Neuroprotection in hypothermia linked to redistribution of oxygen in brain

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Submitted 19 December 2002; accepted in final form 21 February 2003

Sakoh, Masaharu, and Albert Gjedde. Neuroprotection in hypothermia linked to redistribution of oxygen in brain. Am J Physiol Heart Circ Physiol 285: H17–H25, 2003; 10.1152/ajpheart.01112.2002.—Hypothermia improves the outcome of acute ischemic stroke, traumatic injury, and inflammation of brain tissue. We tested the hypothesis that hypothermia reduces the energy metabolism of brain tissue to a level that is commensurate with the prevailing blood flow and hence allows adequate distribution of oxygen to the entire tissue. To determine the effect of 32°C hypothermia on brain tissue, we measured the sequential changes of physiological variables by means of PET in pigs. Cerebral blood flow and oxygen consumption (cerebral metabolic rate of oxygen) declined to 50% of the baseline in 3 and 5 h, respectively, thus elevating the oxygen extraction fraction to 140% of the baseline at 3 h. The results are consistent with the claim that cooling of the brain to 32°C couples both energy metabolism and blood flow to a lower rate of work of the entire tissue.

AT NORMAL TEMPERATURE, neurons do not survive an oligemic insult that causes metabolic variables to decline below specific thresholds (49, 51, 58). The rate of the decline limits the period of therapeutic opportunity (51, 57) to no more than 3 h, which is not sufficient for attempted reperfusion of severely oligemic tissue in clinical situations. Preoperative introduction of hypothermia significantly extends this period (51, 50), but the mechanism responsible for this effect is not known with certainty.

Hypothermia is known to provide remarkable protection against the insults of low blood flow (oligemia) to the brain, particularly when thermoregulatory responses are suppressed by sedation or anesthesia. Thus sedation plays an important role in the effect. As exemplary beneficiaries of the remarkably neuroprotective effect of combined sedation and hypothermia, two heavily sedated adult women, unresponsive and apparently lifeless at respective core temperatures of 23°C and 26°C several days after unrelated suicide attempts, tolerated prolonged absences of measurable blood pressure and pulse rate without apparent ill effects, one proceeding to pass a university matriculation test within weeks of the attempt (18).

iatrogenic hypothermia is used to protect brain tissue during surgery (55) and appears to improve the clinical course of patients with acute ischemic stroke and traumatic brain injury (10, 37, 50, 54). In animals, hypothermia has improved the outcome from acute cerebral ischemia, traumatic brain injury, and inflammatory changes in the brain (1, 5, 6, 27, 38, 62).

The conventional explanation of the neuroprotective effect of hypothermia holds that the hypothermic decline of the cerebral metabolic rate of oxygen (CMRO2) maintains the aerobic metabolism. The same mechanism would explain how hypothermia may substantially attenuate the release of neurotoxic excitatory amino acids during ischemia (29). Older studies, however, led to the claim that the neuroprotection afforded by moderate hypothermia somehow exceeds the effect predicted from the decrease in CMRO2 (5).

The claim that hypothermia protects brain tissue by preserving cellular energy stores and aerobic metabolism is based on observations that CMRO2, like other enzymatically catalyzed biological processes, declines monoeXponentially with temperature according to the formula (4, 40, 41)

\[
J_{O_2} = J_{O_2o} (Q_{10})^{-0.1(T_o - T)}
\]

where \(Q_{10}\) is the temperature coefficient, \(T\) and \(T_o\) are two different temperatures, and \(J_{O_2}\) and \(J_{O_2o}\) are the values of CMRO2 at \(T\) and \(T_o\), respectively. \(Q_{10}\) is close to 2 for enzymatically catalyzed reactions but greater for nonenzymatic reactions. Between 27°C and 37°C, the mean of \(Q_{10}\) measurements is 2.2 for CMRO2 (4). Assuming a baseline brain temperature of 38°C, this formula predicts that CMRO2 is 25% lower at 34.4°C and 50% lower at 29.4°C. There is a question as to whether reductions of this magnitude explain the neuroprotective effect of hypothermia.

Models of nonlinear flow-metabolism coupling have been formulated since the publication of much of the classic literature on brain metabolism in hypothermia.
To test whether declines of cerebral blood flow (CBF) and metabolism in hypothermia generally are consistent with these models of nonlinear flow-metabolism coupling, we measured the sequential changes of CBF, cerebral oxygen consumption or CMRO2 and oxygen extraction fraction (OEF) with PET before and every hour during the 6 h after the initiation of hypothermia at 32°C in Danish landrace pigs, as well as in control brain tissue not subjected to hypothermia. From the measurements, we calculated the capillary oxygen tensions necessary to supply the brain tissue by means of the mathematical expression of a recently reported mechanism of nonlinear flow-metabolism coupling. The results of the test allowed us to predict the effect of hypothermia superimposed on a simulated oligemic insult and to show that the effect fully explained the maintenance of oxidative metabolism in hypothermia, despite the decline of CBF, which was similar in magnitude to that seen in oligemic insults.

NONLINEAR FLOW-METABOLISM COUPLING

To test the null hypothesis of adequate oxygen supply to brain tissue during hypothermia, we applied a model of flow-metabolism coupling, which allowed us to compare the available oxygen in brain capillaries to the oxygen required to supply the entire tissue, both in plain hypothermia and in hypothermia superimposed on oligemia of the tissue.

We previously formulated a mechanism of nonlinear flow-metabolism coupling in normothermia (16, 20, 60) based on the observation of diffusion-limited transport of oxygen to brain tissue (21, 34), and we validated the formulation for the cases of visual cortex activation (20), cortical motor activation (19), and arterial hypoxemia (17). The mechanism dictates the rate of blood flow needed to supply the tissue with an adequate amount of oxygen

\[ F = \left( \frac{f_0}{2C_{Hb}} \right) \left[ 1 + \left( \frac{f_0^2}{L \cdot P_{50}} \right)^{n_H} \right] \left[ 1 + \left( \frac{P_{50}}{P_{A0}} \right)^{n_H} \right] \] (1)

where \( F \) is the blood flow to the tissue (per unit weight or volume), \( f_0 \) is the average oxygen metabolism of a fraction of the tissue (per unit weight or volume of that fraction), assuming the rest of the tissue does not consume any oxygen, \( j \) is that fraction of the tissue, ranging in theory from zero to infinity, that has \( f_0 \) (per unit weight or volume). \( C_{Hb} \) is the arterial hemoglobin concentration, \( L \) is oxygen diffusibility, \( P_{50} \) is the half-saturation oxygen tension of hemoglobin, \( n_H \) is Hill's coefficient (2.65 in pigs; Ref. 48) and \( P_{A0} \) is the arterial oxygen tension. Values of \( f \) exceed unity when the supply of oxygen exceeds the minimum requirement of the tissue as a whole.

The derivation of Eq. 1 was motivated by the well-known absence of a meaningful geometric description of the jumbled capillary network in the brain. Instead, Eq. 1 arose from the simple assumption that every segment of the capillary bed “feeds” the same amount of brain tissue, i.e., that every fraction \( xyz \) of the tissue is served by the same fractions of capillary density and oxygen diffusibility and uses the same fraction of the total oxygen consumption. Along the axial dimension of the capillary bed (z), the OEF is proportional to the fraction of capillary segments traversed by the blood flow, and the segmental oxygen tension is given by this corresponding segmental extraction fraction, according to the Hill equation of the relationship of oxygen tension and oxygen-hemoglobin dissociation, as shown in Fig. 1, of the normothermic baseline determined in the present study. The linearity of the extraction fraction as a function of the capillary segmentation shows that the entire axial dimension can be projected onto a single transaxial plane placed at the segmental fraction 0.5, where the extraction fraction is exactly one-half of the total net extraction and where the total oxygen flux to the tissue is the product of the oxygen diffusibility (L) and the oxygen partial pressure at that point. This product is the “available” oxygen, because no more oxygen can enter the tissue than is dictated by the product of the weighted average oxygen tension in the capillary and the weighted average oxygen diffusibility.

In Eq. 1, the whole tissue oxygen consumption (CMRO2) is the product of \( f \) and \( j_{02} \) if \( f < 1 \) and is just \( j_{02} \) if \( f \geq 1 \). The introduction of \( f \) allows the tissue to respond to contingencies either by changing the metabolic rate of all cells (constant \( f \)) or by changing the fraction of the cells (variable \( f \)) that maintain the baseline metabolic rate. In reality, combinations of both may occur, of course, but for the purposes of this study they are presumed to be either one or the other.

Equation 1 predicts how low blood flow and low oxygen consumption, or low nonmetabolizable tissue fraction, would be coupled nonlinearly. In the equation, the term \( f^2j_{02}/L \cdot P_{50} \) raised to the power of \( n_H \) greatly exceeds zero when \( f^2j_{02} \gg L \cdot P_{50} \). The term \( P_{E0}/P_{A0} \), raised to the power of \( n_H \), on the other hand, remains close to zero when \( P_{E0} \ll P_{A0} \). These relations explain why blood flow must change much more when the rate of oxygen metabolism is changed than when the arterial oxygen tension is changed. Applied to oligemia, the nonlinear flow-metabolism couple predicts that lowering of the fraction of fully oxygenated tissue (\( f \)), caused by blood flow restriction, can be prevented by an alternative reduction of \( j_{02} \), caused by hypothermia.

Equation 1 follows from the relationship between oxygen supply and oxygen consumption. The utilization of the oxygen supply by a given fraction \( x \) of the tissue volume can be

\[ \frac{P_{E0}}{P_{A0}} \] as function of axial segmentation of cortical capillary bed, calculated for baseline state (0th h of hypothermia) of current study. \( E_{O2}(z) \), tension \( P_{E0}(z) \), and \( L(z) \) as functions of axial segment of capillary bed (z) were calculated from the relationships \( E_{O2}(z) = 2j_{O2}[P_{E0}]/P_{A0} \), \( P_{E0}(z) = P_{E0}[1/E_{O2}(z) - 1]^{1/n_H} \), and \( L(z) = j_{O2}P_{E0}(z) \), where \( j_{O2} \) is the measured cerebral metabolic rate of oxygen (\( CMRO2 \); 201.4 \( \mu \)mol·h·g⁻¹·min⁻¹). F the measured blood flow (46.4 ml·h·g⁻¹·min⁻¹), \( C_{O2} \), the measured arterial oxygen consumption (6.7 mM), \( P_{E0} \), the calculated half-saturation tension of oxygen (30.3 mmHg at baseline), and \( n_H \) the Hill coefficient (2.65). \( P_{E0}(0) \) was assumed to equal the arterial oxygen tension (\( P_{A0} \); 131 mmHg).

Fig. 1. Oxygen tension, oxygen extraction fraction (OEF; \( E_{O2} \)), and oxygen diffusibility (\( L \)) as function of axial segmentation of cortical capillary bed, calculated for baseline state (0th h of hypothermia) of current study. \( E_{O2}(z) \), tension \( P_{E0}(z) \), and \( L(z) \) as functions of axial segment of capillary bed (z) were calculated from the relationships \( E_{O2}(z) = 2j_{O2}[P_{E0}]/P_{A0} \), \( P_{E0}(z) = P_{E0}[1/E_{O2}(z) - 1]^{1/n_H} \), and \( L(z) = j_{O2}P_{E0}(z) \), where \( j_{O2} \) is the measured cerebral metabolic rate of oxygen (\( CMRO2 \); 201.4 \( \mu \)mol·h·g⁻¹·min⁻¹). F the measured blood flow (46.4 ml·h·g⁻¹·min⁻¹), \( C_{O2} \), the measured arterial oxygen consumption (6.7 mM), \( P_{E0} \), the calculated half-saturation tension of oxygen (30.3 mmHg at baseline), and \( n_H \) the Hill coefficient (2.65). \( P_{E0}(0) \) was assumed to equal the arterial oxygen tension (\( P_{A0} \); 131 mmHg).

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predicted as the difference between the available oxygen (60) and the oxygen consumption of the fraction \( x \)

\[
RO_2(x) = \left[ \frac{LP_{50}}{f} \right] \left( \frac{2}{E_{O_2}} - 1 \right) - xjO_2
\]

where \( RO_2(x) \) is the rest of the oxygen available to the remaining fraction \( 1 - x \) of the tissue, and \( E_{O_2} \) is the OEF, equal to \( fjO_2/FCaO_2 \), where \( CaO_2 \) is the arterial oxygen concentration. Equation 2 is a line of slope \(-jO_2\) and ordinate intercept \( LP_{50}/f \sqrt{2/\left(2E_{O_2}\right)} - 1/f \).

The ratio of \( P_{cap} \) to \( P_{min} \) is the tissue fraction \( f \) at which the available oxygen is exhausted

\[
f^2 = \left( \frac{LP_{50}}{jO_2} \right) \left( \frac{2}{E_{O_2}} - 1 \right)
\]

where \( E_{O_2} \), as above, equals \( fjO_2/FCaO_2 \). Values of \( f \) less than unity indicate situations in which the tissue exhausts the entire oxygen supply, whereas values of \( f \) higher than unity represent the situations in which the oxygen supply exceeds the demand. Equation 3 can be rearranged to Eq. 1 when \( CaO_2 \) is expressed as a function of the arterial oxygen tension by means of the Hill equation of the oxygen hemoglobin dissociation curve.

The oxygen from brain capillaries causes the capillaries to change their shape in response to the arterial oxygen tension at the arterial end of capillaries to the venous oxygen tension at the venous end. The diffusion pressure of oxygen is the difference between the capillary and mitochondrial tensions. The average driving pressure, equal to the average capillary oxygen tension of all segments of the capillary bed, is the ratio \( fRO_2(x)/L \) for \( x = 0 \) in Eq. 2 (60)

\[
P_{cap}(0) = P_{50} \left( \frac{2}{E_{O_2}} - 1 \right)
\]

where \( f \) is the fraction of the tissue \( CMR_0 \). It follows from the definition of \( f \) that the mitochondrial oxygen tension is effectively zero for \( f \leq 1 \). Thus, for increased extraction fractions, either \( f \) or \( jO_2 \) must decline. If \( jO_2 \) does not decline, \( f \) must.

MATERIALS AND METHODS

The research project and procedures, including the infusion of pancuronium bromide to reduce shivering, was approved by the Danish National Committee on Animal Research Ethics (DaNCARE). Twenty-one female country-bred Yorkshire pigs weighing 38–45 kg were used; ten pigs underwent sequential measurement of physiological variables after hypothermia, and eleven pigs underwent control measurements of CBF and CMRO_2.

The use of the porcine model in pathophysiological and experimental tomography has been established in this laboratory and reported in publications detailing the induction, prolonged anesthesia, and PET of the physiology and pathophysiology of major organs of the Danish Yorkshire pig under control conditions as well as in experimental models of functional brain activation, Parkinson’s disease, deep-brain stimulation, hepatic encephalopathy, and stroke (13, 48).

In brief, the pigs initially were sedated by intramuscular injection of 20 ml of midazolam (5 mg/ml). After intravenous injection of a mixture of 1.0 mg of fentanyl, 50 mg of midazolam, and 4.0 mg of pancuronium, the pigs were intubated and artificially ventilated (Engström Ventilator, Stockholm, Sweden) with a 70% N_2O-30% O_2 mixture. Anesthesia was maintained by continuous infusion of 0.05 mg·kg\(^{-1}\)·h\(^{-1}\) fentanyl, 1.25 mg·kg\(^{-1}\)·h\(^{-1}\) midazolam, and 0.2 mg·kg\(^{-1}\)·h\(^{-1}\) pancuronium bromide to reduce shivering. The adequacy of the anesthesia was continuously assessed by monitoring the systolic and diastolic arterial blood pressures and the electrocardiogram. The body of the pig was shaved to accelerate hypothermia. Indwelling femoral arterial and venous catheters were installed surgically for injection of tracers and arterial blood sampling. A probe advanced into the left internal jugular vein monitored the temperature of the brain.

The pigs were positioned supine in the scanner (Siemens/CTI ECAT EXACT HR) with the head in a custom-made head holder. PET studies were performed before and for 6 h after the initiation of hypothermia. Throughout the experiments, brain temperature, blood pressure, heart rate, and expired-air CO_2 levels were monitored continuously, and arterial blood samples were withdrawn and analyzed (ABL 300; Radiometer, Copenhagen, Denmark) every hour to monitor blood gases and whole blood acid-base parameters. Disturbances in body fluid and gas balances were corrected by appropriate procedures (e.g., forced ventilation and/or changes in infusion rates) to maintain physiological variables within normal bounds, except for body and brain temperature.

Hypothermia. Eight hours of hypothermia were induced by a forced-air cooling system (Bair Hugger model 600 PolarAir Hyper/Hypothermia System; Augustine Medical). The air of the cooling system was (additionally) cooled to −20°C with dry ice. The brain temperature was reduced to 32°C and maintained at that level during the experiments.

Both oxygen diffusibility and the half-saturation tension of oxygen binding to hemoglobin (P_50) decline in hypothermia. The decline of oxygen diffusibility was deemed to be negligible (305°C/310°C = 0.984). P_50 was assumed to decline according to the published relationships shown in Fig. 2. The linear relation between the values of P_50 in units of millimeters of mercury and the temperature in units of degrees Celsius found in three different studies (4, 11, 23) obeyed the expression

\[
P_{50}(\text{mmHg}) = 1.54 (\pm 0.20) (\text{mmHg}/^\circ\text{C}) T - 28 (\pm 7) \text{ (mmHg)}
\]

where \( T \) is the temperature in degrees Celsius.

Positron emission tomography. CBF was estimated by intravenous bolus injection of 800 MBq H_2^{15}O according to the established method (45) used routinely in this laboratory (13, 48). Oxygen consumption was measured by single-breath inhalation of 1 liter of 1,200 MBq [^{15}O]_2O followed by 10-s breath holding according to the similarly established method.
(46) used routinely in this laboratory (13, 48). Regional OEF values were calculated as the ratio of the rates of unidirectional clearance of oxygen and water, equal to the ratio of the measured oxygen consumption (CMRO₂) to the product of the measured arterial oxygen content and the CBF rate, as described below Eq. 2.

\[ \text{OEF} = \frac{\text{CMRO}_2}{\text{CMRO}_2 \times \text{CBF}} \]

The CBF, CMRO₂, and OEF variables were measured before and every hour for 6 h after the onset of hypothermia. A sequence of 21 arterial blood samples (12, 6, and 3 samples during the first, second, and third minutes, respectively), and 12 brain image frames (6, 4, and 2 images/min, respectively) were obtained. In all experiments, total radioactivity was measured in the blood samples. Brain image and arterial data were corrected for the decay of 15O (half-life 123 s).

The PET images were reconstructed as three-dimensional images by using the two-dimensional data acquisition mode providing 47 contiguous 3.2-mm slices with a Hanning filter with a cutoff frequency of 0.5 pixel⁻¹, resulting in a spatial resolution of 4.6 mm (full width at half-maximum). Correction for attenuation was made on the basis of a transmission scan acquired before the injection of radioactivity.

Regions of interest. The MR imaging used a GE Signa Horizon 1.0-tesla imager (GE Medical Systems, Milwaukee, WI). After a sagittal scout, a longitudinal relaxation time-weighted three-dimensional spoiled gradient echo sequence (time of repetition = 8 ms, time of echo = 1.5 ms, 20° flip angle) was acquired for later coregistration of MRI and PET data. With REGISTER (Montreal Neurological Institute, McGill University, Montreal, Canada), regions of interest (ROIs) were selected in cortex of the middle cerebral artery territory and averaged. The ROIs, once defined for each pig, were used in all subsequent PET studies.

Data analysis. Statistical analysis was carried out by one-way repeated-measures ANOVA to determine whether physiological parameters and/or (absolute) values for CBF, CMRO₂, and OEF measured by PET changed significantly over time. A paired t-test with Bonferroni correction for planned comparisons was used to determine whether the effects varied significantly over time. For the statistical significance of each change from baseline to 6 h after hypothermia, the Wilcoxon signed-ranks test was used. A probability threshold of 0.01 was used for statistical significance.

RESULTS

Basic physiological variables and P₅₀. The changes of CBF and CMRO₂ in normothermic control animals maintained on the same regimen and for the same period of time as the hypothermic animals are shown in Fig. 3. CBF increased by no more than 10% over this time, and CMRO₂ declined by no more than 5%.

The changes of brain temperature before and after hypothermia are shown in Fig. 4. Brain temperature decreased significantly to 32°C in 3 h (P < 0.01) and then remained stable during the rest of the experiment. The resulting values of P₅₀, calculated according to Eq. 6, are also shown in Fig. 4. As expected from the equation, P₅₀ closely followed the change in temperature.

Table 1 lists the physiological variables before and after hypothermia. At the baseline and 6 h after the induction of the hypothermia, all physiological variables were in the normal range and, with one exception, did not change during the hypothermia. However, arterial PCO₂ decreased significantly after 3 h of hypothermia.

Sequential changes of CBF, CMRO₂, and OEF in hypothermia. The sequential changes of CBF, CMRO₂, and OEF before and during hypothermia are shown in Fig. 3. The changes of cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO₂), and OEF in normothermic pigs maintained for 6 h.
Fig. 5. CBF decreased to 50% of the baseline in 3 h \( (P < 0.01) \) and then remained stable. CMRO\(_2\) decreased to 50% of the baseline over the 6 h \( (P < 0.01) \). OEF rose to 140% of the baseline in the first 3 h \( (P < 0.01) \) and then gradually returned to the baseline.

The calculated values of Q\(_{10}\) are shown in Fig. 6. The values averaged 2.24 ± 0.06 (SE) in the first 3 h and then rose markedly, as the temperature remained constant but the CMRO\(_2\) continued to fall.

**Flow-metabolism couple.** Setting \( f \) equal to unity in Eq. 3, the oxygen diffusibility \( (L) \) was calculated from the baseline values to be 5.05 \( \mu \text{mol} \cdot \text{h}^{-1} \cdot \text{mmHg}^{-1} \), under the assumption of oxygen availability to the entire tissue in the baseline state. This value was used in subsequent calculation of variables during the hypothermia, including calculation of \( f \) according to Eq. 3 in the non-baseline states. Assuming \( L \) to remain constant, the fraction of tissue supplied with oxygen briefly declined to a little less than unity but eventually exceeded unity slightly when the extraction fractions again tended toward normal.

The correlation between blood flow and oxygen consumption is shown in Fig. 7, in which the continuous curve shows the prediction from Eq. 1 and the points show the actual measurements using the values of \( P_{50} \) and \( L \) calculated above. The predicted values closely matched the measured values except for the lowest points, at which the measured flow values slightly, and according to the correlation coefficient insigificantly, exceeded the calculated values despite the apparent constancy.

The mean capillary oxygen pressure heads supplying the tissue with oxygen, calculated by means of Eq. 4 as a function of time spent in hypothermia, are shown in Fig. 8. This capillary pressure first declined as the extraction fractions increased but subsequently tended to return to normal as the extraction fractions declined. Figure 8 also shows the mitochondrial oxygen tensions calculated by means of Eq. 5. The tensions had negligible values (“zero”) during the first 4 h but eventually slightly exceeded zero when the extraction fractions tended toward normal.

**DISCUSSION**

Hypothermia has a protective effect on neurons at risk of anoxic damage \( (1, 5, 6, 27, 31, 38, 41, 62) \). Well-described neuroprotective effects of hypothermia include less elevated intracranial pressure, maintained mitochondrial function, and suppressed release of excitatory amino acids (glutamate) or glycine \( (2, 8, 12, 14, 33, 36, 47) \). However, the fundamental mechanism underlying the improvement is still unknown.

Insufficient perfusion of brain tissue impairs the oxygen supply but does not reduce the need for oxygen. Hence it is possible that the available oxygen is exhausted by a subset of brain cells with normal metabolic demands, leaving the rest of the cells without oxygen. We tested the claim that hypothermia can correct this imbalance.

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Table 1. General physiological variables before and during hypothermia

<table>
<thead>
<tr>
<th>Time, h</th>
<th>MABP, mmHg</th>
<th>HR, min(^{-1})</th>
<th>Arterial pH</th>
<th>( P_{\text{CO}_2}, \text{mmHg} )</th>
<th>( P_{\text{O}_2}, \text{mmHg} )</th>
<th>( [\text{K}^-], \text{mM} )</th>
<th>( C_{\text{glc}}, \text{mM} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>104 ± 9</td>
<td>103 ± 13</td>
<td>7.45 ± 0.04</td>
<td>35.0 ± 1.2</td>
<td>131 ± 13</td>
<td>3.64 ± 0.15</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>1</td>
<td>109 ± 8</td>
<td>110 ± 13</td>
<td>7.44 ± 0.04</td>
<td>33.5 ± 1.1</td>
<td>137 ± 11</td>
<td>3.55 ± 0.22</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>3</td>
<td>116 ± 10</td>
<td>108 ± 16</td>
<td>7.42 ± 0.03</td>
<td>33.1 ± 1.3*</td>
<td>138 ± 9</td>
<td>3.46 ± 0.26</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td>107 ± 12</td>
<td>110 ± 11</td>
<td>7.41 ± 0.03</td>
<td>32.7 ± 0.7*</td>
<td>136 ± 8</td>
<td>3.44 ± 0.18</td>
<td>6.0 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. MABP, mean arterial blood pressure; HR, heart rate; \( C_{\text{glc}} \), plasma glucose concentration. *Significantly different from baseline value \( (P < 0.01, \text{ANOVA}) \).

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Fig. 5. Sequential changes of CBF, CMRO\(_2\), and OEF before and after hypothermia in pigs. x-Axis indicates time after induction of hypothermia. *Significantly different from all other measurements. NS, not significantly different from succeeding measurement of same variable only.

Fig. 6. Values of the temperature coefficient \( Q_{10} \) during hypothermia calculated for temperature changes indicated by temperature graph. Numbers indicate hourly interval of \( Q_{10} \) calculation.
The current measurements of flow and metabolism are consistent with the null hypothesis that the neurroprotective effect is the result of redistribution of oxygen to a greater fraction of the tissue when the total oxygen supply is limited. The hypothesis is based on the observation that normal nonlinear oxygen supply is limited. The hypothesis is based on oxygen to a greater fraction of the tissue when the total protective effect is the result of redistribution of are consistent with the null hypothesis that the ne-

The inverse relationship between OEF and oxygen diffusibility (\(f_j\), with values of \(CMRO_2\) (Eq. 4) and Fig. 5 and values of \(L\) (5.05 \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\)-mmHg\(^{-1}\)), \(n_{15}\) (2.65), and \(P_{ax}\) as listed in Table 1 and averaged for intervening times.

which primary energy demands are not likely to be lower than under normal circumstances. In the case of hypothermia superimposed on oligemia, the hypothesis claims that the energy needs of more nerve cells in the threatened tissue are satisfied at the prevailing perfusion, causing more cells to receive amounts of oxygen that are adequate for their needs. Because of the uniform effect of hypothermia on most enzymatically catalyzed processes in brain (the range of mea-

Fig. 7. Flow-metabolism couple in hypothermia. x-Axis indicates average values of \(f_j\) (CMRO\(_2\)) shown in Fig. 5. y-Axis indicates average CBF values measured and predicted by Eq. 1 with values of \(P_{50}\) and \(C_{mb}\) shown in Fig. 4, average values of \(f_j\) (CMRO\(_2\)) shown in Fig. 5, and values of \(L\) (5.05 \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\)-mmHg\(^{-1}\)), \(n_{15}\) (2.65), and \(P_{ax}\) as listed in Table 1 and averaged for intervening times.

The novel element in the revisions is the claim that CBF rates are homeostatically regulated to maintain an adequate mean driving pressure of oxygen in the capillary bed as a whole (7, 16), necessitated by the well-established diffusion-limited oxygen delivery to brain mitochondria (3, 7, 17, 21, 34, 60, 61). In hypothermia, the couple works to downregulate blood flow rates when the energy demands of all neurons are reduced by the low temperature.

It is important to emphasize that low “brain” \(P_{O_2}\) in hypothermia, somewhat vaguely defined as an appropriately weighted average of capillary and mitochondrial oxygen tensions, is a physiological response to the lowered metabolism and blood flow, rather than a sign of oxygen deprivation (22). The flow rates observed in hypothermia approximate those measured during normothermic oligemia, in

Fig. 8. Average \(P_{capO_2}\) and mitochondrial oxygen tension \((P_{mitO_2})\) during the course of hypothermia. x-Axis indicates time after induction of hypothermia. y-Axis indicates average \(P_{capO_2}\) and \(P_{mitO_2}\), calculated from Eqs. 4 and 5, with values of \(CMRO_2\) (Eq. 4) and \(E_0\) shown in Fig. 5 and values of \(L\) (5.05 \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\)-mmHg\(^{-1}\)), \(n_{15}\) (2.65), and \(P_{tax}\) as listed in Table 1 and averaged for intervening times.

The inverse relationship between OEF and oxygen diffusibility \((f_j\), with values of \(CMRO_2\) (Eq. 4) and Fig. 5 and values of \(L\) (5.05 \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\)-mmHg\(^{-1}\)), \(n_{15}\) (2.65), and \(P_{tax}\) as listed in Table 1 and averaged for intervening times.

Fig. 9. Available remaining oxygen as function of tissue volume fraction supplied, according to Eq. 2. The coordinate system represents projection of axial dimension on single transaxial plane at capillary fractional segment 0.5, shown in Fig. 1, based on variables measured or calculated \((E_0)\) at 0 h [baseline (control)]: \(F = 46\) ml-hg\(^{-1}\)-min\(^{-1}\), \(j_o = 201.4\) \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\), 6 h (hypothermia): \(F = 21\) ml-hg\(^{-1}\)-min\(^{-1}\), \(j_o = 105\) \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\), and a generic case of oligemia at normothermia (oligemia: \(F = 20\) ml-hg\(^{-1}\)-min\(^{-1}\), \(j_o = 201.4\) \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\)). x-Axis indicates fractional tissue segment in \(x\) direction. y-Intercept is product of weighted average oxygen tension, calculated according to Eq. 4, and weighted average oxygen diffusibility \((L = 5.05\) \(\mu\)mol-hg\(^{-1}\)-min\(^{-1}\)-mmHg\(^{-1}\)), calculated in the baseline case by setting \(f = 1\) in Eq. 3, as shown in Fig. 1, and assumed unchanged in all other conditions.
tissue reserves of oxygen were calculated from and oligemic anoxia are simulated in Fig. 9, in which
20 or 21 ml for reductions of blood
°
50% in 5 h. Fifty percent reduction in CMRO₂ was also
continued to decline during most of the study, reaching
the hypothermia both contribute to the ef
ical magnitude, which prevents or delays irreversible
damage to the ischemic cortex.

The periods of time spent inducing and maintaining the hypothermia both contribute to the efficacy of the treatment. In the present study in pigs, the time required to cool the brain to 32°C was 3 h, in agreement with previous reports in humans (38, 53). CMRO₂ continued to decline during most of the study, reaching 50% in 5 h. Fifty percent reduction in CMRO₂ was also reported for dogs during 30°C hypothermia (42). In patients with head injury, 32.5°C hypothermia reduced CMRO₂ by 45% (38), close to the 40–50% of baseline that is believed to be the viability threshold of oligemia at normal temperature (25, 49, 51, 58).

Previously reported changes of OEF during hypothermia are equivocal (35, 43), perhaps because the changes were determined only once during hypothermia. In the present study, OEF rose gradually during the first 4 h of hypothermia, during which the measured flow changes closely approximated the changes predicted by the flow-metabolism couple expressed in Eq. 1 (Fig. 7). Thereafter, OEF tended to decline toward the baseline value.

The decline of OEF occurred when oxygen consumption continued to decline but temperature and blood flow remained apparently constant after 3 h. This discrepancy led to a marked increase of the calculated value of $Q_{10}$ (Fig. 6).

Values of $Q_{10}$ above 2.5 tend to signify the sensitivity of nonenzymatic reactions related to the physicochemical properties of the internal milieu, speculatively including longer-term changes of membrane permeability, cytochrome oxidase affinity, or any of a host of other factors too complex to identify here. The apparent constancy of blood flow after 3 h may represent a failure of physiological flow-metabolism coupling below 20 ml·hg⁻¹·min⁻¹.

The different roles of increased OEF in hypothermia and oligemic anoxia are simulated in Fig. 9, in which tissue reserves of oxygen were calculated from Eq. 2 for the control situation and for reductions of blood flow to 20 or 21 ml·hg⁻¹·min⁻¹ in hypothermia and normothermia, under the assumption that the basic neuronal demand for oxygen is undiminished in the oligemic brain, whereas it is reduced in hypothermia as measured in the present study. The portions of the tissue supplied with oxygen in the two situations (f) were calculated by means of Eq. 1, and the corresponding capillary and mitochondrial oxygen tensions were calculated by means of Eqs. 4 and 5, once f had been determined, from the value of $L$ estimated in the baseline. The calculation showed that the oligemic reduc-
tion to 20 ml·hg⁻¹·min⁻¹ deprived as much as 30% of the tissue of oxygen, whereas the superimposition of hypothermia at 32°C caused the oxygen to be redistributed to the entire tissue, despite an even further reduction of the driving pressure of oxygen caused by the reduction of $P_{SO₂}$.

Thus, in hypothermia, the reduction of the oxygen driving pressure matched the diminished demand for oxygen. In ischemia, the low oxygen pressure in the capillary bed is imposed by the reduced blood flow to the oligemic brain with normal metabolic demands for oxygen. The simultaneous reduction of CMRO₂ and elevation of OEF in oligemic brain tissue demonstrates the need for the neuroprotective lowering of metabolism of the brain tissue at risk (24, 51).

The observation that hypothermia causes CBF to decrease is not new; CBF declined by 5.2%/°C in patients with head injury (36) and 60–70% in monkeys cooled to 29°C (39). Also in the present study, the CBF decreased to a plateau defined by the brain temperature.

Reduction of CBF (and cerebral blood volume) tends to diminish intracranial pressure (ICP) and intracranial hypertension, which are clinically life-threatening complications of brain damage (1, 36), but at least one study did not show the decrease of CBF thought to explain the reduction in ICP during hypothermia in patients with head injury (38). The effect of hypothermia on CBF and ICP may be related in part to decreased nitric oxide levels (9) and maintenance of the blood-brain barrier (15, 53, 56). These effects of hypothermia can prevent reperfusion injury after acutely ischemic stroke and carotid arterectomy or perfusion pressure breakthrough after removal of large arteriovenous malformations (26, 30, 57).

There is a question as to why reduction of metabolism by pharmacological agents at normothermia generally gives much less improvement of the outcome after an oligemic or ischemic insult. One explanation may be the different effects of normothermia and hypothermia on $P_{SO₂}$. In normothermia, $P_{SO₂}$ is normal and at the same low levels of flow and metabolism lead to large increases of mitochondrial oxygen tension that may cause free radical formation and result in damage to the tissue.

We conclude that rapid cooling of the brain to 32°C could be neuroprotective, because it restores a physiological relationship between cerebral metabolism and perfusion above the viability threshold of brain tissue, which would otherwise be at risk at normal temperature.

This study was funded by the Scandinavia-Japan Sasakawa Foundation, Danish Medical Research Council Grants 9305246, 9305247, 9601888, and 9802833, and the Institute of Experimental Clinical Research, University of Aarhus, Denmark. The Center of Functionally Integrative Neuroscience is funded by the National Science Foundation of Denmark.

REFERENCES
1. Angstwurm K, Reuss S, Freyer D, Arnold G, Dirnagl U, Schumann RR, and Weber JR. Induced hypothermia in ex-

AJP-Heart Circ Physiol • VOL 285 • JULY 2003 • www.ajpheart.org
Hypothermia redistributes oxygen.


