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

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SUMMARY Cerebral vascular carbon dioxide (CO₂) reactivities were compared in normotensive (NTR) and hypertensive (SHR) rats. Cerebral bloodflow (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.

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 mechani-
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 each experiment, the blood pressure was continuously monitored. Arterial samples were taken before and 60 minutes after hyperventilation or normoventilation.

Table 1 Mean Arterial Pressure (MAP) and Arterial pCO₂, pO₂ and pH in Normotensive (NTR) and Spontaneously Hypertensive Rats (SHR) Before and One Hour After Hyperventilation (HV)

<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>apH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR</td>
<td>22</td>
<td>129 ± 4</td>
<td>38.9 ± 1.2</td>
<td>129.0 ± 8.8</td>
<td>7.393 ± 0.016</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>
<td>7.568 ± 0.035§</td>
</tr>
<tr>
<td>SHR</td>
<td>22</td>
<td>191 ± 7</td>
<td>40.0 ± 1.4</td>
<td>141.0 ± 8.5</td>
<td>7.341 ± 0.022</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>
<td>7.453 ± 0.032*</td>
</tr>
</tbody>
</table>

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

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 pCO₂ was significantly lowered to 18.6 mmHg (range 12.5–26.8) in NTR and to 20.7 mmHg (range 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/100 g/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 pCO₂ to CBF in cortex and thalamus. The average increase in cortical CVR per unit decrease in pCO₂ (ranging from 15.8 to 48.8 mmHg) was 0.12 mmHg/ml/100g/min mmHg pCO₂ in NTR, being twice as large as the value of 0.06 in SHR. When pCO₂ 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 (pCO₂ 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 pCO₂ 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 CVR in extreme hypocapnia averaged 2.86 mmHg/ml/100g/min, significantly lower than that of 5.57 in severe hypocapnia, indicating that the constricted vessels tended to dilate in extreme hypo-
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**CORTEX**

**NTR**

\[ y = -0.12x + 6.99 \]
\[ \text{paC0}_2 \geq 15.8 \text{mmHg} \]
\[ r = -0.665 \]
\[ p < 0.001 \]

**SHR**

\[ y = -0.06x + 5.84 \]
\[ \text{paC0}_2 \geq 15.8 \text{mmHg} \]
\[ r = -0.388 \]
\[ p < 0.05 \]

**FIGURE 1. Relationship between cortical CVR and paC02 in SHR and NTR. CVR was proportionally increased as paC02 decreased within a range of 15.8–48.8 mmHg. Increase in CVR per unit reduction of paC02 was greater in NTR than in SHR. Below 15.7 mmHg of paC02, CVR tended to decrease in both SHR and NTR.**

There were, however, no significant differences in CVR in the thalamus in NTR or in the cortex and thalamus in SHR when one compared extreme and severe hypocapnia.

### 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 pC02 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 paO2 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 paC02 is depicted in figure 3. Lactate started to increase at a paC02 below 20 mmHg in both NTR and SHR. At a paC02 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 paC02 ranging between 10 to 20 mmHg was 0.47 mM/kg/mmHg of paC02 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
Table 3 Percent Increase of Cerebrovascular Resistance (CVR) Per Unit Reduction of \( \text{paCO}_2 \) Ranging 15.8–48.8 mm Hg in Normotensive (NTR) and Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>( \Delta \text{CVR}/\text{resting CVR} \times \text{paCO}_2 \times 100 ) (%/mm Hg of ( \text{paCO}_2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR</td>
<td>Cortex: 5.9 ± 1.1* Thalamus: 5.4 ± 1.3</td>
</tr>
<tr>
<td>SHR</td>
<td>Cortex: 3.2 ± 0.7 Thalamus: 4.6 ± 1.0</td>
</tr>
</tbody>
</table>

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

Figure 2. Relationship between thalamic CVR and \( \text{paCO}_2 \) in SHR and NTR. An increase in CVR per unit reduction of \( \text{paCO}_2 \) was almost the same between the two groups.

Table 4 Cerebrovascular Resistance (CVR) at \( \text{paCO}_2 \) 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>( \text{pCO}_2 ) (mm Hg)</th>
<th>Cortex</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21.0–15.8</td>
<td>&lt; 15.7</td>
</tr>
<tr>
<td>NTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.86 ± 0.31 (6)*</td>
<td>2.96 ± 0.44 (8)</td>
<td>3.01 ± 0.53 (6)</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.87 ± 0.23 (2)</td>
<td>4.21 ± 0.64 (7)</td>
<td>3.30 ± 1.23 (3)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.01 (vs \( \text{pCO}_2 \) 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>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>
</tr>
<tr>
<td>HV</td>
<td>16</td>
<td>116 ± 4*</td>
<td>17.1 ± 1.2†</td>
<td>148.3 ± 13.6</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>
</tr>
<tr>
<td>HV</td>
<td>19</td>
<td>168 ± 5*</td>
<td>18.9 ± 1.1†</td>
<td>145.2 ± 10.7</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>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>2.39 ± 0.08</td>
<td>17.6 ± 1.2</td>
<td>2.61 ± 0.11</td>
</tr>
<tr>
<td>HV</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>2.63 ± 0.04</td>
<td>18.7 ± 2.5</td>
<td>2.56 ± 0.17</td>
</tr>
<tr>
<td>HV</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).

Cerebral flow measured by the hydrogen clearance method may, however, contain some underlying subcortical flow yielding an apparent lower flow value. Such flow differences were also demonstrated in a study using an autoradiographic method.20 Arteries on the brain surface supplying the cortex are more densely innervated by adrenergic nerve fibers than the arterial branches penetrating the brain parenchyma.21,22 Such autonomic innervations may have some effect on the cerebral vasoreactivities.

There have been many reports describing an increase in lactate in the brain as well as the cerebrospinal fluid during hyperventilation.3,4,23,24 According to MacMillan and Siesjö's observations,23 cerebral lactate in rats was increased to 3.42 mM/kg at pCO2 21.1 mmHg and 5.25 mM/kg at pCO2 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 pCO2 was significantly small. However, the increases in lactate did not affect ATP levels in either NTR or SHR.

In extreme hypocapnia were pCO2 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),25 and which stimulates glycolysis by the activation of phosphofructokinase.24 Some investigators, however, do not support the concept that alkalosis determines hypocapnia-induced lactate production.23,25

The constricted cerebral vessels began to dilate in extreme hypocapnia. The cortical CVR in NTR at a pCO2 below 15.7 mmHg was rather lower than that at pCO2 in the range of 15.8 to 21.0 mmHg. A similar phenomenon has been documented in humans as well as animals.2,26 Wasserman and his co-workers2 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
19. Heistad DD, Marcus ML, P<br>...
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