Effects of Bilateral Carotid Artery Ligation on Brain Lactate and Pyruvate Concentrations in Normotensive and Spontaneously Hypertensive Rats

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Abstract: Effects of Bilateral Carotid Artery Ligation on Brain Lactate and Pyruvate Concentrations in Normotensive and Spontaneously Hypertensive Rats

Brain lactate, pyruvate, and arterial acid-base balance were measured in normotensive rats (NTR) and spontaneously hypertensive rats (SHR) 60 minutes after bilateral carotid artery ligation. Brain lactate and lactate-pyruvate ratios were significantly increased in both SHR and NTR following carotid occlusion, although lactate increase in the former was six and one-half times greater than in the latter. These findings suggest that bilateral carotid occlusion in SHR may cause more severe circulatory changes which result in increased anaerobic metabolism. Furthermore, higher brain lactate was concomitant with lower arterial carbon dioxide tension. The mechanism of spontaneous hyperventilation following cerebral ischemia was discussed.

Additional Key Words
- spontaneous hyperventilation
- cerebral ischemia
- hypocapnia
- anaerobic metabolism

Introduction

A significantly higher mortality occurs in spontaneously hypertensive rats (SHR) than in normotensive Wistar rats (NTR) after bilateral carotid artery ligation. These findings suggest that bilateral carotid occlusion may cause more severe ischemic damages to the brain in SHR. To assess this possibility, the present study was undertaken to analyze cerebral lactate and pyruvate, as a reflection of anaerobic metabolism, following carotid artery ligation in anesthetized SHR and NTR. Furthermore, a relationship between cerebral anaerobic metabolic and arterial acid-base balance was also studied to clarify the mechanism of spontaneous hyperventilation, frequently observed with acute cerebrovascular disease.

Methods

Thirteen NTR weighing 200 to 340 gm and 13 SHR of F15 to F28 generations weighing 250 to 350 gm of either sex were anesthetized with intraperitoneally administered amobarbital (100 mg per kilogram). They breathed spontaneously. In each rat, one femoral artery was cannulated for blood pressure recording, with an electromanometer, and for anaerobic samplings of blood for Pco2, Pao2, and pH determinations (IL meter of Model 113). A plastic funnel was fitted into a skin incision over the skull bone for subsequent freezing of brain tissue in situ.

EXPERIMENTAL GROUP

In seven NTR and seven SHR the bilateral carotid arteries were dissected free from the vagosympathetic trunk in the neck, and were ligated by double sutures. Prior to ligation, 0.5 ml of arterial blood was taken for gas analysis.

CONTROL GROUP

Six NTR and six SHR were operated on in a similar manner as the experimental group, but the carotid arteries were not occluded. One sample of arterial blood was obtained for gas analysis after the operation was completed.

When Pao2 ranged between 31 and 49 mm Hg and the Pco2 was above 60 mm Hg, the experiment was continued; otherwise the anoxic, hypocapnic or hypercapnic rat was discarded. The second arterial blood sample of 0.5 ml for gas analysis was taken 60 minutes after bilateral carotid ligation in the experimental group, or 60 minutes after the first blood sampling in the control group.

Thereafter, the heads were frozen in liquid nitrogen and the entire supratentorial portion of brain was chiseled out in the frozen state. In rapid sequence, the frozen brain was weighed and ground after the addition of cold perchloric acid. The tissue homogenate, maintained at 0°C to 4°C, was centrifuged and neutralized with potassium hydroxide at pH between 4.5 and 5.0. Lactate and pyruvate concentrations in the tissue homogenate were determined by standard enzymatic methods. In 14 of 26 rats, the lactate concentration...
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of arterial blood taken immediately before freezing the heads was measured by the same methods.

Results

BLOOD PARAMETER

Mean arterial blood pressure (MAP) and blood gas data are given in table 1. An average MAP was 126 mm Hg in NTR and 170 mm Hg in SHR in the nonligated control group, and a final average MAP was 124 mm Hg and 169 mm Hg, respectively. Arterial Pco₂ at the 60-minute period was 42.7 mm Hg in NTR and 38.0 mm Hg in SHR. Inversely, arterial pH was lower in NTR than in SHR, presumably due to differences in Paco₂. Arterial Po₂ was 80.9 mm Hg in NTR and 92.8 mm Hg in SHR.

In both NTR and SHR of the experimental group, MAP was raised immediately after ligation of the carotid artery and reached the peak level of 154 mm Hg and 231 mm Hg within approximately 15.8 minutes and 8.7 minutes, respectively. The MAP returned to the preligation level in 60 minutes after ligation. The average MAP at the end of the experiment was 132 mm Hg and 171 mm Hg in NTR and SHR, respectively. These values were not significantly different from the nonligated group.

Mean values of arterial lactate concentration in 14 rats studied were 1.35 mM per liter in nonligated NTR and SHR, 1.59 mM per liter in NTR and 1.84 mM per liter in SHR of the ligated group. These values were not significantly different.

BRAIN TISSUE METABOLITES

Table 2 summarizes brain lactate, pyruvate and lactate/pyruvate ratios (L/P) in both NTR and SHR from the nonligated and ligated groups.

As shown in figures 1 and 2, mean value of brain lactate for control NTR of 1.64 mM per kilogram of brain weight was lower than control SHR as was the L/P of 12.1 while the pyruvate was slightly higher.

Carotid artery ligation led to slight but significant rises in lactate and L/P in NTR, whereas pyruvate remained unchanged. Mean increase of lactate in NTR was 0.62 mM per kilogram, or a 38% rise, and L/P was 9.8, or 81% above the preligating value. In SHR brain lactate 60 minutes after carotid occlusion increased markedly to 14.8 mM per kilogram, a 675%
Brain Lactate, Pyruvate and Lactate/Pyruvate Ratio in NTR and SHR With or Without Bilateral Carotid Artery Ligation

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactate (mM/kg)</th>
<th>Pyruvate (mM/kg)</th>
<th>L/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (nonligation)</td>
<td>NTR (6) 1.64 ± 0.05</td>
<td>SHR (6) 1.91 ± 0.09</td>
<td>12.1 ± 1.1</td>
</tr>
<tr>
<td>Experimental (ligation)</td>
<td>NTR (7) 2.26 ± 0.19</td>
<td>SHR (7) 14.80 ± 0.79</td>
<td>77.8 ± 6.8</td>
</tr>
</tbody>
</table>

**Statistical analysis**

<table>
<thead>
<tr>
<th></th>
<th>Lactate</th>
<th>Pyruvate</th>
<th>L/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control NTR : control SHR</td>
<td>2.54 &lt; 0.05</td>
<td>0.68 NS</td>
<td>1.84 NS</td>
</tr>
<tr>
<td>Control NTR : experimental NTR</td>
<td>3.00 &lt; 0.025</td>
<td>1.05 NS</td>
<td>2.21 &lt; 0.05</td>
</tr>
<tr>
<td>Control SHR : experimental SHR</td>
<td>14.97 &lt; 0.001</td>
<td>2.96 &lt; 0.025</td>
<td>11.05 &lt; 0.001</td>
</tr>
<tr>
<td>Experimental NTR : experimental SHR</td>
<td>15.49 &lt; 0.001</td>
<td>3.47 &lt; 0.005</td>
<td>9.10 &lt; 0.001</td>
</tr>
</tbody>
</table>

NS: not significant.

increase over the control value, depicted in figure 1. Brain L/P rose from 16.9 to 77.8, a 360% increase, shown in figure 2, and pyruvate increased from 0.125 to 0.197 mM per kilogram, a 58% increase.

Figure 3 depicts the relationship between brain lactate and Paco₂. Only one of seven NTR with carotid occlusion had a lowered Paco₂ of 33.5 mm Hg. In SHR of the ligated group, a marked increase in brain lactate was accompanied by an apparent fall of Paco₂. All but one SHR of the ligated group were hypocapnic with a Paco₂ below 30 mm Hg.

**Discussion**

The amount of decrease in cerebral blood flow (CBF) after bilateral carotid ligation in SHR has not been reported. In NTR, however, Eklöf and Siesjö

Brain lactate concentration in normotensive rats (NTR) and spontaneously hypertensive rats (SHR) with or without bilateral carotid artery ligation. An increase in brain lactate was more predominant in SHR than in NTR 60 minutes after ligation.

Brain lactate/pyruvate ratio in NTR and SHR with or without bilateral carotid artery ligation. An increase in L/P ratio was much greater in SHR than in NTR 60 minutes after bilateral carotid occlusion.
In the present study, the higher brain lactate and L/P in SHR following bilateral carotid occlusion suggest that a greater reduction of blood flow occurred in SHR than in NTR, even though MAP remained unchanged before and 60 minutes after carotid ligation in each group. Possible explanations for the presumed lower CBF in SHR might be as follows: first, the actual fall of perfusion pressure to the territory supplied through the carotid artery, mainly to the frontoparietal regions, would be much greater in SHR than in NTR following carotid ligation. Second, collateral circulation to the ischemic regions from the vertebrobasilar system following carotid ligation might diminish more in SHR than in NTR. Third, a pronounced hyperventilation following carotid ligation in SHR, resulting in lowered Paco₂, could cause cerebral vasoconstriction and reduce CBF.

Carotid ligation per se reduces the inflow pressure, and in addition, a rise of the outflow pressure due to postischemic brain swelling may cause a further decrease of cerebral perfusion pressure defined as the cerebral inflow pressure minus the cerebral venous pressure. A greater lowering of perfusion pressure may reduce CBF more severely in SHR than in NTR, resulting in a more pronounced increase in anaerobic carbohydrate metabolites of the brain in SHR than NTR, as shown in the present study.

Brain swelling or brain edema has been frequently observed in patients with severe cerebral infarction as well as in animals with induced infarction by arterial occlusion or by experimental cerebral embolism. O'Brien and Waltz, who have measured the intracranial pressure in animals with experimental infarction following unilateral middle cerebral artery occlusion, found that the intracranial pressure rose to a maximum of 74 mm Hg on the infarcted side, suggesting a concomitant rise in cerebral venous pressure. Such rises in intracranial pressure undoubtedly interrupt collateral circulation from non-occluded cerebral arteries to the ischemic regions.

Strandgaard et al. have recently described in humans that the lower blood pressure limit of cerebral autoregulation in the hypertensive patient was on the average 120 mm Hg, considerably above the level of 60 to 70 mm Hg in normotensive subjects. The shift to a higher blood pressure level of CBF autoregulation in hypertensive patients is associated with a proportional rise of the limit of critical blood pressure, below which CBF was reduced as blood pressure was lowered, causing relative hypoxia of the brain. It has been reported that the critical MAP producing brain hypoxia is 68 mm Hg in hypertensive subjects and 40 mm Hg in normotensive subjects, or a reduction of CBF to 73% and 66%, respectively.

In the present study, an inverse relation between brain lactate and Paco₂ in SHR with carotid ligation suggests that brain lactic acidosis led to hyperventilation. Hyperventilation associated with hypoperfusion...
of the brain is presumably a reaction to brainstem acidosis caused by acid metabolite or CO₂ accumulation. However, hypocapnia resulting from hyperventilation seems unlikely to cause the additional decrease in CBF since Harper¹³ found in dogs that CO₂ reactivity of brain vessels was completely abolished when MAP was reduced to 50 mm Hg. Furthermore, vascular response to CO₂ changes in the ischemic areas was also impaired because of the postischemic brain tissue acidosis causing vasoparalysis.¹⁴

The more pronounced hypoxic changes in SHR brain with bilateral carotid occlusion may result from the combined effects of a fall in inflow pressure and a rise in outflow pressure, causing a lowered perfusion pressure to regions supplied by the carotid artery. A fall in inflow pressure due to carotid ligation may be an important factor in reducing CBF since the critical autoregulation range of CBF may be shifted to a higher level in SHR. There is no direct proof of this in our animal experiment. A rise in outflow pressure resulting from increased intracranial pressure is another factor which may reduce CBF.

Our previous study¹⁵ as well as Lane et al.¹⁶ have shown in patients with acute cerebrovascular diseases that there was a significant inverse relationship between cerebrospinal fluid lactate and PaCO₂, suggesting that hyperventilation, as reflected by hypocapnia, was related to increased CSF lactate. The mechanism of spontaneous hyperventilation in acute stroke might be explained by three possible causes: hypoxia, CSF acidosis and neurogenic drive. It seems unlikely that hypoxia is an important cause, since there is no correlation between Pao₂ and PaCO₂, but hypoxia may potentiate the effect of other respiratory stimuli.

CSF acidosis appears to lead to ventilatory stimulation and this acid-base sensitive receptor is thought to be located on the lateral surface of the medulla.¹⁷ CSF acidosis due to increased acidic metabolites such as lactic and pyruvic acids is frequently seen in acute cerebral insults, especially intracranial hemorrhages.¹⁸ Despite the accumulation of lactate, however, a normal or slightly alkaline CSF is commonly observed in nonhemorrhagic stroke, indicating that the resulting hyperventilation is not caused by CSF acidosis only.

Central neurogenic hyperventilation has been seen in brain stem lesions.¹⁷ There was a negative correlation found between CSF pH and PaCO₂, namely, a relatively alkaline CSF pH associated with a reduced Pco₂ of both blood and CSF in nonhemorrhagic stroke.³ Our data indicate that hyperventilation and reduced PaCO₂ cannot be readily explained. Neither a metabolic drive due to CSF lactic acidosis nor a neurogenic drive stimulating the respiratory center could be excluded from the present study.

Acknowledgments
The authors are indebted to Miss Y. Sonoda and Miss K. Shirozu for technical assistance.

References
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Stroke. 1975;6:62-66
doi: 10.1161/01.STR.6.1.62

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