Brain Tissue pH after Global Brain Ischemia and Barbiturate Loading in Rats

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SUMMARY Studies were done on rats to determine whether thiopental loading after complete, transient, global brain ischemia causes more rapid postischemic normalization of brain tissue pH. Fifteen halothane-anesthetized rats were subjected to 16 min of complete global brain ischemia by a combination of systemic arterial hypotension (40 torr) and a high pressure (1500 torr) neck cuff. Brain tissue pH was continuously monitored for up to 2 hour postischemia with microelectrodes (tip diameters of one to two \( \mu m \)) inserted about 500 \( \mu m \) into the parietal cortex. During ischemia, brain pH fell rapidly within the first 5 min from 7.0 to 6.2 and changed little thereafter. With restoration of arterial pressure and deflation of the neck cuff, pH did not immediately begin to rise back towards normal. Instead, after a few minutes, it transiently fell to even lower values before beginning to increase indicating increased tissue lactic acidosis when the brain is resaturated with glucose upon reperfusion. Beginning at 5 min postischemia, 7 of the 15 rats were infused with thiopental (90 mg/kg, IV) over 60 min. At 30 min postischemia, brain tissue pH was similar in both groups and by 60 min, back to preischemic values. We conclude that thiopental loading postischemia does not improve normalization of brain pH. The transient decrease in brain pH with reperfusion is discussed.

Methods

**Fabrication of pH Microelectrodes.** Spear-type microelectrodes were fabricated according to the methods described by Hebert. Briefly, micropipettes were made from glass codes 0150 (pH sensitive) and 0120 (soft glass) (Corning Glass Works, Medfield, MA) using a double barreled microelectrode puller (Narishige, Inc., Tokyo, Japan). The tips of the 0120 micropipettes were cut at a diameter of about 50 \( \mu m \). Sixty to 100 \( \mu m \) sections of the tips of the 0150 micropipettes were lodged and heat-sealed in the 0120 micropipettes (fig. 1). Intact 0120 micropipettes were used as reference electrodes. Both pH and reference electrodes were filled by boiling in distilled, deionized water for 60 min. After cooling to room temperature, 2.5 M KCl was flushed into the microelectrodes which were then stored over distilled water. One to 2 days later, the microelectrodes were tested for pH sensitivity. Low sensitivity was usually corrected by immersion in Nochromix for several hours and a slow response by alternate immersion in 0.1 N NaOH and 0.1 N HCl. All microelectrodes used had a sensitivity of 55 mV/pH or greater.

**pH Measurement System.** All pH measurements were made in a double-walled aluminum-screened Faraday cage (fig. 1). The reference and pH microelectrodes were connected via 2.5 M KCl to balanced Ag/AgCl electrodes which were connected to an MPA-6 differential DC preamplifier fed by an MPS-15 DC power supply (Transidyne General Corp., Ann Arbor, MI). The output of the preamplifier was fed into an Accudata 122 DC amplifier (Honeywell, Inc., Denver, CO) and recorded on a strip chart recorder (Model 29, Linear Instruments Corp., Irvine, CA).

**Microelectrode Calibration**

The pH microelectrodes were calibrated at 37°C in 0.05 molar, certified potassium phosphate monobasic-sodium hydroxide buffers (Fisher Scientific Company,
**Figure 1.** Physical configuration of microelectrode pH recording system. Both reference and pH electrodes were inserted approximately 500 μm into the parietal cortex of the rats' brains. Insert shows magnified illustration of the pH microelectrode tip. The rats were electrically isolated from ground while the stereotaxic apparatus in which their heads were fixed was earthed.

Fairlawn, NJ) at pHs of 6.98 and 5.98. Calibrations were done before and after each study (fig. 2). An average baseline drift of 0.195 pH occurred during the studies with unchanged sensitivity. All brain pH measurements were corrected for linear baseline drift as verified in vitro.

**Experimental Procedures**

Sprague-Dawley albino rats (300-500 grams body weight) were maintained ad libitum on rat Purina chow and water up to the time of the experiments. Anesthesia was induced with 4 percent halothane in oxygen and maintained on halothane, one percent in oxygen. The rats were mechanically ventilated after immobilization with pancuronium bromide (0.2 mg, IM). Catheters were inserted into a femoral artery and vein. The rats heads were fixed in a stereotaxic device (David Kopf, Inc.), and the skin removed from the dorsum of the calvarium. Cranionotomies were carefully made over the left cerebral hemisphere and the pH and reference microelectrodes inserted about 500 μm into the frontoparietal cortex. The cranionotomies were sealed with agar-agar in 0.9 percent NaCl. Arterial blood samples (0.5 ml) were obtained to verify normal arterial blood gas values (i.e., $P_{aO_2}$ > 300 torr; $P_{aCO_2}$, 35-45 torr; pH, 7.3-7.4; and base excess ± five mEq/L). All arterial samples were replaced in equal volumes by blood from a donor rat. End-tidal CO$_2$ and rectal temperatures were controlled at 5 to 6 percent and 37-39°C, respectively. Thereafter, a 30 min stabilization period was allowed and followed by a second arterial blood sample withdrawn immediately before ischemia.

**Results**

Physiological variables in control (table 1) and thiopental (table 2) groups were similar. $P_{aO_2}$ was maintained well above 300 torr while $P_{aCO_2}$ was controlled between 35 and 45 torr. A preischemic base deficit of about minus 5 mEq/1 increased to minus 9 mEq/1 at 15 min postischemia. Hematocrits were constant throughout the studies in both groups. Rectal temperatures were regulated at about 37°C. Preischemia, mean brain tissue pH ranged between 7.03 and 7.06 (figs. 3, 4). A slight fall in brain pH oc-
curred with trimethaphan arterial hypotension. With ischemia (cuff on), brain pH fell rapidly and within 5 min, attained near minimum plateau levels ranging between 6.1 and 6.2. Little further decrease occurred between 5 and 16 min of ischemia.

With restoration of MAP in control rats, mean brain pH remained unchanged for the first one to 3 min, rapidly increased when MAP rose to 90 and 155 torr and continued to rise until 5 min postischemia. Between 6 and 10 min, it plateaued at about 6.5 which seemed to correlate with a slight fall in MAP from about 115 to 105 torr. A second plateau at pH 6.8 occurred between 15 and 30 min postischemia before gradually rising to or slightly higher than preischemic levels by 60 min.

In thiopental rats, MAP was 120 torr within one min after recirculation, resulting in an early increase in brain pH from 6.1 to 6.3. Between one and 10 min postischemia, brain pH plateaued at about 6.3 which again appeared to correlate with a slight transient fall in MAP as observed in the control rats. However, these transient reductions in MAP were not statistically significant (p > 0.05). After 10 min postischemia, brain pH increased linearly and by 60 min was back to preischemic values.

In each of the studies of both control and thiopental groups, brain pH remained unchanged for the first one to 3 min after restoration of MAP then transiently decreased by 0.2 to 0.6 pH before beginning to increase (figs. 3, 4). However, these changes were not reflected in the mean data (figs. 3, 4) because of slight variations in time course in each rat.

Discussion

Whether barbiturates are beneficial in the treatment of global ischemic brain damage warrants some dis-

<table>
<thead>
<tr>
<th>Time</th>
<th>$P_{aO_2}$ (mm Hg)</th>
<th>$P_{aCO_2}$ (mm Hg)</th>
<th>pHa</th>
<th>BE mEq/L</th>
<th>Hct</th>
<th>Temp °C</th>
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</thead>
<tbody>
<tr>
<td>Postischemia 15 min</td>
<td>347</td>
<td>42</td>
<td>7.22</td>
<td>-9.0</td>
<td>38</td>
<td>37.0</td>
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<tr>
<td>SD</td>
<td>35</td>
<td>7</td>
<td>0.08</td>
<td>2.6</td>
<td>4</td>
<td>0.8</td>
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<tr>
<td>30 min</td>
<td>361</td>
<td>41</td>
<td>7.23</td>
<td>-8.1</td>
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<td>SD</td>
<td>45</td>
<td>4</td>
<td>0.11</td>
<td>2.6</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>60 min</td>
<td>359</td>
<td>41</td>
<td>7.23</td>
<td>-8.3</td>
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<td>SD</td>
<td>20</td>
<td>11</td>
<td>0.12</td>
<td>3.0</td>
<td>4</td>
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<tr>
<td>90 min</td>
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<td>-6.5</td>
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<td>4</td>
<td>0.5</td>
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</tbody>
</table>

*Eight rats subjected to 16 min transient global brain ischemia induced by systemic arterial hypotension and a high pressure neck tourniquet.

*Seven rats infused with thiopental 90 mg/kg IV beginning at 5 min postischemia. One third the total dose was infused over 5 min and the remaining two-thirds over 35 min.

**FIGURE 3. Mean arterial pressure (MAP) and brain tissue pH in halothane anesthetized rats subjected to 16 min of complete global brain ischemia by arterial hypotension and a high pressure (1500 torr) neck cuff. Inflation of the neck cuff (cuff on) immediately followed trimethaphan hypotension. During ischemia, MAP was controlled at 40 torr. One min before the end of ischemia, norepinephrine (0.016 mg/ml) was infused IV to begin restoration of MAP to preischemic values. Postischemia, MAP was maintained by continued infusion of norepinephrine.
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**Figure 4.** Mean arterial pressure (MAP) and brain pH during global brain ischemia with postischemic thiopental (90 mg/kg) infusion. Thiopental infusion was begun at 5 min postischemia with one third the total calculated dose infused between 5 and 10 min postischemia and the remaining two thirds in the ensuing 55 min. See figure 2 for additional details.

Discussion in light of the current controversy.11, 30 Wright and Ames4 and Goldstein, Wells and Keats,12 reported that barbiturate anesthesia increased the tolerance of the cat and dog, respectively, to global brain ischemia, but their studies were criticized for the lack of monitored postischemic physiologic variables.11 Bleyaert et al13 provided 24 hour intensive care and life support for 7 days in monkeys subjected to 16 min global brain ischemia and found that thiopental loading early postinsult markedly improved neurologic recovery.

Steen and Michenfelder11 were unable to reproduce the results of Goldstein et al.10 possibly because they prevented stress-induced hyperglycemia and cerebral hypermetabolism22 with 70 percent N2O analgesia in addition to lidocaine infiltration of surgical sites in control dogs, while Goldstein et al. used only the latter with immobilization. Glucose loading of the brain worsens neurologic deficit after cerebral ischemia anoxia23 and hypermetabolism probably accelerates pathologic changes during ischemia. The degree of neurologic damage observed by Steen and Michenfelder was less than observed by Goldstein et al. resulting in a reduction in the severity of neurologic damage between control and pentobarbital dogs. Snyder et al.20 reported that postinsult thiopental administration after asphyxial arrest in dogs failed to improve neurologic recovery. However, thiopental treated dogs suffered episodes of severe arterial hypotension with MAPs as slow as 20 torr during thiopental infusion. Also, it is likely that at 3 to 4 hours postresuscitation, the adequacy of spontaneous ventilation and maintenance of arterial pressure in thiopental dogs was less than in control dogs. For these reasons we do not believe that their study was a valid evaluation of the efficacy of thiopental in the treatment of global ischemic anoxic damage. It should also be mentioned that in our experience, dogs are much more sensitive to thiopental than monkeys, resulting in severe cardiac abnormalities with thiopental loading.

Complete global brain ischemia produced by arterial hypotension and the high pressure neck cuff was verified in the monkey19 and evaluated in the rat by visual inspection and biochemically (unpublished observations). Removal of the brain from the calvarium during neck cuff ischemia in heparinized rats revealed that blood trickled into the calvarium only when systolic arterial pressure exceeded 150 torr. Thus, an MAP between 40 and 50 torr should ensure complete ischemia. Comparison of the rate and magnitude of brain cyclic-AMP changes during global ischemia by decapitation and the neck cuff indicates that the latter produces complete ischemia. Finally, the rapid fall and plateau in brain pH indicates complete ischemia since continued perfusion would have resulted in a protracted decline.

The duration of ischemia and effects of thiopental loading in monkeys may not be comparable to their effects in rats. However, 15 min of complete global ischemia in the rats results in prolonged stupor or coma and a sustained significant decrease in brain energy charge potential with severe EEG abnormalities.21 Thus, 16 min of ischemia should result in severe neurologic dysfunction. Although unknown for thiopental, the pentobarbital requirement for surgical anesthesia in monkeys and rats is similar at 30 mg/kg IV.22 Thus, our study should reveal whether thiopental loading improves normalization of brain pH after transient global ischemia.

During one percent halothane anesthesia, brain extracellular pH was about 7.1. This value is similar to that reported by other investigators a) using micro-electrodes with tip diameters of one to 5 μm,4, 7 b) calculated,15, 16 c) DMO28 and d) pH indicator29 estimated brain pH values suggesting a small pH gradient across brain cell membranes. During ischemia, the fall in brain pH approximates the time course of the rise in brain lactate and fall in brain glucose and high energy phosphates.13, 30 After the first 5 min of ischemia, little further change occurred indicating that the acidosis was almost entirely due to
lactate accumulation derived from brain glucose stores.

The transient fall in brain pH with recirculation reflects the relative permeabilities of the blood-brain barrier (BBB) to glucose and lactate and the status of brain oxidative metabolism early postischemia. The BBB is 5 to 6 times more permeable to glucose than to lactate. Recirculation rapidly resaturates the brain with glucose. It is quickly converted to lactate by activated glycolytic enzymes causing a further fall in brain pH. The severe tissue acidosis during ischemia and early postischemia impedes lactate clearance from the brain, while inhibition of brain oxidative metabolism retards oxidation of accumulated lactate. When high energy phosphates and ionic homeostasis are restored, glycolytic activity falls and brain pH gradually returns to normal.

Postischemic thiopental loading did not accelerate brain pH normalization. Unless attributable to the insignificant and transient decreases in MAP, it even appeared to delay pH normalization while in normal brain it increases brain pH and reduces lactate. During ischemia, lactate rapidly accumulates due to a combination of arrested oxidative metabolism and activated glycolytic enzymes attributable to a release of ATP inhibition, activation by ADP and Na⁺ and K⁺ intracellular-extracellular shifts. With recirculation, energy charge potential is restored to 99 per-

**Figure 5-7:** Mean arterial pressure (MAP) and brain tissue pH (pial cortex) in rats during and after 16 min global brain ischemia illustrating the transient decrease in pH preceding the increase with recirculation.
cent of normal within 5 min postischemia. Subsequent restoration of ionic homeostasis inhibits glycolysis, phosphofructokinase activity. Barbiturates apparently only inhibit oxidative metabolism associated with neuronal activity. Thiopeptil may inhibit ATPase activity, the restoration of ionic homeostasis and, therefore, the inhibition of glycolysis. These effects may explain the delay in brain pH normalization and greater brain lactate excretion with thiopental infusion early postischemia. In summary, barbiturate loading after global brain ischemia does not result in more rapid normalization of brain pH. The administration of thiopental in large doses early postischemia appears to delay the normalization of brain pH at least in the first 30 min postischemia. In addition, our results show that the initial reaction of brain pH to recirculation following global ischemia is a further fall in brain pH presumably attributable to rapid conversion of glucose to lactate.

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References

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