Reduction of the Cerebral Protective Effect of Hypothermia by Oligemic Hypotension During Hypoxia in the Rat

M. Mehdi Keykhaa, M.D.,* Frank A. Welsh, Ph.D.,† Magnus Hagerdal, M.D.,‡ and James R. Harp, M.D.§

SUMMARY The effect of arterial hypotension on cerebral cortical tissue levels of adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and reduced nicotinamide adenine dinucleotide (NADH) was studied in male Wistar rats with unilateral carotid ligation exposed to arterial hypoxia (Pao2 25 torr) for 20 min. while the body temperature was maintained at 32°C and 27°C. Brain metabolite levels were normal in normotensive hypothermic animals exposed to hypoxia, but reduction in arterial pressure to 75 torr caused a significant (p < 0.05) decrease in ATP and PCr values and a significant increase in lactate and NADH levels. These changes were comparable to those of normothermic normotensive, hypoxic animals. Furthermore, there was no significant difference in the brain metabolite levels between the two hypotensive hypothermic hypoxic groups.

These results indicate that arterial hypotension severely alters the cerebral protective effect of hypothermia against injury caused by hypoxia, and that further reduction in body temperature (from 32°C to 27°C) will not prevent the harmful effect of hypoxia upon the brain in hypotensive rats.

Stroke, Vol 13, No 2, 1982

It has been well established that hypothermia increases the tolerance of brain tissue to total ischemia and hypoxia. Because of this protective effect, hypothermia has been used in clinical management of head injury, metabolic coma, carotid endarterectomy, and post cardiac arrest brain injury.

Hypothermia has been combined with deliberate hypotension in order to reduce likelihood of ischemic brain injury. Development of fatal mesentric thrombosis in a patient exposed to hypothermia-hypotension led us to question the rationale of this technique.

Mitchenfelder was unable to demonstrate any protective effect from hypothermia during regional ischemia produced by middle cerebral artery ligation in squirrel monkeys. This study was, therefore, designed to examine the importance of the level of arterial pressure on hypothermic protection of brain tissue from injury caused by exposure to hypoxia in the Levine rat model with unilateral carotid ligation. The brain tissue protection was evaluated by measurements of adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and nicotinamide adenine dinucleotide (NADH).

Methods

Forty eight hours after unilateral carotid artery ligation under halothane oxygen anesthesia, unstarved male Wistar rats (250-350 g) were anesthetized with halothane 2-3% in oxygen. Following tracheostomy a French catheter was placed in the trachea and endotracheal anesthesia was maintained with halothane 0.5-1.5%, in nitrous oxide 70%, and oxygen, during surgical preparation. Respiration was controlled with a Harvard small animal ventilator. Both femoral arteries were cannulated with PE50 catheters, for blood gas analysis, exsanguination, and continuous blood pressure monitoring via P23DB Statham Transducer. One femoral vein was cannulated for drug administration. The head was immobilized in a stereotaxic head holder and the skull was exposed through a midline incision in the scalp for placement of a plastic freezing funnel. Fronto occipital silver-silver chloride needle electrodes were inserted for continuous monitoring of electroencephalogram.

Upon completion of surgical preparation, halothane was discontinued d-tubocurarine 1 mg/kg was given intravenously and ventilation was adjusted to maintain arterial blood carbon dioxide tension at 35-40 torr prior to exposure to hypoxia. All animals' lungs were ventilated with a 70% nitrous oxide, 30% oxygen mixture for at least 30 min before exposure to hypoxia. Hypoxia was induced by replacing oxygen with nitrogen to produce an arterial oxygen tension (Pao2) of 25-26 torr. Hypothermia was induced by surface cooling and body temperature was maintained at predetermined level by servo controlled heat lamp connected to a rectal temperature probe. Hypothermia was induced by bleeding the animals into 10 ml heparinized glass syringe. Blood was reinfused when necessary to maintain a mean arterial blood pressure (MAP) of 75 torr in the hypotensive group.

Six groups with six animals in each were studied. Group 1, with 37°C body temperature, MAP greater than 120 torr and Pao2 of 25 torr was the control group. Groups 2 and 3 were hypothermic with body temperature 32 and 27°C respectively and MAP greater than 120 torr (hypothermic-normotensive...
groups). Although the body temperature was kept at 32°C and 27°C in groups 4 and 5 respectively, the MAP was maintained at 75 torr throughout the hypoxic period in these groups (hypothermic-hypotensive groups). In group 6 body temperature was maintained at 37°C with MAP greater than 150 torr. The PaO₂ was kept over 150 torr. These animals' lungs were ventilated with nitrous oxide 70% and oxygen for 20 min.

Arterial blood gases and pH were measured before then three times during the experiment and the values were corrected for temperature differences. Total arterial oxygen content was measured at the same points by the method of Fabel and Lubbers.

After 20 min of hypoxia and the corresponding period in group 6, the brain was frozen in situ by pouring liquid nitrogen into the affixed freezing funnel. Ventilation was continued during freezing and the arterial pressure was maintained at desired levels for at least 1–2 min. The frozen brain was removed en bloc and stored in liquid nitrogen until cortical tissue samples were prepared separately from cerebral hemispheres for microfluorometric analysis of adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and nicotinamide adenine dinucleotide (NADH).

Statistical analysis of the results employed analysis of variance with unpaired Student t test for comparison of individual means in groups having significant F values with p < 0.05 considered significant.

Results

Physiologic parameters

All other groups are compared with group 1, the normothermic, hypoxic series which defines the standard stress. Values for all parameters in this control group are comparable to results of previous studies from this laboratory.

As seen in table 1, body temperature was well controlled in all series, where MAP was not controlled, values agree with earlier studies with hypothermic (group 2, 3) and normothermic (group 6) animals having significantly higher MAP than normothermic, hypoxic animals. In experimental groups 4 and 5 MAP was well controlled at 75 torr.

The third dependent variable, PaO₂, was identical in groups 1–5; PaCO₂ adjusted to 35 torr during normoxia was markedly reduced in group 1 in association with metabolic acidosis. The level of metabolic acidosis is lower in groups 2 and 3 (hypothermic–hypoxia) in accordance with results of earlier studies. However, when MAP was 75 torr (groups 4 and 5), metabolic acidosis was more severe in group 4 and comparable with group 1.

The effect of hypoxic metabolic acidosis upon oxygen transport is seen by comparing arterial oxygen content (CaO₂) in groups 2 and 3 with that of groups 4 and 5. Group 4 has higher CaO₂ than group 1, and this difference relates to the effect of hypothermia upon oxyhemoglobin dissociation. Hemoglobin values were similar in all groups.

Cerebral cortical tissue metabolites

For purposes of reference the metabolite values presented in group 6 are considered normal in our laboratory. The values for ATP and PCr from the right cortices (hemolateral to the ligated carotid artery) and that of lactate and NADH from both cortices in group 1 (normothermic-hypoxia) were significantly (p < 0.05) different from group 6. These low metabolite levels in hypothermic–hypotensive groups (groups 2 and 3) were significantly higher than group 1 and this difference relates to the effect of hypothermia upon oxyhemoglobin dissociation in hypoxia metabolic injury in the cerebral cortex in group 1 animals. Compared with group 1, the values for ATP from the right cortices was significantly (p < 0.05) higher in hypothermic-normotensive groups (groups 2 and 3). The PCr values from both cortices were significantly higher while lactate and NADH values were significantly lower in groups 2 and 3 than in group 1. The metabolite values in hypothermic-hypotensive groups (groups 4 and 5) were similar to those normothermic-hypoxia groups indicating injury due to hypoxia. The lactate value in group 5 was significantly lower than group 1 in the left cortex (controlateral to the ligated carotid artery). In both cortices from group 4 the

<table>
<thead>
<tr>
<th>Rectal temperature (°C)</th>
<th>MAP (torr)</th>
<th>PaO₂ (torr)</th>
<th>PaCO₂ (torr)</th>
<th>pHa</th>
<th>CaO₂ (ml/dl)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>37.1 ± 0.1</td>
<td>122 ± 5</td>
<td>25.7 ± 0.6</td>
<td>26.6 ± 1.4</td>
<td>7.27 ± 0.2</td>
<td>3.97 ± 0.7</td>
</tr>
<tr>
<td>Group 2</td>
<td>31.8 ± 0.008</td>
<td>134.1 ± 5</td>
<td>26.7 ± 0.6</td>
<td>23.6 ± 1.1</td>
<td>7.42 ± 0.02</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>Group 3</td>
<td>26.5 ± 0.2</td>
<td>151 ± 7</td>
<td>26.5 ± 0.9</td>
<td>32.8 ± 2.6</td>
<td>7.41 ± 0.01</td>
<td>12.6 ± 0.2</td>
</tr>
<tr>
<td>Group 4</td>
<td>31.9 ± 0.7</td>
<td>75 ± 1.3</td>
<td>26.3 ± 0.4</td>
<td>29.4 ± 2.1</td>
<td>7.28 ± 0.02</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>Group 5</td>
<td>26.9 ± 0.1</td>
<td>75 ± 0.6</td>
<td>25.1 ± 1.9</td>
<td>35.2 ± 1.5</td>
<td>7.30 ± 0.02</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td>Group 6</td>
<td>37.0 ± 0.1</td>
<td>174 ± 5</td>
<td>162 ± 8</td>
<td>34.7 ± 1.5</td>
<td>7.41 ± 0.02</td>
<td>19.8 ± 0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*Significantly different from Group 1, p < 0.05.
Table 2. Cerebral Cortical Tissue Concentrations of Adenosine Triphosphate (ATP) Phosphocreatine (PCr), Lactate, and NADH from Right and Left Cortices (Right Cortex Homolateral to Carotid Ligation)

<table>
<thead>
<tr>
<th>Group</th>
<th>ATP (μmol/g)</th>
<th>PCr (μmol/g)</th>
<th>Lactate (μmol/g)</th>
<th>NADH (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right cortex</td>
<td>Left cortex</td>
<td>Right cortex</td>
<td>Left cortex</td>
</tr>
<tr>
<td>Group 1</td>
<td>2.42 ± 0.2</td>
<td>2.82 ± 0.04</td>
<td>2.51 ± 0.6</td>
<td>4.23 ± 0.2</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.95* ± 0.05</td>
<td>2.88 ± 0.05</td>
<td>4.88* ± 0.3</td>
<td>5.09* ± 0.2</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.86* ± 0.04</td>
<td>2.82 ± 0.04</td>
<td>5.34* ± 0.2</td>
<td>5.05* ± 0.2</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.19 ± 0.4</td>
<td>2.41 ± 0.4</td>
<td>2.11 ± 0.6</td>
<td>3.57 ± 0.6</td>
</tr>
<tr>
<td>Group 5</td>
<td>2.41 ± 0.2</td>
<td>2.79 ± 0.08</td>
<td>3.4 ± 0.7</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Group 6</td>
<td>2.79* ± 0.03</td>
<td>2.76 ± 0.02</td>
<td>4.65* ± 0.1</td>
<td>4.37 ± 0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *Significantly different from Group 1, p < 0.05.

Changes in ATP and PCr were significantly (p < 0.05) lower and lactate and NADH were significantly higher than those values in unstressed animals (group 6) while in group 5 only the metabolite values from the right cortex was significantly different from group 6 animals. There also was a significant (p < 0.05) side to side difference for PCr and lactate values in group 1 and group 4, being higher in the right side than the left side. The side to side difference was observed in group 5 only for NADH which was significantly higher on the right side than on the left side (table 2).

Discussion

We selected the Levine rat model with unilateral carotid ligation because the degree of hypoxia necessary to produce cerebral tissue injury without carotid ligation causes cardiovascular failure and arterial hypotension. The acute occlusion of one carotid artery has been shown to decrease cerebral blood flow (CBF) by less than 10 percent in the hemisphere homolateral to the occluded carotid artery. This degree of reduction in CBF does not cause any difference in cerebral tissue levels of ATP, PCr, and lactate between the two cerebral hemispheres. Furthermore, the unilateral carotid ligation reduces the compensatory increase in cerebral blood flow induced by hypoxia thus producing profound changes in the energy state of the brain tissue homolateral to the ligated carotid artery compatible with neuronal death without cardiovascular complications encountered during severe hypoxia. Other studies have shown that an increase in the lactate level and decrease in PCr value are the first indication of hypoxic brain injury and they occur prior to decrease in ATP value.

Our results in group 1 confirm that exposure to arterial hypoxia (P_{aO_2} 25 torr) for 20 min, produces changes in the brain energy state indicative of cerebral tissue injury in normothermic animals. Thus while the levels of NADH and lactate increased, PCr and ATP values decreased significantly in group 1 in comparison with normoxic animals in group 6. These changes were more profound in the cortices on the side of the ligated carotid artery than in the contralateral cortices in group 1 (table 2). These results are in agreement with those reported by Salford et al, using similar experimental protocol and may indicate the same degree of neuronal damage in this group.

Reduction in body temperature by 5°C in group 2 prevented the harmful effect of hypoxia on the cerebral metabolite levels which occurred in normothermic animals. These results are comparable to those of normothermic-normoxic animals in group 6, demonstrating the cerebral protective effect of hypothermia from hypoxia. Further reduction in body temperature to 27°C produced the same degree of protection against hypoxia with regard to brain tissue metabolite levels.

These results are in agreement with those of Carlsson et al demonstrating complete cerebral protection from hypoxia with hypothermia at 27°C using rat model with no carotid ligations and with earlier studies from this laboratory using the Levine model rats with unilateral carotid ligation.

However, in the present study when the arterial pressure was lowered to 75 torr, hypothermia, 32°C, failed to protect the brain tissue from hypoxia, and further reduction in body temperature to 27°C did not improve the situation. Thus in both groups 4 and 5 changes which occurred in cerebral tissue metabolites were similar to those of normothermic-hypoxic animals in group 1.

It is quite possible that lack of cerebral blood flow (CBF) autoregulation caused by hypoxia makes the systemic arterial pressure the prime determining factor for maintaining cerebral perfusion. Thus oligemic hypotension would decrease CBF below the critical value needed to maintain brain tissue metabolism in groups 4 and 5.

It has been assumed that hypothermia protects the brain tissue from hypoxic stress by reducing the tissue demand for oxygen. We have shown in previous studies that hypothermia reduces the cerebral meta-
bolic rate for oxygen ($\text{CMR}_02$) in Wistar rat by 5 percent for each degree centigrade,^{21-22} thus at 32°C $\text{CMR}_02$ decreases by 25 percent. An impressive brain protection from hypoxia was achieved at this body temperature in group 2 in the present study. The mild elevation of lactate should not be associated to any neuronal injury in this group.\(^9\)

Hypothermia also greatly increases arterial oxygen content ($\text{CaO}_2$) during hypoxia.\(^{10-16}\) This increase is due to the leftward shift of the oxyhemoglobin dissociation curve and the absence of systemic acidosis in hypothermic animals during hypoxia and has been assumed to be one of the causes of hypothermic protection of brain tissue from hypoxia.\(^9\)

Carlsson et al examined the role of increased $\text{CaO}_2$ during hypothermia in hypoxic rats. They exposed one group of animals to $\text{PaO}_2$ of 12 torr at 27°C body temperature producing $\text{CaO}_2$ of 4.48 volumes percent. Metabolite values in that group were similar to those in 32°C normotensive animals in the present study. Furthermore, although similar values for $\text{CaO}_2$ were obtained in 32°C normotensive and 27°C hypotensive groups in the present study (table 1) there were persistent changes in metabolite levels compatible with hypoxic injury in the hypotensive group. These results are in support of our previous study\(^{16}\) indicating that the cerebral protective effect of hypothermia from hypoxic injury does not depend solely on increases in $\text{CaO}_2$ in hypoxic animals.

Our results thus indicate that an impressive degree of cerebral protection can be achieved at 32°C, and while reduction in $\text{CaO}_2$ due to systemic acidosis may have a role in reducing the hypothermic protection during hypotension, changes in cerebral blood flow due to fall in systemic blood pressure must be a more important factor.

Maintenance of MAP during hypoxia which is characteristic of hypothermic animals (see groups 2 and 3, table 1) may be a very important element of the "protective" effect of hypothermia. This suggests that hypothermia might not be helpful in ischemic stress.

References

15. Keykhah MM, Hägerdal M, Welsh FA: Effect of high vs. low arterial blood oxygen content on cerebral energy metabolism levels during hypoxia with normothermia and hypothermia in the rat. Anesthesiology 52: 492-495, 1980
Reduction of the cerebral protective effect of hypothermia by oligemic hypotension during hypoxia in the rat.
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Stroke. 1982;13:171-174
doi: 10.1161/01.STR.13.2.171

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