passive avoidance test was first carried out, and bilateral common carotid arteries were clamped for 5 min. The latency between the training session and cerebral ischemia was 2, 5 or 24 hr (Fig. 1b).

Histopathology of the hippocampus: Animals used for the histopathological examination were anesthetized with ether 7 days after the bilateral common carotid arteries had been occluded, and the brains were perfused with a 10% buffered formalin solution given through the left cardiac ventricle. The hippocampal region was cut coronally into 3- to 4-mm thick slices, embedded in paraffin and processed using the step section technique. The slides were stained with hematoxylin and eosin, and stained with cresyl violet.

Ischemic neuronal damage in the hippocampal CA1 neurons was graded on a scale of 0–3: score 0 (−), normal neurons; score 1 (+), a few neurons damaged (as few as one neuron damaged); score 2 (++), many neurons damaged; and score 3 (+++), majority of neurons damaged.

Drugs: Drugs to be tested were dissolved in saline or suspended in a 5% gum arabic solution. The injected drug was diluted with saline. Minaprine was given p.o. by intubation 30 min before the occlusion. All other drugs used were given i.p. 30 min before the occlusion. Nicardipine was given 15 min before the occlusion followed by post ischemia maintenance treatment (0.5 mg/kg, i.p., twice daily). The following drugs were used: minaprine, pentobarbital (Nembutal, Dainippon), diazepam (Wako), idebenone (Takeda), ethylapovincamine (Covex), Ca²⁺-hopantenate (Hopate, Tanabe) and nicardipine (Sigma).

Statistical analysis: Results of the passive avoidance experiment were analyzed by the Mann-Whitney U-test.

Results

Characteristics of cerebral ischemia-induced behavioral deficits in the passive avoidance test: In the first experiment (Fig. 1a), the training and test sessions of the passive avoidance were carried out 2 and 3 days after bilateral common carotid artery occlusion, respectively. As shown in Fig. 2, the response latency in the test trial decreased when the duration of the ischemia increased. In particular, when the duration of the ischemia exceeded 2 min, the response latency was significantly shorter than that of sham-operated gerbils.

In the second experiment (Fig. 1b), the interval between the training session and the cerebral ischemia was changed. In the sham-operated gerbils of schedule D, 7 out of 10 Mongolian gerbils did not step down within 60 sec, and the response latency in the test trials was 53.6±3.8 sec (mean±S.E.). In all groups subjected to a 5-min carotid artery occlusion, even when the interval between the training session and the cerebral ischemia was 2, 5 or 24 hr, the response latency was markedly shortened (Fig. 3).

Effect of minaprine and the other drugs on a passive avoidance task: The training session was carried out 2 days after the 5-min occlusion. When minaprine was administered 30 min before the occlusion, the reduction in response latency was significantly attenuated...
at the doses of 50 and 75 mg/kg. When the training session was carried out 24 hr before the occlusion, minaprine also led to a significant dose-related attenuation of the response latency reduction. With 75 mg/kg minaprine, the effect of ischemia was significantly prevented (Fig. 4).

The effects of the other drugs on the passive avoidance task after ischemia are shown in Fig. 5. Pentobarbital and diazepam increased the response latency significantly. The effects of pentobarbital and diazepam were statistically significant. Ethylapovincamine in a dose of 200 mg/kg, but not 100 mg/kg, significantly increased the latency. Idebenone, Ca**-hopantenate and nicardipine had no apparent effect on the latency in the passive avoidance response.

Histopathological changes in CA1 neurons in the hippocampus: In the sham operated gerbils, the entire population of CA1 neurons in the hippocampus was easily recognized. At 7 days of recirculation after a 5 min carotid artery occlusion, the CA1 neurons in the hip-
The hippocampus appeared to be destroyed and were not evident by light microscopic examination. The severity of neuronal damage was dependent on the duration of ischemia, namely 10%, 55.6% and 100% in the 1, 2, 3 and 4 min occlusion groups, respectively, the scale being 3 (+++), indicating that the majority of the neurons were damaged. Destruction and disappearance of the hippocampal CA1 neurons were partially prevented when minaprine was administered 30 min before the occlusion (Fig. 6). Twenty percent of the group given 50 mg/kg of minaprine showed no alteration in CA1 neurons, and 20% showed a slight alteration. In a dose of 75 mg/kg, 70% of the minaprine-injected group possessed the entire population of CA1 neurons in the hippocampus. Severe alteration was recognized in 20% (Table 1).

Discussion

We reported that severe impairment of the passive avoidance task was detected 2 days after bilateral carotid artery occlusion-induced ischemia (5). In the present experiments, cerebral ischemia was carried out 5 min, 2 hr, 5 hr or 24 hr after the training session. The passive avoidance deficit was recognized even when cerebral ischemia was produced 24 hr after the training session. These observations differ from findings of passive avoidance deficit in the case of four-vessel occlusion-induced ischemia in rats. Yamazaki et al. (12) reported that when cerebral
Fig. 5. Effect of various drugs on latency in the passive avoidance task as induced by 5-min bilateral common carotid artery occlusion in Mongolian gerbils. Training for the passive avoidance was carried out 24 hr before cerebral ischemia. The test trial was given 48 hr after cerebral ischemia. All drugs except for nicardipine were given i.p. 30 min before the occlusion. Nicardipine was given i.p. 15 min before the occlusion followed by post ischemia maintenance treatment. N=9 or 10, *P<0.05, **P<0.01, significantly different from the control group.
ischemia was induced by four-vessel occlusion, ischemia produced within 20 min after the avoidance acquisition led to amnesia, while ischemia produced later than 1 hr after the acquisition did not. The discrepancies from the findings of Yamazaki et al. (12) could be explained by the differences of the species and ischemic conditions. Indeed, a 5-min occlusion is severe for Mongolian gerbils, and there is a marked destruction of the CA1 neurons in the hippocampus. Yamazaki et al. (12) used rats and occluded

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**Fig. 6.** Effect of minaprine on histopathological changes in the Mongolian gerbil hippocampus after 5-min cerebral ischemia. a: saline administered gerbil. b: gerbil administered minaprine in a dose of 75 mg/kg. Hematoxylin and eosin, stain. ×40.

**Table 1.** Effects of various drugs on histopathological changes in the Mongolian gerbil hippocampus 7 days after 5-min cerebral ischemia

<table>
<thead>
<tr>
<th>Drugs</th>
<th>dose (mg/kg, i.p.)</th>
<th>n</th>
<th>Incidence of neuronal changes (%)</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td></td>
<td>10</td>
<td>100 (%), 0 (%), 0 (%), 0 (%)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>10</td>
<td>0 (%), 0 (%), 0 (%), 0 (%)</td>
</tr>
<tr>
<td>Minaprine</td>
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<td>0 (%), 0 (%), 0 (%), 100 (%)</td>
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<tr>
<td></td>
<td>50 (p.o.)</td>
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<td>20 (%), 20 (%), 10 (%), 50 (%)</td>
</tr>
<tr>
<td></td>
<td>75 (p.o.)</td>
<td>10</td>
<td>70 (%), 0 (%), 10 (%), 20 (%)</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>10 (p.o.)</td>
<td>10</td>
<td>0 (%), 0 (%), 0 (%), 100 (%)</td>
</tr>
<tr>
<td></td>
<td>20 (p.o.)</td>
<td>9</td>
<td>0 (%), 0 (%), 0 (%), 100 (%)</td>
</tr>
<tr>
<td></td>
<td>50 (p.o.)</td>
<td>9</td>
<td>88.9 (%), 11.1 (%), 0 (%)</td>
</tr>
<tr>
<td>Diazepam</td>
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<td></td>
<td>60 (p.o.)</td>
<td>10</td>
<td>100 (%), 0 (%), 0 (%), 0 (%)</td>
</tr>
<tr>
<td>Idebenone</td>
<td>100 (p.o.)</td>
<td>10</td>
<td>0 (%), 0 (%), 0 (%), 100 (%)</td>
</tr>
<tr>
<td></td>
<td>200 (p.o.)</td>
<td>10</td>
<td>0 (%), 0 (%), 20 (%), 80 (%)</td>
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<tr>
<td>Ethylapovincamine</td>
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<td>10</td>
<td>10 (%), 10 (%), 0 (%), 80 (%)</td>
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<td>20 (%), 0 (%), 10 (%), 70 (%)</td>
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<td>Nicardipine</td>
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<td>10</td>
<td>0 (%), 0 (%), 0 (%), 100 (%)</td>
</tr>
</tbody>
</table>

Ischemic neuronal damage was graded on a scale of 0–3: score 0 (−), normal neurons; score 1 (+), a few neurons damaged (as few as one neuron damaged); score 2 (++), many neurons damaged; and score 3 (+++), majority of neurons damaged.
four vessels for 300 sec. The CA1 neurons in the hippocampus may not be destroyed under this condition.

We assumed that hippocampal damage, especially in the CA1 neurons, might be related to the passive avoidance deficits. In the present experiment, when the destruction of CA1 neurons was prevented by minaprine, pentobarbital, diazepam and ethylapovincamine, the passive avoidance deficit was also significantly attenuated. On the contrary, with Ca**-hopantenate, nicardipine and idebenone, the destruction of CA1 neurons was not prevented, and the passive avoidance deficit was not ameliorated. Furthermore, when bilateral occlusion of common carotid arteries was applied for 1, 2, 3, 4 or 5 min, the response latencies in the test trial decreased as a function of the duration of ischemia. The ischemic cell changes in the CA1 neurons in the hippocampus also became severe as the duration of ischemia was increased. These results indicate that hippocampal damage, especially of the CA1 neurons as revealed by histopathological findings, may relate to behavioral deficits indicated by impairment of the passive avoidance tasks. However, there were instances that a destruction of the CA1 neurons was apparent in a gerbil that showed good performance in the passive avoidance test. Conversely, there were cases where in spite of the prevention of CA1 neuronal destruction, the latency in the passive avoidance task was short. As the passive avoidance test was performed 2 or 3 days after the occlusion and the animals were killed 7 days after the occlusion, this time lag may relate to the discrepancies of passive avoidance deficit and destruction of CA1 neurons. We are currently performing detailed investigations on the relationship between passive avoidance deficit and the destruction of CA1 neurons in the hippocampus.

References
2 Kirino, T.: Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res. 239, 57-69 (1982)