Chronic Cerebral Intracellular Alkalosis Following Forebrain Ischemic Insult in Rats

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We measured cerebral intracellular pH using in vivo phosphorus-31 nuclear magnetic resonance spectroscopy during 1 week after forebrain ischemia or sham operation in eight and seven rats, respectively. Mean maximum pH was significantly higher (p<0.003) in the ischemic group than in the sham-operated group (7.34±0.03 and 7.19±0.02, respectively). The difference between mean maximum pH and baseline pH (7.08±0.01 in each group) was significantly greater (p<0.02) in the ischemic group than in the sham-operated group. In the ischemic group, alkalosis occurred primarily after 48–72 hours of recirculation. We speculate that brain tissue alkalosis occurring chronically after ischemia is associated with delayed ischemic neuronal death. (Stroke 1990;21:463–466)

Cerebral intracellular pH is an important physiologic parameter that changes significantly during and after ischemia. During global cerebral or forebrain ischemia, brain tissue becomes acidic, dropping to a pH of approximately 6.1.1,2 Brain pH usually returns to preischemic values during the first 40 minutes of recirculation, and brain tissue becomes transiently alkalotic during the first hours of recirculation.3–6 A recent in vivo phosphorus-31 nuclear magnetic resonance (31P NMR) study of stroke patients found cerebral tissue alkalosis days after the ischemic event, with a significant correlation between alkalosis and poor clinical outcome.7,8 Likewise, positron emission tomographic data from stroke patients show significant intracellular alkalosis in the infarcted region 10–19 days after the onset of symptoms.9 These data underscore the need to measure brain pH chronically in experimental animals after a controlled ischemic event. To our knowledge, these measurements have not been reported.

Materials and Methods

We used 15 male Wistar rats weighing 220–300 g. All rats were fasted overnight preceding surgery but were allowed free access to water. We subjected eight rats to 8 minutes of forebrain ischemia and the other seven rats to sham operation without the induction of ischemia. Near-complete forebrain ischemia was induced by occluding both common carotid arteries and lowering systemic arterial blood pressure to 50 mm Hg. This model has been described in detail by Smith et al.10,11

The rats were anesthetized 24–48 hours before surgery with 0.5% halothane in 70% N2O and 30% O2 using a face mask and placed in the NMR magnet to obtain baseline pH values. Further measurements were initiated 24 hours after surgery so as to carefully control the induction of ischemia and limit postsurgical stress. In vivo pH was measured using 31P NMR 24, 48, 72, 96, and 168 hours after surgery, with the rat anesthetized as above. During each measurement the rat's body temperature was maintained at 37° C using a heating pad, and the duration of the measurement never exceeded 30 minutes.

In vivo 31P NMR studies were conducted using a 400-MHz, wide-bore (89-mm) Varian VXR 400 spectrometer (Palo Alto, California). A two-turn insulated 6-mm o.d. surface coil was centered near the suture line over the forebrain of each rat. Experiments performed in our laboratory using this coil confirmed no detectable signal contamination from scalp tissue. Spectral acquisition parameters selected to optimize sensitivity were 40 μsec pulse length, 12,000 data points, and 12 kHz spectral width. A recycle delay of 110 msec was used, giving a total interpulse interval of 603 msec. All spectra were processed using either 10- or 20-Hz line broadening and were zero-filled to 16,000 data points.

Intracellular pH was calculated from the chemical shift between phosphocreatine and inorganic phosphate (Pi)12 as pH=6.72+log((δ−3.39)/(5.70−δ)),

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where $\delta$ is the chemical shift in parts per million between the Pi and phosphocreatine peaks. This form of the Henderson-Hasselbach equation was derived from a titration curve generated on our spectrometer, using solutions of known pH similar to those used by Petroff et al. Maximum correction to pH values over the biologic range of cation concentrations is 0.05 pH units.

The groups were compared using Student's unpaired two-tailed $t$ test, and the maximum and baseline pH values within a group were compared using the paired $t$ test; $p<0.05$ was considered to indicate a significant difference.

## Results

Table 1 lists the arterial pH, blood gas tensions, and serum glucose concentrations for the ischemic and sham-operated groups. No significant differences between the groups were detected for any parameter.

Table 1. Arterial pH, Blood Gas Tensions, and Serum Glucose Concentrations for Rats Before and 30 Minutes After Forebrain Ischemia or Sham Operation

<table>
<thead>
<tr>
<th>Time</th>
<th>Sham-operated group</th>
<th>Ischemic group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<tr>
<td></td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>pH</td>
<td>7.38±0.03</td>
<td>7.36±0.03</td>
</tr>
<tr>
<td>PacO₂ (mm Hg)</td>
<td>31.5±3.4</td>
<td>34.6±3.8</td>
</tr>
<tr>
<td>PacO₂ (mg/dl)</td>
<td>150±22</td>
<td>135±22</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>166±21</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are mean±SD.

Figure 1 shows the baseline spectrum and those acquired 24 and 168 hours after ischemia for a representative rat. Each spectrum contains narrow resonances from metabolites within the brain, superimposed upon a broader resonance originating primarily from immobile phosphates in the calvaria. The shift of the Pi peak (peak 2) to the left by 24 hours after ischemia indicates alkalosis.

Figures 2 and 3 are plots of cerebral intracellular pH for the eight rats subjected to forebrain ischemia and for the seven sham-operated rats, respectively. Data from individual rats, as opposed to mean values, are plotted so as not to obscure the individual temporal dependence of the alkalotic shifts; means±SEMs are given in the legends.

All ischemic rats exhibited an increase in pH above baseline values at some time after ischemia (Figure 2). Mean baseline pH in this group was 7.08±0.01; pH increased to a mean maximum of 7.34±0.03 ($p<0.005$). Only two rats exhibited alkalosis 24 hours after ischemia, while the remaining six rats achieved maximum pH either 48 ($n=5$) or 72 ($n=1$) hours after ischemia. In five rats, alkalosis did not last 24 hours.

In the sham-operated group mean baseline pH was 7.08±0.01 (Figure 3), and pH increased to a mean maximum of 7.19±0.02 ($p<0.004$). Maximum pH values were evenly distributed between 24, 48, and 96 hours, with one rat achieving a maximum pH at 72 hours. For the sham-operated group the deviation of the mean±SEM pH values at any time was ≤0.04±0.02 pH units relative to any other time, and the uniformity of the means and SEMs over all times suggests that the observed alkalosis peak may be attributed to experimental error in pH resolution and to inherent biologic variation.

The differences between the maximum and baseline pH values were calculated for both groups; the difference was significantly greater in the ischemic group ($p<0.02$). The mean maximum pH in the ischemic group was also significantly greater than that in the sham-operated group ($p<0.003$).

## Discussion

Our data demonstrate that after 8 minutes of forebrain ischemia, cerebral intracellular pH is significantly higher than baseline and significantly higher than after sham operation. Alkalosis tends to occur $>24$ hours after ischemia, with six of eight rats achieving maximum pH values 48 or 72 hours after ischemia. Other investigators have also noted acute alkalosis 1–3 hours after ischemia, but this alkalosis resolves within hours. Yoshida et al found a relation between poor metabolic recovery and intracel-
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7.5; 7.4; 7.3; 7.2; 7.1; 7.0; 6.9; 6.8

FIGURE 2.  Plot of cerebral intracellular pH from phosphorus-31 nuclear magnetic resonance vs. time after ischemia for eight rats subjected to 8 minutes of forebrain ischemia. Time 0, baseline pH measured 24–48 hours before ischemia. Mean±SEM pH values are 7.08±0.01 at 0 hours, 7.10±0.06 at 24 hours, 7.27±0.05 at 48 hours, 7.14±0.04 at 72 hours, 7.03±0.01 at 96 hours, and 7.03±0.02 at 168 hours.

lular alkalosis in rat brain; pH in the dorsal thalamus increased to 7.48±0.10 after 1 hour of complete cerebral ischemia followed by 4 hours of recirculation. These authors hypothesized that severe tissue alkalosis may be a biochemical marker of advanced tissue injury during recirculation. The ischemic model that we used invariably causes moderate to severe neuronal damage, with neuronal loss in the cortex, hippocampus, and striatum.11,14 Thus, alkalosis may also be indicative of cerebral ischemic cell damage.

Recent clinical studies also support a relation between chronic brain tissue alkalosis and tissue injury. A positive correlation between the degree of postischemic alkalosis and neurologic damage was found in 28 patients suffering an ischemic insult to the middle cerebral artery territory.13,14 Syrota et al9 also found an alkaline shift, to a maximum of 0.45 pH unit, in infarcted human cerebral tissue; a significant correlation between the increase in intracellular pH and the decrease in oxygen extraction fraction was found, suggesting aerobic glycolytic activity concurrent with luxury perfusion in the infarcted region. Aerobic glycolysis has been associated with the presence of active phagocytic cells, which infiltrate ischemic tissue 24 hours after the onset of stroke.15 Human brain tumors also exhibit significant alkalosis compared with normal brain tissue, and alkalosis within brain tumors has been associated with stimulation of cell growth.16 Alkalosis has also been reported in brain subjected to a photodynamically induced insult17; within 1–2 days after treatment the cerebral tissue progresses to necrosis, and a concomitant brain tissue alkalosis is measured.

There appears to be two distinct cycles of alkalosis after an ischemic insult. The first one (as noted by other investigators) occurs and resolves early after an ischemic event3–6 and the second is delayed, occurring most frequently in the present model 48 hours after an ischemic event. Acute intracellular alkalosis may be associated with cytotoxic edema mediated by activation of a Na+/H+ antiporter.18,19 The influx of Na+ into a cell may activate efflux of H+ to maintain electroneutrality. Delayed alkalosis is consistent with the time course of delayed neuronal necrosis noted in rat models of forebrain ischemia20 and may signal severe and irreversible cell damage. Vasogenic edema, delayed with respect to the ischemic insult, may lead to expansion of the extracellular space and

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possible equilibration of the intracellular and extracellular pH.

Alkalosis itself may contribute to cell death. K+ channels in cardiac cells are strongly pH-dependent and could be cytoprotective against ischemia.21 The opening of K+ channels by decreasing intracellular pH causes a rapid cellular hyperpolarization and limits Ca2+ entry. A decrease in intracellular pH, in conjunction with the opening of K+ channels by arachidonic acid, also contributes to more rapid repolarization of cells. Conversely, alkalosis decreases K+ movement into the intracellular space. Cameron22 also found that extracellular alkalosis causes K+ to move into the intracellular space. It is therefore possible that moderate acidosis after ischemia may have a protective role in ischemic cell damage, whereas alkalosis, by decreasing K+ conductance, may itself be detrimental to cell survival.

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References


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