Influence of Sex on Cerebral Ischemia Following Bilateral Carotid Occlusion in Spontaneously Hypertensive Rats: A Metabolic Study

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SUMMARY Cerebral lactate, pyruvate and adenosine triphosphate (ATP), and acid-base balance were measured in male and female spontaneously hypertensive rats (SHR) before, and 1, 3 and 5 hours after bilateral carotid occlusion.

In male SHR, cerebral lactate and the lactate/pyruvate (L/P) ratio progressively increased after occlusion, while cerebral ATP decreased. In female SHR, an increase in lactate and the L/P ratio was less marked than in male SHR. Cerebral ATP remained unchanged 5 hours after occlusion. These data suggest that bilateral carotid artery occlusion may cause more pronounced ischemic changes in the brain in male SHR than in female SHR, resulting in a greater increase in lactate with a concomitant decrease in ATP in male SHR. Results suggest that female SHR are more resistant to cerebral ischemia following bilateral carotid occlusion than male SHR. Blood pressure and gonads in the susceptibility to cerebral ischemia are discussed.

Methods

Adult SHR (Okamoto and Aoki)$^8$, aged 5–10 months, were used for the present study. Male SHR, weighing 300–450 g (339 ± 9 g: mean ± sem), and female SHR, weighing 200–300 g (221 ± 6 g), were anesthetized with intraperitoneal amobarbital 10 mg/100 g body weight. Both common carotid arteries were exposed through a ventral midline incision in the neck, the arteries were separated from the vagosympathetic trunk, and ligated simultaneously. After the skin incision was closed with silk sutures, the animals were returned to their cages except for those subjected to a 1 h study of carotid artery occlusion.

All SH rats were reanesthetized with amobarbital (10 mg/100 g) at 1 h before sacrificing. One femoral artery was cannulated for blood pressure recording with an electromanometer, and for anaerobic sampling to determine pH, Pco2 and Po2 with an IL meter (Model 113). The rectal temperature was maintained close to 37°C, and the animals spontaneously breathed room air. Support of ventilation was not required since the animals tended to over-rather than underventilate.

After arterial blood sampling, the head was immediately frozen in situ with liquid nitrogen. Animals were sacrificed at 1, 3 and 5 h intervals following bilateral carotid artery occlusion. Liquid nitrogen was applied to the skull using a funnel. The frozen whole brain was chiselled out and separated grossly into the
supra- and infratentorial portions.

Each part of the brain was weighed, rapidly ground and homogenized after the addition of 12 ml of ice-cold 1.0 N perchloric acid to the supratentorial tissue, and 5 ml to the infratentorial tissue. The tissue homogenate, maintained at 0° to 4°C, was centrifuged and neutralized with 3 N potassium hydroxide at a pH between 4.5 and 5.0. The supernatants were added to 0.5 M glycine buffer pH 9.0 for lactate analysis, and to 0.5 M triethanolamine buffer pH 7.6 for ATP. Substrates were determined by the enzymatic fluorometric techniques as follows: lactate and pyruvate by the lactate dehydrogenase reaction, and ATP by phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, phosphotriose isomerase and glycerol-1-phosphate dehydrogenase reactions. In control animals which had their carotid arteries exposed but not occluded, cerebral metabolites and arterial acid-base parameters were measured in a similar manner.

All data were expressed as mean ± standard error and statistical differences were calculated by Student's small sample t-test.

Results

1. Arterial Parameters

Average values for mean arterial pressure (MAP) and acid-base balance at various time intervals following carotid occlusion are summarized in table 1. In males, MAP rose at 1 h and arterial PCO₂ decreased with a reciprocal increase in pH. Then, MAP and arterial acid-base parameters returned to the control level or below at the 3 h or longer interval. In contrast, arterial PO₂ was unchanged at each time interval.

In females, arterial acid-base balance and MAP changed in a similar manner but less markedly in comparison with those in males.

2. Cerebral Metabolites

Mean values for lactate, L/P ratio and ATP of the supratentorial tissue at the various time intervals are summarized in table 2. In male SHR, supratentorial lactate greatly increased to 7 times the control at 1 h, 10 times at 3 h and 12 times at 5 h after occlusion. In a similar manner to the changes in lactate, the L/P ratio increased. Conversely, ATP markedly decreased at 1 h and to a lower level at 5 h after occlusion.

In female SHR, supratentorial lactate and the L/P ratio increased following carotid artery occlusion but not as much as they did in male SHR, and ATP remained unchanged. Between male and female groups, there were significant differences of lactate at 1 h and 5 h (P < 0.05), and ATP at 3 h and 5 h (P < 0.005). The difference of L/P ratio did not reach statistical significance.

Mean values for infratentorial lactate, L/P ratio and ATP are summarized in table 3. In males, infratentorial lactate and the L/P ratio progressively increased as the time passed, while ATP remained unchanged at 3 h but decreased at 5 h. In females, metabolic changes were minimal. There were no significant differences in infratentorial metabolites between male and female rats at each time interval. Low ATP values are, in part, due to autolytic changes. The differences observed for male SHR compared to female cannot be accounted for by autolytic artifact.

3. Pre-occluding MAP and Supratentorial Metabolites

Correlation between pre-occluding MAP and supratentorial lactate or ATP at 1 h after occlusion was investigated in all 12 SHR; 6 males and 6 females. There was a tendency to a greater increase in lactate and a decrease in ATP in the animals having a higher MAP, although this correlation was not significant (r = 0.51, P > 0.1 for lactate, r = -0.35, P > 0.2 for ATP).

Discussion

The pathogenetic mechanism of cerebral ischemia following bilateral carotid occlusion in SHR has been studied by Fujishima et al. who found that the lower blood pressure limit for cerebral autoregulation

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**Table 1** Mean Arterial Pressure (MAP) and Arterial Acid-Base Parameters at Various Time Intervals Following Bilateral Carotid Occlusion in Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control</th>
<th>Time interval following carotid occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of rats</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>178 ± 8</td>
<td>197 ± 7</td>
</tr>
<tr>
<td>pH</td>
<td>7.418 ± 0.013</td>
<td>7.623 ± 0.039</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>37.1 ± 2.2</td>
<td>22.0 ± 2.3</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>87.0 ± 6.2</td>
<td>90.0 ± 4.8</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of rats</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>152 ± 6</td>
<td>176 ± 4</td>
</tr>
<tr>
<td>pH</td>
<td>7.358 ± 0.002</td>
<td>7.508 ± 0.073(4)</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>39.5 ± 0.9</td>
<td>28.5 ± 3.2</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>87.3 ± 4.7</td>
<td>91.1 ± 12.2</td>
</tr>
</tbody>
</table>

Number in parenthesis denotes number of rats. Values are mean ± s.e.m.
TABLE 2  Supratentorial Metabolites at Various Time Intervals Following Bilateral Carotid Occlusion in SHR

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control</th>
<th>Time interval following carotid occlusion</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
</tr>
<tr>
<td>Male</td>
<td>No. of rats</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Lactate (mM/Kg)</td>
<td>1.82 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>L/P ratio</td>
<td>13.5 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>ATP (mM/Kg)</td>
<td>2.20 ± 0.11</td>
</tr>
<tr>
<td>Female</td>
<td>No. of rats</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lactate (mM/Kg)</td>
<td>1.99 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>L/P ratio</td>
<td>17.7 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>ATP (mM/Kg)</td>
<td>2.14 ± 0.12(5)</td>
</tr>
</tbody>
</table>

L/P ratio: lactate/pyruvate ratio. Number in parenthesis denotes number of rat, values are mean ± SEM, * statistical significance between males and females at \( P < 0.05 \), ** \( P < 0.005 \).

TABLE 3  Infratentorial Metabolites at Various Time Intervals Following Bilateral Carotid Occlusion in SHR

<table>
<thead>
<tr>
<th>Sex</th>
<th>Time interval following carotid occlusion</th>
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<tbody>
<tr>
<td></td>
<td>1 hour</td>
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<tr>
<td>Male</td>
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<td>Lactate (mM/Kg)</td>
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<tr>
<td></td>
<td>ATP (mM/Kg)</td>
</tr>
</tbody>
</table>

Number in parenthesis denotes number of rat. Values are mean ± SEM.

is shifted to a higher level in SHR than in normoten-
sive rats, while the cerebral perfusion pressure is
lowered to a greater extent after bilateral carotid
occlusion in SHR. They concluded that hypertension
and its related increase in cerebrovascular resistance
play an important role in the susceptibility to cerebral
ischemia in these animals.

In the present study, ischemic changes in brain
metabolism were more pronounced in male than in
female SHR. Although the level of blood pressure
differed between the 2 groups, i.e. higher in males than
females, there was no significant correlation
between the pre-occluding blood pressure level and 1 h
post-occlusion supratentorial lactate or ATP. These
metabolic differences are not explained merely by the
differences in blood pressure.

The influence of gonadal hormones must be con-
sidered as another possible causal factor for these
differences of susceptibility to cerebral ischemia. Eisenberg et al.\(^\text{13}\) have reported that oxygen uptake of the brain is higher in a castrated male rat than in a control. Gordan\(^\text{14}\) has reported that in typical pre-
adolescent testicular eunuchoidism or children, both
cerebral blood flow and cerebral oxygen uptake are
significantly higher than those in the normal adult
human. Payan,\(^\text{15}\) in the study of rats with adrenalec-
tomy and/or castration, found that brain ischemia
following bilateral carotid occlusion is significantly in-
fluenced by hormonal functions. Yamori et al.\(^\text{16}\) have
demonstrated in stroke-prone SHR that long-term ad-
ministration of estrogen, which attenuates hyperten-
sion, delays the development and also decreases the
incidence of stroke in males, while androgen, which
augments hypertension, accelerates and increases the
incidence of stroke in females. They also suggested
that estrogen treatment may directly affect collagenous protein synthesis in the aorta. Similarly, Kirk\(^\text{17}\) has reported that the lower concentrations of
vascular enzymes in the female aorta may be
associated with the lower susceptibility of premeno-
pausal women to atherosclerosis than mature men.

It is concluded that 2 main factors, blood pressure
and gonads, seem to be responsible for the sex
difference in susceptibility to cerebral ischemia follow-
ing bilateral carotid artery occlusion in SHR. From
the present study, however, it is not known whether
gonadal hormones may affect hypertension per se or
directly act upon cerebral blood flow and metabolism.

Acknowledgment

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Yamaguchi for technical assistance and also Miss Junko Hirakawa
for preparing this manuscript.

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concentrations in normotensive and spontaneously hyperten-
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Stroke-Associated Deaths in Washington County, Maryland, with Special Reference to Water Hardness

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K. J. HELSING, B.S., AND E. L. GOLDBERG, Sc.D.

SUMMARY Deaths associated with strokes from 1963 to 1975 among 36,860 adult residents of Washington County, Maryland, were studied in relation to the hardness of drinking water at home, assessed on the basis of 1,569 water samples taken during this period. There was no satisfactory evidence that water hardness was related to stroke mortality. Age was a strongly related factor. There was little or no association with sex, marital status, socio-economic status as reflected by education or housing, smoking history, or frequency of church attendance.

DEATH RATES from stroke have shown considerable variation by state and by region of the United States for the last 4 census years. The highest rates have tended to occur in the South Atlantic or East South Central regions, and the lowest in the Middle Atlantic or Mountain regions. Over this period, the ratio of rates in the highest to lowest states has been in the neighborhood of 2.0 and the ratio for regions a little less than 1.5. A study of death certification practices concluded that these differences in mortality from stroke were largely real.

Among the factors looked at as possible explanations of these geographic differences has been hardness of drinking water. In his pioneering study of water characteristics and strokes, Kobayashi reported a close relationship between death rates from apoplexy in Japan and a measure of water acidity, the ratio of sulfates to carbonates. Schroeder attempted to confirm this finding in the United States, but found only a slight correlation of the sulfate-bicarbonate ratio with coronary heart disease. Total hardness, however, showed significant associations with a number of cardiovascular causes of death. In his first paper, Schroeder merely mentioned that the correlation of stroke deaths with water hardness was significant for the age group 55–64. A later paper during the same year reported a correlation of −0.33 between the age-adjusted death rates from stroke in 1949–51 and the weighted average of the hardness of finished water by states. A slightly smaller negative correlation was noted in 1960 for the 88 largest cities in the U.S. When Schroeder looked at the 1960 mortality data for 201 standard metropolitan statistical areas by race, sex, and age, he found correlations of cerebral thrombosis death rates to be nonsignificant while those for cerebral hemorrhage were significantly negative only
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