Brain Metabolism Following Bilateral Carotid Occlusion in 2 Different Models of Experimental Hypertensive Rats

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SUMMARY Brain metabolites and arterial acid-base measurements were made one hr after bilateral carotid artery occlusion in 2 different models of hypertensive rats. Animals used included renovascular hypertensive rats (RHR) with an altered renin-angiotensin system and desoxycorticosterone hypertensive rats (DHR) with low plasma renin activity (PRA). The mean value for supratentorial lactate of 7.41 mM/kg in RHR was significantly higher than in DHR (3.90 mM/kg) or in control normotensive rats (3.10 – 2.56 mM/kg). Concomitantly, the lactate/pyruvate ratio tended to increase and ATP to decrease in RHR only. In these same rats (RHR) infratentorial lactate was also increased.

The results suggest that bilateral carotid occlusion leads to anaerobic metabolism of the brain in RHR but not in DHR, suggesting that the renin-angiotensin system may play some role in the susceptibility to cerebral ischemia following carotid occlusion in the hypertensive rats.

OUR PREVIOUS studies1, 2, 3 have shown a great increase in cerebral lactate and in the lactate/pyruvate ratio with a concomitant decrease in ATP following bilateral carotid artery occlusion in spontaneously hypertensive rats (SHR) and minimal metabolic changes in normotensive rats (NTR), suggesting that the hypertensive rats are more susceptible to cerebral ischemia than NTR. This has been proved by the pathological study which showed more diffuse and severe ischemic lesions of the brain in SHR.4,5

Brunner et al.6 have reported that hypertensive patients with high plasma renin activity (PRA) tend to have more severe vascular complications, such as stroke and myocardial infarction, than patients with normal or low PRA.

The present study was undertaken to clarify whether the susceptibility to cerebral ischemia following carotid artery occlusion differs between 2 different varieties of hypertensive rats: renovascular hypertensive rats and desoxycorticosterone hypertensive rats. In both types the duration and grade of hypertension was identical. The RHR rat has high-renin hypertension and the latter (DHR) rats have low-renin hypertension. In these animals, we measured lactate, pyruvate and ATP concentrations in the brain as an indicator of ischemic metabolism and also determined arterial acid-base measurements one hour after bilateral carotid occlusion.

Materials and Methods

Production of Hypertension

Male Wistar rats, weighing 150 to 200 g, were anesthetized with intraperitoneal amobarbital (10 mg/100 g body weight). In one group of animals the left renal artery was exposed through a dorsal incision of the abdomen and constricted with a silver clip to 0.2 mm in diameter. The contralateral kidney and its artery were left intact. After the operation, animals were fed with either a regular diet containing 0.6% NaCl or low salt diet containing 0.1% NaCl. Blood pressure was measured weekly by a tail-cuff technique. The animals in which blood pressure exceeded 160 mmHg on more than one occasion during 6 postoperative weeks were used in this study as renovascular hypertensive rats (RHR).

In another group of animals, left unilateral nephrectomy was performed. These rats were fed with a regular diet and ad libitum 1% saline solution and 20 mg of desoxycorticosterone acetate (DOCA) was administered subcutaneously weekly. The animals having a higher than 160 mmHg systolic pressure during the postoperative 6 weeks were defined as DOCA-salt hypertensive rats (DHR).

Three groups of normotensive rats (NTR) were prepared; in the first group, sham operation was performed without constricting the renal artery and a low sodium diet was given (Sham NTR : S-NTR). In the second group, unilateral nephrectomy was performed and a 1% saline solution was given without DOCA administration (Nephrectomized-salt NTR : N-NTR), and in the last group, no operation was performed and the normotensive rats were fed by regular diet and tap water (Control NTR : C-NTR).

Experimental Procedure

The animals were anesthetized with intraperitoneal amobarbital (10 mg/100 g) at the time of final study. One femoral artery was cannulated for blood pressure recording with an electromanometer and sampling of arterial blood. One femoral vein was cannulated for blood sampling and fluid infusion. Both common
carotid arteries were exposed through a ventral midline incision in the neck, and separated carefully from vagosympathetic trunks. Body temperature was kept close to 37°C. Each animal breathed room air spontaneously throughout the experiment.

One sample of 0.5 ml venous blood for PRA was taken in polyethylene tubes containing 0.2 ml of a 9% EDTA solution and kept in an ice bath. Then, angiotensin II analogue (1-Sar-8 Ile-angiotensin II) of 100 µg/kg body weight was infused intravenously and arterial blood pressure was monitored continuously for 40 min or longer until it returned closely to the pre-infusion level.

After the angiotensin II analogue test was completed, the first sample of arterial blood was obtained for gas analysis, and both carotid arteries were doubly ligated by silk sutures at the same time in all groups of rats but C-NTR. Thereafter, the animal was fixed in a head-holder and a plastic funnel was placed on the skull. Sixty min later, a second sample of arterial blood was withdrawn for gas analysis and the animal's head was frozen in situ by filling a plastic funnel with liquid nitrogen. The whole brain was chiselled out in a frozen state and separated grossly into supra- and infratentorial portions. In a rapid sequence, each part of the brain was mounted in cold perchloric acid for determinations of brain metabolites. After the animal was sacrificed, the heart was removed and weighed.

Determinations

PRA was determined by radioimmunoassay using homologous renin substrate, and expressed as nanograms of angiotensin I produced per ml of plasma per hr. Arterial pH, PCO₂ and PO₂ were determined by IL meter (model 113). Brain metabolites such as lactate, pyruvate and ATP were measured by standard enzymatic methods.

The animals which showed hypoxemia or hypercapnea during the experiment were excluded from the results. Excluding the effect of drug-induced transient hypotension on cerebral metabolism, the angiotensin II analogue was not administered in 2 RHR, so that only metabolic data were available in these animals. However, a statistical analysis was made as a whole, since the values in these 2 animals did not differ from those in the remaining RHR.

Results

Table 1 summarizes the average values for mean arterial pressure (MAP) and relative heart weight in each group. There was no difference in these measurements between RHR and DHR, indicating that the severity and duration of hypertension were almost identical.

PRA averaged 163.3 ± 55.1 (sd) ng/ml/hr in 6 RHR and 62.8 ± 11.2 ng/ml/hr in 5 DHR, its difference being significant (p < 0.02). MAP changes in response to angiotensin II analogue infusion are shown in figure 1. Immediately after the infusion in RHR, MAP started to fall to approximately 70% of

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**Table 1. Mean Arterial Pressure (MAP) and Relative Heart Weight (RHW)**

<table>
<thead>
<tr>
<th>Group</th>
<th>RHR (n=6)</th>
<th>DHR (n=6)</th>
<th>S-NTR (n=5)</th>
<th>N-NTR (n=4)</th>
<th>C-NTR (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>164 ± 18</td>
<td>161 ± 19</td>
<td>129 ± 11</td>
<td>144 ± 29</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>RHW (%)</td>
<td>0.42 ± 0.05</td>
<td>0.47 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>0.31 ± 0.02</td>
</tr>
</tbody>
</table>

RHR: renovascular hypertensive rats; DHR: desoxycorticosterone-salt hypertensive rats; S-NTR: sham-operated normotensive rats; N-NTR: unilateral nephrectomy-salt normotensive rats; C-NTR: control normotensive rats.

Values are mean ± sd.
the control level, and remained low for the next 20 min, followed by a gradual return to the preinfusion level, but DHR as well as NTR showed substantially no change. This suggests that the mechanism of hypertension was different, in that hypertension in RHR was produced by action of the renin-angiotensin system but not in DHR.

Average values for brain metabolites 60 min after carotid occlusion are summarized in Table 2. Supratentorial lactate averaged 7.41 mM/kg in RHR and 3.90 mM/kg in DHR, this difference being significant \( (p < 0.05) \). The former value (7.41 mM/kg) (RHR) was significantly higher than that for S-NTR rats \( (p < 0.01) \) or C-NTR rats \( (p < 0.005) \) and the latter value, (3.90 mM/kg) (DHR) was not different from that in N-NTR rats \( (p > 0.2) \) but significantly higher than that in control rats (NTR) \( (p < 0.05) \). Figure 2 shows individual values for lactate in each group.

In RHR, the lactate/pyruvate \( (L/P) \) ratio, an indicator of the redox state of cytoplasmatic NADH/NAD\(^+\), was increased but did not differ statistically from that in DHR. ATP value in RHR was significantly lower than that in C-NTR \( (p < 0.05) \) although it did not differ from that in DHR. Infratentorial metabolites changed little in all groups of animals but the RHR, in which lactate was higher than that in S-NTR \( (p < 0.05) \).

As shown in Table 2, arterial \( P_{CO_2} \) was consistently decreased after carotid occlusion in both RHR or DHR, although there was no difference between the two groups. In a similar manner, \( pH \) increased in some rats, but the levels did not differ between RHR and DHR. Arterial \( P_O_2 \) remained unchanged before and after carotid occlusion.

**Discussion**

RHR, a model of high PRA hypertension, showed a marked reduction of blood pressure in response to the angiotensin II analogue infusion, while DHR, a model of low PRA hypertension, showed no such change, indicating that the maintenance of hypertension in RHR was effected through the renin-angiotensin system, while in DHR by another mechanism. Although the duration, grade and severity of hypertension were almost identical, ischemic changes in brain metabolism following bilateral carotid occlusion were pronounced in RHR but not in DHR. Myers and Yamaguchi\(^*\) have reported that levels of lactate in the ischemic brain are a function of glucose levels in the blood, but there was no evidence of hyperglycemia in RHR or hypoglycemia in DHR.\(^{10}\) The present results suggest that the renal hypertensive rat is susceptible to cerebral ischemia following bilateral carotid occlusion, while the desoxycorticosterone hypertensive rat resists cerebral ischemia.

Brunner et al.\(^*\) concluded from their clinical observations that plasma renin may cause vascular changes

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**Table 2** Supratentorial and Infratentorial Metabolites Following Bilateral Carotid Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>RHR ( (n = 8) )</th>
<th>DHR ( (n = 8) )</th>
<th>S-NTR ( (n = 5) )</th>
<th>N-NTR ( (n = 4) )</th>
<th>C-NTR* ( (n = 6) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supratentorial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (mM/Kg)</td>
<td>7.41 ± 3.35*(^*)</td>
<td>3.90 ± 1.21*</td>
<td>2.56 ± 0.80</td>
<td>3.10 ± 0.67</td>
<td>2.42 ± 0.56</td>
</tr>
<tr>
<td>Pyruvate (mM/Kg)</td>
<td>0.206 ± 0.04*</td>
<td>0.192 ± 0.037*</td>
<td>0.158 ± 0.050</td>
<td>0.188 ± 0.032</td>
<td>0.132 ± 0.025</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>23.3 ± 7.5</td>
<td>20.5 ± 7.6</td>
<td>17.3 ± 5.9</td>
<td>18.6 ± 2.8</td>
<td>19.9 ± 6.9</td>
</tr>
<tr>
<td>ATP (mM/Kg)</td>
<td>1.64 ± 0.57*</td>
<td>2.11 ± 0.44</td>
<td>2.22 ± 0.25</td>
<td>2.18 ± 0.31</td>
<td>2.35 ± 0.28</td>
</tr>
<tr>
<td><strong>Infratentorial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (mM/Kg)</td>
<td>3.74 ± 0.87(^*)</td>
<td>3.72 ± 0.90</td>
<td>2.77 ± 0.41</td>
<td>3.06 ± 0.34</td>
<td>2.86 ± 0.53</td>
</tr>
<tr>
<td>Pyruvate (mM/Kg)</td>
<td>0.223 ± 0.083</td>
<td>0.221 ± 0.037</td>
<td>0.161 ± 0.056</td>
<td>0.222 ± 0.027</td>
<td>0.135 ± 0.027</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>18.7 ± 6.1</td>
<td>17.1 ± 4.0</td>
<td>20.0 ± 10.7</td>
<td>13.9 ± 1.4</td>
<td>24.9 ± 7.3</td>
</tr>
<tr>
<td>ATP (mM/Kg)</td>
<td>1.72 ± 0.53</td>
<td>1.90 ± 0.33</td>
<td>1.95 ± 0.25</td>
<td>2.17 ± 0.05</td>
<td>2.00 ± 0.33</td>
</tr>
</tbody>
</table>

**Note:** L/P ratio: lactate/pyruvate ratio. Values are mean ± SD.

\( *: p < 0.05 \) (RHR vs DHR), \( a: p < 0.05 \) (DHR vs S-NTR), \( b: p < 0.01 \) (RHR vs S-NTR), \( c: p < 0.05 \) (RHR vs C-NTR), \( d: p < 0.05 \) (DHR vs C-NTR), \( *: control rats without carotid occlusion. \)
such as angionecrosis or vasculopathy. Furthermore, Nagaoka and coworkers\(^{11}\) have recently demonstrated that stroke-prone SHR (SHR-SP), whose blood pressure was further raised by clipping the unilateral renal artery, have a higher incidence of stroke and a more marked vasculopathy in the brain than do the SHR-SP, whose blood pressure was raised by administration of DOCA-salt. They proposed the presence of an unknown factor of renal origin causing the spontaneous development of stroke in hypertensive rats.

Other than the action of renin on the vessels, the vascular response to humoral substances differs between RHR and DHR. The latter has more sensitivity to angiotensin II and renin, while the former is more sensitive to noradrenaline, tyramine and vasopressin.\(^{12,13}\) Such differences in vascular reactivity to humoral substances might be responsible in part for ischemic damage in the brain in RHR.

Although its mechanism is still uncertain from the present study, it is concluded that increased renin activity may affect the susceptibility to cerebral ischemia following carotid occlusion in hypertensive rats.

### Acknowledgment

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### References


### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>pH</th>
<th>Pco₂ (mm Hg)</th>
<th>Pco₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>7.457 ± 0.022</td>
<td>79.2 ± 12.6</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7.540 ± 0.094</td>
<td>76.8 ± 12.6</td>
</tr>
</tbody>
</table>

*Values are mean ± s.d. Paired test between C and E, *p < 0.05, **p < 0.005, ***p < 0.001.

The second arterial sample (E) was obtained at one hour interval from the first one (C) in C-NTR of which carotid arteries were not occluded.

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