0031-3998/93/3403-0370$03.00/0
PEDIATRIC RESEARCH

Vol. 34, No. 3, 1993
Printed in U.S.A.

The Effect of Hypercarbia on Age-Related Changes in Cerebral Glucose Transport and Glucose-Modulated Agonal Glycolytic Rates

RONALD J. T. CORBETT, ABBOT R. LAPTOOK, RICK STERETT, GREG TOLLEFSBOL, AND DAMIAN GARCIA

Ralph Rogers and Mary Nell Magnetic Resonance Center, Department of Radiology [R.J.T.C.] and Pediatrics [A.R.L., R.S., G.T., D.G.], University of Texas Southwestern Medical Center, Dallas, Texas 75235-9085

ABSTRACT. This study examined the effect of hypercarbia on cerebral agonal glycolytic rates and brain lactate accumulation after complete ischemia induced by cardiac arrest. Before cardiac arrest, the blood plasma glucose concentration in seven newborn (113 d postconception; normal gestation, 115 d) and seven 1-mo-old (144 d postconception) piglets was adjusted to a specific value (range, 1 to 64 mM), and then inspired ventilation gases were changed to 10:50:40 CO_2:O_2:N_2 for 20 min. The agonal glycolytic rate was measured by monitoring the rate of cerebral lactate formation *in vivo* using proton nuclear magnetic resonance spectroscopy, and postmortem brain lactate concentrations were measured biochemically in tissue extracts obtained 40 to 45 min after cardiac arrest. These data were compared with 21 normocarbic piglets of similar age, nine examined as part of the present study and 12 examined previously (Corbett RJT, Laptook AR, Ruley JI, Garcia D: Pediatr Res 30:579–586, 1991). There was a nonlinear relationship between the final postmortem brain lactate concentration and preischemia blood plasma glucose concentration that was most prominent in newborn piglets and previously had gone unnoticed. When analyzed using a steady-state model for glucose transport, this relationship revealed that normocarbic newborns had a lower preischemia affinity constant for the transport mechanism for glucose (2.8 ± 1.5 mM) and lower cerebral glucose utilization rate relative to transport rate (0.12 ± 0.04), compared with 1-mo-olds (4.5 ± 1.4 mM and 0.30 ± 0.03, respectively). In the presence of hypercarbia, these differences diminish, suggesting that newborn and 1-mo-olds had nearly identical affinity constants of transport mechanism for glucose (3.7 ± 0.8 and 4.0 ± 0.4 mM, respectively) and identical cerebral glucose utilization rate relative to transport rate (0.21 ± 0.03 and 0.23 ± 0.01, respectively). For 1-mo-olds, hypercarbia substantially decreased the maximal rate of agonal glucose utilization (3.93 ± 0.55 to 1.75 ± 0.11 $\mu mol \cdot g^{-1} \cdot min^{-1}$) and decreased the concentration of plasma glucose (6.86 ± 3.00 to 1.27 ± 0.41 mM) at which the half maximal rate of utilization occurs, whereas in newborns the relative decrease produced by hypercarbia was not as prominent (1.46 ± 0.14 to 1.12 ± 0.22 $\mu mol \cdot g^{-1} \cdot min^{-1}$ and 0.93 ± 0.66 to 0.80 ± 1.14 mM, respectively). To the extent that lactic-acidosis enhances irreversible tissue damage, hypercarbia could be beneficial for either age group because hypercarbia reduces both agonal glycolytic rate and brain tissue glucose concentration in newborns and substantially decreases agonal glycolytic rate in 1-mo-olds. (*Pediatr Res* 34: 370–378, 1993)

Abbreviations

AGR, initial agonal glycolytic rate during complete ischemia
$[glucose]_{brain}$, brain tissue glucose concentration
$[glucose]_{plasma}$, arterial blood plasma glucose concentration
HCT, hematocrit
HR, heart rate
^1H NMR, proton nuclear magnetic resonance
k_{lac}, first-order rate constant for lactate accumulation
K_m, blood plasma glucose concentration where glycolytic rate is half maximal
K_t, affinity constant of transport mechanism for glucose
$[lactate]_{final}$, final postmortem brain lactate concentration
$[lactate]_{initial}$, preischemia brain lactate concentration
$[lactate]_{plasma}$, blood plasma lactate concentration
MAP, mean arterial blood pressure
T_{max}, maximal cerebral glucose transport rate before ischemia
V_{glu}, cerebral glucose utilization rate before ischemia
V_{max}, maximum initial AGR during complete ischemia
pH_a, arterial blood pH
NMR, nuclear magnetic resonance
$pH_{intracellular}$, intracellular brain pH

Lactic acidosis associated with ischemia or hypoxic-ischemia in the presence of hyperglycemia is thought to enhance irreversible brain tissue damage (1). However, hypercarbia also frequently occurs as a component of hypoxic-ischemic encephalopathy in newborns (2). It has been suggested that elevated tissue CO_2 could have a beneficial moderating effect on biochemical disturbances associated with hypoxia, ischemia, or seizures (3). This hypothesis stems from the observation that hypercapnia slows the cerebral metabolic rate for glucose consumption (3). If the rate of lactate accumulation during ischemia is also slowed by hypercarbia, then intracellular tissue acidosis formed in parallel with lactate production will be reduced, thereby diminishing irreversible damage associated with acidosis (1). However, with the exception of one study (4), the observation that hypercapnia reduces glucose utilization is based on measurements of the uptake and turnover of radioactively labeled glucose or analogues in well-perfused brain under aerobic conditions. During complete ischemia, brain tissue oxygen tension rapidly drops to zero

Received November 30, 1992; accepted April 23, 1993.
Correspondence and reprint requests: Ron Corbett, Department of Radiology, 5801 Forest Park Road, University of Texas Southwestern Medical Center, Dallas, TX 75235-9085.
Supported by 90G-075 and 92R-075 Grant-in-Aids awarded by the American Heart Association Texas Affiliate, and Southwestern *In Vivo* Biotechnology Resource Facility Grant 5-P41-RR02584.

and glycolytic rates are accelerated by an order of magnitude (5, 6). It is unclear whether hypercarbia slows AGR during anaerobic conditions to the same extent as observed during aerobic conditions. A second consideration is that $[glucose]_{brain}$ is increased by hypercarbia (2, 7). Therefore, the presence of hypercarbia and hyperglycemia may combine to increase lactic acidosis during ischemia. Furthermore, it has been demonstrated that in newborn swine (8) and adult rabbit brain (9), AGR is enhanced by increased $[glucose]_{plasma}$. It is conceivable that the putative beneficial effect of hypercarbia on reducing AGR could be offset if an elevated $[glucose]_{brain}$ also increases AGR or the degree of tissue lactic acidosis. The effect of hypercarbia on glycolysis is further complicated by the observation that the $[glucose]_{plasma}$ for K_m is lower for newborn piglets compared with 1-mo-old piglets, and therefore the rate of lactate formation in newborns is not as strongly accelerated by hyperglycemia in the former compared with the latter (8). Overall, these considerations make it difficult to predict what effect hypercarbia has on $[glucose]_{brain}$ and on AGR in the developing brain.

The objective of the present study was to evaluate the effects of hypercarbia on brain glucose transport and AGR in developing swine brain over a range of $[glucose]_{plasma}$. This investigation was performed in newborn and 1-mo-old animals because our previous study showed that significant increases in cerebral energy metabolism occur over this period (8). The specific questions addressed are does cerebral glucose transport change with increased age, does hypercarbia affect glucose transport, and does hypercarbia affect glucose-modulated cerebral glycolytic rates and are there age-related differences?

MATERIALS AND METHODS

Experimental Protocol. The surgical procedures and experimental protocol were approved by the University of Texas Southwestern Medical Center Institutional Review Board for Animal Research. A total of 14 miniature swine (Sinclair strain) from five different litters were studied during hypercarbia. To evaluate the effects of hypercarbia, we have made extensive comparisons with normocarbic newborn and 1-mo-old piglets who were exposed to the same protocol as described below, except that CO_2 was not included in the inhalation gases. Some results for normocarbic newborns ($n = 6$) and 1-mo-old piglets ($n = 6$) were described previously (8). To further examine the relationship between $[glucose]_{plasma}$, $[glucose]_{brain}$, and AGR, five additional newborn ($n = 11$) and four 1-mo-old piglets ($n = 10$) were studied during normocarbia.

The piglets studied during hypercarbia were divided into two groups at postconceptual ages ranging from 113 to 114 d (newborn) and 141 to 148 d (1-mo-old). Normal term gestation for this species is 115 d. For all five litters, birth was initiated after 111 to 112 d gestation via prostaglandin-induced labor (10) as described previously (8). On the day of the study, an animal was surgically prepared, wrapped in a blanket warmed with circulating water, placed in the magnet, and allowed to attain stable blood gas readings and rectal temperature of 38°C during a 1-h period before collecting control data. Control data to assess baseline brain lactate levels and systemic physiologic status consisted of a ^1H NMR spectrum, the drawing of a blood sample to measure P_{O_2} and P_{CO_2} tensions, pH_a and HCT, plasma glucose and lactate concentrations, and the recording of HR and MAP. Next, a continuous i.v. infusion of glucose (10 to 50 $mg \cdot kg^{-1} \cdot min^{-1}$), or bolus injection of insulin (2 to 4 IU/kg) was given to adjust the $[glucose]_{plasma}$ of individual piglets to a predesignated value in the range of 1 to 64 mM. In a previous study (8), we determined that 30 to 40 min was sufficient for $[glucose]_{plasma}$ to reach a steady state. In the present study, blood samples to monitor blood gases and substrate concentration were drawn 30 to 40 min after the injection of insulin or infusion of glucose. This was followed by 20 min of hypercarbia, achieved by altering the ventilation gases to 8–10:50:42–40, CO_2:O_2:N_2. Blood samples were obtained at one or two intervals during hypercarbia (see Results). Two min after the final blood sample measured during hypercarbia, complete cerebral ischemia was induced via cardiac arrest by an i.v. injection of 4 mL of 4 M KCl directly to the heart. The rate of lactate accumulation was measured by collecting 50 individual ^1H NMR spectra every 0.6 min for 30 min, starting 1 min before cardiac arrest. At 29 min after cardiac arrest, NMR data collection was terminated and the animal was removed from the magnet. The portion of the cerebral cortex immediately below the NMR coil was removed 40 to 45 min postmortem, and stored at −70°C for subsequent measurements of brain lactate concentration.

Animal Preparation. Short-acting anesthetics were used in the present study so that when experimental data collection began the affects of these agents on cerebral energy metabolism would be negligible. After premedication with ketamine (15 to 20 mg/kg, intramuscular) and infiltration of the surgical sites with 1% xylocaine, the animal was tracheotomized and ventilated with 70:30 N_2O:O_2 (Harvard small animal respirator). Intravascular catheters were placed in the common carotid artery and right atrium via an external jugular vein. D-tubcurarine Cl (0.10 mg/kg) and nalbuphine (0.15 mg/kg) were administered i.v. for muscle relaxation and analgesia, respectively. A second injection of nalbuphine was given 15 to 60 min before initiating cardiac arrest. The presence of nalbuphine for analgesia during the experiment was not expected to affect cerebral metabolism because narcotic opiates can relieve pain but have no or only mild depressive effects on CNS activity (11). Skin overlying the skull was retracted so the NMR radiofrequency coil rested directly on the skull. After surgery, the animal was transported to the magnet and ventilated with 70:30 N_2:O_2 and stabilized for 1 h before collecting control *in vivo* NMR and physiologic readings.

NMR Spectroscopy. Protocols for the collection and processing of ^1H NMR data remained unaltered from our earlier report (8). Briefly, ^1H NMR spectroscopy was performed using a General Electric Omega operating system and 40-cm-diameter bore 4.7 Tesla Oxford superconducting magnet. ^1H NMR spectra from the cerebral cortex were measured using a radiofrequency coil (5 × 3 cm) tuned to 200 MHz. ^1H NMR data were collected using a $1\bar{3}3\bar{1}$-τ-$2\bar{6}6\bar{2}$-τ-Ac, spin-echo–pulse sequence, where "1" corresponds to the first portion of a binomial pulse equal to 1/8 of the 90° pulse width; the other pulses are multiples of this, and the bar indicates 180°-phase inversion. The prepulse delay time was 1 s and τ, the delay time for echo formation, was 150 ms. The accumulated free induction decay (16 transients collected every 36 s) was processed by applying direct current offset baseline correction, a 10-Hz exponential apodization function, Fourier transformation, and zero-order phasing. The phase angle applied was determined from the setting that gave the maximal positive peak for the final postmortem β-lactate ^1H NMR signal. The ^1H NMR spectra measured at control were subtracted from all subsequent spectra collected during ischemia to yield difference spectra corresponding to the change in cerebral lactate concentration after cardiac arrest. The relationship between the *in vivo* NMR lactate signal height and brain lactate concentration was established by using the postmortem brain lactate NMR signal and tissue lactate concentration as a calibration factor (8). The validity of this method for measuring changes in brain lactate concentration has been previously discussed (8, 9, 12). Briefly, the calibration relies on the assumption that the postmortem brain lactate concentration has reached a plateau after 30 min of ischemia, that the relaxation properties of the lactate-^1H NMR signal do not change during ischemia, and that there are no ^1H NMR signals from other compounds that could interfere with the quantitation of the signal from lactate. The data presented in Figure 1 were used to verify that brain lactate concentration had reached a plateau within 30 min using the same procedure described previously (8). Throughout this period, there were no significant changes in the line width of the lactate peak suggesting that the apparent spin-spin relaxation properties

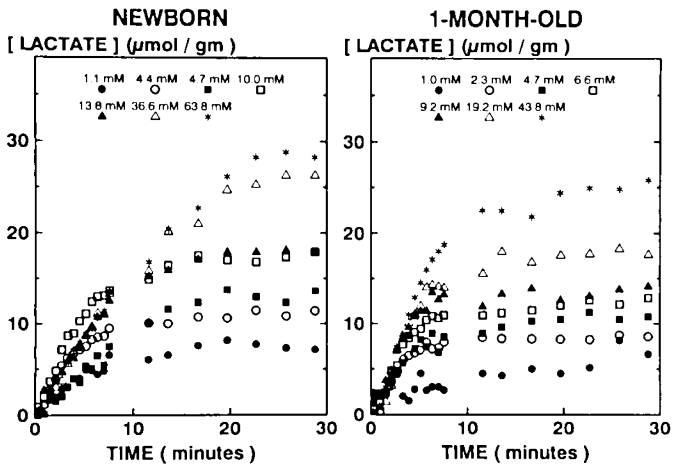

Fig. 1. Rates of agonal lactate formation after complete ischemia. Plots of the change in lactate concentration *versus* time are shown for both hypercarbic newborn and 1-mo-old piglets. The blood plasma glucose concentration for individual piglets immediately before cardiac arrest (time = 0) are indicated in the figure *inset*.

of the β-lactate protons do not change. The spin-lattice relaxation properties of the β-lactate protons do not change between control and ischemia insofar as that control brain lactate concentration derived from the postmortem calibration of the β-lactate ^{1}H NMR signal is in good agreement with brain lactate concentration measured from perchloric acid extracts made from brain tissue frozen under control conditions (13). Interference from the ^{1}H NMR signals of other compounds such as lipids and proteins was discounted because we used a long delay time (150 ms) in the spin-echo–pulse sequence and because no peaks other than that from lactate at 1.33 ppm were observed from 1.7 to 0.9 ppm in ^{1}H NMR difference spectra.

Physiologic Monitoring and Biochemical Measurements. MAP and HR were measured via the catheter in the carotid artery using a Gould (Cleveland, OH) pressure transducer and a Coulbourn (Lehigh Valley, PA) polygraph. The catheter in the carotid artery also was used to obtain blood for monitoring pH_a, blood gases, $[lactate]_{plasma}$, $[glucose]_{plasma}$, and HCT. The catheter in the jugular vein was used for the administration of nalbuphine, glucose, insulin, and 4 M KCl. The pH_a, P_{CO_2}, and P_{O_2} were measured from a 200-μL sample using an Instrumentation Laboratory model 1306 pH/blood gas analyzer (Lexington, MA) blood gas analyzer. Plasma lactate concentration was measured using the lactate dehydrogenase assay after deproteinization of 200 μL of plasma with 8% perchloric acid and centrifugation for 1 min at 1500 × *g*. Plasma glucose was determined by the glucose oxidase/peroxidase assay after deproteinization of 50 μL of plasma with 2% perchloric acid and centrifugation.

Frozen postmortem cortical brain samples (5 to 10 g per piglet) were ground to a powder and mixed thoroughly to ensure a homogeneous sample representative of the entire brain region measured by the NMR radio frequency coil. Brain tissue lactate concentrations were determined using the lactate dehydrogenase enzymatic assay of the neutralized aqueous portion from 0.5 g of brain powder, homogenized, and extracted in 5% perchloric acid (14).

Data Analysis. All statistical tests and regression fits were performed using SPSS/PC (Version 3.1, Statistical Package for the Social Sciences Inc., Chicago, IL), loaded on an AST Premium 286 personal computer (AST Research Inc., Irvine, CA), and upgraded with a Super SX-386 board (Super Computers Inc., Readmond, WA). Tests for differences between multiple groups were made by a one-way analysis of variance using Duncan's multiple comparison test (15) to identify statistically significant differences ($p < 0.05$) between specific pairs of means.

Cerebral glucose transport. Time-dependent changes in $[glucose]_{brain}$ can be expressed as the sum of Michaelis-Menten equations (16):

$$\frac{d[glucose]_{brain}}{dt} = \frac{T_{max} \cdot [glucose]_{plasma}}{[glucose]_{plasma} + K_t} - \frac{T_{max} \cdot [glucose]_{brain}}{[glucose]_{brain} + K_t} - \frac{V_{glu} \cdot [glucose]_{brain}}{[glucose]_{brain} + K_{hex}} \quad (1)$$

The first term on the right-hand side of equation 1 represents glucose movement into the brain, the second term represents glucose movement out of the brain, and the third term represents glucose utilization by the brain. Brain glucose was evaluated indirectly by measuring the final concentration of brain lactate achieved after cardiac arrest. Analogous to earlier studies that used decapitation to induce complete ischemia (4–6), cardiac arrest converts the brain into a closed system in which all glucose and other glycosyl units are converted into lactate (see below). T_{max} equals the maximal rate of glucose transport; K_t equals the affinity constant of glucose for the transport mechanism. T_{max} and K_t represent the averaged transport properties of brain endothelial cells located on the luminal and contraluminal membranes (16). V_{glu} and K_{hex} are the Michaelis-Menten constants for glucose utilization, which are assumed to equal the maximal velocity and affinity constant for the hexokinase catalyzed conversion of glucose to glucose-6-phosphate, respectively. The affinity constant for glucose to hexokinase is assumed to equal approximately 0.05 mM (16), and, therefore, $K_{hex} \ll [glucose]_{brain}$ and the last term in equation 1 is approximately equal to V_{glu} (16). The results presented below for hypercarbic animals and our previous study of normocarbic piglets (8) indicate that $[glucose]_{plasma}$ was constant for 10 to 20 min before ischemia; therefore, a steady state of glucose flux between brain tissue and blood plasma is assumed to exist for all animals. If $[glucose]_{plasma}$ is constant, then $d[glucose]_{brain}$ divided by dt equals 0, and equation 1 can be rearranged to give an expression with $[glucose]_{brain}$ on the left-hand side:

$$[glucose]_{brain} = \frac{-K_t\{(V_{glu}/T_{max}) - [[glucose]_{plasma}/(K_t + [glucose]_{plasma})]\}}{1 + \{(V_{glu}/T_{max}) - [[glucose]_{plasma}/(K_t + [glucose]_{plasma})]\}} \quad (2)$$

Nonlinear regression analysis using equation 2 was performed for sets of $[glucose]_{brain}$ *versus* $[glucose]_{plasma}$, measured for different groups of piglets to obtain best-fit estimates of K_t and the ratio V_{glu}/T_{max}.

To estimate $[glucose]_{brain}$, we have made the following two assumptions. The postmortem brain lactate concentration minus the control brain lactate concentration ($[lactate]_{final}$) equals two times the sum of preischemia intracellular concentrations of glucose plus endogenous nonglucose (glycogen and sugar phosphates) reserves of glycosyl units ($[glycosyl]_{initial}$). The intercept from a plot of postmortem lactate *versus* preischemia $[glucose]_{plasma}$ equals the sum of endogenous nonglucose glycosyl units present in brain tissue (see Results). If these assumptions are true, then:

$$[glucose]_{brain} = ([lactate]_{final} - \text{INTERCEPT})/2 \quad (3)$$

The numerator in equation 3 is divided by 2 because 2 mol of lactate are generated per mol of glucosyl unit consumed. The first assumption is justified given that cardiac arrest essentially converts the brain into a "closed system," with no exchange between blood and brain because blood flow is 0 (17). The second assumption implies that $[glycosyl]_{initial}$ is independent of acute changes in $[glucose]_{plasma}$, and therefore the intercept can be subtracted from the $[lactate]_{final}$ determined for individual animals with different $[glucose]_{plasma}$ at the point where complete ischemia is initiated (see Discussion).

Agonal glycolytic rates. The k_{lac} was determined by the analysis

of the change in brain lactate concentrations ([lactate]$_t$) measured at different times during the first 5 min after cardiac arrest using the following equation:

$$[\text{lactate}]_t = [\text{lactate}]_{\text{final}} (1 - e^{(-k_{lac} \cdot t)}) \quad (4)$$

where [lactate]$_{final}$ is equal to the postmortem brain lactate concentration minus control brain lactate concentration (1.5 μmol·g^{-1}) because the lactate signal at control is not represented in the ^{1}H NMR different spectra we used to measure lactate accumulation. Nonlinear regression analysis was performed to obtain the best estimate and SD of the rate constant for lactate production, k_{lac}. A discussion of the use of this model, the use of nonlinear regression analysis to evaluate k_{lac}, and an error analysis have been discussed previously (8).

The AGR equals the maximum rate of lactate production (*i.e.* [lactate]$_{final}$ multiplied by k_{lac}) divided by 2 because two lactate molecules are produced per molecule of glucose consumed during glycolysis:

$$\text{AGR} = -\text{d}[\text{glucose}]_{\text{brain}}/\text{dt} = (\text{d}[\text{lactate}]/\text{dt})/2 = ([\text{lactate}]_{\text{final}} \cdot k_{lac})/2 \quad (5)$$

AGR was related to the substrate concentration, [glucose]$_{plasma}$, via the Michaelis-Menten equation:

$$\text{AGR} = (V_{max} \cdot [\text{glucose}]_{\text{plasma}})/([\text{glucose}]_{\text{plasma}} + K_m) \quad (6)$$

V_{max} represents the maximal cerebral glycolytic rate with respect to blood glucose concentration, and K_m is the apparent Michaelis-Menten constant for glucose corresponding to the [glucose]$_{plasma}$ where half maximal reaction velocity is reached. Nonlinear regression analysis was performed to obtain best-fit estimates of the parameters V_{max} and K_m.

RESULTS

Physiologic readings. The mean physiologic readings for the newborn and 1-mo-old piglets under control conditions and 18 ± 2 min after inducing hypercarbia are summarized in Table 1. One-mo-old piglets had significantly higher body weight, MAP, P_{O_2}, and lower [lactate]$_{plasma}$, compared with newborns. With the exception of a slightly higher control [glucose]$_{plasma}$ and lower P_{CO_2} for newborns (*i.e.* 1.3 kPa), the prehypercarbia control physiologic readings were not significantly different from the control physiologic readings for normocarbic newborn and 1-mo-old swine measured previously (8), or the nine additional normocarbia animals studied as part of the present study (data not shown). For both newborn and 1-mo-old piglets, changing the ventilation gas mixture to include 8 to 10% CO_2 resulted in a significant decrease in pH_a and increase in P_{CO_2} compared with control values. The magnitude of change in pH_a and P_{CO_2} did not differ between the two age groups. There also was a decrease in HR and an increase in MAP that were of similar magnitude for both age groups, and a slight decrease in P_{O_2} for 1-mo-old piglets. We judged that the slight alterations and differences in HR, MAP, and P_{O_2} would have a negligible impact on cerebral energy metabolism measured during complete ischemia.

The measurement of glucose in blood plasma obtained at different stages in the protocol suggest that piglets had steady state levels of [glucose]$_{plasma}$ for at least the full 20-min exposure to CO_2 in the ventilation gas mixture before ischemia. For a subgroup of six animals, [glucose]$_{plasma}$ was measured 30 to 40 min after injecting insulin or infusing glucose and again 18 min after exposure to CO_2; the difference between the two values was not significant (paired *t* test). In a different subgroup of seven animals, two sets of physiologic readings were measured 12 and 18 min after exposure to CO_2. There were no significant differences between any mean physiologic readings with the exception of slightly lower pH_a, which was 0.02 units lower after 18 min of hypercarbia compared with 12 min of hypercarbia (paired *t* test, $p = 0.007$). For one hypoglycemic 1-mo-old animal, we were unable to draw an arterial blood sample during hypercarbia, and so we estimated [glucose]$_{plasma}$ to equal 1 mM based on a blood sample drawn 40 min after injecting insulin and from venous blood taken during hypercarbia.

After the injection of KCl, HR dropped to 0 within one or two beats, with a concurrent drop in MAP, resulting in a well-defined point at which complete cerebral ischemia was initiated.

Kinetics of lactate formation. In vivo lactate-^{1}H NMR spectra collected in the present study did not differ substantially in quality or signal-to-noise compared with spectra presented previously (8, 12). ^{1}H NMR difference spectra of hypercarbia minus control indicated no peaks detectable above the noise, suggesting that hypercarbia did not alter brain lactate concentrations to within the limits of detectability of the NMR measurement (approximately 1 μmol·g^{-1}). This is consistent with previous *in vitro* determinations of control lactate concentration from piglet brain frozen during normocarbia or hypercarbia, which revealed no significant differences from each other (1.5 *versus* 1.4 μmol·g^{-1}) (13). Serial difference spectra collected after cardiac arrest (*i.e.* ischemia minus control) revealed a rapid increase in the lactate-^{1}H NMR signal at 1.33 ppm corresponding to the β-protons of lactate; no other peaks were present in the difference spectra. Within 20 to 29 min, the lactate-^{1}H NMR signal reached a plateau and NMR data collection was terminated.

The approximately 40-min postmortem [lactate]$_{final}$ measured in cortical brain tissue was used to calculate a calibration factor

Table 1. *Comparison of physiologic data for newborn and 1-mo-old piglets before (pre-CO_2) and during (CO_2) hypercapnia**

	Newborn (n = 7)		1-mo-old (n = 7)	
	Pre-CO_2	CO_2	Pre-CO_2	CO_2
Age (days)	113 ± 1		144 ± 3†	
Wt (kg)	0.95 ± 0.08		5.31 ± 0.46†	
[Glucose]$_{plasma}$ (mM)	7.3 ± 2.6	1–64‡	6.7 ± 1.5	1–44‡
[Lactate]$_{plasma}$ (mM)	2.2 ± 0.6	1.6 ± 1.0	1.4 ± 0.4†	1.4 ± 0.6
pH_a	7.44 ± 0.06	7.03 ± 0.06§	7.45 ± 0.05	7.04 ± 0.02§
P_{CO_2} (kPa)	4.8 ± 0.3	14.1 ± 1.1§	4.4 ± 0.4	13.6 ± 0.9§
P_{O_2} (kPa)	14.7 ± 4.5	15.6 ± 3.3	21.1 ± 2.0†	17.6 ± 3.2§
HCT (vol%)	29 ± 8	32 ± 8	36 ± 4	37 ± 5
HR (beats/min)	234 ± 24	185 ± 48§	237 ± 34	192 ± 32§
MAP (mm Hg)	70 ± 5	83 ± 12§	99 ± 12†	138 ± 5§‖

* Values are mean ± SD.

† Significant difference compared with mean measured for newborns before hypercarbia (unpaired *t* test, $p < 0.05$).

‡ Ranges are given because [glucose]$_{plasma}$ was adjusted by injecting insulin or infusing glucose before inducing hypercarbia (see Materials and Methods).

§ Significant difference compared with prehypercarbia (paired *t* test, $p < 0.05$).

‖ Significant difference compared with mean for newborns during hypercarbia (unpaired *t* test, $p < 0.05$).

to convert lactate-^{1}H NMR signal measured at earlier times after cardiac arrest into a brain tissue lactate concentration (Fig. 1). Nonlinear regression analysis was used to fit equation 4 to the first 5 min of lactate *versus* time data sets to evaluate the k_{lac}. The k_{lac} values corresponding to the best fit ranged from 0.067 to 0.22 min^{-1} for hypercarbic newborns and from 0.13 to 0.30 min^{-1} for hypercarbic 1-mo-old piglets. Previously, we had noted a negative linear correlation between k_{lac} and $[glucose]_{plasma}$ for newborn normocarbic piglets but not for 1-mo-old piglets (8). Plots of k_{lac} *versus* $[glucose]_{plasma}$ (data not shown) suggested a trend ($p \approx 0.1$) for a negative linear correlation for both hypercarbic newborns (slope = -0.00167 ± 0.001 min^{-1}/mM; $r = -0.61$) and 1-mo-old (slope = -0.0029 ± 0.0012 min^{-1}/mM; $r = -0.73$) piglets. These slopes were not significantly different from each other. The slopes of k_{lac} *versus* $[glucose]_{plasma}$ for hypercarbic newborn or 1-mo-old piglets were significantly lower than their normocarbic counterparts (Duncan multiple comparison test, $p < 0.05$). The inclusion of data for two additional normocarbic animals in both age groups did not substantially alter the slopes for newborns (slope = -0.0032 ± 0.0006 min^{-1}/mM; $r = -0.90$) or 1-mo-old piglets (slope = $+0.0003 \pm 0.0023$ min^{-1}/mM; $r = 0.05$) compared with our previous report (8).

Cerebral glucose transport. The increased number of animals and higher range of $[glucose]_{plasma}$ examined in the present study revealed a nonlinear relationship between $[lactate]_{final}$ and $[glucose]_{plasma}$ that we previously did not detect (Fig. 2). This was most evident in either group of newborns. For $[glucose]_{plasma}$ concentrations less than 20 mM, the formation of $[lactate]_{final}$ for a given prearrest $[glucose]_{plasma}$ was steep, as demonstrated by slopes of 0.91 ± 0.15 ($n = 9$) and 0.87 ± 0.14 ($n = 5$) for normocarbic and hypercarbic newborns, respectively. In contrast, slopes calculated using the full set of data were 0.58 ± 0.07 and 0.30 ± 0.04, respectively. The nonlinear relationship between $[lactate]_{final}$ and $[glucose]_{plasma}$ was less pronounced for 1-mo-old animals. Linear fits to the subset of data where $[glucose]_{plasma}$ <20 mM gave a significantly higher slope compared with linear fits calculated using the full set of animals for hypercarbic 1-mo-olds (0.59 ± 0.08 *versus* 0.40 ± 0.04) but not for normocarbic 1-mo-olds (0.43 ± 0.08 *versus* 0.39 ± 0.06). To further test for nonlinearity, we compared fits obtained using a first-order (*i.e.* linear) equation *versus* a second-order (*i.e.* nonlinear) polynomial equation. For both newborn groups, the fit obtained using a second-order equation (*solid* and *dashed lines*, Fig. 2) was a significantly better than the fit obtained using a first-order equation, as judged by the lower residual sums of squares (F test for the significance of added parameters, $p <$ 0.05). Second-order fits also were significantly better than first-order fits for hypercarbic 1-mo-olds but not for normocarbic 1-mo-olds.

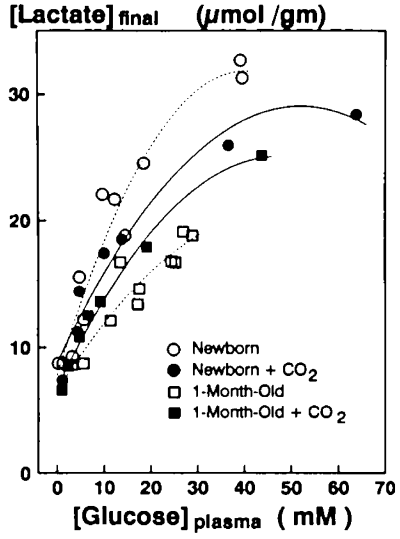

Fig. 2. The relationship between maximal brain lactate concentration ($[Lactate]_{final}$) and blood plasma glucose concentration ($[Glucose]_{plasma}$) for newborn (*circles*) and 1-mo-old piglets (*squares*) in the presence (*closed symbols*) and absence (*open symbols*) of hypercarbia. The *solid lines* represent the best fit of a second-order polynomial equation ($A \cdot [glucose]_{plasma}^2 + B \cdot [glucose]_{plasma} + C$) to each of the four groups. The A and B coefficients ranged from −0.015 to −0.0061 and from 1.2 to 0.59, respectively. The intercept equaled 7.8 ± 1.4, 8.8 ± 1.3, 6.6 ± 1.9, and 6.8 ± 0.6 $\mu mol \cdot g^{-1}$ for newborn normocarbic, newborn hypercarbic, 1-mo-old normocarbic, and 1-mo-old hypercarbic groups, respectively. Regression coefficients ranged from 0.87 to 0.99.

The constancy in the brain lactate-^{1}H NMR signal from 20 to 30 min after cardiac arrest (Fig. 1) indicates that cerebral reserves of glucose and glycogen were exhausted within the first 20 min of complete cerebral ischemia. Previous experiments have shown that the concentrations of glycolytic intermediates after ischemia remain less than 0.2 $\mu mol \cdot g^{-1}$ with the exception of glycerol-P that increased in parallel with lactate at a ratio of 1:24, glycerol-

It is important not to overinterpret the meaning of the *solid curves* presented in Figure 2. Our main purpose in using the second-order polynomial was to test our contention that the relationship between $[lactate]_{final}$ and $[glucose]_{plasma}$ is nonlinear and to provide optimal estimates for the value of the intercept for the plots shown in Figure 2, using the entire set of experimental data (see below). The underlying mechanism responsible for the nonlinear relationship between $[lactate]_{final}$ and $[glucose]_{plasma}$ is not provided by polynomial equations. To interpret the nonlinear relationship between $[lactate]_{final}$ and $[glucose]_{plasma}$ in terms of physiologically meaningful parameters, we performed an analysis based on the theory for cerebral glucose transport using equation 2. The intercepts from the above mentioned second-order fits (see legend to Fig. 2) were used in the estimation of $[glucose]_{brain}$ using equation 3, and plots of $[glucose]_{brain}$ *versus* $[glucose]_{plasma}$ were made for the four different groups (Fig. 3). Using nonlinear regression analysis, equation 2 was fit to these data (*dashed* and *solid lines* in Fig. 3) to evaluate K_t and T_{max} relative to V_{glu}. The results of these fits (Table 2) reveal substantially lower K_t and V_{glu}/T_{max} ratio for normocarbic newborns compared with 1-mo-olds. These differences disappear for the hypercarbic newborn and 1-mo-old piglets, whose K_t and V_{glu}/T_{max} ratios were not different from each other. Hypercarbia produced changes in K_t and V_{glu}/T_{max} compared with normocarbia for either age group, but the changes are in the opposite direction. Whereas hypercarbic 1-mo-olds had a lower K_t and V_{glu}/T_{max} ratio (25% and 12%, respectively) compared with normocarbic 1-mo-olds, for newborns K_t and the V_{glu}/T_{max} ratio appeared to increase by 75% and 30%, respectively.

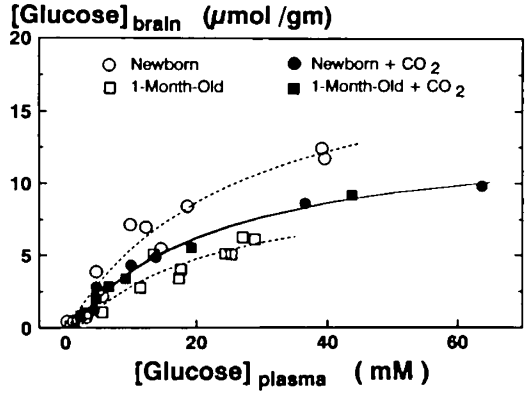

Fig. 3. The relationship between brain glucose concentration ($[Glucose]_{brain}$) and blood plasma glucose concentration ($[Glucose]_{plasma}$) for newborn and 1-mo-old piglets in the presence and absence of hypercarbia. The *solid lines* represent the best fit by nonlinear regression analysis using equation 2. The best fit V_{glu}/T_{max} and K_t values used to generate the *solid lines* are summarized in Table 2.

Table 2. *Comparison of V_{glu}/T_{max} and K_t for newborn and 1-mo-old piglets during normocarbia and hypercarbia**

	Normocarbia		Hypercarbia	
	Newborn (n = 11)	1-mo-old (n = 10)	Newborn (n = 7)	1-mo-old (n = 7)
V_{glu}/T_{max}	0.12 ± 0.04†‡§	0.30 ± 0.03‡‖§	0.21 ± 0.03†‖	0.23 ± 0.01†‖
K_t (mM)	2.8 ± 1.5†	4.5 ± 1.4‖	3.7 ± 0.8	4.0 ± 0.4

* Values are best-fit value ± SD.
† Significant difference compared with 1-mo-old normocarbic piglets (Duncan multiple comparison test, $p < 0.05$).
‡ Significant difference compared with newborn hypercarbic piglets (Duncan multiple comparison test, $p < 0.05$).
§ Significant difference compared with 1-mo-old hypercarbic piglets (Duncan multiple comparison test, $p < 0.05$).
‖ Significant difference compared with newborn normocarbic piglets (Duncan multiple comparison test, $p < 0.05$).

P:lactate (5). These results suggest that errors in the calculation of $[glucose]_{brain}$ due to incomplete hydrolysis of intracellular glucose or high postmortem concentrations of glycolytic intermediates would be negligibly small. However, glucose in whole blood undergoes slow glycolysis at a rate of approximately 3% per h in adults and approximately 26% per h in newborns (18); therefore, we expected some glucose to still be present in the brain tissue at the end of the NMR experiment. Enzymatic assays of postmortem brain tissue confirmed that $[glucose]_{brain}$ ranged from 0.2 to 4 $\mu mol \cdot g^{-1}$ and increased as a function of the preischemic $[glucose]_{plasma}$. The concentration of glucose remaining was consistent with that expected from glucose present in the cerebral vasculature, assuming a cerebral blood volume corresponding to 3% to 6% of the total brain volume. We considered the possibility that glucose converted to lactate in the cerebral vasculature after the initiation ischemia could result in an overestimation of $[glucose]_{brain}$ because $[glucose]_{brain}$ was calculated from $[lactate]_{final}$ (see equation 3). Because hypercarbia increases cerebral blood volume, this overestimation would be higher in animals that were made hypercarbic before ischemia. To estimate the maximum effect this could have on the transport constants in Table 2, we assumed that, as a worst case, the fraction of brain volume corresponding to cerebral blood volume equaled 0.04 and 0.09 for normocarbic and hypercarbic animals, respectively (19), and that all glucose remaining in the blood was converted to lactate within 40 min. We then corrected the calculated $[glucose]_{brain}$ values by subtracting the preischemia $[glucose]_{plasma}$ by one of these two fractions, and refit equation 2 to the revised $[glucose]_{brain}$ *versus* $[glucose]_{plasma}$ data sets for the four groups. The revised V_{max}/T_{max} ratios were only slightly increased in all four age groups by 0.02 to 0.05 units compared with the values reported in Table 2. The revised K_t values for normocarbic animals also were slightly decreased (K_t = 2.6 ± 1.4 and 3.9 ± 1.3 mM for newborn and 1-mo-old piglets, respectively). For hypercarbic animals, the revised K_t values showed larger reductions (K_t = 1.9 ± 1.2 and 2.6 ± 0.2) because the correction factor for cerebral blood volume was approximately 2.2 times more than applied to the data collected for normocarbic animals. However, the use of "corrected" $[glucose]_{brain}$ values does not alter the major conclusions made in the preceding paragraph; normocarbic 1-mo-olds have significantly higher K_t and V_{max}/T_{max} ratios compared with normocarbic newborns, and these age-related differences diminish in the presence of hypercarbia.

Agonal glycolytic rates. The AGR calculated using equation 4 was dependent on $[glucose]_{plasma}$ for both newborn and 1-mo-old hypercarbic piglets with distinct differences compared with each other and compared with normocarbic piglets (Fig. 4). Nonlinear regression analysis of AGR *versus* $[glucose]_{plasma}$ using equation 5 revealed no significant differences in the V_{max} and K_m values measured for normocarbic and hypercarbic newborns (Table 3). In contrast, 1-mo-old hypercarbic piglets had significantly lower V_{max} and K_m values compared with normocarbic 1-mo-old piglets. The hypercarbic 1-mo-old piglets had significantly higher V_{max} values compared with hypercarbic newborns, whereas K_m was not different. Normocarbic 1-mo-old piglet had significantly higher V_{max} and K_m values compared with normocarbic newborns in agreement.

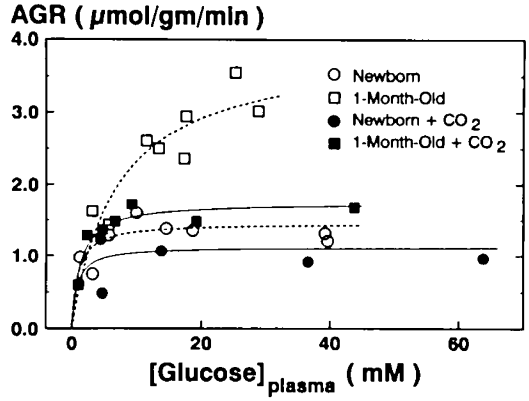

Fig. 4. Dependence of AGR on plasma glucose concentration ($[Glucose]_{plasma}$) at the initiation of total ischemia. Different symbols are used to represent piglets of four different age groups (see Table 1). The *solid lines* represent the best fit by nonlinear regression to the data using equation 5; best fit V_{max} and K_m values are summarized in Table 3.

DISCUSSION

Differences in physiologic readings for newborn and 1-mo-old piglets during hypercarbia were small and unlikely to produce differential effects on cerebral glucose transport measured before ischemia or AGR measured during ischemia. We drew similar conclusions previously for normocarbic newborns and 1-mo-old piglets (8). The $[glucose]_{plasma}$ of both newborn and 1-mo-old piglets was constant throughout the 20-min period of hypercarbia, and the degree of hypercarbia resulting from the inhalation of 8% to 10% CO_2 (P_{CO_2} approximately 14 kPa) was identical and constant for both age groups. Overall, these results show that the severity of hypercarbia was well matched for the two age groups and that a steady state of elevated P_{CO_2} and modified $[glucose]_{plasma}$ was achieved. As discussed previously (8, 16) 10 to 20 min is considered sufficient time to ensure equilibration of glucose exchange between blood and brain before ischemia.

The additional data collected for normocarbic piglets in the present study lead us to change our perspective about age-related differences in the potential for brain lactate formation during complete ischemia. Previously, we concluded that hyperglycemic-normocarbic newborn and 1-mo-old piglets would generate similar high concentrations of lactate during complete ischemia (8). This conclusion was based on the similarity of slopes measured for different age groups of piglets from plots of the postmortem $[lactate]_{final}$ *versus* preischemia $[glucose]_{plasma}$ made for the entire range of $[glucose]_{plasma}$ studied (8). As mentioned in Materials and Methods, the postmortem $[lactate]_{final}$ can be used as direct indicator of preischemia $[glucose]_{brain}$ because the procedure to induce complete ischemia (cardiac arrest) converts the brain into a closed system. In the present study, the slope of

Table 3. *Comparison of V_{max} and $[glucose]_{plasma}$ for K_m for newborn and 1-mo-old piglets during normocarbia and hypercarbia**

	Normocarbia		Hypercarbia	
	Newborn ($n = 8$)	1-mo-old ($n = 8$)	Newborn ($n = 7$)	1-mo-old ($n = 7$)
V_{max} ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	1.46 ± 0.14†	3.93 ± 0.55‡§‖	1.12 ± 0.22†‖	1.75 ± 0.11†§
K_m (mM)	0.93 ± 0.66†	6.86 ± 3.00‡§‖	0.80 ± 1.14†	1.27 ± 0.41†

* Values are best-fit value ± SD.

† Significant difference compared with 1-mo-old normocarbic piglets (Duncan multiple comparison test, $p < 0.05$).

‡ Significant difference compared with newborn normocarbic piglets (Duncan multiple comparison test, $p < 0.05$).

§ Significant difference compared with newborn hypercarbic piglets (Duncan multiple comparison test, $p < 0.05$).

‖ Significant difference compared with 1-mo-old hypercarbic piglets (Duncan multiple comparison test, $p < 0.05$).

plots of $[lactate]_{final}$ *versus* preischemia $[glucose]_{plasma}$ for the subset of data where $[glucose]_{plasma}$ <20 mM was significantly higher for newborns compared with 1-mo-old piglets, implying that higher levels of $[glucose]_{brain}$ were present in newborns compared with 1-mo-old piglets. This is further supported by recent *in vitro* assays of glucose in brain tissue funnel-frozen under control conditions for normoglycemic piglets of different ages. Whereas the $[glucose]_{brain}/[glucose]_{plasma}$ concentration ratio equaled 0.36 ± 0.03 for 1-mo-old normoglycemic piglets, this ratio equaled 0.74 ± 0.16 for newborns (20). Overall, these results suggest that steady state $[glucose]_{brain}$ levels are higher in normocarbic newborns compared with 1-mo-old piglets, especially for $[glucose]_{plasma}$ in the range of 1 to 20 mM (Fig. 3). The trend for relatively smaller increases in $[lactate]_{final}$ when $[glucose]_{plasma}$ >20 mM tends to obscure this difference between the two age groups, as in our previous study where the data were considered for the entire range of $[glucose]_{plasma}$ (8). We interpret the similarity of the intercepts obtained from plots of $[lactate]_{final}$ *versus* $[glucose]_{plasma}$ for normocarbic *versus* hypercarbic groups to suggest that endogenous nonglucose reserves of glycosyl units were unaffected by acute hyperglycemia or hypercarbia. This is consistent with previous studies of rats in which no significant acute changes in brain glycogen concentration were observed after exposure to 10% or 20% CO_2 (4, 7, 21), or for mice and rats infused with glucose (22). This, plus the observation of high linear correlations between $[lactate]_{final}$ and $[glucose]_{plasma}$ when $[glucose]_{plasma}$ <20 mM, supports the assumption that preischemic $[glucose]_{brain}$ can be calculated from the $[lactate]_{final}$ measured in postmortem brain tissue samples using equation 3.

Recently, lactate and glycogen determinations were made for newborn and 1-mo-old piglets from brain tissue frozen under control conditions (20). For both age groups, $[lactate]_{brain}$ equaled approximately 1.4 $\mu mol \cdot g^{-1}$, whereas $[glycogen]_{brain}$ equaled approximately 1.0 and 1.8 $\mu mol \cdot g^{-1}$ for newborn and 1-mo-olds, respectively. Given this baseline $[lactate]_{brain}$ and assuming that 2.9 lactate molecules are generated per molecule of glycogen during anaerobic glycolysis (5), we estimated contribution by these two parameters to $[lactate]_{final}$ equal to 4.3 and 6.6 $\mu mol \cdot g^{-1}$ for newborn and 1-mo-olds, respectively. The latter concentration is in excellent agreement with the intercepts estimated for 1-mo-olds in Figure 2. The former concentration is approximately 4 $\mu mol \cdot g^{-1}$ lower than the intercepts estimated for newborns, suggesting that approximately 2 $\mu mol \cdot g^{-1}$ of glycosyl units contribute to $[lactate]_{final}$ from a source not originating from preischemic cellular glucose, glycogen, and lactate. This implies that the sum of preischemia concentration of other hexoses or glycolytic intermediates is higher in newborn compared with 1-mo-old piglets.

Nonsteady state studies of ^{14}C-glucose transport in the brain of adult and suckling rats indicate that K_t does not change with age (23, 24). It is important to note that the K_t values in Table 2 are not directly comparable to the values reported in these two studies because the latter were derived from nonsteady state tracer studies (16). Steady state measurements of K_t are available only for adult brain and range from 2 to 7 mM, with fairly prominent species variations (16). The steady state measurement of K_t in the present study for normocarbic newborns was lower than 1-mo-olds, suggesting that the affinity of the glucose transporter may increase slightly over this interval. It is conceivable that this could reflect ontogenetic changes in the brain glucose transporter (25). However, the relatively low values for K_t in both newborn and 1-mo-olds during either hypercarbia or normocarbia suggests that under normoglycemic or hyperglycemic conditions, the glucose transporter will be nearly saturated and operating at its maximal rate, T_{max}.

The present study does not provide direct information on age-related changes in either T_{max} or V_{glu} because only the ratio V_{glu}/T_{max} is calculated. Non-steady state measurements of glucose transport suggest that T_{max} doubles from 2 to 3 wk of age to adulthood (23, 24). There also is substantial evidence that V_{glu} increases with brain maturation (2, 5, 6, 20). For either hypercarbic or normocarbic newborn or 1-mo-old piglets, T_{max} was eight to three times more than V_{glu}, suggesting that glucose transport into the brain was not the rate-limiting step for glucose utilization, consistent with previous reports of adult brain (16).

The functional form of equation 2 illustrates an important point about the relationship between $[glucose]_{brain}$, T_{max}, and V_{max}; steady-state levels of $[glucose]_{brain}$ critically depend on the ratio V_{glu}/T_{max}. Changes in this ratio have a profound impact on $[glucose]_{brain}$, as indicated by the $[lactate]_{final}$ measured in the present experiments. For example, newborns had a larger increase in $[lactate]_{final}$ as $[glucose]_{plasma}$ increases compared with 1-mo-olds. This phenomenon is primarily a consequence of a 2.5 times higher V_{glu}/T_{max} ratio in 1-mo-olds compared with newborns. The higher rate of glucose utilization relative to transport in 1-mo-olds creates a stronger "metabolic sink" that lowers $[glucose]_{brain}$ relative to $[glucose]_{plasma}$ (16). Thus, under normoglycemic and hyperglycemic conditions ($[glucose]_{plasma} \approx 6$ to 20 mM), normocarbic newborn piglets subsequently exposed to cardiac arrest generate $[lactate]_{final}$ concentrations ranging from 10 to 25 $\mu mol \cdot g^{-1}$ compared with 9 to 15 $\mu mol \cdot g^{-1}$ for 1-mo-olds (Fig. 2). An important consequence is that newborn piglets subjected to complete ischemia for a sufficient duration could have brain lactate concentrations associated with acidosis-enhanced irreversible tissue damage in adults ($[lactate]_{final}$ >20 $\mu mol \cdot g^{-1}$ and $pH_{intracellular}$ <6.2) (1) when $[glucose]_{plasma}$ exceeds approximately 11 mM, whereas for 1-mo-old piglets a $[glucose]_{plasma}$ of approximately 30 mM would be required. Similar levels of brain lactate during complete ischemia result in similar levels of brain acidosis for either newborn or 1-mo-old piglets because the physiochemical acid buffering capacity of brain does not change during this period (12). Thus, it would seem prudent to maintain $[glucose]_{plasma}$ below 10 mM in clinical situations where complete or near-complete ischemia could occur.

Hypercarbia produced substantially different changes in the relationship between $[glucose]_{brain}$ and $[glucose]_{plasma}$ in newborns compared with 1-mo-old piglets (Fig. 3). For newborn and 1-mo-old piglets, hypercarbia had no significant effect on K_t. However, whereas hypercarbia decreased the V_{glu}/T_{max} ratio by approximately 23% in 1-mo-olds, this ratio increased by approximately 75% in newborns. The effect that hypercarbia has on $[glucose]_{brain}$, through its effect on V_{glu}/T_{max}, becomes most pro-

nounced during hyperglycemia. This is illustrated in Figures 2 and 3; when $[glucose]_{plasma}$ exceeds 10 mM, the difference between $[lactate]_{final}$ or $[glucose]_{brain}$ in hypercarbic *versus* normocarbic animals was most prominent. In newborns, hypercarbia lowered $[glucose]_{brain}$ and hence lowered $[lactate]_{final}$ in animals subsequently exposed to ischemia. This could translate into a greater beneficial effect for hypercarbic newborns compared with normocarbic newborns because lactic acidosis would be decreased in the former compared with the latter. For example, a $[lactate]_{final}$ equal to 20 $\mu mol \cdot g^{-1}$ will be reached at a $[glucose]_{plasma}$ of approximately 18 mM in hypercarbic newborns, whereas this same concentration of lactate is reached at only 11 mM in normocarbic newborn piglets. In contrast to newborns, hypercarbia moderately raises $[glucose]_{brain}$ for hyperglycemic compared with normocarbic 1-mo-old piglets. Higher preischemia $[glucose]_{brain}$ may be detrimental because this would result in higher levels of lactic acidosis during complete ischemia. However, the slower formation of detrimental levels of lactic acidosis in hypercarbic 1-mo-olds compared with normocarbic 1-mo-olds could provide a more important beneficial effect for acute ischemic episodes (see below).

Relatively few studies have been performed to measure glucose utilization during complete ischemia. This reflects the technical difficulties and the cost associated with accurately measuring the time course of lactate formation using *in vitro* sampling techniques that supply one time point per animal (5, 9, 20). We are aware of only one previous study (4) that measured AGR in adult rat brain after complete ischemia induced via decapitation during steady states of normocapnia (5.2 kPa) *versus* mild hypercapnia (8.1 kPa). AGR was 6.24 ± 0.82 and 4.80 ± .96 $\mu mol \cdot g^{-1} \cdot min^{-1}$ in normocarbic and hypercarbic groups, respectively, corresponding to a 23% reduction. Given the milder degree of hypercapnia compared with the present study and noting that $[glucose]_{plasma}$ for the two groups was 10.6 and 12.8 mM, respectively, the reduction observed in the previously mentioned study (4) is similar to that observed for 1-mo-old piglets in the present study.

Glucose utilization during ischemia was measured after a 20-min period when $[glucose]_{plasma}$ was constant; therefore, K_m and V_{max} reflect rate-limiting reactions for agonal glycolysis inside the cell. Hexokinase is thought to be a key enzyme controlling the increased glycolytic rate during ischemia (5, 16). The binding affinity of glucose for hexokinase (K_{hex}) is approximately 0.05 mM, and, therefore, the K_m values in Table 3 are much higher than K_{hex} expected in brain. However, K_m was determined from plots of AGR *versus* $[glucose]_{plasma}$, not $[glucose]_{brain}$. Therefore, based on the theoretical relationships presented in Figures 3 and 4, we calculated a set of 15 AGR *versus* $[glucose]_{brain}$ data points for each of the four groups of piglets and determined V_{max} and K_m using equation 6. The relationship between AGR and $[glucose]_{brain}$ was similar to the plots shown in Figure 4, with the exception that the curves for the four different groups are steeper and are shifted to the left. V_{max} was not substantially different from the values shown in Table 3 (1.34 ± 0.04 and 3.59 ± 0.29 $\mu mol \cdot g^{-1} \cdot min^{-1}$ for newborn and 1-mo-old normocarbic animals, respectively, and 1.03 ± 0.02 and 1.48 ± 0.06 $\mu mol \cdot g^{-1} \cdot min^{-1}$ for newborn and 1-mo-old hypercarbic animals, respectively), but K_m was shifted to lower values for all four groups (0.32 ± 0.05 and 1.31 ± 0.29 mM for newborn and 1-mo-old normocarbic animals, respectively, and 0.12 ± 0.02 and 0.09 ± 0.03 mM for newborn and 1-mo-old hypercarbic animals, respectively). The correction for potential overestimation of $[glucose]_{brain}$ due to the conversion of cerebral blood glucose to lactate (see Results) resulted in slightly lower K_m estimates for normocarbic animals (0.29 and 1.27 mM for newborns and 1-mo-olds, respectively), and slightly higher estimates for hypercarbic animals (0.27 and 0.18 mM for newborns and 1-mo-olds, respectively). All calculated K_m values are higher than literature estimates of K_{hex}, especially for normocarbic 1-mo-olds. Presently, it is unclear whether the difference between K_m and K_{hex} is a reflection of species differences in the value of K_{hex} in swine compared with rodents, experimental differences in the manner in which K_m was calculated (*in vivo versus in vitro*), or that there is a control point for the glucose modulation of AGR other than hexokinase. Further experiments to evaluate the enzymatic properties of hexokinase in swine brain could help clarify this issue.

As illustrated in Figure 4, AGR is strongly influenced by $[glucose]_{plasma}$ to different degrees depending on both age and the absence or presence of hypercarbia. The additional data collected for two hyperglycemic normocarbic newborn and two 1-mo-old piglets resulted in slightly higher estimates of K_m and V_{max} compared with our previous study (8). However, as in the previous study, both V_{max} and K_m were significantly higher in 1-mo-old normocarbic piglets compared with normocarbic newborns. Whereas hypercarbia decreases V_{max} and K_m slightly in newborns, these quantities showed prominent decreases in 1-mo-olds. Thus, during ischemia in hyperglycemic animals ($[glucose]_{plasma}$ >20 mM), hypercarbia reduces the rate of lactate accumulation by up to 55% in 1-mo-old piglets but only by 23% in newborns. For normoglycemic or hypoglycemic animals ($[glucose]_{plasma}$ <6 mM), hypercarbia will decrease rates of lactate accumulation to the about same degree in newborn and 1-mo-old animals. Several recent *in vivo* ^{31}P NMR studies of the cerebral metabolic consequences of hypercarbia using adult animal models from various species have been reported (26–29). These studies all report a decrease in $pH_{intracellular}$ that should inhibit glycolysis and an increase in inorganic phosphate that should stimulate glycolysis (30). Given the observation that hypercarbia decreases AGR in adult rats (4), it is possible that the metabolic effects of a decrease in $pH_{intracellular}$ predominate over the increase in inorganic phosphate to produce a net inhibitory effect. Less certain is the effect of hypercarbia on cerebral ADP and AMP concentrations, with one study suggesting an increase (26), two suggesting no change (27, 28), and one suggesting a decrease (29). Decreases in $pH_{intracellular}$ and increases in inorganic phosphate also have been reported for immature brain (31, 32); however, changes in ADP and AMP have not been reported. Currently, it is unclear whether age-related difference in the magnitude of changes in pH, inorganic phosphate, ADP, or AMP induced by hypercarbia could account for the difference that hypercarbia had on AGR for newborn *versus* 1-mo-old piglets in the present study. Further studies of potential age-related differences in the effect that hypercarbia has on $pH_{intracellular}$ or phosphorylated metabolite concentrations using *in vivo* ^{31}P NMR could help clarify this issue.

In conclusion, these results demonstrate that both brain maturation and $[glucose]_{plasma}$ are critical factors to consider when assessing the effects of hypercarbia. To the extent that it reduced the rate of lactate formation and $[lactate]_{final}$, hypercarbia could prove beneficial because both $[glucose]_{brain}$ and AGR were reduced in newborns and AGR was reduced in 1-mo-olds with only a marginal increase in $[glucose]_{brain}$. These effects were most prominent in animals who were made hyperglycemic before ischemia was initiated. Under these conditions, hypercarbia could help reduce brain lactic acidosis to levels below the critical threshold thought to be associated with enhanced irreversible tissue damage (1). However, the putative benefit realized from the direct effects of hypercarbia on brain lactate formation must be interpreted with caution because in clinical settings acute hypercarbia is normally viewed as undesirable (2). Hypercarbia often implies serious cardiovascular instability and could be detrimental by increasing the incidence of intraventricular hemorrhage or altering blood flow regulation.

Acknowledgment. Dr. Corbett thanks the Departments of Pediatrics and Radiology for their continuing support, which made this study possible.

REFERENCES

1. Rehncrona S 1985 Brain acidosis. Ann Emerg Med 14:770–776
2. Volpe JJ 1987 Hypoxic-ischemic encephalopathy: basic aspects and fetal assessment. In: Volpe JJ (ed) Neurology of the Newborn. WB Saunders, Philadelphia, pp 160–195
3. Miller AL 1985 Carbon dioxide narcosis. In: McCandless DW (ed) Cerebral Energy Metabolism and Metabolic Encephalopathy. Plenum Press, New York, pp 143–162
4. Kogure K, Busto P, Scheinberg P, Reinmuth O 1975 Dynamics of cerebral metabolism during moderate hypercapnia. J Neurochem 24:471–478
5. Lowry OH, Passonneau JV, Hasselberger FX, Schultz DW 1964 Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J Biol Chem 239:18–30
6. Holowach-Thurston J, McDougal DB 1969 Effect of ischemia on metabolism of the brain of the newborn mouse. Am J Physiol 216:348–352
7. Folbergrova J, MacMillan V, Siesjo BK 1972 The effect of hypercapnic acidosis upon some glycolytic and Krebs cycle-associated intermediates in the rat brain. J Neurochem 19:2507–2517
8. Corbett RJT, Laptook AR, Ruley JI, Garcia D 1991 The effect of age on glucose dependent changes in agonal cerebral glycolytic rates. Pediatr Res 30:579–586
9. Petroff OAC, Prichard JW, Ogino T, Shulman RG 1988 Proton magnetic resonance spectroscopy studies of agonal carbohydrate metabolism in rabbit brain. Neurology 38:1569–1574
10. Linderkamp O, Betke K, Guntner M, Jap GH, Riegel KP, Walser K 1981 Blood volume in newborn piglets: effects of time of natural cord rupture, intra-uterine growth retardation, asphyxia, and prostaglandin-induced prematurity. Pediatr Res 15:53–57
11. Benedetti C, Butler SH 1990 Systemic analgesia. In: Benica JJ (ed) The Management of Pain, Volume II Lea & Febiger, Philadelphia, pp 1640–1675
12. Corbett RJT, Laptook AR, Garcia D, Ruley JI 1992 Cerebral acid buffering capacity at different ages measured *in vivo* by ^{31}P and ^{1}H nuclear magnetic resonance spectroscopy. J Neurochem 59:216–226
13. Corbett RJT, Laptook AR 1990 Acid homeostasis and partial ischemia in neonatal piglet brain. J Neurochem 54:1208–1217
14. Lowry OH, Passonneau JV 1972 A Flexible System of Enzymatic Analysis. Academic Press, New York
15. Duncan DB 1955 Multiple range and multiple *F* tests. Biometrics 11:1–42
16. Lund-Andersen H 1979 Transport of glucose from blood to brain. Physiol Rev 59:305–352
17. Gatfield PD, Lowry OH, Schultz DW, Passoneau JV 1966 Regional energy reserves in mouse brain and changes with ischemia and anaesthesia. J Neurochem 13:185–195
18. Meites S, Bohman N 1963 *In vitro* stabilization of blood glucose with water. Clin Chem 9:594–599
19. Mosley ME, Chew WM, White DL, Kucharczyk J, Litt L, Derugin N, Dupon J, Brasch RC, Norman D 1992 Hypercarbia-induced changes in cerebral blood volume in the cat: a ^{1}H MRI and intravascular contrast agent study. Magn Reson Med 23:21–30
20. Corbett RJT, Laptook AR, Garcia D, Ruley JI 1993 Energy reserves and utilization rates in developing brain measured *in vivo* by ^{31}P and ^{1}H nuclear magnetic resonance spectroscopy. J Cereb Blood Flow Metab 13:235–246
21. Miller AL, Hawkins RA, Veech RL 1975 Decreased rate of glucose utilization by rat brain *in vivo* after exposure to atmospheres containing high concentrations of CO_2. J Neurochem 25:553–558
22. Nelson SR, Schultz DW, Passoneau JV, Lowry OH 1968 Control of glycogen levels in brain. J Neurochem 15:1271–1279
23. Cremer JE, Cunningham VJ, Pardridge WM, Braun LD, Oldendorf WH 1979 Kinetics of blood-brain barrier transport of pyruvate, lactate and glucose in suckling, weanling, and adult rats. J Neurochem 33:439–445
24. Daniel PM, Love ER, Pratt OE 1978 The effect of age upon the influx of glucose into the brain. J Physiol 274:141–148
25. Asano T, Shibasaki Y, Kasuga M, Kanazawa Y, Takaku F, Akanuma Y, Oka Y 1988 Cloning of a rabbit brain glucose transporter and alterations of glucose transporter mRNA during tissue development. Biochem Biophys Res Commun 154:1204–1211
26. Nioka S, Chance B, Hilberman M, Subramanian HV, Leigh JS, Veech RL, Forster RE 1987 Relationship between intracellular pH and energy metabolism in dog brain as measured by ^{31}P NMR. J Appl Physiol 62:2094–2102
27. Petroff OAC, Prichard JW, Behar KL, Rothman DL, Alger JR, Shulman RG 1985 Cerebral metabolism in hyper- and hypocarbia: ^{31}P and ^{1}H nuclear magnetic resonance studies. Neurology 35:1681–1688
28. Nishimura M, Johnson DC, Hitzig BM, Okunieff P, Kazemi H 1989 Effects of hypercapnia on brain pH_i and phosphate metabolite regulation by ^{31}P-NMR. J Appl Physiol 66:2181–2188
29. Litt L, Gonzalez-Mendez R, Severinghaus JW, Hamilton WK, Rampil IJ, Shuleshko J, Murphy-Boesch J, James TL 1986 Cerebral intracellular ADP concentrations during hypercarbia: an *in vivo* ^{31}P nuclear magnetic resonance study in rats. J Cereb Blood Flow Metab 6:389–392
30. Erecinska M, Silver IA 1989 ATP and brain function. J Cereb Blood Flow Metab 9:2–19
31. Cady EB, Chu A, Costello AMD, Delpy DT, Gardiner RM, Hope PL, Reynolds EOR 1987 Brain intracellular pH and metabolism during hypercarbia in the newborn lamb. J Physiol 382:1–14
32. Corbett RJT, Laptook AL, Hassan A, Nunnally RL 1988 Quantitation of acidosis in neonatal brain tissue using the ^{31}P NMR peak of phosphoethanolamine. Magn Reson Med 6:99–106