Pulmonary Hemorrhage in Experimental Cerebral Ischemia in Mongolian Gerbils

Brain Metabolism and Lung Pathology

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SUMMARY

Pulmonary changes in acute cerebral ischemia were studied in anesthetized Mongolian gerbils, in which both carotid arteries were occluded simultaneously. Lactate, pyruvate and adenosine triphosphate (ATP) in the brain were measured as indicators of the severity of cerebral ischemia. Microscopic changes in the lung were arbitrarily scored from 0 (normal) to 3 points (severely affected) by the grade and the extent of lesions. Mean arterial pressure (MAP) was also measured through the cannulated femoral artery before and after carotid artery occlusion in a separate group of animals.

Cerebral lactate was increased while ATP decreased in ischemic animals in which pulmonary changes such as intra-alveolar hemorrhages were prominent and frequent. The lung pathology score averaged 1.3 in animals with severe ischemia (lactate ≥ 10 mM/Kg), 0.7 in moderate ischemia (5–10 mM/Kg) and 0.3 in mild or no ischemia (<5 mM/Kg), respectively, suggesting that severe brain ischemia may cause fulminant pulmonary changes. The mechanism of pulmonary lesions in acute cerebral ischemia is discussed.

Additional Indexing Words:
Brain metabolism Neurogenic pulmonary edema

FULMINANT pulmonary edema, characterized by marked vascular congestion, intra-alveolar hemorrhage and protein-rich edema fluid, develops in association with a variety of acute, often fatal, central nervous system...
injuries including head trauma, cerebral hemorrhage,\textsuperscript{11,12} non-hemorrhagic stroke,\textsuperscript{13} convulsive disorders, brain tumors and increased intracranial pressures. Acute pulmonary edema has been experimentally produced in laboratory animals by the intracisternal injection of fibrin,\textsuperscript{41} electrolytic lesions of the hypothalamus\textsuperscript{51,61} or by a rapid and extensive increase in cerebrospinal fluid pressure,\textsuperscript{71,81} although it is an unusual finding in cases of ischemic brain damage.

The present investigation was undertaken to evaluate lung pathology in acute cerebral ischemia induced by bilateral carotid occlusion in Mongolian gerbils. Brain metabolites were determined as an indication of the severity of cerebral ischemia.

\textbf{Methods}

A total of 42 Mongolian gerbils of either sex, weighing 60 to 100 gm, were used for this study. They were anesthetized with intraperitoneal amobarbital 10 mg/100 gm body weight. Through a ventral midline incision in the neck, both common carotid arteries were exposed and separated from the vagosympathetic trunk. These arteries were doubly ligated by silk sutures at the same time in experimental animals and remained unligated in sham-operated control ones.

Brain metabolism: At 1 or 3 hour intervals following carotid occlusion or sham operation, the animals were decapitated into liquid nitrogen for immediate freezing of the brains. The entire supratentorial portion of the brain was then chiselled out, weighed in the frozen state and ground in rapid sequence. After the addition of cold perchloric acid, the tissue was homogenized and centrifuged. The supernatant was neutralized with potassium hydroxide at a pH of between 4.5 and 5.0. Lactate, pyruvate and ATP concentrations in the tissue homogenate were determined by standard enzymatic methods.

Lung pathology: The lungs were excised after decapitation and fixed in 10\% formalin for 1 week. Microscopic examinations were made by one of the authors (S.S.) using a blind technique. The severity of lung pathology was arbitrarily scored 0 to 3 points according to the extent and degree of the lesions; no change of the lung as 0, a slight change as 1, moderate as 2 and severe as 3 points.

Blood pressure: In a separate group of 12 anesthetized animals one femoral artery was cannulated with PE 10 polyethylene tubing connected to an electrical pressure transducer. Arterial blood pressure was continuously recorded before and after carotid artery occlusion or sham operation.
RESULTS

Brain metabolism: Mean values for lactate, lactate/pyruvate (L/P) ratio and ATP of the brain in ischemic and sham-operated animals are summarized in Table I. By 1 hour following carotid occlusion, lactate and the L/P ratio were increased by 3.5 and 3.0 times the control values, respectively. ATP decreased to 31% of control. By 3 hours after occlusion, lactate and L/P ratio were also increased by 2.1 and 3.1 times and ATP decreased to 36% of the corresponding control. All of these differences were of statistical significance.

Lung pathology: Histologic examinations of the lung showed focal or diffuse pulmonary hemorrhage and congestion in a majority of the ischemic animals, although edema fluid was rarely observed in the alveolar spaces. Neither rupture nor necrosis of the pulmonary vessels was evident in these lungs. Fig. 1 depicts the normal lung of a control animal, defined as 0 point.

Table I. Supratentorial Lactate, Lactate/Pyruvate (L/P) Ratio, and ATP in Mongolian Gerbils Following Bilateral Carotid Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Carotid artery</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>1 hr</td>
<td>sham</td>
<td>9</td>
<td>2.65±0.33</td>
<td>39.4±3.2</td>
<td>1.45±0.33</td>
</tr>
<tr>
<td></td>
<td>occluded</td>
<td>8</td>
<td>9.31±0.73***</td>
<td>116.7±11.1***</td>
<td>0.45±0.09*</td>
</tr>
<tr>
<td>3 hrs</td>
<td>sham</td>
<td>11</td>
<td>4.13±0.23</td>
<td>52.3±7.6</td>
<td>1.79±0.23</td>
</tr>
<tr>
<td></td>
<td>occluded</td>
<td>14</td>
<td>8.79±0.57***</td>
<td>160.8±43.0**</td>
<td>0.65±0.18***</td>
</tr>
</tbody>
</table>

sham = sham-operated. * p<0.02, ** p<0.005, *** p<0.001 (vs the corresponding sham-operated animals), values are mean±SEM.

Fig. 1. Normal lung (0 point). H & E, x120
Fig. 2. Lung with moderate pathologic changes (2 points). There are focal intra-alveolar hemorrhages.  H & E, $\times 190$

Fig. 3. Lung with severe pathologic changes (3 points). Intra-alveolar hemorrhages are more pronounced and diffuse.  H & E, $\times 140$

Fig. 2 demonstrates moderate changes in the lung after 1 hour of ischemia (2 points) and Fig. 3 shows pronounced intra-alveolar hemorrhages in an animal with ischemia of 3 hours duration (3 points). Mean lung pathology scores were 0.2 after 1 hour and 0.3 after 3 hours in sham-operated animals, while those in ischemic animals were 0.9 at 1 hour and 0.6 at 3 hours.

Relationship between brain metabolism and lung pathology: Severity of brain ischemia was divided into three grades by lactate levels as follows; severe ischemia ($10 \text{ mM/Kg}$ of lactate or more), moderate ischemia (between 5 and
Table II. Relationship between Cerebral Lactate Level and Pulmonary Changes in Gerbils with or without Bilateral Carotid Occlusion

<table>
<thead>
<tr>
<th>Lactate level (mM/Kg)</th>
<th>No. of rats</th>
<th>Mean lung score (points)*</th>
<th>No. of rats with abnormal lung**</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥10</td>
<td>7</td>
<td>1.3</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>5-10</td>
<td>17</td>
<td>0.7</td>
<td>9 (57%)</td>
</tr>
<tr>
<td>&lt;5</td>
<td>18</td>
<td>0.3</td>
<td>6 (33%)</td>
</tr>
</tbody>
</table>

* The severity of lung pathology was scored from 0 (normal) to a maximum of 3 points (severely affected), ** pulmonary changes of 1 point severity or more (see text for further explanations).

Fig. 4. Mean arterial pressure (MAP) following bilateral carotid artery occlusion in anesthetized gerbils. An initial abrupt elevation of MAP, followed by a late-occurring progressive rise, is observed in animals with carotid occlusion (closed circles). In contrast, MAP in control animals changed little (open circles).

10 mM/Kg) and mild or no ischemia (below 5 mM/Kg). As shown in Table II, abnormalities of the lung greater than 1 point were observed in 71% of the severely ischemic, 57% of the moderately ischemic, and 33% of mildly or non-ischemic animals, respectively, indicating a relation between the pulmonary changes and cerebral ischemia.

Blood pressure: Following bilateral carotid occlusion, mean arterial pressure (MAP) changed in a biphasic way as shown in Fig. 4. MAP rose
abruptly from 65 to 117 mmHg within 10 min after occlusion, followed by a further late-occurring progressive rise. In contrast, MAP in sham-operated animals remained unchanged during the first 1 hour, followed by a slight increase with time, probably due to recovery from anesthesia.

**Discussion**

None of the Mongolian gerbils used in our laboratory survived for 24 hours following bilateral carotid occlusion, while 70% survived beyond 24 hours following unilateral carotid occlusion (unpublished observations), these data being compatible with a majority of the previously reported mortality and survival rates in gerbils with carotid occlusion. In the present study, we found severe pathologic changes in the gerbil lung, mainly intra-alveolar hemorrhage, following bilateral carotid occlusion, suggesting that cerebral ischemia may directly or indirectly cause pulmonary hemorrhage.

In brain injuries, the hypothalamus has been implicated as a site of the central nervous system responsible for the development of pulmonary hemorrhage. Gamble and Patton have reported that either an electrolytic lesion in the basal portion of the preoptic nucleus or midline destruction of the periventricular system caudal to the preoptic region produces fulminant pulmonary edema as well as hemorrhage in rats. Maire and Patton suggested that a pulmonary edemagenic center resides in the post-chiasmatic area of the hypothalamus, which is normally inhibited by the preoptic nucleus of the hypothalamus. Reynolds, however, denied either the presence of an edemagenic center or the importance of an inhibitory mechanism. Instead, he postulated that a concomitant irritation of the surrounding tissue, when produced by electrolytic lesions in the hypothalamus, may stimulate sympathetic fibers descending through the hypothalamus, resulting in fulminant pulmonary edema. Furthermore, Chen et al observed that decerebration had no effects on the cardiovascular or pulmonary changes induced by cerebral compression and they concluded that these changes must principally derive from activation of the neural structures below the decerebration such as the medulla oblongata or the spinal cord. The present observations indicate that the degree of pulmonary hemorrhage was closely related to the severity of the brain damage. However, an ischemic model of the gerbil is known to develop extensive lesions in both hemispheres, including the hypothalamus. Therefore, it is difficult to identify the primary site of the edemagenic center in this ischemic brain and further study is necessary to clarify this problem.

It has been noted that cerebral compression causes systemic hypertension known as the Cushing phenomenon. An ischemic reaction of the
vasomotor center in the medulla oblongata leads to sympathetic overactivation, peripheral vasoconstriction and venoconstriction, resulting in a massive shift of blood into the low resistance systems such as the pulmonary circulation. These hemodynamic changes may cause severe heart strain and pulmonary hypertension. In the present investigation, bilateral carotid occlusion caused a biphasic rise in systemic arterial pressure. We regarded the late-occurring hypertension as a Cushing phenomenon that was due to a sympathetic pathway mediated ischemic reaction of the brainstem secondary to severe supratentorial ischemia. In addition to the centrally-induced sympathetic discharge, alterations of pulmonary permeability seem to be another causal mechanism of neurogenic pulmonary edema. It is noted that pulmonary edema associated with heroin overdose resembles neurogenic pulmonary edema. From the present results, however, it is not clear whether pulmonary changes in cerebral ischemia are simply due to a neurogenic mechanism or if there is an additional factor influencing pulmonary permeability.

Acute and severe stroke is often accompanied by an excessive rise in blood pressure and pulmonary dysfunction with hypoxemia, although most stroke patients tend to hyperventilate. Our findings are clinically important for the treatment of these patients. In general, pulmonary involvement in acute stroke is diagnosed as bronchopneumonia and treated with antibiotics. We must bear in mind the possibility of neurogenic pulmonary edema in patients with ischemic stroke.

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REFERENCES