Age-Related Vulnerability to Cerebral Ischemia in Spontaneously Hypertensive Rats

Hiroshi Yao, MD; Seizo Sadoshima, MD; Hiroaki Ooboshi, MD; Yuji Sato, MD; Hideyuki Uchimura, MD; and Masatoshi Fujishima, MD

Background and Purpose: We sought to determine the effects of aging on regional cerebral blood flow and ischemic brain damage in transient cerebral ischemia in rats.

Methods: Five adult (5–6 months) and five aged (18–22 months) female spontaneously hypertensive rats were subjected to 20 minutes of bilateral carotid occlusion and 60 minutes of recirculation under amobarbital anesthesia (100 mg/kg i.p.). Regional cerebral blood flow in the hippocampus and striatum was measured using the hydrogen clearance method. Nine adult and 14 aged rats were subjected to 20 minutes of bilateral carotid occlusion or were sham-operated under ether anesthesia. Seven days after 20 minutes of cerebral ischemia, the rats' brains were perfusion fixed. Ischemic damage in the hippocampus and striatum was graded (0 [normal] to 3 [majority of neurons damaged]).

Results: After 20 minutes of bilateral carotid occlusion, striatal cerebral blood flow decreased to 9.1±1.5 and 3.9±2.0 ml/100 g/min in aged and adult rats, respectively, and hippocampal cerebral blood flow decreased to 8.6±2.4 and 5.7±2.4 in aged and adult rats, respectively. Although these ischemic cerebral blood flow values were not significantly different between the two age groups, scores for ischemic damage in the hippocampus CA-1 subfield and striatum were significantly higher in aged than in adult rats (p<0.05, Kruskal-Wallis' h test with Bonferroni correction).

Conclusions: We conclude that aging may be a primary factor in the development of greater ischemic neuronal damage observed in aged hypertensive rats. (Stroke 1991;22:1414-1418)
as previously described.2,4,5,9,10 Teflon-coated platinum CBF electrodes were placed stereotaxically in the right striatum (0.5 mm anterior, 2.5 mm lateral to the bregma and 4.5 mm from the brain surface) and in the right hippocampus CA-1 subfield (4 mm posterior, 1.5 mm lateral to the bregma and 3.3 mm from the brain surface). The reference Ag-AgCl electrode was inserted under the skin. After completion of the surgery, 30 minutes was allowed before the experiment. After two or three baseline CBF values were determined, the right common carotid artery was ligated tightly for 20 minutes followed by 60 minutes of recirculation by removing the ligature. Cerebral blood flow was determined after 10 and 20 minutes of carotid occlusion, and 10, 30, and 60 minutes of recirculation.

Fourteen aged (18–22 months old; weight, 195–241 g) and nine adult (5–6 months old; weight, 200–240 g) female SHRs were used for histological examination. Systolic blood pressure was determined with a tail-cuff method (model MK-1000, Muromachi-Kikai, Tokyo) without anesthesia. Transient cerebral ischemia was induced in SHRs by bilateral carotid occlusion as previously described. Briefly, the rats were anesthetized with ether, both common carotid arteries were exposed through a ventral midline incision in the neck and occluded with Sugita aneurysm clips for 20 minutes, or were sham-operated. Eight of the 14 aged rats and five of the nine adult rats were randomly assigned to bilateral carotid occlusion. At the end of 20 minutes of bilateral carotid occlusion, carotid blood flow was restored by releasing the clips. Two aged rats that died during the ischemic or posts ischemic period were excluded from the study. Seven days after the 20-minute bilateral carotid occlusion, rats were anesthetized with amobarbital (100 mg/kg i.p.) and the brains were perfused with 4% paraformaldehyde in 1/15 M phosphate buffer (pH 7.3) via the left ventricle of the heart after a brief wash-out period with heparinized saline. The brains were removed and fixed in 4% neutral formaldehyde for 7 days. Paraffin sections were taken at the level of the striatum and hippocampus in each rat and were stained with hematoxylin and eosin. The sections were examined under a light microscope. Ischemic neuronal damage in the striatum and hippocampus CA-1 subfield was graded from 0 to 3 (3, majority of neurons damaged; 2, many neurons damaged; 1, a few neurons damaged; or 0, normal) by one of the authors (Y.S.) without any knowledge about the age of the SHR and experimental conditions.

All values were expressed as mean±SEM. Differences in physiological parameters, mean arterial blood pressure, carotid back pressure, and CBF between adult and aged SHR were analyzed using the unpaired t test. Mean scores for ischemic damage among aged and adult ischemic or sham-operated groups were compared using the nonparametric Kruskal-Wallis’ h test with Bonferroni correction; differences in systolic blood pressure were compared using analysis of variance and Scheffe’s test.

Table 1 summarizes mean values for arterial acid-base parameters, hematocrit, and blood glucose before and 20 minutes after carotid occlusion and 60 minutes of recirculation.

## Results

Table 1 summarizes mean values for arterial acid-base parameters, hematocrit, and blood glucose before and at 20 minutes of ischemia and 60 minutes of recirculation. There were no differences in physiological parameters between adult and aged SHRs except for Pco2 at 60 minutes of recirculation. Resting mean arterial blood pressure in aged SHRs was 193±9 mm Hg, which tended to be higher than 173±5 mm Hg in adult SHRs (not significant). Resting carotid back pressure of 128±10 mm Hg in aged SHRs was significantly higher (p<0.05) than 93±10 mm Hg in adult SHRs (Figure 1). After 10 minutes of carotid occlusion, carotid back pressure decreased markedly to 33±5 and 34±10 mm Hg in aged and adult SHRs, respectively, and returned toward the pres ischemic levels after the release of the ligature. The mean values for carotid back pressure in aged SHRs at 30 and 60 minutes of recirculation were also significantly higher than those in adult SHRs. Resting striatal and hippocampal blood flow values were not significantly different between the two age groups (Figure 2). At 20 minutes after carotid occlusion, striatal blood flow decreased to 9.1±1.5 and 3.9±2.0 ml/100 g/min in aged and adult SHRs, respectively, and hippocampal blood flow decreased to 8.6±2.4 and 5.7±2.4 ml/100 g/min in aged and adult SHRs, respectively. Cerebral blood flow reductions during ischemia and also CBF recoveries during 60 minutes of recirculation were not significantly different between the two age groups.

Systolic blood pressure of the sham-operated groups was significantly higher in aged (235±5 mm Hg) than adult SHRs (195±11, p<0.05, analysis of variance and Scheffe’s test); however, systolic blood pressure of the ischemic groups was not signif-
FIGURE 1. Changes in mean arterial blood pressure (MABP) and carotid back pressure (CBP) during ischemia and after recirculation. Bars represent SEM (n=5). *p<0.05 vs. adult spontaneously hypertensive rats. Resting carotid back pressure/MABP ratios were 0.66±0.02 and 0.53±0.05 in aged and adult rats, respectively, being significantly higher in aged than in adult rats (p<0.05). , Aged; , adult.

FIGURE 2. Changes in cerebral blood flow during ischemia and recirculation. Bars represent SEM (n=5). Resting striatal blood flow values were 57.1±7.2 and 57.8±5.6 ml/100 g/min in adult and aged spontaneously hypertensive rats, respectively. Resting hippocampal blood flow values were 47.9±11.0 and 34.5±3.5 ml/100 g/min in adult and aged rats, respectively. Cerebral blood flow values during cerebral ischemia were not significantly different between the two age groups. Although the extents of hyperemia in the striatum and hippocampus at 10 minutes of recirculation were higher in adult than in aged rats, the differences were not statistically significant. , Aged; , adult.

FIGURE 3. Scores for ischemic damage. 3, Majority of neurons damaged; 2, many neurons damaged; 1, a few neurons damaged; 0, normal. Bars represent mean values of scores for ischemic damage in aged ischemic spontaneously hypertensive rats; 1.92±0.42 and 0.92±0.23 for hippocampus CA-1 and striatum, respectively. *p<0.05 vs. adult ischemic rats and aged sham-operated (Sham-op) rats, Kruskal-Wallis' h test with Bonferroni correction. , Aged; , adult.

Unilaterally moderate damage in one each, slight bilateral damage in three rats, and no damage in one. Figure 3 summarizes histological findings in the hippocampus CA-1 subfield and striatum. Scores for ischemic damage in the CA-1 subfield and striatum of
aged SHRs were significantly higher than those of adult SHRs. Typical histological findings are demonstrated in Figure 4.

Discussion

According to neuropathological studies on the postischemic brain damage, the most vulnerable sites for cerebral ischemia are the CA-1 pyramidal cells of the hippocampus, the medium to small sized neurons of the striatum, the Purkinje neurons of the cerebellum, and the cells in layer 3, 5, and 6 of the cerebral cortex. Our present study revealed that aging is an important factor for the development of neuronal damage induced by cerebral ischemia in such vulnerable brain regions as the hippocampus and striatum. A greater reduction in CBF could be expected in aged than in adult rats following carotid occlusion, although both striatal and hippocampal CBF were not significantly different between aged and adult female SHRs, as shown in this study and our previous study. Cerebral blood flow in aged SHRs begins to decrease at a higher blood pressure level under controlled hemorrhagic hypotension, which is caused by a more marked upward shift of the lower limit of CBF autoregulation in aged SHRs. Our present study, however, indicated that the degree of CBF reduction after bilateral carotid occlusion was not different between the two age groups of SHRs. This is probably caused by a marked bilateral carotid occlusion-induced fall in the perfusion pressure represented in carotid back pressure, which is probably too low to maintain CBF in both age groups. The critical values of lower carotid back pressure limit are 25 and 50 mm Hg in normotensive and hypertensive rats, respectively.

Further study is needed to exclude the possibility that more severely deranged energy metabolisms, such as reduction in high energy phosphates, occur in aged SHRs in comparison to adult SHRs during cerebral ischemia of a similar grade. Another possibility explaining the mechanism of neuronal damage in aged SHRs would be a reduction in the diffusion of O2 through the vessel wall due to long-standing hypertension rather than the aging process.
It is generally accepted that a release of the excitatory neurotransmitter glutamate in association with severe ischemia is one of the critical factors in the development and progression of ischemic neuronal damage in the CA-1 pyramidal neurons. In the striatum, released dopamine rather than glutamate may be responsible for the ischemic striatal damage. Dopamine depletion protects the striatal neurons from ischemia-induced cell death. These results suggest that both neurotransmitters, glutamate and dopamine, released during ischemia aggravate ischemic tissue damage in selectively vulnerable brain regions such as the hippocampus and striatum. Thus, it might be necessary to compare the amounts of striatal dopamine and hippocampal glutamate released during ischemia in adult and aged SHRs.

Ischemia-induced changes in receptors for the released neurotransmitters, then, are also of interest, since ischemic neuronal damage may largely depend on the ischemia-induced neurotransmitter release or neural transmission as discussed above. Striatal dopamine D1 receptors and hippocampal glutamate receptors decrease 7 days after 20–30 minutes of forebrain ischemia. These decreased receptor densities might reflect the loss of neurons with the specific receptors. Therefore, further studies on ischemia-induced changes in the densities of receptors in aged as well as in adult SHRs are also needed to clarify the pathophysiology for age-related vulnerability to cerebral ischemia.

In conclusion, our study revealed that aging may be the primary contributor to the development of ischemic neuronal damage in the hippocampus and striatum in hypertensive rats.

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