Response of the Cerebral Circulation to Profound Hypocarbia in Neonatal Lambs

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Hyperventilation to extremely low arterial carbon dioxide tension (Paco$_2$) has been used in the management of persistent pulmonary hypertension in newborn infants. With progressive hypocarbia, cerebral vasoconstriction occurs, raising the concern that extreme hypocarbia may result in cerebral oxygen deprivation. Therefore, I evaluated regulation of the cerebral circulation during acute hypocarbia in 10 newborn lambs. Whole-brain and regional blood flows measured using radioactive microspheres, arterial and venous (sagittal sinus) blood gases, and oxygen contents were measured in each lamb at four arterial carbon dioxide tensions. Whole-brain oxygen delivery, oxygen consumption, and fractional oxygen extraction were calculated. Finally, arterial and venous lactate concentrations were measured to assess cerebral lactate production. Whole-brain blood flow (CBF) decreased in a nonlinear fashion as Paco$_2$ ranged from 46 to 12 mm Hg [ln(CBF) = 0.025(Paco$_2$) + 3.38; r=0.70, p<0.001]. Similar responses were demonstrated for all regional blood flows examined. Cerebral fractional oxygen extraction (E) increased in a nonlinear fashion [ln(1-E) = 0.023(Paco$_2$)-1.37; r=0.80, p<0.001], and cerebral metabolic rate for oxygen was unchanged with hypocarbia. Cerebral venous lactate concentration increased significantly (3.49 ±0.23 vs. 2.01 ±0.22 mM, p<0.001) during severe hypocarbia (Paco$_2$ of <22 mm Hg), and the arterial-venous lactate concentration difference became negative. These results demonstrate uniform responses of whole-brain and regional blood flows and stable cerebral oxygen consumption during moderate and severe hypocarbia. Although there is evidence for cerebral lactate production during severe hypocarbia, this is not likely to indicate cerebral hypoxia as oxygen consumption does not change. (Stroke 1988;19:1365-1370)

Hyperventilation to extremely low arterial carbon dioxide tension (Paco$_2$) is an effective therapeutic modality for the management of persistent pulmonary hypertension in newborn infants. However, decreasing Paco$_2$ results in cerebral vasoconstriction in newborn animals, raising the possibility that a potential consequence of this therapy is cerebral ischemia. This concept is supported by work in adult animals and humans showing decreases in cerebral tissue oxygen tension (Po$_2$), the development of abnormalities in electroencephalographic (EEG) recordings, and decreased cerebral metabolic rate for oxygen (CMRO$_2$) during hyperventilation. As a result, concerns have been raised about the safety of marked hypocarbia for the developing brains of newborn infants.

In the neonatal period, the response of (whole-brain) cerebral blood flow (CBF) is linear over a wide range (31-71 mm Hg) of Paco$_2$. However, studies that have measured CBF at lower Paco$_2$ have demonstrated a nonlinear response of CBF to changes in Paco$_2$. Specifically, the change in CBF per millimeter of mercury change in Paco$_2$ decreases as Paco$_2$ decreases into the hypocarbic range. This general response pattern is seen when one considers both CBF and regional cerebral blood flow (rCBF). However, heterogeneity in rCBF responses has also been demonstrated in several studies, suggesting that certain brain regions may be more prone to oxygen deprivation during hypocarbia than others. Finally, metabolic changes during hypocarbia, in particular changes in CMRO$_2$, have also been studied in newborn animals, demonstrating decreases in CMRO$_2$ during acute hypocarbia. To the extent that a decrease in CMRO$_2$ may imply cerebral oxygen deprivation, this issue is central to the safety of inducing hypocarbia in newborn infants.

My investigation was designed to quantitatively address several issues. I used newborn lambs to
investigate the effect of acute, severe hypocarbia on CMRO$_2$. I examined the means by which the cerebral circulation regulates CMRO$_2$ by assessing the responses of CBF, cerebral oxygen delivery (OD, CBF x arterial oxygen content), and cerebral fractional oxygen extraction (E) during acute hypocarbia. I sought supporting evidence for hypocarbia-induced tissue hypoxia by examining cerebral arterial-venous lactate concentration differences. Finally, I measured rCBF to assess regional variation in hypocarbia-induced cerebral vasoconstriction.

**Materials and Methods**

Ten 1-7-day-old newborn lambs were operated on under pentobarbital anesthesia. In each lamb, polyvinyl chloride catheters (0.034 in. i.d. x 0.054 in. o.d.; Martech Medical Products, Lansdale, Pennsylvania) were placed in the left ventricle via an axillary artery, in the brachiocephalic artery via an axillary artery, in the abdominal aorta via a femoral artery, in the inferior vena cava via a femoral vein, and in the posterior sagittal sinus proximal to the confluence of the veins via a 1-in. diameter burr-hole in the middle proximal to the lambdoid sutures. The catheters entering the lamb’s extremities were protected in a pouch on the abdomen; the sagittal sinus catheter was cut, pinned, and sutured to the lamb’s scalp. The lambs were returned to their mothers and allowed 24 hours to recover. At the time of study, all lambs were standing and feeding normally. Previous work has demonstrated that this recovery period is adequate to eliminate any pentobarbital effect on CBF.$^{12}$

CBF and rCBF were measured using the reference organ radiolabeled microsphere technique as described.$^{12,16}$ The reference organ (blood) was withdrawn through the brachiocephalic artery catheter into a counting vial at 2.47 ml/min by a precalibrated pump (Harvard Apparatus, Dover, Massachusetts). After completion of the study, the lambs were killed with T-61 Euthanasia Solution (American Hoechst, Summerville, New Jersey), the position of the catheters was checked, and the brains were removed and placed in formalin for 1 week and then divided into brainstem, left (L) and right (R) cerebellum, L and R midbrain/diencephalon, L and R frontal lobe, L and R temporal lobe, L and R occipital lobe, and L and R parietal lobe samples. Gray matter samples were obtained from the caudate nuclei and white matter samples from the corpus callosum and internal capsule. Radioactivity in each sample was determined using a three-channel gamma counter (Tracor Analytic, Des Plaines, Illinois), and rCBF was calculated as described.$^{12}$ CBF was calculated using the sum of the radioactive counts and the sum of the regional brain weights for all samples rostral to the pons. Adequate central mixing of microspheres using left ventricular injection has been confirmed in newborn lambs.$^{16}$ All reference organ blood samples and all tissue samples (except white matter) contained >400 microspheres$^{19}$; white matter samples contained 200-400 microspheres.

OD, CMRO$_2$, and E were calculated as described.$^{12,20}$ Sagittal sinus Cvo$_2$ represents venous drainage from primarily cerebral cortical structures and therefore is not precisely representative of the entire tissue mass used for the calculation of CBF. However, data from previous studies show that CBF determined using microspheres and cerebral perfusion assessed by I/(Cao$_2$-Cvo$_2$), which is a measure of cerebral perfusion for only the region drained by the sagittal sinus, behave similarly.$^{12,20}$

Blood samples for pH, P$_{O_2}$, carbon dioxide tension (Paco$_2$), and oxygen content were withdrawn anaerobically into heparinized Natelson glass pipettes from the brachiocephalic artery and sagittal sinus catheters. pH, P$_{O_2}$, and Paco$_2$ were measured at 39.5°C using a Radiometer BMS3 MK2 blood gas analyzer (Copenhagen, Denmark). Blood hemoglobin concentration expressed at oxygen capacity and oxyhemoglobin saturation were measured colorimetrically in duplicate using a hemoximeter (Radiometer, Denmark), and oxygen content was calculated as the product of hemoglobin concentration and oxyhemoglobin saturation since the contribution of physically dissolved oxygen to oxygen content is negligible. Blood samples for lactate concentration were drawn into iced syringes from the brachiocephalic artery and sagittal sinus catheters, were immediately deproteinized in perchloric acid, and were assayed using a fluorometric method.$^{21}$ Blood pressure and heart rate were continuously monitored in the abdominal aorta (Gould Instruments, Oxnard, California); blood pressure was referenced to the right atrium.

On the day of study, the lambs were paralyzed with 0.1 mg/kg pancuronium, anesthetized with fentanyl (20 μg/kg bolus followed by 10 μg/kg/hr infusion), and ventilated using an infant ventilator (Bird Co., Palm Springs, California) with a gas mixture that provided a Pao$_2$ of 80-120 mm Hg and a Paco$_2$ of 35-40 mm Hg. Pancuronium has been shown to have no effect on CBF or CMRO$_2$. Fentanyl has been shown in this model to have no effect on baseline CBF or CMRO$_2$ and to have no effect on the hypoxic and autoregulatory responses of the cerebral circulation.$^{22}$ No painful procedures were performed during the course of the study.

One measurement each of CBF, arterial and venous pH, P$_{O_2}$, Paco$_2$, oxygen content, and lactate concentration were made in each lamb after a 30-minute control period. Paco$_2$ was then altered by adjusting the ventilator rate; Pao$_2$ was maintained with small adjustments in FiO$_2$. Each lamb had CBF, pH, P$_{O_2}$, Paco$_2$, oxygen content, and lactate concentration measured at three or four levels of Paco$_2$. The order of the conditions [control (Paco$_2$ of >30 mm Hg, n = 10), moderate hypocarbia (Paco$_2$ of 23-30 mm Hg, n = 20), and severe hypocarbia (Paco$_2$ of >22 mm Hg, n = 11)] were varied from lamb to lamb. Each lamb was allowed to stabilize.
for 30 minutes at each PaCO2 level. After measurements were made at each condition, the lamb was returned to control PaCO2 for 30 minutes. Blood pressure and heart rate were continuously monitored throughout the entire study period. After the last measurements, the lamb was killed.

The responses of CBF, rCBF, OD, and E to changes in PaCO2 were evaluated using least-squares linear regression analysis after natural log transformation. For rCBF, L and R samples were combined since they did not differ. After determination of the standard error of the slope, significance of the slope compared with \( b = 0 \) was assessed at \( p<0.05 \) using \( t \) tests. rCBF slopes were also compared with each other using two-tailed \( t \) tests and a \( p<0.05 \) level of significance. The response of CMRO2 to changes in PaCO2 was evaluated without transformation. Paco2, CBF, OD, CMR02, E, mean arterial blood pressure (MABP), heart rate, venous lactate concentration, and arterial-venous lactate concentration difference were compared using one-way analysis of variance. If the overall \( F \) test was significant, individual conditions were compared using paired \( t \) tests and the Bonferroni correction for multiple comparisons.

Results

The response of CBF to changes in Paco2 over the range 12–46 mm Hg is depicted in Figure 1. The relation is nonlinear and is described by the equation \( \ln(\text{CBF}) = 0.025(\text{Paco2}) + 3.38 \) (\( r = 0.70, \ p<0.001 \)). The response of rCBF was quite similar. For all brain regions examined the slopes were between 0.020 and 0.026 ln(ml/min/100 g/mm Hg), with \( r \) between 0.61 and 0.72, all significant at \( p<0.001 \) (Table 1). The response of OD to changes in Paco2 is depicted in Figure 2 and is described by \( \ln(\text{OD}) = 0.026(\text{Paco2}) + 1.50 \) (\( r=0.73, /XO.001 \)). The relation between E and Paco2 (Figure 3) is described by \( \ln(1 - \text{E}) = 0.023(\text{Paco2}) - 1.37 \) (\( r=0.80, p<0.001 \)).

In contrast to CBF, OD, and E, CMRO2 did not change with Paco2 [CMRO2 = 0.0065(Paco2) + 4.39; \( r=0.08, p>0.05 \)] (Figure 4).

Table 2 gives hemodynamic and physiologic data during control, moderate hypocarbia, and severe hypocarbia conditions. There were no significant differences in CMRO2, MABP, and heart rate. CBF and OD decreased and E increased significantly with progressive hypocarbia. Venous lactate concentration increased significantly only during severe hypocarbia. The arterial-venous difference in lactate concentration was positive during the control and moderate hypocarbia conditions and negative (indicating cerebral lactate production) during severe hypocarbia.

Discussion

My study examining the response of the newborn lamb cerebral circulation to progressive hypocarbia yielded several important results. The responses of CBF and rCBF to hypocarbia were nonlinear, with no significant differences among regions. More importantly, CMRO2 was unchanged even during severe hypocarbia.
Previous studies of the cerebral circulation in neonatal animals have demonstrated nonlinear decreases in CBF similar to that seen in newborn lambs. However, discrepancies are evident when data examining rCBF responses to hypocarbia are considered. Hansen et al., using newborn piglets, demonstrated a greater percent decrease in CBF compared with cerebellar, thalamic, or brainstem regional blood flows as PaCO$_2$ decreased from 35 to 15 mm Hg. Shapiro et al., using newborn puppies, were able to show significant decreases only in subcortical white matter regional blood flow as PaCO$_2$ decreased from 34 to 22 mm Hg; multiple gray matter regions examined failed to yield significant regional blood flow decreases. Young and Yagel, also using newborn puppies, were unable to demonstrate a decrease in cortical regional blood flow with hypocarbia but demonstrated decreases in diencephalon, brainstem, and spinal cord regional blood flows. There are several plausible explanations for these discrepancies, including methodology and species differences. Specific methodology concerns include differences in study design, in techniques used to measure blood flow, in anesthesia, and difficulty in demonstrating significance when absolute changes are small and experimental variability is high. Regarding species differences, newborn lambs have a more mature brain at birth than either piglets or puppies. It is most likely that the differences noted relate to methodology rather than species-specific maturational factors. This is supported by the fact that the work of Reuter and Disney in newborn puppies demonstrated rCBF responses similar to that in newborn lambs. rCBF decreased in a nonlinear fashion and to a similar degree with progressive hypocarbia in all brain regions examined. This most recent study using puppies is more similar to my study in experimental design and method of blood flow measurement than is earlier work.

My data also add to published information on the response of the newborn lamb cerebral circulation to changes in PaCO$_2$. Of interest is the lack of regional differences in the responses of several brain regions to hypocarbia. Previously, rCBF response differences were shown during hypercarbia. The response of brainstem regional blood flow to hypercarbia was greater than that seen in the cerebellum, the cerebral cortex, and the gray matter, which in turn demonstrated greater responses than the white matter. An obvious difference between the two studies is that the earlier work was

**TABLE 2. Physiologic and Metabolic Parameters Under Conditions of Hypocarbia in 10 Hyperventilated Newborn Lambs**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>PaCO$_2$ (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>CMRO$_2$ (ml/100 g/min)</th>
<th>OD (ml/100 g/min)</th>
<th>E</th>
<th>MABP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Lacate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 37.5±1.5</td>
<td>81.7±7.2</td>
<td>4.73±0.27</td>
<td>13.08±1.08</td>
<td>0.38±0.03</td>
<td>82±4</td>
<td>218±8</td>
<td>2.01±0.22</td>
</tr>
<tr>
<td>Moderate hypocarbia</td>
<td>20 27.9±0.7*</td>
<td>58.9±5.2*</td>
<td>4.36±0.23</td>
<td>8.96±0.75*</td>
<td>0.50±0.03</td>
<td>77±3</td>
<td>215±10</td>
<td>2.34±0.35</td>
</tr>
<tr>
<td>Severe hypocarbia</td>
<td>11 17.8±0.7*§</td>
<td>47.5±1.9*§</td>
<td>4.58±0.15</td>
<td>7.50±0.31*§</td>
<td>0.61±0.01*</td>
<td></td>
<td>80±2</td>
<td>234±7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PaCO$_2$, arterial carbon dioxide tension; CBF, whole-brain cerebral blood flow; CMRO$_2$, cerebral metabolic rate for oxygen; OD, cerebral oxygen delivery; E, cerebral fractional oxygen extraction; MABP, mean arterial blood pressure; HR, heart rate; V, venous; A-V, arterial-venous difference.

*tp<0.001, 0.01 different from control.

§§t§p<0.001, 0.05, 0.01 different from moderate hypocarbia.
performed in awake lambs, while I used paralyzed, anesthetized, ventilated lambs. However, if one looks at the response of CBF in a comparable region in both studies (Paco$_2$ of 28-40 mm Hg), the slopes are 2.38 and 2.44 ml/100 g/min/mm Hg Paco$_2$ (current and previous, respectively). This, coupled with data showing equal hypoxic responses in awake and similarly anesthetized lambs as well as normal autoregulation with changes in blood pressure, are consistent with no effect of fentanyl and pancuronium on regulation of the cerebral circulation. A second possible explanation for the findings is that, with the smaller changes in rCBF with hypocapnia, true regional differences are obscured.

CMRO$_2$ has also been assessed in several studies of newborn animals. To the extent that a decrease in CMRO$_2$ may imply central nervous system (CNS) oxygen deprivation, these measurements provide important information about the safety of hyperventilation in newborn infants. Hansen et al$^{13}$ studied changes in CMRO$_2$ in newborn piglets during both acute and more prolonged (2 hours) hypocapnia. After 30 minutes of hypocapnia, both CBF and CMRO$_2$ were significantly decreased; however, by 60 minutes CMRO$_2$ had returned to baseline. In newborn puppies only acute hypocapnia has been evaluated, with demonstration of decreased CMRO$_2$.$^4$ In contrast, my data is the first evidence in a newborn model of stable CMRO$_2$ during acute, severe hypocapnia.

Analysis of the data in Figures 1 and 3 in a manner different from that presented in "Results" provides some interesting information about regulation of the cerebral circulation during hypocapnia to preserve CMRO$_2$. Although the data in the figures is best presented from statistical and biologic standpoints as a continuous function, there is a point (22 mm Hg Paco$_2$) below which regulation of cerebral oxygenation changes. This point was determined by linear regression analysis without transformation of the data above and below various levels of Paco$_2$. The best fits were obtained using 22 mm Hg Paco$_2$, below which no further decrease in CBF [CBF = 0.024(Paco$_2$) +0.70; r = 0.009] or in E [E= -0.003(Paco$_2$) + 0.67; r= -0.17] occur. Thus, above 22 mm Hg Paco$_2$, CMRO$_2$ is maintained by an increase in E as the cerebral vascular bed constricts. With more severe hypocarbia, CMRO$_2$ is maintained by attenuation of cerebral vasoconstriction, with no further increases in E. Several mechanisms can be proposed to explain the attenuation of hypocarbic cerebral vasoconstriction at very low Paco$_2$. Progressive vasoconstriction as well as decreased oxygen availability during alkalosis (the Bohr effect) may combine to cause tissue hypoxia. The net result is a compensatory vasodilation due to elaboration of local mediators or due to the effects of hypoxia-induced acidosis. This mechanism is supported by data showing decreases in cerebral cortical Po$_2$ and CMRO$_2$, when adult animals and humans are hyperventilated$^{15,6,8,9}$ and changes in the dominant frequency of the EEG when Paco$_2$ is <20 mm Hg.$^7$ In contrast, my data demonstrate stable CMRO$_2$, even during extreme hypocapnia. Furthermore, work in both newborns and adults has demonstrated normal levels of high-energy phosphates with Paco$_2$ as low as 12 mm Hg.$^{15,25,26}$ Another explanation that is consistent with my data (increased venous lactate concentration despite normal CMRO$_2$) is the observation that glycolytic activity increases with respiratory alkalosis.$^{27}$ Furthermore, incubation of brain tissue in alkalotic solutions leads to increased concentrations of lactate even in the presence of hyperbaric oxygen.$^{28}$ This increase in glycolytic activity may be the result of stimulation of phosphofructokinase.$^{26}$

Despite several studies now available, disagreement still exists about whether reduction of CBF with hypocapnia is harmful. My data as well as that of Hansen et al$^{13}$ (prolonged hypocapnia with neonatal piglets) suggest not. With hypocapnia, CMRO$_2$ is maintained initially by an increase in E as CBF decreases. With more severe hypocapnia, E increases no further and there is attenuation of cerebral vasoconstriction, possibly related to cerebral lactate production. These data are consistent with outcome data in neonates who have been hyperventilated for treatment of persistent pulmonary hypertension.$^{10,11}$ In general, despite representing an extremely high-risk group for CNS damage, their outcome is generally favorable, suggesting that hypocapnia does not contribute to CNS injury in newborn infants.

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