Sodium, ATP, and Intracellular pH Transients During Reversible Complete Ischemia of Dog Cerebrum

Scott M. Eleff, MD; Yuichi Maruki, MD; Lee H. Monsein, MD; Richard J. Traystman, PhD; R. Nick Bryan, MD, PhD; and Raymond C. Koehler, PhD

We tested the hypotheses that with the onset of cerebral ischemia, massive cellular sodium influx does not occur until adenosine triphosphate is fully depleted and that on reperfusion, neuronal sodium efflux does not occur until adenosine triphosphate is fully restored. We examined the temporal relationships among transcellular sodium, energy metabolism, and intracellular pH with sodium and phosphorus magnetic resonance spectroscopy in a new, hemodynamically stable, brain stem-sparing model of reversible, complete cerebral ischemia in eight anesthetized dogs. Inflation of a neck tourniquet after placement of glue at the tip of the basilar artery resulted in decreased blood flow to the cerebrum from 29 ± 5 to 0.3 ± 0.5 ml/min/100 g. Medullary blood flow was not significantly affected, and arterial blood pressure was unchanged. Sodium signal intensity decreased and did not lag behind the fall in adenosine triphosphate. After 12 minutes of ischemia, reperfusion resulted in a more rapid recovery of sodium intensity (12.4 ± 4.8 minutes) than either adenosine triphosphate (16.5 ± 3.7 minutes) or intracellular pH (38.9 ± 1.8 minutes). Because intracellular sodium has a weaker signal than extracellular sodium, the decreased sodium intensity is interpreted as sodium influx and indicates that sodium influx does not require full depletion of adenosine triphosphate. Rapid recovery of sodium intensity during early reperfusion may represent sodium efflux, although increased plasma volume and sodium uptake from plasma may also contribute. If our interpretation of the sodium signal is correct, delayed recovery of adenosine triphosphate may be due to the utilization of adenosine triphosphate for the restoration of transcellular sodium gradient. (Stroke 1991;22:233-241)

The application of magnetic resonance spectroscopy (MRS) to in situ brain allows the time course of ischemically perturbed cerebral bioenergetics as reflected by adenosine triphosphate (ATP), phosphocreatine, and intracellular pH1-2 and transcellular sodium gradient3 to be examined. The potential interactions of brain sodium gradients, intracellular pH, and bioenergetics are many. Maintenance of a normal sodium gradient, necessary for normal neurologic function, is an ATP-dependent process and accounts for 40% of the metabolic demand of the brain as assessed by high-dose lidocaine during barbiturate coma.4 Maintenance of a normal intracellular pH is critical for proper functioning of most enzymatic processes. With the onset of complete ischemia, intracellular pH falls as glucose stores are converted to lactic acid and ATP is hydrolyzed.5 Soon after ATP depletion, extracellular Na+ activity decreases.6 However, Na+ influx may precede the sudden decrease in extracellular Na+ activity as measured by microelectrodes if this early Na+ influx is accompanied by sufficient water to maintain a constant sodium activity. On reperfusion, limited ATP generation may be diverted into restoring ionic gradients instead of maintaining neuronal integrity. It is unclear whether the transcellular sodium gradient is restored only after ATP levels are normalized, or if the ATP utilization required to restore the gradient delays normalization of ATP concentration and thereby delays ATP availability for other metabolic processes.

We tested two hypotheses. First, with the onset of ischemia, massive Na+ influx does not occur until...
ATP is fully depleted. Second, on reperfusion, Na+ efflux does not occur until ATP concentrations are fully restored to normal levels. We used phosphorus-31 and sodium-23 MRS to directly measure ATP, intracellular pH, and changes in detectable sodium MRS signal with 1-minute time resolution together with measurements of cerebral blood flow and oxygen consumption before, during, and after 12 minutes of global cerebral ischemia. Interpretation of the 23Na MRS signal is based on intracellular sodium being relatively undetectable by MRS. A decrease in 23Na MRS signal will occur when extracellular sodium goes intracellular (collapse of the neuronal sodium gradient). However, a change in total intracranial sodium, as might occur with models using cerebrospinal fluid infusion or hemorrhage-induced changes in intracranial blood volume, will also affect the 23Na MRS signal. For this reason, we developed a new model of complete, reversible ischemia, which we characterized both angiographically and with regional blood flow measurements in the present study. In contrast to the carotid system, the dog's spinal–basilar arterial system is resistant to compression via reversible application of a rapid-filling, high-pressure neck tourniquet. Angiographically guided, selective embolization of the tip of the basilar artery prevents filling of the circle of Willis via the basilar artery. We find that this results in a model of global cerebral ischemia with brain stem sparing and a stable blood pressure.

Materials and Methods

Dogs were anesthetized with high-dose fentanyl (50 μg/kg i.v.) plus low-dose pentobarbital (10 mg/kg i.v.). We infused additional pentobarbital during the surgery and experiment (3 mg/kg/hr) to maintain a constant level of reduced cerebral oxygen consumption.2 Animals were intubated and ventilated to maintain normal blood gases, and a tracheostomy was performed to maintain ventilation during neck cuff inflation. Catheters were placed in axillary arteries and in the left ventricle via the femoral artery for microsphere measurements as previously described.2 A 7F tapered to a 5F catheter was introduced into the right femoral artery through a 7F sheath. The catheter was positioned in the proximal left vertebral artery. Under fluoroscopic guidance, a Tracker-18 2.7F catheter was passed through this catheter over a Seeker-14 guidewire and then was negotiated successively through the vertebral, third spinal branch, and ventral spinal arteries into the basilar artery above the origin of the caudal cerebellar arteries. A mixture of 2 ml N-butyl cyanoacrylate glue, 500 mg tantalum powder, and 0.5 ml Pantopaque was injected until the mixture was just seen exiting the tip of the catheter, which then was withdrawn. Distal migration of the glue before complete setting was minimized by about 10 seconds of cardiac arrest obtained with 200–300 μg/kg acetylcholine chloride injected into the left ventricular catheter. The time between glue injection and the onset of experimentally induced cerebral ischemia was about 2 hours.

Temporalis muscle and skin were fully retracted from the skull so there was no muscle contamination in the MRS spectra. A midline burr hole was made in the skull, and a catheter was inserted in the sagittal sinus. The animal was fitted with a high-pressure, cuffed bladder around the neck for later inflation and was placed in a copper-lined cradle with the head fixed in position by a stereotactic frame and a warm water-perfused blanket wrapped around the abdomen.

Oxygen tension (PO2), CO2 tension (PCO2), and pH of blood samples were measured with Radiometer ABL 3 electrodes and analyzer (Copenhagen). Oxygen saturation, hemoglobin concentration, and oxygen content were measured with a CO-oximeter (model 282, Instrumentation Laboratories, Lexington, Mass.). Blood glucose levels were analyzed with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Arterial blood pressure and sagittal sinus pressure were measured with transducers and were recorded continuously on a Gould-Brush polygraph (Gould Instruments, Cleveland, Ohio).

Cerebral blood flow from the entire cerebrum (grey and white matter), but excluding caudate nucleus, thalamus, and lower structures, which were measured separately, was measured with radioabeled microspheres (15-μm diameter) injected through the left ventricular catheter as previously described.2 Cerebral oxygen consumption was calculated by multiplying the arterial–sagittal sinus venous oxygen content difference by cerebral blood flow.

MRS spectra were obtained on a CSI spectrometer (General Electric, Fremont, Calif.) interfaced with a 40-cm bore, 4.7 T magnet (Oxford Instruments, Oxford). A 2-cm3 glass tube containing inorganic phosphate and the sodium salt of the shift reagent dysprosium–triethylentetraminehexaacetic acid was placed on the skull. Signal acquisition was limited to the cortex by the use of an appropriately sized MRS surface coil.7 A 5-cm-diameter, inductively coupled, single-turn surface coil was multiply tuned for 23Na (53 MHz), 31P (81 MHz), and 1H (200 MHz) and was placed over the skull and external standard. The MRS free induction decays were averaged in separate memory bins for 1 minute. Sodium free induction decays were collected every 500 ms (120 per minute); phosphorus free induction decays were collected every 3 seconds (20 per minute). Typical pulse lengths for 31P and 23Na were 100 and 125 μsec, respectively. Intracellular pH was calculated from the chemical shift of inorganic phosphate from phosphocreatine.8 The area under β-ATP after 20-Hz line broadening was used for calculating changes in ATP from control levels. Sodium signal intensity was calculated from the peak height after a 10-Hz line broadening was applied.

We studied 11 dogs; eight underwent the complete ischemia protocol. Baseline 31P and 23Na MRS mea-
measurements were accumulated, arterial and sagittal sinus blood samples were obtained, and microsphere injections were made. Additional 1-minute MRS spectra were collected, after which the neck tourniquet was inflated to 400 mm Hg for 12 minutes. Spectra were collected in 1-minute averaged blocks for the next 60 minutes. Microsphere blood flow measurements and arterial and sagittal sinus blood samples were obtained at 8 minutes of ischemia and 8, 30, and 60 minutes of reperfusion. Arterial PCO₂ was maintained at 35 mm Hg throughout the experiment.

Intracellular pH, ATP, and sodium were considered to have recovered when they regained 86% (two time constants) of signal strength of the difference from control and end-ischemia. A log transformation of recovery time was used to reduce skew. One-factor analysis of variance with repeated measures was applied to test for significance at the 5% level. Data are presented as mean±SEM. The Newman-Keuls multiple range test was applied to test for differences between sodium, ATP, and intracellular pH recovery.

Results

We evaluated infratentorial and supratentorial blood flow angiographically and with radiolabeled microspheres. The top left panel of Figure 1 illustrates the right and left carotid circulation and the basilar system forming the circle of Willis. The bottom left panel shows the effects of inflating the neck tourniquet in the presence of a patent basilar system, demonstrating normal filling of the circle of Willis and hence flow to the cerebrum even though there is no flow through the common carotid arteries. After glue embolization of the rostral basilar artery tip, selective vertebral artery injection of contrast demonstrates normal filling of posterior fossa vessels with no flow to the supratentorial compartment (Figure 1, top right panel).
TABLE 1. Effect of Glue Embolization on Cerebral Hemodynamic and Arterial Blood Analyses

<table>
<thead>
<tr>
<th></th>
<th>Pre-glue</th>
<th>Post-glue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow (ml/min/100 g)</td>
<td>35.4±4.1</td>
<td>28.8±5.0</td>
</tr>
<tr>
<td>Medullary blood flow (ml/min/100 g)</td>
<td>25.7±2.6</td>
<td>20.8±3.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>126±8</td>
<td>107±5</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.38±0.02</td>
<td>7.38±0.03</td>
</tr>
<tr>
<td>Arterial PCO₂ (mm Hg)</td>
<td>35±3</td>
<td>34±3</td>
</tr>
<tr>
<td>Arterial PO₂ (mm Hg)</td>
<td>132±17</td>
<td>135±13</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.5±1.0</td>
<td>15.6±1.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=7).

We quantified cerebral blood flow with radiolabeled microspheres (Table 1). Because blood flow determined by radiolabeled microspheres is limited to six time points, Table 1 includes data from four of the eight dogs used in the full protocol and an additional three dogs used for testing the stability of the model. After glue embolization, cerebral and medullary blood flow were 87±17% and 80±9% of preembolization values, respectively. These changes were not significant (p>0.4 and p>0.1, respectively).

Inflation of the neck tourniquet resulted in essentially no flow to the cerebrum (Table 2), whereas flow to the medulla was maintained above 12 ml/min/100 g, a level sufficient to maintain brain stem auditory evoked responses. Mean arterial pressure remained unchanged. At 8 minutes of reperfusion, a significant increase in blood flow was seen in the cerebrum but not in the medulla. Cerebral hypoperfusion was evident at 60 minutes of reperfusion, whereas medullary blood flow remained unchanged. Cerebral oxygen consumption was significantly reduced throughout reperfusion. There was no significant change in arterial blood gases (Table 3).

Table 4 summarizes the recovery results. Recovery of the sodium signal was significantly faster than recovery of ATP, which was significantly faster than recovery of intracellular pH. Figure 4 shows the time course of all three parameters before and during ischemia and for the first 25 minutes after ischemia. We tried to synchronize the induction of cerebral ischemia with the beginning of minute -12 (minute zero represents start of reperfusion). Our accuracy was approximately ±20 seconds. Sodium and ATP signals fell in parallel to within 10% of their eventual minimum within 4-6 minutes. Intracellular pH, which started at a control value of 7.09±0.04, continued to decrease throughout ischemia, reaching a value of 6.19±0.07 at 12 minutes of ischemia. With reperfusion, recovery of sodium preceded recovery of ATP. Both recovery curves were qualitatively similar in shape, but ATP had a greater initial delay. The ATP concentration remained at about 10% of control value for the first 3 minutes, at which time sodium had recovered 50%. Intracellular pH recovery lagged significantly behind reperfusion, both Na and ³¹P MRS spectra had returned to baseline (Figures 2D and 3D).

Table 2 summarizes the effects of 12 minutes of ischemia on cerebral hemodynamics.

Table 2. Effects of 12 Minutes of Ischemia on Cerebral Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Post-glue (n=8)</th>
<th>Ischemia (n=8)</th>
<th>Reperfusion (min) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>97±3.9</td>
<td>106±8.2</td>
<td>89±6.9</td>
</tr>
<tr>
<td>CBF (ml/min/100 g)</td>
<td>28.6±5.4</td>
<td>0.3±0.5*</td>
<td>52.6±5.5*</td>
</tr>
<tr>
<td>MBF (ml/min/100 g)</td>
<td>20.6±3.4</td>
<td>16.3±2.4</td>
<td>22.8±3.7</td>
</tr>
<tr>
<td>CMRO₂ (ml/min/100 g)</td>
<td>3.4±0.47</td>
<td>...</td>
<td>2.45±0.42*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; CBF, cerebral blood flow; MBF, medullary blood flow; CMRO₂, cerebral metabolic rate of oxygen consumption. Note MBF and MAP are unaffected in this brain stem-sparing model of global cerebral ischemia.

*Significantly different from control.

Figure 2. One-minute ²³Na magnetic resonance spectroscopic scans from our model of basilar tip embolization and reversible neck tourniquet ischemia of dog brain. Trace A is control, trace B is minute 12 of ischemia, trace C is minute 12 of reperfusion, and trace D is minute 70 of reperfusion. The decrease in sodium amplitude in trace B reflects net movement of sodium from the extracellular to the intracellular spaces. Return of sodium amplitude in traces C and D represents restoration of the sodium gradient.
sodium and ATP recovery. At 17 minutes, intracellular pH was 6.60±0.06 (approximately half recovered). At 43 minutes of recovery, intracellular pH was nearly fully recovered (7.02±0.04).

Discussion

We believe these results do not confirm our initial hypotheses that sodium influx into cells occurs abruptly only when cerebral ATP levels reach zero during complete ischemia or that sodium efflux during reperfusion occurs only when cerebral ATP levels are restored to normal. Instead, the sodium MRS signal decreased in parallel with the decrease in ATP during ischemia, and an increase in sodium MRS signal preceded full ATP recovery during reperfusion.

Our assumption that changes in $^{23}$Na MRS signal are proportional to changes in interstitial and intracellular sodium is critically dependent on keeping total intracranial sodium nearly constant. Changes in total sodium, as occurs with edema formation, would increase the $^{23}$Na MRS signal even when the sodium gradient does not change. For this reason, we chose a model in which total intracranial sodium should remain constant during ischemia. Additionally, increased tissue uptake of sodium during reperfusion has generally been observed after longer ischemic durations than presently used. Whether there is significant tissue uptake immediately after 12 minutes of ischemia is unclear. It should be recognized that the use of $^{23}$Na MRS in the brain is a very new technique with many potential artifacts, such as a changing intracellular detectability factor.

Models of complete global cerebral ischemia in the dog include ventricular fibrillation, aortic occlusion, and increased intracranial pressure with controlled hemorrhage. All of these models may invoke secondary effects from other organ systems, changing total intracranial sodium and factitiously altering the sodium MRS signal, which is sensitive both to the total amount (not concentration) of sodium ions and to their distribution (intracellular versus extracellular). Four-vessel occlusion models, which work well in the rat, have variable results in the dog. This is due to numerous collateral vessels even when both internal carotid and vertebral arteries are ligated. The most important of these collaterals is the large spinal-basilar system in the dog. The numerous vertebral branches anastomose with the spinal artery and form the basilar artery. The bony encasement of these vessels prevents compression when a neck tourniquet is inflated. In fact, neck tourniquet inflation with compression of the carotid arteries has little effect on brain blood flow in the dog. Angiographic placement of glue is clinically performed in certain vascular abnormalities of the brain. We adapted this technique to prevent blood flow from the basilar artery to the circle of Willis. Exact placement of the glue is critical, and for this reason, we injected acetylcholine into the heart to cause 10–20 seconds of cardiac arrest. The circulatory half-life of acetylcholine is brief and should not cause any persistent effects on the vasculature. Placement of glue at the basilar tip has an insignificant effect on cerebral blood flow. This is to be expected because the vertebral-basilar and carotid systems of the dog are physiologically independent. Subsequent rapid inflation of a neck tourniquet to 400 mm Hg stops all carotid and supratentorial blood flow without venous hypertension and without affecting the spinal basilar system (Table 2). The lack of a posts ischemic increase in medullary blood flow and the stable arterial blood pressure during cuff inflation are indicative of adequate brainstem perfusion.

Unlike $^{31}$P MRS, in which the compounds of interest resonate at different frequencies secondary to covalent bonds, intracellular and extracellular sodium contribute to the same peak. Because $^{23}$Na has spin $\frac{1}{2}$ ($^{31}$P and $^1$H are simple spin $\frac{1}{2}$ nuclei), interactions between charged protein in the intracellular space and quadrupolar relaxation mechanisms make intracellular sodium only partially detectable by MRS, although this may be due to instrumental limitations. Changes in sodium concentration would have minimal effects on detectability. This has been confirmed and recently reviewed for many tissue types. The finding that intracellular sodium is partially undetectable has been confirmed in gerbil and cat brains and is also seen in sodium magnetic resonance imaging studies of human brain. Therefore, if total tissue sodium is constant, a decrease in sodium MRS signal implies a shift from extracellular to intracellular sodium, that is, a collapse of the transneuronal gradient.

Because total tissue sodium should be conserved when there is no blood flow during the onset of complete ischemia, sodium influx into cells can be estimated. Assuming a baseline intracellular sodium concentration of 30 mM, intracellular volume of 80%, extracellular sodium concentration of 154 mM, and extracellular volume of 20%, then 56% of tissue sodium is extracellular. In cell systems, sodium MRS detectability is in the range of 40–70%. Assuming an average intracellular sodium MRS detectability of 50% in brain in vivo, the observed drop in sodium MRS signal to 86.8% of control (Table 4) implies a decrease in extracellular sodium from 56% to 36% of tissue sodium. Thus, we estimate that 37% of extracellular sodium moved intracellularly. Therefore, relatively small percent changes in sodium signal intensity represent substantial Na+ flux because of the amplification by the MRS detectability factor.

The assumptions in this calculation were subjected to error sensitivity. A 20% overestimation of the pres ischemic intracellular or extracellular amount of sodium led to only a 7% error in estimated sodium influx. Therefore, estimated sodium influx is relatively insensitive to assumptions of the initial conditions of sodium partitioning. On the other hand, the calculation is sensitive to the MRS detectability factor. If the intracellular sodium detectability factor ranges 40–60%, then the amount of sodium influx...
FIGURE 3. One-minute $^{31}$P magnetic resonance spectra acquired concurrently with Figure 2 traces. The three peaks on the right side of the spectrum in trace A are the gamma, alpha, and beta phosphorus nuclei of adenosine triphosphate (ATP); the next peak to the left is phosphocreatine, then a diester peak (PDE), inorganic phosphate (Pi), and phosphomonoesters (PME) composed mainly of sugar phosphates. Notice complete absence of ATP and intracellular pH of 6.2 in trace B, partial return of ATP but intracellular pH of 6.5 in trace C, and return to baseline intracellular pH of 7.1 in trace D.

ranges 29–48% of the preischemic extracellular sodium. Nonetheless, the relative changes in sodium distribution would still be correct. Because water diffuses more freely than sodium, a 29–48% range of sodium influx is comparable in magnitude to the approximately 50% shrinkage in the extracellular space estimated by other techniques.24,25 Furthermore, a 29–48% decrease in extracellular water would result in as much as a 7–12% increase in intracellular volume. These volume changes represent an upper bound because potassium efflux will mitigate the effect of sodium influx on water shifts. Therefore, our data are consistent with a significant shift of water into the intracellular space during ischemia.

Ion-sensitive electrode measurements of extracellular sodium activity indicate little change over the first few minutes of complete ischemia, followed by an abrupt decrease coincident with depolarization, which presumably occurs when ATP is completely lost.26 In contrast, the sodium MRS signal began to decrease in parallel with ATP. This apparent difference can be reconciled because the change in sodium MRS signal represents sodium influx, whereas micro-electrodes are sensitive to concentration. In a frog skin model system, intracellular and extracellular activity coefficients are similar, implying that sodium is readily exchangeable.19 If the initial sodium influx is accompanied by water, extracellular sodium activity may remain unchanged until there is massive potassium efflux and extracellular water retention. Our finding differs from those of Naritomi et al,3 in which graded hypotension in the gerbil had minimal effects on the sodium MRS signal until after $^{31}$P MRS measured decreases in ATP. However, measurements on the small gerbil brain required 10 minutes for each data point, and only 3–6 minutes are needed to deplete ATP. In addition, graded, incomplete ischemia may produce different transient changes than a one-step, single decrease in cerebral blood flow to zero. It is also possible that the initial drop in sodium signal at 30 seconds represents a decrease in

TABLE 3. Arterial and Sagittal Sinus Blood Samples

<table>
<thead>
<tr>
<th></th>
<th>Post-glue (baseline)</th>
<th>Ischemia</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.40±0.02</td>
<td>7.37±0.03</td>
<td>7.34±0.02</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>34±2</td>
<td>37±4</td>
<td>37±2</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>174±20</td>
<td>156±25</td>
<td>156±22</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.3±1.1</td>
<td>15.5±0.8</td>
<td>15.8±1.1</td>
</tr>
<tr>
<td>Oxygen content (ml/dl)</td>
<td>20.9±1.6</td>
<td>20.9±1.3</td>
<td>21.6±1.7</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>69±3</td>
<td>76±4</td>
<td>88±5</td>
</tr>
<tr>
<td>Sagittal sinus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>54±3</td>
<td>...</td>
<td>55±6</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>29±3</td>
<td>...</td>
<td>53±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
Table 4. Time in Minutes to 86.5% Recovery After 12 Minutes of Global Cerebral Ischemia

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Sodium</th>
<th>ATP</th>
<th>pH</th>
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<tr>
<td>1</td>
<td>21</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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<td>3</td>
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<td>7</td>
<td>3</td>
<td>10</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>12</td>
<td>39</td>
</tr>
</tbody>
</table>

Average recovery: 12.4±4.8 16.5±3.7* 38.9±1.8t
End-ischemic value: 86.8±2.8 0±0 6.19±0.07 (% control)

*Significantly different from sodium recovery.
†Significantly different from sodium and ATP recovery.

cerebral blood volume, because venous drainage from the cerebrum through the spinal venous plexus might persist with cuff inflation. However, this would not negate our observation that the subsequent fall in sodium signal parallels that of ATP, rather than occurring only after ATP is fully depleted.

The finding that the $^2$Na MRS signal, indicative of the sodium gradient, begins to recover before ATP recovery is consistent with the following facts. First, the single greatest energy-requiring function of the neuron is maintenance of the transneuronal ionic gradient. Maintenance of ionic gradients requires approximately 50% of total oxygen consumed. The free energy for ATP hydrolysis ($\Delta G_{ATP}$ = 14.7 kcal/mol) exceeds that for Na$^+$-K$^+$ ATPase ($\Delta G_{Na,K}$ = 8.3-9.9 kcal/mol, neuron-glia, respectively). Therefore, the Na$^+$-K$^+$ pump operates far from equilibrium. In fact, even at the $K_m$ for ATP of the Na$^+$-K$^+$ pump of 0.2-0.4 mM, the pump is still far from equilibrium. During the first few minutes of recovery, the maximum rate of ATP synthesis may be slow secondary to decreased mitochondrial function. Therefore, the recovery of the sodium gradient, an energy-requiring process, might be expected to act as a clamp on ATP concentration by preventing ATP from rising above the Na$^+$-K$^+$/ATPase $K_m$ of 0.2-0.4 mM (7-13% control ATP) until the rate of ATP synthesis from partially stunned mitochondria exceeds maximum pump usage. Furthermore, sodium itself can potentiate calcium inhibition of state 3 respiration in isolated mitochondria. This analysis of the free energies involved in sodium transport does not explain the apparent decrease in the sodium gradient before significant loss of ATP. Perhaps there is a large increase in inward Na$^+$ permeability preceding the full loss of ATP during ischemia.

There are several alternative explanations for the rapid recovery of the sodium MRS signal. First, the initial increase in the sodium MRS signal during the first minute of reperfusion may represent refilling of the plasma space, particularly if the estimated 37% decrease in extracellular sodium occurred in both interstitial and plasma spaces. Moreover, postischemic hyperemia may also contribute to the faster recovery of the sodium signal. Nevertheless, even if the initial sodium recovery during the first minute represents a vascular effect, the remaining recovery paralleled ATP recovery. Thus, our hypothesis that recovery of the transcellular sodium gradient lags behind recovery of ATP is still not confirmed even when vascular effects are considered. A second possibility is that cell lysis during early reperfusion allows intracellular sodium to become extracellular and therefore fully detectable by MRS. However, if there was significant lysis, ATP recovery would be decreased proportionately, and we did not observe this.

Finally, we cannot exclude the possibility that sodium may be transported down its concentration gradient from plasma to interstitial fluid during early reperfusion. However, this would probably lead to an overshoot of sodium MRS signal because total tissue sodium would increase. We did not observe an overshoot in this study, which was limited to the first hour of reperfusion. In most studies demonstrating

Figure 4. Effects of 12 minutes of cerebral ischemia on measurable values from magnetic resonance spectroscopy. Notice postischemic recovery of sodium precedes recovery of adenosine triphosphate (ATP), which precedes recovery of intracellular pH (pHi). Data are scaled so that zero represents control values and 100 represents the minimum value obtained for an individual experiment (mean values in Table 4).
large increases in total tissue sodium, measurements were made either after longer durations of permanent focal ischemia or incomplete global ischemia or after more prolonged complete ischemia and several hours of reperfusion. Ito et al did not observe substantial increases in tissue sodium during reperfusion in the gerbil until the duration of carotid occlusion was extended beyond 30 minutes. However, with 15 minutes of severe forebrain ischemia in rats, significant edema formation was detected between 5 and 15 minutes of reperfusion. In our study, the $^{23}$Na MRS intensity largely recovered by 6 minutes of reperfusion. Therefore, we believe that Na$^+$ efflux out of neurons and glia is the major contributing factor to the rapid recovery of the sodium MRS signal during the early minutes of reperfusion after 12 minutes of complete ischemia, although tissue sodium uptake may also contribute as reperfusion is prolonged.

In this study, as in our previous study, intracellular pH recovery lags substantially behind ATP recovery. This observation is consistent with the concept that lactic acidosis persists during early reperfusion. This observation is consistent with the concept that lactic acidosis persists during early reperfusion. In this study, as in our previous study, intracellular pH recovery lags substantially behind ATP recovery. This observation is consistent with the concept that lactic acidosis persists during early reperfusion.

Acknowledgment

We gratefully acknowledge the fine technical assistance of Adrian Lawrence.

References


**KEY WORDS** • animal models • nuclear magnetic resonance • cerebral blood flow • dogs
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