The Effects of Variations in PaCO_2 on Brain Blood Flow and Cardiac Output in the Newborn Piglet

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ABSTRACT. The acute effects of normoxemic hypocarbia and hypercarbia were examined in six newborn piglets. Brain blood flow was maintained during hypocarbia until extremely low PaCO_2 (<15 mm Hg) levels were achieved at which time total brain and cerebral blood flow decreased significantly from baseline values. Blood flow to the thalamus, cerebellum and brain stem was unchanged from baseline conditions during hypocarbia. This suggests that the newborn brain is relatively insensitive to moderate degrees of hypocarbia. Extreme hypocarbia ($\text{PaCO}_2 <15$ mm Hg) was associated with a significant increase in heart rate, accompanied by a significant decrease in mean arterial blood pressure; however, cardiac output was not significantly different from baseline determinations. Hypercarbia with normoxemia was associated with significant increases in total brain blood flow, with greater blood flow to the brain stem, cerebellum, and thalamus than to the cerebrum. The percentage of cardiac output received by the brain was also significantly increased, although total cardiac output was unchanged. This demonstrates that the newborn cerebral vasculature is sensitive to hypercarbia and that regional differences in sensitivity may account for the greater increments in blood flow to the caudal portions of the brain than that to the cerebrum. (*Pediatr Res* 18:1132-1136, 1984)

Hyperventilation to extremely low PaCO_2 levels has been advocated as a form of therapy for pulmonary vasospasm in the human neonate (7, 22). Hyperventilation has also been used in adults as a method of restoring autoregulation of the brain blood flow following brain injury (21). In the adult, there is no further reduction in cerebral blood flow below carbon dioxide tensions of approximately 20 mm Hg, presumably because ischemia induces metabolic changes in the brain that override the CO_2 control of the cerebral vasculature (3, 10, 26). Studies in the newborn human and animals have reported either an increased (18, 25) or decreased response (12, 27-29) of the cerebral vasculature to variations in arterial carbon dioxide tensions. Furthermore, many of these studies have been done in newborn

species whose brain maturation differs significantly from that of the term human neonate. The maturation of the newborn piglet brain is comparable to a human infant of 36-38 wk gestation (6, 23). Therefore, we utilized the newborn piglet to assess the acute effects of variations in PaCO_2 on brain blood flow and cardiac output, with a comparison of regional blood flow within the brain and the percentage of cardiac output received by the brain during hypo- and hypercarbia.

MATERIALS AND METHODS

Six, 1- to 4-day-old farm-bred piglets were subjects of this study. All piglets remained with the sows until the morning of the study. The weight of the piglets was 1.52 ± 0.42 kg (mean $\pm$ SD).

Surgical procedures. All surgical procedures were performed under nitrous oxide inhalation anesthesia with local anesthesia using 1% xylocaine. A tracheotomy was performed and the piglets were ventilated with a pressure-limited Amsterdam infant ventilator using a gas mixture of 70% nitrous oxide and 30% oxygen. Polyvinyl catheters (OD127 ID86) were placed in the left axillary artery, abdominal aorta via the femoral artery and inferior vena cava via the femoral vein. The left ventricle was catheterized via the right axillary artery with a $3\frac{1}{2}$ French vascular catheter (Argyle, Sherwood Medical Industries) and placement was verified by pressure tracings and at autopsy.

Experimental protocol. All animals received a gas mixture of compressed air and 25-30% oxygen following surgery and a 90-min stabilization period was allowed to elapse following surgery. Paralysis was induced with 0.5 mg/kg of pancuronium and the ventilator was adjusted to obtain a predetermined PaCO_2 range of 35-45 mm Hg for the baseline blood flow measurement. Following the baseline determination, progressive hypocarbia was induced by increasing the ventilatory rate to achieve the predetermined PaCO_2 ranges of 25-35, 15-25, and less than 15 mm Hg. Following these three hypocarbic measurements, the ventilatory rate was returned to baseline and 15 min later, hypercarbia was induced by adding 15% CO_2 to the inspired gases. Two hypercarbic measurements were made at the predetermined PaCO_2 ranges of 55-70 and greater than 70 mm Hg. Each piglet was studied under hypo- and hypercarbic conditions. Hypocarbia was always induced prior to hypercarbia due to previous work demonstrating that hypercarbia may alter autoregulation in the brain (9, 14). Each blood flow determination was performed after the PaCO_2 range.

Heart rate and systemic arterial blood pressure were monitored continuously using a Hewlett-Packard transducer (Lexington, MA) and recorded on a Hewlett-Packard polygraph (7754A series, Lexington, MA) via the abdominal aorta catheter. Arterial

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blood gases were measured using a Corning blood gas analyzer 175 (Corning Scientific Instrument, Medford, MA). Hematocrits were measured using a microhematocrit method. Just prior to each blood flow determination, the arterial blood gas, hematocrit, systolic, diastolic, and mean arterial blood pressure, and heart rate were measured. After each measurement, blood withdrawn was replaced via the inferior vena cava catheter with an equal amount of whole blood, of similar hematocrit, obtained from an age-matched piglet donor.

Blood flow was measured with microspheres, $15 \pm 5 \mu\text{m}$ in diameter, labeled with one of the following radionuclides: ^{51}Cr , ^{113}Sn , ^{46}Sc , ^{95}Nb , ^{57}Co , and ^{103}Ru (New England Nuclear, Inc., Boston, MA). Approximately 9×10^5 microspheres suspended in 1.2 ml of 10% dextran and 0.02% Tween were continuously agitated and injected into the left ventricle over 30 s and the catheter was flushed with 2 ml 0.9% saline. A reference sample of blood from the axillary artery catheter was collected continuously for 2 min beginning 10 s prior to the microsphere injection, at a withdrawal rate of 1.03 ml/min using a constant withdrawal pump (Harvard Apparatus, Millis, MA).

The position of each catheter was confirmed at autopsy. The brain was separated from the spinal cord at the level of the first cervical vertebra and weighed prior to and following adequate fixation (10% formalin). The brain was then divided into sections representing cerebrum, cerebellum, thalamus-hypothalamus, and brain stem. All sections were weighed and placed in counting vials to a height of 1 cm. The heart, liver, spleen, gastrointestinal tract, kidneys, adrenal glands, and remaining carcass were then separately incinerated at 270°C for 72 h. The resulting ash was uniformly packed to 1-cm height into counting vials. Radioactivity from all tissue samples and the reference blood samples were measured in a γ well counter (Packard Autogamma Scintillation Spectrometer, Packard Instrument Co., Downers Grove, IL). A computer (PdP-11/34 Digital Equipment Corp., Maynard, MA) was used to correct for spillover counts from the different isotopes and to calculate blood flow (13). Blood flow to the various tissues was calculated according to the equation: organ blood flow = cpm organ/cpm reference blood $\times$ rate of withdrawal of reference blood sample (13). Cardiac output was calculated by summation of the absolute blood flow to each organ and the carcass and corrected for body weight. Blood flow to the individual organs and regional sections of the brain was expressed as ml/min/100 g wet (nonfixated) weight. All blood and tissue specimens contained at least 400 microspheres, and usually greater than 700 microspheres. In no case was any change in arterial blood pressure or pulse rate noted as the result of the microsphere injection. Two blood flow determinations were discarded due to technical problems with blood withdrawal at PaCO₂ ranges of <15 and >70 mm Hg, and one because of arrhythmias noted during the microsphere injection (PaCO₂ range, 55–70). Studies in our laboratory have documented the absence of significant right to left ductal shunt in 2-day-old piglets (16).

Data analysis. Analysis of variance for repeated measurements was used to detect statistical significance. If a significant difference was found, the Dunnett's multiple range *t* test was used to

compare the means to the baseline values. Analysis of variance was used to detect significant regional brain blood flow differences within a given predetermined PaCO₂ range with the multiple comparison Newman-Keuls *post-hoc* test. The least squares method of linear regression was also used. All values were expressed as mean $\pm$ standard deviation.

RESULTS

Table 1 summarizes the values for arterial blood gases and hematocrit obtained at predetermined PaCO₂ ranges. The PaCO₂ and pH values varied as was expected during hypocarbia and hypercarbia with corresponding respiratory alkalosis and acidosis. The PaO₂ and hematocrit did not vary significantly between any of the blood flow determinations.

Figure 1 demonstrates heart rate, mean arterial blood pressure, and cardiac output at each of the predetermined PaCO₂ ranges. The mean heart rate was significantly higher than baseline at PaCO₂ range less than 15 mm Hg and significantly lower than baseline at PaCO₂ range greater than 70 mm Hg ($p < 0.05$). Similarly, the mean arterial blood pressure was significantly lower than baseline at the PaCO₂ range of less than 15 mm Hg and significantly higher than baseline at the PaCO₂ range greater than 70 mm Hg. The changes counterbalanced each other and, thus, the cardiac output did not vary significantly from baseline at any of various PaCO₂ ranges.

Figure 2 shows the relationship between total brain blood flow and PaCO₂. Because the relationship was nonlinear, log transformation of brain blood flow was performed prior to using least squares methods of linear regression. This resulted in a correlation coefficient of 0.95 ($p < 0.01$). Although the data are not

Fig. 1. Heart rate (HR), mean arterial blood pressure (MAP), and cardiac output (CO) at the various predetermined PaCO₂ ranges.

Table 1. Arterial blood gas and hematocrit values in study subjects*

	Baseline (n = 6)	Hypocarbica			Hypercarbia (n = 5)	
		n = 6	n = 6	n = 5		
Predetermined PaCO ₂ range (mm Hg)	35–45	25–35	15–25	<15	55–70	>70
PaCO ₂ (mm Hg)	41 $\pm$ 2	29 $\pm$ 1	20 $\pm$ 1	13 $\pm$ 1	64 $\pm$ 3	89 $\pm$ 4
pH	7.40 $\pm$ 0.5	7.56 $\pm$ 0.02	7.66 $\pm$ 0.04	7.78 $\pm$ 0.03	7.18 $\pm$ 0.01	7.04 $\pm$ 0.04
PaO ₂ (mm Hg)	78 $\pm$ 7	81 $\pm$ 5	82 $\pm$ 3	75 $\pm$ 5	85 $\pm$ 7	89 $\pm$ 10
Base excess (mEq/liter)	2.7 $\pm$ 1.3	3.6 $\pm$ 2.3	4.5 $\pm$ 2.0	3.5 $\pm$ 2.2	-2.4 $\pm$ 1.5	-4.4 $\pm$ 1.0
Hematocrit (%)	23 $\pm$ 3	24 $\pm$ 3	23 $\pm$ 2	24 $\pm$ 3	24 $\pm$ 2	23 $\pm$ 2

* Values are mean $\pm$ SD.

presented, the correlation between pH and total brain blood flow was also curvilinear with little change in brain blood flow at pH values of 7.40 and greater ($r = 0.89$, $p < 0.05$).

Figure 3 shows the mean blood flow at each of the predeter-

Fig. 2. Curvilinear relationship between total brain blood flow and PaCO_2 .

Fig. 3. Total brain blood flow at the various predetermined PaCO_2 ranges.

Fig. 4. Regional brain blood flow at the various predetermined PaCO_2 ranges. Flow is expressed as per cent change in blood flow as compared to baseline blood flow to that region. The asterisk designates a significant difference ($p < 0.05$) in per cent change in blood flow to the cerebrum as compared to the other regions within a given PaCO_2 range. The dagger designates a significantly ($p < 0.05$) greater change in per cent blood flow to the brain stem compared to the other regions at the $\text{PaCO}_2 > 70$ mm Hg range.

mined PaCO_2 ranges. Total brain blood flow differed significantly ($p < 0.05$) from baseline only at the hypocarbic determination of less than 15 mm Hg and at both hypercarbic determinations. However, if the three hypocarbic ranges are compared to each other, there are no significant differences among the <15, 15-25, and 25-35 mm Hg values.

Figure 4 compares the regional changes in brain blood flow within a given PaCO_2 range, expressed as per cent change in blood flow from baseline flow to that particular region. There were no significant regional differences within the PaCO_2 ranges of 25-35 and 15-25 mm Hg. Within the PaCO_2 range of <15 mm Hg, the per cent decrease from baseline blood flow to the cerebrum was significantly greater than to the other regions ($p < 0.05$). At both hypercarbic ranges, blood flow to the various regions significantly increased when compared to baseline. The per cent increase in regional flow to the cerebrum was significantly lower than the per cent increase to the cerebellum, thalamus, and brain stem ($p < 0.05$). At the PaCO_2 range >70 mm Hg, the per cent increase above baseline was significantly greater in the brain stem than to any other brain regions within this CO_2 range ($p < 0.05$).

Figure 5 examines the blood flow expressed as per cent cardiac output to the carcass, the visceral organs (heart, gastrointestinal tract, liver, spleen, adrenal glands, and kidneys) and the brain at the various CO_2 levels. The carcass and visceral organs did not show significant changes in the per cent cardiac output received by any of the CO_2 ranges due to large variation in individual organ flow. The brain showed a significant increase in the per cent cardiac output received at both hypercarbic ranges ($p < 0.05$).

The data are not shown but none of the organs (heart, liver, spleen, gastrointestinal tract, kidneys, adrenal glands, and carcass) other than the brain showed significant changes in blood flow at any of the predetermined CO_2 ranges when compared with baseline determinations.

DISCUSSION

In the present study, hypocarbica (PaCO_2 range >15 mm Hg) and hypercarbica ($\text{PaCO}_2 > 70$ mm Hg) resulted in statistically significant alterations in mean arterial blood pressure and heart rate from baseline conditions. However, there were no statistically significant alterations in cardiac output throughout the PaCO_2 ranges studied. It has previously been shown that hypoventilation induced by positive pressure ventilation may result

Fig. 5. Per cent cardiac output to the carcass, visceral organs (heart, liver, gastrointestinal tract, adrenal glands, and kidneys) and brain at the various predetermined Paco₂ ranges.

in decreased venous return to the heart and reduced cardiac output (30) and hypercarbia may produce hypertension and reflex bradycardia (24, 29). In our study, both of these effects occurred in our animals but compensatory changes in heart rate and arterial blood pressure resulted in a counterbalance phenomenon which resulted in unaltered cardiac output.

The baseline values for brain blood flow and cardiac output are somewhat higher in our acute preparation than in more chronic newborn piglet preparations, both in our laboratory (20) and others (34). The effects of anesthesia and muscle paralysis must also be considered. Nitrous oxide anesthesia may elevate cerebral blood flow but has less effect than barbiturates or halothane on cerebral blood flow (11), and all piglets were given a stabilization period of 90 min after anesthesia prior to the baseline determinations of blood flow. We have demonstrated previously in our laboratory that muscle paralysis with curare (15) has no effect on cerebral blood flow and others have demonstrated similar findings with pancuronium (2).

The correlation between total brain blood flow and Paco₂ was similar to that described in other neonatal and adult animal species (26, 27, 29). Specifically, we demonstrated that the relationship becomes nonlinear below CO₂ tensions of 20–25 mm Hg. This implies that the newborn piglet's cerebral vasculature has a response similar to that seen in mature animals, such that during extreme hypocarbica there is decreasing Paco₂ vascular sensitivity due to the release of local vasodilating substances such as adenosine, lactate, and/or potassium (3). We cannot determine on a local basis whether the response of cerebral vasculature was due to variations in Paco₂ or other local factors that were altered in response to changing Paco₂ levels. For instance, the change in pH as a result of changes in Paco₂ may produce an effect on cerebral blood flow. Whether metabolic acidosis will result in increased cerebral blood flow in neonatal animals is controversial (5, 33).

Total brain blood flow and blood flow to the cerebrum decreased significantly from baseline values only at the Paco₂ range of <15 mm Hg. This cut-off point is somewhat arbitrary; however, the Paco₂ ranges examined were selected prior to the study. The observation that there were no significant differences in brain blood flow among the three hypocarbica ranges suggests that there is a gradual decline in brain blood flow throughout the hypocarbica range that reaches a statistically significant de-

crease at the Paco₂ <15 mm Hg range. Our study confirms the findings of Shapiro *et al.* (29), in newborn puppies, that there is little decrease in brain blood flow during hypocarbica. However, it should be noted that the Paco₂ range of <15 mm Hg did result in a significant reduction in cerebral blood flow when compared with baseline. The magnitude of reduction is also rather significant (40% of baseline); thus, beyond this range of hyperventilation, significant cerebral ischemia may occur which may warrant concern.

The increases in brain blood flow with hypercarbia is consistent with the observation of others (4, 27, 29, 32). We did not extend our observations to Paco₂ ranges beyond 100 mm Hg; thus, we cannot determine whether the slope of the brain blood flow-Paco₂ curve would show a similar decrease to that observed in adult animals (26). In addition, the per cent cardiac output received by the brain increased significantly from baseline levels during hypercarbia. This is consistent with previous observations in newborn animals during other stresses associated with hypercarbia such as asphyxia where preservation of brain blood flow occurs at the expense of decreased blood flow to less vital organs (1). In our animal model of hypercarbia with normoxemia, it is interesting to note brain blood flow increased in spite of the stable cardiac output and presumably stable O₂ delivery to the brain. This ultimately results in hyperperfusion of the brain which, as has been suggested by many authors (31), may be a factor in the pathogenesis of cerebral hemorrhage in the newborn infant. We did not observe any signs of cerebral hemorrhage at autopsy in these newborn piglets but one would not expect to find intraventricular hemorrhage because the germinal matrix has resolved in this animal model at birth.

The regional differences observed in our piglets within a given Paco₂ range follow the same pattern observed in other newborn animal species following hypercarbia (28, 29), thus, the per cent change in blood flow to the brain stem > cerebellum > cerebrum which follows the hierarchy of maturation in the newborn's brain. This same hierarchy of preservation of blood flow has been observed following asphyxia and/or hypotension in the newborn piglet (15, 16). During hypocarbica, no regional changes were observed until total brain blood flow decreased significantly, at which time blood flow to the cerebrum was significantly less than blood flow to the other regions of the brain. This suggests that the cerebrum in the newborn piglet is less metabolically active than other brain regions and when brain blood flow decreased with hypocarbica, the other more metabolically active regions of the brain may preserve blood flow by producing local vasodilating substances.

The data are not presented but there were no significant changes in blood flow to individual organs (heart, liver, spleen, stomach, small intestine, colon, kidney, adrenal glands, and carcass) other than the brain with Paco₂ variation. This was true whether the flow was expressed as ml·min⁻¹·100 g⁻¹ or as per cent cardiac output (Fig. 5). In large part, this was due to large variations among animals in organ blood flow. There have been no previous studies to our knowledge in newborn animals concerning the effects of hypocarbica and hypercarbia without hypoxia on organ blood flow other than to the brain. Even in adult animals, the effects of hypo- and hypercarbia with normoxemia on organ blood flow vary widely from study to study (8, 17, 19).

In summary, brain blood flow in the newborn piglet did not decrease significantly from baseline values until extreme degrees of hypocarbica (Paco₂ <15 mm Hg) were achieved. Regional brain blood flow to the cerebrum decreased significantly when total brain blood flow fell, whereas blood flow to the brain stem, thalamus, and cerebellum was preserved.

This hierarchy of regional brain blood flow may be due to regional differences in maturation of the newborn brain with less metabolically active and mature regions having altered vascular responses to various stimuli.

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Muscarinic Cholinergic Receptors in Developing Rat Lung

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ABSTRACT. Muscarinic cholinergic receptors were identified and partially characterized in crude membrane fractions of rat lung and trachea from day 17 of gestation to adulthood using (–)-[³H]quinuclidinyl benzilate (QNB). (–)-[³H]QNB binding to rat lung membrane was characteristic of muscarinic cholinergic receptor sites. Binding

capacity of muscarinic receptors sites was relatively low in rat lung compared to that in other tissues. The number of (–)-[³H]QNB-binding sites (binding capacity) decreased progressively and significantly from 79 ± 8 fmol·mg^{–1} protein on days 17–18 of gestation to 21 ± 3 fmol·mg^{–1} mean $\pm$ SEM on days 21–22 of gestation, $p < 0.01$. Binding capacity did not vary thereafter from birth to adulthood. Affinity of (–)-[³H]QNB binding for lung membranes did not change with age (K_D approximately 70 pM). (–)-[³H]QNB-binding sites were significantly higher in membrane preparations of trachea or tracheal-bronchial tissue than in lung parenchyma from both the adult and newborn rats. (–)-[³H]QNB binding was undetectable in crude membrane preparations of cultured purified type II epithelial cells isolated from the adult rat lung.

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