Mechanism of Increased Sensitivity to Cerebral Ischemia Following Carotid Artery Occlusion In Stroke-prone Spontaneously Hypertensive Rats: Importance of Genetic Factors

MASAHIRO SUNO, PH.D., MITSURU KAKIHANA, M.S., MASAKI SHIBOTA, PH.D., AND AKINOBU NAGAOKA, PH.D.

SUMMARY Neurological symptoms and cerebral metabolism following bilateral carotid artery occlusion (BCAO) were observed in stroke-prone spontaneously hypertensive rats (SHRSP), stroke-resistant SHR (SHRSR), normal Wistar-Kyoto rats (WKY) and the F₁ and F₂ hybrids between SHRSP and SHRSR or WKY.

Systolic blood pressure recorded before BCAO was found to rank in the following order: SHRSP > F₁ (SHRSP × SHRSR) > SHRSR > F₁ (SHRSP × WKY) > WKY. The effect of BCAO in these rats tended to be proportional to the blood pressure. F₁ (SHRSP × WKY) was more sensitive to brain ischemia than SHRSR. In addition, though none of the SHRSR (average blood pressure 155 mm Hg) developed acute stroke symptoms, many animals of the F₁ generations, in which the blood pressure was equal to or lower than that of SHRSR, developed stroke symptoms. Lactate and ATP changes in the F₁ generations did not correlate with the blood pressure.

The results suggest that genetic factors may play an important role in susceptibility to brain ischemia in the stroke-prone rats.

BILATERAL CAROTID artery occlusion (BCAO) caused severe ischemic damages of the brain in spontaneously hypertensive rats (SHR), but not in normotensive Wistar-Kyoto rats (WKY). In the brain of SHR, blood flow was markedly reduced. Anaerobic metabolites were increased, and adenosine triphosphate (ATP) was decreased after BCAO. These facts suggest that hypertension accelerates cerebral ischemia following carotid artery occlusion. Fujishima et al. and Ogata et al. speculated that hypertension and its related increase in cerebral vascular resistance play important roles in the higher susceptibility to brain ischemia in SHR.

The stroke-prone strain of spontaneously hypertensive rats (SHRSP), established by Okamoto et al., showed a steeper rise of blood pressure at an early stage of hypertension and a much higher incidence of spontaneous stroke than did a stroke-resistant strain of the rats (SHRSR). In the present study, neurological deficit and changes in cerebral metabolites due to BCAO were observed in SHRSP, SHRSR and normal WKY, and the F₁ and F₂ hybrids in order to elucidate the involvement of genetic factors in the higher susceptibility to cerebral ischemia.

Materials and Methods

Male stroke-prone SHR (SHRSP), stroke-resistant SHR (SHRSR), control Wistar-Kyoto rats (WKY), F₁ (SHRSP × SHRSR), F₁ (SHRSP × WKY), F₂ (SHRSP × SHRSR) and F₂ (SHRSP × WKY) hybrids, at 10 weeks of age, were used for the present study. The source of the parental strains of rats and mating procedures have been previously described. The animals were anesthetized with ether. Both common carotid arteries were exposed by a ventral middle cervical incision, separated carefully from vagosympathetic trunks and ligated with silk sutures simultaneously. The total operative time was less than 7 min. Sham-operated animals were used as controls.

Behavioral Observation. The animals were observed for 8 hours and at 24 hours after BCAO. Neurological symptoms observed in SHRSP are shown in table 1.

In rats that had no ischemic seizure within 8 hours the time was recorded as 480 minutes.

Cerebral Metabolites. A rat was placed in a microwave applicator (Toshiba, Model TMW-6402) to irradiate the head with microwaves at 5 kw for 1.5 sec. The brain was removed and a piece of parieto-frontal cortex and adjacent white matter (about 400 mg) were cut out, weighed and placed into a chilled tube containing 4 ml of 0.3 N perchloric acid and homogenized. The tissue homogenate, kept at 0°C to 4°C, was centrifuged and neutralized with 1.5 N potassium carbonate at pH 6.0-7.0. Lactate and ATP were determined by standard enzymatic methods. The results were expressed as mmol per kg wet weight of tissue.

Blood Pressure Measurement and Statistical Analysis. Systolic blood pressure was measured in the tail artery of conscious animals the day before the experiment using a pulse-pick up method. Results were expressed...
TABLE 1 Neurological Symptoms Induced by Brain Ischemia Following BCAO in SHRSP

<table>
<thead>
<tr>
<th>Average time (min) after BCAO</th>
<th>Neurological symptoms</th>
</tr>
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<tbody>
<tr>
<td>0-30</td>
<td>normal slight decrease of spontaneous motor activity or circling behavior no walking and slight weakness of hind limb or asymmetrical posture</td>
</tr>
<tr>
<td>30-60</td>
<td>weakness of fore and hind limbs, but righting reflex still present weakness of fore and hind limbs, and no righting reflex</td>
</tr>
<tr>
<td>60-150</td>
<td>piloerection and tremor jumping, impulsive running and seizure (ischemic seizure)</td>
</tr>
<tr>
<td>150-420</td>
<td>dyspnea, somnolence or coma death</td>
</tr>
</tbody>
</table>

as mean ± SEM and statistical analysis was performed by Student's t-test, Fisher's exact-test or linear regression analysis.

Results

1. Blood Pressure and Neurological Symptoms in the Parental Strains and the F1 hybrids

Systolic blood pressure in the animals used for the neurological and biochemical observations is shown in tables 2 and 3. The blood pressure was lower in the following rank order: SHRSP > F1 (SHRSP X SHRSR) > SHRSR > F1 (SHRSP X WKY) > WKY. The blood pressure of the F1 groups was intermediate between those of their corresponding parental strains.

The time course of onset of an ischemic seizure following BCAO is shown in figure 1A. In SHRSP, all animals showed a decrease in spontaneous motor activity, weakness of hind limb or circling behavior within 30 min, and marked weakness of fore and hind limbs within 60 min after BCAO. The incidence of ischemic seizures was 40% at one hour and 100% at 3 hours after the occlusion. In SHRSR and WKY, no ischemic seizure was observed within 8 hours. F1 (SHRSP X SHRSR) and F1 (SHRSP X WKY) were intermediate in the frequency of ischemic seizures between their corresponding parental strains. The time course of mortality following BCAO is shown in figure 1B. All SHRSP had dyspnea and coma, and died from respiratory arrest within 8 hours following BCAO. The mortality rate in F1 (SHRSP X SHRSR) and F1 (SHRSP X WKY) was 70% and 15% respectively 8 hours after BCAO. No animals of WKY or SHRSR died within 8 hours. The mortality rate 24 hours after BCAO in WKY, SHRSR, F1 (SHRSP X SHRSR) and F1 (SHRSP X SHRSR) was 5, 20, 40 and 95%, respectively.

The mean values of the time required for the onset of ischemic seizure and death are summarized in table 3, in which statistical analysis was performed on the frequency of behavioral changes. The sensitivity to cerebral ischemia decreased in the following rank order: SHRSP > F1 (SHRSP X SHRSR) > F1 (SHRSP X WKY) > SHRSR > WKY. The frequency of ischemic seizure for F1 (SHRSP X WKY) was higher (p = 0.053) compared with that in SHRSR, although the blood pressure of the F1 rats was significantly lower than that of SHRSP.

2. Cerebral Metabolites in the Parental Strains and the F1 Hybrids

Changes in cerebral lactate concentration are shown in figure 2. In SHRSP, cerebral lactate increased 6 times as high as the control level at 30 min and 10 times at 2 hours after BCAO. In SHRSR, the lactate concentration increased slightly the increment being 3 times 4 hours after the occlusion. No change in the lactate level was observed in WKY. F1 (SHRSP X WKY) showed a greater change than did the SHRSR. The differences between F1 (SHRSP X WKY) and SHRSR were significant (p < 0.05) at 1 and 2 hours.

Cerebral ATP in SHRSP markedly decreased to 10% of the control level 2 hours after the occlusion. In F1 (SHRSP X SHRSR), the concentration of ATP

TABLE 2 Systolic Blood Pressure and Times Required for the Onset of an Ischemic Seizure and Death in SHRSP, SHRSR, WKY, and the F1 Hybrids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number</th>
<th>Blood pressure (mm Hg)</th>
<th>Ischemic seizure (min)</th>
<th>Death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRSP</td>
<td>20</td>
<td>187 ± 2 [ &lt; 0.001]</td>
<td>83 ± 9 [ &lt; 0.01]</td>
<td>219 ± 19</td>
</tr>
<tr>
<td>F1 (SHRSP X SHRSR)</td>
<td>20</td>
<td>166 ± 2 [ &lt; 0.01]</td>
<td>241 ± 22 [ &lt; 0.01]</td>
<td>385 ± 23</td>
</tr>
<tr>
<td>SHRSR</td>
<td>20</td>
<td>155 ± 3 [ &lt; 0.01]</td>
<td>&gt; 480 [ &lt; 0.1]</td>
<td>&gt; 480</td>
</tr>
<tr>
<td>F1 (SHRSP X WKY)</td>
<td>20</td>
<td>145 ± 2 [ &lt; 0.001]</td>
<td>430 ± 24 [ &lt; 0.1]</td>
<td>470 ± 7</td>
</tr>
<tr>
<td>WKY</td>
<td>20</td>
<td>115 ± 1 [ &lt; 0.01]</td>
<td>&gt; 480 [ &lt; 0.1]</td>
<td>&gt; 480</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
Blood pressure was measured the day before BCAO.

[ ] p value for statistical difference between each successive group. Blood pressure was analyzed by Student's t test, and ischemic seizure and death were tested in the incidence by Fisher's exact test at 8 hr after BCAO except ischemic seizure in SHRSP and F1 (SHRSP X SHRSR) at 3 hr.
Table 3  Cerebral Lactate Concentration 2 Hours after BCAO in SHRSP, SHRSR, WKY and the F₁ Hybrids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Blood pressure (mm Hg)</th>
<th>Lactate (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Occlusion</td>
</tr>
<tr>
<td>SHRSP</td>
<td>182 ± 2 (12)</td>
<td>1.7 ± 0.6 (4)</td>
</tr>
<tr>
<td></td>
<td>( p &lt; 0.001)</td>
<td>19.6 ± 0.4 (8)**</td>
</tr>
<tr>
<td>F₁ (SHRSP × SHRSR)</td>
<td>166 ± 2 (9)</td>
<td>1.7 ± 0.2 (4)</td>
</tr>
<tr>
<td></td>
<td>( p &lt; 0.05)</td>
<td>10.6 ± 0.5 (5)**</td>
</tr>
<tr>
<td>SHRSR</td>
<td>158 ± 3 (10)</td>
<td>1.8 ± 0.1 (4)</td>
</tr>
<tr>
<td></td>
<td>( p &lt; 0.01)</td>
<td>4.3 ± 0.5 (5)*</td>
</tr>
<tr>
<td>F₁ (SHRSP × WKY)</td>
<td>142 ± 4 (10)</td>
<td>1.8 ± 0.1 (5)</td>
</tr>
<tr>
<td></td>
<td>( p &lt; 0.001)</td>
<td>7.1 ± 1.1 (5)*</td>
</tr>
<tr>
<td>WKY</td>
<td>120 ± 2 (8)</td>
<td>2.0 ± 0.5 (4)</td>
</tr>
</tbody>
</table>

Values are mean ± s.e. ( ) number of animals.
* p < 0.01 vs control, ** p < 0.001 vs control.
[ ] p value for statistical differences between each successive group.

Figure 1. Time course of occurrence of an ischemic seizure (A) and death (B) following BCAO. SHRSP, • — •; F₁ (SHRSP × SHRSR), O—O; SHRSR, O—O; F₁ (SHRSP × WKY), ▲ — ▲; WKY, △ — △. Number of animals and statistical treatment are shown in table 2.

Discussion

SHRSP rats were much more sensitive to cerebral ischemia caused by carotid artery occlusion than SHRSR and WKY. All of SHRSP died within 8 hours after BCAO; the mean survival time was about 3.5 hours. Yamori et al. reported that the mortality decreased moderately and reached 50% of the control level 2 hours after the occlusion. In WKY, SHRSR and F₁ (SHRSP × WKY), the ATP remained unchanged, indicating that the change in ATP was less marked than in lactate.

In table 3, the average values for blood pressure and cerebral lactate concentration are summarized. The value for lactate determined 2 hours after the occlusion declined in the following order: SHRSP > F₁ (SHRSP × SHRSR) > F₁ (SHRSP × WKY) > SHRSR ≥ WKY. This order was comparable to that observed for neurological changes.

3. Relation Between Blood Pressure and Neurological or Biochemical Changes in F₁ Generations

The blood pressures ranged from 180 to 120 mm Hg in F₁ (SHRSP × WKY) and from 200 to 140 mm Hg in F₁ (SHRSP × SHRSR). The average blood pressure of the F₁ generations was 143 ± 2 and 166 ± 2 mm Hg, respectively. The times required for onset of an ischemic seizure and death following BCAO were 421 ± 17 and 459 ± 9 min in F₁ (SHRSP × WKY) and 277 ± 23 and 379 ± 16 min in F₁ (SHRSP × SHRSR), respectively. These values were comparable to those of their F₁ generations.

The relation between blood pressure and onset of an ischemic seizure in F₂ (SHRSP × WKY) and F₂ (SHRSP × SHRSR) are presented in figure 3 and for comparative purposes the data in SHRSR and F₁ (SHRSP × WKY) are also shown. It was noteworthy that 6 rats having blood pressure lower than 150 mm Hg developed an ischemic seizure. In F₂ (SHRSP × SHRSR), 16 of 22 F₂ hybrids, whose blood pressure was in the same range as the SHRSR (140-170 mmHg), developed an ischemic seizure.

The mean value of cerebral lactate concentration, measured 2 hours after the occlusion, was 10.9 ± 0.8 mmol/kg in F₂ (SHRSP × SHRSR) and 4.9 ± 0.5 mmol/kg in F₂ (SHRSP × WKY). No significant correlation was observed between the lactate concentration and blood pressure ( r = 0.14, p > 0.05) and between ATP concentration and blood pressure ( r = 0.10, p > 0.05) in the 2 F₂ generations.
our experiments, the carotid artery was exposed under light ether anesthesia, and in their experiments the carotid artery was ligated under pentobarbital anesthesia. The protective effect of barbiturates against ischemic damages in cerebral tissues has been repeatedly described.13,14

Choki et al.1 have shown that bilateral carotid artery ligation caused a much more pronounced decrease of cerebral blood flow in SHR than in WKY. In our SHRSP, cerebral blood flow was reduced markedly and was accompanied by a significant increase in lactate after BCAO. The pronounced reduction of blood flow in SHRSP is likely to be responsible for the higher frequency of an ischemic seizure and mortality.

The pathogenetic mechanism of cerebral ischemia caused by BCAO in SHR has been studied by Fujishima et al.4–6. They found that the increased vascular resistance due to persistent high blood pressure in SHR might be responsible for an upward shift of cerebral blood flow autoregulation and a marked reduction of the cerebral perfusion pressure after BCAO, resulting in severe brain ischemia. Ogata et al.7 showed that the distribution of cerebral arteries including the circle of Willis was essentially the same between SHR and WKY. This finding suggests that a functional mechanism in cerebral circulation rather than a morphological one may account for the increased sensitivity to brain ischemia in the SHRs.

In the present study, the average blood pressure was correlated with the degrees of neurological deficit and cerebral metabolic changes in SHRSP, SHRSR, WKY, and the hybrids, although there were some exceptions. These findings indicated that hypertension is responsible for the susceptibility to cerebral ischemia in the SHR. Such a relationship was not found for lactate change and the onset-time of an ischemic seizure in SHRSR and F1 (SHRSP × WKY). Therefore, F1 (SHRSP × WKY) hybrid, which had SHRSP-genes, appears to be more sensitive to the brain ischemia than SHRSR although the blood pressure was lower in F1 (SHRSP × WKY). This suggests an important role of genetic factors in the sensitivity to brain ischemia in the hypertensive rats. The speculation was

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**Figure 2.** Time course of changes in cerebral lactate concentration following BCAO. SHRSP, •—•; F1 (SHRSP × SHRSR), ○—○; SHRSR, ○—○; F1 (SHRSP × WKY), △—△; WKY, △—△. Each point represents mean ± SEM of 4–8 animals. The difference of lactate between SHRSR and F1 (SHRSP × WKY) is statistically significant (p < 0.05) at one and two hours after BCAO.

rate after BCAO was markedly higher in SHRs than in WKY, but the survival time was comparable in SHRSP (35 hours) with SHRSR (33 hours). This difference in the survival times of SHRSP seems to be due partly to the difference in the anesthetics used. In
supported by the following evidence: 1) ischemic seizures were observed in some of $F_2$ (SHRSP × WKY) rats whose blood pressures were lower than 150 mm Hg, but none of SHRSR, lower than 150 mm Hg, but none of SHRSR (average blood pressure; 155 mm Hg) developed an ischemic seizure, 2) 16 out of 22 $F_2$ (SHRSP × SHRSR) animals with the same range of blood pressure as the SHRSR developed an ischemic seizure, and 3) the correlations between blood pressure and cerebral metabolic changes in both $F_2$ generations were not significant. These results suggest that genetic factors in addition to those controlling blood pressure are involved in the susceptibility to the brain ischemia in SHRSP.

Nagaoka et al. have studied the relation of hypertension and onset of cerebrovascular lesions (stroke) in SHRSP. They demonstrated that the frequency of stroke was associated with SHRSP-gene concentration rather than with the level of blood pressure. Together with the present finding and their findings, hypertension-related cerebrovascular disorders in SHRSP appear to be caused by some special predisposition which may be determined by genetic factors in SHRSP.

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