Effect of hyperglycemia on brain pH levels in areas of focal incomplete cerebral ischemia in monkeys

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The adverse effect of a minimal cerebral blood flow (CBF) in models of global ischemia has been noted by many investigators. One factor believed important in this situation is the level of blood glucose, since a continued supply of this metabolite results in increased tissue lactate, decreased brain pH, and increased cell damage. The authors have extended these observations to a model of focal incomplete ischemia. Brain pH was measured in fasted squirrel monkeys in regions of focal incomplete ischemia after transorbital occlusion of the middle cerebral artery (MCA). In both control and hyperglycemic animals, CBF was reduced to less than 30% of baseline. At 3 hours after MCA occlusion, brain pH in the control group was 6.66 ± 0.68 as compared to 6.27 ± 0.26 in the glucose-treated group. This difference was statistically significant by Student's unpaired t-test (p < 0.05). Thus, hyperglycemia results in decreased tissue pH in regions of focal incomplete cerebral ischemia in monkeys.

Key Words: cerebral metabolism • cerebral ischemia • brain pH • hyperglycemia • monkey

It has long been known that neural tissue has a limited ability to tolerate ischemia. Much experimental and clinical work has defined the parameters of blood flow reduction that lead to electrical neuronal failure, ionic dysequilibrium, and cell death. It would seem intuitively obvious that the severity of neural damage would relate primarily to both the degree and the duration of blood flow reduction. Thus, if ischemia is complete, one might expect a more severe injury than with a lesser degree of ischemia for the same period of time.

However, a number of observations have been made which indicate that global incomplete ischemia with maintenance of less than 10% of normal cerebral blood flow (CBF) can be more detrimental to neural tissue than global complete ischemia. In mongrel dogs, rats, rabbits, monkeys, and cats, global incomplete ischemia with CBF maintained at less than 10% of control levels resulted in poorer recovery than did global ischemia whether measured by energy state, metabolic parameters, electrophysiological phenomena, or neurological outcome. Three hypotheses have been advanced to explain this observation: 1) during incomplete ischemia, slowly moving blood might aggregate in the microcirculation, thereby preventing reperfusion following restoration of flow; 2) neuronal or glial membranes may be damaged by free radicals, which are formed in greater abundance during incomplete ischemia; and 3) excessive lactate production from continued supply of substrate for anaerobic glycolysis may lead to excessive tissue acidosis and enhance tissue damage.

The purpose of the present study was to examine the last hypothesis and determine the effect of increased serum glucose concentrations on tissue pH in an area of focal cerebral ischemia using the model of middle cerebral artery (MCA) occlusion in the squirrel monkey.

Materials and Methods

General Experimental Design

Twelve squirrel monkeys (Saimiri sciureus), each weighing between 700 and 1000 gm, were used in this study. Six of the 12 were assigned to a control group that received only saline as a placebo. The other six animals were made hyperglycemic by infusion of glucose as 5% dextrose in water (D5W). In both groups, measurements were made of intracellular brain pH, cortical tissue perfusion, and serum glucose prior to and at hourly intervals for 3 hours during focal cerebral ischemia produced by occlusion of the MCA. Systemic pCO2 was kept constant at 40 torr and mean arterial...
blood pressure (MABP) was monitored throughout the experiment.

**Animal Preparation**

The monkeys were fasted overnight, although water was available. They were anesthetized with 4% inspired halothane, operated on under 1.5% halothane, and then studied under 1.0% halothane. All animals underwent tracheostomy and placement of femoral arterial and venous catheters. A Harvard respirator* was used for ventilation, and pancuronium bromide (0.15 mg/kg) was given to abolish respiratory efforts. Body temperature was maintained at 37°C with a warming pad.

The skin, subcutaneous tissue, and muscle were excised over the right parietal area to expose the skull. The underlying bone was removed with a high-speed air drill, and the dura was opened with the aid of an Olympus operating microscope. A thin plastic sheet (Saran Wrap) was substituted for the dura to keep the brain moist and prevent surface oxygenation. The MCA was isolated at its origin from the internal carotid artery by an intraorbital approach. Gold-plated electroencephalograph (EEG) electrodes were inserted at three points on each side of the animal's skull in the frontal, temporal, and posterior regions.

Following surgical preparation, the animals were moved from the operating table to the microscope stage. Preocclusion control measurements of cortical perfusion and tissue pH were taken. The MCA was then occluded, and measurements were continued during 3 hours of focal ischemia. Brain pH was also measured at the time of death from airway occlusion. In the placebo group, saline was infused at a rate of 1.5 ml/hr starting 15 minutes after MCA occlusion. In the glucose-treated animals, D5W was infused at a rate of 1.5 ml/hr starting 15 minutes after occlusion of the MCA.

**Brain Intracellular pH and Cortical Perfusion**

Umbelliferone (7-hydroxycoumarin), a lipid-soluble pH-sensitive fluorescent indicator that is nontoxic and freely diffusible across the blood-brain barrier, was used to determine brain pH. The molecular and ionic forms of umbelliferone are both fluorophors but with different fluorescent characteristics. Therefore, it is possible to create a nomogram relating pH to the ratio of the 450-nm fluorescent curves of the indicator from 340- and 370-nm excitation. The use of this nomogram to measure brain pH and an example of pH calculation can be found in a recent article from our laboratory. Each pH measurement was accompanied by simultaneous determinations of cortical perfusion, blood gas, and serum glucose levels. Artifacts related to an alteration of the pH of the indicator in a lipid-soluble environment, the concentration of the indicator, changes in the indicator redox potential, and the solvent effect with differential quenching have been reported in detail by Sundt and Anderson.

Cortical tissue perfusion was determined by the clearance curve of the molecular form of the indicator using the 1-minute initial-slope index. The change in indicator cortical tissue perfusion obtained by this method corresponded closely to the values measured with krypton-85 in other experiments performed in this laboratory on monkeys.

The umbelliferone was injected intravenously for a period of 2 minutes during the two control measurements. This also resulted in ample penetration of the indicator into the ischemic zone via collateral circulation. Brain pH was calculated at 30-second intervals over 90 seconds for a total of three determinations. The average value was used for the final determination. The three consecutive values did not vary more than 0.010 pH units, indicating a stable measurement.

**Instrumentation for pH Determination**

The microspectrofluorometer used for this study was equipped with optics for bright field illumination which allowed high-sensitivity recording of low-intensity excitation energy from a small avascular area of cortex (80 μm in diameter); a high-speed filter wheel with four interference filters which permitted synchronization of the emission fluorescent signal at 450 nm with the 340- and 370-nm excitation bands; and an emission-recording system consisting of a high-sensitivity thermoelectrically cooled photomultiplier tube coupled to a high-efficiency grating monochromator. Fluorescent emission measurements were amplified by a cascaded electrometer amplifier and directed into a photodetector synchronized with the filter wheel. The fluorescence washout curves were recorded on a two-channel strip-chart recorder. Details of this instrumentation are described in previous reports from this laboratory.

**Results**

The data from the two groups of animals are summarized in Fig. 1. During incomplete ischemia from MCA occlusion, the brain pH in the placebo group decreased from a mean control value ± standard error of the mean (SEM) of 6.95 ± 0.03 to 6.74 ± 0.09 at 15 minutes and to 6.66 ± 0.08 at 3 hours. Cortical tissue perfusion in this same group decreased to 30% of control at 15 minutes after MCA occlusion and was 23% of control at 3 hours. The serum glucose level was 279 ± 45 mg/dl prior to MCA occlusion, increasing steadily to 483 ± 82 mg/dl at 3 hours after MCA occlusion.

In the glucose-treated group, brain pH during complete ischemia fell from a mean control value of 6.91 ± 0.03 to 6.65 ± 0.06 at 15 minutes and to 6.27 ± 0.26 at 3 hours. Cortical tissue perfusion decreased to 32% of control at 15 minutes after MCA occlusion and to 15% of control at 3 hours. The serum glucose level was 210 mg/dl prior to occlusion, increasing to 737 ± 42 mg/dl.

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* Respirator manufactured by Harvard Apparatus Co., South Natick, Massachusetts.
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mg/dl at 3 hours after MCA occlusion. Mean arterial pCO₂ and MABP in both groups were 38.7 ± 1.0 mm Hg and 107 ± 7 mm Hg, respectively.

There was no significant difference in cortical tissue perfusion, brain pH, and serum glucose levels between the two groups prior to and during MCA occlusion. There was a significant difference (p = < 0.05 > 0.01) between serum glucose levels and brain pH in the two groups at 1, 2, and 3 hours according to Student’s unpaired t-test. In both groups the pH fell at death, to 6.05 for the placebo group and to 6.01 for the glucose-treated group. The severity of the EEG change correlated with the reduction in cortical tissue perfusion and the change in brain pH.

Discussion

The model of focal incomplete ischemia used in this study, which involved transorbital occlusion of the MCA, reduced perfusion to less than 30% of baseline values in both the control and glucose-infused groups. Both groups of animals showed a tendency for further decline in tissue perfusion over the course of the experiment. These values are in accordance with previous findings of Sundt and Waltz. In the control group, the brain pH declined quickly but stabilized at 6.7 and remained constant until late in the experiment. The brain pH of the glucose-treated animals did not plateau until 2 hours after MCA occlusion. At this time serum glucose levels were still rising (Fig. 1).

While the causes for increasing brain acidosis during ischemia are many, one of the prime factors is the progressive accumulation of lactic acid. A number of investigators have shown that any remaining flow during a period of global cerebral ischemia results in increasing tissue lactate levels due to the brain’s inability to oxidatively metabolize the continued supply of the metabolite glucose. In addition to the rate at which exogenous substrate is supplied to the ischemic tissue, the state of endogenous glucose present at the onset of the ischemia is also important. Thus, primates deprived of food recovered better than primates intravenously infused with dextrose solutions prior to cerebral ischemia, and the outcome correlated with the level of brain tissue lactate that had accumulated. The magnitude of lactate accumulation may then be dependent on several factors including: first, the baseline level of brain glycogen or glucose stores; second, the serum glucose level at a given density and duration of residual blood flow; and third, the density and duration of residual blood flow at a given serum glucose level.

During complete global ischemia, the normal glycogen and glucose stores are sufficient to increase tissue lactate levels to about 15 µmol/gm brain. If there is residual flow, and particularly if there is coincident hyperglycemia, lactate levels will rise above 20 µmol/gm and may attain 35 to 40 µmol/gm. When tissue lactate levels rise above 20 µmol/gm tissue water, there is an increased incidence of ischemic cell damage. Whether the detrimental effects are due to the acidosis itself or to the simultaneous increase in tissue osmolality is unknown.

Previous models used to evaluate the effect of glucose on brain pH or lactate levels utilized global cerebral ischemia. Our results extend these observations to the more commonly encountered clinical situation of focal incomplete ischemia. In this model, pH continued to fall for more than 2 hours following MCA occlusion due to an ongoing supply of glucose to the ischemic tissue. At its nadir, tissue pH was 6.3, a level that corresponds to a tissue lactate level of nearly 20 µmol/gm tissue water. Presumably, the equilibrium achieved represented the balance between substrate delivery and removal of the products of metabolism which the residual blood flow could sustain.

There are two useful clinical suggestions that may be inferred from these data. If hypotension or temporary occlusion of a major cerebral vessel is necessary, one might refrain from infusing glucose since this might aggravate any tendency for ischemic cell damage that might develop. Finally, anesthetic agents that tend to accentuate hyperglycemia might be avoided during neurosurgical procedures in which there is an expected perturbation of CBF.

References

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