Changes in Locomotor Activity and Passive Avoidance Task Performance Induced by Cerebral Ischemia in Mongolian Gerbils

Yasuko Karasawa, PhD; Hiroaki Araki, PhD; Susumu Otomo, PhD

Background and Purpose. We investigated changes in locomotor activity, passive avoidance task performance, and hippocampal CA1 neurons induced by cerebral ischemia in Mongolian gerbils to examine the relationship between these behavioral changes and CA1 neuronal damage.

Methods. Spontaneous locomotor activity was measured using the open field method before and 1, 3, 7, 14, or 28 days after 1- to 5-minute occlusion of the bilateral common carotid arteries. Locomotor activity after the second episode of 5-minute ischemia was also measured at 1-month intervals. The passive avoidance task was performed 7 or 28 days after induced ischemia. Histopathological changes in CA1 neurons after ischemia were assessed.

Results. Locomotor activity was increased 1 and 3 days after induced ischemia but not 14 and 28 days later. When the gerbils were again subjected to 5-minute ischemia 1 month after the initial 5-minute induced ischemia, locomotor activity even 1 day later was significantly increased. In contrast, passive avoidance impairment depended on the duration of ischemia, as determined 7 and 28 days after induced ischemia. Hippocampal CA1 neuronal damage was progressive, that is, changes in CA1 neurons were apparent even 1 day after 5 minutes of induced ischemia, and the CA1 neurons disappeared 7 days after 5 minutes of ischemia.

Conclusions. Passive avoidance impairment after ischemia is related to damage of CA1 neurons. Changes in locomotor activity after induced ischemia do not seem to be linked to CA1 neuronal damage. (Stroke. 1994;25:645-650.)

Key Words • cerebral ischemia • hippocampus • neuronal death • gerbils

Neurons in the CA1 region of the hippocampus in Mongolian gerbils are selectively vulnerable to ischemia, and these neurons often show a delay in morphologically obvious cell death termed "delayed neuronal death" after reversible ischemia. The hippocampus is an area of the brain related to memory and habitation, and lesions of this region lead to behavioral abnormalities. We reported that severe behavioral abnormality in the passive avoidance task was apparent when tests were performed 3 or 14 days after the bilateral induction of 5 minutes of ischemia in Mongolian gerbils. Changes in locomotor activity after cerebral ischemia have also been reported. However, there are differences in the data on locomotor activity. Mileson and Schwartz found that locomotor activity was increased 1, 4, and 28 days after 5 minutes of ischemia in gerbils, whereas Kuroiwa et al reported that hyperactivity occurred 7 hours after 5 minutes of ischemia in gerbils.

In the present experiment we investigated locomotor activity and passive avoidance task performance after cerebral ischemia induced in Mongolian gerbils. It has been reported that learning ability, locomotion, and emotion can have effects on performance of the passive avoidance task. This study was warranted because it is important to know whether passive avoidance impairment induced by cerebral ischemia contributes to post-ischemic hyperactivity and whether these behavioral changes are related to damage of hippocampal CA1 neurons. The relation among changes in locomotor activity, passive avoidance task performance, and CA1 neuronal damage was also examined.

Materials and Methods. Male Mongolian gerbils supplied by Shin Nihon Dobutsu and weighing 60 to 90 g were housed in an air-conditioned room at 22±1°C. Light was provided on a 12-hour light/dark cycle with lights off at 7 PM. Food and water were provided ad libitum. All the animals had become thoroughly familiar with being handled. Each group consisted of eight to 10 animals.

The gerbils were anesthetized 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 sympathetic nerves were separated, as described. The arteries were clamped with aneurysm clips for 1, 2, 3, 4, or 5 minutes; the clips were then removed, and the skin was sutured. Sham-operated animals were treated in the same manner except for the absence of clamping. The rectal temperature was kept close to 37°C during ischemia and until 3 hours after ischemia, using a heating lamp and a heating pad.

The gerbils were trained in a conventional step-down type of passive avoidance apparatus that was divided into safe and grid parts. The experimental chamber (22.5x20.0x19.5 cm) was made of acrylic fiber. The floor had a grid of stainless-steel rods and a 2-mA scrambled shock generator (Neuroscience, Inc). The safe part (20.0x9.5x3.0 cm) was made of acrylic fiber and was fixed at one side of the chamber. Training in passive avoidance was carried out 6 or 27 days after induced ischemia. Each animal was placed initially on the safety platform. When the gerbil stepped down onto the grid floor, it received a foot shock. Although the gerbils repeatedly stepped
The hippocampal region, cut coronally into 3- to 4-mm-thick slices, was embedded in paraffin and processed for histological examination. The CA1 neurons in the hippocampus were readily visible. Light microscopic examination of the hippocampus at 1 day of recirculation after 5 minutes of ischemia revealed that the neuronal damage was 1(+) in 50% of the gerbils, thereby indicating that a few neurons were damaged. In the 1-, 2-, 3-, and 4-minute occlusion groups, no histopathological changes of CA1 neurons were observed. When duration of ischemia exceeded 3 minutes, the severity of neuronal damage was 3(++) in 100% of gerbils at 7 days after ischemia (Table).

Results

The training sessions for the passive avoidance task were carried out after bilateral carotid artery occlusion. When the test trial was performed 7 days later, the response latency was 59.3±0.7 seconds (mean±SE) in the sham-operated gerbils. In gerbils subjected to common carotid artery occlusion, the response latency in the 7-day test trial decreased and depended on ischemic duration. When duration of ischemia exceeded 2 minutes, the response latency was significantly shorter than that in sham-operated gerbils (P<0.01) (Fig 3, top panel). In contrast, the response latency in the sham-operated gerbils was 55.9±4.1 seconds (mean±SE) when the test trial was performed 28 days after induced ischemia. Decrement in the response latency depended on the duration of ischemia and was significantly different from findings in sham-operated gerbils subjected to 3 minutes of ischemia (P<0.01), 4 minutes of ischemia (P<0.05), or 5 minutes of ischemia (P<0.01) (Fig 3, bottom panel).

Locomotor activity was measured before and 1, 3, 7, 14, or 28 days after ischemia in the first experiment. At 1 day after ischemia locomotor activity increased and depended on duration of ischemia. In the gerbils subjected to 4 or 5 minutes of ischemia, locomotor activity was significantly different from that in the sham-operated gerbils (P<0.05, P<0.01, respectively). At 3 or 7 days after ischemia the increase in locomotor activity was also apparent but was less than that on 1 day after ischemia. On days 14 or 28 after ischemia no significant changes in locomotor activity were evident (Fig 4). In the second experiment the gerbils were subjected to two 5-minute sessions of ischemia at 1-month intervals. After the second ischemic episode, the entire population of CA1 neurons in the hippocampus was readily visible. Light microscopic examination of the hippocampus at 1 day of recirculation after 5 minutes of ischemia revealed that the neuronal damage was 1(+) in 50% of the gerbils, thereby indicating that a few neurons were damaged. In the 1-, 2-, 3-, and 4-minute occlusion groups, no histopathological changes of CA1 neurons were observed. When duration of ischemia exceeded 3 minutes, the severity of neuronal damage was 3(++) in 100% of gerbils at 7 days after ischemia (Table).
FIG 2. Photographs show grade of neuronal damage in hippocampal CA1 neurons. Top left, 0(-): normal neurons; top right, 1(+): a few neurons damaged (as few as one neuron damaged); bottom left, 2(++): many neurons damaged; bottom right, 3(+++): majority of neurons damaged. Neurons in the hippocampal CA1 region showed degeneration of Nissl bodies, destruction, or disappearance (arrowheads). DG indicates dentate gyrus. Original magnification x10.
Discussion

The severity of hippocampal CA1 neuronal damage was progressive and depended on the duration of ischemia. Destruction of CA1 neurons appeared from 1 or 3 days after 5 minutes of ischemia and disappeared for the most part within 7 days. At 7 days after the ischemic insult, 100% of those in the groups with more than 3 minutes of ischemia were graded 3(++++), thereby indicating that the majority of neurons were damaged. It would thus appear that 3 minutes of ischemia is the mildest insult leading to a severe alteration in CA1 neurons.

When the passive avoidance test was performed 7 or 28 days after ischemia, passive avoidance impairment was evident and depended on duration of ischemia. We reported that passive avoidance impairment also occurred 3 days after the ischemia and depended on duration of ischemia. Passive avoidance impairment was evident in the group with neuronal damage in the hippocampal CA1 region. All these results suggest that hippocampal damage, especially in CA1 neurons, might be related to impairment in passive avoidance task performance.

Because an increase in locomotor activity may possibly account for this impairment in passive avoidance task performance, we investigated changes in locomotor activity after cerebral ischemia. Locomotor activity in the open field method increased, dependent on ischemic duration, at 1 and 3 days after the ischemic insult. However, this increase in locomotor activity gradually disappeared and at 14 and 28 days after ischemia locomotor activity did not differ from that in the sham-operated group in all ischemic groups. These results suggest that passive avoidance impairment does not contribute to increases in locomotor activity. The increase in locomotor activity does not appear to be related to destruction and disappearance of CA1 neurons.

In addition to histopathological changes in hippocampal CA1 neurons, the relation of functional changes of CA1 neurons to locomotor activity should be considered. Suzuki et al. reported that CA1 neurons showed hyperactivity on the first day after 5 minutes of ischemia. In our study we examined the effect of two separate ischemic insults on locomotor activity to determine whether functional change to CA1 neurons would alter the locomotion. On the 28th day after the first 5 minutes of ischemia, no change in locomotor activity was evident. When the gerbils were subjected to the second ischemic insult, hyperlocomotion was apparent even the next day, and the hippocampal CA1 neurons had completely disappeared.
Histopathological Changes in CA1 Neurons in Hippocampus After Cerebral Ischemia in Mongolian Gerbils

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Neuronal damage was graded on a scale of 0 to 3: 0(−), normal neurons; 1(+), a few neurons damaged (as few as one neuron damaged); 2(++), many neurons damaged; and 3(+++), majority of neurons damaged.

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Fig. 5. Line graph shows effects of two 5-minute episodes of ischemia on locomotor activity in Mongolian gerbils. The interval between sessions was 1 month. Locomotor activity was measured 1 day before and 1, 3, and 7 days after the second ischemic episode induced by bilateral common carotid artery occlusion (occl). Locomotor activity was assessed as total moving distance (in centimeters) for 3 minutes \( (n=8) \). *\( p<0.05 \), **\( p<0.01 \) significantly different from sham-sham-operated group (the first and second ischemic episodes were sham operated).
on that same day. We propose that functional abnormality in CA1 neurons may not be the cause of hyperlocomotion after cerebral ischemia.

The nucleus accumbens or striatum is related to motor function.18 Miyamoto et al17 reported that continuous hyperactivity was induced after bilateral lesioning of the nucleus accumbens. Shreve and Uretsky16 reported that locomotor activity can be elicited by excitatory amino acids in the lateral preoptic area. In contrast, it was found that neurotransmitters such as excitatory amino acids, dopamine, and norepinephrine are released during and after ischemia.19-21 In addition, various types of receptors were seen to undergo changes in the presence of cerebral ischemia. Benfenati et al22 stated that mRNA of D2 dopamine receptors decreased after transient cerebral ischemia in the rat striatum, and Nishino and Davis23 found that [3H]prazosin binding to gerbil forebrain membranes was reduced during cerebral ischemia. These changes in the release of some neurotransmitters in regions such as nucleus accumbens or striatum may relate to the increase in locomotor activity after cerebral ischemia. We are currently investigating mechanisms involved in hyperlocomotion after cerebral ischemia.

Acknowledgment

We thank M. Ohara for editorial assistance.

References

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Editorial Comment

Two of the most important end points in experimental cerebral ischemia are histological changes and the functional impairment. The article by Karasawa and colleagues deals with the important issue of trying to connect the functional impairment induced by ischemia to the histologically demonstrable neuronal damage. In gerbils subjected to ischemia by bilateral carotid artery occlusion, there is progressive damage to the CA1 neurons in the hippocampus. The animals display behavioral changes manifested by increased locomotor activity that is transient and impaired passive avoidance. Karasawa and colleagues show that the impairment in passive avoidance correlates with the neuronal damage in the hippocampus, suggesting that the two are causally related, whereas the changes in locomotor activity bear no relation to the hippocampal neuronal damage and hence are probably due to other mechanisms. This type of functional histological correlation may facilitate understanding of research results in experimental cerebral ischemia.

Hermes A. Konto, MD, PhD
Associate Editor for Basic Science
School of Medicine
Medical College of Virginia
Richmond, Va
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Y Karasawa, H Araki and S Otomo

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