Edema, Cation Content, and ATPase Activity After Middle Cerebral Artery Occlusion in Rats

G.Y. Yang, MD; S.F. Chen, PhD; H. Kinouchi, MD; P.H. Chan, PhD; and P.R. Weinstein, MD

Background and Purpose: Reduction of cerebral blood flow results in several acute metabolic disturbances, including a reduction in Na,K-ATPase activity. The relation between this reduction and the onset of edema is unknown, as is the effect of restoration of blood flow. Therefore, we investigated the role of decreased Na,K-ATPase activity in the pathogenesis and time course of ischemic brain edema and reperfusion.

Methods: The middle cerebral arteries of rats were occluded by cannulation with a nylon suture for 30, 60, 120, or 240 minutes. The animals were then decapitated (permanent occlusion) or the suture was withdrawn to allow 24 hours of reperfusion before decapitation (temporary occlusion). Na,K-ATPase activity and Na⁺, K⁺ and water contents were measured at various intervals.

Results: In the ischemic hemisphere, Na,K-ATPase activity was significantly decreased at 30, 60, 120, and 240 minutes of permanent occlusion (p<0.05). There was also a significant decrease in rats subjected to 60 or 120 minutes of temporary occlusion followed by 24 hours of reperfusion. Water content increased after 60, 120, or 240 minutes of permanent occlusion (p<0.01); after 24 hours of reperfusion, water content remained elevated (p<0.01). The Na⁺ content increased after both permanent and temporary occlusion, and the K⁺ content decreased only after permanent occlusion. Increases in water content correlated with decreases in Na,K-ATPase activity after temporary occlusion and with the Na⁺:K⁺ ratio after permanent occlusion.

Conclusion: Reduction in Na,K-ATPase activity resulting in disruption of cellular ionic homeostasis may account for early development of cytotoxic brain edema after permanent occlusion of the middle cerebral artery. Such edema is also present 24 hours after 60 and 120 but not 30 minutes of temporary occlusion.

KEY WORDS • brain edema • cerebral ischemia • reperfusion • rats

Oclusion of the middle cerebral artery (MCA) causes metabolic disturbances in the brain that result in edema and infarction,¹,² which may be exacerbated rather than reversed even if ischemia is followed by reperfusion.³ The temporal sequence of these disturbances is not precisely known; however, interruption of cerebral blood flow is generally believed to result first in depletion of energy stores and next in disruption of ion homeostasis, release of neurotransmitter amino acids, and release of free fatty acids.⁴ These physiological and biochemical alterations are the primary determinants of cell survival or death.

Decreased energy metabolism results in dysfunction of all adenosine triphosphate (ATP)-dependent enzymes. One such enzyme is Na,K-ATPase, a catalyst for the Na⁺ pump, which normally maintains intracellular and extracellular Na⁺ and K⁺ concentrations. The transmembrane homeostasis of these ions helps to regulate cell volume through a process described by Donnan's pump and leak hypothesis.⁵ Although available data are limited, the activity of Na,K-ATPase during cerebral ischemia is thought to be decreased, leading to cellular edema, which may ultimately contribute to cell death.⁶

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A decrease in Na,K-ATPase activity has been reported during ischemia, but the results of in vivo studies have varied.⁷ Depletion of ATP precedes reduction of Na,K-ATPase activity in brain tissue affected by transient global ischemia, but it has not been proven that this ATP depletion is the primary factor causing the decreased enzyme function.⁹ Furthermore, Na,K-ATPase activity after regional ischemia followed by reperfusion has not been investigated. To clarify the relation between Na⁺ pump failure and the formation of brain edema, the correlations between changes in water content, cation concentration, and Na,K-ATPase activity were measured after occlusion of the MCA in rats.

To occlude the MCA either permanently or temporarily, our technique using intraluminal blockage of the internal carotid artery (ICA) with a nylon suture was used,¹⁰ rather than other published methods.¹¹,¹² This
 technique reliably produces both cortical and striatal ischemia for comparison with any changes that might occur in the contralateral, normally perfused hemisphere. Previous studies document that permanent MCA occlusion with this method reproducibly produces ischemic infarction in the distribution of the MCA, and withdrawal of the suture results in recirculation of the MCA.\textsuperscript{10} Laser Doppler studies indicate that cortical cerebral blood flow is reduced to 10–15% of the control level by this method of occlusion (J. Chen, personal communication). Because this method does not require craniectomy, the surgical mortality rate is low. Furthermore, reperfusion is easily accomplished by withdrawal of the suture.

**Materials and Methods**

The protocol used in this study was approved by the Committee on Animal Research at the University of California, San Francisco. Fifty-four adult male Sprague-Dawley rats weighing 260–320 g were anesthetized with 35 mg/100 g body wt i.p. chloral hydrate. A PE-50 catheter was introduced into the femoral artery to allow continuous monitoring of arterial blood pressure and sampling of blood for analysis of blood gases and pH. The rats were ventilated with a mixture of 30% O\textsubscript{2} and 70% N\textsubscript{2}O via an inhalation mask to maintain anesthesia and a Pao\textsubscript{2} level of 100 mm Hg or higher. Blood gases were analyzed three times during the experiment. Blood pressure was maintained above 90 mm Hg by reducing the anesthetic dose, and body temperature was maintained at 37°C with a heating pad.

The MCA was occluded by the method of Zea Longa et al\textsuperscript{10} with some minor changes. Under an operating microscope, the left common carotid artery (CCA) was exposed through a midline incision. The branches of the external carotid artery (ECA), including the occipital, terminal lingual, and maxillary arteries, were isolated and coagulated. The ICA was then isolated and its extracranial branch, the pterygopalatine artery, was ligated with 5-0 silk suture near its origin. The ICA was thus the only extracranial branch of the CCA that remained patent. Next, a 5-cm length of 4-0 nylon suture with a slightly enlarged and rounded tip was introduced into the transected lumen of the ECA and a slight curving of the advancing suture verified that the blunt tip of the suture had reached the proximal segment of the anterior cerebral artery (ACA). Resistance and a slight curving of the advancing suture verified that the blunt tip of the suture had reached the proximal segment of the anterior cerebral artery (ACA). The suture remained in this position during occlusion of the MCA. The distance from the tip of the suture to the bifurcation of the CCA was 22 mm. To restore ICA-MCA perfusion, the suture was withdrawn back into the ECA.

Water content, Na\textsuperscript{+} and K\textsuperscript{+} concentrations, and Na,K-ATPase activity were measured in rats subjected to MCA occlusion with or without reperfusion. The rats were divided into nine groups of six rats each. Two groups underwent a sham surgery protocol consisting of exposure without cannulation of the ICA and ligation of the ECA and pterygopalatine artery, with decapitation of sections (2 mm posterior to the frontal pole) at 240 minutes. Sections from the two hemispheres were separated, and one pair of sections (2 mm posterior to the frontal pole) was frozen in liquid nitrogen and stored at −70°C for a subsequent assay of Na,K-ATPase activity. The pair of slices immediately posterior to this slice was used to measure water content and Na\textsuperscript{+} and K\textsuperscript{+} concentrations.

The tissue samples were weighed on an automatic electrobalance (CAHN 25, Caton Division, Vertron Corp., Paramount, Calif.) with 0.01 mg precision to obtain wet weight (W). They were then dried in a desiccating oven (Fisher Isotemp, Santa Clara, Calif.) at 105°C for 24 hours and reweighed to obtain dry weight (D). The water content was calculated as \((W-D)/W\times100\). The dehydrated sections were digested in 4 ml of 2N nitric acid for 1 week. A 0.2-ml aliquot of the solution was then removed and diluted to 10 ml with deionized water. The Na\textsuperscript{+} and K\textsuperscript{+} contents were measured in this solution by atomic absorption spectroscopy (model 5600, Perkin-Elmer Corp., Norwalk, Conn.) based on our previously described method.\textsuperscript{13} Absorption was measured at 589.6 and 766.5 nm, using a hollow Na\textsuperscript{+}–K\textsuperscript{+} cathode lamp, a promix burner, and an acetylene–air flame. The flame conditions and detection wavelengths were optimized for sensitivity and linearity.

The activity of Na,K-ATPase was measured according to the method of Chan et al.\textsuperscript{14} The assay medium contained 80 mM imidazole, 100 mM NaCl, 20 mM KCl, 5 mM MgCl\textsubscript{2}, and 5 mM ATP at pH 7.5. The specimens were thawed and homogenized in buffer (10 ml/g wet wt). The assay was carried out at 37°C for 30 minutes. KCl was omitted and 0.1 mM ouabain was added to the incubation medium to assess ATPase activity not inhibited by ouabain (Mg-ATPase activity). This amount was subtracted from the total ATPase activity to obtain the Na,K-ATPase activity. The protein content of each homogenate was determined by the method of Lowry et al.\textsuperscript{15} and the activity of Na,K-ATPase was expressed in micromoles of inorganic phosphorus per milligram of protein per minute (\(\mu\)mol P\textsubscript{i}/mg protein/min).

In each experimental group, the contralateral hemispheres served as controls for comparing cation and water contents. Because of variations observed in the contralateral hemispheres of rats subjected to ischemia, values from sham-operated rats were used as controls for comparing Na,K-ATPase activity from the ischemic hemisphere in the experimental groups. The sham-operated rats were also used as controls for comparing physiological data. The data are presented as mean±standard error of the mean (SEM). The statistical significance of differences in water content, Na\textsuperscript{+} and K\textsuperscript{+} concentrations, and Na,K-ATPase activity was determined using analysis of variance. Correlations between water content, the Na\textsuperscript{+}:K\textsuperscript{+} ratio, and Na,K-ATPase activity in each group were evaluated by linear regression analysis. Probability values less than 0.05 were considered to represent significant differences.

**Results**

There were no significant differences in mean arterial blood pressure, blood gases (Pao\textsubscript{2}, Paco\textsubscript{2}), or blood pH among rats in permanently occluded, reperfused, and
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TABLE 1. Physiological Parameters in Rats During Middle Cerebral Artery Occlusion and Reperfusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham-operated controls</th>
<th>Occlusion duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>MAP</td>
<td>103.7±8.5</td>
<td>102.2±11.3</td>
</tr>
<tr>
<td>PaO₂</td>
<td>125.0±10.2</td>
<td>122.8±5.0</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>36.4±3.8</td>
<td>41.4±3.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.337±0.019</td>
<td>7.336±0.024</td>
</tr>
<tr>
<td>Temporary occlusion and 24 hours reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>106.0±6.4</td>
<td>106.3±8.2</td>
</tr>
<tr>
<td>PaO₂</td>
<td>122.8±5.7</td>
<td>128.8±5.4</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>39.2±6.1</td>
<td>39.1±3.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.335±0.013</td>
<td>7.339±0.011</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure. Values are mean±SEM.

Sham-operated groups (Table 1). Animals were excluded from the studies when physiological parameters were not in the normal range during an experiment.

Water content measurements in the contralateral hemisphere were similar at 30, 60, 120, and 240 minutes of permanent occlusion (Figure 1, top). In the ischemic hemisphere at 30 minutes of permanent occlusion, there was no significant difference between water content in the ischemic and contralateral hemispheres. However, beginning 60 minutes after occlusion, the water content was higher in the ischemic hemisphere (p<0.01).

After 30 minutes of temporary occlusion and 24 hours of reperfusion, water contents in the ischemic and contralateral hemispheres were similar (Figure 1, bottom). After 60 or 120 minutes of temporary MCA occlusion and 24 hours of reperfusion, however, the water content was greater in the ischemic hemisphere than in the contralateral hemisphere (p<0.01).

Both Na⁺ (Figure 2, top) and K⁺ (Figure 3, top) levels were similar in the control and ischemic hemispheres at 30 minutes of permanent occlusion, and all levels were in the normal range as determined in previous studies. At 60 minutes of permanent occlusion, the Na⁺ concentration was 19.6% higher in the ischemic hemisphere than in the contralateral hemisphere (432.2±46 versus 361.4±32 meq/kg dry wt, p<0.05). At 240 minutes of permanent occlusion, the Na⁺ concentration was 62.2%
higher in the ischemic hemisphere (594.3±51 versus 366.3±26 meq/kg dry wt, *p<0.01). At 60 minutes, the K+ concentration was 12.0% less in the ischemic hemisphere than in the contralateral hemisphere (455.9±13 versus 517.8±17 meq/kg dry wt, *p<0.05). At 240 minutes, the K+ concentration was 18.8% less in the ischemic hemisphere (412.3±20 versus 507.5±14 meq/kg dry wt, **p<0.01).

After 60 minutes of temporary occlusion and 24 hours of reperfusion, the level of Na+ in the ischemic hemisphere was slightly, but not significantly, higher than in the contralateral hemisphere (Figure 2, bottom). After 120 minutes of temporary occlusion followed by 24 hours of reperfusion, the Na+ concentration was increased in the ischemic hemisphere (*p<0.05). There were no significant changes in K+ concentration in the ischemic hemisphere after 30, 60, or 120 minutes of temporary MCA occlusion (Figure 3, bottom).

In the brains of the sham-operated rats, the Na,K-ATPase activity was 0.605±0.055 μmol Pi/mg protein/min. At 30 minutes of permanent MCA occlusion, this activity was decreased in the ischemic hemisphere to 67% of the control level (Figure 4, top). Despite slight increases after occlusion for 60 or 120 minutes, Na,K-ATPase activity in the ischemic hemisphere was decreased to 58% of the control level after 240 minutes of permanent occlusion. For each duration of permanent occlusion, the Na,K-ATPase activity was significantly lower than in the sham-operated rats.

The Na,K-ATPase activity 24 hours after the onset of reperfusion was significantly lower than the control level after 60 and 120 minutes of temporary MCA occlusion, but not after 30 minutes (Figure 4, bottom). The activity after 30, 60, and 120 minutes of MCA occlusion followed by 24 hours of reperfusion was 86.6%, 80.0%, and 76.9% of the control level in sham-operated rats, respectively.

After temporary occlusion of the MCA, regression analysis demonstrated a correlation between increases in water content and decreases in Na,K-ATPase activity in the ischemic hemisphere (r= -0.52, *p<0.05; Figure 5, top). In all groups subjected to permanent MCA occlusion, there was a correlation between increases in water content and the Na+:K+ ratio (r=0.59, *p<0.05; Figure 5, bottom). After permanent occlusion, no correlation was seen between changes in water content and Na,K-ATPase activity.

**Discussion**

This study demonstrates that the water content and Na+ level increase progressively with duration of ischemia and that the K+ level and Na,K-ATPase activity decrease in rat brains after both permanent and temporary occlusion of the MCA. The changes observed in cation levels are consistent with previously reported changes in the levels of extracellular Na+ and K+ in focal brain ischemia. These acute changes may produce substantial ion gradients between the lesion site and the surrounding brain tissue, cerebrospinal fluid, and the intravascular compartment. Diffusion of Na+ and K+ across these gradients results in influx of Na+ into the ischemic region and efflux of K+ out of it. Because these extracellular ion shifts coincide with the development of
edema, it is reasonable to suggest that they are involved in the evolution and propagation of ischemic edema.

In cellular edema, Na\(^+\) in the extracellular space exchanges with K\(^+\) in the cells, resulting in an increase in intracellular Na\(^+\) and a decrease in intracellular K\(^+\) without overall changes in net ion concentration or increases in water content. This explains our findings of decreased Na,K-ATPase activity without changes in water content or cation concentration after 30 minutes of permanent MCA occlusion. The occurrence of measurable alterations in these values after longer durations of ischemia probably reflects delayed onset of vasogenic edema formation from increased blood-brain barrier (BBB) permeability.\(^\text{18}\) The lack of correlation between increases in water content and changes in cation concentration 24 hours after temporary MCA occlusion may also reflect the presence of cellular edema with partial resolution of vasogenic disturbances.\(^\text{8}\) Other processes such as diffusion, glial cell uptake, or ion exchange between the lesion site and surrounding tissue or cerebrospinal fluid may also contribute to the observed normalization of K\(^+\) and, to a lesser extent, Na\(^+\) concentrations.\(^\text{17,19}\)

Reduction in Na,K-ATPase activity after 30 minutes of permanent occlusion not associated with changes in water and cation content suggests a possible relation between Na\(^+\) pump failure and cellular edema formation. The correlation observed between changes in Na,K-ATPase activity and water content 24 hours after temporary MCA occlusion also supports this hypothesis with respect to the pathogenesis of persistent postischemic cellular and vasogenic edema. The lack of such a correlation in the permanent occlusion groups may be a result of experimental variability in measurements of enzyme activity.

Our results are compatible with the hypothesis that Na\(^+\) pump failure is an important cause of edema during early ischemia. Complete interruption of the cerebral circulation rapidly depletes the energy reserves of the brain and decreases Na,K-ATPase activity, leading to cerebral edema and cell death.\(^\text{20,21}\) If circulation is restored promptly and completely, cerebral energy stores can normalize rapidly, even after 60 minutes of complete ischemia.\(^\text{16,22}\) Our observation that Na,K-ATPase activity was still reduced after posts ischemic reperfusion only after longer durations of temporary occlusion suggests that the recovery of enzyme function depends on the duration of MCA occlusion. Thus, the 30-minute temporary occlusion group may represent a transient ischemic attack in which enzyme activity recovered and edema was not present after 24 hours of reperfusion. The persistent inactivation of Na,K-ATPase could be a result of mitochondrial damage during ischemia that prevents restoration of ATP levels.\(^\text{14}\)

The increase in water content observed 24 hours after 60 or 120 minutes of temporary MCA occlusion indicates that edema, like reduced Na\(^+\) pump activity, is not completely reversed by reperfusion. Cation concentrations, in contrast, returned to normal except for Na\(^+\) after 120 minutes of occlusion. Although Hossmann et al\(^\text{23}\) found that postischemic brain swelling is reversed by reperfusion and that cation concentrations return to control levels or nearly to control levels within 2–3 hours after temporary global ischemia lasting as long as 60 minutes, delayed recurrence of brain edema has also been described.\(^\text{24}\) Our results indicate that 24 hours after focal temporary ischemia lasting 60 or 120 minutes, edema may still be present either because irreversible damage has occurred or because the edema has not yet resolved.

Betz et al\(^\text{19,25}\) proposed a polarity of Na\(^+\) movement across the BBB, facilitated by Na,K-ATPase at luminal sites, where Na\(^+\) is pumped from the blood into the brain in exchange for K\(^+\). However, ischemic alteration of enzyme activity at this site alone would not explain the substantial increase in Na\(^+\) concentration observed after regional ischemia resulting from permanent occlusion of the MCA. Nevertheless, our results do not exclude the possibility of a partial breakdown in the permeability of the BBB to plasma Na\(^+\) after temporary or permanent MCA occlusion. Further experiments to evaluate the selective permeability of the BBB in this model are required to elucidate the relative importance of these mechanisms of edema formation.

Early cellular edema resulting from cerebral ischemia is a complex process that may be reversible before cell necrosis occurs. Further research for interactions between energy-dependent mechanisms of enzymatic regulation of cation and water contents should provide additional explanations for the pathogenesis of ischemic edema.

References


Editorial Comment

The authors have used their own, fine model of permanent or temporary middle cerebral artery occlusion in the rat to investigate the time course of Na,K-ATPase activity and Na⁺, K⁺, and H₂O content in ischemic brain. Their findings may not be very surprising, but confirmation of what were previously only hypotheses or suspicions is very useful. With permanent occlusion between 30 and 120 minutes, Na,K-ATPase activity was approximately 60% of control; after 24 hours of reperfusion this was approximately 80%. It should be borne in mind, however, that for the assay of ATPase activity, a sufficient amount of ATP is provided; actual activity during ischemia, when ATP is depleted, is probably much lower. The correlation between water content and Na,K-ATPase after 24 hours of reperfusion does not necessarily indicate a causal relation, as 80% of activity of an enzyme system would seem more than sufficient to maintain homeostasis. The degree of increase in water content and decrease in ATPase activity may just be similar indicators of the severity of the injury to multiple homeostatic systems. Nevertheless, this study lays the groundwork for more research in the important area of brain edema during and after an ischemic insult, and the authors' model will be a useful adjunct.

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