Regulation of Cerebral Blood Flow After Asphyxia in Neonatal Lambs

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In a postasphyxia neonatal lamb model, the responses of the cerebral circulation to hypoxic hypoxia and changes in systemic arterial blood pressure were examined. Ventilated newborn lambs (n = 14) were subjected to a gradual asphyxial insult, resuscitated, and returned to control ventilator settings. During the time 2-5 hours after asphyxia, the responses of cerebral blood flow (CBF), cerebral oxygen delivery (OD), cerebral oxygen consumption (CMRO2), and cerebral fractional oxygen extraction (E) to changes in either arterial oxygen content (CaO2) or mean arterial blood pressure (MAP) were assessed. These data were compared with measurements from nonasphyxiated lambs (n = 7). With hypoxia (n = 7), cerebral blood flow increased (CBF = 646/CaO2 + 44) compared with nonasphyxiated lambs (CBF = 1121/CaO2 + 11). In asphyxiated lambs, cerebral oxygen delivery decreased (OD = 0.41 CaO2 + 6.87), but cerebral oxygen consumption remained stable due to a proportional increase in cerebral fractional oxygen extraction (E = -0.014 CaO2 + 0.65). In nonasphyxiated lambs, cerebral oxygen delivery, consumption, and fractional extraction were unchanged with hypoxia. In response to alterations in blood pressure, both cerebral blood flow (CBF = 0.84 MAP + 6.62) and oxygen delivery (OD = 0.13 MAP + 0.77) were pressure-passive. With hypotension, cerebral fractional oxygen extraction increased (E = -0.003 MAP + 0.69) but not enough to prevent a decrease in cerebral oxygen consumption (CMRO2 = 0.042 MAP + 1.79). In nonasphyxiated lambs, cerebral blood flow, oxygen delivery, consumption, and fractional extraction did not vary with blood pressure. My data demonstrate impaired cerebral vasodilation in response to hypoxia and hypotension after an asphyxic insult. In a second study (n = 10), the role of cerebral edema in postasphyxia abnormalities of cerebral blood flow regulation was assessed. The impairment of postasphyxia cerebral vasodilation could not be attributed to the evolution of gross cerebral edema. (Stroke 1988;19:239–244)
vena cava via a femoral vein, and in the posterior sagittal sinus proximal to the confluence of the veins. The sagittal sinus catheter was placed through a 1-in.-diameter burr hole in the midline proximal to the lambdoidal 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 a 24-hour recovery period. At that time, all lambs were standing and feeding normally. Previous work has demonstrated that 24 hours is adequate to eliminate any pentobarbital effect on CBF.

**Physiologic Measurements**

CBF was measured using the reference organ radiolabeled microspheres technique as previously described. The reference organ was withdrawn through the brachiocephalic artery catheter into a counting vial by a precalibrated pump (2.47 ml/min; Harvard Apparatus, Dover, Massachusetts). After completion of the study, lambs were killed with T-61 Euthanasia Solution (American Hoechst, Summitville, New Jersey), position of the catheters was checked, and the brains were removed. Brains were placed in formalin for 1 week and then divided into left and right frontal, parietal, occipital, and temporal cortex as well as midbrain-diencephalon, cerebellum, medulla-pons, caudate nuclei, and hippocampi. The radioactivity in each sample was determined using a three-channel gamma counter (Tracor Analytic, Des Plaines, Illinois), and regional blood flows were calculated as previously described. Whole-brain blood flow was calculated using the sums of the radioactive counts and regional brain weights for all regions rostral to the pons. Adequate central mixing of microspheres using the left ventricular injection site has previously been confirmed in newborn lambs. All reference blood samples and all tissue samples contained >400 microspheres.

Blood samples for pH, carbon dioxide tension (PCO₂), oxygen tension (PO₂), and oxygen content were withdrawn anerobically into heparinized Natelson glass pipettes from the brachiocephalic artery and sagittal sinus catheters. PO₂, PCO₂, and pH were measured at 39.5°C using a Radiometer RMS3 MK2 blood gas analyzer (Radiometer, Copenhagen, Denmark). Hemoglobin concentration expressed at oxygen capacity and oxygen saturation were measured colorimetrically in duplicate by a hemoximeter (Radiometer), and oxygen content was calculated as the product of hemoglobin and oxygen saturation. Blood pressure and heart rate were continuously monitored in the abdominal aorta (Gould Instruments, Oxnard, California). Blood pressure was referenced to the right atrium.

**Assessment of Cerebral Edema**

Brains evaluated for the presence of cerebral edema were examined morphologically at the time of removal for flattening of cerebral convolutions, depression of the superior surface of the cerebellum, herniation of cerebellar tonsils and vermis, and mesial compression of the brainstem. Percent water was assessed by comparing wet and dry weights using the method of Elliott and Jasper. Brain specific gravity was determined on a kerosene-bromobenzene gradient column using the methods of Nelson et al.

**Experimental Procedures**

On the day of study, the lambs were paralyzed with 0.1 mg/kg pancuronium, anesthetized with 20 μg/kg initial dose of fentanyl followed by an infusion of 10 μg/kg/hr, intubated, and ventilated with an infant ventilator (Bird, Co., Palm Springs, California) with a baseline gas mixture of 30–35% O₂ and 65–70% N₂, to provide Pao₂ of 80–120 mm Hg and a baseline ventilator rate of 25–35 breaths/min to provide Paco₂ of 35–40 mm Hg. Pancuronium has been shown to have no effect on CBF and CMRO₂. Preliminary studies in our lab have also shown that at the dose used, fentanyl does not affect CBF and CMRO₂ in lambs (see "Discussion").

Fourteen lambs were then subjected to a gradual asphyxial insult by altering inspired gas concentrations and ventilator rate as previously described. In a stepwise fashion over 30 minutes, Pao₂ was lowered from baseline to 15–22 mm Hg, CaO₂ was lowered to 1.5–2.5 vol%, and Paco₂ was increased to 60–70 mm Hg by decreasing the ventilator rate to 6–10 breaths/min and adjusting the gas mixture to 10% O₂ and 90% N₂. Over the next 30–40 minutes heart rate and blood pressure remained stable, but during the final 10–15 minutes of the insult the lambs became bradycardic (heart rate <100 beats/min) and hypotensive (mean arterial blood pressure, MAP 20–35 mm Hg). The lambs were then returned to baseline ventilator settings and fractional inspired oxygen concentration. Initial CBF (microspheres), arterial and venous blood gases, and oxygen contents were measured 2 hours after termination of the asphyxial insult.

In hypoxic asphyxiated lambs (n = 7), inspired oxygen concentration was varied to induce hypoxic hypoxia. CaO₂ ranged from 3.5 to 25.0 vol%. CBF, arterial and venous blood gases, and oxygen contents were measured three times at different CaO₂ in each lamb. The lambs were maintained at a given inspired oxygen concentration for 15 minutes before the measurements. After each measurement, lambs were returned to baseline ventilator settings and allowed to stabilize for 30 minutes before the next change in CaO₂. The order of treatments was randomized.

Hypotensive asphyxiated lambs (n = 7) were exposed to varying blood pressures by removal or infusion of the lamb’s own blood. MAP ranged from 22 to 100 mm Hg, but 21 of the 22 determinations ranged from 42 to 100 mm Hg. When blood pressure stabilized at a new level, CBF, arterial and venous blood gases, and oxygen contents were measured. The lambs were then taken to a new blood pressure by either reinfusion of blood or further hemorrhage. Each lamb had three or four sets of measurements, the order of which was randomized.
Seven lambs were handled in a similar fashion and served as nonasphyxiated controls. Each had baseline CBF, arterial and venous blood gases, and oxygen contents measured and then had two measurements at different CaO2 (3.4–22.0 vol%) and two at different blood pressures (45–100 mm Hg). In all 21 lambs, the microsphere determination of CBF was bracketed by measurements of arterial and venous oxygen contents and blood gases. At the study’s completion, all lambs were killed and the brains were removed for microsphere analysis.

In a second study, 10 additional lambs were used for cerebral edema determination. Five lambs were paralyzed and anesthetized as above; blood gases measured every 30 minutes were maintained in the normal range until killing. These lambs served as edema controls. Five lambs were asphyxiated as previously described, resuscitated, and returned to baseline blood gases. Blood gases and arterial and venous oxygen contents were measured at 5 minutes, at 30 minutes, and every 30 minutes hence to 4 hours after asphyxia, at which time the lambs were killed. The blood gases and arteriovenous differences in oxygen content were measured to assure that the cerebral circulation after asphyxia in these lambs was comparable to those in which CBF was measured with microspheres. Brains of the five control and five asphyxiated lambs were evaluated for the presence of cerebral edema.

Data Analysis

CMR02, OD, and E were calculated as previously described.7,13 The arterial and venous oxygen contents measured before and after microsphere CBF determination were averaged for these calculations. Cerebral vascular resistance (CVR) was calculated by dividing MAP by whole-brain blood flow. The responses of CBF, CMR02, OD, and E to hypoxia or hypotension as well as the response of CVR to changes in MAP in the asphyxiated and nonasphyxiated groups were analyzed using least-squares linear regression analyses. The relation between CaO2 and CBF was not linear, so the reciprocal of CaO2 versus CBF was analyzed. The slopes for both asphyxiated and nonasphyxiated groups were compared against the null hypothesis 1^ =  0 for significance using \( t \)-test with Bonferroni correction, whereas nonasphyxiated lambs exhibited a greater increase in CBF at comparable levels of hypoxia (CBF = 1121/CaO2 + 11; \( r = 0.92; \ p < 0.01 \)). The response of CBF in asphyxiated lambs differs significantly (\( p < 0.05 \)) from that in nonasphyxiated controls. OD fell with hypoxia in asphyxiated lambs (OD = 0.41 CaO2 + 6.87; \( r = 0.59; \ p < 0.01 \)). However, CMRO2 (CMRO2 = 0.01 CaO2 + 4.22, \( r = 0.05 \)) was unchanged with hypoxia due to an increase in E (E = 0.014 CaO2 + 0.65; \( r = 0.68; \ p < 0.05 \)) (Figure 2). In nonasphyxiated lambs OD (OD = 0.02 CaO2 + 11.9; \( r = 0.04 \)), E (E = 0.002 CaO2 + 0.51; \( r = 0.19 \)), and CMRO2 were all unchanged with hypoxic hypoxia. Regional CBF demonstrated a similar pattern. Compared with nonasphyxiated lambs, the asphyxiated group had significantly smaller CBF increases with hypoxia in all the cortical regions.

### Results

#### Physiologic Variables

Table 1 depicts blood gases, CaO2, MAP, and heart rate before, during, and 2 hours after asphyxia. There are no significant differences between values obtained before and 2 hours after asphyxia. There are also no significant differences between baseline values in the 14 asphyxiated and the seven nonasphyxiated lambs.

#### Hypoxic Hypoxia

The response of CBF to isocapnic hypoxic hypoxia in asphyxiated and nonasphyxiated lambs is depicted in Figure 1. In asphyxiated lambs, CBF = 646/CaO2 + 44 (\( r = 0.80; \ p < 0.01, \ t \)-test with Bonferroni correction), whereas nonasphyxiated lambs exhibited a greater increase in CBF at comparable levels of hypoxia (CBF = 1121/CaO2 + 11; \( r = 0.92; \ p < 0.01 \)). The response of CBF in asphyxiated lambs differs significantly (\( p < 0.05 \)) from that in nonasphyxiated controls. OD fell with hypoxia in asphyxiated lambs (OD = 0.41 CaO2 + 6.87; \( r = 0.59; \ p < 0.01 \)). However, CMRO2 (CMRO2 = 0.01 CaO2 + 4.22, \( r = 0.05 \)) was unchanged with hypoxia due to an increase in E (E = 0.014 CaO2 + 0.65; \( r = 0.68; \ p < 0.05 \)) (Figure 2). In nonasphyxiated lambs OD (OD = 0.02 CaO2 + 11.9; \( r = 0.04 \)), E (E = 0.002 CaO2 + 0.51; \( r = 0.19 \)), and CMRO2 were all unchanged with hypoxic hypoxia. Regional CBF demonstrated a similar pattern. Compared with nonasphyxiated lambs, the asphyxiated group had significantly smaller CBF increases with hypoxia in all the cortical regions.

| Table 1. Physiologic Variables for Asphyxiated and Nonasphyxiated Lambs |
|------------------|------------------|-----------------|----------------|----------------|------------------|------------------|
|                  | PaO2 (mm Hg)     | PaCO2 (mm Hg)   | pH              | CaO2 (vol %)   | MAP (mm Hg)     | HR (beats/min)   |
| Asphyxiated      |                  |                 |                 |                |                 |                  |
| Baseline         | 109 ± 4          | 38 ± 2          | 7.41 ± 0.02     | 17.2 ± 1.1     | 82 ± 4          | 208 ± 10         |
| Asphyxia         | 18 ± 1*          | 53 ± 2*         | 6.90 ± 0.03*    | 2.2 ± 0.3*     | 31 ± 2*         | 67 ± 4*          |
| 2 hr after asphyxia | 107 ± 3          | 37 ± 1          | 7.32 ± 0.03     | 18.2 ± 1.2     | 75 ± 3          | 235 ± 8          |
| Nonasphyxiated   | 108 ± 4          | 39 ± 3          | 7.38 ± 0.03     | 15.8 ± 1.7     | 78 ± 5          | 212 ± 11         |

PaO2, arterial O2 tension; PaCO2, arterial CO2 tension; CaO2, arterial O2 content; MAP, mean arterial blood pressure; HR, heart rate. All values are mean ± SEM.

*p < 0.01 different from baseline, \( t \)-test with Bonferroni correction.
Variations in Blood Pressure

After asphyxia CBF fell with hypotension (CBF = 0.84 MAP + 6.62; r = 0.83; p < 0.01, t test with Bonferroni correction) (Figure 3) compared with no change in CBF over a MAP range of 45–100 mm Hg in nonasphyxiated lambs (CBF = 0.07 MAP + 72; r = 0.14). To assess whether autoregulation was abolished or partially impaired, the response of CVR to changes in MAP was examined. CVR was unchanged in asphyxiated lambs (CVR = 0.0015 MAP + 0.98; r = 0.16), whereas the anticipated decrease was seen with hypotension in nonasphyxiated lambs (CVR = 0.01 MAP + 0.22; r = 0.77; p < 0.01). After asphyxia, OD fell with hypotension (OD = 0.13 MAP + 0.77; r = 0.79; p < 0.01). E increased (E = −0.003 MAP + 0.69; r = 0.50; p < 0.05), but not enough to prevent a fall in CMRO₂ (CMRO₂ = 0.042 MAP + 1.79; r = 0.50; p < 0.05). Significant decreases in CBF and OD with hypotension were seen in all regions examined. In nonasphyxiated lambs OD, E, and CMRO₂ were all unchanged over the MAP range of 45–100 mm Hg. Similarly, regional CBF and OD were unchanged with changes in MAP. Acute hemorrhage or reinfusion of blood was not associated with a significant change in hematocrit, Paco₂, Pao₂, or CaO₂.

Cerebral Edema

There were no morphologic differences in brains of asphyxiated and control lambs. Mean ± SD brain water content (82.99 ± 0.59% control, 82.77 ± 0.22% asphyxiated lambs) and brain specific gravity (1.0405 ± 0.0009 control, 1.0399 ± 0.0008 asphyxiated lambs) also did not differ.

Discussion

The importance of postischemic events to the genesis of brain injury has been emphasized by work in several ischemia models. Neuronal damage in the neocortex and hippocampus of rats exposed to four-vessel occlusion worsened for hours to days after relatively brief forebrain ischemia, whereas other work has demonstrated the role of postischemic cerebral perfusion in central nervous system damage. My study examines another possible mechanism for extension of brain injury after asphyxia. In a neonatal lamb postasphyxia model, there was markedly impaired vasodilation in response to hypoxic hypoxia, while no cerebral vasodilation was evident with systemic hypotension. These data are the first demonstration of impaired hypoxic vasodilation after asphyxia in a newborn model. The data are consistent, however, with previous results in an adult rat postischemia model. In the adult

![Figure 1](image1.png)

**Figure 1.** Cerebral blood flow (CBF) vs. arterial oxygen content (CaO₂). Points and solid line represent response to hypoxic hypoxia in asphyxiated lambs (n = 7). Dashed line represents data from nonasphyxiated (control) lambs (n = 7, 21 measurements; data available upon request). Slopes differ; p < 0.05.

![Figure 2](image2.png)

**Figure 2.** Cerebral fractional oxygen extraction vs. arterial oxygen content (CaO₂). Points and solid line represent response to hypoxic hypoxia in asphyxiated lambs (n = 7). Dashed line represents data from nonasphyxiated (control) lambs (n = 7, 21 measurements; data available upon request).

![Figure 3](image3.png)

**Figure 3.** Cerebral blood flow (CBF) vs. mean arterial blood pressure. Symbols represent response to changes in blood pressure in individual asphyxiated lambs (n = 7). Regression line is derived from pooled data of all lambs. Dashed line represents data from nonasphyxiated (control) lambs (n = 7, 21 measurements; data available upon request).
model, as in my studies, the hypoxic response of CBF was attenuated in most brain regions examined.  

A greater body of data is available for discussion of postasphyxia cerebral autoregulation. Adult postischemia models have demonstrated intact cerebral autoregulation.  

However, prior work in newborn lambs has demonstrated that cerebral autoregulation is impaired following an hypoxic insult and that the duration of hypoxia is an important determinant of loss of normal autoregulation. These differences between newborns and adults could be the result of maturation or simply due to differences in the experimental cerebral insult.  

Several potential mechanisms exist that can explain the impaired cerebral vasoreactivity after asphyxia in newborn lambs. These include impaired vasodilation caused by obstruction of resistance vessels by cerebral edema, excessive production of cerebral vasocostructor(s) such as thromboxanes and leukotrienes, or vascular endothelial injury secondary to oxygen free radical production during reperfusion. The generation of cerebral edema would explain both the previously described postasphyxia cerebral hyperperfusion as well as the impaired vasoreactivity demonstrated in my study. This hypothesis is attractive since cerebral edema as a complication of neonatal asphyxia has been documented pathologically in Myer’s nonhuman primate asphyxia model. Furthermore, this concept is supported by the data of Grote and Schubert, who demonstrated an attenuated response of CBF to the vasodilating effects of hypoxia in the presence of cerebral edema. However, my data in newborn lambs did not demonstrate evidence to support gross cerebral edema playing a role. It is important to note, however, that histologic studies were not performed, so the presence of edema of glial cells or vascular endothelium still cannot be ruled out as a cause for impaired vasodilation. Other potential mechanisms may be elucidated by future investigation.  

It is unlikely that the paralytic and anesthetic agents I used impaired cerebral vasoreactivity. Pancuronium has previously been shown to have no effect on CBF and CMRO2 in newborn lambs. Preliminary work in our laboratory (unpublished observations) has also demonstrated no effect of fentanyl at the dose used on CBF and CMRO2. My data from nonasphyxiated lambs extend those observations. Cerebral autoregulation was intact, and the hypoxic response of CBF was identical to that previously reported in awake, unanesthetized lambs, despite the administration of both pancuronium and fentanyl.  

My results also provide pertinent data about the adaptive responses of the cerebral circulation under stress. From a theoretical standpoint, the brain has two mechanisms to maintain CMRO2: an increase in CBF, and an increase in E. Under normal circumstances in newborn lambs during hypoxic hypoxia (CaO2 of <4 vol%), CMRO2 is maintained by a sufficient increase in CBF to maintain a stable OD. E therefore remains relatively stable. However, my data demonstrate that when hypoxic vasodilation is attenuated, E does in fact increase, up to at least 0.65, to maintain stable CMRO2. Thus, my data provide evidence to support the theoretical premise that, under circumstances of failed vasodilation, the cerebral circulation will extract more oxygen to maintain stable CMRO2. This is consistent with the general hypothesis that the cerebral circulation is very sensitive to changes in oxygen availability. A similar phenomenon was seen during hypotension. Comparing a decrease in CaO2 and MAP to 33% of baseline (CaO2 = 5.7 vol%, MAP = 27 mm Hg), E increased to the same degree (0.61 at MAP = 27 mm Hg vs. 0.57 at CaO2 = 5.7 vol%). Due to the profound drop in OD with hypotension, CMRO2 could not be maintained. Thus, my data suggest a more severe impairment of vasodilation during hypotension than with hypoxic hypoxia. This concept is supported by the demonstration of no change in CVR as MAP varies. Similarly, regional CBF data demonstrate preservation of OD to the brainstem and deep cerebral structures (caudate nucleus, hippocampus) during hypoxic hypoxia, whereas OD decreases in all structures with hypotension.  

In summary, after asphyxia in newborn lambs, cerebral vasodilation is attenuated in response to hypoxia and is absent in response to hypotension. During hypoxic hypoxia, as cerebral OD falls CMRO2 is maintained due to a compensatory increase in E. However, during hypotension the decrease in OD is such that CMRO2 cannot be maintained despite an increase in E. My data suggest that during the postasphyxia period of cerebral recovery, the neonatal brain may be placed at great risk for extension of injury, in particular by subsequent episodes of hypotension.  

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