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Alterations in Cerebral Blood Flow and Phosphorylated Metabolites in Piglets during and after Partial Ischemia

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ABSTRACT. Ventilated piglets were studied before, during (15 min), and after (90 min) hemorrhagic hypotension to correlate a 60% reduction in cerebral blood flow with cerebral energy state using radiolabeled microspheres ($n = 12$) and *in vivo* ^{31}P nuclear magnetic resonance spectroscopy ($n = 11$). Cerebral blood flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) decreased during hypotension (98 ± 28 to 41 ± 28, $p < 0.05$), increased at 5 min postreperfusion (131 ± 53, $p < 0.05$), and returned to control values by 90 min postreperfusion. Cerebral O_2 uptake was reduced during partial ischemia, remained depressed 5 min postreperfusion, and increased to within 20% of control values at 90 min postreperfusion. Relative to control, hypotension was associated with decreased ($p < 0.05$) phosphocreatine (62 ± 11%), phosphocreatine/inorganic phosphate ratio (41 ± 10%), and nucleoside triphosphate (82 ± 12%) while inorganic phosphate increased (155 ± 32%, $p < 0.05$). During ischemia intracellular pH dropped from 7.06 ± 0.07 to 6.59 ± 0.31 ($p < 0.05$) and the cerebral arteriovenous difference of glucose increased. Phosphorylated metabolites returned to within 10% of control 15 min after blood reinfusion and remained constant thereafter. Based on calculations of ATP synthesis and utilization rates during control and hypotension, we speculate that the rate of energy utilization of the brain during ischemia is reduced 18–49% relative to the control utilization rate. (*Pediatr Res* 23: 206–211, 1988)

Abbreviations

MABP, mean arterial blood pressure
CBF, cerebral blood flow
^{31}P NMR, ^{31}phosphorus nuclear magnetic resonance
PCr, phosphocreatine
Pi, inorganic phosphate
NTP, nucleoside triphosphate
pHa, arterial pH
pHpi, cerebral intracellular pH based on the chemical shift of Pi
pHet, cerebral intracellular pH based on the chemical shift of phosphomonoester
ANOVA, analysis of variance

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Cerebral metabolic effects of partial ischemia are pertinent to multiple problems in sick human neonates. Events such as asphyxia, tension pneumothorax, intracranial hemorrhage, and hypovolemia may result in alterations in cerebral perfusion pressure with or without changes in blood O_2 tension. Although scant data are available concerning CBF measurements in sick human neonates (1–4), partial cerebral ischemia may be of greater clinical concern than complete cerebral ischemia in view of the prevalence of events contributing to the former compared to the latter. Despite this, relatively little research has been done on perinatal brain ischemia and the most frequently used animal model has been complete cerebral ischemia via decapitation (5–7).

Inasmuch as partial ischemia encompasses a spectrum of cerebral perfusion pressure and CBF, multiple investigations in adult animals have examined the reduction in CBF necessary to alter energy metabolism. Siesjo and Zwetnow (8) used hypovolemic hypotension in adult rats and reported unaltered brain ATP and PCr when MABP >40 mm Hg. Although CBF was not measured, the results were attributed to maintenance of CBF at the lower limit of autoregulation. Similarly, reduction in cerebral perfusion pressure by increased CSF pressure was associated with unaltered adenine nucleotides until cerebral perfusion pressure <30 mm Hg (9). Further investigations of adult rats by Eklof and Siesjo (10) revealed that 50–60% decreases in CBF were necessary to decrease cerebral ATP. Welsh *et al.* (11) suggested that in adult animals there is a critical range of cerebral perfusion pressure at which CBF is sufficiently diminished and energy metabolism can no longer be maintained. Additional supportive evidence for this was provided by the observation that graded reductions of cerebral perfusion pressure from 60 to 30 mm Hg in adult cats resulted in an increasing number of fluorescent patches of NADH within cerebral tissue with progressive alterations in ATP, PCr, and lactate concentration (12). Recently combined CBF and ^{31}P NMR measurements have established that the flow threshold for energy failure is in the range of 12–20 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ in adult gerbils (13, 14).

Extrapolation from adult investigations to newborns is difficult because the neonatal period is characterized by lower cerebral O_2 uptake (15), greater tolerance to hypoxia and ischemia (5), and enhanced transport of alternate cerebral energy substrates (16). We have demonstrated that piglets may serve as a model of partial cerebral ischemia in the neonatal period since a 60% fall in MABP is associated with a 50% reduction in CBF (17). We used this experimental model to determine if similar changes in CBF are associated with altered cerebral energy metabolites in the neonatal period. Cerebral energy metabolism was assessed *in vivo* using ^{31}P NMR spectroscopy to perform measurements in the same animal before, during, and after hypotension.

METHODS

Twenty-six miniature swine were studied at 10 ± 4 (mean ± SD) days of age after birth and weighed 1.76 ± 0.42 kg. Piglets remained with the sow until the morning of the study at which time one piglet was removed, premedicated with ketamine (5 mg/kg intramuscularly), tracheotomized, and ventilated (Harvard Apparatus Rodent Respirator, model 680) with 70% nitrous oxide and 30% O_2. For blood flow determinations (n = 12) polyethylene catheters were placed in the left ventricle (via the left common carotid artery), abdominal aorta (via a femoral artery), inferior vena cava (via a femoral vein), left axillary artery, and the sagittal sinus after exposure of the midline calvarium and creation of a 1-cm burr hole. For ^{31}P NMR measurements (n = 14) polyethylene catheters were placed in the left common carotid artery, abdominal aorta, and inferior vena cava. In five of the piglets used for ^{31}P NMR, a catheter was inserted into the sagittal sinus.

After catheter placement inspired gases were changed to 70% nitrogen and 30% O_2, 0.3 mg/kg D-tubocurarine was administered intravenously, and a 90-min stabilization period commenced. Piglets used for ^{31}P NMR were wrapped in a heating pad (rectal temperature 38.5–39.5° C), positioned supine with their heads resting on a single turn 4-cm diameter surface coil, and stabilized after placement within a superconducting magnet (1.89 Tesla, 30-cm bore diameter, Oxford Instruments), with vascular catheters routed outside the magnet. For this investigation the coil was applied to the intact scalp overlying both parietal and a portion of both frontal lobes of the brain. The contribution of skin to the *in vivo* spectrum was evaluated by examining the spatial variation in coil sensitivity as reported by Tofts *et al.* (18), determination of the total quantity of perchloric acid soluble phosphorus/gm of skin and cortical tissue, and analysis of NMR spectrum from skin biopsies obtained during normo- and hypotension. We calculate that 90% of the total phosphorus signal is derived from gray and white matter of the cerebral cortex underneath the coil and the remaining 10% from skin. During hypotension phosphorylated metabolites of skin do not undergo major changes in relative concentrations. Finally visual inspection of the tissue underneath the surface coil reveals the absence of muscle. We conclude that the contribution of skin and muscle to the evaluation of changes in brain phosphorylated metabolites is negligible.

Twenty-three piglets (n = 12 for microspheres, n = 11 for NMR), underwent an experimental protocol consisting of three sequential study periods: control, hypotension, and postreperfusion. Hypotension was induced by withdrawing blood from the vena caval catheter into heparinized syringes until MABP <45 mm Hg, a pressure less than the lower limits of cerebral autoregulation (17). The mean estimated blood volume removed was 34 ± 4 ml/kg and hypotension was maintained for 15 min. Recirculation was achieved by reinfusion of all withdrawn blood over a 3-min period and followed by a 90-min postreperfusion period. Throughout the protocol the inspired O_2 concentration was 30% and the ventilator rate was adjusted to maintain arterial isocapnia. In three additional piglets the effects of lengthening the duration of hypotension was evaluated. Hemorrhagic hypotension was used to maintain a reduced MABP for 35 min and NMR data are reported for spectrum obtained in the last 8 min of this interval. Recovery was not studied in these three animals.

CBF was determined during control, the last 3 min of hypotension, and 5 and 90 min after the onset of reperfusion. Before CBF measurements blood was sampled for cerebral arteriovenous differences of O_2 content, blood gases, and pH (total volume of blood removed at each CBF determination was 5.4 ml). Blood loss was immediately replaced using sterile packed red blood cells previously obtained from a littermate and stored in acid-citrate-dextrose anticoagulant. For piglets studied by ^{31}P NMR, spectrum were obtained during the control period, the last 8 min of hypotension, and 11 times sequentially postreperfusion commencing after reinfusion of withdrawn blood. All animals studied by ^{31}P-NMR were sampled for arterial blood gases and pH at the midpoint of spectrum collected during control, hypotension, and at 5, 30, 60, and 90 min postreperfusion. In five piglets cerebral arteriovenous differences of O_2 content and plasma concentrations of glucose and lactic acid were determined using the catheter in the sagittal sinus to collect cerebral venous blood. The total volume of blood removed for cerebral arteriovenous difference samples was 2 ml and each piglet's own packed red blood cells were reinfused after removal of plasma for glucose and lactic acid concentrations.

Vascular pressures, blood gases, pH, and O_2 content were measured as previously described (17). Lactic acid concentrations were determined using a lactate dehydrogenase enzymatic assay (Sigma Chemical Co) on plasma (200 μl) after deproteinization with 8% perchloric acid. Glucose determinations were performed on plasma (50 μl) after deproteinization with 2% perchloric acid using a glucose-oxidase enzymatic method (Sigma). CBF was measured using the reference organ radiolabeled microsphere technique as described by Heymann *et al.* (19). For each measurement approximately 8×10^5 microspheres suspended in 3.5 ml of dextran were injected into the left ventricle over a 30-s period after which the catheter was flushed with 1.2 ml of isotonic saline. Starting before the microsphere injection arterial reference samples were withdrawn from the axillary artery and abdominal aortic catheters into counting vials under oil at a rate of 1.03 ml/min for 2 min with a Harvard pump. All brain tissue and reference samples contained more than 400 microspheres.

At study completion the piglets were sacrificed (Nembutal, 150 mg/kg intravenously, Abbot Laboratories) and catheter placement verified. For piglets injected with microspheres the entire brain was removed, cut into sections, and the weight of each section determined. All brain tissue and blood reference samples were counted in a Packard 3-Channel Auto-Gamma Spectrometer (model 5385) and CBF computed as previously described (19). Wet tissue weight was used to express blood flow as $ml \cdot min^{-1} \cdot 100\ g^{-1}$.

Calculated variables included cerebral O_2 delivery, O_2 uptake, and O_2 extraction. Cerebral O_2 delivery ($mmol \cdot min^{-1} \cdot 100\ g^{-1}$) is derived from the product of CBF and arterial O_2 content. By the Fick principle, cerebral O_2 uptake ($mmol \cdot min^{-1} \cdot 100\ g^{-1}$) is equivalent to the product of CBF and the cerebral arteriovenous difference of O_2 content. Cerebral O_2 extraction is calculated from the ratio of cerebral O_2 uptake/cerebral O_2 delivery.

In vivo ^{31}P NMR data were collected on a Nicolet NT-80 NMR spectrometer. After shimming on the proton signal from the brain, phosphorus spectrum were obtained using an observe frequency of 32.5 MHz, a 50-μs excitation pulse length, and a 3.4-s recycle time. Each NMR spectrum had 4096 data points and 140 transients were averaged (approximately 8 min/spectrum). An external standard, methylene diphosphonate (20 mM), was used in each study as a chemical shift reference. Quantitation of ^{31}P NMR was based on peak heights, normalized to the external standard, and corrected for partial saturation by comparison to spectrum collected using a 20-s recycle time. Spectral analysis over the course of a study compared normalized peak heights during hypotension and postreperfusion to values at control and results were expressed as percent of control. Intracellular pH was calculated from the chemical shift of the phosphomonoester (pHet) or Pi peak (pHpi). Appropriate titration curves were used to formulate the following equations from which intracellular pH was calculated (20):

$$pHpi = 6.757 + \log 10((x - 3.282)/(5.698 - x)$$
$$pHet = 5.625 + \log 10((y - 3.190)/(6.946 - y)$$

were x and y refer to the chemical shift of Pi and phosphomonoester peak, respectively. Results were analyzed with a one-way ANOVA with repeated measures and regression analysis. Significant interactions on the ANOVA ($p < 0.05$) were localized by

a Newman-Keuls multiple comparison test. Values reported are the mean and SD.

RESULTS

Analysis of arterial and cerebral venous pressure, pH, and blood gases from piglets used for blood flow determinations and ^{31}P NMR were similar and hence results for the 23 animals were pooled (Fig. 1). Arterial hypotension was associated with alterations in cerebral venous pressure, arterial and cerebral venous pH, and cerebral venous blood gases. Cerebral venous O_2 tension decreased during hypotension from 32 ± 7 to 19 ± 6 mm Hg, increased at 5-min postreperfusion (63 ± 16 mm Hg) and returned to control values by 30-min postreperfusion. Arterial PCO_2 decreased to 28 ± 9 mm Hg during hypotension and was associated with an elevation in cerebral venous CO_2 tension. By 90-min postreperfusion all physiologic variables had returned to control levels.

During hypotension CBF fell 56 ± 31% then rose 33 ± 30% above control at 5-min postreperfusion, and returned to preischemic levels at 90 min postreperfusion (Table 1). The fall in CBF contributed to disproportionate reductions in cerebral O_2 delivery and O_2 uptake so that cerebral fractional extraction of O_2 rose. The increase of CBF and arterial O_2 content 5-min postreperfusion caused cerebral O_2 delivery to return to preischemic values, but O_2 uptake remained decreased and gave rise to a low cerebral fractional extraction of O_2 and the previously noted cerebral venous hyperoxia. At 90-min postreperfusion cerebral O_2 delivery again decreased reflecting a reduced arterial O_2 content (due to a lowered hematocrit) and not an alteration of CBF. Cerebral O_2 uptake was 20% below control levels and cerebral fractional O_2 extraction was comparable to control.

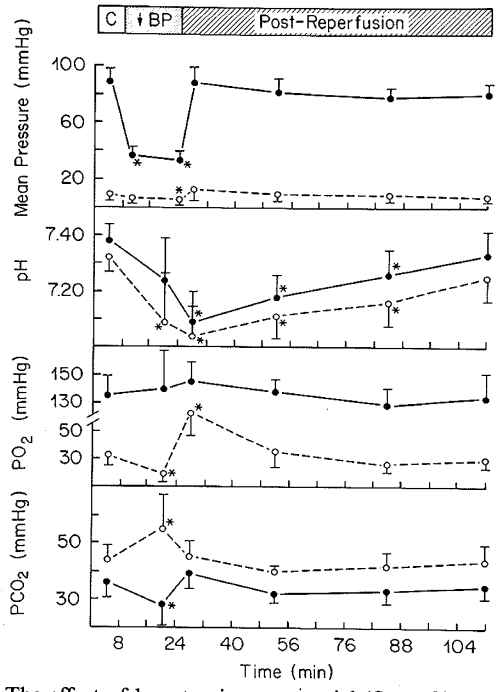

Fig. 1. The effect of hypotension on arterial (●---●) and cerebral venous (○---○) pressure, pH, PO_2, and PCO_2. Data are mean ± SD of 23 piglets studied with either microspheres or ^{31}P NMR during control (*c*), hypotension (*BP*), and postreperfusion. *$p < 0.05$ *versus* control.

In piglets used for ^{31}P NMR studies arterial and cerebral venous plasma glucose concentrations (Table 2) rose during hypotension. For the five animals with a sagittal sinus catheter, the cerebral arteriovenous difference of glucose widened from control to hypotension, 0.4 ± 0.01 to 4.0 ± 2.7 mmol/liter, respectively ($p < 0.05$). Elevations of arterial plasma lactic acid concentration occurred concurrent with decreased pHa. Cerebral arteriovenous differences of lactic acid concentration were negligible at control (0.0 ± 0.10 mmol/liter) and subsequent arteriovenous differences did not change.

Figure 2 displays representative ^{31}P NMR spectrum from a piglet during control, hypotension, and postreperfusion. Seven resonance peaks are detectable and include the β, α, and γ peaks of NTP, PCr, phosphodiester, Pi, and phosphomonoester. The peak height ratio of PCr/Pi at control for 11 piglets was 2.0 ± 0.6. Changes during hypotension included an increase in Pi and a decrease in PCr, pHpi, and PCr/Pi (0.8 ± 0.3 for the latter, $n = 11$, $p < 0.05$). Within 16–24 min of reinfusion the ^{31}P NMR spectrum is not different than control.

In Figure 3 pHpi and percent changes in PCr, Pi, and NTP are plotted *versus* time. The pHpi was 7.06 ± 0.07 at control, decreased to 6.59 ± 0.31 during hypotension, and remained at a

Table 1. *Effect of hypotension on CBF, O_2 delivery, O_2 uptake, and fractional extraction of O_2 in piglets studied with microspheres (mean ± SD)*

			Postreperfusion	
	Control	Hypotension	5 min	90 min
CBF (ml·min^{-1}·100 g^{-1})	98 ± 27	41 ± 27*	131 ± 53*	83 ± 37
O_2 delivery (mmol·min^{-1}·100 g^{-1})	0.44 ± 0.07	0.15 ± 0.12*	0.48 ± 0.13	0.33 ± 0.11
O_2 uptake (mmol·min^{-1}·100 g^{-1})	0.26 ± 0.05	0.12 ± 0.08*	0.13 ± 0.04*	0.21 ± 0.06*
O_2 extraction	0.59 ± 0.11	0.86 ± 0.08*	0.30 ± 0.10*	0.65 ± 0.10

* $p < 0.05$ *versus* control.

Table 2. *Effect of hypotension on arterial (a) and cerebral arteriovenous differences (av) of plasma concentrations of glucose and lactic acid in piglets studied with ^{31}P NMR (mean ± SD)*

			Postreperfusion			
	Control	Hypotension	5 min	30 min	60 min	90 min
Glucose (a) (mmol/liter, $n = 5$)	7.2 ± 0.6	14.8 ± 5.7*	12.0 ± 4.0*	10.9 ± 3.6	9.9 ± 3.5	8.6 ± 2.4
Glucose (av) (mmol/liter, $n = 5$)	0.4 ± 0.1	4.0 ± 2.7*	0.6 ± 0.4	0.2 ± 0.1	0.7 ± 0.5	0.6 ± 0.4
Lactic acid (a) (mmol/liter, $n = 5$)	1.4 ± 0.6	10.7 ± 1.8*	10.1 ± 0.8*	8.3 ± 1.5*	7.0 ± 2.3*	5.6 ± 2.7*
Lactic acid (av) (mmol/liter, $n = 5$)	0.0 ± 0.1	−0.1 ± 0.2	0.1 ± .3	0.1 ± 0.3	0.1 ± 0.1	0.0 ± 0.2

* $p < 0.05$ *versus* control.

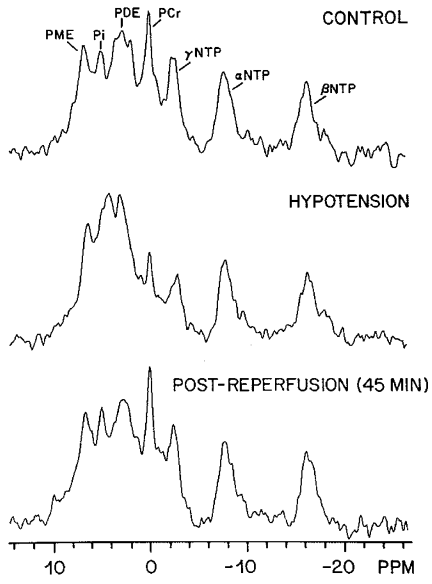

Fig. 2. Representative ^{31}P NMR spectrum of a piglet during control (*top*), hypotension (*middle*), and 45 min postreperfusion (*bottom*). Resonance peaks are labeled as follows: β, α, γ, NTP-β, α, and γ peaks of nucleoside triphosphate, PCr, phosphodiester (*PDE*), Pi, and phosphomonoester (*PME*).

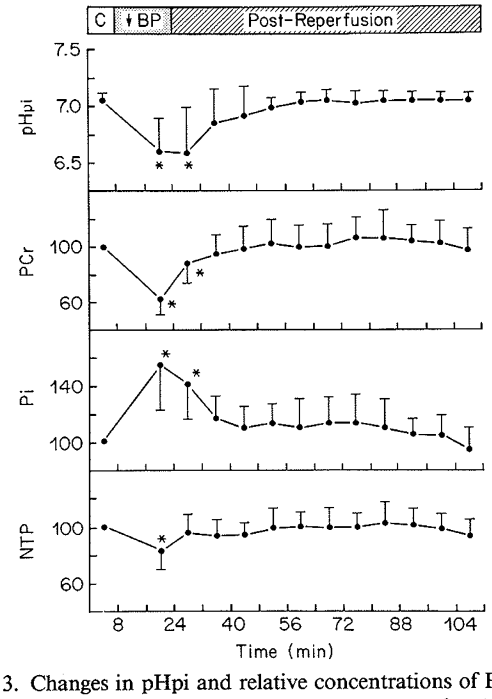

Fig. 3. Changes in pHpi and relative concentrations of PCr, Pi, and nucleoside triphosphate (*NTP*) before, during, and after hypotension. Data are mean ± SD and the phosphorylated metabolites are represented as the percent change relative to control. NTP represents the mean of the β, α, and γ peaks. $^*p < 0.05$ compared to control.

similar value of 6.58 ± 0.44, during the first spectrum (0–8 min) after blood reinfusion. Subsequently there was rapid recovery of pHpi to control values. Intracellular pHet calculated from the chemical shift of the phosphomonoester peak revealed quantitatively similar changes between control and hypotension with a Δ pHet of −0.39 ± 0.24. PCr fell to 62 ± 11% of control during hypotension and within 8 min of blood reinfusion PCr increased to 88 ± 14% of control. In all subsequent spectrum PCr returned to control values. Pi increased during hypotension to 155 ± 32% and remained elevated (141 ± 26%) 8 min after blood reinfusion.

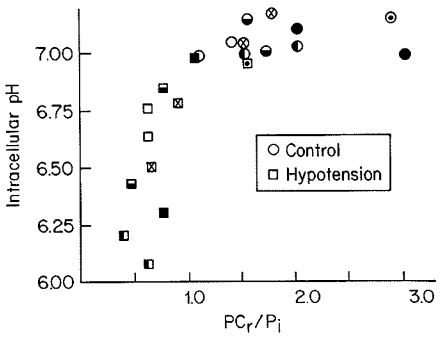

Fig. 4. Relationship between pHpi and the PCr/Pi ratio during control and hypotension. Littermates are designated by similar *symbols*.

In subsequent spectrum Pi returned to control values. During hypotension less prominent changes occurred in NTP (82 ± 12% of control) and was rapidly restored to baseline values after blood reinfusion. In Figure 4 pHpi is plotted *versus* PCr/Pi for data collected during control and hypotension. The relationship is nonlinear with a steep decline in pHpi when PCr/Pi is less than 1. The PCr/Pi ratio for individual piglets during control correlated well with their subsequent PCr/Pi during hypotension ($r = 0.83$, $p < 0.01$), but did not correlate well with either pHpi or percent reduction in NTP during hypotension ($r = 0.39$ and 0.50, respectively).

In three additional piglets the duration of hypotension was increased to 35 min with a decrease in MABP from 76 ± 5 to 28 ± 5 mm Hg. ^{31}P NMR spectrum obtained during the last 8 min of this interval revealed reductions in PCr (40 ± 20% of control), NTP (53 ± 17% of control), pHpi (7.05 ± 0.04 to 6.16 ± 0.12), and increases in Pi (262 ± 105% above control).

DISCUSSION

In preterm and term human neonates positron-emission tomography and xenon clearance have provided preliminary CBF data implicating an important role for ischemia after intraventricular hemorrhage (1) and asphyxia (2, 3). Limitations of current methodology, such as spatial resolution, prevent accurate distinction of cerebral tissue subjected to complete or incomplete ischemia. Despite the apparent importance of cerebral ischemia in neonatal disease, few animal investigations have examined both cerebral vascular and metabolic effects of ischemia and of those performed most have used *in vitro* freeze clamp techniques in newborn rats and mice to assess complete ischemia (5–7). Norwood *et al.* (21) studied complete cerebral ischemia *in vivo* using isolated perfused neonatal rat brain and ^{31}P NMR. To our knowledge brain energy metabolism during partial ischemia and the postischemic interval have not been investigated in an intact newborn animal using noninvasive methodology. ^{31}P NMR offers the ability to monitor *in vivo* cerebral phosphorylated metabolites over time, permitting measurements to be acquired before, during, and after ischemia in individual animals. The use of different animals for separate study time points and potential alterations induced by brain freezing for *in vitro* analysis are obviated.

The results of our investigation demonstrate that a 50–60% reduction in CBF is associated with reversible perturbations of cerebral O_2 uptake, high energy phosphorylated metabolites, and intracellular pH. As indicated by the three piglets studied for 35 min of hypotension, if the duration of partial ischemia is prolonged, progressively larger alterations in cerebral phosphorylated metabolites are observed. Thus, an absolute CBF of approximately 40 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ was associated with failure to maintain control levels of cerebral high energy metabolites. This CBF is higher than the critical flow threshold for energy failure in adult animals of other species such as gerbils (13, 14). When

expressed as a relative reduction in CBF, energy failure in piglets occurs at similar flow decrements as in adult rats (10) and gerbils (14). However, our data do not allow us to draw conclusions concerning differences in ischemic threshold between newborn and adult animals because we did not study adult miniature swine and we did not examine a spectrum of CBF reduction to accurately identify a threshold for energy failure.

Based on biochemical analysis of extracts from brain frozen *in situ*, complete ischemia in newborn and adult animals impairs oxidative phosphorylation and results in reduced high energy phosphorylated metabolites (5–7, 22–24). The reduction in cerebral O_2 uptake herein is consistent with a decrease in cerebral oxidative metabolism. The results of the ^{31}P NMR experiments directly support the contention that a 50–60% decrease in CBF perturbs energy metabolism because an increase in Pi and reductions in PCr, NTP, and pHpi were observed. The reduction in pHpi, NTP, and the increase in cerebral arteriovenous difference of glucose are consistent with a change in cellular metabolism to a combination of aerobic plus anaerobic energy production during partial ischemia. Interestingly, the fall in pHpi was of similar magnitude to estimates made for complete ischemia models (21, 25). For piglets subjected to longer intervals of partial ischemia further decreases in pHpi occurred, reflecting continued substrate delivery, and glycolysis, thus contributing to higher tissue lactate concentrations (26, 27).

The 20% fall in NTP in piglets must be interpreted cautiously in view of potential regional variations in brain metabolism. Welsh *et al.* (11) reported a regional evaluation of cerebral ATP concentrations during ischemia revealing few values between 30 and 80% of control ATP. Thus, a 20% reduction in ATP may correspond to 80% of subregions with normal ATP and 20% with total depletion of ATP. Welsh *et al.* (11) speculated that an apparent threshold for ATP concentrations during ischemia reflects rapid energy utilization relative to low energy reserves.

However, Welsh *et al.* (11) also noted that energy utilization may be altered during ischemia as evidenced by changes in electroencephalographic activity. We have used our results to estimate brain energy utilization during ischemia. The cerebral O_2 uptake at control, 2.6 $\mu mol \cdot min^{-1} \cdot g^{-1}$ (Table 1), is sufficient to metabolize the entire cerebral glucose uptake (0.39 $\mu mol \cdot min^{-1} \cdot g^{-1}$ calculated from control data in Tables 1 and 2) by oxidative phosphorylation. This gives a maximal ATP production rate of 14 $\mu mol \cdot min^{-1} \cdot g^{-1}$ at control. Inasmuch as ATP levels at control are constant, ATP production and utilization must be equivalent. During hypotension cerebral O_2 uptake is decreased to 1.2 $\mu mol \cdot min^{-1} \cdot g^{-1}$ and is sufficient for 7.2 $\mu mol \cdot min^{-1} \cdot g^{-1}$ of ATP production provided that a cerebral glucose uptake of at least 0.2 $\mu mol \cdot min^{-1} \cdot g^{-1}$ is attained. From the CBF and arteriovenous difference during hypotension we calculate a cerebral glucose uptake of 1.6 $\mu mol \cdot min^{-1} \cdot g^{-1}$, in excess of the above requirement. Even if all the remaining glucose was metabolized by anaerobic glycolysis, we estimate a maximal ATP production rate of 10 $\mu mol \cdot min^{-1} \cdot g^{-1}$. If the rate of ATP utilization during hypotension remained the same as the control rate, then total depletion of high energy metabolites would occur within 1 to 5 min, depending on the production rate (7.2–10 $\mu mol \cdot min^{-1} \cdot g^{-1}$) and available brain energy reserves (6 to 20 $\mu mol/g$) assumed (5).

The above calculated time for total energy depletion does not agree with the observed 20 and 38% reductions in ATP and PCr levels, respectively, after 15 min of hypotension. To account for the observed drops in brain high energy metabolites, brain energy utilization may be only fractionally (1–15%) greater than production. Thus, if our estimated rate of ATP production is correct (7.2–10 $\mu mol \cdot min^{-1} \cdot g^{-1}$), then the rate of energy utilization during hypotension would be reduced by at least 18–49% compared to control energy utilization. We point out two important limitations that make these estimates speculative: 1) use of the Fick principle to accurately calculate substrate utilization requires a steady state of blood flow, arteriovenous differences, and tissue metabolism (28) and this may not be the case during ischemia and 2) NMR measurements derive a signal from a localized region of tissue which may not be representative of the bulk tissue giving rise to CBF and arteriovenous difference measures.

Reinfusion of blood was followed by reestablishment of control levels of cerebral phosphorylated metabolites and pHpi. Even after profound partial or complete cerebral ischemia, restoration of PCr and ATP to within 80% of control values have been reported (29, 30). The changes in cerebral phosphorylated metabolites paralleled an increase in cerebral O_2 delivery although cerebral O_2 uptake and cerebral fractional extraction of O_2 remained low. This observation allows for alternative speculations such as: 1) further reductions in brain energy requirements after reperfusion, 2) continued energy production from anaerobic metabolism, or 3) the occurrence of an elevated cerebral O_2 uptake in the first minutes after ischemia providing sufficient O_2 to restore high energy metabolites followed by a fall in O_2 uptake at the time of our measurements. Steen *et al.* (31) reported observations in adult dogs to support this.

It has been suggested that PCr/Pi represents a bioenergetic reserve of tissue because PCr helps maintain ATP concentrations as the latter is converted to ADP and Pi during hypoxia (32). The decrease in PCr and increase in Pi with small reductions in NTP during ischemia (Fig. 3) supports this concept. As noted by others (25), a pool of PCr persists when ATP decreased during ischemia, suggesting compartmentation of PCr in the brain as speculated for muscle (33). Alternative explanations include differential rates of fall of PCr and ATP during intracellular acidosis (25) or creatine phosphokinase inhibition secondary to brain ischemia. Herein the PCr/Pi ratio at control (2.0 ± 0.6) agrees with preliminary data by Delivoria-Papadopoulos *et al.* (34) of a PCr/Pi ratio of 1.7 in piglets. Similar PCr/Pi ratios have been reported for 10- to 16-day-old rabbits (2.3) and neonatal lambs (1.4) (35, 36). Considerable variation existed among piglets for the control PCr/Pi ratios but within a litter values tend to cluster (Fig. 4). Hilberman *et al.* (37) reported that in adult dog brain a PCr/Pi of 1.0 represents a critical value and when less than one profound metabolic alterations occurred. The results in Figure 4 are consistent with this; however, when PCr/Pi <1 the magnitude of change in pHpi varied at a given PCr/Pi. Although PCr/Pi may represent one bioenergetic reserve, we were unable to use the control PCr/Pi to predict the extent of alterations in cerebral phosphorylated metabolites and pHpi during partial ischemia for individual animals.

The results of this investigation demonstrate that changes in cerebral phosphorylated energy metabolites occur at a CBF of 40 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ which represents a 56% reduction of CBF relative to control. The alterations in cerebral energy metabolism were reversible as demonstrated by serial ^{31}P NMR measurements. Combined CBF and ^{31}P NMR measurements should be useful in further defining ischemic thresholds for irreversible energy failure.

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