The Detrimental Effects of Prolonged Hypothermia and Rewarming in the Dog

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The authors had previously observed a deleterious cerebrovascular effect of prolonged hypothermia in primates and cats. In this study they examined the systemic as well as cerebral hemodynamic and metabolic effects of 24 hours of hypothermia in the dog. With decreases in temperature to 29 C, cardiac output (Q) and whole-body oxygen consumption (V\textsubscript{O\textsubscript{2}}) initially decreased 52 and 42 per cent, respectively. Thereafter, despite a stable temperature, both Q and V\textsubscript{O\textsubscript{2}} continued to decrease, and at 24 hours values were 7 and 28 per cent of control, respectively. Cerebral blood flow (CBF) and cerebral oxygen consumption responded similarly. At 24 hours inhomogeneous perfusion of both brain and skeletal muscle was observed. With rewarming, cardiovascular collapse with severe tissue hypoxia and acidosis developed; CBF became grossly inadequate, resulting in depletion of brain energy stores. (Key words: Anesthetic techniques: hypothermia, induced. Brain: blood flow; metabolism. Heart: cardiac output. Hypothermia. Metabolism: oxygen consumption.)

It is generally accepted that hypothermia offers an effective means of increasing tolerance of the brain for periods of decreased or absent oxygen delivery. In this sense, hypothermia is considered beneficial, and for acute applications lasting as long as a few hours this appears to be valid. The effects of prolonged hypothermia are not so readily evaluated; reported results have been inconsistent and, because of the lack of valid controls, difficult to assess. We had previously observed a detrimental effect of 48 hours of hypothermia (29 C) on a primate stroke model, which was confirmed by a subsequent 48-hour study in primates and cats. These investigations were concerned primarily with the cerebrovascular effects of prolonged hypothermia. The present study was designed to extend these observations to include evaluation of both systemic and cerebral hemodynamic and metabolic effects of prolonged hypothermia. The dog was selected for these experiments because of extensive past experience with this model, including a previous study concerned with the effects of acute hypothermia on canine whole-body and cerebral metabolism and hemodynamics.

Materials and Methods

Twelve unmedicated, fasting female mongrel dogs weighing 9–14 kg were studied in the prone position. Five dogs were used for continuous hemodynamic and metabolic studies during 24 hours of hypothermia, followed by rewarming, four dogs were used for cerebral biopsies with measurements of metabolites after 24 hours of hypothermia, and three dogs were used for cerebral carbon black infusions following 24 hours of hypothermia. Anesthesia was induced and maintained during the preparatory surgical procedure with halothane, 1 per cent, in nitrous oxide, 70 per cent, and oxygen. Succinylcholine, 40 mg, was given to facilitate intubation of the trachea with auffed endotracheal tube, and ventilation was controlled with a Harvard pump. A femoral artery was exposed and cannulated for pressure measurements and blood sampling. During the 24-hour hypothermic period, all animals received pancuronium, 0.05 mg/kg/hr, diazepam, 0.1 mg/kg/hr, and 5 per cent dextrose in 0.45 per cent saline solution, 2.5 ml/kg/hr. Additional pancuronium was given as needed to avoid any shivering, and the effectiveness was monitored with a nerve stimulator.

In five dogs used for continuous hemodynamic and metabolic studies, a pulmonary-artery catheter (5-Fr) with right atrial and pulmonary-artery ports was placed via the right superficial jugular vein. A right atrial thermostor was placed via the same route for measurements of body temperature. The dogs were heparinized, nitrous oxide was replaced by nitrogen in the inspired gases, and halothane was discontinued for at least 20 min prior to control normothermic measurements. Thereafter the temperature was decreased to 29 C by surface cooling with ice packs, and all measurements were repeated. For the subsequent 24 hours the dogs were maintained on an intensive care protocol with continuous attendance by a physician or by technicians under the supervision of a physician. Ventilation was controlled such that P\textsubscript{A\textsubscript{CO\textsubscript{2}}} values were maintained near 35 torr (temperature-corrected). The temperature was maintained near 29 C with ice packs or heat lamps as needed. Arterial blood pressure and temperature were monitored...
continuously. Mean arterial pressure (MAP), central venous pressure (CVP), heart rate (HR), temperature, and urinary output were recorded every 30 min, and EEG and ECG every two hours. Cardiac output (Q; ml/min/kg) was measured every four hours by dye dilution, using cardiogreen dye. At the same time, pulmonary arterial pressure (PAP), blood-gas values, hemoglobin, and hematocrit were measured. Blood oxygen content values were calculated from measurements of oxyhemoglobin concentration ($I_{p}-282$ CO-oximeter) and oxygen tension values (IL electrodes).9 Mixed venous blood was obtained from the pulmonary artery, and whole-body oxygen consumption ($V_{O_2}$; ml/min/kg) was calculated as the product of $Q$ and arterial–mixed venous blood oxygen content differences ($\Delta V_{O_2}$).

At the end of the 24-hour period, nitrous oxide was substituted for nitrogen in the inspired gases and the sagittal sinus was exposed, isolated and cannulated as previously described10,11 for direct measurement of cerebral blood flow (CBF). Cerebral metabolic rate for oxygen (CMR$_{O_2}$) was calculated as the product of CBF and arterial—sagittal sinus blood oxygen content differences. Brain temperature was measured with a parietal epidural thermistor and maintained equal to body temperature. Following a complete set of measurements, rewarming was initiated using heat lamps and heating blankets. In the first three dogs an epinephrine infusion was used as needed in an effort to maintain MAP above 60 torr during rewarming. This failed consistently to maintain blood pressure, and in the last two dogs blood pressure control was effected by transfusion of crossmatched blood. All measurements were repeated at $31, 33, 35$, and $37 \text{ C}$. After reaching $37 \text{ C}$, the dura overlying the cerebral hemispheres was excised and four biopsy specimens of brain were taken by a technique that deposits a sample of brain (200–400 mg) into liquid nitrogen within 1 sec. The tissue was stored at $-76 \text{ C}$ and prepared for analysis in a refrigerated box ($-25 \text{ C}$) as described by Folbergrova et al.12 Tissue extracts were analyzed by enzymatic fluorometric methods for phosphocreatine (PCr), adenosinetriphosphate (ATP), adenosinediphosphate (ADP), adenosine monophosphate (AMP), and glucose,13 lactate, and pyruvate.14 The sum of the adenine nucleotides was calculated as $\sum Ad = [ATP] + [ADP] + [AMP]$ and the energy state of the tissues was expressed as the energy charge potential: ECP = [ATP] + 0.5 [ADP]/$\sum Ad$.15

Seven additional hypothermic dogs were followed using the same intensive care protocol for 24 hours, but without pulmonary arterial catheterization or sagittal sinus cannulation. In these dogs body temperature was measured with an esophageal thermistor and a femoral vein was catheterized for drug and fluid administration. In four of these dogs, at the end of the 24 hours of hypothermia, a craniotomy was performed, the dura excised, and four brain biopsies were taken. In the third other dogs carbon black was infused at the end of the hypothermic period. For this purpose, the thorax was opened, the ascending aorta was cannulated, and the dogs were exsanguinated. Both carotid arteries were then rapidly cannulated and together perfused with 1 liter of isotonic saline solution, followed by infusion of a filtered suspension of colloidal carbon (Pelikan Werke) at a perfusion pressure of 120 torr. After the first 300 ml of this solution was infused, formalin, 10 per cent, was simultaneously infused until an additional volume of carbon black and formalin, 500 ml of each, had been given. The brains were then allowed to set for 30 min before they were removed and replaced in formalin, 10 per cent. The brain surface was thereafter inspected for filling defects (pale areas), as were coronal brain sections. Control studies in normothermic dogs had previously demonstrated that this technique of infusion resulted in homogeneous staining of the gray and white matter of the brain, as well as the extra-cerebral tissues.

All blood-gas values are reported corrected for temperature. All values are presented as means ± SEM. Control data for CBF, CMR$_{O_2}$, and cerebral metabolites from a previous study16 are presented to allow for comparison of results. The Student t test for unpaired data was used for comparison of different groups of animals. The Student t test for paired data was used for statistical comparison of data obtained from the same animals. $P < 0.05$ was regarded as significant.

Results

There was no important difference in hemodynamic or metabolic values among the five animals during active cooling or the 24 hours of maintained hypothermia. None of the measured variables changed at the end of the 24 hours when nitrous oxide was substituted for nitrogen in the inspired gases. During rewarming the three dogs given epinephrine received doses of as much as 2 g/kg/min, while the two other dogs given transfusions received 60 and 68 ml/kg. One of the epinephrine-treated dogs died at 36 C. Except for the better blood pressure control, the two dogs that received transfusions differed from the others only in that they tended to have less metabolic acidosis, accounted for
at least in part by the added buffer capacity in the transfused blood.

With cooling, whole-body oxygen consumption ($\dot{V}_{O_2}$) initially decreased to 58 per cent of control (at 29 C), while cardiac output decreased to 48 per cent of control (fig. 1). In the subsequent 24 hours, temperature remained stable, but both $\dot{V}_{O_2}$ and $Q$ continued to decrease, the former to 28 per cent of control, while $Q$ decreased to only 7 per cent of control. The ratio $Q/\dot{V}_{O_2}$ remained below control throughout the hypothermic period. During rewarming, no significant increase in $Q$ occurred, whereas $\dot{V}_{O_2}$ increased progressively, but at 37 C $\dot{V}_{O_2}$ was only 50 per cent of control.

At the end of the 24-hour hypothermic period, CBF was 15 per cent, while $CMR_{O_2}$ was 39 per cent, of previously established control values (fig. 2). With rewarming, both increased progressively, but at 37 C, CBF and $CMR_{O_2}$ were only 26 and 72 per cent of control, respectively. The ratio CBF/$CMR_{O_2}$ remained less than the corresponding control ratio throughout the rewarming period.

Among the other measured variables there were no changes in $P_{aO_2}$ or $P_{aCO_2}$ (corrected for temperature) throughout the entire study (table 1). A slight stable metabolic acidosis was present throughout the hypothermic period; with rewarming, a severe metabolic acidosis developed. Mixed venous oxygen tension ($P_{VO_2}$) and saturation ($S_{VO_2}$) decreased progressively during the hypothermic period, reflecting the decrease in $Q/\dot{V}_{O_2}$. With rewarming, $S_{VO_2}$ continued to decrease, again reflecting a further de-
crease in $Q/V_{O_2}$, while $P_{\text{O}_2}$ did not decrease further due to the rightward shift of the oxygen–hemoglobin dissociation curve produced by both the temperature and the progressive acidosis (mixed venous blood $pH$ was 7.01 ± 0.05 at 37°C). Mean arterial pressure decreased about 20 per cent with cooling, but remained relatively stable without support until rewarming. An adequate MAP could be maintained during rewarming only by the use of extreme measures (see above). The more than 12-fold increase in calculated systemic vascular resistance (SVR) after 24 hours of hypothermia reflects the extreme decrease in $Q$ in the presence of a relatively stable MAP. Pulmonary arterial pressure and CVP did not change significantly throughout the study. The EEG pattern was stable throughout the 24 hours and consistent with moderate sedation secondary to both hypothermia and diazepam.

At the end of the 24-hour hypothermic period, brain biopsies revealed that the cerebral energy stores were normal, as was brain lactate (table 2). In the four animals that were successfully rewarmed to 37°C after 24 hours of hypothermia, brain biopsy specimens were abnormal. Mean values for $P_{\text{C}}$, ATP, and ECP were significantly lower than those measured in both hypothermic and control animals. Brain ADP, AMP, lactate and L/P ratio values were not different among the dogs.

Cerebral perfusion defects were apparent in two of the three dogs infused with carbon black at the end of 24 hours of hypothermia. In these two animals, there were areas of cerebral cortex varying in size to 6–7 mm in diameter that were unstained by carbon black. The brain of the third dog was homogeneously stained and not different from that observed in control normothermic dogs. In all three dogs there was a

### Table 1. Physiologic Variables in Dogs during 24 Hours of Hypothermia Followed by Rewarming

<table>
<thead>
<tr>
<th></th>
<th>Control $(n = 5)$</th>
<th>Hypothermia $(n = 5)$</th>
<th>Rewarming $(n = 5)$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature (C)</strong></td>
<td>37.1 ± 0.1</td>
<td>29.1 ± 0.2</td>
<td>32.1 ± 0.1</td>
</tr>
<tr>
<td>$P_{\text{O}_2}$ (torr)</td>
<td>167 ± 5</td>
<td>155 ± 7</td>
<td>145 ± 11</td>
</tr>
<tr>
<td>$P_{\text{CO}_2}$ (torr)</td>
<td>34 ± 1</td>
<td>35 ± 1</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>$pH$</td>
<td>7.40 ± 0.1</td>
<td>7.32 ± 0.02*</td>
<td>7.33 ± 0.01*</td>
</tr>
<tr>
<td>Buffer base (mEq/l)</td>
<td>43 ± 1</td>
<td>38 ± 1*</td>
<td>38 ± 1*</td>
</tr>
<tr>
<td>$P_{\text{O}_2}$ (torr)</td>
<td>69 ± 2</td>
<td>53 ± 2*</td>
<td>39 ± 1*</td>
</tr>
<tr>
<td>$S_{\text{O}_2}$ (per cent)</td>
<td>88.4 ± 0.8</td>
<td>87.4 ± 0.9</td>
<td>68.5 ± 2.7*</td>
</tr>
<tr>
<td>Mean arterial pressure (torr)</td>
<td>133 ± 11</td>
<td>109 ± 10*</td>
<td>116 ± 7*</td>
</tr>
<tr>
<td>Pulmonary arterial pressure (torr)</td>
<td>15 ± 2</td>
<td>15 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Central venous pressure (torr)</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>208 ± 27</td>
<td>123 ± 4*</td>
<td>100 ± 6*</td>
</tr>
<tr>
<td>Systemic vascular resistance (dyne sec cm$^{-5}$)</td>
<td>3,036 ± 323</td>
<td>5,458 ± 911*</td>
<td>18,050 ± 2,915*</td>
</tr>
<tr>
<td>Hemoglobin (mg/100 ml)</td>
<td>16.9 ± 0.8</td>
<td>19.1 ± 1.1*</td>
<td>16.4 ± 1.5</td>
</tr>
</tbody>
</table>

* $P < 0.05$. 

**Table 2. Cerebral Metabolites in Dogs after 24 Hours of Hypothermia and after Rewarming Compared with Control Values**

<table>
<thead>
<tr>
<th></th>
<th>Control $(n = 7)$</th>
<th>Hypothermia $(n = 4)$</th>
<th>Rewarmed $(n = 4)$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phosphocreatine (umol/g)</strong></td>
<td>3.04 ± 0.17</td>
<td>3.15 ± 0.15</td>
<td>2.03 ± 0.34†</td>
</tr>
<tr>
<td><strong>ATP (umol/g)</strong></td>
<td>2.14 ± 0.10</td>
<td>2.07 ± 0.07</td>
<td>1.70 ± 0.12</td>
</tr>
<tr>
<td><strong>ADP (umol/g)</strong></td>
<td>0.299 ± 0.012</td>
<td>0.259 ± 0.003</td>
<td>0.340 ± 0.026</td>
</tr>
<tr>
<td><strong>AMP (umol/g)</strong></td>
<td>0.059 ± 0.010</td>
<td>0.030 ± 0.003</td>
<td>0.080 ± 0.024</td>
</tr>
<tr>
<td><strong>Energy Change Potential</strong></td>
<td>0.92 ± 0.01</td>
<td>0.93 ± 0.00</td>
<td>0.88± ± 0.01</td>
</tr>
<tr>
<td><strong>Glucone (umol/g)</strong></td>
<td>2.21 ± 0.18</td>
<td>3.91 ± 0.30</td>
<td>4.02 ± 0.40</td>
</tr>
<tr>
<td><strong>Lactate (umol/g)</strong></td>
<td>1.04 ± 0.14</td>
<td>1.16 ± 0.30</td>
<td>4.40 ± 0.67</td>
</tr>
<tr>
<td><strong>Lactate/Pyruvate Ratio</strong></td>
<td>17 ± 1</td>
<td>24 ± 4</td>
<td>67 ± 4</td>
</tr>
</tbody>
</table>

* Previously reported.†

$† P < 0.05$. 

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striking patchy non-perfusion in the muscle overlying the skull. In control normothermic dogs, these extracerebral tissues were always deeply and homogeneously stained with carbon black.

Discussion

This study represents a logical extension of two previous reports from this laboratory. Initially, in a primate study concerned with acute stroke, we were surprised to find that, whereas 48 hours of barbiturate therapy ameliorated the cerebral effects of permanent middle cerebral artery occlusion, 48 hours of hypothermia had a clearly deleterious effect. This was followed by an extensive study of prolonged hypothermia (48 hours) in primates and cats both with and without regional cerebral ischemic lesions. Here, an apparent species difference was observed, in that even normal cats failed to survive rewarming following hypothermia, whereas in monkeys this was only consistently lethal when a middle cerebral artery was occluded. However, even normal monkeys demonstrated a hypothermic “lesion,” in that survival was not 100 per cent, neurologic recovery was remarkably slow in survivors, cerebral perfusion was inhomogeneous prior to rewarming, and CBF did not recover acutely with rewarming, resulting in depletion of brain high-energy stores.

The present study was designed to examine both systemic and cerebral effects of prolonged hypothermia in greater detail. Dogs were selected for several reasons: an established technique for direct measurement of CBF and CMRO₂ was available, with known control values; the acute effects of hypothermia on both canine whole-body and canine cerebral hemodynamics and metabolism were known from a previous study; and finally, we wanted to determine to what extent, if any, the dog differed from either the cat or the monkey. The answer to the latter question was quickly apparent: the “lesion” of hypothermia was readily produced in the dog and was apparently fully manifested by 24 hours.

The systemic effects of hypothermia were striking. With initial cooling to 29°C the decreases in both Q and VO₂ were of the expected magnitude. Thereafter, further decreases in both continued with time despite stable body temperature, vital signs and acid–base balance. That MAP was stable can be accounted for only by a progressive and large increase in resistance (to 12-fold). The initial increase in resistance can be primarily accounted for by increased blood viscosity (an 8·C decrease should increase viscosity approximately 23 per cent).

Thereafter, resistance changes can be accounted for only by changes in the vascular beds. Since VO₂ continued to decrease and a progressive systemic metabolic acidosis did not occur (during hypothermia), we conclude that perfusion of various vascular beds ceased in a progressive and presumably random manner. This was supported by the consistently abnormal results of carbon black perfusion studies, wherein much of the muscle overlying the skull was not stained. That mixed venous blood O₂ tension (and saturation) continued to decrease with time also indicates that resistance in the perfused vascular beds was increasing, but not sufficient to result in a worsening metabolic acidosis.

With rewarming, the previously stable vital signs and acid–base balance rapidly deteriorated. The severe metabolic acidosis can be accounted for both by inadequate perfusion of those vascular beds still open and by the opening of previously closed vascular beds with an out-pouring of acidic metabolites. That VO₂ returned only to 50 per cent of control at 37°C reflects the grossly inadequate systemic perfusion.

These observations are largely but not entirely consistent with the limited number of published studies available for comparison. Fisher et al. found that cardiac output was decreased to 5 per cent of the normothermic value after 24 hours at 25°C in spontaneously ventilating dogs, but in a later study by one of the same authors, cardiac output decreased only to 68 per cent of the normothermic value after 24 hours at 25°C. In this study, however, arterial oxygen saturation was decreased to half the initial value despite controlled ventilation with 95 per cent oxygen. Azancot et al. found a decrease to 16 per cent of the normothermic value after 24 hours at 26°C in spontaneously ventilating dogs. The discrepancy in results is not easily explained by the information given in these reports. The decrease in Q that we observed occurred despite a more moderate decrease in temperature, small changes in hematocrit, and rigorous control of blood-gas values. The decreases reported by Fisher et al. and Azancot et al. can therefore not be explained solely on the basis of hemoconcentration or a deterioration of the preparation due to inadequate ventilation. Patchy perfusion during hypothermia has been found in microscopic studies of the mesentery of dogs and rats where cessation of blood flow in some vessels was seen when preparations were studied at 28 and 20°C, respectively. Systemic collapse upon rewarming has also previously been found by Blair et al. in dogs and Popovic in rats.
MacPhie et al.\textsuperscript{27} found that recovery from hypothermia increased the work done by the adrenal cortices by three to four times, and spontaneously ventilating dogs exposed to 72 hours of hypothermia (26–29 °C) failed to recover with rewarming.\textsuperscript{27}

The cerebral effects of prolonged hypothermia largely paralleled the systemic effects, but were somewhat less severe. Whereas approximately 50 per cent decreases in CBF and CMR\textsubscript{O\textsubscript{2}} are expected with an acute decrease in brain temperature to 29 °C,\textsuperscript{a} these values were decreased to 15 and 39 per cent, respectively, after 24 hours. Judging from the cerebral metabolites, CBF was marginally adequate for metabolic needs, but regions of very low or no flow were apparent in the carbon black perfusion studies. These observations were entirely consistent with the previous primate study\textsuperscript{7} wherein patchy areas of very low or absent flow were observed using an autoradiographic technique (after 48 hours of hypothermia). With rewarming, CBF increased only moderately, while CMR\textsubscript{O\textsubscript{2}} increased at the appropriate rate (Q\textsubscript{O\textsubscript{2}} max for canine brain is 2.2\textsuperscript{b}), but to only 72 per cent of control, either because of the low starting value for CMR\textsubscript{O\textsubscript{2}} at 29 °C or because of an inadequate global or regional flow. The depletion in brain energy stores following rewarming is consistent with an inadequate CBF.

It is generally agreed that hypothermia of brief duration is well tolerated by both man and animals and increases the brain's tolerance for periods of decreased or absent O\textsubscript{2} delivery. The consistently deleterious effect of prolonged hypothermia that we observed in laboratory animals is not generally recognized to occur in man. Possibly man is resistant to these effects. Species differences among laboratory animals is certainly apparent, with primates appearing to be most resistant. However, it is interesting that the once common application of prolonged hypothermia for the treatment of head injury or post-cardiac arrest has been largely abandoned. A reason for this change in practice is not evident from a review of the medical literature, but it is reasonable to assume, at the least, that no meaningful improvement in morbidity or mortality rates was apparent with hypothermia in these patient groups. It is also of interest that in Fays\textsuperscript{28} pioneer application of prolonged hypothermia, in 1940, for the treatment of patients with metastatic carcinoma, the mortality rate was not negligible. In 169 "treatments" (124 patients), the mortality rate was 11.4 per cent. Strikingly, of these, only two patients died while hypothermic, four died during rewarming, and the remaining 13 died in the first 24 hours following rewarming. We know of no published report concerning the effects of prolonged hypothermia in acute stroke; however, in one unpublished series\textsuperscript{f} of ten of 12 such patients died following rewarming after four to seven days at 28–30 °C. Based upon the above considerations and the results of our studies in animals, we question the beneficial effects of prolonged hypothermia in man, and suspect that there may well be serious undesirable consequences instead. That the latter is true for laboratory animals seems certain.

\textbf{References}

9. Theye RA: Calculation of blood O\textsubscript{2} content from optically determined Hb and HbO\textsubscript{2}. \textit{Anesthesiology} 33:653–657, 1970

\footnote{\textsuperscript{a} Q\textsubscript{O\textsubscript{2}} max for CMR\textsubscript{O\textsubscript{2}} equals the ratio of the CMR\textsubscript{O\textsubscript{2}} values obtained at two temperatures differing by 10 °C (value at high temperature/value at low temperature). This is presumed to reflect temperature effects only and will not be affected by different anesthetics.}

\footnote{\textsuperscript{b} Fields WS, Department of Neurology, University of Texas, Houston, Texas: Personal communication.}