

## Cerebral Blood Flow Response to $P_{aCO_2}$ during Hypothermic Cardiopulmonary Bypass in Rabbits

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Differences in cerebral blood flow (CBF) between alpha-stat and pH-stat management depend on preserved responsiveness of the cerebral vasculature to changes in arterial carbon dioxide tension ( $P_{aCO_2}$ ). We tested the hypothesis that hypothermia-induced reductions in CBF would decrease the CBF response to changing  $P_{aCO_2}$  ( $\Delta CBF/\Delta P_{aCO_2}$ ). Anesthetized New Zealand white rabbits were randomly assigned to one of three temperature groups—group 1 (37° C, n = 9); group 2 (31° C, n = 10); or group 3 (25° C, n = 10)—and were cooled using cardiopulmonary bypass. After esophageal temperature equilibration (~ 40 min), oxygenator gas flows were serially varied to achieve  $P_{aCO_2}$  values of 20, 40, and 60 mmHg (temperature-corrected). All animals were studied at all three  $P_{aCO_2}$  levels in random order. At each level of  $P_{aCO_2}$ , CBF and masseter blood flow were determined using radiolabeled microspheres. There were no significant differences between groups with respect to mean arterial pressure (~ 80 mmHg), central venous pressure (~ 4 mmHg), or hematocrit (~ 22%). Prior normothermic studies have found  $\Delta CBF/\Delta P_{aCO_2}$  to be proportional to CBF. Nevertheless, in this study, with hypothermia-induced reductions in CBF,  $\Delta CBF/\Delta P_{aCO_2}$  was not significantly different between temperature groups. Thus, hypothermia either increased the sensitivity of the cerebral vasculature to carbon dioxide and/or increased the effective level of cerebrospinal fluid respiratory acidosis produced by each increment of temperature-corrected  $P_{aCO_2}$ . This latter possibility is consistent with "alpha-stat" acid-base theory, wherein such increments of actual (temperature-corrected)  $P_{aCO_2}$  would produce increasing degrees of "respiratory acidosis" (measured at 37° C) with progressive hypothermia. When we compared  $\Delta CBF/\Delta P_{aCO_2}$  between temperature groups using  $P_{aCO_2}$  values measured at 37° C, we found that  $\Delta CBF/\Delta P_{aCO_2}$  did decrease with hypothermia-induced reductions in CBF. Over the  $P_{aCO_2}$  range of 40–60 mmHg (measured at 37° C),  $\Delta CBF/\Delta P_{aCO_2}$  equalled  $0.97 \pm 0.60 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$  at 37° C versus  $0.54 \pm 0.23 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$  at 25° C, ( $P = 0.02$ ). These findings suggest that the CBF response to changes in  $P_{aCO_2}$  is sensitive to variations around electrochemical neutrality and not to absolute levels of  $P_{aCO_2}$ . Although hypothermia lead to marked reductions in CBF, extracranial blood flow (in the masseter muscle) was little affected. This alteration of intracranial/extracra-

nial flow distribution could lead to underestimation of CBF with xenon clearance methods used during hypothermic cardiopulmonary bypass, if the detection systems used overlies extracranial muscle. (Key words: Anesthesia: cardiovascular. Brain: blood flow; carbon dioxide response. Cardiopulmonary bypass: blood gas management. Temperature: hypothermia.)

CONTROVERSY surrounds the effect of hypothermia and acid-base management upon the brain during cardiopulmonary bypass. Using the "alpha-stat" approach, arterial carbon dioxide tension ( $P_{aCO_2}$ ) and pH are maintained at 40 mmHg and 7.4 respectively, measured at 37° C, without regard to the patient's temperature (blood gases are not temperature-corrected). With this technique, it is believed the dissociation state of the  $\alpha$ -imidazole of histidine is preserved, better maintaining the structure and function of biologic molecules under hypothermic conditions.<sup>1-3</sup> In contrast, with "pH-stat" management,  $P_{aCO_2}$  and pH are maintained at 40 mmHg and 7.4 respectively, when corrected to actual patient temperature (blood gases are temperature-corrected).<sup>1-3</sup> Due to increased carbon dioxide solubility in plasma with hypothermia, pH-stat management produces relative hypercapnia and acidemia compared to that produced by alpha-stat management. A fundamental physiologic issue underlying the alpha-stat versus pH-stat controversy concerns the cerebral blood flow (CBF) response to  $P_{aCO_2}$  during hypothermic bypass—specifically, what controls the response and which acid-base strategy produces "better" physiologic conditions.

Prior studies both in humans and in animals (without cardiopulmonary bypass) have shown brain regions with lower baseline CBF undergo less absolute change in CBF in response to changes in  $P_{aCO_2}$  ( $\Delta CBF/\Delta P_{aCO_2}$ ) than do regions with higher CBF values.<sup>4-8</sup> Anesthetic and age-induced reductions in baseline CBF also appear to decrease  $\Delta CBF/\Delta P_{aCO_2}$ .<sup>8,9</sup> An explanation for these observations was offered by Ackerman,<sup>4</sup> who found  $\Delta CBF/\Delta P_{aCO_2}$  in awake normothermic humans to be described by the following relationship:

$$\Delta CBF/\Delta P_{aCO_2} \approx k \cdot CBF/MAP$$

where MAP is mean arterial pressure and k is a constant. Because the CBF/MAP ratio has the units of conductance, Ackerman suggested that  $\Delta CBF/\Delta P_{aCO_2}$  varies with the "basal tone" of the cerebrovascular bed. The author postulated that tissue metabolism, by means of its influence

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on cerebrovascular smooth muscle tone, modulates the response of the cerebral vasculature to carbon dioxide. Regions having lower CBF at any given MAP would have lower conductance and, consequently, lower  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  than regions with higher CBF at the same MAP. Hypothermia is known to decrease CBF and metabolism and would be expected, at constant arterial pressure, to result in decreased cerebrovascular conductance. We hypothesized that if  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  varies with cerebrovascular conductance,  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ , like CBF, would be lower during hypothermia as compared to normothermia. This experiment was designed to test this hypothesis using our previously described rabbit model of cardiopulmonary bypass.<sup>10,11</sup>

### Materials and Methods

The experimental protocol was approved by the Animal Care Committee of the University of Iowa. Animal preparation, vascular access, cardiopulmonary bypass techniques, and microsphere blood flow methodology were as previously described.<sup>11</sup> Anesthesia was induced with halothane in oxygen in 33 New Zealand White rabbits (weight  $4.0 \pm 0.4$  kg [mean  $\pm$  standard deviation]). After tracheal intubation, the animals were paralyzed with a succinylcholine infusion, and to achieve normocapnia the lungs were ventilated with a gas mixture containing 1.5% halothane in 33% oxygen/balance nitrous oxide. Temperature was measured with an esophageal thermistor. Central venous pressure was measured *via* the right external jugular vein, and both brachial arteries were cannulated (PE-160) for microsphere reference blood samples. Teflon catheters (14-G, 32 mm long) were inserted into each femoral artery, and after systemic heparinization (300 U/kg), an 18-Fr right atrial catheter was secured using a purse-string suture. Just prior to bypass, halothane and nitrous oxide were discontinued, and anesthesia was maintained with a loading dose and constant infusion of fentanyl and diazepam (fentanyl: loading dose 100  $\mu\text{g}/\text{kg}$ , infusion  $2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; diazepam: loading dose 2 mg/kg, infusion  $50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), and muscle relaxation was achieved with pancuronium (0.1 mg/kg).

The bypass circuit consisted of a venous reservoir, a centrifugal blood pump, a membrane oxygenator/heat exchanger, and a variable temperature water pump. Priming fluid consisted of 300 ml 6% hydroxyethyl starch in normal saline (Hetastarch<sup>®</sup>, E. I. Du Pont, Bannockburn, IL) to which was added 250 mg calcium chloride, 1000 U heparin, and  $\sim 150$  ml filtered fresh donor rabbit red blood cells to achieve a total volume of  $\sim 450$  ml and a hematocrit of  $\sim 20\%$ . A continuous in-line blood gas analysis sensor (model 200, Cardiovascular Devices, Irvine, CA), placed distal to the oxygenator, was calibrated against blood samples analyzed by standard blood gas

analysis. Bypass was initiated and maintained at a flow rate of  $100 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , monitored with a calibrated in-line electromagnetic flow meter. The pulmonary artery was clamped. *Via* purse-string suture, a 16-G left ventricular catheter allowed passive drainage to the venous reservoir. Arterial pressure was measured from the left brachial arterial catheter. Arterial pressure was a dependent variable in each experiment; *i.e.*, no pharmacologic or mechanical method was used specifically to control it.

Animals were randomly assigned to one of three temperature groups: group 1 ( $37^\circ \text{C}$ ,  $n = 11$ ), group 2 ( $31^\circ \text{C}$ ,  $n = 11$ ), or group 3 ( $25^\circ \text{C}$ ,  $n = 11$ ); the water-pump temperature was adjusted accordingly. The oxygenator was ventilated initially with a variable mixture of oxygen and nitrogen to achieve a  $\text{PaCO}_2$  of 40 mmHg, corrected to the animal's actual esophageal temperature.¶¶ After temperature equilibration to the desired level ( $\sim 40$  min of bypass), oxygenator gas flows were serially changed to achieve randomly preassigned levels of temperature-corrected  $\text{PaCO}_2$ , namely, 20, 40, and 60 mmHg. After equilibration for  $\sim 15$  min at each  $\text{PaCO}_2$ , hemodynamic and blood gas measurements were made, and organ blood flows were determined by the microsphere technique. All animals had organ blood flow measurements made at the end of each of the three periods of random-ordered temperature-corrected  $\text{PaCO}_2$ , and the order of determination, (*e.g.*,  $\text{PaCO}_2 = 20, 60, 40$ , or  $40, 20, 60$ , *etc.*) was also recorded. Sodium bicarbonate was given to maintain base excess no greater than  $-4$  mEq/l, when measured at  $37^\circ \text{C}$ . At the completion of the experiment, animals were killed by discontinuation of bypass and intracardiac administration of saturated potassium chloride solution.<sup>12</sup>

Organ blood flows were determined by radioactive microsphere technique. Isotopes included  $^{141}\text{Ce}$ ,  $^{95}\text{Nb}$ ,  $^{153}\text{Gd}$ ,  $^{46}\text{Sc}$ ,  $^{85}\text{Sr}$ , and  $^{113}\text{Sn}$  (New England Nuclear, Boston, MA). Two hundred microliters of stock microspheres ( $\sim 900,000$  microspheres), vigorously mixed for 2–4 min prior to withdrawal, were diluted in 1.5 ml suspending solution (10% dextran-40 in normal saline with 0.5% [volume/volume] Tween-80) and mixed an additional 60 s. Microspheres were injected over 30 s into the arterial perfusion tubing just proximal to its bifurcation into the two femoral inflow cannulas. Starting 15 s before microsphere injection and continuing 90 s thereafter, blood was simultaneously withdrawn from each brachial arterial catheter *via* a calibrated withdrawal pump (1.96 ml/min).

¶¶ All reported blood gas values were measured on an IL 1304 pH/blood gas analyzer at an electrode temperature of  $37^\circ \text{C}$  (Instrumentation Laboratory, Lexington, MA). Temperature corrections were performed by the internal blood gas correction program of the device (National Committee for Clinical Laboratory Standards: Definition of quantities and conventions related to blood pH and gas analysis. Catalog #C12-T).

After the experiment, the brain was removed and dissected into the following regions: right and left cerebral hemispheres, cerebellum, midbrain (including the thalamus, pons, and cerebral peduncles), and medulla. Right and left masseter muscles also were sampled. Fresh tissue samples were weighed and placed in counting tubes, and with reference blood samples, each was counted for 5 min in a  $\gamma$  counter. Isotope separation, background and overlap corrections, and organ blood flow calculations (milliliters per 100 grams per minute) were performed by standard techniques.<sup>13-15</sup> Weight-averaged values for right and left masseter blood flow and weight-averaged regional CBFs were used to calculate mean masseter blood flow and global CBF, respectively, at each  $P_{aCO_2}$ .  $\Delta CBF/\Delta P_{aCO_2}$  was calculated for each animal as the difference in global CBF between  $P_{aCO_2}$ s of 20 and 40 mmHg and 40 and 60 mmHg, divided by the differences in temperature-corrected  $P_{aCO_2}$ .

#### STATISTICS

Hemodynamic and blood gas measurements were compared using two-way repeated-measures analysis of variance, with  $P_{aCO_2}$  as the repeated measure in each animal. Differences between temperature groups in  $\Delta CBF/\Delta P_{aCO_2}$  between  $P_{aCO_2}$ s of 20 and 40 mmHg and between  $P_{aCO_2}$ s of 40 and 60 mmHg were tested using separate two-way (group, order of determination) analysis of variance. Order of determination was classified in each case as either  $P_{aCO_2}$  increasing (e.g., going from 20 to 40 or 40 to 60 mmHg) or  $P_{aCO_2}$  decreasing (e.g., from 40 to 20 or 60 to 40 mmHg). The difference between  $\Delta CBF/\Delta P_{aCO_2}$  for  $P_{aCO_2}$ s between 20 and 40 mmHg and  $\Delta CBF/\Delta P_{aCO_2}$  for  $P_{aCO_2}$ s between 40 and 60 mmHg was compared using pooled data from all temperature groups in a paired  $t$  test. Significance was assumed for  $P < 0.05$ . All results are expressed as mean  $\pm$  standard deviation.

#### Results

One animal from group 1, which had severe airway obstruction with anesthesia induction and prolonged cyanosis prior to intubation, was excluded. Three other animals were excluded from the final data analysis on statistical grounds. Three CBF data points were found to have studentized residuals exceeding three standard deviations above the mean. Each of the data points originated from one animal in each temperature group. The paired  $t$  test, which evaluated the dependence of  $\Delta CBF/\Delta P_{aCO_2}$  on  $P_{aCO_2}$  in each animal, required the presence of all data points. Therefore, the exclusion of one data point necessitated exclusion of all other related data. Thus, a total of four animals were excluded, such that  $n = 9$  in group 1,  $n = 10$  in group 2, and  $n = 10$  in group 3. Paired right and left microsphere reference counts were well matched ( $r = 0.88$ ), indicating adequacy

of microsphere mixing and distribution. There were no right-left blood flow asymmetries between either the cerebral hemispheres or masseter muscles in any temperature group at any  $P_{aCO_2}$ .

Physiologic data are presented in table 1. Within each temperature group there were no differences in temperature among the three  $P_{aCO_2}$ s (20, 40, and 60 mmHg). There were no significant differences within or among temperature groups in bypass duration, mean arterial pressure, central venous pressure, or hematocrit at any  $P_{aCO_2}$ . There were no significant differences among temperature groups with respect to temperature-corrected  $P_{aCO_2}$  or temperature-corrected  $pH_a$ . Arterial oxygen tension differed significantly among temperature groups, increasing significantly with increasing  $P_{aCO_2}$  ( $P < 0.0001$ ) and with decreasing temperature ( $P < 0.0001$ ).

Cerebral and masseter blood flow data are shown in table 2. CBF varied both with temperature ( $P < 0.0001$ ) and with  $P_{aCO_2}$  ( $P < 0.0001$ ). Masseter blood flow was significantly different among temperature groups ( $P = 0.04$ ) but was not affected by  $P_{aCO_2}$  variations ( $P = 0.24$ ).

The CBF response to alterations in temperature-corrected  $P_{aCO_2}$  is shown in figure 1.  $\Delta CBF/\Delta P_{aCO_2}$  between  $P_{aCO_2}$ s of 20 and 40 mmHg equalled  $0.65 \pm 0.53$ ,  $0.57 \pm 0.41$ , and  $0.89 \pm 0.42$   $ml \cdot 100 g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$  for temperature groups 1, 2, and 3, respectively (group:  $F = 1.86$ , degrees of freedom = 2,  $P = 0.18$ ; order:  $F = 0.21$ , degrees of freedom = 1,  $P = 0.65$ ).  $\Delta CBF/\Delta P_{aCO_2}$  between  $P_{aCO_2}$ s of 40 and 60 mmHg equalled  $0.97 \pm 0.60$ ,  $0.99 \pm 0.64$ ,  $1.54 \pm 1.23$   $ml \cdot 100 g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$  for temperature groups 1, 2, and 3, respectively (group:  $F = 1.15$ , degrees of freedom = 2,  $P = 0.34$ ; order:  $F = 0.04$ , degrees of freedom = 1,  $P = 0.85$ ). Because of unexpectedly large variance in CBF at  $P_{aCO_2} = 60$  at 25° C, we had insufficient statistical power, given our group sizes, to demonstrate an increase in  $\Delta CBF/\Delta P_{aCO_2}$  with increasing  $P_{aCO_2}$  for each temperature group. Hence, the difference between  $\Delta CBF/\Delta P_{aCO_2}$  determined between  $P_{aCO_2} = 20-40$  mmHg and that determined between  $P_{aCO_2} = 40-60$  mmHg was compared using pooled data from all temperature groups. By such a comparison,  $\Delta CBF/\Delta P_{aCO_2}$  from  $P_{aCO_2} = 20-40$  mmHg was significantly less than  $\Delta CBF/\Delta P_{aCO_2}$  from  $P_{aCO_2} = 40-60$  mmHg ( $n = 29$ ,  $t = -2.6$ ,  $P = 0.016$ ).

#### Discussion

The CBF response to changes in  $P_{aCO_2}$  is believed to be mediated, at least in part, by changes in perivascular  $pH$ .<sup>16-18</sup> Because carbon dioxide readily crosses the blood-brain barrier, hypercapnia leads to respiratory acidosis in cerebral perivascular tissue. This tissue acidosis leads to decreased vascular smooth muscle tone, cerebral vasodilation, and increased CBF.

TABLE 1. Physiologic Parameters during Cardiopulmonary Bypass

Group	Temperature (°C)	CO <sub>2</sub> level (mmHg)	PaCO <sub>2</sub> (mmHg)		pHa		PaO <sub>2</sub> (mmHg)		Mean Arterial Pressure (mmHg)	Central Venous Pressure (mmHg)	Hematocrit (%)	Bypass Duration (min)
			Corrected	37° C*	Corrected	37° C*	Corrected	37° Ct				
1 (37° C)	36.9 ± 0.3	20	23 ± 1	7.54 ± 0.06	7.49 ± 0.05	152 ± 82	82	78 ± 16	4 ± 3	21 ± 2	75 ± 21	
	36.9 ± 0.2	40	40 ± 2	7.35 ± 0.04	7.27 ± 0.03	192 ± 93	93	82 ± 12	3 ± 2	21 ± 1	57 ± 20	
	37.0 ± 0.4	60	62 ± 2	7.20 ± 0.04	7.14 ± 0.05	226 ± 119	119	81 ± 12	4 ± 2	21 ± 2	76 ± 17	
2 (31° C)	30.4 ± 0.8	20	22 ± 2	7.58 ± 0.06	7.49 ± 0.05	190 ± 89	89	80 ± 15	3 ± 2	20 ± 3	74 ± 29	
	30.6 ± 0.8	40	41 ± 2	7.36 ± 0.03	7.14 ± 0.05	215 ± 79	79	78 ± 10	3 ± 2	20 ± 2	74 ± 14	
	30.6 ± 0.8	60	62 ± 4	7.22 ± 0.05	7.40 ± 0.04	314 ± 169	169	78 ± 7	3 ± 2	21 ± 2	68 ± 21	
3 (25° C)	25.0 ± 0.2	20	22 ± 2	7.58 ± 0.04	7.40 ± 0.04	320 ± 55	55	77 ± 10	3 ± 3	21 ± 2	80 ± 27	
	25.0 ± 0.2	40	67 ± 4	7.36 ± 0.03	7.20 ± 0.02	317 ± 83	83	79 ± 9	3 ± 3	22 ± 2	78 ± 29	
	25.0 ± 0.2	60	59 ± 2	7.22 ± 0.04	7.07 ± 0.03	436 ± 128	128	79 ± 10	4 ± 3	22 ± 2	79 ± 29	

Mean ± SD. Group 1 (n = 9); groups 2 and 3 (n = 10).

\* Temperature groups significantly different only when parameter was measured at 37° C.

P = 0.0001.

† Temperature groups significantly different, P = 0.0001. PaO<sub>2</sub> increased with increasing PaCO<sub>2</sub>, P = 0.0001.

The CBF response to PaCO<sub>2</sub> during hypothermic bypass has been studied directly<sup>19-22</sup> and also can be inferred from other studies that compared CBF between alpha-stat and pH-stat management.<sup>23,24</sup> In these studies, the slope of the carbon dioxide response curve ( $\Delta\text{CBF}/\Delta\text{PaCO}_2$ ) approximates 0.5-0.9 ml·100 g<sup>-1</sup>·min<sup>-1</sup>·mmHg<sup>-1</sup>, depending on whether non-temperature-corrected or temperature-corrected values for PaCO<sub>2</sub> are used, respectively. These slopes are less than the usual normothermic value of 1.5-2.0 ml·100 g<sup>-1</sup>·min<sup>-1</sup>·mmHg<sup>-1</sup> measured in humans<sup>8,25</sup> and animals.<sup>6,7,26</sup> Because numerous studies have shown that  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  varies with baseline CBF, we hypothesized that hypothermia-induced reductions in CBF would result in a decrease in  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ .

arterial pressure was constant with varying PaCO<sub>2</sub>, carbon dioxide-induced increases in CBF must have been the result of cerebral vasodilation, i.e., decreased vascular smooth muscle tension, increased vessel radius and, therefore, increased conductance. In agreement with Ackerman,<sup>4</sup> it appears that at any given constant temperature, decreased CBF (and cerebrovascular conductance) is associated with decreased  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ . In sharp contrast, when CBF decreased with hypothermia, this relationship did not hold. Because arterial pressure was constant between the three temperature groups, hypothermia-induced reductions in CBF at each PaCO<sub>2</sub> level must have been accompanied by increased smooth muscle tension, decreased vessel radius, and decreased conductance. If  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  is proportional to cerebrovascular conductance, as suggested by Ackerman,<sup>4</sup> one would expect  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  to decrease with hypothermia. *It did not.* Clearly, hypothermia changed the interaction between the cerebral vasculature and PaCO<sub>2</sub>. That  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  remained constant, despite hypothermia-induced reductions in CBF, suggests 1) that hypothermia increased the cerebrovascular smooth muscle response to PaCO<sub>2</sub>, and/or 2) that hypothermia increased the effect (i.e., cerebrospinal fluid [CSF] respiratory acidosis) produced by a given level of temperature-corrected PaCO<sub>2</sub>.

The latter hypothesis is consistent with predictions from alpha-stat hypothermic acid-base theory. With alpha-stat management, if pHa = 7.40 and PaCO<sub>2</sub> = 40 mmHg when measured at 37° C, then pHa and PaCO<sub>2</sub> are "physiologically appropriate," maintaining the dissociation state of the  $\alpha$ -imidazole group of histidine, regardless of the patient's actual temperature.<sup>1-3</sup> With this strategy, departures from normal pHa and PaCO<sub>2</sub> when measured at 37° C represent acidemia or alkalemia and hypercapnia or hypocapnia, respectively. If, however, the pH-stat approach is used, departures from pHa = 7.40 and PaCO<sub>2</sub> = 40, when corrected to the subject's actual hypothermic temperature, constitute acid-base disturbances. Our experiment was deliberately conducted using the latter ap-

TABLE 2. Cerebral and Masseter Blood Flow with Varying Temperature and PaCO<sub>2</sub>

Group	Temperature-corrected PaCO <sub>2</sub>		
	20	40	60
Cerebral blood flow (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )*			
1 (37° C)	68 ± 7	80 ± 14	101 ± 11
2 (31° C)	38 ± 12	49 ± 8	70 ± 17
3 (25° C)	25 ± 5	41 ± 5	69 ± 25
Masseter blood flow (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )†			
1	14 ± 5	15 ± 9	13 ± 6
2	10 ± 5	9 ± 4	9 ± 4
3	20 ± 13	16 ± 11	16 ± 9

Mean ± SD. Group 1 (n = 9); groups 2 and 3 (n = 10).

\* Cerebral blood flow varied with temperature ( $P < 0.0001$ ), and PaCO<sub>2</sub> ( $P < 0.0001$ ).

† Masseter blood flow varied with temperature group ( $P = 0.04$ ), but not PaCO<sub>2</sub> ( $P = 0.24$ ) (repeated-measures ANOVA). There were no significant differences in masseter blood flow between groups at any PaCO<sub>2</sub> with *post hoc* testing.

proach; *i.e.*, PaCO<sub>2</sub> was temperature-corrected. According to alpha-stat theory, fixed values of temperature-corrected PaCO<sub>2</sub> would produce progressively greater degrees of "respiratory acidosis" as temperature is reduced. Indeed, when the CBF *versus* PaCO<sub>2</sub> relationship is presented with uncorrected PaCO<sub>2</sub> values (measured at 37° C), the severity of respiratory acidosis appears to have varied between groups, increasing with progressive hypothermia (fig. 2).  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  can be compared between temperature groups only at equivalent degrees of respiratory acidosis, which, as shown in figure 2, in this experiment would be

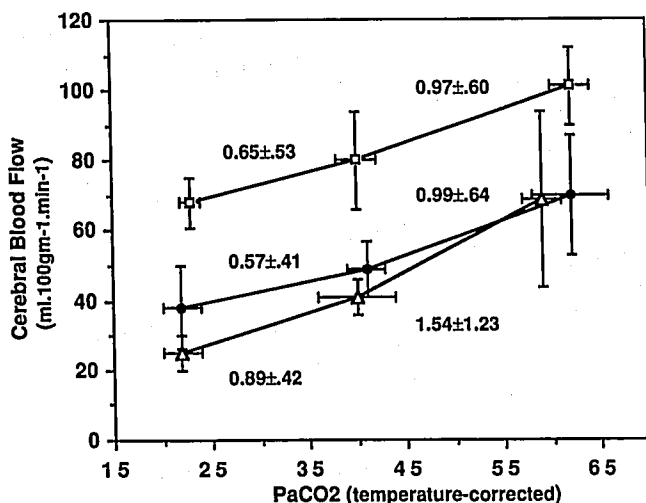


FIG. 1. Cerebral blood flow response to changes in temperature-corrected PaCO<sub>2</sub> (mean ± SD). Numeric values correspond to the slope of the carbon dioxide response curve (ml · 100 g<sup>-1</sup> · min<sup>-1</sup> · mmHg<sup>-1</sup>) between PaCO<sub>2</sub>s of 20 and 40, and 40 and 60 mmHg. The slope of CBF *versus* PaCO<sub>2</sub> was not significantly different among groups. Squares = Group 1 (37° C, n = 9); circles = group 2 (31° C, n = 10); triangles = group 3 (25° C, n = 10).

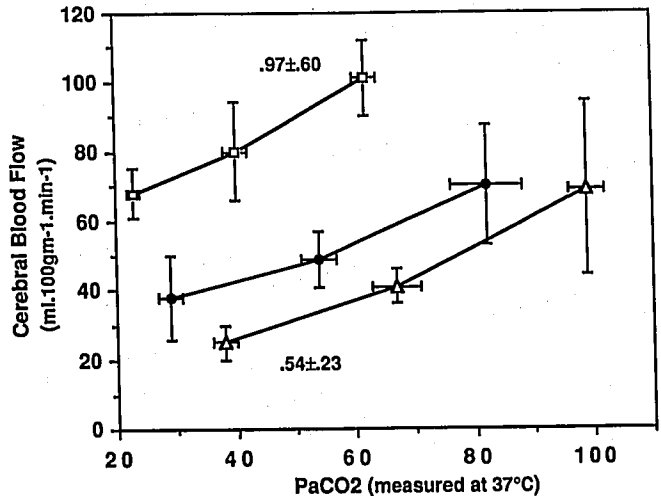


FIG. 2. Cerebral blood flow response to changes in PaCO<sub>2</sub> (measured at 37° C). Numeric values correspond to the slope of the carbon dioxide response curve (ml · 100 g<sup>-1</sup> · min<sup>-1</sup> · mmHg<sup>-1</sup>) within the overlap range of approximately 40–60 mmHg for groups 1 and 3. The slopes are significantly different,  $P = 0.02$ . Squares = group 1 (37° C, n = 9); circles = group 2 (31° C, n = 10); triangles = group 3 (25° C, n = 10).

in the range of PaCO<sub>2</sub> = 40–60 mmHg (measured at 37° C). Only groups 1 and 3 have overlapping data points in this range. When compared in this fashion, the slope of the carbon dioxide response curve ( $\Delta\text{CBF}/\Delta\text{PaCO}_2$ ) was, as predicted, significantly less during hypothermia (group 3, 25° C) as compared to normothermia (group 1, 37° C)— $0.54 \pm 0.23$  *versus*  $0.97 \pm 0.60$  ml · 100 g<sup>-1</sup> · min<sup>-1</sup> · mmHg<sup>-1</sup>, respectively ( $P = 0.02$ ).§§ This finding is consistent with the aforementioned studies that demonstrated global and regional carbon dioxide responsiveness to vary with baseline CBF values and cerebrovascular conductance. Thus, the dependence of  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  on baseline CBF is compatible with known normothermic physiology when alpha-stat definitions of acid-base physiology are used, but *not* when pH-stat definitions are used.

Hitzig compared ventilatory responses to changes in CSF pH in turtles and goats at various body temperatures.<sup>27</sup> The author concluded that ventilatory responses were not directed toward maintenance of any fixed CSF pH but rather directed toward maintenance of a CSF pH that maintained constant the dissociation of the histidine  $\alpha$ -imidazole group. In other words, ventilatory responses to changing PaCO<sub>2</sub> appeared directed toward maintenance of alpha-stat "neutrality."

Based on our findings, we propose that CBF responses to carbon dioxide are similarly tied to the fractional dissociation of the histidine  $\alpha$ -imidazole group. We believe that it is likely that the "receptor" linking cerebral peri-

§ § The Kolmogorov-Smirnov test was used because of small group sizes and the potential for different population variances between the two groups.

vascular hydrogen ion concentration with cerebrovascular tone is subject to the same increase in *p*K with hypothermia as are other biologic molecules and, therefore, that this "receptor" automatically changes its "set-point" as temperature decreases. This resetting of physicochemical neutrality toward greater *p*H values with hypothermia would have the effect of increasing the *relative degree* of perivascular acidosis produced by a constant level of temperature-corrected PaCO<sub>2</sub>. Because  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  concomitantly varies with CBF, simultaneous reductions in CBF induced by hypothermia would be expected to limit increases in  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ .

Accordingly, in this experiment we observed that the absolute value of  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ , at fixed values of temperature-corrected PaCO<sub>2</sub>, remained constant in the presence of hypothermia.  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  followed a more consistent pattern, varying with CBF (and cerebral conductance) *both* at constant and variable temperature, *only* when alpha-stat definitions of acid-base physiology were used. In total, these observations suggest the ionization state of the  $\alpha$ -imidazole of histidine is a factor in regulation of the CBF response to PaCO<sub>2</sub>.

Recently, Prough and co-workers reported that the order of determination (*i.e.*, low to high PaCO<sub>2</sub> *vs.* high to low PaCO<sub>2</sub>) influenced  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  during hypothermic cardiopulmonary bypass.<sup>22</sup> They found that CBF decreased with increasing bypass duration, the effect being to augment  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  with reductions in PaCO<sub>2</sub> and to diminish  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  with increases in PaCO<sub>2</sub>. Our experiment was not originally designed to study the effect of order (*i.e.*, bypass duration), and although randomized, the order of CBF determination was not stratified. Simultaneous analysis of both slopes (20–40 mmHg and 40–60 mmHg) would have increased our power to detect differences in  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  as a function of order. However, multivariate analysis of variance was not appropriate in our experiment because the order term was different for the two slopes. Thus, although we did not find an effect of order on  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ , our statistical power was low. We cannot rule out a possible effect of order (bypass duration) on  $\Delta\text{CBF}/\Delta\text{PaCO}_2$ .

With a few exceptions,<sup>28,29</sup> human studies have used xenon clearance techniques, with external detectors, to measure CBF during hypothermic bypass.<sup>19–24,30–33</sup> Because isotope is introduced into the systemic circulation, clearance curves are distorted by low-flow extracranial sources. At normothermia, CBF values in regions of high flow are little affected by the low-flow contribution of extracerebral counts present in the clearance curve, whereas CBF values from low-flow regions tend to be underestimated due to this contamination.<sup>34,35,¶¶</sup> These

problems are eliminated by the use of microsphere technique. Our data indicate that extracranial blood flow (in the masseter muscle) does not decrease with hypothermia, whereas intracranial blood flow is dramatically reduced. Consequently, the extracerebral component might be expected to contribute a greater proportion to the overall clearance signal during hypothermia as compared to normothermic conditions, artifactually lowering the calculated CBF value. Whether this is a significant effect in human CBF measurements is unknown. Of note, two recent studies in humans of CBF during hypothermic bypass have reported CBF values obtained by the Kety-Schmidt technique<sup>28,29</sup> that are considerably higher than those obtained by xenon clearance.

A potential methodologic problem in this experiment concerns the presence of moderate hyperoxia and the variation in PaO<sub>2</sub> between groups at assigned PaCO<sub>2</sub>s. Hyperoxia is known to decrease CBF, such that CBF values and responses to increasing PaCO<sub>2</sub> may have been diminished. However, the magnitude of the vasoconstrictive response to moderate hyperoxia is small (breathing 100% oxygen results in a 6–12% decrease in CBF compared to room-air baseline<sup>36,37</sup>), and moderate hyperoxia is not known to affect carbon dioxide responsiveness. In addition, we have performed subsequent studies using this preparation to gain insight into this question. CBF was independent of PaCO<sub>2</sub> in the range of 100–550 mmHg at 25° C (unpublished data). We do not believe that variations in PaCO<sub>2</sub> are likely to have significantly affected the results.

In summary, in agreement with prior studies, at constant temperature between 25 and 37° C it appears that  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  varies as a function of CBF (and cerebral conductance). In contrast,  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  remains constant when CBF varies as a function of temperature. Specifically, when temperature-corrected blood gas values are used,  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  does not decrease with hypothermia-induced reductions in CBF. Either 1) hypothermia increases the response of cerebrovascular smooth muscle to carbon dioxide, or 2) hypothermia increases the effect (CSF respiratory acidosis) of fixed levels of actual (temperature-corrected) PaCO<sub>2</sub>. The alpha-stat theory of hypothermic acid-base physiology, wherein PaCO<sub>2</sub> is measured at 37° C, predicts this latter possibility. Accordingly, when uncorrected PaCO<sub>2</sub> values were used,  $\Delta\text{CBF}/\Delta\text{PaCO}_2$  followed a more consistent pattern, appearing to vary with CBF regardless of temperature. Together, these observations suggest that the "receptor" that sets cerebrovascular tone is sensitive to variations around electrochemical neutrality between 25 and 37° C and not to absolute levels of PaCO<sub>2</sub>.

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