Differences in Intraischemic Temperature Influence Neurological Outcome After Deep Hypothermic Circulatory Arrest in Newborn Dogs

Dennis J. Mujise, MD; Javad Towfighi, MD; Daniel F. Heitjan, PhD; Robert C. Vannucci, MD

Background and Purpose Hypothermia to core temperatures ranging from 16°C to 24°C has become an established procedure to extend the "safe" interval of cardiac arrest during open heart surgery in human infants. The present experiment was designed to ascertain whether differences in core (rectal) temperature during hypothermic circulatory arrest influence the presence and extent of ischemic brain damage.

Methods Newborn dogs (postnatal age, 3 to 5 days) were anesthetized with halothane (4% induction; 0.5% maintenance), intubated, paralyzed, and artificially ventilated with 70% nitrous oxide/30% oxygen. Thereafter, the dogs were surface cooled with ice packs to either 16°C (n=6), 20°C (n=8), or 24°C (n=6). The dogs then were subjected to circulatory arrest for 1.75 hours by the intravenous injection of KCl, following which they were resuscitated with intravenous NaHCO3 and epinephrine, artificial ventilation, and closed chest cardiac massage. Those dogs that survived for 8 hours of recovery (n=16) underwent neurobehavioral examination followed by perfusion-fixation of their brains for pathological analysis.

Results All newborn dogs were successfully resuscitated after 1.75 hours of cardiac arrest, rewarmed to 37°C, and ultimately weaned from anesthesia and ventilatory support. Four dogs sustained secondary systemic complications with death at 4 to 7 hours. All surviving dogs remained stable, with systemic blood pressure, heart rate, arterial oxygen, and acid-base balance within the normal, normothermic range. Of the 16 surviving dogs, all except 1 showed histological evidence of brain damage at 8 hours of recovery. Morphometric analysis of the number of necrotic neurons in the vulnerable gray matter structures showed the greatest damage to cerebral cortex at 24°C and the least damage to this structure at 16°C by either regression analysis (r=.62; P=.01) or a repeated-measures model (P=.008). The extent of damage to the caudate nucleus was similar in the three temperature groups, while damage to the amygdaloid nucleus was greater at 24°C compared with 20°C but with no difference in the severity of damage between 20°C and 16°C. A close correlation existed between neurobehavioral deficits in the surviving dogs and the severity of damage to the cerebral cortex (r=.72; P=.001).

Conclusions The findings indicate that differences in intraischemic core temperature during deep hypothermic circulatory arrest influence the severity of damage to the cerebral cortex of newborn dogs. Specifically, the lower the temperature below 24°C, the more protected the structure from ischemic injury. Furthermore, the greater the cortical damage, the more severe the neurobehavioral deficits. Such was not the case for the amygdaloid nucleus and especially for the caudate nucleus. Accordingly, differences in core temperature, even at very low levels, appear critical for optimal protection of the newborn brain during hypothermic circulatory arrest. (Stroke. 1994;25:1433-1442.)

Key Words • heart arrest • hypothermia • newborn • dogs

See Editorial Comment, page 1442

Hypothermia to a core temperature of 16°C to 24°C combined with either complete circulatory arrest or low circulation ("low flow state"), accomplished with cardiopulmonary bypass, is the established procedure for the surgical correction of congenital heart defects.1-3 Investigations in infants and children have documented the efficacy and safety of hypothermic circulatory arrest with arrest times varying from 75 to 90 minutes.4-6 What presently is not established is the optimal core temperature during ischemia due to complete arrest in newborns.7-10 Such core temperatures allow complete recovery of all organs, including the brain, without untoward postoperative complications. Too warm a core temperature shortens the safe interval for surgical repair during circulatory arrest; too cold a temperature increases the risk of cellular injury arising from hypothermia per se as well as greater difficulty in reestablishing cardiac activity. In both adult and immature animals, minor reductions (2°C to 4°C) in core or brain temperature from normothermia dramatically improve neuropathologic outcome after complete or incomplete cerebral ischemia.7-10 Similar experiments have not been accomplished at the much lower temperatures required to prolong the cerebral ischemia produced by circulatory arrest necessary to allow adequate time for a surgical procedure. Accordingly, we have conducted experiments in a newborn dog model of hypothermic circulatory arrest to determine the effect of small variations in...
core temperature between 16°C and 24°C on neurological outcome and the presence and extent of ischemic brain damage.

Materials and Methods

Animal Preparation

Pregnant mongrel dogs were purchased from a local breeder and housed in individual kennels. After spontaneous vaginal delivery, the newborn puppies were kept with their bitches until time of experimental manipulation at 3 to 5 days of postnatal age. The newborn dogs were anesthetized with halothane (4% induction; 1% to 1.5% maintenance), following which they underwent endotracheal intubation and muscular paralysis with succinylcholine (15 mg/kg body wt). Thereafter, the animals were artificially ventilated with a gas mixture of 0.5% halothane/70% nitrous oxide/29.5% oxygen. Under local anesthesia (1% procaine HCl), a femoral artery was cannulated with polyethylene tubing (PE-50), which was connected via a Statham transducer (Gould, Inc) to a dynographic recorder (model R 711; Beckman Instruments, Inc) to monitor systemic heart rate and blood pressure. A side arm of the catheter allowed for intermittent collection (0.2 mL) of arterial blood for analysis of oxygen and acid-base status on a blood gas microanalyzer (model ABL30; Radiometer America, Inc). Oxygen and acid-base balance were maintained within a narrow range (PaCO$_2$, 35 to 42 mm Hg; pH, 7.35 to 7.42; PaO$_2$, >60 mm Hg) by small adjustments of tidal volume and ventilatory rate. PaCO$_2$, PaO$_2$, and pH were measured at 37°C during both normothermia and hypothermia. Plasma glucose was measured intermittently on a glucose microanalyzer (Glu- costat; Beckman Instruments). A femoral vein also was cannulated as a route for injections of drugs and infusion of glucose. Body temperature was monitored by means of a rectal probe attached to a servo-controlled heating lamp and was initially maintained at 37.0±0.2°C.

Induction of Hypothermia

Once steady-state arterial normoxia and acid-base balance were achieved, the newborn dogs were gently positioned prone on a plastic bag containing crushed ice, which extended around the sides of the animal. Ice was not applied to the midline portion of the back or to the head. Rectal temperature was continuously monitored during the cooling period, and the ice packs were removed when the temperature reached either 16°C, 20°C, or 24°C. Thereafter, the temperature was maintained within a narrow predetermined level (±0.5°C) either by reapplying the ice packs near the sides of the animal or by automatic activation of the heating lamp. No adjustments in tidal volume or ventilatory rate were made during or after the cooling process. The interval of cooling necessary to lower brain temperature between 16°C and the desired hypothermic level ranged from 60 to 90 minutes. A previous investigation demonstrated that once a stable level of systemic hypothermia is achieved for 30 minutes, brain temperature remains 1°C to 2°C above rectal temperature.11

Circulatory Arrest and Resuscitation

Once hypothermia to the predetermined level was achieved for 30 minutes, the heart of each newborn dog was arrested with the injection of KCl (25 mmol/L IV) in a small volume (2 to 3 mL). Artificial ventilation was discontinued simultaneously with the cardiac arrest. Complete circulatory arrest was verified by the absence of spontaneous heart rate and systemic blood pressure monitored on the dynograph. All animals remained asystolic for 1.75 hours. Thereafter, the animals were resuscitated by (1) resumption of artificial ventilation with 70% nitrous oxide/30% oxygen at 1.5 times the prearrest ventilatory rate without change in tidal volume; (2) body warming with the heating lamp; (3) injection of NaHCO$_3$ (2.0 mmol/kg IV); (4) injection of 1/10,000 epinephrine (0.02 mg/kg IV); and (5) closed chest cardiac massage at a rate of 60 compressions per minute. These maneuvers resulted in spontaneous heart action in all animals, with progressively increasing heart rate and systemic blood pressure thereafter. Plasma glucose, hemato crit, arterial oxygen, and acid-base status were determined at 15 minutes and 1, 2, 4, and 8 hours of recovery. Hypoglycemia (blood glucose <90 mg/dL) was treated with a constant but variable infusion of 10% glucose in water. Metabolic acidemia (pH <7.25) was treated with bolus injections of NaHCO$_3$ (10 mmol/kg). Hypocapnia (PaCO$_2$ <25 mm Hg) was corrected by adjustments in ventilatory rate or tidal volume. Once rewarming to 37°C was complete, the animals were weaned from the ventilator as the effect of the succinylcholine subsided. Once a stable, spontaneous respiratory pattern was achieved, the endotracheal tube was removed. Thereafter, the puppies were placed in an infant warmer with an environmental temperature of 34°C and continued to receive an intravenous infusion of 10% glucose in water to maintain optimal glucose and fluid homeostasis. The animals were maintained for 8 hours, at which time they underwent perfusion-fixation of their brains (see "Neuropathologic Methods").

Four anesthetized, paralyzed, and ventilated newborn dogs served as controls. They were either maintained at 37°C (1 dog), rendered hypothermic to 20°C for 1.75 hours (1 dog), or rendered hypothermic to 16°C for 1.75 hours (2 dogs), followed thereafter by warming to 37°C for an additional 8 or more hours. These animals did not undergo circulatory arrest.

Neurological Assessment

Immediately before death at 8 hours, each newborn puppy underwent an assessment of functional impairment as reflected in alterations of consciousness, tone, and spontaneous activity.12 Righting, sucking, and nociceptive withdrawal reflexes as well as tone and equilibrium were assessed and individually graded from 0 (absent) to 3 (active) for an optimal total score of 15. The presence, frequency, and character of convulsive activity also were recorded.

Neuropathologic Methods

At 8 hours of recovery from hypothermic circulatory arrest, each newborn dog was deeply anesthetized with pentobarbital (50 mg/kg IV), at which time they underwent perfusion-fixation of their brains with formaldehyde/glacial acetic acid/absolute methanol (FAM; 1:11:8) for 30 minutes. Cerebral perfusion was performed by opening the abdomen and chest and inserting a metal cannula through the apex of the left ventricle into the proximal ascending aorta. After clamping the cannula in place, the right atrium was opened and heparinized saline infused through the ascending aorta at room temperature for approximately 30 seconds or until the return to the right atrium was clear. Meanwhile, the abdominal aorta was clamped. The saline infusion was followed by a solution of FAM for an additional 30 minutes. The pressure of perfusion was adjusted to that of the expected normal mean arterial blood pressure (MAPB) for each animal. At the completion of the perfusion-fixation, the scalp was removed and the brain together with the entire head was fixed an additional 24 hours in FAM, following which it was removed entirely from the skull and fixed in FAM for 1 week. Each FAM-fixed brain was cut coronally in 2- to 3-mm-thick slices, which were individually embedded in paraffin. Sections (6 μm thick) were stained with hematoxylin-eosin and examined blindly (J.T.) at the light microscopic level.

To ascertain quantitatively the extent of neuronal necrosis, the populations of damaged neurons per 1 mm$^2$ area of cerebral cortex, caudate nucleus, and amygdaloid nucleus were determined. These regions of brain were chosen because of their known vulnerability to ischemic damage in the newborn dog.13,14 To ensure consistency, only the damaged neurons showing eosinophilic perikarya and nuclear karyorrhexis or pyknosis were counted. For cerebral cortex, a coronal section...
at the level between the infundibulum and the mamillary bodies was selected for study. To ensure a wide sampling in each hemisphere of the section, five equidistant points between the longitudinal fissure and the rhinal fissures (A through E) were marked on the cerebral cortex (Fig 1). The cortex adjacent to these points was studied on both sides (Fig 2) by using an eyepiece grid calibrated for the ×40 objective of the microscope. To avoid counting a neuron more than once and to cover a wider area, the following rules were used: (1) the neurons touching the left and top of the grid were not counted; (2) by using a modified microscope stage mover, the slide was moved roughly perpendicular to the cortical layers, and strips of one ocular grid in width and one fifth ocular grid apart were counted; (3) depending on the thickness of the cortex, 1 to 2 mm² was covered for each cortical point; and (4) the outermost layer was not included in the study because this area is never damaged.13,14

For the study of the caudate nucleus and amygdaloid nucleus, the coronal section through the body of the anterior commissure was used for the caudate nucleus and the section at the infundibular level was used for the amygdaloid nucleus. The entirety of each of these structures within both cerebral hemispheres was counted for the number of damaged neurons per area of each structure in square meters.

**Statistical Analyses**

All experiments were conducted on the puppies cooled to 16°C and 24°C on the same day and time as littermates undergoing circulatory arrest at 20°C for 1.75 hours. The 20°C temperature and 1.75-hour arrest interval were chosen because our previous investigations characterized the presence and distribution of brain damage using these two variables during circulatory arrest in newborn dogs.13,14 Our initial hypothesis was that a temperature higher than 20°C would be associated with greater brain damage and that a temperature lower than 20°C would be associated with less brain damage.
TABLE 1. Characteristics of 20 Newborn Dogs Subjected to Hypothermic Circulatory Arrest

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Age, d</th>
<th>Weight, g</th>
<th>Resuscitation Time, min</th>
<th>Rewarming Time, min</th>
<th>Seizures</th>
<th>Behavior Score</th>
<th>Comments</th>
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<tr>
<td>16°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (10)</td>
<td>4</td>
<td>360</td>
<td>9</td>
<td>68</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2 (12)</td>
<td>5</td>
<td>532</td>
<td>5</td>
<td>84</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3 (12)</td>
<td>5</td>
<td>697</td>
<td>3</td>
<td>98</td>
<td>0</td>
<td>...</td>
<td></td>
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<tr>
<td>4 (13)</td>
<td>5</td>
<td>569</td>
<td>22</td>
<td>126</td>
<td>+</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5 (13)</td>
<td>5</td>
<td>600</td>
<td>11</td>
<td>119</td>
<td>+++</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>6 (14)</td>
<td>5</td>
<td>582</td>
<td>20</td>
<td>66</td>
<td>0</td>
<td>4</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>4.8±0.4</td>
<td>557±111</td>
<td>11±8</td>
<td>93±25</td>
<td>8.0±2.9*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20°C</td>
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<td></td>
<td></td>
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<tr>
<td>7</td>
<td>3</td>
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<td>5</td>
<td>60</td>
<td>0</td>
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<td>5</td>
<td>613</td>
<td>16</td>
<td>115</td>
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<tr>
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<td>91</td>
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<tr>
<td>10</td>
<td>4</td>
<td>312</td>
<td>6</td>
<td>67</td>
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<td>8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>588</td>
<td>18</td>
<td>54</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>4.1±1.0</td>
<td>513±130</td>
<td>11±6</td>
<td>85±26</td>
<td>7.7±2.1†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (7)</td>
<td>3</td>
<td>462</td>
<td>6</td>
<td>90</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>16 (8)</td>
<td>5</td>
<td>645</td>
<td>4</td>
<td>94</td>
<td>++</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>17 (8)</td>
<td>5</td>
<td>560</td>
<td>10</td>
<td>79</td>
<td>+++</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>18 (9)</td>
<td>3</td>
<td>379</td>
<td>2</td>
<td>90</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>19 (11)</td>
<td>3</td>
<td>510</td>
<td>20</td>
<td>72</td>
<td>0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>20 (14)</td>
<td>5</td>
<td>545</td>
<td>6</td>
<td>66</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>4.0±1.1</td>
<td>517±91</td>
<td>8.0±6.5</td>
<td>82±11</td>
<td>4.0±1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to littermates cooled to 20°C. +, ++, +++ indicate increasing severity and duration of seizures. *P<.05, †P<.01 compared with 24°C.

Statistical analysis of the data was accomplished using ANOVA for sequential data with Dunnott's correction, the paired Student's t test, and linear regression analysis. The brain damage data also were analyzed using three repeated-measures models: (1) a model assuming no temperature effect, (2) a model assuming a linear regression of damage score on temperature, and (3) a model assuming that there are temperature effects but that they are not necessarily linear. These models accounted for correlation within litters and were fit to the data to confirm the other analyses. Because the data were not balanced, ie, not all three temperatures were done in each litter, the repeated-measures models were fit by maximum likelihood.

Institutional Approval
The experiments described here were reviewed by the Animal Care and Use Committee of The Pennsylvania State University College of Medicine–The Milton S. Hershey Medical Center and approved on August 8, 1987.

Results

General Findings
A total of 20 newborn dogs from seven litters were cooled to a body temperature of either 24°C (n=6), 20°C (n=8), or 16°C (n=6) (Table 1). To minimize intralitter and interlitter variability, the puppies underwent hypothermic circulatory arrest in either pairs (n=4) or triplets (n=4); in each group 1 animal was rendered hypothermic to 20°C. All animals were successfully resuscitated and weaned from muscular paralysis and artificial ventilation within 3 to 4 hours after resuscitation, at which time they were extubated. There...
was no difference in the duration from resuscitation to weaning among the three groups. The interval from onset of cardiopulmonary resuscitation to the appearance of a spontaneous heart rate ranged from 2 to 22 minutes and was not significantly different among the three groups. The duration of rewarming required to attain normothermia also was similar among the three groups and averaged 80 to 90 minutes. Seven puppies developed seizures after extubation, consisting of tonic-clonic movements with associated gasping or apnea lasting up to 1 minute. Diazepam (0.1 mg/kg IV) promptly controlled the convulsive activity in all except 1 animal (see below). Sixteen puppies survived 8 hours of recovery and underwent perfusion-fixation for neuropathologic analysis.

Four newborn dogs died before anticipated perfusion-fixation at 8 hours of recovery (Table 1). Cause of death in 3 animals (1 from each temperature group) appeared to be an abdominal catastrophe, characterized by an abrupt decrease in hematocrit to <15 (normal, 38 to 42) and the concurrent development of a severe metabolic acidosis (pH <7.01) and a progressive systemic hypotension. None of these animals exhibited premorbid seizures. Postmortem examination revealed hemoperitoneum in all 3 without hemorrhage within other organs. The fourth puppy (24°C) exhibited a severe encephalopathy consisting of intermittent seizures, tremulousness, opisthotonos, flaccidity, gasping respirations, and ultimately apnea, leading to cardiopulmonary arrest.

Systemic physiological variables of the 16 surviving newborn dogs are presented in Table 2 and Fig 3. Relatively minor changes in pHa, Paco2, HCO3~, and plasma glucose occurred in the transition from normothermia to hypothermia; MABP decreased to 85%, 60%, and 45% of normothermia in puppies cooled to 24°C, 20°C, and 16°C, respectively. Proportionately greater percent reductions in heart rate occurred with decreasing core temperatures. After successful completion of cardiopulmonary resuscitation (ie, spontaneous heartbeat) from 1.75 hours of circulatory arrest, MABP was already within 80% of baseline normothermia by 15 minutes of recovery and was completely normalized in all three groups by 1 hour, at which interval body temperature ranged from 33°C to 37°C. Heart rate responded more slowly to rewarming but, like MABP, was not different from normothermic control rates by 1 hour. Paco2 at 15 minutes of recovery was increased compared with preischemic tensions in all three groups, whereas HCO3~ was decreased in the 24°C group and increased in the 16°C group. As a consequence, pHa was similar to normothermic control only in the 16°C group. Paco2 was within the normal range by 1 hour of recovery, but HCO3~ was decreased or unchanged (24°C), indicating a mild metabolic acidemia in all three groups. The acidemia occurred concurrent with a 25% to 35% increase in plasma glucose. In this regard, we previously showed that posts ischemic hyperglycemia is associated with lactacidemia, which presumably arises from enhanced anaerobic glycolysis by muscle.13 By 8 hours of recovery, all systemic variables returned to their prearrest, normothermic values.

Neuropathologic Findings

The brains of the hypothermic control newborn dogs did not show any gross or microscopic abnormalities, and their brains were not different from those of the normothermic control.

Neuropathologic examination of the newborn dogs at 8 hours of recovery from hypothermic circulatory arrest
for 1.75 hours showed minor degrees of choroid plexus and ventricular hemorrhage. Histologically, there was evidence of brain damage at 8 hours of recovery in all animals except 1 arrested at 16°C. As previously described,13,14 tissue injury was focused on the cerebral cortex, caudate nucleus, putamen, claustrum, and amygdaloid nucleus. In the regions of brain damage, the process of ischemic cell change of neurons was well advanced. Tissue injury took the form of selective necrosis of individual neurons that exhibited eosinophilic cytoplasm and either pyknotic or karyorrhexic nuclei (Fig 4). Within cerebral cortex, damage was localized predominantly in layers 3 and 5 plus 6 with occasional involvement of layers 2 and 4. Patchy foci of selectively damaged neurons within cortex were more prominent in posterior than in anterior sections, with an accentuation at the lateral and superior aspects of the cerebral hemispheres. Within striatum (caudate nucleus and putamen), damaged neurons were located mainly in the dorsal region, and the putamen was more severely involved than the caudate nucleus. Within the amygdaloid nucleus, the main site of damage was the lateral aspect. The hippocampus, cerebral hemispheric white matter, brain stem, and cerebellum showed no alterations except in 2 animals with severe cerebral cortical damage. One of these animals, arrested at 20°C (animal 10), had mild neuronal necrosis limited to the outer zones of the subiculum, CA1, and CA2 sectors of the hippocampus. The other animal, arrested at 24°C (animal 18), exhibited extensive neuronal necrosis involving most of the pyramidal cells in the CA2 and CA3 regions and the outer layer of the CA1 and subiculum. A few neurons in the fascia dentata were also damaged. In this animal, the thalamus and different regions of the brain stem showed scattered eosinophilic neurons in various nuclei. In thalamus the centrolateral nucleus was mainly involved, and in brain stem the vestibular nucleus, motor nucleus of the trigeminal nerve, and caudal pontine reticular nucleus were most severely involved. The cerebellum was not involved in any of the animals.

Histological grading of the extent of damage within cerebral cortex, caudate nucleus, and amygdaloid nucleus in relation to intraschismic body temperature is shown in Fig 5. For cerebral cortex, all of 4 surviving animals subjected to cardiac arrest at 24°C showed greater damage than their respective littermate controls arrested at 20°C (P<.05, paired t test). Four of 5 animals subjected to cardiac arrest at 16°C showed less brain damage than their respective littermate controls arrested at 20°C (P=.07). Regression analysis showed a significant relation between the number of damaged neurons within cerebral cortex and intraschismic body temperature between 16°C and 24°C (r=.62; P=.01). Repeated-measures modeling also showed a positive correlation of temperature on the damage score, and the overall test for any differences among the three temperature groups was significant at P=.008.

The extent of damage to the caudate nucleus was similar in the three temperature groups, with a regression analysis yielding r=.33 and P=.05. Repeated-measures analysis also revealed no significant differences among the three temperature groups. An explanation for the absence of any difference in the extent of damage to the caudate nucleus might relate to the small number of neurons damaged in any individual animal (Fig 5), thereby precluding the occurrence of any statistically significant differences in the three temperature groups. For amygdaloid nucleus, the extent of injury was greater in puppies subjected to cardiac rest at 24°C compared with their respective littermate controls arrested at 20°C (P<.05, paired t test), but there was no difference in the extent of injury between the 16°C and 20°C groups. Regression analysis of the amygdaloid nucleus damage yielded r=.37 and P>.05. Repeated-measures analysis also revealed no significant difference among the three temperature groups.

Four animals with hypothermic circulatory arrest did not survive for 8 hours of recovery and therefore were not graded histologically. Histological examination of the brains of these animals showed moderate to severe damage in 3 puppies with core temperatures of 20°C or 24°C, but no damage was seen in the brain of the puppy that had been cooled to 16°C. The distribution and severity of brain damage were similar to those that survived 8 hours (see above).

### Neurobehavioral Status

All surviving animals were subjected to a neurobehavioral examination immediately before death at 8 hours of recovery. A spectrum of neurobehavioral deficits was seen that correlated closely with the severity of
FIG 4. Photomicrographs of cerebral cortex from a newborn dog surviving 8 hours of hypothermic circulatory arrest (20°C) showing damaged neurons (arrows) with nuclear pyknosis and karyorrhexis. Damaged neurons are located primarily in layers III and V (hematoxylin-eosin; bars=20 μm (left) and 50 μm (right)).

damage to the cerebral cortex (Fig 6). Specifically, the lower the neurobehavioral score, the more extensive the brain damage. Postischemic seizures occurred in 6 of 16 puppies, especially those with the greatest neurobehavioral deficits and brain tissue injury.

Discussion

The findings of the present investigation indicate that differences in intraischemic core temperature during deep hypothermic circulatory arrest influence the severity of damage to the cerebral cortex of newborn dogs. Specifically, the lower the temperature below 24°C, the more protected the structure from ischemic injury. Furthermore, the greater the damage, the more profound the neurobehavioral deficits, a not unexpected correlation. For the amygdaloid nucleus, brain damage was greater in animals arrested at 24°C compared with 20°C but not at 20°C compared with 16°C. The caudate nucleus showed a similar severity of damage at core temperatures ranging from 16°C to 24°C.

Studies in adult animals, especially dogs, subjected to hypothermic circulatory arrest also suggest that the lower the intraischemic temperature, the better the ultimate neurological outcome. In this regard, Fisherman et al.15 using a model of hemorrhagic shock combined with hypothermia to produce circulatory arrest for 2 hours,16 found that body cooling to 15°C (esophageal temperature) combined with selective head cooling to <10°C (tympanic membrane temperature) was associated with an improved neurological outcome and less brain damage than in control dogs in which tympanic membrane temperature was maintained at 15°C. Body cooling to <10°C esophageal temperature led to severe cardiopulmonary complications (see also References 17 and 18). Of additional importance is the fact that adult dogs cooled to 15°C for 90 to 120 minutes exhibit cerebral cortical damage,16 which is only slightly greater than the severity of damage seen in newborn dogs subjected to hypothermic circulatory arrest at 16°C for 105 minutes (present study). Although the methods of quantitative neuropathologic assessment were different in the two studies, the similar severities of brain damage suggest that the extent of cerebral protection afforded by deep hypothermia is comparable in newborn and adult dogs despite major age-related differences in cerebral metabolism at normothermia (see below).19

The temperature coefficient (Q10) is a value that expresses the ratio of the rate of a reaction at one temperature (eg, 37°C) to that observed with a 10°C change in temperature (ie, 27°C). The relation between Q10 and cerebral metabolism, as reflected by the cerebral metabolic rate for oxygen (CMRO2) is controversial (for review, see Reference 20). Some investigations have shown a linear relation between CMRO2 and Q10,21,22 whereas others have shown that the relation is better defined in terms of log CMRO2.23,24 Assuming that a curtailment of cerebral oxidative metabolism is the predominant factor that protects the brain from the damaging effect of ischemia during hypothermia,20,25,26 a
Fig 5. Repeated-measures analysis of brain damage scores of cerebral cortex (top), caudate nucleus (middle), and amygdaloid nucleus (bottom) of newborn dogs subjected to circulatory arrest at 16°C, 20°C, or 24°C. Shown are box plots, which are a graphic display for comparing the center, spread, and symmetry of data across groups. The box encloses the central half of the data, extending from the first quartile (25th centile) to the third quartile (75th centile), with the white stripe at the median. The dashed lines give an indication of the lengths of the tails and the symmetry of the distribution.

Regression analyses of neuropathologic data from the present investigation suggest that newborn dogs exhibit progressively less severe ischemic damage to the cerebral cortex as body temperature is reduced from 24°C to 16°C. The finding supports the notion that there is a linear rather than an exponential relation between $Q_{10}$ and cerebral metabolism or other factors responsible for protecting the brain from ischemic damage. Indeed, collective analysis of data from investigations in adult dogs suggests that $Q_{10}$ and CMRO$_2$, and by inference a resistance to cerebral ischemia, are linearly related ($r=.95; P<.001$). Alternative processes that might precipitate or perpetuate neuronal injury during or after cerebral ischemia include the generation of oxygen free radicals, disruption of intracellular calcium homeostasis, and excitatory amino acid neurotoxicity (for review, see Reference 32). A reduction in or inhibition of these processes by hypothermia would be anticipated to increase the resistance of the brain to ischemic damage.

As in our previous neuropathologic studies, those regions of newborn dog brain most vulnerable to the ischemia produced by hypothermic circulatory arrest included cerebral cortex, caudate nucleus, and amygdaloid nucleus. First, the evolution of ischemic injury is quite rapid in the newborn dog brain such that major lesions are recognizable even at 4 hours of recovery from hypothermic circulatory arrest. Accordingly, there is little difference in the extent of the ischemic lesions between 4 and 24 hours. Second, hippocampal and brain stem injury occurs only in association with severe cerebral cortical injury, whereas the cerebellum and white matter structures are never damaged. In addition, there is no evidence of a delayed neuronal necrosis, especially of the hippocampus. The topography of the ischemic neuronal necrosis in the newborn dog mimics that of human infants subjected to hypothermic circulatory arrest, al-
though in humans damage to white matter (periventricular and subcortical leukomalacia) and to the brain stem is also encountered. The histologically apparent lesions in surviving newborn dogs appear to be the direct consequence of cerebral ischemia occurring only during the period of circulatory arrest, since postischemic complications do not occur (see present data) and cerebral blood flow is restored promptly after resuscitation. In human infants, brain lesions, when they occur, might relate to cerebral ischemia beyond the safety margin promoted by hypothemic cardiac arrest or to an intraoperative or postoperative complication of the surgical procedure (e.g., air emboli or postoperative hypotension).

The present data in newborn dogs have additional relevance to the human situation. In newborn infants, circulatory arrest is accomplished at core temperatures ranging from 15°C to 24°C and even higher, depending on the experience and judgment of individual surgical teams. The experimental findings presented here indicate that core temperatures as low as 16°C provide optimal protection during at least 60 minutes of cerebral cortex without adversely influencing other organs, especially the heart. In addition, even small increments in core (and brain) temperature during hypothermic circulatory arrest increase the likelihood for brain injury to occur, especially when the arrest interval is extended beyond any previously defined safety margin. Strict attention to preischemic, intraischemic, and postischemic body temperature should minimize the ever-present potential of ischemic brain damage.

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References

Differences in intraschemic temperature influence neurological outcome after deep hypothermic circulatory arrest in newborn dogs.
D J Muijse, J Towfighi, D F Heitjan and R C Vannucci

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