Effect of Plasma Glucose Concentration on Cerebral Metabolism During Partial Ischemia in Neonatal Piglets

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We used neonatal piglets to determine the influence of plasma glucose concentration on cerebral energy metabolism during and immediately after partial ischemia. We assessed cerebral metabolism using in vivo phosphorus-31 magnetic resonance spectroscopy. Arterial plasma glucose concentration was increased in four piglets by systemic infusions of dextrose in water for comparison with infusions of saline in four controls or decreased in eight piglets by fasting for 24-48 hours for comparison with four fed piglets. Plasma glucose concentration showed a significant linear correlation with intracellular pH \( r = -0.7, p < 0.05 \). Piglets that developed hypoglycemia during partial ischemia had a smaller reduction in intracellular pH and a larger increase in inorganic phosphate content than piglets that were normoglycemic or hyperglycemic during ischemia. Similar differences persisted during the first 5 minutes of postischemic reperfusion. Subsequently, the cerebral concentrations of phosphorylated compounds returned to normal in all piglets. Our results demonstrate that 1) arterial plasma glucose concentration influences cerebral energy metabolism and intracellular pH during ischemia, 2) neonatal piglets can develop profound brain acidosis, and 3) brain acidosis during ischemia does not influence the restoration of cerebral phosphorylated compounds to control levels during the first 90 minutes after ischemia. (Stroke 1990;21:435-440)

The influence of blood glucose concentration on cerebral metabolism during hypoxia and/or ischemia is an important determinant of cell damage.1-3 Food-deprived juvenile monkeys and adult rats tolerated circulatory arrest functionally and pathologically better than animals infused with glucose before arrest.2,4 A mechanism postulated for irreversible brain damage associated with glucose administration is the development of severe brain acidosis and the accumulation of lactate, with threshold concentrations of approximately 20 μmol/g.3,5,6 In contrast, a deleterious effect of excessive glucose administration before hypoxia-ischemia has not been noted for neonatal rodents.7 Biochemical correlates of these observations revealed mean brain lactate concentrations of <20 μmol/g.8 It is possible that excessive brain lactate (>20 μmol/g) never accumulates during hypoxia and/or ischemia in neonatal animals, implying that a deleterious effect of excessive glucose on neonatal cerebral metabolism does not exist.9 However, using neonatal piglets we have recently demonstrated that during partial ischemia brain lactate concentration exceeded 20 μmol/g, profound intracellular acidosis occurred with values <6.0, and there was a strong linear correlation between the extent of brain acidosis and lactosis \( r = 0.94 \).10 Our current study addresses the following questions: 1) Do different plasma glucose concentrations during partial ischemia influence cerebral intracellular pH \( (pH_i) \) and the concentrations of phosphorylated energy metabolites? 2) Do alterations in cerebral \( pH_i \) during ischemia effect the recovery of cerebral phosphorylated compounds immediately after ischemia?

Materials and Methods

We studied 20 piglets at a mean±SD postnatal age of 8±3 days and a mean±SD weight of 1.37±0.25 kg. The protocol for preparation of the animals, the anesthesia used, and the placement of catheters has been described.11 An inflatable vascular occluder (3 mm lumen diameter and 8 mm cuff width) was positioned around the right common carotid artery. After preparation, the piglets were ventilated with 70% \( N_2O \).
and 30% O₂ and 0.3 mg/kg i.v. d-tubocurarine chloride was administered. A 90-minute stabilization period commenced after the piglets were wrapped with a heating blanket to maintain rectal temperature at 38.5–39.5°C. Each piglet was positioned supine with its head resting on a single-turn 4-cm-diameter surface coil and placed in a superconducting magnet (1.89 T, 30-cm bore diameter, Oxford Instruments, Oxford, England). The scalp was retracted and the calvaria rested on the coil, which extended over both cerebral hemispheres from the midfrontal to the midoccipital region.

Two experimental protocols were used. In protocol 1, eight piglets were studied during control, during a 10-minute intravenous infusion (10 ml/kg) of either 10% dextrose in water (D₁₀W, n=4) or equimolar saline (n=4), during 25 minutes of partial ischemia, and during 90 minutes of postischemic reperfusion. Ischemia was induced by inflation of the vascular occluder (resulting in bilateral carotid artery occlusion; the left common carotid artery was ligated for catheter insertion) combined with hemorrhagic hypotension as previously described. Postischemic reperfusion commenced after deflation of the vascular occluder and reinfusion of all withdrawn blood over 3 minutes. All eight piglets remained with their respective sow and litter until the morning of the experiment. In protocol 2, plasma glucose concentrations of the remaining 12 piglets were altered by leaving the piglets with their sow until the morning of the study (n=4) or by fasting them for 24–48 hours with free access to water (n=8). This experiment also consisted of a 10-minute control period, 25 minutes of partial ischemia, and 90 minutes of postischemic reperfusion and was performed as described above. During both experiments the inspired O₂ concentration was 30% and the ventilator rate was adjusted to maintain arterial isocapnia.

In both protocols blood was sampled from the carotid artery and sagittal sinus for O₂ content, blood gases, pH, and plasma concentrations of glucose and lactic acid as previously described. In protocol 1, blood was also sampled for electrolyte concentrations and osmolality. In all 20 piglets, blood was sampled twice during the control period, after 15 and 22 minutes of partial ischemia, and 5, 30, 60, and 90 minutes after the initiation of postischemic reperfusion. In protocol 1, blood was also sampled 1 and 5 minutes following completion of the infusion.

In vivo phosphorus-31 magnetic resonance (P-31 MR) spectra were obtained in triplicate during the control period, during the last 12 minutes of partial ischemia, and at nine intervals during postischemic reperfusion commencing immediately after reinfusion of the withdrawn blood. Data were collected on a Nicolet NT-80 spectrometer (Madison, Wisconsin). After shimming on the proton signal from the brain, spectra were obtained using an observed frequency of 32.5 MHz, an excitation pulse length of 50 μsec, and a recycle time of 2 seconds. Each spectrum had 4,096 data points, and 128 transients were averaged (approximately 5 min/spectrum). An external standard, 20 mM methylene diphosphonate, was used in each study as a chemical shift reference. Peak heights were normalized to the external standard and corrected for partial saturation by comparison with a spectrum collected during the control period using a 20-second recycle time. Normalized peak heights during partial ischemia and postischemic reperfusion were expressed as percentages of the height during the control period. Differences in peak height reflect changes in metabolite concentration only if resonance peak line width remains constant. We assumed that the spin–spin relaxation time, which may change peak line width, was constant for the phosphorus-31 resonance peaks. The total phosphate concentration was assessed by cutting out the spectra and weighing them; the weight was taken as a measure of the total area. This was performed on single spectra from the control period, the ischemic period, and the end of the reperfusion period to assess if the concentration of P-31 MR-visible phosphorylated compounds changed. pH was calculated from the chemical shift of the inorganic phosphate (Pᵢ) peak as \[ \text{pH} = 6.757 + \log \left[ \frac{x-3.282}{5.698-x} \right] \], where \( x \) is the chemical shift of Pᵢ. There is ample evidence that calculated pH agrees with pH measured with non-MR methodology. During and after ischemia Pi may increase and phosphorus may be lost from cells, possibly rendering pH, a composite measure of both intracellular and extracellular pH. This seems unlikely since immediately following brain ischemia, the extracellular fluid volume of the brain is small and since the concentration of Pi in the extracellular fluid is relatively low, even in pathologic conditions.

Repeated-measures analysis of variance was used to examine differences between or among groups within a protocol over time. In protocol 2, not all fasted piglets became hypoglycemic during ischemia; therefore, they were grouped post hoc according to their arterial plasma glucose concentration during ischemia. Thus, in protocol 2 three groups of piglets (designated fed, fasted, and fasted without hypoglycemia) were analyzed. Significant interactions \( (p<0.05) \) were localized by the unpaired \( t \) test. Linear regression analysis was employed to examine the results. Values are reported as mean±SD.

Results

In protocol 1, all measured physiologic and biochemical variables were similar between the groups before the infusions of D₁₀W or saline. Infusion of D₁₀W increased the arterial plasma glucose concentration from 119±31 mg% during the control period to 273±36 mg% 5 minutes after the infusion \( (p<0.05) \), while infusion of saline did not alter it \( (132±32 \text{ and } 140±43 \text{ mg% during the control period and 5 minutes after the infusion, respectively}) \). In spite of different arterial plasma glucose concentrations following infusions, similar concentrations were measured during partial ischemia (343±52 and 340±95 mg% in D₁₀W- and saline-infused piglets, respectively). During partial ischemia, mean arterial
During the control period, plasma glucose concentration (Figure 1) differed in fed and fasted piglets (117±14 and 75±20 mg%, respectively) and was intermediate in fasted piglets without hypoglycemia (100±13 mg%). During partial ischemia, it fell in fasted piglets (range 25–47 mg%), rose in fed piglets (range 171–271 mg%), and remained approximately unchanged in fasted piglets without hypoglycemia (range 89–217 mg%). These ranges were used to group the piglets post hoc. Differences in arterial plasma glucose concentration persisted during posts ischemic reperfusion but were less pronounced. Similar patterns of MABP occurred (Figure 1). During partial ischemia, $\text{PaCO}_2$ decreased similarly in the three groups in spite of our reducing the ventilator rate (from 35.7±1.9 to 26.4±4.0, from 32.8±4.4 to 28.0±8.1, and from 36.2±1.7 to 33.3±5.1 mm Hg during the control period and partial ischemia in the fed, fasted, and fasted without hypoglycemia groups, respectively). In contrast, during partial ischemia cerebral venous $\text{PCO}_2$ ($\text{PcvCO}_2$) rose less in fasted piglets than in fed piglets or fasted piglets without hypoglycemia (Figure 1). There were no differences in arterial or cerebral venous pH, $\text{PO}_2$, or $\text{O}_2$ content.

In protocol 2, the cerebral arteriovenous difference in plasma glucose concentration [(A–V)$_g$] was 10.5±3.0, 9.1±2.8, and 8.6±2.5 mg% for the fasted, fed, and fasted without hypoglycemia groups, respectively, during the control period. During partial ischemia, (A–V)$_g$ was correlated directly with arterial glucose concentration and (A–V)$_g$ was correlated directly with arterial $\text{PCO}_2$ concentration for the fed piglets and for the fasted piglets without hypoglycemia (Figure 1). There were no differences in arterial or cerebral venous pH, $\text{PO}_2$, or $\text{O}_2$ content.

Due to the results from protocol 1, fasting was employed in protocol 2 to study reduced arterial plasma glucose concentrations during brain ischemia. During the control period, glucose concentration (Figure 1) differed in fed and fasted piglets (117±14 and 75±20 mg%, respectively) and was intermediate in fasted piglets without hypoglycemia (100±13 mg%). During partial ischemia, it fell in fasted piglets (range 25–47 mg%), rose in fed piglets (range 171–271 mg%), and remained approximately unchanged in fasted piglets without hypoglycemia (range 89–217 mg%). These ranges were used to group the piglets post hoc. Differences in arterial plasma glucose concentration persisted during posts ischemic reperfusion but were less pronounced. Similar patterns of MABP occurred (Figure 1). During partial ischemia, $\text{PaCO}_2$ decreased similarly in the three groups in spite of our reducing the ventilator rate (from 35.7±1.9 to 26.4±4.0, from 32.8±4.4 to 28.0±8.1, and from 36.2±1.7 to 33.3±5.1 mm Hg during the control period and partial ischemia in the fed, fasted, and fasted without hypoglycemia groups, respectively). In contrast, during partial ischemia cerebral venous $\text{PCO}_2$ ($\text{PcvCO}_2$) rose less in fasted piglets than in fed piglets or fasted piglets without hypoglycemia (Figure 1). There were no differences in arterial or cerebral venous pH, $\text{PO}_2$, or $\text{O}_2$ content.

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FIGURE 2. Examples of phosphorus-31 magnetic resonance spectra during control period (lower spectra) and at completion of ischemia (upper spectra) for fasted and fed piglets. Seven resonance peaks identified in control spectra: PME, phosphomonoester; Pi, inorganic phosphate; PDE, phosphodiesters; PCr, phosphocreatine; and γ, α, and β-NTP, gamma, alpha, and beta peaks of nucleotide triphosphate, respectively. Horizontal axis, frequency; vertical axis, peak intensity.

DISCUSSION

Previous studies of piglets using unilateral carotid artery occlusion and hemorrhagic hypotension demonstrated a 50–60% reduction in cerebral blood flow (CBF). We used bilateral carotid artery occlusion plus hemorrhagic hypotension to induce more severe partial ischemia (60–75% reduction in CBF, unpublished observations); our technique was associated with selective neuronal necrosis (unpublished observations).

To examine the effects of two arterial plasma glucose concentrations on cerebral metabolism during partial ischemia, we initially used infusions of D₁₀W and saline. In spite of different glucose concentrations following the infusions, ischemia was associated with similar changes in the glucose con-
Our results demonstrate that arterial plasma glucose concentration influences cerebral pH, and the concentration of phosphorylated metabolites during ischemia. Piglets that were hypoglycemic during partial ischemia (fasted) had smaller decreases in pH, but greater increases in Pi concentrations than piglets that remained normoglycemic (fasted without hypoglycemia) or became hyperglycemic (fed). However, no significant differences among the three groups were detected in ATP concentrations during ischemia. Differences in Pi but not ATP concentrations may at first appear inconsistent. Hydrolysis of ATP during ischemia increases the concentrations of adenosine diphosphate (ADP), adenosine monophosphate (AMP), Pi, and adenosine. The precise stoichiometry between reductions in ATP concentration and increases in Pi concentration depends on the relative activity of phosphorylases and kinases that govern whether ATP is dephosphorylated stepwise to ADP and then AMP or directly to AMP. Multiple investigations have demonstrated that during ischemia the change in ATP concentration is modest (two- to threefold increase) while increases in AMP concentrations range between 15- and 100-fold. Thus, a stoichiometric relation between 2–3 moles of Pi generated per mole of ATP hydrolyzed depends on the extent of further AMP hydrolysis and provides an explanation for our observations for Pi and ATP. During the control period, the signal-to-noise ratio for ATP is approximately 5:1 and reductions in its concentration during ischemia limit the discrimination of differences of 30–40% between groups. Conversely, increases in the Pi concentration enhance its signal-to-noise ratio and facilitate the detection of intergroup differences.

Changes in pH, reflect continued brain glucose delivery in fed piglets and fasted piglets without hypoglycemia compared with fasted piglets. Glucose is thus available in the first two groups for anaerobic metabolism, resulting in lactate production, ATP generation, and coupling to hydrolysis of ATP. This results in reutilization of ADP, AMP, and Pi with a simultaneous expanding pool of H+ and a reduction in pH. In contrast, the fasted piglets have insufficient substrate to readily generate ATP, leading to an expanding pool of Pi with limited generation of H+. Differences among the groups in PaCO₂ during ischemia support the notion that brain glucose delivery was different. Presumably, substrate is more readily available for the remaining oxidative phosphorylation of partially ischemic brain in animals without hypoglycemia, leading to increased CO₂ production and a higher PaCO₂ than in animals with hypoglycemia.

In contrast to our results, glucose administration during partial ischemia in 1–4-week-old lambs had no effect on brain pH or concentrations of phosphorylated compounds and lactate. An important difference in the experimental protocols is that Hope et al administered glucose midway through a 40-minute period of ischemia. An effect of additional glucose would depend on appreciably greater quantities of it entering the brain, which may not occur during severe ischemia. In an investigation of 7-day postnatal rats pretreated with glucose or saline before hypoxia-ischemia, Vannucci et al found no differences in the concentrations of brain phosphorylated metabolites and lactic acid between groups; pH was not measured. It remains unclear whether it is appropriate to compare cerebral metabolic responses during ischemia with those during hypoxia-ischemia. However, our results do agree with observations from adult animals in which partial ischemia with hyperglycemia leads to a more pronounced intracellular acidosis than in animals with hypoglycemia.

FIGURE 4. Scatterplot of arterial plasma glucose concentration (n=20) and cerebral arteriovenous difference in glucose concentration (n=17) vs. pH at completion of ischemia for piglets. △, fed; ○, fasted; ●, fasted without hypoglycemia; ×, dextrose infusion; □, saline infusion. Only 17 values are plotted for arteriovenous difference because of malfunction of sagittal sinus catheter during ischemia.
We also demonstrated that despite differences in P-31 MR spectra during partial ischemia, all piglets showed improvement in the cerebral energy state during postischemic reperfusion. For adult animals, hyperglycemia augments ischemic brain damage assessed histologically24,25 and functionally.24 Restitution of CBF26 and cerebral ATP, PCR, and lactate concentrations during 90 minutes of postischemic reperfusion in adult cats was severely impaired in glucose-pretreated animals compared with controls.3 In contrast, hyperglycemia and/or brain acidosis does not affect the recovery of cerebral phosphorylated metabolites in piglets during the first 90 minutes following ischemia. Whether this difference reflects varying severities of brain ischemia or an effect of maturation is unclear.

The relation between arterial plasma glucose concentration and the change in cerebral energy metabolism and pH during partial ischemia does not delineate a mechanism for these observations. We have assumed that similar reductions in MABP result in similar decreases in CBF among groups. This may not be true since glucose administration is associated with a decrease in CBF in adult animals27 and with a reduction in cerebral arteriolar diameter in newborn animals.28 It is also unknown whether different levels of brain glucose or pH modulate cerebral metabolic rate. Nevertheless, in contrast to observations in neonates of other species, our results suggest that during ischemia there is a direct relation between arterial plasma glucose concentration, brain energy metabolism, and pH, in piglets. Investigations in neonatal animals to demonstrate different neuropathologic effects of ischemia with differing brain pHs have not been performed. Brain ischemia in piglets during hypoglycemia and hyperglycemia provides a model to answer this question.

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References


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