Time Course of Early Brain Edema Following Reversible Forebrain Ischemia in Rats

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Cerebral ischemia is known to be accompanied by brain edema. This increase in brain tissue water content probably influences the final outcome of an ischemic insult negatively. Despite extensive investigations on different aspects of brain edema, information on edema development during the early recirculation period following ischemia is sparse. We assessed changes in brain water content, as reflected by changes in tissue density, during the early recirculation period following severe forebrain ischemia. Fasted rats were subjected to 5, 15, or 30 minutes of ischemia and 5 to 180 minutes of recirculation. The specific gravity of specimens from the caudoputamen, frontoparietal cortex, hippocampus, and mesencephalon were measured with a Percoll linear density gradient. Five minutes of ischemia followed by recirculation did not produce any significant regional brain edema. However, following 15 minutes of ischemia, transient edema developed in the caudoputamen, frontoparietal cortex, and hippocampus. This edema was maximal after 30 minutes of reperfusion and was normalized after 180 minutes of reperfusion. Similar edema was seen following 30 minutes of ischemia. In the mesencephalon (where blood flow is approximately 50% of control during the ischemic insult) no brain edema was noted following 5, 15, or 30 minutes of ischemia. We discuss to what extent this transient regional brain edema may influence the selective neuronal vulnerability and cell damage observed in rats subjected to reversible forebrain ischemia and how these findings may correlate with neurochemical alterations observed during the early recirculation period. (Stroke 1989;20:1565-1570)
The operative procedure has been described in detail.16 In short, isoflurane (Abbott Laboratories Ltd., Kent, U.K.) in a mixture of nitrous oxide/oxygen (70%/30%) was used to induce (3%) and maintain (1-1.5%) anesthesia. The rats were endotracheally intubated and connected to an animal ventilator. The common carotid arteries were exposed via a ventral midline incision, and a silicone catheter was inserted into the right jugular vein to allow withdrawal of blood for induction of hypotension during ischemia. The tail artery was cannulated for continuous blood pressure monitoring and blood sampling. The electroencephalogram (EEG) was monitored by placing needle electrodes bilaterally in the temporal muscles via a small skin incision. The rats were then heparinized (50 IU) and allowed a 30-minute steady-state period. Skull and rectal temperatures were measured with a thermometer (A-05, Ellab, Copenhagen, Denmark) and a standard mercury thermometer, respectively, and were maintained at close to 37°C by a distant heating lamp (55 W). Blood pressure was maintained at 100–130 mm Hg. PaO2 and PaCO2 were maintained at >90 mm Hg and 35–40 mm Hg, respectively, by adjusting ventilation. At that PaCO2, plasma pH was kept between 7.35 and 7.45. Plasma glucose levels were measured with a Beckman Glucose Analyzer 2 (Beckman Instruments Inc., Los Angeles, California).

Isoflurane administration was discontinued, and 1 minute later near-complete forebrain ischemia was induced by bilateral clamping of the common carotid arteries, combined with exsanguination to achieve a blood pressure of approximately 50 mm Hg. Under these conditions EEG activity rapidly became isoelectric.16 Ischemia was maintained for 5, 15, or 30 minutes, and the clamps were then removed and the withdrawn blood was reinfused.

Fifty-six rats were subjected to 15 minutes of ischemia and assigned to one of seven groups (n=8 in each group). Rats in one group were killed immediately upon termination of ischemia (i.e., the clamps were not removed and no blood was reinfused), and rats in the other six groups were decapitated after 5, 15, 30, 60, 90, or 180 minutes of recirculation. Sixteen additional rats were subjected to 5 or 30 minutes of ischemia and 30 or 60 minutes of recirculation (n=4 in each group). The same procedures were followed in nine control rats, except that ischemia was not induced.

Following decapitation of the rats, the brains were rapidly transferred to a closed chamber with a relative humidity of >90% at ambient temperature. Samples of approximately 25 mg from the frontoparietal cortex, the caudoputamen, the dorsal hippocampus, and the mesencephalon were dissected bilaterally on an ice-cold plate immediately before being submerged in a density gradient of polyvinyl pyrrolidone–coated silica particles (Percoll, Pharmacia AB, Uppsala, Sweden) as described by Tengvar et al.17,18 Glass spheres of known densities (1.0300, 1.0350, 1.0400, 1.0450, and 1.0500 g/cm3, Scientific Glass, Bloomfield, New York) were introduced into the column. The position of these spheres in the gradient reflects the linearity of the column, and when equidistantly spaced, these spheres provide a simple and reliable calibration for the continuous gradient. The tissue samples were introduced into the gradient, left hemisphere samples first, and their position was measured 3 minutes later, thus allowing determination of their specific gravity (SG) in relation to the positions of the glass spheres. Thereafter, the same procedure was undertaken for the right hemisphere samples.

The data were subjected to one-way analysis of variance. Significance was assumed when p<0.05. When significance was reached, Dunnet's test was used to determine which groups differed.

Results

We found all rats to be normoxic, normocapnic, normotensive, normoglycemic, and without major plasma acid–base disturbances before the induction of ischemia (Table 1) and during recirculation. The temperature in the rectum and on the skull remained at 37°C.

We found the density gradients to be linear. SG of corresponding tissue samples from the two sides of the brain did not differ in any group, so we calculated the mean SG for brain regions.

Data from the 56 rats subjected to 15 minutes of ischemia and from the controls are presented in Figure 1. In the rats with 0 minutes of recirculation, no significant changes in SG were found in the frontoparietal cortex, the caudoputamen, or the hippocampus compared with control. In the frontoparietal cortex, SG decreased significantly after 15 minutes of ischemia followed by 15 minutes of recirculation compared with control (Figure 1, top; a nonsignificant change in SG was present after 5 minutes of recirculation). After 30 minutes of recirculation, SG had decreased further and reached the lowest value observed. After 60 and 90 minutes of recirculation, SG was still lower than control but was gradually returning to normal. After 180 minutes of recirculation, no significant difference in SG was observed compared with control. In the caudoputamen and hippocampus, the decreases in SG were similar to that in the frontoparietal cortex, with the lowest value observed after 30 minutes of recirculation. In the caudoputamen and hippocampus, SG then gradually increased, normalizing after 180 minutes of recirculation (Figure 1, top). The maximal change in SG was similar in these three brain regions. As an example, after 30 minutes of recirculation, the decrease in SG of the frontoparietal cortex was 0.0026 g/cm3.

Five minutes of ischemia followed by 30 or 60 minutes of recirculation did not change SG significantly (Figure 2). Thirty minutes of ischemia followed by 30 minutes of recirculation decreased SG in the frontoparietal cortex, the caudoputamen, and
the hippocampus (Figure 3, left); the change was similar in magnitude to that caused by 15 minutes of ischemia, but the SG decrease remained unchanged or was slightly more accentuated after 60 minutes of recirculation. Thus, there was an indication that edema following 30 minutes of ischemia is more sustained than that following 15 minutes of ischemia.

In the mesencephalon, no significant change in SG was found during recirculation following 15 minutes of ischemia (Figure 1, bottom). Following 30 minutes of ischemia, there was a tendency toward an increase in SG during 60 minutes of recirculation (Figure 3, right), but this did not reach significance.

Discussion

Linear density gradients, as first described by Nelson et al., have proved to be reliable for measuring SG and brain edema. Changes in SG are assumed to primarily reflect changes in the water content of brain tissue, and a decrease in SG is usually interpreted as an increase in the amount of water. Clearly, changes in blood content are important for the SG of a tissue and should be taken into account during studies of ischemia and reperfusion, during which drastic changes in cerebral blood flow and blood volume occur. However, the postischemic changes in SG we observed are partly inverse to previously mapped postischemic changes in cerebral blood flow, that is, SG decreased during the period of known postischemic hyperperfusion. Since blood always has a higher density than brain tissue, the changes in SG we observed cannot be explained by changes in blood content.

It has previously been shown that in the ischemic model we used, blood flow in the forebrain (including the frontoparietal cortex, caudoputamen, and hippocampus) decreases to <5% of normal. In our study, 5 minutes of severe forebrain ischemia resulted in no observable regional edema during recirculation. When the ischemic period was prolonged to 15 minutes, edema developed and was of similar severity in the frontoparietal cortex, the caudoputamen, and the hippocampus. Edema developed gradually and reached a maximum after 30 minutes of recirculation. The edema then gradually resolved in these three regions; SG was normalized after 180 minutes of recirculation. Following 30 minutes of ischemia, edema was more sustained in time but was not more prominent in magnitude than after a 15-minute ischemic insult. Thus, 15 minutes of forebrain ischemia seems to be adequate for studying early reversible postischemic brain edema.

In the mesencephalon, where blood flow is approximately 50% of normal during this ischemic insult, no changes in brain water content were noted after 15 minutes of ischemia. However, following 30 minutes of ischemia, SG increased progressively after 30 and 60 minutes of recirculation. The most likely explanation for this is an increased relative blood content. Postischemic hypoperfusion may cause a relative redistribution of blood to brain regions less severely affected by the ischemic insult, such as the mesencephalon.

All extra fluid in the brain is ultimately derived from blood; hence, if there is no blood flow, there will be no increase in tissue water content. In fact, in our study SG did not change in any forebrain region in rats decapitated immediately following

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**TABLE 1. Physiologic Parameters Before Ischemia in Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ischemia 15 min</th>
<th>Ischemia 5 min recirculation</th>
<th>Ischemia 15 min recirculation</th>
<th>Ischemia 30 min recirculation</th>
<th>Ischemia 60 min recirculation</th>
<th>Ischemia 90 min recirculation</th>
<th>Ischemia 120 min recirculation</th>
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<tr>
<td>Rectal temperature (°C)</td>
<td>37.2±0.3</td>
<td>36.9±0.3</td>
<td>37.0±0.2</td>
<td>37.0±0.3</td>
<td>37.0±0.5</td>
<td>37.1±0.3</td>
<td>36.9±0.4</td>
<td>36.9±0.4</td>
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<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>115±10</td>
<td>114±10</td>
<td>120±20</td>
<td>120±9</td>
<td>112±12</td>
<td>117±12</td>
<td>119±15</td>
<td>118±16</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>110±5</td>
<td>105±10</td>
<td>105±10</td>
<td>108±12</td>
<td>112±4</td>
<td>106±7</td>
<td>113±8</td>
<td>108±10</td>
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<tr>
<td>PaCO₂ (mm Hg)</td>
<td>36±2</td>
<td>35±1</td>
<td>36±2</td>
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<td>35±2</td>
<td>36±2</td>
<td>37±2</td>
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</tr>
<tr>
<td>Arterial pH</td>
<td>7.39±0.04</td>
<td>7.38±0.03</td>
<td>7.41±0.04</td>
<td>7.41±0.03</td>
<td>7.40±0.02</td>
<td>7.40±0.03</td>
<td>7.41±0.02</td>
<td>7.40±0.03</td>
</tr>
<tr>
<td>Blood glucose concentration (mmol/l)</td>
<td>6.9±1.0</td>
<td>7.7±2.2</td>
<td>7.1±1.0</td>
<td>7.3±1.5</td>
<td>8.0±1.8</td>
<td>9.1±3.0</td>
<td>8.6±2.2</td>
<td>8.9±0.8</td>
</tr>
</tbody>
</table>

Data are mean±SD.
ischemia, which confirms that the ischemic model we used produces very severe forebrain ischemia.16,24

Our findings are complementary to previous results. In our laboratory, Warner et al25 showed that forebrain ischemia in rats with comparably severe insults led to biphasic edema. The first sample was taken after 90 minutes of recirculation, and these authors then observed an edema that persisted for up to 6 hours of recirculation before complete normalization; after 24 hours, edema recurred. We have shown that the initial edema noted by Warner et al25 is probably most pronounced after 30 minutes of recirculation and that after 90 minutes of recovery this edema is already partially resolved.

Available information on edema development during the early recirculation period following an ischemic insult is sparse. Avery and coworkers26 exposed gerbils, animals with an incomplete circle of Willis, to very profound ischemia (60 minutes of bilateral carotid artery occlusion), which caused 94% to die after ≤24 hours of recirculation. The authors showed a rapid decrease of SG after 5 minutes of recirculation in all regions investigated (including the cortex, corpus striatum, thalamus, and hippocampus).26 The change in SG progressed, being maximal after 1–2 hours of recirculation. After 3 hours, SG returned to normal in all regions except the hippocampus. Using another model of very severe ischemia in rats (four-vessel occlusion for 30 minutes), Petito et al27 showed a transient 1% increase in total brain water content after 15 and 30 minutes of recirculation. Although still elevated, brain water content was returning to normal after 60 minutes of recovery. These authors, however, did not detect any regional edema in the striatum, middle cerebral artery cortex, or hippocampus at any postischemic interval.27 Finally, Todd and coworkers,28,29 using
four-vessel occlusion in rats for 15, 30, or 60 minutes, suggested a decrease in mean cortical SG after 30 minutes of recirculation followed by normalization after 180 minutes of recirculation.

Conditions for the development of brain edema clearly exist during the early recirculation period following severe ischemia. Experiments have shown that severe ischemia is accompanied by a rapid decrease in extracellular Na⁺, Cl⁻, and Ca²⁺ contents, concomitant with an increase in cortical impedance. This is generally interpreted as reflecting a cellular influx of Na⁺ and Cl⁻ together with osmotically obliged water, leading among other things to a decrease in extracellular space. Cerebral ischemia is also known to be accompanied by a significant increase in tissue osmolality. Since no (or very little) loss of osmoles into the circulating blood takes place during ischemia, an osmotic pressure gradient develops between the blood and the brain. It is also generally accepted that transient incomplete cerebral ischemia is followed by a "hyper-hypo-perfusion" sequence. When recirculation begins, this early hyperperfusion together with the osmotic pressure gradient may thus induce a rapid shift of water from the blood to the brain, which promotes the development of edema. However, the nature of the extra osmoles remains to be shown. Furthermore, there must be clarification of the forces by which water is translocated from the blood to the extracellular fluid (ECF), that is, how the electrolyte composition of the ECF is altered during the immediate postischemic period.

Since brain edema following cerebral ischemia has some specific properties, the term ischemic edema has been proposed to replace both of the more commonly used terms cytotoxic/cellular edema and vasogenic edema. Ischemic edema is initially primarily cytotoxic, whereas vasogenic edema, with leakage of high-molecular-weight indicators of blood-brain barrier dysfunction, is a late phenomenon that develops hours or days after the early edema. The early transient edema shown in our study is probably of the so-called cytotoxic type, that is, it primarily reflects the intracellular accumulation of water.

In summary, following 5 minutes of severe forebrain ischemia, no regional edema was observed during the early recirculation period. Fifteen minutes of ischemia was followed by transient brain edema in the frontoparietal cortex, the caudoputamen, and the hippocampus. This edema reached a maximum after approximately 30 minutes of recirculation and normalized after 180 minutes of recirculation. Following 30 minutes of ischemia, the development of edema was similar, albeit more sustained in time. It cannot be excluded that this early postischemic edema, although resolved within some hours, has an impact on metabolically perturbed cells during recirculation and thereby on the final outcome of the ischemic insult. Whether of pathologic significance or not, this edema is one parameter to take into account when considering the postischemic neurochemical events that contribute to ischemic cell necrosis.
Acknowledgment

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References


Key Words • brain edema • cerebral ischemia • rats