Cerebral Autoregulation During Moderate Hypothermia in Rats

Marleen J.J. Verhaegen, MD; Michael M. Todd, MD; Bradley J. Hindman, MD; and David S. Warner, MD

**Background and Purpose:** Little is known about the effects of hypothermia on cerebral autoregulation. The present study was designed to examine cerebral blood flow responses to controlled hemorrhagic hypotension in normothermic and hypothermic rats.

**Methods:** Cortical blood flow was measured with a laser-Doppler flowmeter in halothane-anesthetized rats assigned to one of three groups: normothermic group 1 (n=8) with a pericranial temperature of ≈36.5°C or hypothermic group 2 (n=8) or group 3 (n=8) with a pericranial temperature of ≈30.5°C. In group 2, a PaCO$_2$ of ≈40 mm Hg was maintained without correction for body temperature. To evaluate the role of PaCO$_2$, in group 3 animals PaCO$_2$ was kept at ≈40 mm Hg as corrected for body temperature. In all animals, the mean arterial blood pressure was reduced by hemorrhage in increments of 10 mm Hg every 2 minutes.

**Results:** In group 1 animals, a typical autoregulatory curve was observed with cerebral blood flow first falling at or below 75% of baseline at a mean arterial pressure of 57±15 mm Hg (mean±SD). Absolute normotensive cerebral blood flow in group 2 fell to ≤75% of baseline at a mean arterial pressure of 73±21 mm Hg. In group 3, no evidence of autoregulation was seen. Cerebral blood flow reached values ≤75% of baseline at a mean arterial pressure of 82±14 mm Hg, whereas calculated cerebrovascular resistance failed to show any compensatory vasodilation as the mean arterial pressure decreased.

**Conclusions:** Different PaCO$_2$ management schemes used during hypothermia may have profound effects on cerebral blood flow and on autoregulation. If PaCO$_2$ is maintained at 40 mm Hg after correction for temperature, autoregulation is abolished. If uncorrected PaCO$_2$ is maintained at ≈40 mm Hg, some degree of autoregulation is preserved, albeit with a right-shifted “knee.”

**Key Words** • autoregulation • cerebral blood flow • hypothermia • ultrasonics • rats

In the last few years there has been a resurgence of interest in the cerebral effects of moderate hypothermia. This stems from observations showing that small reductions in brain temperature (2-6°C) dramatically protect against temporary cerebral ischemia in experimental animals.1-3 In clinical practice, therapeutic hypothermia has been limited to the operating room, largely to procedures that use cardiopulmonary bypass, and although many studies have been published concerning cerebral physiology during hypothermic cardiopulmonary bypass, remarkably little is known about basic cerebrovascular changes produced by hypothermia without bypass conditions. Cerebral blood flow (CBF) and cerebral metabolic rate are known to decrease during cooling.6-9 and responsiveness to PaCO$_2$ is preserved.10 However, we have been unable to find information regarding autoregulatory patterns during hypothermia without cardiopulmonary bypass. If hypothermia is to be more widely used, characterization of hypothermic cerebral physiology in the absence of circulatory support is required.

The current study was undertaken to examine the influence of moderate hypothermia (rectal temperature, ≈31°C) on cerebral autoregulation. This was done by using laser-Doppler flowmetry (LDF) to measure moment-to-moment cortical CBF as mean arterial pressure (MAP) was reduced by progressive controlled hemorrhage. In addition, we also evaluated the impact of different schemes for hypothermic acid–base management, since this may have a marked effect on CBF and autoregulation. These different schemes included 1) “alpha-stat” management, where PaCO$_2$ and pH are maintained at 40 mm Hg and 7.40 without correction for body temperature and 2) “pH-stat” management, where these parameters are maintained at 40 mm Hg and 7.40 after correction for temperature.

**Materials and Methods**

All aspects of this study were approved by the University of Iowa Animal Care and Use Committee. Sixteen male Sprague-Dawley rats weighing 301–365 g, with free access to food and water before surgery, were anesthetized with 4% halothane in oxygen. After tissue

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infiltration with 1% lidocaine, a tracheotomy was performed, and mechanical ventilation was started (tidal volume, \( \approx 3.3 \text{ ml} \); 40–50 breaths per minute). During surgery, anesthesia was maintained with 1–1.5% halothane in an oxygen/air mixture (FiO\(_2\), \( \approx 0.5 \)), and muscle paralysis was achieved with 0.9 mg \( d \)-tubocurarine chloride given subcutaneously. A rectal thermistor was placed, and temperature was maintained at 38±0.5°C with a warming blanket. In each rat, one femoral vein and both femoral arteries were cannulated, again after tissue infiltration with 1% lidocaine. One arterial catheter was used for continuous blood pressure monitoring and withdrawal of arterial blood samples; the other was used for withdrawal of blood during hemorrhagic hypotension. The animal was then turned prone, and the head was fixed in a stereotactic frame (David Kopf Instruments, Tujunga, Calif.). A right-sided parietal craniectomy (\( \approx 3 \times 3 \text{ mm} \)) was performed by a high-speed electric drill with the aid of a surgical microscope. The drilling site was irrigated with cool saline to avoid thermal injury to the cortex, and care was taken to leave the dura intact. A pericranial needle thermistor (model 524, Yellow Springs Instrument Co., Yellow Springs, Ohio) was then placed adjacent to the craniectomy. Cranial temperature was thereafter maintained at 36.5±0.5°C using an overhead heating lamp. Throughout the experiment, MAP was kept at \( >80 \) mm Hg (6% hastastarch given intravenously if necessary). Hematocrit and arterial blood gases were measured at regular intervals, and ventilation was adjusted to maintain P\(_{a02}\) at \( >100 \) mm Hg and P\(_{aCO2}\) between 38 and 42 mm Hg. When surgery was completed, anesthesia was maintained with an inspired halothane concentration of 0.7% (as verified with a Datex anesthetic agent monitor, model 222, Puritan Bennett Co., Wilmington, Mass.). Heparin (50 units) was given intravenously. Surgical preparation time was approximately 50 minutes.

**CBF Measurement**

CBF was monitored by LDF (model BPM 403A blood perfusion monitor, Vasomedics, St. Paul, Minn.). Values displayed by the laser-Doppler flowmeter are not absolute and will be designated CBF\(_{LDF}\). Although the Vasomedics unit reports flow as \( \text{microvascular ml/100 g/min,} \) we have chosen to report all CBF\(_{LDF}\) values here either without specific units (i.e., as arbitrary \( \text{flow units}\)) or as a percentage of the CBF\(_{LDF}\) value obtained under baseline conditions (see below).

A probe with an outer diameter of 0.8 mm was positioned over the cortex, avoiding large dural or pial blood vessels. By use of a micromanipulator, the probe was placed on the dura without indenting it. The craniectomy site was then slowly, but continuously, rinsed with saline to avoid dehydration of the dura and accumulation of blood under the probe. The CBF\(_{LDF}\) signal was recorded on paper along with MAP (model 79 polygraph, Grass Instruments Co., Quincy, Mass.). Before placing the probe on the dura, a zero-flow mark was made by use of a zero-flow signal from the instrument. Hypocarbia was then briefly induced by hyperventilation to verify that appropriate CBF changes could be detected. If necessary, the probe was repositioned until this criterion was met. Thereafter, ventilation was readjusted to obtain normocarbia. To permit the use of a heating lamp after placement of the laser-Doppler probe (since light from the heating lamp interferes with the laser-Doppler signal), each animal was shielded with aluminum foil. From this point on, even the slightest probe displacement was carefully avoided.

Animals were randomized to normothermic (group 1, \( n=8 \)) and to hypothermic (group 2, \( n=8 \)) groups. In the normothermic group, rectal temperature was maintained at 38±0.5°C while pericranial temperature was kept at 36.5±0.5°C. The animals in the hypothermic group were cooled with ice packs on the body to achieve rectal and pericranial temperatures of \( \approx 31°C \). In both groups ventilation was adjusted to maintain P\(_{aCO2}\) between 38 and 42 mm Hg, as directly measured by the blood gas analyzer at an electrode temperature of 37°C. No effort was made to correct the reported pH or P\(_{aCO2}\) for body temperature. Note that this approach to hypothermic pH/CO\(_2\) management is referred to as alpha-stat (see below).\(^{11-13}\)

Approximately 2½ hours after induction of anesthesia, the left femoral artery was opened to a saline-filled reservoir that was suspended at a predetermined height above the animal. This allowed us to set an identical stable baseline MAP of 110 mm Hg in each rat. MAP was kept at this level for 10 minutes. Thereafter, MAP was reduced stepwise in increments of \( \approx 10 \) mm Hg every 2 minutes by progressively lowering the reservoir. After a final MAP of \( \approx 20 \) mm Hg had been reached, the animal was killed by intravenous injection of 1 ml saturated KCl. After death, return of the CBF\(_{LDF}\) signal to the zero-flow mark was verified.

**Additional Studies**

**Hypothermia with pH-stat management and halothane 0.7% (group 3).** There has been extensive debate concerning the proper method of managing P\(_{aCO2}\) and pH during hypothermia.\(^{11-13}\) One method is that used above (group 2), where a P\(_{aCO2}\) of \( \approx 40 \) mm Hg and pH of \( \approx 7.40 \) are maintained without correction for temperature. This approach is referred to as alpha-stat. The other method attempts to maintain a P\(_{aCO2}\) of \( \approx 40 \) mm Hg and pH of \( \approx 7.40 \) as back-corrected to the animal’s body temperature. This has been referred to as pH-stat management. pH-stat–managed animals are always hypercarbic relative to alpha-stat–managed animals when P\(_{aCO2}\) is reported in equivalent temperatures. Since the choice of P\(_{aCO2}\) management might play an important role in autoregulation, eight additional rats were studied during hypothermia with pH-stat management. Surgical preparation was as described above. Animals in this group were cooled to \( \approx 31°C \), anesthesia was maintained with 0.7% halothane, and arterial pH and P\(_{aCO2}\) were corrected for body temperature.\(^{14}\) The temperature-corrected P\(_{aCO2}\) was kept between 38 and 42 mm Hg. Temperature-corrected P\(_{aCO2}\) was calculated by the following equation: P\(_{aCO2}\) (temperature-corrected) = P\(_{aCO2}\) (at 37°C) \( \times 10^{\Delta P\(_{aCO2}\)} \), where \( t^\circ \text{C} \) is temperature. Temperature-corrected pH was calculated using the following equation: pH (temperature-corrected) = pH (at 37°C) – (\( \Delta \text{pH}\)\( \Delta T \))/(7.4–pH [at 37°C]).

**Hypothermia with pH-stat management and halothane 0.45% (group 4).** Since hypothermia to 31°C increases the solubility of halothane in blood and brain and increases anesthetic potency by approximately 35% in rats,\(^{15}\) three hypothermic rats were anesthetized with
0.45% halothane during hypothermia to provide a dose of halothane that would be equipotent to 0.7% halothane at 38°C. PaCO₂, corrected for body temperature (pH-stat management), was maintained between 38 and 42 mm Hg.

Note that this group (and group 3) was added to the study immediately after the completion of studies in groups 1 and 2.

**Data Analysis**

Since each animal generated a great number of CBF_LDF/MAP data pairs (one every 12 seconds for over 20 minutes), a method was needed to simplify the results to allow statistical evaluation. First, to ensure that all measurements were made under relatively stable conditions, only CBF_LDF values recorded during the second minute of each 2-minute stage were used. Second, MAP was divided into 10 mm Hg bins (25–34, 35–44, and 45–54 mm Hg, etc.). For each animal, CBF_LDF values (expressed both as absolute numbers and as percentage of baseline) were averaged within each bin, yielding nine flow values for each rat. CBF_LDF was then plotted versus the midpoint of each MAP bin (30, 40, and 50 mm Hg, etc.). All subsequent statistical comparisons were carried out on CBF_LDF values expressed as percentage of baseline. Since CBF_LDF expressed in this manner is a ratio and hence is not normally distributed, these values were subjected to a logarithmic transformation before analysis. Groups were then compared using a two-way univariate repeated-measures analysis of variance (ANOVA), with experimental groups treated as a “between-groups” variable and MAP treated as a “within-groups” variable. Since only three rats were entered into the hypothermic/pH-stat(0.45%) halothane group (group 4), we made no attempt to include this group in the statistical analysis.

To define the lower “knee” of the autoregulatory curves, CBF_LDF at each point in the experiment was calculated as percentage of baseline, and then a running average of three sequential flow values (each 12 seconds apart) was calculated for the entire experiment (to compensate for moment-to-moment variability). This was then inspected, and the MAP at which this running average first reached or fell below 75% of baseline was recorded. This MAP value was compared between groups using a one-way ANOVA. In addition, a one-way repeated-measures ANOVA was performed within each group, and Dunnett’s t test was performed to determine the MAP bin at which CBF_LDF was first significantly different from baseline. A similar process was used to examine changes in calculated cerebrovascular resistance (CVR_LDF), which was calculated as absolute CBF_LDF/MAP.

Physiological variables recorded under baseline conditions were compared between groups by one-way ANOVA. A value of p<0.05 was considered significant.

All statistical tests were performed using STATVIEW II and SUPERANOVA programs for the Macintosh computer (Abacus Concepts, Inc., Berkeley, Calif.).

**Results**

Baseline physiological variables are shown in Table 1. Weight, MAP, and PaO₂ were not significantly different between groups. Hematocrit was slightly higher in the three hypothermic groups. As intended, rectal and cranial temperatures were significantly different between normothermic and hypothermic groups but were identical for the three hypothermic groups. PaCO₂ and pH varied as intended.

Baseline CBF_LDF values (which are not absolute values) are also found in Table 1. CBF_LDF in group 2 (hypothermic/alpha-stat) was significantly less than in

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**Table 1. Baseline Physiological Variables**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (normothermia, halothane 0.7% (n=8))</th>
<th>Group 2 (hypothermia, alpha-stat, halothane 0.7% (n=8))</th>
<th>Group 3 (hypothermia, pH-stat, halothane 0.7% (n=8))</th>
<th>Group 4 (hypothermia, halothane 0.45% (n=3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>361±17</td>
<td>345±16</td>
<td>327±17</td>
<td>328±21</td>
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<tr>
<td>Mean arterial pressure (mm Hg)*</td>
<td>127±13</td>
<td>126±10</td>
<td>120±10</td>
<td>127±5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41±2</td>
<td>43±2</td>
<td>43±2</td>
<td>45±2</td>
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<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal</td>
<td>38.1±0.5</td>
<td>31.0±0.8</td>
<td>30.6±0.3</td>
<td>30.8±0.4</td>
</tr>
<tr>
<td>Cranial</td>
<td>36.5±0.4</td>
<td>30.8±0.7</td>
<td>30.3±0.2</td>
<td>30.8±0.3</td>
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<tr>
<td>pH (units)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Temperature uncorrected</td>
<td>7.41±0.03</td>
<td>7.41±0.03</td>
<td>7.31±0.02</td>
<td>7.30±0.03</td>
</tr>
<tr>
<td>Temperature corrected</td>
<td>7.39±0.03</td>
<td>7.49±0.03</td>
<td>7.41±0.05</td>
<td>7.38±0.03</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Temperature uncorrected</td>
<td>40.1±2</td>
<td>40.3±2.3</td>
<td>52.2±3.3</td>
<td>52.9±5.9</td>
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<tr>
<td>Temperature corrected</td>
<td>42±2.2</td>
<td>31.5±2.4</td>
<td>39.4±2.7</td>
<td>40.6±4.2</td>
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<tr>
<td>PaO₂ (mm Hg)</td>
<td>182±32</td>
<td>208±40</td>
<td>181±36</td>
<td>218±67</td>
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<tr>
<td>CBF_LDF (no units)</td>
<td>90±35</td>
<td>49±23</td>
<td>94±33</td>
<td>113±25</td>
</tr>
</tbody>
</table>

Values are mean±SD. The terms alpha-stat and pH-stat are defined in the text. Temperature corrected values are corrected for the animals’ actual rectal temperatures; temperature uncorrected values are taken directly from the blood gas monitor machine (set at 37°C). CBF_LDF, cerebral blood flow monitored by laser-Doppler flowmetry.

*Mean arterial pressure is listed prior to opening the blood reservoir.

tp<0.05 for group 2 vs. groups 1 and 3.
Figure 1. Cerebral blood flow monitored by laser-Doppler flowmetry (CBF<sub>LDF</sub>) plotted against mean arterial pressure (MAP) for groups 1–3. Each MAP value on the x axis represents the midpoint of a 10 mm Hg MAP bin (e.g., 30 represents 25–34 mm Hg, 40 represents 35–44 mm Hg, etc.). CBF<sub>LDF</sub> values, expressed in “flow units” as displayed on the laser-Doppler flowmeter, are mean ± SD values for each MAP bin. Note that only selected SD bars are shown to avoid excessive overlap and clutter.

Changes in mean CBF<sub>LDF</sub> versus MAP for the three primary experimental groups (1–3) are shown in Figure 1, and CBF<sub>LDF</sub> is expressed as percentage of baseline in Figure 2. In normothermic animals (group 1), CBF<sub>LDF</sub> was stable at a MAP of ≈65 mm Hg and then decreased. In these animals, CBF<sub>LDF</sub> reached 75% of baseline at a MAP of 57±15 mm Hg, and CBF<sub>LDF</sub> was first significantly different from the baseline value at a MAP of 45–54 mm Hg. In group 2 (hypothermic/alpha-stat), the MAP-CBF<sub>LDF</sub> curve was quite shallow with no obvious plateau. CBF<sub>LDF</sub> reached 75% of baseline at a MAP of 73±21 mm Hg, and significance relative to baseline was reached at a MAP of 75–84 mm Hg. In contrast, the curve for group 3 (hypothermic/pH-stat) was relatively steep, declining almost monotonically with decreasing MAP. CBF<sub>LDF</sub> reached 75% of baseline at a MAP of 82±14 mm Hg. The CBF<sub>LDF</sub> value became significantly different from the baseline value at a MAP of 85–94 mm Hg. Two-way ANOVA (with MAP treated as a repeated measure) performed on log-transformed CBF<sub>LDF</sub> values (expressed as percentage of baseline) indicates that the three curves are statistically distinct (as defined by the A×B interaction). Furthermore, when pairwise comparisons are performed (group 1 versus group 2, group 1 versus group 3, etc.), the A×B interactions were all highly significant.

Figure 2. Cerebral blood flow monitored by laser-Doppler flowmetry (CBF<sub>LDF</sub>), expressed as percentage of baseline CBF<sub>LDF</sub> (determined at mean arterial pressure [MAP] of ≈110 mm Hg), plotted against MAP. Each MAP value on the x axis represents the midpoint of a 10 mm Hg MAP bin (e.g., 30 represents 25–34 mm Hg, 40 represents 35–44 mm Hg, etc.). CBF<sub>LDF</sub> values are mean ± SD values for each MAP bin.

CBF<sub>LDF</sub> reached 75% of the baseline value at a MAP of 83±14 mm Hg.

One-way repeated-measures ANOVA with post hoc Dunnett’s test comparison shows that CBF<sub>LDF</sub> in group 1 is significantly lower than the baseline value between MAP bins of 75–84 and 45–54 mm Hg but not at pressures above or below this range. In group 2, CBF<sub>LDF</sub> is significantly less than baseline at all pressures below the MAP bin of 55–64 mm Hg. In contrast, in group 3 CBF<sub>LDF</sub> is significantly greater than the baseline CBF<sub>LDF</sub> value at all pressures below 55–64 mm Hg.

Discussion

CBF normally varies little between MAPs of ≈50 and 150 mm Hg. Although it is simple to demonstrate the stability of CBF within the midposition of the autoregulatory curve, defining the upper and lower limits is more difficult. Intermittent CBF measurement methods (e.g., <sup>133</sup>Xe or H<sub>2</sub>O washout techniques) require stable physiological conditions for many minutes. These conditions may be difficult to maintain during hypotension and hypertension. More “instantaneous” methods such as the use of radioactive microspheres don’t require such prolonged stability, but only a limited number of measurements can be made (ranging from one to six). Hence, most investigators collect only a few data points for each subject and then pool data from a large number of subjects. Although this approach has been valuable, autoregulation would be better studied using continuous flow measurements, allowing the construction of a nearly complete curve in each animal.

This idea is not new. Rapela and Green used a venous outflow method to examine autoregulation in the dog as early as 1967, and Phillis et al used a postglomerular vein outflow technique in rats. Unfortunately, these methods are surgically complex. The intro-
duction of LDF has made continuous blood flow measurements readily available, albeit in small tissue volumes.²⁰,²¹ LDF has been well validated for the measurement of CBF. The absolute flow values differ from those yielded by other methods and probably should be considered as unitless, but there is little doubt that LDF is an accurate method for quantitating changes in CBF relative to some reference point.²²–²⁵

Some caution must be exercised with respect to our results. For example, we have reported absolute CBF\textsubscript{LDF} values in Figure 1 and in Table 1. This was done to compare baseline flows among groups. Although CBF\textsubscript{LDF} is best used to examine changes (and most of our statistical comparisons were done on data expressed as percentage of baseline flow), we feel that these intergroup comparisons are reasonable. The observed CBF\textsubscript{LDF} values are normally distributed with a coefficient of variability similar to that seen with other flow methods.²⁶ There are also clear differences between group 1 and group 2, the magnitudes of which are similar to those reported by others.¹⁰ Our measurements were also made through the intact dura. This was done to avoid changing the cortical microenvironment and to limit the potential for injury. This approach has been taken by others, particularly in the rat, in which the dura is thin and transparent. Finally, we produced hypotension by hemorrhage. This method has been used by others.²⁷,²⁸ Although it allowed us to control MAP, it could be argued that the results obtained might not apply to other conditions. Hemorrhage is associated with increases in the plasma concentration of a number of vasoconstricting compounds (e.g., catecholamines). There is no obvious way to eliminate the possible influence of such events, although increases in circulating catecholamines and peptide hormones may also be seen with hypotension produced by other methods (e.g., aortic balloons and nitroprusside). In fact, a study of these biochemical events during hypothermia might provide some explanation for the different curves seen. Hemorrhage also resulted in a small reduction in arterial hematocrit during the 20-minute experimental period. To rule out the possibility that this might be a confounding variable, we measured hematocrit in 15 animals prepared identically to those in groups 1–3 (five in each group). Whereas baseline hematocrit was greater in both the hypothermic groups, it declined by an identical 20% in all three groups. It seems unlikely that this could explain the intergroup differences in autoregulation, although it remains possible that the changing hematocrit might influence flow and autoregulation in all groups. Hemoglobin, either free or contained in red blood cells, is a scavenger of endothelin-derived relaxing factor.²⁹,³⁰ Hence, a reduction in hematocrit might increase the amount of endothelin-derived relaxing factor present, which might alter autoregulation. A reduction in blood viscosity caused by a falling hematocrit, with accompanying increases in the velocity of flow over the endothelium, might also act to release vasodilatory compounds.³¹

The autoregulatory curve in our normothermic nor- mocarbic rats is similar to that reported by many workers. It is a bit difficult to define the lower limit of autoregulation, since this point is not as discrete as often believed. However, the MAP at which CBF\textsubscript{LDF} first reached 75% of baseline was 57±15 mm Hg, and the first significant decrease in CBF\textsubscript{LDF} (relative to baseline) occurred in the 45–54 mm Hg MAP range. These are similar to values found by others. For example, Barry et al.²⁷ found the first significant decrease in CBF below baseline at a MAP of 50–69 mm Hg. Hoffman et al.²⁸ found a similar result using radioactive microspheres. Other groups have used LDF. Eyre et al.²² found a lower limit of autoregulation ranging from 43 to 70 mm Hg in rabbits. Dirnagl and Pulsinelli²³ concluded that the lower limit (defined as the MAP at which cortical blood flow decreased to 80% of baseline) in halothane-anesthetized rats was 80 mm Hg, a value not inconsistent with our own, since they used spontaneously hypertensive rats and defined their “baseline” flow as that seen at a MAP of 130 mm Hg. One feature of LDF-determined autoregulation does deserve special comment. This concerns the remarkable moment-to-moment variability in CBF\textsubscript{LDF} in a given animal. This finding is not new²⁴,³³ but reinforces the idea that autoregulation, particularly in a given individual, cannot be accurately described by the concise sharp curves found in many reviews and texts.

Hypothermia reduces the cerebral metabolic rate in a fairly predictable fashion. Decreasing body temperature by 10°C will typically reduce the cerebral metabolic rate by a factor of 2.5. However, the effects of hypothermia on CBF are far more variable. Much of this variability stems from nonuniformity in blood gas management during hypothermia.³⁴ It has long been known that CO\textsubscript{2} responsiveness is maintained during hypothermia.³⁵ More recent studies conducted during hypothermic cardiopulmonary bypass indicate that CO\textsubscript{2} dramatically influences CBF.³⁵,³⁶ and there has been considerable argument about what constitutes a “normal” Paco\textsubscript{2} during hypothermia. When discussing hypothermic acid–base strategy, it is important to remember that the pH of electrochemical neutrality (pN, where [H\textsuperscript{+}]=\[\text{OH}\textsuperscript{-}\]) varies with temperature, because the ionization constant of water is temperature dependent.¹¹–¹³ pN increases with hypothermia, becoming more “alkalotic” relative to normothermic values. The rate of this change (∆pH/∆T) is approximately −0.017 pH units°C\textsuperscript{-1}. For example, if water, with an initial pH of 7.00 at 25°C, is cooled anaerobically to 0°C, the pH of neutrality will increase to 7.45 as the activity of H\textsuperscript{+} decreases. Poikilothermic animals maintain intracellular pH near pN irrespective of temperature. The pK of the imidazole group of histidine varies with temperature in an almost identical fashion to water: ∆pK/∆T=−0.016 pK units°C\textsuperscript{-1}. By preserving intracellular pN, poikilotherms maintain the ionization state of the histidine-imidazole group (called alpha) at a constant level—hence the term alpha-stat. The alpha-stat hypothesis predicts that the histidine-imidazole ionization state is the primary determinant of the charge state and pH-dependent functions of many of the body’s proteins. Thus, with alpha-stat acid–base management, changing temperature does not alter histidine ionization, such that protein charge state, structure, and function are preserved.³⁷ When hypothermic, poikilotherms achieve alpha-stat conditions by establishing a “respiratory alkalo- sis” relative to normothermic (37°C) blood gas values, when blood gases are measured at in vivo temperature. Alpha-stat management can be approximated in homeo- therms by maintaining arterial pH at 7.40 and Paco\textsubscript{2} at 40 mm Hg as measured at normothermia (37°C), regardless of the subject’s actual in vivo temperature (i.e., blood
gases are not temperature corrected). This was the strategy used in group 2. Because the solubility of CO₂ in blood increases with decreasing temperature (=4.5%/°C), true in vivo PacO₂ is less than 40 mm Hg. Blood gases analyzed at the subject’s actual in vivo temperature reveal a low PacO₂ and high pHa (respiratory alkalosis).* Alpha-stat proponents suggest that this respiratory alkalosis is physiologically appropriate during hypothermia and will better preserve normal physiological conditions, even though homeotherms are not normally hypothermic.

The other acid–base strategy, referred to as pH-stat, mimics that of hibernating species. pH-stat management attempts to maintain arterial pH at 7.40 and PacO₂ at 40 mm Hg as measured at the subject’s actual in vivo temperature (blood gases are temperature corrected). pH-stat management produces relative hypercarbia and acidemia compared with alpha-stat management. Which hypothermic blood gas strategy is “physiologically correct” for nonhibernating homeotherms? The question is unresolved, but a growing body of data, including the results from the current study, suggests that the alpha-stat approach may be more reasonable.

Our results clearly indicate that CO₂ management during hypothermia profoundly affects cerebral autoregulation. As discussed above, normothermic group 1 rats autoregulated normally. CBF-pdf values were relatively stable until MAP decreased to values of 55–64 mm Hg. In stark contrast, when PacO₂ was kept at a temperature-corrected value of 40 mm Hg (group 3, pH-stat management), baseline CBF-pdf was not different from that seen during normothermia, but no evidence of autoregulation was seen (Figures 1 and 2). The difference between groups 1 and 3 can also be seen if one plots an index of CVR-pdf, i.e., MAP/CBF-pdf versus MAP (Figure 3). In the normothermic group, CVR-pdf decreased steadily until MAP was 45–54 mm Hg and then rose. This pattern has been well described and might serve as a better definition of normal autoregulation than changes in CBF per se.\textsuperscript{16,17} Since CVR-pdf in group 3 progressively increased during hypotension, it is reasonable to conclude that pH-stat conditions were associated with a failure of normal compensatory vaso-dilation. CBF-pdf values for group 2 (hypothermia/alpha-stat) do not show a distinct plateau phase as in group 1. Nevertheless, when CBF-pdf is expressed as percentage of the baseline value obtained at 110 mm Hg (Figure 2), the curve is seen to have a distinct convexity (as compared with the linear fall in group 3). Examination of the CVR-pdf versus MAP curve (Figure 3) also demonstrates that, as in normothermic animals, CVR-pdf decreased during hypotension. This indicates cerebral vasodilator response with alpha-stat management and suggests that autoregulation is at least partially preserved.

Why should cerebral autoregulation curves for the two hypothermic groups differ so markedly? The most likely explanation is that pH-stat management results in an unphysiological hypercarbia. It has long been known that hypercarbia impairs or abolishes autoregulation, presumably because CO₂-induced vasodilation limits

*If a sample of blood having a pH of 7.40 and PacO₂ of 40 mm Hg at 37°C is corrected to a patient temperature of 30°C, pHa and PacO₂ will be reported as 7.50 and 29 mm Hg, respectively.\textsuperscript{14}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure3}
\caption{
Cerebrovascular resistance (CVR-pdf) was calculated as mean arterial pressure (MAP)/cerebral blood flow monitored by laser-Doppler flowmetry and plotted against MAP. CVR-pdf values are mean±SD. Each MAP value on the x axis represents the midpoint of a 10 mm Hg MAP bin (e.g., 30 represents 25–34 mm Hg, 40 represents 35–44 mm Hg, etc.). Only selected SD bars are shown to avoid excessive overlap and clutter.
}
\end{figure}

the vessels’ capacity to dilate further.\textsuperscript{18,38,39} Since hypothermic animals with pH-stat CO₂ management (group 3) are relatively hypercarbic compared with alpha-stat hypothermic animals (group 2), it is possible that vasodilation was maximal under baseline conditions in group 3 animals. What is unclear is why baseline CBF-pdf and CVR-pdf values that appear to be identical with those seen during normothermia should represent “maximal vasodilation” during hypothermia. The only possible explanation is that factors other than vessel diameter and wall tension must be playing some role. Since our work does not help in resolving this issue, we will only refer the reader to the excellent discussion presented by Paulson et al.\textsuperscript{17}

Our results are consistent with the findings and conclusions of studies conducted during hypothermic cardiopulmonary bypass. The CBF response to PacO₂ is preserved during bypass, and pH-stat–managed subjects have greater CBF values than those treated with an alpha-stat approach. The cerebral vasodilation of pH-stat management has also been found to eliminate cerebral autoregulation.\textsuperscript{40–43} Whereas with a few exceptions,\textsuperscript{44} most investigators report autoregulation to be preserved with alpha-stat management.\textsuperscript{41,45,46} Ongoing studies in our laboratory, using a rabbit cardiopulmonary bypass model, have shown preservation of normothermic cerebral oxygen supply/demand relations (oxygen extraction ratio, =0.42) when alpha-stat management is used (27°C), whereas with pH-stat management, the oxygen extraction ratio decreases to lower values, suggesting an excess of CBF.\textsuperscript{47}

A brief mention needs to be made concerning group 4. These animals were added to the study because of the known interaction of hypothermia and anesthetics. In 1974, Vitez et al\textsuperscript{15} demonstrated that anesthetic requirements for halothane were reduced (4.82%/°C) during hypothermia in rats. Since studies in goats, rhesus
monkeys, and baboons show impaired cerebral autoregulation with halothane, 58-60 we felt it was possible that the loss of autoregulation in group 3 animals was due to a relatively higher dose of halothane than in group 1 animals. Although this is unlikely, because of the partial maintenance of autoregulation in group 2 animals (which also received 0.7% halothane), we also found that the autoregulatory curve for the group 4 animals was almost superimposable on that for the group 3 animals. Therefore, it appears that small differences in halothane concentration between hypothermic and normothermic animals do not explain our findings.

In conclusion, these experiments indicate that the blood gas management scheme chosen during hypothermia may have profound cerebrovascular effects. The evidence collected suggests that the maintenance of normal PaCO2 and pH as measured at normal body temperature (alpha-stat) results in decreased baseline CBF values and at least partial preservation of autoregulation. By contrast, when pHa and PaCO2 are maintained at 7.40 and 40 mm Hg, respectively, after correction for temperature (pH-stat), hypothermic animals behave as if significantly hypercarbic and autoregulation is abolished. These findings support the idea that alpha-stat management of PaCO2 represents a more physiological approach. Investigators should keep these results in mind when designing studies of cerebral physiology during hypothermia and clearly define and report their blood gas management strategies.

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Editorial Comment

The authors have provided an extremely well-performed and well-controlled study that shows one benefit of the alpha-stat approach to arterial CO2 and pH levels during hypothermia. The rats maintained a relatively normal autoregulatory pattern. Rats managed with the pH-stat approach had failure of autoregulation, presumably because of the dilation caused by the hypercarbic acidic state produced by pH-stat. The authors’ data confirm three other cited articles, but their study is unique in that it alone uses animals without cardiopulmonary bypass.

The authors remind us that the autoregulatory “plateau” is often a gentle slope, certainly at its low end, where the precise “lower limit” could not be sharply delineated.

The authors briefly discuss a possible role for endothelium-derived relaxing factor (EDRF) in the phenomenon they report. They consider the complex relation of anemia (produced by hemorrhagic hypotension) to EDRF levels. The relation of reduced hematocrit to increased velocity is mentioned, and it is stated that increased EDRF release might result. This may be incorrect since EDRF release is shear dependent, shear is both velocity dependent and viscosity dependent, and viscosity decreases as hematocrit decreases. Hematocrit reduction, by decreasing the viscosity, can cause a decreased shear and reduce EDRF. This may account for the decrease in pial arterial diameters reported by others1 during hematocrit reduction. In any case, the authors are probably correct in saying that hematocrit reduction is not responsible for the paralyzing effect of pH-stat on autoregulation. They correctly point out that hematocrit reduction occurred to an equal extent in both the alpha-stat and the pH-stat groups.

However, EDRF may be the basis for the pH-stat effect. Recent studies suggest that EDRF synthesis may increase as pH is lowered by hypercarbia.2–4 If proved correct, this could explain the dilation that occurs during pH-stat, a dilation that may “fix” the vessels in a dilated state. It would be interesting to know whether these vessels could be dilated by anything other than further reductions in pH. The authors suggest that the vessels are maximally dilated but have not tested this assertion.

In any case, although a role for EDRF in hypercarbic dilation is vigorously investigated and debated, the authors’ study will remain an important addition to our knowledge concerning the use of hypothermia as a cryoprotective technique. Their data certainly suggest that it may be harmful to attempt to maintain a PaCO2 of 40 mm Hg after correction for body temperature. A PaCO2 of 40 mm Hg measured at 37°C may be preferable.

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