Cerebral Blood Flow and Ischemia-Induced Neurotransmitter Release in the Striatum of Aged Spontaneously Hypertensive Rats

Hiroshi Yao, MD; Hiroaki Ooboshi, MD; Setsuro Ibayashi, MD; Hideyuki Uchimura, MD; and Masatoshi Fujishima, MD

Background and Purpose: We found age-related vulnerability to cerebral ischemia in the hippocampus and striatum in spontaneously hypertensive rats. Further study revealed that ischemia-induced release of hippocampal taurine, an inhibitory amino acid, was reduced by 40% in aged rats compared with adult rats, which suggested an impaired inhibitory function against excitotoxicity in aged rats. The purpose of this study was to examine whether ischemia-induced neurotransmitter release is altered in the striatum of aged spontaneously hypertensive rats.

Methods: Five adult (5–6 months) and five aged (18–22 months) female spontaneously hypertensive rats were subjected to 20 minutes of cerebral ischemia induced by bilateral carotid artery occlusions and 120 minutes of recirculation under amobarbital anesthesia (100 mg/kg i.p.). Cerebral blood flow was determined using the hydrogen clearance method, and extracellular concentrations of neurotransmitters were determined with the brain dialysis technique in the striatum.

Results: During ischemia, cerebral blood flow in aged rats decreased to 8.7±1.2 (mean±SEM) mL/100 g per minute (11% of the resting), which was not different from 5.2±1.7 mL/100 g per minute (8% of the resting) in adult rats, and extracellular dopamine and amino acids (glutamate, aspartate, and taurine) increased by approximately 170- and 10-30-fold, respectively, and returned to baseline after 20–40 minutes of recirculation. These values of neurotransmitters, however, were not different between aged and adult rats during ischemia and reperfusion.

Conclusions: It is unlikely that a presynaptic mechanism is involved in age-related vulnerability in the striatum of hypertensive rats.

See Editorial Comment, page 580

I
schastic neuronal damage in the hippocampus and striatum, regions highly vulnerable to ischemia-induced injury, is produced by 20 minutes of transient ischemia in aged but not in adult spontaneously hypertensive rats (SHRs). Because cerebral blood flow (CBF) during ischemia and recirculation was not different between aged and adult SHRs, aging per se (i.e., the aging process of neurons) may be a major factor in the age-related vulnerability to cerebral ischemia in SHRs. In the hippocampal CA-1 subfield, we demonstrated that ischemia-induced release of taurine, one of the inhibitory amino acids, was diminished by 40% in aged SHRs compared with adult SHRs, whereas amounts of released glutamate were the same between the two age groups. Hence, it was inferred that an inhibitory neuromodulator function to protect brain

See Editorial Comment, page 580

against excitotoxicity is impaired in aged SHRs. In the striatum, both dopamine and glutamate released during ischemia play an important role in the development of ischemic striatal damage. Relative glucose hypermetabolism or marked metabolism/flow uncoupling observed in the striatum after transient ischemia is assumed to be the evidence for excitotoxicity, which may be caused by the massive release of neurotransmitters such as glutamate and/or dopamine. The purpose of this study was to examine whether ischemia-induced neurotransmitter release is altered in the striatum of aged SHRs.

Materials and Methods

Five aged (18–22 months, 195–255 g) and five adult (5–6 months, 200–245 g) female SHRs, which were maintained in the Kyushu University Animal Center under specific pathogen-free conditions, were used in this study. Female rats were used because female SHRs live longer than 20 months with a relatively low mortality rate (approximately 30% at 20 months), whereas approximately half of 15-month-old male SHRs die during 15–20 months of age. Under amobarbital anesthesia (100 mg/kg body wt i.p.), one femoral artery was cannulated for recording of mean arterial blood pres-
ure and anaerobic sampling of blood. Both common carotid arteries were exposed through a ventral midline incision in the neck, carefully separated from the vagosympathetic trunks, and loosely encircled with sutures for later ligation. The rat's head was fixed in a head holder, and one burr hole was made on the skull for inserting a CBF electrode and a dialysis probe. The hydrogen clearance technique\textsuperscript{13,14} was chosen for CBF determination. A dialysis probe, 300 \mu m in outer diameter (CMA/10 microdialysis probe, 3-mm dialysis membrane, Carnegie Medicine, Sweden), and a Teflon-coated platinum CBF electrode attached with a thermocouple probe, 300 \mu m in diameter, were placed stereotaxically in the right striatum (0.5 mm anterior and 3.0 mm lateral to the bregma and 4.5 mm from the brain surface) as previously described.\textsuperscript{11-16} The reference Ag–AgCl electrode was inserted under the skin. The dialysis probe was perfused with Ringer's solution (154 mmol/L Na\textsuperscript{+} 147, Ca\textsuperscript{2+} 2.5, K\textsuperscript{+} 4, and Cl\textsuperscript{−} 155.5) at a flow rate of 4 \mu l/min with a microperfusion pump (EP-60, Eicom Corp., Kyoto, Japan). Perfusates were collected every 20 minutes into plastic tubes. Brain temperature in the striatum was kept at 36–37°C before carotid occlusion with a heat lamp and monitored but not controlled during cerebral ischemia and recirculation. Rectal temperature was kept close to 37°C throughout the experiment. After completion of the surgery, 60 minutes was allowed before measurements of baseline parameters. During the next 60 minutes of the resting period, two baseline CBF values were determined and three baseline perfusates were collected. Then both common carotid arteries were ligated tightly for 20 minutes. CBF was determined at 20 minutes of ischemia and 10, 30, 60, and 120 minutes of recirculation. We confirmed the locations of CBF and dialysis probes and the absence of macroscopic trauma at the end of the experiment.

Dopamine and amino acid concentrations were determined using high performance liquid chromatography as previously described. Briefly, 20 \mu l of the perfusate was injected directly into a high performance liquid chromatograph with an electrochemical detector immediately after sampling for determination of dopamine and its metabolites (3,4-dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA]). The chromatographic system consisted of an L-6000 pump (Hitachi Ltd., Tokyo) set to flow at a rate of 1.0 mL/min, a reverse-phase column (Eicompak MA-50DS, 4.6x150 mm, Eicom), and a fluorescent detector (RF-535, Shimazu). The mobile phase was 0.1 M sodium phosphate (pH 6.0) containing 30% (vol/vol) methanol.

Values are mean±SEM (n=5 in each group). SHRs, spontaneously hypertensive rats; MABP, mean arterial blood pressure.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>Recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aged SHRs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>177±4</td>
<td>179±5</td>
<td>152±4</td>
</tr>
<tr>
<td>PCO\textsubscript{2} (mm Hg)</td>
<td>44.3±0.8</td>
<td>29.7±1.7</td>
<td>43.2±2.5</td>
</tr>
<tr>
<td>Po\textsubscript{2} (mm Hg)</td>
<td>73.6±3.4</td>
<td>99.0±4.9</td>
<td>77.8±4.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.01</td>
<td>7.54±0.01</td>
<td>7.41±0.01</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42±2</td>
<td>43±3</td>
<td>41±3</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>74±5</td>
<td>123±14</td>
<td>117±17</td>
</tr>
<tr>
<td>Adult SHRs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>175±4</td>
<td>166±13</td>
<td>156±3</td>
</tr>
<tr>
<td>PCO\textsubscript{2} (mm Hg)</td>
<td>42.5±0.3</td>
<td>27.5±3.6</td>
<td>43.1±2.0</td>
</tr>
<tr>
<td>Po\textsubscript{2} (mm Hg)</td>
<td>81.6±1.8</td>
<td>100.8±4.2</td>
<td>79.4±2.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.40±0.01</td>
<td>7.54±0.04</td>
<td>7.38±0.02</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42±1</td>
<td>41±2</td>
<td>40±2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>78±8</td>
<td>112±13</td>
<td>109±14</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The statistical differences in physiological parameters, CBF, and extracellular dopamine or amino acids between aged and adult SHRs were analyzed by the unpaired t test.

Results

There were no differences in physiological parameters between aged and adult SHRs (Table 1). Changes in striatal CBF and extracellular concentrations of dopamine and amino acids are summarized in Table 2. Striatal CBF decreased to 8.7±1.2 mL/100 g per minute (11% of the resting) and 5.2±1.7 ml/100 g per minute (8% of the resting) after bilateral carotid artery occlusion in aged and adult SHRs, respectively. Striatal brain temperature dropped by 1.1–1.8°C during 20 minutes of cerebral ischemia and returned to baseline level after recirculation. Extracellular dopamine concentrations increased by approximately 170-fold (approximately 0.9 \mu M in the perfusate) during ischemia in both age groups (Table 2). During 20 minutes of ischemia, glutamate concentrations increased by 24- and 29-fold (approximately 6–7 \mu M) and taurine increased by 10- and 12-fold (9.5 \mu M) in aged and adult SHRs, respectively. These concentrations returned to baseline by 20–40 minutes of reperfusion. No significant differences in the concentrations of dopamine and amino acids were demonstrated between aged and adult SHRs. The “excitotoxic index”\textsuperscript{6} data calculated as (glutamate)×(glycine–threonine peak)/(taurine) were not different between the two age groups. Changes in metabolites of dopamine (DOPAC and HVA) during ischemia and recirculation did not reach statistical significance compared with the respective resting values.

Discussion

CBF in aged SHRs begins to decrease at a higher blood pressure level under controlled hemorrhagic hy-
potension. However, the present results as well as our previous study demonstrated that striatal CBF reduction during 20 minutes of bilateral common carotid artery occlusions was not different between adult and aged SHRs. It seems somewhat paradoxical that the reductions in CBF after carotid occlusion were not different between the two age groups because cerebral vasoreactivity is known to be impaired in chronic hypertension. In SHRs, vascular hypertrophy and remodeling result in encroachment, endothelium-dependent vasodilator response is impaired, and CBF through collaterals is decreased compared with normotensive rats. However, despite hypertrophy and remodeling of cerebral arteries in hypertensive rats, Baumbach et al showed that the distensibility of the arteriolar wall is increased during long-standing hypertension. They concluded that cerebral arteries may adapt to long-standing hypertension with minimal impairment of functional characteristics to protect brain from reduced perfusion pressure or cerebral ischemia. Although further study is needed to clarify the pathophysiology of age-related vulnerability to cerebral ischemia. Another factor in addition to excitotoxicity must be considered as a mechanism responsible for vulnerability to cerebral ischemia in aged SHRs. It is known that the metabolism of dopamine or serotonin through monoamine oxidase, the activity of which is increased in aged brain, produces potentially toxic hydrogen peroxide. In the latter study, an increased survival rate of rats was associated with a marked decrease in extracellular DOPAC and HVA during reperfusion. Our present study, however, failed to demonstrate significant elevations of extracellular DOPAC and HVA above preischemic levels during reperfusion in both age groups. Thus, our results do not support the concept that hydrogen peroxide produced through enhanced monoamine oxidase activity in aged SHRs is involved in age-related striatal vulnerability. In conclusion, ischemia-induced changes in extracellular dopamine, glutamate, and taurine were not different between adult and aged SHRs in the striatum. It is unlikely that a presynaptic mechanism is involved in age-related vulnerability of the striatum in aged hypertensive rats.

In the hippocampal CA-1 subfield, ischemia-induced taurine release was selectively reduced among several amino acids measured in aged SHRs. However, the amounts of ischemia-induced neurotransmitters released in the striatum were not different between aged and adult SHRs in the present study. Therefore, a presynaptic mechanism or altered neurotransmitter release is not involved in the striatal vulnerability of aged SHRs. However, age-related changes in receptors for the released neurotransmitters should be considered because ischemic neuronal damage may depend largely on neural transmission, and increased sensitivity to excitotoxic substances in aged SHRs could provide an explanation for the age-related vulnerability. Age-related sensitivity to kainate neurotoxicity has been reported, suggesting that kainate and N-methyl-D-aspartate receptors could be sensitive mediators for excitotoxic pathology in the aged brain. Further studies on age-related changes in glutamate and dopamine receptors in SHRs are needed to clarify the pathophysiology of age-related vulnerability to cerebral ischemia. Another factor in addition to excitotoxicity must be considered as a mechanism responsible for vulnerability to cerebral ischemia in aged SHRs. It is known that the metabolism of dopamine or serotonin through monoamine oxidase, the activity of which is increased in aged brain, produces potentially toxic hydrogen peroxide. In the latter study, an increased survival rate of rats was associated with a marked decrease in extracellular DOPAC and HVA during reperfusion. Our present study, however, failed to demonstrate significant elevations of extracellular DOPAC and HVA above preischemic levels during reperfusion in both age groups. Thus, our results do not support the concept that hydrogen peroxide produced through enhanced monoamine oxidase activity in aged SHRs is involved in age-related striatal vulnerability. In conclusion, ischemia-induced changes in extracellular dopamine, glutamate, and taurine were not different between adult and aged SHRs in the striatum. It is unlikely that a presynaptic mechanism is involved in age-related vulnerability of the striatum in aged hypertensive rats.

**References**


Editorial Comment

This study represents an extension of previously published work from this laboratory with respect to the effects of aging on ischemic outcome. In a previous publication, Dr. Yao and colleagues reported hippocampal and striatal vulnerability after 20 minutes of transient global ischemia in aged but not in adult spontaneously hypertensive rats (SHRs). To determine whether age-related striatal vulnerability was due to neurochemical or hemodynamic differences, the authors compared ischemia-induced neurotransmitter release and ischemic cerebral blood flow in adult and aged SHRs. Their data indicate that ischemic severity and elevations in extracellular dopamine, glutamate, aspartate, and taurine are similar in the two age groups. The major implications of this work are that increased ischemic vulnerability of aged SHRs is not due to differences in ischemic severity or altered neurotransmitter release.

Although this is a negative study, these data are important in that they suggest that mechanisms other than presynaptic events are involved in age-related ischemic vulnerability. In this regard, the authors provide speculation concerning age-related changes in neuronal receptor sensitivity to neurotransmitters. Such speculation is consistent with previous reports indicating that the density and distribution of glutamate and N-methyl-D-aspartate (NMDA) binding sites as well as degrees of NMDA-induced neurotoxicity change during brain development. Finally, this article underscores the need to conduct experimental investigations using animal models that incorporate stroke risk factors (age and hypertension) in an attempt to more closely mimic the clinical conditions of stroke.

W. Dalton Dietrich, PhD, Guest Editor Department of Neurology University of Miami School of Medicine Miami, Fla.

References


Cerebral blood flow and ischemia-induced neurotransmitter release in the striatum of aged spontaneously hypertensive rats.
H Yao, H Ooboshi, S Ibayashi, H Uchimura and M Fujishima

*Stroke*. 1993;24:577-580
doi: 10.1161/01.STR.24.4.577

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/24/4/577

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/