Response of the Cerebral Circulation to Profound Hypocarbia in Neonatal Lambs

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Hyperventilation to extremely low arterial carbon dioxide tension (Paco₂) 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 Paco₂ ranged from 46 to 12 mm Hg \( \text{ln}(\text{CBF}) = 0.025(\text{Paco}_2) + 3.38; r=0.70, p<0.001 \). Similar responses were demonstrated for all regional blood flows examined. Cerebral fractional oxygen extraction (E) increased in a nonlinear fashion \( \text{ln}(1-E) = 0.023(\text{Paco}_2)-1.37; r=0.80, p<0.001 \), 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 (Paco₂ 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 (Paco₂) is an effective therapeutic modality for the management of persistent pulmonary hypertension in newborn infants.1 However, decreasing Paco₂ results in cerebral vasoconstriction in newborn animals,2-4 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 (P_0₂), the development of abnormalities in electroencephalographic (EEG) recordings, and decreased cerebral metabolic rate for oxygen (CMRO₂) during hyperventilation.5-9 As a result, concerns have been raised about the safety of marked hypocarbia for the developing brains of newborn infants.10-11

In the neonatal period, the response of (whole-brain) cerebral blood flow (CBF) is linear over a wide range (31-71 mm Hg) of Paco₂.12 However, studies that have measured CBF at lower Paco₂ have demonstrated a nonlinear response of CBF to changes in Paco₂. Specifically, the change in CBF per millimeter of mercury change in Paco₂ decreases as Paco₂ decreases into the hypocarbic range. This general response pattern is seen when one considers both CBF and regional cerebral blood flow (rCBF).34 However, heterogeneity in rCBF responses has also been demonstrated in several studies,31-34 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 CMRO₂, have also been studied in newborn animals, demonstrating decreases in CMRO₂ during acute hypocarbia.41-42 To the extent that a decrease in CMRO₂ 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 vasocostriction.

**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 bunc-hole in the middle proximal to the lamboid 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.$^{2-16,18}$ 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 Hoochst, 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.$^{18}$ 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 (Pco$_2$), and oxygen content were withdrawn anaerobically into heparinized Natelson glass pipettes from the brachiocephalic artery and sagittal sinus catheters. pH, P$_O_2$, and Pco$_2$ 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), 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$_2$. The order of the conditions [control (Paco$_2$ of ±2 mm Hg, n=11), 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 CMR₀₂ to changes in PaCO₂ was not change with PaCO₂ [CMR₀₂ = 0.0065(PaCO₂) + 4.39; $r=0.08$, $p>0.05$] (Figure 4).

Table 2 gives hemodynamic and physiologic data during control, moderate hypocarbica, and severe hypocarbica conditions. There were no significant differences in CMR₀₂, MABP, and heart rate. CBF and OD decreased and E increased significantly with progressive hypocarbica. Venous lactate concentration increased significantly only during severe hypocarbica. The arterial-venous difference in lactate concentration was positive during the control and moderate hypocarbica conditions and negative (indicating cerebral lactate production) during severe hypocarbica.

**FIGURE 1. Scatterplot of cerebral blood flow (CBF) versus arterial carbon dioxide tension (PaCO₂) in 10 hyperventilated newborn lambs.**

**FIGURE 2. Scatterplot of cerebral oxygen delivery (OD) versus arterial carbon dioxide tension (PaCO₂) in 10 hyperventilated newborn lambs.**

**TABLE 1. Regression Equations for PaCO₂ Versus Whole-Brain and Regional Blood Flows for 10 Hyperventilated Newborn Lambs**

<table>
<thead>
<tr>
<th>Equation</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In (Whole brain) = 0.025(PaCO₂) + 3.38</td>
<td>0.70</td>
</tr>
<tr>
<td>In (Brainstem) = 0.026(PaCO₂) + 3.25</td>
<td>0.72</td>
</tr>
<tr>
<td>In (Cerebellum) = 0.024(PaCO₂) + 3.53</td>
<td>0.69</td>
</tr>
<tr>
<td>In (Midbrain/diencephalon) = 0.024(PaCO₂) + 3.37</td>
<td>0.70</td>
</tr>
<tr>
<td>In (Frontal cortex) = 0.023(PaCO₂) + 3.01</td>
<td>0.70</td>
</tr>
<tr>
<td>In (Temporal cortex) = 0.024(PaCO₂) + 3.01</td>
<td>0.67</td>
</tr>
<tr>
<td>In (Occipital cortex) = 0.020(PaCO₂) + 3.53</td>
<td>0.61</td>
</tr>
<tr>
<td>In (Parietal cortex) = 0.022(PaCO₂) + 3.47</td>
<td>0.68</td>
</tr>
<tr>
<td>In (Gray matter) = 0.026(PaCO₂) + 3.50</td>
<td>0.72</td>
</tr>
<tr>
<td>In (White matter) = 0.020(PaCO₂) + 3.28</td>
<td>0.64</td>
</tr>
</tbody>
</table>

n=40, $p = 0.001$ for all equations. PaCO₂ arterial carbon dioxide tension.

**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 $\ln(\text{CBF}) = 0.025(\text{PaCO}_2) + 3.38$ ($r=0.70$, $p<0.001$). 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 $\ln(\text{OD}) = 0.026(\text{PaCO}_2) + 1.50$ ($r=0.73$, $p<0.001$). The relation between E and PaCO₂ (Figure 3) is described by $\ln(1 - \text{E}) = 0.023(\text{PaCO}_2) - 1.37$ ($r=0.80$, $p<0.001$).

In contrast to CBF, OD, and E, CMR₀₂ did not change with PaCO₂ [CMR₀₂ = 0.0065(PaCO₂) + 4.39; $r=0.08$, $p>0.05$] (Figure 4).

**Discussion**

My study examining the response of the newborn lamb cerebral circulation to progressive hypocarbica yielded several important results. The responses of CBF and rCBF to hypocarbica were nonlinear, with no significant differences among regions. More importantly, CMR₀₂ was unchanged even during severe hypocarbica.
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 Paco₂ 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 flow as Paco₂ 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 Paco₂. 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>n</th>
<th>Paco₂ (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>CMRO₂ (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>Lactate V(mM)</th>
<th>A-V(mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>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>
<td>0.23±0.12</td>
</tr>
<tr>
<td>Moderate hypocarbia</td>
<td>20</td>
<td>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>
<td>0.02±0.07</td>
</tr>
<tr>
<td>Severe hypocarbia</td>
<td>11</td>
<td>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>80±2</td>
<td>234±7</td>
<td>3.49±0.23*</td>
<td>0.20±0.03*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Paco₂, arterial carbon dioxide tension; CBF, whole-brain cerebral blood flow; CMRO₂, 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 (Paco2 of 28-40 mm Hg), the
slopes are 2.38 and 2.44 ml/100 g/min/mm Hg Paco2 (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 pancuron-
im 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.

CMRO2 has also been assessed in several studies of newborn animals. To the extent that a decrease in CMRO2 may imply central nervous system (CNS) oxygen deprivation, these measurements provide important information about the safety of hyperventilation in newborn infants. Hansen et al15 studied changes in CMRO2 in newborn piglets during both acute and more prolonged (2 hours) hypocarbia. After 30 minutes of hypocarbia, both CBF and CMRO2 were significantly decreased; however, by 60 minutes CMRO2 had returned to baseline. In newborn puppies only acute hypocarbia has been evaluated, with demonstration of decreased CMRO2. In contrast, my data is the first evidence in a newborn model of stable CMRO2 during acute, severe hypocarbia.

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 hypocarbia to preserve CMRO2. 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 Paco2) below which regulation of cerebral oxygenation changes. This point was determined by serial linear regression analysis without transformation of the data above and below various levels of Paco2. The best fits were obtained using 22 mm Hg Paco2, below which no further decrease in CBF [CBF = 0.024(Paco2)2 + 0.70; r = 0.009] or increase in E [E= -0.003(Paco2)2 + 0.67; r= -0.17] occur. Thus, above 22 mm Hg Paco2, CMRO2 is maintained by an increase in E as the cerebral vascular bed constricts. With more severe hypocarbia, CMRO2 is maintained by attenuation of cerebral venoconstriction, with no further increases in E. Several mechanisms can be proposed to explain the attenuation of hypocarbic cerebral vasoconstriction at very low Paco2. Progressive vasoconstriction as well as decreased oxygen availability during alkalo-
osis (the Bohr effect) may combine to cause tissue hypoxia. The net result is a compensatory vasodila-
tion due to elaboration of local mediators or due to the effects of hypoxia-induced acidosis. This mech-
anism is supported by data showing decreases in cerebral cortical Po2 and CMRO2 when adult animals and humans are hyperventilated16 and changes in the dominant frequency of the EEG when Paco2 is <20 mm Hg. In contrast, my data demonstrate stable CMRO2, even during extreme hypocarbia. Furthermore, work in both newborns and adults has demonstrated normal levels of high-energy phos-
phates with Paco2 as low as 12 mm Hg. Another explanation that is consistent with my data (in-
creased venous lactate concentration despite normal CMRO2) is the observation that glycolytic activity increases with respiratory alkalosis. Furthermore, incubation of brain tissue in alkalotic solutions leads to increased concentrations of lactate even in the presence of hyperbaric oxygen. This increase in glycolytic activity may be the result of stimulation of phosphofructokinase.

Despite several studies now available, disagreement still exists about whether reduction of CBF with hypocarbia is harmful. My data as well as that of Hansen et al15 (prolonged hypocarbia with neo-

natal piglets) suggest not. With hypocarbia, CMRO2 is maintained initially by an increase in E as CBF decreases. With more severe hypocarbia, 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 pulmo-


nary hypertension. In general, despite representing an extremely high-risk group for CNS damage, their outcome is generally favorable, suggesting that hypocarbia does not contribute to CNS injury in newborn infants.

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