Regional Cerebral Blood Flow and Glucose Utilization during Hypothermia in Newborn Dogs

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To ascertain the effect of profound hypothermia on brain function and metabolism, newborn dogs were subjected to surface cooling during which regional cerebral blood flow (rCBF) and glucose utilization (rCGU) were measured with iodinated-C[14]antipyrine and 2-deoxy-D-[14]glucose, respectively. Puppies were anesthetized with nitrous oxide, paralyzed, and their lungs artificially ventilated to maintain arterial normoxia (P_{aO2} > 60 mmHg) and normal acid-base balance (P_{aCO2} = 35–41 mmHg; pH = 7.34–7.42). When rectal temperature was decreased from 37 to 20°C, mean arterial blood pressure (MAP) and heart rate decreased from 238 to 64 beats/min (P < 0.001). Arterial P_{aCO2} was reduced from 38 to 31 mmHg (P < 0.001) (corrected to 37°C), whereas pH was unchanged from control (7.40). The electroencephalogram slowed progressively and became isoelectric at 22–25°C. During normothermia (n = 6) blood flow to 16 component structures of the brain varied from 17 (occipital white matter) to 65 (medulla) ml·100 g⁻¹·min⁻¹, whereas during hypothermia (n = 6) blood flow was lower in all regions (P < 0.001) at remarkably uniform levels (6.3–10.3 ml·100 g⁻¹·min⁻¹). Thus, the greatest reductions (range, 16–48% of control) in CBF occurred in those structures with the highest intrinsic flows during normothermia and were proportionately less in low flow structures. Regional CGU also decreased in all brain regions (P < 0.001). Normothermic CGU (n = 5) varied from 9 (occipital white matter) to 24 (cerebellum) µmol·100 g⁻¹·min⁻¹, whereas during hypothermia rCGU (n = 5), like rCBF, was within a narrow range (0.47–0.57 µmol·100 g⁻¹·min⁻¹). The percent reductions in rCGU (range, 2.0–6.5% of control) were always greater than corresponding reductions in rCBF. The findings indicate that cerebral glucose utilization is globally depressed during profound hypothermia but that CBF remains more than adequate to support the energy needs of the immature brain. (Key words: Anesthesia; Pediatric; Brain; Cerebral blood flow; Cerebral metabolic rate of glucose; Cerebral oxygen consumption. Temperature: hypothermia.)

HYPOTHERMIC CARDIAC ARREST has become an established procedure to allow the operative correction of heart defects, especially in infants and children. The rationale for this surgical approach relates to the well-known observation that acute brain damage secondary to systemic hypoxia, hypotension, or cardiac arrest is prevented or substantially reduced by prior or concurrent hypothermia.¹⁻⁵ However, it is not known how long an infant can tolerate hypothermic circulatory arrest without sustaining ischemic brain injury. Clinical practice suggests a "safe" interval of 60–70 min.⁶⁻⁸ Furthermore, infants occasionally sustain brain damage even when the duration of cardiac arrest is well within the "therapeutic window."⁹,¹⁰ It remains to be determined whether such brain damage arises as a complication of the surgical procedure itself (e.g., postoperative hypotension, air emboli) or as a result of cerebral ischemia beyond the safety margin for any specific individual.

The present communication describes our initial investigations to clarify the protective influence of hypothermia on the immature brain subjected to total ischemia produced by cardiac arrest. In the study described here, we ascertained the regional cerebrovascular and metabolic responses of newborn dogs surface cooled to a core temperature of 20°C but without cardiac arrest. The newborn dog was chosen for study because its size allows for the continuous monitoring of systemic physiologic variables prior to and during hypothermia, and its brain maturation is comparable to that of the newborn infant at 34–36 weeks of gestational age.¹¹

Materials and Methods

The experiments described here were reviewed by the Animal Care and Use Committee of the Milton S. Hershey Medical Center and approved on August 8, 1987.

Animal Preparation

Pregnant mongrel dogs were purchased from a local breeder and housed in individual kennels. Following spontaneous vaginal delivery, the newborn puppies were kept with their bitches until the time of experimental manipulation at 2–7 days of postnatal age. In each puppy anesthesia was induced with 4% halothane. Following tracheostomy the dogs were paralyzed with pancuronium bromide (1.0 mg/kg intraperitoneally), and their lungs were mechanically ventilated with a gas mixture of 30% oxygen/70% nitrous oxide at an initial tidal volume of 1 ml/100 g body weight and rate of 50 breaths/min. Duration of halothane exposure never exceeded 5 min. After local anesthesia (1% procaine HCl), a femoral artery was cannulated with polyethylene tubing (PE 50), which was con-
Regional cerebral blood flow (rCBF) was measured by the indicator fractionation technique as originally described by Sakurada et al. Regional cerebral glucose utilization (rCGU) was measured in separate newborn dogs by a modification of the 2-deoxyglucose (2-DG) technique as originally described by Sokoloff et al. The methods to measure rCBF and rCGU are described in detail in the Appendix.

Timing of the rCBF and rCGU Experiments

The hypothermic newborn dogs undergoing measurements of rCGU were injected with 2-[14C]-DG as soon as their rectal temperatures reached 20°C. The animals then were killed 45 min later. To coordinate temporally the rCBF and rCGU experiments, those hypothermic puppies undergoing measurements of rCBF were maintained at 20–21°C for 45 min prior to the injection of iodo-[14C]-antipyrine. The animals were killed 1 min later. Control animals were maintained at 37°C for approximately the same interval (1.5–2.0 h) required to complete the blood flow and metabolic measurements in the hypothermic animals.

Statistical Analysis

Statistical analysis of the data was performed using analysis of variance (ANOVA), and unpaired and paired two-tailed Student t tests.

Results

Time Course of Hypothermia

A preliminary study was conducted in three newborn dogs to ascertain the changes in core and brain temperature during surface cooling. All three animals responded similarly; therefore, a representative animal will be described (fig. 1). During normothermia brain temperature (36.1°C) was nearly identical to rectal temperature (36.7°C). Brain temperature remained 1–2°C above rectal temperature during profound hypothermia. Mean arterial blood pressure increased transiently at the onset of cooling, decreasing thereafter to a low of 34 mmHg by 2 h. In contrast, heart rate followed closely the changes in rectal temperature, even to the occurrence of a slight increase upon rewwarming from 18 to 20°C. The electroencephalogram (EEG) slowed progressively and became isoelectric between 22 and 25°C.
and subcortical white matter the lowest flow values.\textsuperscript{15,16} CBF decreased significantly in all brain regions during hypothermia to remarkably uniform levels, ranging from 8.3 to 10.3 ml·100 g\textsuperscript{−1}·min\textsuperscript{−1}. Thus, the greatest reductions in CBF (range, 16–48% of control) occurred in those structures with the highest intrinsic flows during normothermia and were proportionately less in low flow structures.

**Regional Cerebral Glucose Utilization**

As with CBF, CGU was variable among individual structures in newborn dog brain (fig. 3). Control values ranged from nine (frontal and occipital white matter) to 24 (cerebellum) μmol·100 g\textsuperscript{−1}·min\textsuperscript{−1}. Glucose utilization tended to be higher in hindbrain (brain stem and cerebellum) than in forebrain structures (cerebral gray matter, subcortical white matter, and diencephalon).\textsuperscript{11,16} During hypothermia CGU decreased to low and relatively uniform rates, ranging from 0.47 to 0.57 μmol·100 g\textsuperscript{−1}·min\textsuperscript{−1}. The reductions in rCGU (range, 2.0–6.5% of control) were on the average eightfold to tenfold greater than the corresponding reductions in rCBF.

**Flow: Metabolism Couple**

As is known to exist in adult animals and humans,\textsuperscript{14,17} a direct correlation between rCBF and rCGU was present during normothermia in newborn dogs. This relationship can be defined in terms of “relative flow,” which is represented by CBF·CGU\textsuperscript{−1} (ml/μmol) or ml of blood flow required for every μmol of glucose consumed by component structures of brain (fig. 4). During normothermia the flow:metabolism couple was relatively constant (1.8–2.8 ml/μmol; \( P = 0.06 \)) among the four major regions of brain. Relative flow increased in all regions during hypothermia (\( P < 0.001 \)) but to a lesser extent in white matter compared with forebrain gray matter, brain stem, and cerebellum (\( P = 0.01 \)).

### Table 1. Systemic Physiologic Variables during Normothermia and Hypothermia in Newborn Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normothermia (37° C)</th>
<th>Hypothermia (20° C)</th>
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<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>238 ± 8</td>
<td>64.0 ± 1.0*</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>75.0 ± 5.0</td>
<td>47.0 ± 2.0*</td>
</tr>
<tr>
<td>( pH_a )</td>
<td>7.40 ± 0.01</td>
<td>7.40 ± 0.01</td>
</tr>
<tr>
<td>( P_{aCO_2} ) (mmHg)</td>
<td>37.9 ± 1.0</td>
<td>30.5 ± 1.0*</td>
</tr>
<tr>
<td>( P_{aO_2} ) (mmHg)</td>
<td>93.0 ± 9.4</td>
<td>157 ± 14†</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>184 ± 11</td>
<td>197 ± 15</td>
</tr>
<tr>
<td>Blood lactate (mM)</td>
<td>1.97 ± 0.28</td>
<td>2.30 ± 0.28</td>
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Values represent the mean ± SEM for 11 normothermic and 11 hypothermic newborn dogs.

*\( P < 0.001 \) versus normothermia (two-tailed unpaired t test).
†\( P < 0.01 \) versus normothermia (two-tailed unpaired t test).
**FIG. 2.** Regional cerebral blood flow (rCBF) during normothermia (37° C) and hypothermia (20° C) in newborn dogs. Bars represent the means of six animals; vertical lines denote ± 1 SE. All hypothermic values were significantly different from control (normothermia) with $P < 0.001$ (unpaired t test). Abbreviations: FC = frontal cortex; PC = parietal cortex; OC = occipital cortex; Hippo = hippocampus; FW = frontal white; PW = parietal white; OW = occipital white; CC = corpus callosum; CN = caudate nucleus; Th = thalamus; Hyth = hypothalamus; Coll = colliculi; MO = medulla oblongata; CH = cerebellar hemisphere; CV = cerebellar vermis.

**Discussion**

The present findings indicate substantial reductions in rCBF and in rCGU during profound hypothermia in newborn dogs. Although blood flow and metabolism were not measured at intermediate temperatures between 37 and 20° C, the percent reductions in CBF and CGU per degree change in temperature can be calculated, assuming a linear relationship between the variables. 18-20 CBF to cerebral cortical gray matter (average of three regions) decreased by 2.2 ml·100 g$^{-1}$·min$^{-1}$·° C$^{-1}$ or by 4.8%·° C$^{-1}$, whereas CBF to subcortical white matter decreased by 0.8 ml·100 g$^{-1}$·min$^{-1}$·° C$^{-1}$ or by 3.5%·° C$^{-1}$. Other structures exhibited varying percent changes in blood flow proportionate to their intrinsic flows at normothermia. The percent change in CGU was always greater than that of CBF for all individual structures. In cerebral cortex CGU decreased by 1.0 µmol·100 g$^{-1}$·min$^{-1}$·° C$^{-1}$ or by 5.6%·° C$^{-1}$. CGU in subcortical white matter was reduced by 0.5 ml·100 g$^{-1}$·min$^{-1}$·° C$^{-1}$ or by 5.2%·° C$^{-1}$. The percent changes·° C$^{-1}$ in CGU are similar to those (approximately 5%) reported for the cerebral metabolic rate for oxygen (CMRO$_2$) and the cerebral metabolic rate for glucose (CMR$_{glucose}$) in adult animals. 19,20

Although research studies pertaining to hypothermia in immature animals have been limited, a rather extensive literature does exist regarding the cerebrovascular and metabolic responses of adult animals to hypothermia. Five separately published investigations in adult dogs demonstrate that hypothermia produces progressive reductions in both CBF and CMRO$_2$ (fig. 5). 19,21-24 At core temperatures ranging from 28 to 37° C, the percent changes in CBF and CMRO$_2$ are comparable, whereas at temperatures below 28° C the percent reduction in CMRO$_2$ is greater than that of CBF. The data of Steen et al. 24 are

**FIG. 3.** Cerebral glucose utilization (rCGU) during normothermia (37° C) and hypothermia (20° C) in newborn dogs. Bars represent the means of five animals; vertical lines denote ± 1 SE. All hypothermic values were significantly different from control (normothermia) with $P < 0.001$ (unpaired t test). Abbreviations: FC = frontal cortex; PC = parietal cortex; OC = occipital cortex; Hippo = hippocampus; FW = frontal white; PW = parietal white; OW = occipital white; CC = corpus callosum; CN = caudate nucleus; Th = thalamus; Hyth = hypothalamus; Coll = colliculi; MO = medulla oblongata; CH = cerebellar hemisphere; CV = cerebellar vermis.
Fig. 4. Relationship between cerebral blood flow (CBF) and cerebral glucose utilization (CGU) or "relative flow" in four major regions of newborn dog brain during normothermia and hypothermia. Relative flow was calculated according to the equation: CBF/CGU^1 (ml/µmol). Bars represent the means of 2-7 structures; vertical lines denote ± 1 SE. Forebrain gray matter included frontal cortex, parietal cortex, occipital cortex, hippocampus, caudate nucleus, thalamus, and hypothalamus; forebrain white matter included frontal white, parietal white, occipital white, and corpus callosum; brain stem included colliculi, pons, and medulla; cerebellum included cerebellar hemisphere and vermis. Interregional differences in relative flow during normothermia and hypothermia were statistically analyzed by ANOVA with Dunnet t correction.

especially relevant to the present investigation to the extent that CBF and CMRO_2 in adult dogs cooled to 18°C were 45% and 9% of the normothermic values, respectively. These percent reductions are remarkably similar to those for CBF and CGU observed in newborn puppies at 20°C. Unfortunately, CMR_glucose was not measured in the experiments of Steen et al., but measured in other studies, the percent changes were equal to or minimally less than that of CMRO_2, indicating that alternate organic substrates do not contribute to oxidative metabolism during hypothermia. Thus, despite the lower CBF and CMRO_2 of the newborn dog relative to the adult at normothermia, the cerebrovascular and metabolic responses of the immature brain to hypothermia are near identical to that of its adult counterpart.

Three factors potentially contributed to the decreases in rCBF during hypothermia, specifically, systemic hypotension, hypocapnia, and the hypothermia per se. In this regard, Young et al. have shown in normothermic newborn dogs that arterial hypotension does not adversely influence CBF until MABP falls to or below 20 mmHg. In the present study, MABP never fell below 38 mmHg during hypothermia. Hypocapnia in the newborn dog to PacO_2 of 31 mmHg (present study) would be expected to lower blood flow to cerebral cortex, subcortical white matter, and brain stem by no more than 25%, 4%, and 36%, respectively, compared with the observed reductions of 82%, 65%, and 83% of normothermic values, respectively. Thus, the widespread reductions in CBF during hypothermia were primarily the direct consequence of a global curtailment in oxidative metabolism as reflected by the decreases in CGU.

Of the 16 newborn dog brain regions analyzed during hypothermia, all but three white matter structures exhibited a close correspondence between CBF and CGU (fig. 4). Even in white matter, the percent reduction in CGU was greater than that of CBF, although not to the extent noted in other regions. It follows that no structure would be injured by hypothermia as long as systemic blood pressure and oxygenation are maintained. Of necessity, a mismatch between blood flow and metabolism will occur during hypothermic cardiac arrest because in the absence of cerebral perfusion, metabolism must continue at a basal rate to maintain ion gradients. Given the proposed vulnerability of immature white matter to systemic hypoxia and/or hypotension, it is reasonable to assume that these regions of brain might also be the least resistant to the damaging effects of cerebral ischemia produced by prolonged hypothermic circulatory arrest. Neuropathologic studies are now in progress to confirm or deny this speculation.

Fig. 5. Cerebral blood flow (CBF) and the cerebral metabolic rate for oxygen (CMRO_2) during hypothermia in adult dogs. Values represent the means as percent of values obtained during normothermia; connecting vertical lines denote simultaneous measurements in the same animals. The newborn dog data are those of the present study for cerebral cortex (average of frontal, parietal, and occipital); the open triangle represents cerebral glucose utilization rather than CMRO_2. Data for adult dogs are derived from Michenfelder and Theye, Pesna et al., Lafferty et al., Astrup et al., and Steen et al.
Appendix

MEASUREMENT OF REGIONAL CEREBRAL BLOOD FLOW

Regional cerebral blood flow (rCBF) was measured by the indicator fractionation technique as originally described by Sakurada et al. Specifically, 50 μCi of iodo-[14C]-antipyrine (New England Nuclear) diluted in 3.8 ml 1N NaCl was constantly infused intravenously over 1 min via an infusion pump, and 0.05 ml arterial blood samples were collected into heparinized capillary tubes at 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 s during the isotopic infusion. Exactly at 1 min, pentobarbital (>100 mg/kg) in a solution containing 50 mg/ml was rapidly injected intravenously to produce cardiac arrest within 5 s. Thereafter, the brain was rapidly removed from its skull and sectioned fresh in the coronal plane. Specimens of tissue (20–40 mg) were dissected from 16 distinct regions of brain and placed in preweighed scintillation vials. Reweighing of the vials ascertained the tissue wet weight, to which was added 1.0 ml Soluene-350. The whole blood samples were immediately centrifuged for 5 min at 3,000 Xg, and a precise aliquot (0.01 ml) of the separated plasma was transferred to scintillation vials to which was added 1.0 ml of Soluene-350. Additional aliquots (0.01 ml) of plasma collected at 0, 15, 30, and 45 min were analyzed for glucose concentration on a microglucose analyzer (Beckman Glucostat). The vials containing the brain and blood specimens were agitated overnight in a mechanical shaker, following which the extracts were combined with 10 ml of Dimilune-36. The solutions then were counted in a liquid scintillation spectrometer with appropriate blanks and standards.

Several rate constants are incorporated into the operational equation of Sokoloff et al. These rate constants include: \( K_i = 2-DG \) transport into brain; \( K_e = 2-DG \) efflux from brain into blood; and \( K_s = \) the tissue phosphorylation of 2-DG to 2-DG-6-phosphate (2-DG-6-P). Use of these rate constants, which differ from those of glucose, is required to estimate the amount of unmetabolized 2-DG present in brain at the time of killing of the animal. Without use of the constants, calculated rCGU would overestimate the true value because isotopic counting of brain tissue specimens (or autoradiography) includes both 2-DG and 2-DG-6-P activities. The 2-DG rate constants have not been determined for newborn dog brain, but it is reasonable to assume that they differ substantially from those of adult rat brain. Furthermore, it is likely that the rate constants change during hypothermia, given the anticipated lower rate of cerebral metabolism under this condition. Accordingly, we devised an experiment to determine the extent to which radioactive 2-DG in brain was metabolized to 2-DG-6-P under hypothermic conditions. Knowledge of this relationship circumvents the need to use the 2-DG rate constants in the Sokoloff equation.

Four hypothermic (20℃) newborn dogs were surgically prepared and injected with [2-14C]-DG as described above. Between 35 and 45 min of isotope circulation, the scalp of the animal was incised in the longitudinal plane under local anesthesia. A bottomless plastic funnel then was positioned over the exposed skull and sutured in place. Immediately following the collection of the 45-
min blood sample, the animal received an iv infusion of pentobarbital (15–20 mg/kg) during which time transcalvarial freezing of the entire brain was accomplished by irrigating the scalp funnel with liquid nitrogen. A lethal dose of pentobarbital then was administered, the animal decapitated, and the isolated head stored at −70°C. Thereafter, the brain was bisected in the coronal plane under liquid nitrogen with a cooled hacksaw, and tissue samples (50–60 mg) obtained from the cerebral cortex and subcortical white matter at −20°C. Neutralized (pH = 7.0) perchloric acid (PCA) extracts of each tissue sample then were prepared as previously described.

An 0.3 ml aliquot of each brain extract diluted in 0.7 ml of distilled water was added to 10 ml of Dimilume-36 and the solution counted in the scintillation spectrometer, while another aliquot was assayed for glucose concentration using an enzymatic, fluorometric technique. The remaining portion of the extract (0.5 ml) was passed over an ion-exchange column form form (Biorad Econocolumn) and eluted with 3 ml water. The eluent, containing only neutral compounds, specifically 2-14C-DG and glucose, then was isotopically counted. From these procedures, the amount of 2-14C-DG-6-P was determined by subtracting the [14C] in the eluent from the [14C] in the original tissue extract, using the appropriate dilution factors. From the data it was determined that the percent 2-14C-DG metabolized to 2-14C-DG-6-P during hypothermia was 3.5% and 4.8% for gray and white matter, respectively. These ratios then were substituted for those components of the original Sokoloff equation containing K1, K2, and K3.

A standard curve previously has been developed that relates the percent of 2-14C-DG metabolized to 2-14C-DG-6-P in either gray or white matter in control, warm, normothermic newborn dogs at plasma glucose concentrations ranging from 0.6 to 13.8 mmol/L. K4, (dephosphorylation of 2-DG-6-P by glucose-6-phosphatase) was assumed to be zero.

The lumped constant (LC) is a value that defines a composite of six individual constants representing the following: 1) the ratio of the distribution volumes (λ) for 2-DG and glucose in brain, 2) the relative activity of brain glucose-6-phosphatase (φ), and 3) the apparent Michaelis-Menten constants (Km and Vm) and maximal velocities (Vm and Vm) of brain hexokinase for glucose and 2-DG, respectively.

The LC already has been determined to be 0.42 for nitrous oxide anesthetized and paralyzed newborn dogs, a value similar to that (0.46) reported for unanesthetized adult rats. The LC was not measured directly during hypothermia; rather, the LC was ascertained in the four animals that underwent the determination of the 2-14C-DG-6-P · total [14C]−1 ratios described above. Cerebral cortical gray and subcortical white matter/plasma glucose ratios were obtained in each animal, which then were applied to a nomogram published by Partridge et al. for determination of individual LC in adult rat brain. Therefore, a total of eight values (range, 0.48−0.53) provided an average LC of 0.48 ± 0.01 (SEM).

As mentioned previously, we used a modification of the original Sokoloff equation to measure rCGU in newborn dog brain by substituting the measured values of the percent 2-DG metabolized for the rate constants. Thus:

\[
\text{CGU (μmol} \cdot \text{100 g}^{-1} \cdot \text{min}^{-1}) = \frac{\text{Gr}^\text{*}(T) \times (2\text{-[14C]-DG-6-P} \cdot \text{Total [14C]}^{-1}) \times 100}{(\text{LC}) \int_0^\infty \frac{\text{Cp}^\text{*}}{\text{Cp}} \, dt}
\]

where:

- Gr^* (T) = the concentration of the tracer in brain at time T (dpm/g);
- Cp^* = the concentration of the tracer in plasma (dpm/ml);
- Cp = the concentration of glucose in plasma (μmol/ml);
- LC = lumped constant.

References

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