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 posts ischemia with microelectrodes (tip diameters of one to two µm) inserted about 500 µm 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 posts ischemia, 7 of the 15 rats were infused with thiopental (90 mg/kg, IV) early postischemia and incomplete ischemia, an excessive increase in brain lactate is believed to be responsible for the worsened biochemical derangements and recovery of neurologic function.

Bleyaert et al.,4 recently showed in monkeys that thiopental loading (90 mg/kg, IV) early posts ischemia 5 days after 16 min of complete global brain ischemia supporting earlier findings in cats5 and dogs6 with barbiturate pretreatment. The mechanism of barbiturate amelioration of ischemic brain damage is unknown, but improved brain oxygenation secondary to a reduction in oxygen consumption has been suggested.7,8,9,10 Thio pental loading after global brain ischemia in the rat did not improve normalization of brain PO2, but a direct metabolic effect causing more rapid recovery of brain pH through a mechanism unrelated to brain oxygenation could not be excluded.11,12

In normal brain, barbiturates increase pH and reduce lactate.13,14 The aim of this study was to determine whether postischemic thiopental loading of the rat brain subjected to 16 min complete, transient, global brain ischemia enhances normalization of brain tissue pH. Our findings show it does not.

SUBSTANTIAL EVIDENCE indicates that brain acidosis plays an important role in the pathogenesis of ischemic encephalopathy. First, the severity of brain edema appears to correlate with the degree of tissue acidosis. Second, preischemic glucose loading of the brain worsens neurologic recovery after global ischemia.8,9 Third, incomplete compared to complete global brain ischemia results in greater metabolic derangements.10,11 In both preischemic glucose loading and incomplete ischemia, an excessive increase in brain lactate is believed to be responsible for the worsened biochemical derangements and recovery of neurologic function.


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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. Craniotomies were carefully made over the left cerebral hemisphere and the pH and reference microelectrodes inserted about 500 μm into the frontoparietal cortex. The craniotomies 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., Pao₂ > 300 torr; Paco₂, 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 CO₂ 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.

Global brain ischemia was induced by a method previously described in monkeys. Briefly, thiopental 0.2 mg, was injected intravenously to decrease abruptly mean arterial pressure (MAP) to 40 to 50 torr. Immediately thereafter, a high pressure neck tourniquet, loosely wrapped around the neck, was inflated to 1500 torr for 16 min. Throughout ischemia, MAP was maintained at 50 torr by manipulation of inspired halothane and positive end-expiratory pressure. After 15 min of ischemia, norepinephrine (0.016 mg/ml) was infused intravenously to restore MAP gradually to about 125 torr by the end of ischemia. All measurements were continued for up to 2 hours postischemia.

Statistical comparisons were done using t-tests for unpaired data. Unless stated otherwise, all significant differences have a p value of 0.05 or less.

Results

Physiological variables in control (table 1) and thiopental (table 2) groups were similar. Pao₂ was maintained well above 300 torr while Paco₂ 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 plateaud 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. 5–7). 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-
Complete global brain ischemia produced by arterial hypotension and the high pressure neck cuff was verified in the monkey 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 rat results in prolonged stupor or coma and a sustained significant decrease in brain energy charge potential with severe EEG abnormalities. 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. 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, b) calculated, c) DMO 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. 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-
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. Thiopental 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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