Effects of Hyperventilation on Cerebral Blood Flow and Brain Tissue Metabolism in Normotensive and Spontaneously Hypertensive Rats

Takao Ishitsuka, M.D., Masatoshi Fujishima, M.D., Yasuo Nakatomi, M.D., Kinya Tamaki, M.D., and Teruo Omae, M.D.

SUMMARY Cerebral vascular carbon dioxide (CO₂) reactivities were compared in normotensive (NTR) and hypertensive (SHR) rats. Cerebral blood flow (CBF) in cortex and thalamus were evaluated before and during one hour of hyperventilation. After one hour of hyperventilation brain lactate, pyruvate, and ATP concentrations were also determined. Significant and similar reductions of CBF due to hyperventilation induced hypocapnia were found in both NTR and SHR groups. In contrast the percent increase in cerebrovascular resistance (CVR) per unit decrease in paco₂ was significant, indicating that hypocapnia induced vasoconstriction is greater in NTR than in SHR groups. During hyperventilation the average value for lactate in the NTR group was 3.98 mM/kg. In contrast it was 3.15 mM/kg in the SHR group, a significant difference (p < 0.05). When paco₂ fell below 15 mm Hg the cerebral lactate increased strikingly in the NTR group and cortical CVR was reduced suggesting that an accumulation of the ischemic metabolites caused dilatation of the constricted cerebral vessels. In contrast the SHR group disclosed no such changes. The increase CVR characteristic of SHR appeared to diminish the cerebral vasoconstrictive response to hypocapnia. As a result ischemic metabolites in the brain do not increase in this group to the degree that they do in NTR.

It has been known that hyperventilation leads to vasoconstriction of cerebral arteries with reduced cerebral blood flow (CBF) and cerebral oxygen tension, and consequently alters the brain metabolism. The electroencephalographic changes during hyperventilation are indistinguishable from those observed during cerebral hypoxia.

Our previous studies have demonstrated that spontaneously hypertensive rats (SHR) are more susceptible to cerebral ischemia than normotensive rats (NTR) following bilateral carotid occlusion, suggesting that the cerebral vasoreactivities to changes of the perfusion pressure might be different between SHR and NTR. In fact, the lower limit of cerebral autoregulation is significantly higher in SHR than NTR, and the increased cerebral vascular resistance due to the persistent high blood pressure is responsible for such upward shift of the autoregulation in SHR. These facts suggested that the cerebrovascular response to paco₂ is also different between SHR and NTR.

For this purpose, we studied cerebral circulation and brain metabolism before and during one hour of hyperventilation in hypertensive animals and normotensive controls.

Material and Methods

Adult male Wistar strain NTR and SHR aged 5 months or more, weighing 230 to 580g, were anesthetized with intraperitoneally administered amobarbital (100 mg/kg). After tracheotomy, the animals were immobilized with d-tubocurarine chloride and mechanically ventilated with a gas mixture of 70% N₂O and 30% of O₂. One femoral artery was cannulated to measure blood pressure and for anaerobic sampling of arterial blood for paco₂, po₂, and pH determinations with a Model 113 IL meter. The body temperature, measured in the rectum, was kept close to 37°C throughout the experiment.

The animals were divided into two experimental groups: one for measurements of local CBF and the other for determinations of brain tissue metabolites.

1. Cerebral Blood Flow

Twenty-two NTR and 22 SHR were used for this purpose. The animal’s head was fixed in a head holder, and two small burr holes were made on the skull 2 mm lateral to the bregma on each side for stereotaxic insertion of teflon-coated platinum electrodes, one in the parietal cortex (2mm in depth from the brain surface) and the other in the thalamus. After allowing more than 30 minutes to achieve a steady state, CBF was repeatedly measured using the hydrogen clearance technique with 10% hydrogen gas mixture initially, and at 5, 30, and 60 minute intervals after hyperventilation induced by increasing the animals’ respiratory rate. The blood pressure was continuously recorded and arterial samples were obtained before and 60 minutes after hyperventilation.

After completion of each animal study, the animal’s brain was grossly examined. One animal in which brain hemorrhage was found as a result of electrode insertion was not included in the present results.

2. Brain Tissue Metabolism

Twenty-one NTR and 24 SHR were studied. Each animal’s head was fixed in a head-holder and a plastic funnel was fitted into a skin incision over the bone skull. Following one hour of hyperventilation (16 NTR
and 19 SHR) or one hour of control normoventilation (5 NTR and 5 SHR), each head was frozen in situ by pouring liquid nitrogen into the plastic funnel. The whole brain was then chiselled out in the frozen state. In rapid sequence, the supratentorial portion of the frozen brain was weighed, ground, and homogenized after the addition of cold perchloric acid. The tissue homogenate, maintained at 0°C to 4°C, was centrifuged and neutralized with potassium hydrochloride at a pH of 4.5 to 5.0. Lactate, pyruvate and ATP were determined by a standard enzymatic method. During hyperventilation, MAP was significantly decreased from 64.5 to 34.7 ml/100g/min (54%) at 5 minutes after hyperventilation, and remained unaltered for the following 60 minutes. Thalamic CBF, being generally higher than cortical CBF, was also reduced to 55% in NTR and 50% in SHR after 60 minutes of hyperventilation. The degree of CBF reduction did not differ between the NTR and SHR groups.

On the other hand, cortical CVR was markedly increased to 175% of the resting value in NTR but only to 125% in SHR after 60 minutes of hyperventilation. Similarly, thalamic CVR rose to 174% in NTR but only 145% in SHR.

Figures 1 and 2 demonstrate a linear relation of pCO$_2$ to CBF in cortex and thalamus. The average increase in cortical CVR per unit decrease in pCO$_2$ (ranging from 15.8 to 48.8 mmHg) was 0.12 mmHg/ml/100g/min pCO$_2$ in NTR, being twice as large as the value of 0.06 in SHR. When pCO$_2$ was reduced by 1 mmHg, cortical CVR was increased by 5.9% in NTR but only by 3.2% in SHR (table 3). This difference is statistically significant ($p < 0.05$). There was, however, no thalamic CVR difference between the two groups.

In extreme hypocapnia (pCO$_2$ below 15.7 mmHg), the increased cortical CVR tended to decrease in NTR but not SHR (fig. 1). Therefore, the animals were arbitrarily divided into two subgroups defined by the pCO$_2$ level, i.e. one below 15.7 mmHg (extreme hypocapnia) and another ranging between 15.8 and 21.0 mmHg (severe hypocapnia) as shown in table 4. In NTR, cortical CBF in extreme hypocapnia averaged 2.86 ml/100g/min, significantly lower than that of 5.57 in severe hypocapnia, indicating that the constricted vessels tended to dilate in extreme hypo-

### Results

#### 1. Cerebral Blood Flow

Average values for mean arterial pressure (MAP) and arterial acid-base parameters before and one hour after hyperventilation are given in table 1. At the end of hyperventilation, MAP was significantly decreased in SHR but not in NTR. Arterial pCO$_2$ was significantly lowered to 18.6 mmHg (range 12.5–26.8) in NTR and to 20.7 mmHg (14.2–34.0) in SHR with a reciprocal rise in pH, respectively.

Average values for cortical and thalamic CBF, and cerebrovascular resistance (CVR) calculated from CBF and MAP before and during hyperventilation are tabulated in table 2. Cortical CBF in NTR was significantly decreased from 64.5 to 34.7 ml/100g/min (54% of the resting value) at 5 minutes after hyperventilation. It remained at the same level for a 60 minute period. Similarly, cortical CBF in SHR was significantly decreased from 60.0 to 33.2 ml/100g/min

### Table 1

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>MAP (mm Hg)</th>
<th>pCO$_2$ (mm Hg)</th>
<th>pO$_2$ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR resting</td>
<td>22</td>
<td>129 ± 4</td>
<td>38.9 ± 1.2</td>
<td>129.0 ± 8.8</td>
</tr>
<tr>
<td>HV</td>
<td>20</td>
<td>122 ± 6</td>
<td>18.6 ± 0.9‡</td>
<td>145.0 ± 10.8</td>
</tr>
<tr>
<td>SHR resting</td>
<td>22</td>
<td>191 ± 7</td>
<td>40.0 ± 1.4</td>
<td>141.0 ± 8.5</td>
</tr>
<tr>
<td>HV</td>
<td>17</td>
<td>143 ± 14†</td>
<td>20.7 ± 1.4‡</td>
<td>144.2 ± 12.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, †p < 0.01, ‡p < 0.005, $\ddagger$ p < 0.001 (vs resting value).

### Table 2

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>CBF (ml/100 g/min)</th>
<th>CVF (mm Hg/ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex</td>
<td>Thalamus</td>
</tr>
<tr>
<td>NTR resting</td>
<td>22</td>
<td>64.5 ± 5.5</td>
</tr>
<tr>
<td>HV 5 min</td>
<td>21</td>
<td>34.7 ± 2.3§</td>
</tr>
<tr>
<td>30 min</td>
<td>21</td>
<td>35.3 ± 2.6§</td>
</tr>
<tr>
<td>60 min</td>
<td>20</td>
<td>35.4 ± 2.9§</td>
</tr>
<tr>
<td>SHR resting</td>
<td>22</td>
<td>60.0 ± 4.1</td>
</tr>
<tr>
<td>HV 5 min</td>
<td>18</td>
<td>33.2 ± 4.5§</td>
</tr>
<tr>
<td>30 min</td>
<td>18</td>
<td>32.3 ± 2.8§</td>
</tr>
<tr>
<td>60 min</td>
<td>17</td>
<td>34.1 ± 3.3§</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.02, †p < 0.01, ‡p < 0.005, $\ddagger$ p < 0.001 (vs resting value).
EFFECTS OF HYPERVENTILATION ON CBF AND BRAIN TISSUE/Ishtuka et al.

2. Brain Tissue Metabolism

Average values for mean arterial pressure (MAP) and arterial acid-base parameters after one hour of normoventilation or hyperventilation are given in table 5. MAP was significantly lowered in both hyperventilated NTR and SHR in comparison with those in normoventilated animals. Arterial pCO₂ was significantly reduced to 17.1 (range 10.2–25.2) mmHg in hyperventilated NTR and to 18.9 (12.1–28.5) mmHg in SHR with a reciprocal increase in pH, respectively. There was no difference of pO₂ between normoventilation and hyperventilation.

Mean values for lactate, lactate/pyruvate (L/P) ratio and ATP of the brain are tabulated in table 6. During hyperventilation, lactate was increased to 3.98 mM/kg (167% of normoventilation) in NTR (p < 0.05) and 3.15 mM/kg (120%) in SHR: the latter was not significant. In both NTR and SHR, the L/P ratio tended to increase whereas ATP remained unchanged.

The relationship between lactate and pCO₂ is depicted in figure 3. Lactate started to increase at a pCO₂ below 20 mmHg in both NTR and SHR. At a pCO₂ of less than 15 mmHg, lactate steeply increased to 5.36 ± 0.58 mM/kg in NTR, being greater than that of 3.82 ± 0.18 mM/kg in SHR (p < 0.05).

The amount of lactate increase per unit reduction of pCO₂, ranging between 10 to 20 mmHg was 0.47 mM/kg/mmHg of pCO₂ in NTR and 0.17 in SHR; this difference was also statistically significant (p < 0.05), indicating that in NTR with severe hypocapnia lactate was accumulated excessively in the brain.

Discussion

The present results demonstrate that the cortical and thalamic CBF in both NTR and SHR decreased to approximately 55% of the resting value after one hour of hyperventilation. These findings are compatible with those in human studies of Alexander et al.¹ who found a 50% reduction of CBF in prolonged hypocapnia.

During hyperventilation, the blood pressure fell more markedly in SHR than NTR, probably due to reduction of cardiac output, although the blood pressure reduction did not exceed the lower limit of cerebral autoregulation.¹¹ Therefore, we used CVR, instead of CBF, as an indicator of the cerebrovascular reactivity to hypocapnia in the present study. The cerebrovascular CO₂ reactivity is proportional to vascular conductance,¹³ and a reciprocal of CVR of the resting state. NTR, in which the resting CVR was lower than that in SHR, might have been thought to have a lesser
vasoreactivity to CO₂. In fact, the percent increase of CVR in response to lowering of pacO₂ within a range 15.8 to 48.8 mm Hg was significantly greater in NTR than SHR, indicating that cerebral vessels in NTR are able to constrict more markedly than do those in SHR.

Tominaga et al., 14 however, found no difference of CBF reactivity to pacO₂ in the range of 20 to 55 mm Hg in normotensive and hypertensive humans who had no clinical signs nor angiographic findings of arteriosclerosis. These investigators did not mention CVR changes in hypocapnia in these subjects.

The lesser sensitivity to CO₂ in SHR might be due to the increased CVR, but it is not known from the present study whether an elevated CVR is simply due to morphological changes of the vessel walls such as fibrinoid degeneration 15 or to functional alterations such as sympathetic vascular tone. 16, 17 Johansson and Nilsson 18 who observed an increased CVR during hypercapnia-induced cerebral vasodilatation in SHR have concluded that the stiffness of the resistance vessels in SHR is due to structural changes of the vessel walls. Furthermore, Heistad et al. 19 have recently reported that maximal cerebral vasodilator responses to hypercapnia are diminished in atherosclerotic monkeys.

The CO₂ reactivity of cortical vessels was greater than in the thalamus in spite of the fact that resting cortical CBF was lower than thalamic CBF. The corti-

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**Table 3** Percent Increase of Cerebrovascular Resistance (CVR) Per Unit Reduction of pacO₂ Ranging 15.8-48.8 mm Hg in Normotensive (NTR) and Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th>pCO₂ (mm Hg)</th>
<th>Cortex</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.0-15.8</td>
<td>2.86 ± 0.31 (6)*</td>
<td>3.01 ± 0.53 (6)</td>
</tr>
<tr>
<td>15.7</td>
<td>2.96 ± 0.44 (8)</td>
<td>3.30 ± 1.23 (3)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.01 (vs pCO₂ 21.0-15.8 mm Hg). Number in the parentheses denotes number of rats.

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**Table 4** Cerebrovascular Resistance (CVR) at pCO₂ Ranging 21.0-15.8 mm Hg and Below 15.7 mm Hg in Normotensive (NTR) and Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th>pCO₂ (mm Hg)</th>
<th>Cortex</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.0-15.8</td>
<td>2.86 ± 0.31 (6)*</td>
<td>3.01 ± 0.53 (6)</td>
</tr>
<tr>
<td>15.7</td>
<td>2.96 ± 0.44 (8)</td>
<td>3.30 ± 1.23 (3)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.01 (vs pCO₂ 21.0-15.8 mm Hg). Number in the parentheses denotes number of rats.
Table 5: Mean Arterial Pressure (MAP) and Arterial Acid-base Parameters at One-hour Normoventilation (NV) and Hyperventilation in Normotensive Rats (NTR) and Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>MAP (mm Hg)</th>
<th>PCO₂ (mm Hg)</th>
<th>PO₂ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR NV</td>
<td>5</td>
<td>138 ± 6</td>
<td>36.5 ± 0.7</td>
<td>186.3 ± 28.8</td>
<td>7.327 ± 0.026</td>
</tr>
<tr>
<td>HV</td>
<td>16</td>
<td>116 ± 4*</td>
<td>17.1 ± 1.2†</td>
<td>148.3 ± 13.6</td>
<td>7.584 ± 0.022†</td>
</tr>
<tr>
<td>SHR NV</td>
<td>5</td>
<td>195 ± 4</td>
<td>37.0 ± 0.8</td>
<td>152.4 ± 19.5</td>
<td>7.379 ± 0.024</td>
</tr>
<tr>
<td>HV</td>
<td>19</td>
<td>168 ± 5*</td>
<td>18.9 ± 1.1†</td>
<td>145.2 ± 10.7</td>
<td>7.546 ± 0.019†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.05, †p < 0.001 (vs corresponding NV).

Table 6: Cerebral Lactate, Lactate/Pyruvate (L/P) Ratio and ATP in NTR and SHR with One-hour Normoventilation (NV) and Hyperventilation (HV)

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>Lactate (mM/kg)</th>
<th>L/P ratio</th>
<th>ATP (mM/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR NV</td>
<td>5</td>
<td>2.39 ± 0.08</td>
<td>17.6 ± 1.2</td>
<td>2.61 ± 0.11</td>
</tr>
<tr>
<td>HV</td>
<td>16</td>
<td>3.98 ± 0.36*</td>
<td>23.9 ± 1.9</td>
<td>2.65 ± 0.11</td>
</tr>
<tr>
<td>SHR NV</td>
<td>5</td>
<td>2.63 ± 0.04</td>
<td>18.7 ± 2.5</td>
<td>2.56 ± 0.17</td>
</tr>
<tr>
<td>HV</td>
<td>19</td>
<td>3.15 ± 0.17</td>
<td>23.6 ± 2.3</td>
<td>2.35 ± 0.15</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.05 (vs corresponding NV).

There have been many reports describing an increase in lactate in the brain as well as the cerebrospinal fluid during hyperventilation. According to MacMillan and Siesjö’s observations, cerebral lactate in rats was increased to 3.42 mM/kg at pACO₂ 21.1 mmHg and 5.25 mM/kg at pACO₂ 13.5 mmHg after 20 minutes of hyperventilation. These values are very similar to our present results in NTR but not in SHR; in SHR an increase in lactate per unit reduction of pACO₂ was significantly small. However, the increases in lactate did not affect ATP levels in either NTR or SHR.

In extreme hypocapnia where pACO₂ is below 15 mm Hg, NTR showed a much greater increase in cerebral lactate than did SHR, resulting from a greater reduction of CBF due to more intense vasoconstriction in NTR. In other words, SHR exhibited less sensitivity to CO₂, resulting in a lesser increase in cerebral lactate. It has been noted that extreme hypocapnia leads to a critical reduction of cellular oxygenation consequent with an increase in brain lactate and a moderate derangement of the energy metabolism. Such metabolic changes are mainly due to the reduced CBF, and a resultant increase in arterial pH, which causes a shift of the oxyhemoglobin dissociation curve to the left (Bohr effect), and which stimulates glycolysis by the activation of phosphofructokinase. Some investigators, however, do not support the concept that alkalois determines hypocapnia-induced lactate production.

The constricted cerebral vessels began to dilate in severe hypocapnia. The cortical CVR in NTR at a pACO₂ below 15.7 mmHg was rather lower than that at pACO₂ in the range of 15.8 to 21.0 mmHg. A similar phenomenon has been documented in humans as well as animals. Wasserman and his co-workers found that human CBF did not decrease to below 60% of the control level in extreme hypocapnia, and suggested that some vasodilatory mechanism which antagonizes...
vasoconstriction is operating in profound hypocapnia. From our results, an excess production of cerebral lactate, or tissue acidosis by accumulation of the ischemic metabolites, might be an important factor for vasodilatation under these conditions.

In conclusion, lesser increases in CVR and cerebral lactate in response to hypocapnia in SHR suggest that persistent hypertension causes an increase in vascular resistance of the brain resulting in a decrease in cerebrovascular reactivities to various stimuli, namely a less constrictive response to hypocapnia as shown in the present study and also a less vasodilatory response to hypotension as demonstrated in our previous study.27

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References

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