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Quantitative Relationship between Brain Temperature and Energy Utilization Rate Measured *in Vivo* Using ^{31}P AND ^{1}H Magnetic Resonance Spectroscopy

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ABSTRACT

In neonatal and adult animals, modest reduction in brain temperature (2–3°C) during ischemia and hypoxia-ischemia provides partial or complete neuroprotection. One potential mechanism for this effect is a decrease in brain energy utilization rate with consequent preservation of brain ATP, as occurs with profound hypothermia. To determine the extent to which modest hypothermia is associated with a decrease in brain energy utilization rate, *in vivo* ^{31}P and ^{1}H magnetic resonance spectroscopy (MRS) was used to measure the rate of change in brain concentration of phosphocreatine, nucleoside triphosphate, and lactate after complete ischemia induced by cardiac arrest in 11 piglets (8–16 d). Preischemia metabolite concentrations and MRS-determined rate constants were used to calculate the initial flux of high energy phosphate equivalents (d[~P]/dt, brain energy utilization rate). Baseline physiologic and MRS measurements were obtained at 38.2°C and repeated after brain temperature was adjusted between 28 and 41°C. This was followed by measurement of d[~P]/dt during complete ischemia at 1–2°C increments within this temperature range. Adjusting brain temperature did not alter any systemic variable except for heart rate which directly correlated with brain temperature ($r = 0.95$, $p < 0.001$). Before ischemia brain temperature inversely correlated with phosphocreatine ($r = -0.89$, $p < 0.001$), and reflected changes in the phosphocreatine-ATP equilibrium, because brain temperature inversely correlated with intracellular pH ($r = -0.77$, $p = 0.005$). Brain temperature and d[~P]/dt were directly correlated and described by a linear relationship (slope = 0.61, intercept = −12, $r = 0.92$, $p < 0.001$). A reduction in brain temperature from normothermic values of 38.2°C was associated with a decline in d[~P]/dt of 5.3% per 1°C, and therefore decreases in d[~P]/dt during modest hypothermia represent a potential mechanism contributing to neuroprotection. (***Pediatr Res* 38: 919–925, 1995**)

Abbreviations

d[~P]/dt, cerebral energy utilization rate
MR, magnetic resonance
MRS, magnetic resonance spectroscopy
PCr, phosphocreatine
FID, free induction decay
CMRo$_2$, cerebral O_2 uptake

Recent investigations in neonatal piglets (1) and rat pups (2, 3) have demonstrated that relatively small reductions in temperature (2–3°C) during brain ischemia or hypoxia-ischemia provides partial neuroprotection. There are multiple mechanisms by which modest hypothermia may modulate the extent of ischemia-induced brain injury. For example, modest hypothermia attenuates the extent of brain acidosis during and immediately after brain ischemia in neonatal piglets (4). Modest hypothermia blunts the increase in concentration of excitatory amino acids in brain extracellular fluid during ischemia in adult rats (5). Another potential benefit of intraischemic hypothermia is preservation of brain ATP. The latter has been confirmed in adult and neonatal animals when temperature is reduced less than 30°C (6, 7). Preservation of brain ATP concentration presumably occurs as a result of a decrease in metabolic rate and, accordingly, energy utilization rate. It is unclear whether more modest reductions in intraischemic temperature also preserve brain ATP. In adult cats and rats,

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temperature reductions of 4°C were associated with higher brain ATP concentration initially during ischemia, but by the end of the ischemic interval were similar to values of normothermic controls (8, 9). In our laboratory, an intraischemic temperature difference of 2.9°C between normothermic and modestly hypothermic piglets was associated with a trend for greater preservation of brain ATP in cooler animals (57 *versus* 43% of control for hypothermic *versus* normothermic, $p = 0.11$) (4). These results suggest that even reductions in temperature of 2–3°C may alter brain energy utilization rate. The purpose of this report was to explore this observation further by determining if modest hypothermia is associated with decreases in brain energy utilization rate. A recent application of MRS has been developed in this laboratory to measure the energy utilization rate (10) and was used in these experiments.

METHODS

This study was approved by the Institutional Review Board for Animal Research at the University of Texas Southwestern Medical Center. A total of 11 piglets were acquired after sows were induced [prostaglandin induction (11) at 109–111 d gestation, term is 115 d], and animals were studied at either 8–9 or 15–16 d postnatal age (120 and 126 d postconceptual age, respectively). Piglets were administered ketamine (20 mg/kg, intramuscularly) and pentobarbital (20 mg/kg, i.v. via ear vein) for anesthesia. Catheters were positioned by aseptic technique in the internal and external jugular vein and the left common carotid artery. Catheters were tunneled s.c. to the piglet's back and stored in a pouch. Animals were allowed 24 h to recover from anesthesia and surgery, and were gavage or pan fed sow replacement milk. Preparation for experiments on the day after surgery included the use of pentothal (20 mg/kg, i.v.) to facilitate oral intubation and mechanical ventilation with inspired gases of 70% N_2O and 30% O_2. Muscle paralysis and analgesia were achieved with tubocurare Cl (0.1 mg/kg/h, i.v.) and nubain (0.15 mg/kg, i.v.), respectively. For measurement of brain temperature, a skin flap was retracted over the calvarium, a 5-mm burr hole was created (5 mm lateral and proximal to the sagittal and coronal suture, respectively), the dura was incised, and a fiberoptic temperature probe (outer diameter, 1.4 mm; Luxtron Corporation) was passed to a depth of 10 mm from the cortical surface. The temperature probe was secured in place, the burr hole was filled with bone wax, and the scalp was left retracted. Piglets were wrapped in a thermal blanket, which was attached to a water bath via a roller pump, and allowed control of core body temperature by varying the temperature of the water bath. The piglets were positioned prone in a support pod with their exposed calvarium resting adjacent to a cranial surface coil. The support pod was positioned in the bore of a magnet, and piglets were allowed to stabilize for 60 min.

The experimental protocol consisted of a control period during which brain temperature was maintained between 38 and 38.5°C. We have previously demonstrated a close relationship between brain and core body temperature under nonischemic conditions (<0.5°C difference between sites) (1). Baseline systemic physiologic and brain biochemical variables were obtained in duplicate. The brain temperature was then adjusted by altering core body temperature via changes in the temperature of the water circulating through the thermal blanket wrapped around the piglet. A spectrum of brain temperature was investigated by altering each animal's temperature to a different value so that the group of 11 piglets had brain temperatures within a range of 27.6–41°C at increments of 1–2°C. Systemic physiologic and brain biochemical variables were acquired after a steady state of brain temperature was achieved. Complete cerebral ischemia was then initiated rapidly by cardiac arrest induced by 4 mL of 4 M KCl, i.v. Changes in brain energy metabolites were monitored for 30 min after initiation of complete ischemia. After 30 min the animals were removed from the magnet, and the portion of the cerebral cortex immediately below the surface coil was removed and stored at −70°C for subsequent analysis of brain lactate concentration.

Physiologic assessment consisted of measuring heart rate, mean arterial pressure, and arterial blood samples for blood gases, pH, hematocrit, and plasma concentrations of glucose and lactate as previously described (12). Brain temperature was measured with a Luxtron 750 Fluoroptic Thermometry system. MRS of piglet brain was performed on a General Electric CSI system and a 40-cm diameter bore Oxford superconducting magnet operating at a field strength of 4.7 Tesla. Details have been previously described (10) and will be summarized below. ^{31}P and ^{1}H MR spectra were obtained using a two-turn radiofrequency coil (coil dimensions, 5 × 3 cm), double-tuned to the ^{31}P and ^{1}H resonance frequencies (13). The protocol for data collection consisted of acquiring 16 FID at the ^{1}H frequency for 25 s, followed by the collection of four FID at the ^{31}P frequency for 5 s. A 3-s interval was required for the computer to switch data collection parameters, making the total time to collect a pair of ^{1}H and ^{31}P MR spectra equal to 36 s. To establish a steady state of magnetization before the recording of data, a 90° pulse was applied at 1 s before collecting the four FID at the ^{31}P frequency. ^{31}P MR data collection consisted of a 90° excitation pulse, 256-ms acquisition time, and 700-ms delay before the next excitation pulse; the sweep width was 5000 Hz, and there were 2048 data points per FID. The accumulated ^{31}P FID was processed by applying baseline correction for DC offsets, removing the first three data points by left shifting followed by right shifting to reestablish the phase integrity, applying an exponential apodization function corresponding to 20 Hz line broadening, Fourier transformation, zero-order phasing, and baseline straightening using a spline interpolation routine. Intracellular pH was derived from the chemical shift difference between phosphocreatine and inorganic phosphorus of ^{31}P spectrum as previously described (10). To measure the β-lactate ^{1}H MR spectrum, signals from water and lipids were suppressed using a $1\bar{3}3\bar{1} - \tau - 2\bar{6}6\bar{2} - \tau -$ acquire spin echo pulse sequence, where "1" corresponds to the first portion of a binomial pulse equal to ⅛ of the 90° pulse width; the other pulses were multiples of this; overbars indicate phase inversion (14). The prepulse delay time was 1 s, the delay time for echo formation (τ) equaled 150 ms, the sweep width was 2000 Hz, and there were 2048 data points per FID. The accumulated ^{1}H FID (16 transients) was processed by

applying baseline correction for DC offset, multiplication by an exponential apodization function corresponding to 10 Hz line broadening, Fourier transformation, and zero-order phasing. The phase angle used was determined from the setting that gives the maximal positive peak for the final postmortem β-lactate MR spectrum. To suppress non-lactate ^{1}H MR signal, a control spectrum was subtracted from all subsequent spectra collected during ischemia, yielding a series of difference spectra showing the time course of cerebral lactate accumulation after complete ischemia. To reduce the amount of noise accumulated by virtue of performing difference spectroscopy, the spectra obtained during control and after temperature alterations were obtained as the sum of 160 FID transients. The postmortem brain lactate content measured from the tissue extract was used to calibrate the intensity of the β-lactate signal measured in the final ^{1}H MRS obtained in the protocol summarized above. The validity of using the change in height of the β lactate ^{1}H MRS signal as a measure of change in brain lactate concentration, has been previously discussed (15).

Cerebral energy utilization rate was calculated using an application of MRS previously reported from this laboratory (10). Briefly, complete ischemia was induced to convert the brain into a closed system whereby all energy can only be derived from existing cellular concentrations of energy yielding substrates. The latter can be determined using interleaved *in vivo* ^{31}P and ^{1}H MR spectra to measure the rates of change in cerebral concentrations of PCr, β-NTP, and lactate after complete ischemia. The rate constants for the PCr and β-NTP decline, and lactate increases were determined by nonlinear regression fits to the experimental data, assuming first order kinetics. The kinetic order of the time course of changes in PCr and lactate (converted into equivalent glycosyl units) was assessed by making a time order analysis by plotting the log of the decrease in metabolite concentration per unit time *versus* the log of the metabolite concentration (16). The mean order of these time courses (given by the slope) equaled 1.03 ± 0.95 and 1.25 ± 0.59 for lactate and PCr, respectively (n = 11 animals). The latter was based upon the time course from 0 to 5 min and 0 to 3 min after KCl injection for lactate and PCr, respectively. This analysis supports the use of first order equations to characterize kinetic changes in lactate and PCr throughout the temperature range studied. The time course of changes in NTP follows mixed parallel and series kinetics and therefore is not amenable to this type of analysis. Preischemic concentrations of PCr and NTP were determined previously (10) and the lactate calibration was used to derive lactate concentrations before and during ischemia. The rate constants and preischemic metabolite concentrations were used to calculate the initial flux of high energy phosphate equivalents (~P) which equals cerebral energy utilization rate (d[~P]/dt, μmol of ~P/g/min) when ischemia was initiated. Regression analysis was used to determine relationships between temperature and d[~P]/dt, rate constants, and phosphorylated metabolites. Systemic data acquired during control and after temperature manipulation were compared using a nonpaired t test and regression analysis. All results are expressed as mean ± SD.

RESULTS

Systemic physiologic results at control were acquired at a brain temperature of 38.2 ± 0.2°C (Table 1). After control measurements brain temperature was adjusted with values extending from 27.6 to 41°C. Mean values of systemic physiologic variables over this temperature range did not differ from measurements at 38.2°C except for a small but significant increase in plasma glucose concentration (Table 1). Regression analysis for each systemic variable and brain temperature revealed correlation coefficients which were not significant except for heart rate (Table 1). Heart rate showed a direct correlation with brain temperature (r = 0.95, $p < 0.001$, Fig. 1).

A representative example of a ^{31}P spectra at control is shown in Figure 2 (*top panel*). To determine whether changes in brain temperature before complete ischemia altered the concentration of phosphorylated metabolites, previously determined concentrations of PCr and NTP (2.5 μmol/g of brain for each phosphorylated metabolite) from piglets of similar ages and control conditions were used as reference values for resonance peaks of control spectra acquired at 38.2°C (10). Resonance peak heights of spectra acquired after altering brain temperature were compared with control spectra to derive concentrations of PCr and NTP from the reference values. Results are plotted in the *bottom panel* of Figure 2. As brain temperature decreases there is an inverse relationship with PCr (r = −0.89, $p < 0.001$), but there were no changes in NTP concentration. Coincident with changes in PCr, there is an inverse relationship between intracellular pH and brain temperature (r = −0.77, p = 0.005).

Examples of the effects of extremes of brain temperature on changes in phosphorylated metabolites and lactate concentration during ischemia are plotted in Figure 3. The results were acquired from two experiments performed at 40 and 28.6°C. Rate constants for changes in brain metabolites were derived from the first 10 min of interleaved spectra after cardiac standstill as previously described (10). The rate of decline for both NTP and PCr, and the rate of increase in lactate concentration were 0.56 ± 0.02, 0.47 ± 0.01, and 0.076 ± 0.005 min^{-1}, respectively, at a temperature of 28.6°C, and are mark-

Table 1. *Systemic variables at control and after alteration in brain temperature*

	Control*	After temp** change	r†
Mean arterial pressure (mm Hg)	91 ± 20	94 ± 20	0.38
Heart rate (beats/m)	263 ± 38	228 ± 67	0.95‡
pHa	7.42 ± .09	7.40 ± .08	0.09
PaO_2 (kPa)	20.5 ± .8	20 ± 2.8	0.13
$PaCO_2$ (kPa)	5.3 ± .6	5.6 ± .9	−0.05
Hematocrit (%)	34.1 ± 5.4	35.4 ± 6.1	−0.34
Glucose (mMol)	6.1 ± 1.8	7.7 ± 2.3***	−0.42
Lactate (mMol)	1.4 ± 0.6	1.3 ± 0.5	0.33

* Brain temperature at control was 38.2 ± 0.2°C.

** Brain temperature extended from 27.6–41.0°C.

*** p = 0.02.

† r values represent correlation coefficients from regression analysis of each systemic variable over the range of temperature from 27.6–41°C.

‡ $p < 0.001$.

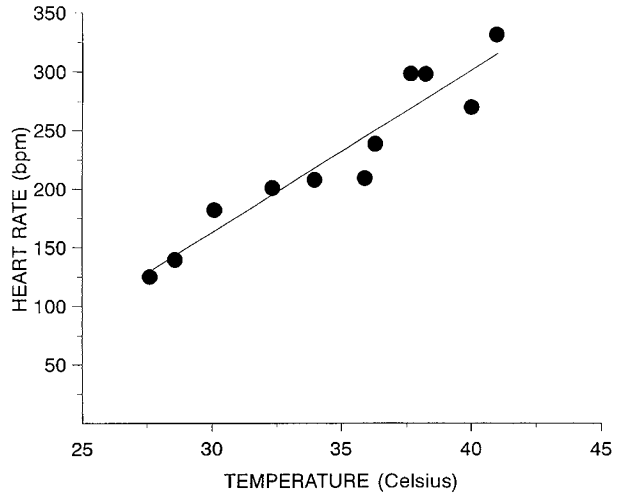

Figure 1. Scatterplot of the relationship between heart rate and brain temperature. The two variables were directly correlated ($r = 0.95, p < 0.001$) and the line of regression is described by heart rate = 13.9 temp − 255.

edly slower when compared with results at 40°C which were 3.31 ± 0.23, 1.09 ± 0.07, and 0.42 ± 0.03 min^{-1} for NTP, PCr, and lactate, respectively. Using the results from the entire range of temperatures examined, 27.6–41.0°C, regressions were performed between temperature and the calculated rate constants for phosphorylated metabolites and lactate. There were significant direct correlations between brain temperature and the rate constants for lactate, NTP, and PCr ($r = 0.94$, 0.94, and 0.86, respectively, all $p < 0.001$).

Knowledge of the rate constants, initial concentrations of phosphorylated metabolites (2.5 μmol/g for PCr and NTP) (10), and final concentration of lactate allowed calculation of cerebral energy utilization rate. There was a direct correlation between brain temperature and cerebral energy utilization rate ($r = 0.92, p < 0.001$, Fig. 4). Regressions were also performed between brain temperature and cerebral energy utilization rate for piglets studied at 8–9 d and 15–16 d. Results were similar for the two age groups and there were no differences in slopes and intercepts. To determine whether brain temperature was stable over the time course of these measurements, brain temperature was measured at 1-min intervals during the first 10 min after KCl administration for six piglets. Brain temperature fell by 0.1°C/min over this interval and was independent of the pre-KCl temperature.

DISCUSSION

The primary purpose of this investigation was to determine whether small reductions in temperature alter brain energy utilization rate, and thus provide one potential mechanism for the neuroprotection associated with modest hypothermia. This was achieved by measuring the brain energy utilization rate over a spectrum of brain temperatures at increments of 1–2°C in piglets whose age and method of instrumentation were identical to animals of our prior report (1). The results of this investigation demonstrate a linear relationship between brain energy utilization rate and brain temperature over a range of 27.6–41°C. Based upon the linear regression analysis, a 1°C reduction in brain temperature from normothermic values of 38.2°C leads to a 5.3% reduction in the brain energy utilization rate. In view of the rapid increase in brain energy utilization rate over the first month of life (10), the relationship between temperature and the brain energy utilization rate was compared in 8–9 and 15–16 d piglets, and no differences were found. However, this study was not designed to examine the influence of age, and there may be important differences between newborn and older animals in the rate of change in phosphorylated metabolites as a function of temperature which deserves further investigation.

The results of this investigation confirm some expected effects of changes in temperature under nonischemic conditions. Because brain temperature was altered by manipulating systemic temperature, the direct relationship between heart rate and brain temperature presumably reflects cardiovascular responses to temperature-associated changes in whole body O_2 consumption and metabolic rate (17). Also evident was an inverse relationship between brain temperature and phosphocreatine concentration. This reflects temperature-associated changes in brain intracellular pH which shifts the equilibrium of the creatine phosphokinase reaction toward phosphocreatine as the temperature is reduced and is consistent with previous reports (18–20).

An important assumption in deriving brain energy utilization rate was the use of an exponential function to fit serial MR results over time and derive a first order rate constant as done in previous studies (10, 21). A first order model assumes that a single rate constant governs the changes in brain energy metabolites during complete ischemia, and thus accounts for changes in glycolytic rate which occur as substrates are depleted and inhibitors of glycolysis accumulate. As stated in "Methods," a time order analysis of lactate accumulation and phosphocreatine degradation indicated that use of first order kinetics is valid. Furthermore, plots of order *versus* temperature did not reveal a significant correlation for either lactate or PCr, suggesting that the order of the reaction kinetics does not change with temperature. Thus, it is appropriate to use the same kinetic equation to analyze the data over the range of temperatures studied.

Previous reports have addressed the effect of normothermia and extreme hypothermia (<25°C) on the kinetics of intraischemic change in phosphorus metabolites in neonatal (22) and adult brain (6, 23). However, the effect of a modest reduction in temperature (2–3°C) on such variables has not been examined and cannot be extrapolated from these reports due to the absence of results at temperatures between 25 and 38°C. This issue is further clouded by the report of Chopp *et al.* (8) in which the extent of change in phosphorylated metabolites during a fixed time interval of ischemia, as determined using ^{31}P MRS in adult cats, was independent of temperature over the range of 26.8–34.6°C. What has been examined is the effect of temperature on brain metabolic rate, using $CMRo_2$ as an index of metabolic activity. All studies in neonates and adults agree that a reduction in core body or brain temperature is associated with a lowering of $CMRo_2$, but the magnitude of reduction in $CMRo_2$ for a given decrease in temperature remains controversial. In human neonates, infants, and children

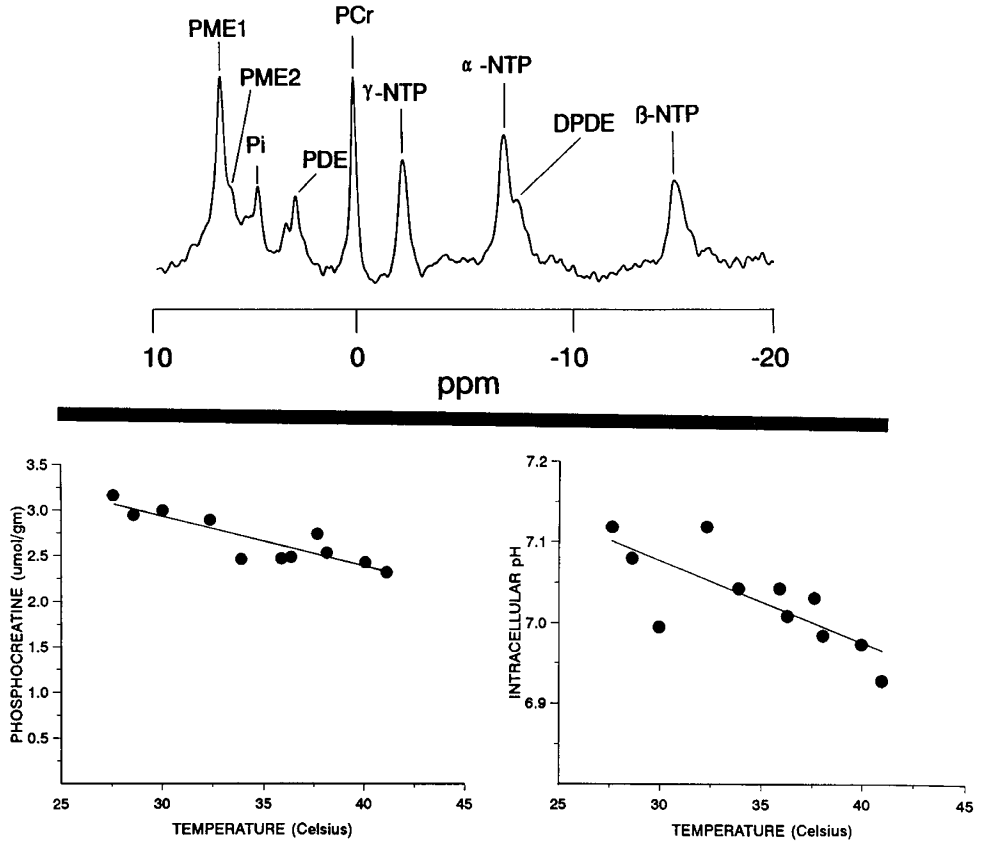

Figure 2. (*Top panel*) A representative ^{31}P MR spectrum under control condition is plotted. The horizontal axis is chemical shift in parts per million (ppm) and the vertical axis is relative peak intensity. Resonance peaks are identified as follows; phosphoethanolamine (*PME1*), phosphocholine (*PME2*), inorganic phosphorus (*Pi*), phosphodiester (*PDE*), phosphocreatine (*PCr*), and γ, α, and β peaks of nucleotide triphosphate (*NTP*), and diphosphodiesters (*DPDE*). (*Bottom panel*) The relationship between brain temperature and both PCr and intracellular pH are plotted. Control spectra were acquired at a mean brain temperature of 38.2 ± 0.2°C, and PCr was assigned a value of 2.5 μmol/g. The intracellular pH at control was 6.99 ± 0.04. Repeat ^{31}P spectra were acquired after brain temperature was altered in the range of 27.6–41°C. Lines of linear regression are included and are described by the following equations: PCr = −0.06 temp + 4.73 ($r = -0.89$, $p < 0.001$) and intracellular pH = −0.01 temp + 7.38 ($r = -0.77$, $p = 0.005$).

undergoing hypothermia and cardiopulmonary bypass, $CMRo_2$ was exponentially related to temperature reduction (24). In contrast, $CMRo_2$ of adult rats decreased linearly with temperature between 37 and 22°C (25). In adult dogs the relationship between $CMRo_2$ and brain temperature was neither linear nor exponential (26). Collectively, these studies have a paucity of results over the temperature range investigated in the present investigation (28–41°C). For example, in both human infants and adults dogs, $CMRo_2$ was determined at 37°C and after reducing the temperature by 9 or 10°C (24, 26). Only when the studies of Hagerdal *et al.* (25) and Carlsson *et al.* (27) are combined are there measurements of $CMRo_2$ in adult rats at five different temperatures over the range of 27–42°C, and they demonstrate a linear relationship between temperature and $CMRo_2$. Conclusions regarding a possible nonlinear relationship between temperature and $CMRo_2$ are based upon $CMRo_2$ measurements at temperatures less than 28°C. The results of the present investigation are in good agreement with the collective results pertaining to adult rats (25, 27). Furthermore, the magnitude of change in the brain energy utilization rate as a function of brain temperature (5.3% per 1°C) is similar to the change in $CMRo_2$ as a function of rectal temperature (5–6% per 1°C) (25).

An important issue not resolved by the current investigation is whether a 16% reduction in cerebral energy utilization which accompanies a 3°C decrease in brain temperature could contribute to the observed neuroprotection associated with modest hypothermia. Presumably, a reduction in cerebral energy utilization may offer neuroprotection by enhancing the maintenance of brain ATP concentration; reduction in ATP is the hallmark of cellular energy failure and is critical for initiating a cascade of cellular events [*e.g.* loss of ionic gradients (28), release of excitatory neurotransmitters (5), and elevated intracellular calcium concentration (29)] which can directly result in tissue damage. Results in adult rats indirectly support this contention, because a reduction in temperature of 1–3°C during hypoxia-ischemia was associated with maintenance of brain ATP concentration, whereas hypoxia-ischemia at a constant rectal temperature was associated with a 50% decrease in brain ATP concentration (30). These results suggest that the differences in ATP concentration are secondary to temperature-associated changes in energy utilization. In our earlier study (4), the brain ATP concentration determined over the last 7 min of a 15-min interval of ischemia was 43 ± 18 and 57 ± 21% of control for normothermic and modestly hypothermic treated animals, respectively ($p = 0.11$). The 16% difference in

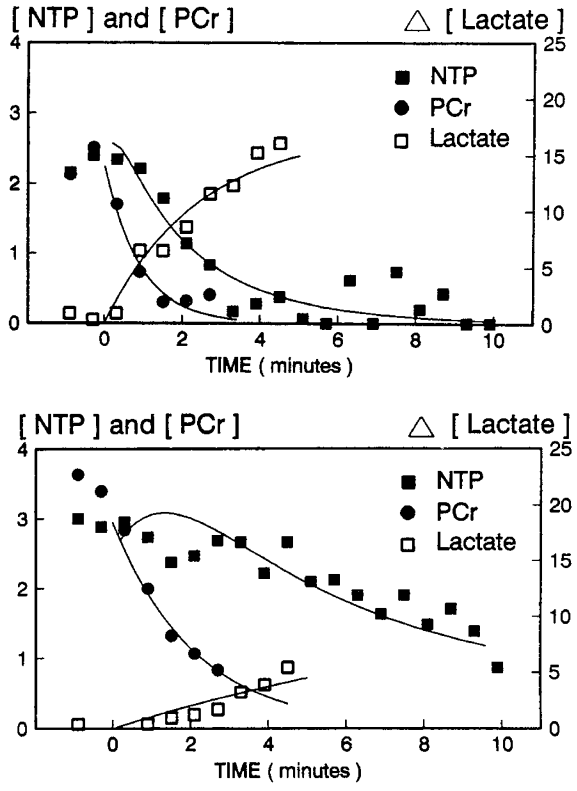

Figure 3. Time course results for NTP, PCr, and lactate concentrations (micromoles/g of brain) measured *in vivo* by ^{31}P and ^{1}H MRS for two 8-d-old piglets after cardiac arrest (time = 0). Before cardiac arrest, rectal temperature was adjusted to either 40.0°C (*top panel*) or 28.6°C (*bottom panel*). The *solid lines* show the best fits of kinetic equations which describe the time course of changes in concentrations of lactate, PCr, and NTP.

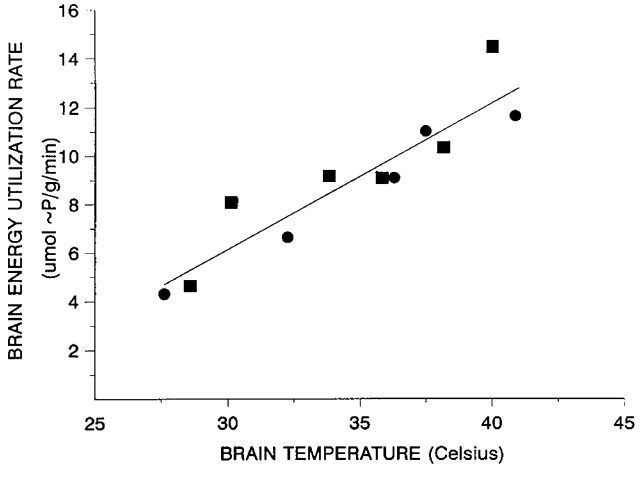

Figure 4. The relationship between brain temperature and energy utilization rate is plotted. *Squares* and *circles* represent piglets of 8–9 and 15–16 d of age, respectively. The regression line for all animals is included and is described by the equation d[~P]/dt = 0.61 temp − 12 ($r = 0.92$, $p < 0.001$).

d[~P]/dt associated with a 2.9°C reduction in temperature may contribute to the observed group ATP concentrations, given that the direction of group ATP changes (hypothermic > normothermic) is consistent with temperature-associated ATP preservation (6, 7). This assumes that differences in d[~P]/dt during the initiation of ischemia persist during the subsequent 15-min interval. It remains to be determined whether there is a threshold concentration of ATP critical for initiating ischemic cellular events, or if progressive changes in ATP concentration coincide with progressive perturbation of cellular homeostasis. For either scenario it remains possible that even a 15% reduction in d[~P]/dt could favorably alter neurologic outcome after short intervals of ischemia by preserving intraischemic brain ATP.

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