Relationship Between Plasma Glucose, Brain Lactate, and Intracellular pH During Cerebral Ischemia in Gerbils

David J. Combs, PhD, Robert J. Dempsey, MD, Mary Maley, BS, David Donaldson, and Charles Smith, MD

The dose-response relation between plasma glucose and brain lactate and the relation of these parameters to intracellular pH during severe cerebral ischemia have not been well characterized over a wide range of plasma glucose levels. Experiments to delineate these relations in the gerbil model of global ischemia were performed by using phosphorus-31 nuclear magnetic resonance spectroscopy to measure intracellular pH and a new method to measure brain lactate. Ischemia increased final brain lactate linearly 4 μmol/g for every 100 mg/dl increase in plasma glucose up to 650 mg/dl (p=0.0001, r²=0.9); beyond 650 mg/dl, saturation of the glucose transport-glycolysis system occurred. Plasma glucose correlated better with ischemic intracellular pH than did brain lactate. However, when brain lactate levels are compared with intracellular pH during ischemia, the relation may be threshold rather than linear. A narrow transition zone, during which ischemic intracellular pH decreased precipitously with increasing brain lactate, was observed between 17 and 22 μmol/g; below 17 μmol/g, intracellular pH remained stable at 6.8-6.9, whereas above 22 μmol/g, intracellular pH decreased maximally to about 6.2. The marked decrease in intracellular pH that occurs when brain lactate surpasses 17 μmol/g suggests that this sudden drop in intracellular pH may account for the "lactate threshold" for increased cerebral ischemic damage. (Stroke 1990;21:936-942)

Since the observation by Myers and Yamaguchi that glucose infusion increased brain injury from cardiac arrest, the ability of hyperglycemia to enhance cerebral ischemic damage after transient ischemia has become well accepted.2–5 The increased ischemic brain damage in the presence of hyperglycemia is believed to be related to increased production of lactate and to the accompanying increase in tissue acidosis. Hyperglycemia produces substantial increases in brain lactate during ischemia,6–10 and treatment with a glycolytic inhibitor can block hyperglycemia-enhanced damage.7 Measurement of interstitial pH and calculation of intracellular pH (pHi) during cerebral ischemia indicate that increased acidosis accompanies increased tissue lactate.8–10

Nevertheless, hyperglycemia studies are often restricted to comparisons of normoglycemic animals with animals that have been made hyperglycemic by one dose of glucose.2–6 The dose-response relation between blood glucose and brain lactate over a wide range of blood glucose levels has not been well described in a model of severe cerebral ischemia. Furthermore, the relation between brain lactate and brain pHi during severe global ischemia has not been well characterized over an extensive range of hyperglycemia. The understanding of such relations is especially important in light of the recent finding that brain pathology may be enhanced more by lactic acidosis than by other types of acidosis.11

In these experiments, the relation between plasma glucose, brain lactate, and intracellular pH is described over a wide range of plasma glucose levels during 20 minutes of global ischemia in the Mongolian gerbil. pHi is measured by use of phosphorus-31 nuclear magnetic resonance (31P NMR) spectroscopy, and a new method for measuring brain lactate is introduced and discussed.
Methods

Two series of experiments were performed in this study. The first series examined the relation between varying plasma glucose levels and brain lactate levels in control gerbils (nonischemic group [n=30]) and in gerbils subjected to bilateral carotid artery occlusion (ischemic group [n=35]). The second series of experiments was performed in a manner similar to the first, except that all gerbils were subjected to ischemia (n=21) and were monitored for brain pH, with \(^{31}\text{P}\) NMR spectroscopy.

In the first experimental series, 65 male Mongolian gerbils (53±1 g [mean±SEM]) were fasted overnight before all experiments. All gerbils were anesthetized with a combination anesthetic (87 mg/kg ketamine hydrochloride plus 13 mg/kg xylazine i.p.) and supplemented as needed. Both common carotid arteries were isolated by means of a lateral incision, and a suture was placed around each artery for easy manipulation. Gerbils were treated intraperitoneally with 0.003 units/kg insulin, saline, or one of four doses of glucose (0.5, 1, 2, or 4 g/kg). All solutions were made up in 0.9% saline, and a total volume of 15 ml/kg was administered to all gerbils. Thirty minutes after treatment, bilateral carotid artery occlusion with vascular clips was performed on 35 gerbils (ischemic group) and was maintained for 20 minutes. After 20 minutes of ischemia, the anesthetized gerbils were decapitated and placed in the magnetic field. The gerbils were treated in a manner similar to the first experimental series to produce varying levels of brain lactate. The heparinized trunk blood was collected from the frontal regions of the frozen brain at room temperature and then returned to the freezer and maintained at −70°C until analysis. The frozen brain samples were weighed and then immediately homogenized in 4 ml sodium fluoride buffer (1 mg/ml sodium fluoride, 0.021 M KH₂PO₄, and 0.029 M NαH₂PO₄·2H₂O at pH 7.0) kept on ice. Duplicate 25-μl aliquots of the homogenate were analyzed for lactate concentration by an enzyme electrode method with a lactate electrode in a glucose analyzer (model 23A, Yellow Springs Instrument Co., Yellow Springs, Ohio). By using the glucose analyzer, we were able to calibrate to two decimal places instead of the one decimal place possible with the lactate analyzer (model 23L, Yellow Springs Instrument Co.). The lactate electrode contains a membrane composed of a layer of glucaraldehyde-cross-linked L-lactate oxidase, which catalyzes a reaction between L-lactate and oxygen to produce hydrogen peroxide. Hydrogen peroxide then reacts at the platinum anode and produces a current that is linearly proportional to the concentration of lactate in the sample. The technique will be referred to as the lactate analyzer technique.

Known solutions of L(+)lactic acid (0.25, 0.5, 0.75, 1.0, and 2.0 mM) in the sodium fluoride buffer were tested in duplicate to generate a standard curve from which final concentration of the tissue homogenate was calculated. Calibration of the lactate analyzer was checked between each duplicate sample by using a 5 mM L-lactate standard (YSI 2327, Yellow Springs Instrument Co.) provided for the analyzer and a zero lactate flush buffer (YSI 2357, Yellow Springs Instrument Co.). Assuming that 1 g brain tissue has a volume of 1 ml, the lactate concentrations of the tissue were calculated by using the following equation: tissue lactate (μmol/g) = (tissue volume + 4 (ml) [lactate concentration of homogenate (μmol/ml)] / tissue weight (g)). The lactate analyzer has previously been used to measure tissue lactate in muscle.\(^{12}\)

To test the accuracy of the lactate analyzer, 12 gerbils (10 ischemic and two nonischemic) were treated in a manner similar to the first experimental series to produce varying levels of brain lactate. The anesthetized gerbils were decapitated and placed in liquid nitrogen either just before or after 20 minutes of ischemia. Brain samples were removed as in the previous experimental series and divided into two portions: one for analysis by the lactate analyzer technique and the other for analysis by a more com-
mon enzymatic fluorometric technique. The two methods were compared by simple regression analysis.

For the enzymatic fluorometric technique, each brain sample was ground to a fine powder at -20°C with a mortar and pestle. The ground sample was then added to a preweighed microcentrifuge test tube containing 1.0 ml cold 10% trichloroacetic acid solution and mixed. The tube was then reweighed to calculate tissue weight and then centrifuged at 3,200g (3,750 rpm) for 5 minutes at 4°C (GRP centrifuge, Beckman Instruments, Inc., Palo Alto, California). The supernatant was then neutralized with a 1-ml mixture of tri-n-octylamine and Freon 113 (22:78, vol/vol), vortexed for 2 minutes, spun at 3,000 rpm for 5 minutes at 4°C, and stored at -70°C until assayed. The twelve samples were then analyzed for lactate content using the l-lactate enzymatic fluorometric method as described by Passonneau. In vivo 31P NMR spectra (80 MHz) were obtained using a 4.7-T horizontal, 25.4 cm free-bore magnetic resonance imager/spectrometer (SISCO, Fremont, California). The gerbils were positioned in the middle of the magnetic field, and a four-turn, 1.8-cm-diameter copper wire transmit/receive surface coil was placed over the dorsal skull between the ears with the anterior end at the midline of the eyes. The skin above the dorsal skull was left intact. Previous work in the gerbil indicated that removal of the tissue over the dorsal skull does not alter normal brain phosphorus spectra and that little extracranial contamination of the phosphorus signal occurs (authors’ unpublished observations).

Magnetic field homogeneity was optimized by shim- ming to less than 60 Hz on the proton resonance (200 MHz) of tissue water. Each accumulated 31P NMR spectrum (spectral width 10,000 Hz, 10,000 data points, 800 repetitions) was obtained by using a nominal 30° pulse and 500-msec repetition time. Intensity correction for saturation was not applied. Sine bell filtering and line broadening of 5 Hz were used in signal processing. The specific peaks followed in these experiments were inorganic phosphate (P1) and phosphocreatine. Average \( \text{pH}_1 \) was calculated by using the position difference (P1 shift) between the P1 and phosphocreatine peaks according to the equation of Petroff et al: \[ \text{pH}_1 = 6.77 + \log[(P_1 \text{ shift} - 3.29)/(5.68 - P_1 \text{ shift})] \]

31P NMR spectra were measured before ischemia and during the final 10 minutes of the 20-minute ischemic episode.

Body temperature was maintained at 37°-38°C (range 36°-38.5°C) inside the magnet by placing the gerbils within a tygon tube coil supplied continuously with warm water (K-Module, Baxter Healthcare Corporation, McGaw Park, Illinois) and by blowing warm air through the magnet’s bore.

The data were analyzed by simple regression analysis with analysis of variance (ANOVA) to test whether or not the slope significantly differed from zero. The slope was considered significantly different from zero if \( p \leq 0.05 \). All analyses were done on a Macintosh SE computer (Apple Computer, Inc., Cupertino, California) using the StatView 512+ statistical package (BrainPower, Inc., Agoura Hills, California).

Results

Brain lactate levels measured by the lactate analyzer compared quite favorably to levels measured in the same gerbils by the more established enzymatic fluorometric technique (Figure 1). Analysis of variance indicated a highly significant slope \( (p=0.0001) \) that was close to unity (slope=1.14). The \( y \) intercept was quite close to the origin (−0.59), and the coefficient of determination \( (r^2=0.96) \) indicated that the two methods were strong predictors of one another.

A summary of plasma glucose and brain lactate values for each treatment group in the first experimental series is presented in Table 1. All remaining discussion of results will focus on data presented for all individual gerbils. Brain lactate did not vary with changes in plasma glucose in nonischemic gerbils; however, the relation between plasma glucose and brain lactate among ischemic gerbils indicated a saturable system (Figure 2). Although a significant slope was found for plasma glucose versus brain lactate among the ischemic gerbils \( (p=0.0001) \), the coefficient of determination \( (r^2) \) was only 0.54 due to a tendency for brain lactate to level off once plasma glucose surpassed 650 mg/dl. Under 650 mg/dl, a very strong linear relation (brain lactate=0.04 plasma...
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Glucose+4.08, p=0.0001, r²=0.9) between plasma glucose and brain lactate, could be demonstrated among ischemic gerbils and indicated that brain lactate increases 4 µmol/g for every 100 mg/dl increase in plasma glucose.

An example of 31P NMR spectra before and during ischemia in a gerbil from the second experimental series is shown in Figure 3. The gerbil was treated with 0.5 g/kg glucose. Plasma glucose was 458 mg/dl, and brain lactate was 20.07 µmol/g at the end of ischemia. Cerebral ischemia caused large reductions in phosphocreatine and adenosine triphosphate peaks, a large increase in the P, peak, and a decrease in brain pH from 7.18 to 6.45. In the overall study, pH, could not be calculated during ischemia in three of the 21 gerbils because the phosphocreatine peak decreased until it was not distinguishable from background noise.

Both plasma glucose and brain lactate were examined as predictors of ischemic pH, using simple linear regression (Figure 4). The slopes of both straight lines were significantly different from zero. However, on the basis of its better coefficient of determination, plasma glucose was a better predictor of ischemic pH, than brain lactate. The equations for these linear regressions are as follows:

\[ \text{pH}_i = (-7.55 \times 10^{-4}) \text{ plasma glucose} + 6.85 \]

\[ \text{pH}_i = (-0.02) \text{brain lactate} + 6.89 \]

Further examination of the relation between brain lactate and pH, suggests that the relatively poor cor-
The simpler and faster lactate analyzer technique proved to be an accurate means of measuring brain lactate. Nonischemic brain lactate levels in the first experimental series (Table 1) and preschismic pHi generated a straight line with a slope not significantly different from zero. Therefore, preschismic pHi was independent of the glycemic state of the gerbil. This stability of preschismic pHi is illustrated in Figure 5 by a plot of the preschismic pHi of all gerbils against each gerbil’s final brain lactate.

### Discussion

The simpler and faster lactate analyzer technique proved to be an accurate means of measuring brain lactate. Nonischemic brain lactate levels in the first experimental series (Table 1) are comparable with levels found in previous studies. Normal lactate levels from rat brains frozen in situ are 1–2 \( \mu \text{mol/g} \). Decapitation of gerbils into liquid nitrogen produces brain lactate levels of 1.62 \( \mu \text{mol/g} \). Furthermore, for ischemic and nonischemic gerbils, good agreement was observed between the lactate analyzer technique and a common enzymatic technique (Figure 1).

Nuclear magnetic resonance spectroscopy of phosphorus-containing compounds has been used by a number of investigators to measure brain pH during ischemia. The technique has many advantages including the ability to measure pH, and to make repeated measurements in the same animal. The \( ^{31} \text{P NMR spectroscopy method used in this study is best for measuring pH values between 6 and 8 since it is within this range that linearity of the P_\beta shift is maintained. This technique is not very sensitive in detecting pH values <6. Therefore, it is possible to miss a very acidic population of cells, such as glial cells, which may be capable of attaining pH levels ≤5.21,22 Although this limitation could account for the leveling off of pHi at about 6.2 as lactate increased above 22 \( \mu \text{mol/g}, other investigators have detected pH changes as low as 5.5 by using NMR spectroscopy. The threshold for marked decreases in pH below 6.8–6.9, as lactate surpassed 17 \( \mu \text{mol/g}, would not be affected by this potential limitation.

Another problem involved in the use of this \( ^{31} \text{P NMR technique is whether or not changes in the intracellular free Mg^{2+} during ischemia could compromise the derivation of pHi from the P shift. Changes in the P shift with changes in acidity are not altered by Mg concentrations ≤2.5 mM. Intracellular free Mg concentrations for brain tissue have been estimated at 0.5–1.0 mM. Using \( ^{31} \text{P NMR spectra, Brooks and Bachelard recently demonstrated in superfused cerebral tissues that the concentration of free intracellular Mg in the brain is 0.33 mM and that severe hypoxia increases its concentration to 0.57 mM. Therefore, it seems unlikely that nonischemic or ischemic pHi measurements were affected by Mg levels in the brain.

The first experimental series revealed a strong linear relation between plasma glucose and brain lactate in ischemic gerbils with plasma glucose levels ≤650 mg/dl. Lactate accumulation during ischemia leveled off with levels of plasma glucose >650 mg/dl. A variety of reasons could account for this plateau in lactate accumulation. Since the \( K_m \) for glucose transport to the brain is about 6–9 mM, the system for transporting glucose into the brain may be approaching saturation at blood glucose levels >650 mg/dl (36.1 mM); thus, at these levels, the availability of substrate for glycolysis would be limited. The reduction of cerebral blood flow to the gerbil forebrain may have also limited substrate availability and, therefore, maximal lactate accumulation. Because cerebral blood flow is reduced to near zero in the cerebral cortex and to 30% of control in the basal ganglia in this model, substrate availability for glycolysis is mainly restricted to glucose trapped in the vasculature of the brain, intracellular glucose, and glycogen. In a zero-flow situation, the contribution of intravascularly trapped glucose would be small and would maximally increase tissue lactate by 0.67 \( \mu \text{mol/g} \) (assuming cerebral blood volume of 6 ml/100 g) for every 100 mg/dl increase in plasma glucose. This is a minor increase compared with the 4 \( \mu \text{mol/g} \) increase actually observed for every 100 mg/dl increase in plasma glucose ≤650 mg/dl. It is clear that much of the increase in brain lactate must come from increased intracellular glucose and glycogen although residual
blood flow may contribute as well. However, even if glucose transport was not saturated and some residual flow existed in the normoglycemic ischemic brain, hyperglycemia might still be self-limiting in generating lactic acid because of the ability of hyperglycemia to reduce regional cerebral blood flow. Duckrow et al.\textsuperscript{28} have demonstrated that regional cerebral blood flow in the normal brain is reduced approximately 7% per 10 mM increment in plasma glucose ≤60 mM.

Although it is clear that increased brain lactate production during ischemia is associated with greater decreases in brain pH,\textsuperscript{8-10} the relation between these parameters over a wide range of lactic acid levels is not as well characterized. Paschen et al.\textsuperscript{29} observed a linear relation between brain lactate and brain pH after cardiac arrest. Brain lactate increased from about 3 μmol/g to about 11 μmol/g over the first 40 seconds of cardiac arrest; brain pH decreased from 7.0 to 6.0.\textsuperscript{29} Blood glucose was not measured or purposely varied in this study. Although this study suggests that the relation between brain lactate and pH\textsubscript{i} during ischemia is linear, this study was done only over the first 40 seconds of the ischemic insult. Our study looked at total lactate produced over 20 minutes and compared that with pH\textsubscript{i} measured during the second half of the ischemic insult. Because cerebral blood flow is reduced to near zero in the cerebral cortex and to 30% of control in the basal ganglia in this model\textsuperscript{27} and because brain lactate is maximum in total ischemia within 2–3 minutes,\textsuperscript{30} it is likely that pH\textsubscript{i} and lactate were stable and relatively constant by 10 minutes into the ischemic insult and not under the state of flux, as in the study of Paschen et al.\textsuperscript{29} Another reason for the differences found in the relation between lactate and brain pH could reside in the different methods of measuring pH. Paschen et al measured tissue pH, not pH\textsubscript{i}, by the umbelliferone technique.

Kraig et al\textsuperscript{a} examined lactate accumulation versus extracellular pH after complete ischemia and observed a relation similar to that found in this study. Extracellular pH decreased to about 6.81 in animals with lactate levels <13 μmol/g and to 6.18 in animals with lactate levels >16 μmol/g.\textsuperscript{9} Between the narrow range of 13 and 16 μmol/g, intermediate changes in interstitial pH were observed.\textsuperscript{9} The changes in pH observed during ischemia in our study were quite similar. Intracellular pH decreased to 6.8–6.9 in brains with lactate levels <17 μmol/g and to about 6.2 in brains with lactate levels >22 μmol/g. Intermediate changes in pH\textsubscript{i} were observed in brains with tissue lactate levels between 17 and 22 μmol/g. It is not clear why there appears to be a nonlinear relation between brain lactate and pH\textsubscript{i}. Since pH\textsubscript{i} does not drop beyond 6.8–6.9 until brain lactate concentration surpasses 17 μmol/g, this lactate level may indicate the point at which hydrogen ion production surpasses intracellular buffering capacity. Although adenosine triphosphate hydrolysis, not lactate production, is the primary source of increased hydrogen ion during anaerobic metabolism, 2 mol hydrogen ion are produced stoichiometrically for every mole of glucose converted to 2 mol lactate.\textsuperscript{31} The brain lactate range of transition from small to large pH changes in our study is not much wider than those of Kraig et al;\textsuperscript{a} however, the level of brain lactate at which pH begins its marked decrease is higher in our study. This difference in lactate threshold for decreased pH may reflect differences in the model or species that was used or differences (i.e., buffering capacity) in the cellular compartment that was monitored.

It is more difficult to speculate why pH\textsubscript{i} fails to decrease further as brain lactate surpasses 22 μmol/g. As mentioned earlier, it is possible that limitations of \textsuperscript{3}P NMR spectroscopy in detecting pH\textsubscript{i} changes below 6 are responsible for this observation. Otherwise, it is difficult to reconcile our observations with those of Kraig et al.\textsuperscript{a} Although Kraig et al hypothesize that ion-transport characteristics of astroglia maintain a steady-state extracellular pH of about 6.2 despite very high lactate levels, our data do not reveal an intracellular compartment that would continue to become acidic as extracellular pH is maintained.

The ability of hyperglycemia to increase cerebral ischemic damage is associated with ischemic brain lactate levels >20 μmol/g.\textsuperscript{32,33} Below this lactate level,
increased lactate accumulation is not accompanied by increased cerebral ischemic damage. Therefore, hyperglycemia-enhanced damage could be described as a threshold effect with no increase in ischemic damage occurring until brain lactate accumulation during ischemia surpasses 20 μmol/g. Our data also support the lactate threshold concept for increased ischemic damage since pH remained stable until brain lactate reached 17 μmol/g and then markedly decreased as brain lactate increased beyond this level. It is possible that it is these large decreases in brain pH that may be responsible for the increased ischemic damage associated with suprathreshold lactate levels.

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References


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