Ischemic Brain Edema With and Without Reperfusion: An Experimental Study in Gerbils

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SUMMARY Tissue water and rCBF from the same area of brain was measured in gerbils with cerebral ischemia. In one experiment we related the severity of ischemia that developed after one hour of carotid occlusion to the amount of edema which formed. In a second experiment brain made ischemic for one hour was reperfused for one hour to assess the effect of reperfusion of ischemic tissue upon edema formation. We identified a critical threshold (10-14 ml·100g⁻¹·min⁻¹) for the reversibility of the ischemic process, above which edema can resolve upon reperfusion. When postocclusion rCBF was less than 10 ml·100g⁻¹·min⁻¹, edema was maximal at the end of occlusion and did not resolve with reperfusion. Autoregulation was preserved in ischemic tissue in which the edema process resolved with reperfusion.

RESTORATION OF BLOOD FLOW to ischemic brain is now clinically feasible. Logic dictates that reperfusion of ischemic brain tissue should improve brain function and might improve outcome from an ischemic event, but clinical and laboratory experience indicate that edema formation associated with ischemia may actually be aggravated by reperfusion. This conflict between logic and experience poses a practical question for clinicians: Should ischemic tissue be reperfused and, if so, under what circumstances?

Both the duration and the severity of ischemia influence the effects of reperfusion. The duration of ischemia from which recovery of function can be expected varies from species to species, and is affected by temperature, drugs, anatomical variants, age and other factors. The severity of ischemia is influenced by the degree of perfusion of a core of tissue with its own exclusive capillary bed and by the perfusion of that tissue through collateral vessels able to react to the ischemic event nearby. Thus, acute occlusion of a major cerebral artery may cause severe or even complete ischemia in a small area, moderate ischemia in a larger adjacent area, and no ischemia in peripheral areas perfused normally by abundant collateral vessels.

We developed an animal model that provides a spectrum of low flow states for one hour after acute carotid occlusion. Measurement of rCBF and tissue water from the same area of brain in this preparation has allowed us to relate the severity of ischemia to the severity of edema which forms during the first hour after occlusion and to identify the effects of reperfusion upon rCBF and edema in the same tissue.

In earlier studies, brain water content was closely related to the severity of ischemia. The rCBF threshold for edema formation, identified in our gerbil model, coincided with that found in the primate model by Symon, et al. The ischemia threshold for edema formation we demonstrated was slightly higher than that.
associated with morphologic changes and severe ionic alterations.7,8

In the current study we test the effects of reperfusion upon the evolution of ischemic edema in an attempt to clarify the relationship between the severity of ischemia and the effects of reperfusion.

Methods
Adult gerbils (40–70 g) were anesthetized with pentobarbital (60 mg kg⁻¹, intraperitoneal). A PE polyethylene catheter was placed in the abdominal aorta through the left femoral artery and connected to a Statham P 23 Db transducer. Systemic arterial blood pressure was continuously monitored. Arterial blood samples were analyzed periodically for PaCO₂, PaO₂, pH and hematocrit. No more than 0.4 ml of blood was withdrawn from any animal during an experiment in order to preserve adequate intravascular volume. The left or both carotid arteries were exposed in the neck with the aid of magnification. Burr holes were made bilaterally over the frontal and parietal cortex, taking care to preserve the integrity of the dura and cortex beneath.

rCBF
Four teflon coated platinum electrodes (100 μm diameter) were inserted stereotactically 0.5 mm into the cerebral cortex and held in place with acrylic cement. Electrodes were polarized +400 mV to a silver-silver chloride reference electrode and allowed to stabilize for one hour. Hydrogen gas (2% in oxygen) was then delivered over the face of the spontaneously breathing animal. Clearance of hydrogen from the cortex was recorded when H₂ administration ceased. rCBF was calculated by the initial slope method.9 Earlier work has shown that artifacts due to recirculation of H₂ fall to 10% within 5 seconds of discontinuation of H₂ inhalation.3 rCBF of zero ("no flow") existed in our study only if H₂ failed to clear from the tissue and the amplitude of the resaturation curve failed to change.

Brain Edema
Brain water was measured by the specific gravity method at the conclusion of each experiment.10,11 Bromobenzene-kerosene columns were prepared and calibrated with solutions of K₂SO₄ of known specific gravity. Columns were used only if a uniform and continuous gradient was present from top to bottom.

Animals were killed by pentobarbital overdose and the brains were removed rapidly. With the aid of magnification and during immersion of the tissue in kerosene to avoid exposure to air, a sample of brain surrounding each electrode (excluding the actual area of electrode implantation) was dissected free and immersed immediately in the column. SG was measured for each of the four samples and was expressed as the mean ± S.D.

Experimental Protocol
Control rCBF, PaCO₂, PaO₂, pH, and hematocrit measurements were made when MAPB, heart rate and ventilation were stable. Thereafter, animals underwent unilateral or bilateral carotid occlusion with vascular clips. rCBF was measured when occlusion began, and at 30 min and 50 min after occlusion. Animals were then divided into two groups:

Group A (n = 20)
16 gerbils had unilateral and 4 had bilateral carotid occlusion for one hour. The animals were then sacrificed and the specific gravity of the tissue adjacent to each electrode was measured.

Group B (n = 24)
18 gerbils had unilateral and 6 had bilateral carotid occlusion for one hour after which clips were removed: the brains were reperfused for one hour. The animals were then sacrificed for measurement of specific gravity of the brain samples.

Control Group
The specific gravity of cerebral cortex was measured immediately after pentobarbital was given in three normal animals to verify consistency of our methods with those established before.3 Tissue around the electrode tips was sampled for water content in two others to assess implantation artifact. Sham carotid occlusions were carried out in three gerbils. rCBF was measured periodically for three hours and water content of brain tissue was assessed terminally three hours after sham occlusion.

Statistical Analysis
Specific gravity values for Group A (occlusion only) and Group B (occlusions plus reperfusion) were related to the corresponding rCBF values during the post-occlusion period. After distribution into equal rCBF bins (eg. 24–20, 19–15 ml·100g⁻¹·min⁻¹) unpaired t-tests were performed between corresponding bins of the two groups.

Results

Control Group
The specific gravity method for measuring tissue water content was consistent with that used by us before in a different laboratory.3 Moreover, tissue water around the implanted electrodes was not altered significantly from control over the course of each individual experiment. Finally, rCBF and tissue water did not change from control values over the three hours of observation after sham carotid occlusion.

Group A
Control rCBF for animals prior to unilateral carotid occlusion was 29.2 ± 4.9 ml·100g⁻¹·min⁻¹. After unilateral occlusion rCBF fell nearly 60% and remained depressed for the duration of occlusion (table 1). Control rCBF was 30.0 ± 4 ml·100g⁻¹·min⁻¹ prior to bilateral occlusion. After bilateral occlusion rCBF fell to 2.8 ± 1.3 ml·100g⁻¹·min⁻¹ and fell further during the one hour occlusion period.

Specific gravity (SC) of the cortex from the hemisphere ipsilateral to the single carotid occlusion was 1.0470 ± 0.0017 after clipping for one hour. This
Table 1  Cerebral Blood Flow: (ml·100g⁻¹min⁻¹, M ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10m'</th>
<th>50m'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unilat.</td>
<td>29.2 ± 4</td>
<td>12.8 ± 5</td>
<td>11.9 ± 7.1</td>
</tr>
<tr>
<td>bilat.</td>
<td>30.0 ± 4</td>
<td>2.8 ± 1.3</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unilat.</td>
<td>30.1 ± 5</td>
<td>11.0 ± 6</td>
<td>10.6 ± 6</td>
</tr>
<tr>
<td>bilat.</td>
<td>31.7 ± 5.8</td>
<td>2.7 ± 1.4</td>
<td>1.4 ± 1.8</td>
</tr>
</tbody>
</table>

decrease of SG from control (1.0500 ± 0.0004) represents a significant (p < .001) accumulation of water in the cortical samples one hour after occlusion. When both carotid arteries were occluded for one hour, SG failed to change from control, indicating no change in tissue water content when rCBF approximated zero.

A further relationship was apparent when rCBF was correlated with regional specific gravity. Water accumulated in brain cortex if rCBF fell below 20 ml·100g⁻¹min⁻¹. Specific gravity fell further as ischemia worsened, reaching its lowest level when flow was about 7 ml·100g⁻¹min⁻¹. When rCBF approximated zero, edema failed to develop and the specific gravity did not differ from control (fig. 1).

MABP and blood gases did not change in animals before and after unilateral carotid occlusion. MABP rose immediately after bilateral occlusion, accompanied by elevated pulse pressure, and remained above control values throughout the period of occlusion.

**Figure 1.** SG values for Group A (occlusion only, unshaded bars) and Group B (occlusion plus reperfusion, shaded bars) were related to the corresponding rCBF values during the period of occlusion: Mean SG (± SD) for each 5 ml·100g⁻¹min⁻¹ interval rCBF were obtained for both groups. In Group A, SG decreased when rCBF fell below 20 ml·100g⁻¹min⁻¹ and further decreased with increasing ischemia. When rCBF was close to zero SG did not differ from control values. In Group B, edema was absent after reperfusion when rCBF during occlusion was more than 19–15 ml·100g⁻¹min⁻¹ and was significantly less than Group A when flow was 14–10 ml·100g⁻¹min⁻¹ during occlusion. Water accumulation occurred after reperfusion in areas of zero flow in Group B.

**Figure 2.** Gerbils in which unilateral carotid occlusion caused rCBF to fall but remain above the threshold of reversibility for edema. At the release of occlusion Mean Arterial Blood Pressure (MABP ± SD) fell temporarily, but rCBF (± SD) returned to normal. Hyperemia did not develop.

Blood gases were not affected significantly by bilateral occlusion for one hour.

**Group B**

The rCBF values for animals with carotid occlusion for one hour followed by reperfusion for one hour are also shown in table 1. Flow values for animals in both Groups A and B were similar during the one hour period of unilateral or bilateral occlusion prior to reperfusion.

rCBF varied substantially when the ischemic hemisphere was reperfused after unilateral occlusion. If flow was greater than 12 ml·100g⁻¹min⁻¹ during occlusion, then rCBF returned to normal within one hour of reperfusion (fig. 2). Hyperemia did not develop in that situation and autoregulation remained intact (fig. 3). When flow was 10 ml·100g⁻¹min⁻¹ or less during occlusion, rCBF during reperfusion was highly variable (fig. 4). For example, clip release in two animals caused MABP to fall initially, accompanied by initial impairment of reperfusion. During the subsequent one hour period or reperfusion, rCBF rose above control values as MABP spontaneously rose in those same animals. In fact, hyperemia was present in all gerbils undergoing reperfusion after severe ischemia and was always associated with MABP exceeding 65 mm Hg.

When both hemispheres were reperfused after one hour of bilateral carotid occlusion, there was an initial fall in systemic arterial pressure after the clips were removed, associated with an initial impairment of reperfusion (fig. 5). When arterial pressure rose spontaneously thereafter, hyperemia appeared consistently and autoregulation was lost predictably. An estimate of cerebrovascular resistance (CVR) was calculated from the MABP/rCBF relationship. Figure 6 shows high resistance to flow associated with an initial decrease in blood pressure and the loss of cerebrovascular reactivity when the initial resistance was overcome.

While the clearance curves observed during the con-
Autoregulation evaluated during reperfusion in areas without edema (B). Autoregulation in a separate series (A) of normal gerbils is shown for comparison (Crockard et al., 1982). Values are mean ± SD.

Mean specific gravity of the cortical samples from animals with unilateral occlusion was not significantly different, whether or not the brains were reperfused for two and even three components during hyperemia. As suggested by Olsen et al. the lowest value was chosen as an expression of CBF in the area.

Mean specific gravity of the cortical samples from animals with unilateral occlusion was not significantly different, whether or not the brains were reperfused for one hour, provided rCBF exceeded 20 ml·100g⁻¹·min⁻¹ at the end of the occlusion period. Edema did appear after reperfusion, but only when rCBF values during the occlusions were less than 14 ml·100g⁻¹·min⁻¹ (fig. 1). Maximum edema was found in areas that sustained a severe ischemic insult (rCBF less than 10 ml·100g⁻¹·min⁻¹). When gerbils were reperfused for one hour after bilateral occlusion, specific gravity fell significantly (p < 0.0001), indicating the accumulation of water in brain tissue (fig. 1).

Discussion

Validity of the Model

Combining both the hydrogen clearance method for rCBF and the analysis of tissue samples for specific gravity allows us to measure rCBF and water content in contiguous areas of brain. While multiple rCBF measurements are possible throughout each experiment, brain tissue specific gravity can be evaluated only at its termination. Because of this methodological limitation, we have assumed water content of the brain samples to be the same in areas of brain from both Groups having the same residual rCBF at the end of the occlusion period. This assumption is validated by the uniformity of rCBF values in both Groups during the period of occlusion and the close relationship between residual flow and amount of edema produced at the end of one hour of occlusion. This approach allows us to study both the dynamics of edema and the kinetics of blood flow in brain tissue during occlusion and after reperfusion. The model also permits us to relate the edema found after reperfusion to the severity of ischemia that existed before reperfusion.

Dynamics of Edema and Blood Flow

Edema developed in our model when blood flow was less than 20 ml·100g⁻¹·min⁻¹ and increased with progressive reduction in blood flow. However, edema did not appear when blood flow was restored to areas in which rCBF was 15–19 ml·100g⁻¹·min⁻¹ during occlusion. Areas with postocclusion rCBF at 10–14 ml·100g⁻¹·min⁻¹ showed significantly (p < .01) less
edema after restoration of flow than comparable areas measured in the absence of reperfusion. An ischemic event whose rCBF was above 10–14 ml·100g⁻¹·min⁻¹ for one hour might, then, be considered a reversible insult for edema. The concept of reversibility, hypothetically dependent upon residual rCBF, implies the existence of a relationship between residual flow and the degree of tissue damage.

The ischemia threshold for disturbance of water homeostasis in our model is about 20 ml·100g⁻¹·min⁻¹. This threshold is identical to that found in baboon cortex. Electrical activity is depressed in animals and in man at approximately the same value of flow. Neurological signs also appear at that degree of ischemia.

The accumulation of substances such as neurotransmitters, lactate and arachidonate, caused to appear by a reduced energy charge, might be responsible for functional paralysis of neurons, the formation of an osmotic gradient between blood and brain, and the development of brain edema. This increase in water content may not be the result of failure of ionic homeostasis, a phenomenon known to occur at values of flow lower than 10–15 ml·100g⁻¹·min⁻¹. In fact, when rCBF falls to 9–11 ml·100g⁻¹·min⁻¹ extracellular K⁺ rises while Na⁺ and water move into the intracellular compartment. Brain impedance increases as the cells swell. When postocclusive rCBF is less than 10 ml·100g⁻¹·min⁻¹ edema is maximal at the end of occlusion and does not resolve with reperfusion.

Hossmann has shown that, during recovery from complete ischemia, edema associated with alterations of Na⁺ and K⁺ homeostasis is a reversible phenomenon when the normal energy charge is restored. On the other hand, Siesjo and Hass suggest that intracytoplasmic accumulation of Ca²⁺ is the triggering event for the onset of irreversible damage. Raised intracytoplasmic Ca²⁺ activity is known to alter cellular biochemistry, disrupt cell membranes and cause free fatty acids to accumulate. Vasactive substances released into tissue by the ischemic insult, may also contribute to formation of edema. Extracellular Ca²⁺ decreases when blood flow falls below approximately 10 ml·100g⁻¹·min⁻¹. It is not surprising that the same flow threshold is associated with development of morphological changes of infarction and irreversibility of edema. Thus the mechanism for edema clearance may operate only in areas in which a moderate decrease in flow causes a moderate energy imbalance, insufficient for normal function but adequate to maintain the structural integrity of the tissue.

When postocclusion rCBF was virtually nil in our experiments, edema was not found but significant edema did develop upon restoration of blood flow to tissue with virtually no flow for an hour. This concept of "no flow-no edema" has already been described. Hossmann's studies indicated that complete ischemia pro-
duces an increase in brain osmolality and a shift of ions and fluids within the brain, but in the absence of intravascular fluids, water cannot enter the brain. Upon recirculation ionic and osmotic gradients between brain and blood equilibrate and edema develops. Edema developed in our model upon reperfusion for one hour of tissue that had no flow at the end of one hour of bilateral carotid occlusion. Initial impairment of reperfusion in the presence of arterial hypertension, which developed consistently on release of bilateral occlusion, may have superimposed upon complete ischemia a period of severe but incomplete, and thus more deleterious ischemia. In fact, preservation of adequate perfusion pressure upon restoration of flow was essential for recovery in those experiments.

Development of hyperemia is a common finding when blood flow is restored to ischemic tissue. In our study, hyperemia and impaired autoregulation were present only in areas in which edema did not resolve, while resolution of edema was associated with preservation of autoregulation and the absence of hyperemia.

References
Ischemic brain edema with and without reperfusion: an experimental study in gerbils.

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