

Arterial Oxygenation Determines Autoregulation of Cerebral Blood Flow in the Fetal Lamb

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Summary

We examined autoregulation of cerebral blood flow (CBF) over the range of oxygen tension commonly observed in the chronic fetal lamb preparation. Seventeen animals were surgically prepared under general anesthesia for chronic *in utero* studies. Based on measured resting arterial P_{O_2} and calculated % saturation 24-48 h after surgery, two groups were defined: a normoxic group of eight with saturation of 57% or higher (our laboratory normal for physiologically stable preparations) and an hypoxic group of nine with saturation less than 57%. Regional CBF was measured with radiolabeled 15- μ m microspheres. Autoregulation of CBF was assessed by measuring the change in CBF when fetal mean arterial blood pressure (MABP) was acutely decreased and increased by withdrawal and reinfusion of fetal blood. In normoxic animals CBF was constant over an MABP range of 42-61 torr in all four areas of the brain examined: cerebral hemispheres, basal ganglia, cerebellum, and brain stem. In hypoxic animals CBF was pressure dependent in all areas over an MABP range of 46-73 torr, *i.e.*, autoregulation was abolished. These studies demonstrate that the mechanism of autoregulation is functionally developed in the mature fetal lamb, but operationally dependent upon arterial oxygen concentration. Below a saturation of approximately 50-60% CBF varies directly with perfusion pressure.

Abbreviations

BP, blood pressure
CBF, cerebral blood flow
cpm, count per min
i.d., internal diameter
MABP, mean arterial blood pressure
rCBF, regional cerebral blood flow

Impairment of cerebrovascular autoregulation is now generally believed to be an important pathophysiologic mechanism in the genesis of hypoxic and hemorrhagic brain injury of the newborn (10, 20). The theoretical model states that with impairment of autoregulation the cerebral microcirculation is rendered vulnerable to changes in arterial blood pressure. Systemic hypotension results in ischemic cerebral blood flow (CBF) and cerebral infarction, whereas hypertension leads to cerebral hyperemia, rupture of poorly supported capillaries in the vulnerable subependymal germinal matrix, and cerebral hemorrhage (10, 15).

Impaired autoregulation has been demonstrated in the severely asphyxiated fetal lamb (12) and in sick human newborns (11). The well oxygenated newborn lamb has been shown to autoregulate (16), and there is evidence that the normoxic fetal lamb has at least partial autoregulatory control of CBF (19). But the functional maturity of the autoregulatory mechanism in the fetal lamb, and its sensitivity to hypoxia, have not been clearly established.

The present studies were designed to examine autoregulation *in utero* in the near term fetal lamb, and to determine its stability over the range of arterial oxygen tension normally encountered in chronically prepared laboratory animals.

MATERIALS AND METHODS

We studied 17 near term fetal lambs estimated to be 130-140 days gestation. Our methods for fetal studies in the chronically prepared sheep have been described in previous publications (12, 19). Surgical preparation was done under halothane/oxygen general anesthesia with controlled ventilation via an endotracheal tube. The ewe was placed in the supine position and femoral arterial and venous catheters inserted. Through a midline abdominal approach a small hysterotomy was made to expose the fetal limbs. A hind limb was delivered and femoral arterial and venous catheters (0.86 mm i.d.) were advanced to the descending aorta and inferior vena cava. A forelimb was then delivered and an axillary artery catheter advanced to the common brachio-cephalic-trunk. The incisions were closed and all catheters were brought subcutaneously to a pocket on the ewe's flank. Prophylactic antibiotics (penicillin and gentamycin) were administered to both mother and fetus.

Studies were performed 24-48 h after surgical preparation. In the laboratory the ewe stood quietly in a cart and was allowed hay and water *ad libitum*. Fetal and maternal arterial blood pressures and fetal venous pressures were continuously recorded with Stratham P23 pressure transducers and an Hewlett Packard dynograph. Maternal femoral and fetal ascending aortic blood gases were measured with a Corning 165 blood gas analyzer at 37°C. Maternal rectal temperature was monitored and averaged 38.5°C.

Percent fetal oxygen saturation was calculated using the equation of Meschia *et al.* (13):

$$\log P_{O_2} = \log(P_{O_2})_{50,7.4} - K_2(pH - 7.4) + 0.368 \log \frac{S}{100 - S}$$

in which $(P_{O_2})_{50,7.4}$ is the oxygen tension at which the hemoglobin is half saturated at pH of 7.4, K_2 and 0.368 are two coefficients and S is the % saturation of hemoglobin with oxygen. The average values of K_2 and $\log (P_{O_2})_{50,7.4}$ for fetal hemoglobin in the lamb are 0.532 and 1.213, respectively.

Fetal regional brain blood flow was measured by the radioactive microsphere technique, using 15- μ m diameter microspheres labeled with gamma emitting [141 Ce], [51 Cr], or [85 Sr] (3M Company). For each measurement approximately 1 million spheres were injected into the inferior vena cava over 30 sec. Arterial blood samples were withdrawn at a fixed rate, 1.94 ml/min., from the brachiocephalic catheters during and for 1.5 min after microsphere injection.

After the ewe had rested quietly in the laboratory for 1-2 h a complete set of control measurements were made. Fetal hypotension was then produced by withdrawal of fetal arterial blood. Sixty to 150 ml of blood (average 92.8) were withdrawn over 5-10 min to reduce MABP by approximately 20%. After stabilization for 5-15 min under hypotensive conditions a complete second set of measurements was made. The heparinized blood was then reinfused and within 15 min a third set of measurements were made.

At the end of the experiment the ewe and fetus were painlessly

killed with intravenous sodium pentothal and saturated KCl solution. The fetus was autopsied to validate catheter placement, and the brain was removed. Tissue samples from both sides were cut, weighed, and packed in counting tubes. The samples included all of the brain stem, and deep cerebral tissue, and about 50% of the cerebral hemisphere of each side. Gamma counts of each label in the weighed tissue samples and in the arterial blood samples were measured by a Searle gamma well counter.

If microspheres are homogeneously distributed in the arterial blood supplying an organ, and are completely removed from blood during passage through the organ, then the following equation calculates organ blood flow (4):

$$F = F_a \times \frac{i_{\text{organ}}}{i_{\text{arterial}}}$$

F, organ blood flow (ml/100 g/min); F_a , withdrawal rate of the reference arterial blood sample; i_{organ} , microsphere content of the organ tissue (cpm/100 g); and i_{arterial} , microsphere content of the total reference arterial blood sample (cpm).

The data was analyzed as paired measurements, with statistical comparison by the student *t* test for paired data.

RESULTS

Seventeen near term fetal lambs were studied. Arterial oxygen tension in blood from the ascending aorta was measured in the resting state 24–48 h after surgical preparation. The range of observed values for P_{O_2} was 12.0–22.0 torr, and for calculated arterial oxygen saturation, 29.5–68.7%. The average P_{O_2} and O_2 saturation for chronically prepared physiologically stable animals in our laboratory is 19 and 57%. Using an arterial O_2 saturation of 57% as the arbitrary dividing point we have defined two groups for comparative purposes: eight animals with an O_2 saturation of 57% or higher are termed the normoxic group and nine animals with less than 57% O_2 saturation are termed the hypoxic group (Table 1). Except for P_{O_2} and O_2 saturation there were no major differences in control state measurements between these groups. Although the arterial pH of the hypoxic group was slightly lower it was well within the normal range.

Table 2 and Figure 1 present the data for the normoxic group during decreasing and increasing blood pressure (BP). Eight animals were subjected to hypotension by blood withdrawal with a mean decrease of 26% in arterial BP. In six animals a mean increase in BP of 41% was then produced by reinfusion of withdrawn blood. Regional cerebral blood flow (rCBF) measurements before and after blood withdrawal and before and after reinfusion were compared. Although BP changes were pronounced (26% and 41%) there were no changes in rCBF in any region of the brain.

Arterial oxygenation did not change although PCO_2 increased significantly and pH fell during hypotension. We ascribe this respiratory acidosis to impairment of placental CO_2 exchange as a result of decreased fetal placental perfusion (18, 19).

In Table 3 and Figure 1 the data for the hypoxic group during decreasing and increasing BP is presented. Eight animals were made hypotensive by blood withdrawal with a mean decrease of 31% in arterial BP. In seven animals BP was increased by reinfusion of withdrawn blood, with a mean increase in BP of 39%. These are very similar to the BP changes in the normoxic group. Again rCBF measurements before and after blood withdrawal and before and after reinfusion were compared. All regions of the brain showed a pressure dependent rCBF, regional perfusion significantly decreasing with hypotension and increasing with hypertension. Not only did the mean values for rCBF change significantly, but every animal in this group demonstrated impaired autoregulation with changing BP. The magnitude of the changes in PCO_2 and pH was similar to the normoxic group.

DISCUSSION

These studies indicate that the mechanism of autoregulation is functionally mature in the near term fetal lamb, and can be demonstrated to be operationally intact in chronically prepared,

Table 1. Comparison of control state measurements of normoxic and hypoxic groups¹

| Variable | Normoxic group (8) | Hypoxic group (9) | Difference | P value |
|---|--------------------|-------------------|------------|---------|
| rCBF (ml·g ⁻¹ ·min ⁻¹) | | | | |
| Brain stem rCBF | 1.92 ± 0.19 | 1.78 ± 0.24 | | NS |
| Cerebellum rCBF | 1.55 ± 0.17 | 1.60 ± 0.19 | | NS |
| Basal ganglia rCBF | 1.76 ± 0.27 | 1.77 ± 0.24 | | NS |
| Cerebral hemispheres rCBF | 1.00 ± 0.11 | 1.13 ± 0.14 | | NS |
| MABP (torr) | 54.6 ± 3.0 | 56.6 ± 2.4 | | NS |
| P_{aO_2} (torr) | 19.8 ± 0.5 | 15.9 ± 0.7 | 3.9 | <0.001 |
| O_2 Saturation (%) | 63.3 ± 1.5 | 45.7 ± 2.9 | 17.6 | <0.001 |
| P_{aCO_2} (torr) | 37.8 ± 0.9 | 38.0 ± 1.4 | | NS |
| pH | 7.41 ± 0.01 | 7.37 ± 0.01 | | <0.02 |

¹ rCBF is regional cerebral blood flow, MABP is mean arterial blood pressure. All blood gas values are for ascending aortic blood, proximal to the ductus arteriosus. Statistical comparison by the *t* test for unpaired samples. All values are expressed as mean ± S.D.M.

Table 2. Autoregulation of fetal rCBF in normoxia¹

| Variable | Decreasing blood pressure (8) | | | | Increasing blood pressure (6) | | | |
|---|-------------------------------|-------------|------------|---------|-------------------------------|--------------|------------|---------|
| | Control | Hypotension | Change (%) | P value | Control | Hypertension | Change (%) | P value |
| rCBF (ml·g ⁻¹ ·min ⁻¹) | | | | | | | | |
| Brain stem rCBF | 1.89 ± 0.19 | 1.90 ± 0.24 | +1 | NS | 1.69 ± 0.25 | 1.76 ± 0.15 | +4 | NS |
| Cerebellum rCBF | 1.57 ± 0.16 | 1.43 ± 0.20 | -9 | NS | 1.20 ± 0.15 | 1.38 ± 0.17 | +15 | NS |
| Basal ganglia rCBF | 1.70 ± 0.27 | 1.65 ± 0.25 | -3 | NS | 1.55 ± 0.23 | 1.68 ± 0.24 | +8 | NS |
| Cerebral hemispheres rCBF | 1.03 ± 0.10 | 0.98 ± 0.13 | -5 | NS | 0.88 ± 0.13 | 0.98 ± 0.10 | +11 | NS |
| MABP (torr) | 56.6 ± 3.7 | 41.8 ± 2.8 | -26 | <0.001 | 43.5 ± 4.5 | 61.3 ± 5.7 | +41 | <0.001 |
| P_{aO_2} (torr) | 19.7 ± 0.5 | 20.8 ± 0.8 | | NS | 20.8 ± 0.8 | 19.6 ± 0.5 | | NS |
| O_2 Saturation (%) | 62.9 ± 1.6 | 63.5 ± 2.1 | | NS | 63.8 ± 2.0 | 59.3 ± 1.8 | | NS |
| P_{aCO_2} (torr) | 37.3 ± 1.0 | 41.1 ± 1.4 | +3.8 | <0.02 | 42.0 ± 1.6 | 41.8 ± 1.8 | | NS |
| pH | 7.41 ± 1.0 | 7.38 ± .01 | -0.03 | <0.02 | 7.38 ± 0.01 | 7.37 ± 0.02 | | NS |

¹ Abbreviations as for Table 1. Statistical comparison by the *t* test for paired samples. Paired measurements of all variables were compared before and after blood withdrawal and before and after reinfusion. In most cases, the measurements made during hypotension served as the controls for the measurements made during hypertension. All values are expressed as mean ± S.D.M.

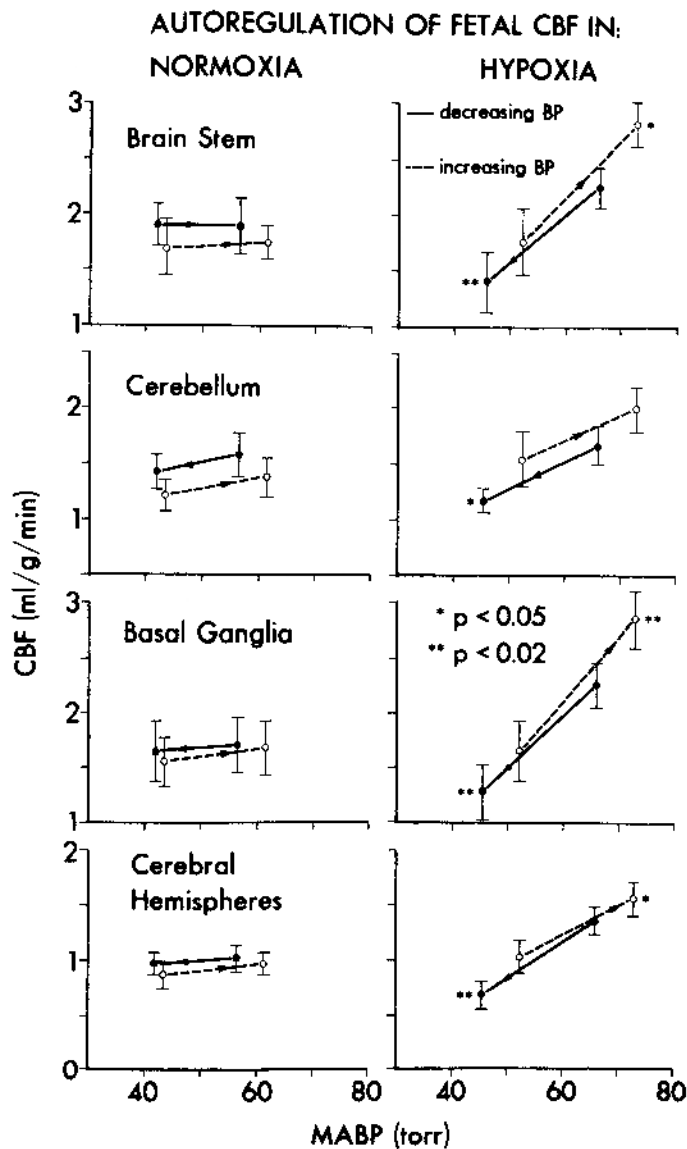


Fig. 1. Autoregulation of fetal regional cerebral blood flow in normoxia and hypoxia. The vertical bars represent the standard deviations of the means. The direction of the arrows indicates decreasing blood pressure (BP) or increasing BP. Thus solid lines are decreasing BP, hatched lines increasing BP. Abbreviations are as in Table 1.

physiologically stable, and well oxygenated animals. It appears, however, that autoregulation is operationally very dependent upon arterial oxygen concentration and, in these experiments, was consistently abolished when arterial oxygen saturation was below approximately 57%. Because fetal hemoglobin in our animals averages 11.5g/dl this corresponds to an arterial O_2 content of 8–9 ml/dl or 3–4 millimoles. This is above the level of oxygenation at which hypoxic hyperemia is observed (1, 6). Jones *et al.* (8) have demonstrated that CBF in the lamb is a function of arterial oxygen concentration over a wide concentration range. In the fetal animal they observed a hyperbolic relationship between CBF and arterial O_2 content when O_2 content was ≈ 3 mM (7); thus, only lower arterial levels of O_2 have an important influence on fetal CBF. It appears that autoregulation in the fetus is abolished at a level of oxygenation a little higher than that required to demonstrate hypoxic hyperemia.

The threshold of hemoglobin O_2 saturation for impairment of autoregulation in the fetus, 50–60%, is also very similar to that which abolishes autoregulation in adult dogs (3).

Although impairment of autoregulation was evident, these experiments do not provide any indication of the time relationships involved. That is, they do not tell us whether autoregulation is functionally oxygen dependent with immediate recovery upon restoration of normoxia or whether hypoxia temporarily disrupts the mechanism of autoregulation with a time delay to restoration of normal function. Previous work from our laboratory suggests that the latter may be the case (2).

In 1969 Purves and James (16) observed autoregulation of CBF in the fetal and newborn lamb. They studied acutely exteriorized fetal lambs with normal arterial blood gases (mean PO_2 , 23–24 torr and mean PCO_2 40 torr) and found that grey matter blood flow was independent of MABP over the range 43–90 torr for exteriorized fetuses and 45–93 torr for newborn lambs. Although they attempted to assess autoregulation with elevated PCO_2 , they did not look at the effect of reduced oxygen tension. Toubas *et al.* (18) also observed that CBF was maintained during hemorrhagic hypotension in the well oxygenated fetal lamb.

The newborn dog (5) and piglet (9), which have immature brains at birth, were also observed to autoregulate between MABPs of 27–97 torr and 50–105 torr, respectively. Although normal autoregulation has not yet been demonstrated in the human newborn, it has been observed in young animals with both mature and immature brains at birth.

In a previously published study (19) we reported that the physiologically stable fetal lamb showed evidence of CBF autoregulation to BP changes of $\pm 20\%$. But autoregulation to hypotension was incomplete, probably because arterial oxygen concentration in some of those animals fell near the threshold for impairment. When normoxia is more rigidly defined and normo-

Table 3. Impaired autoregulation of fetal rCBF in hypoxia¹

| Variable | Decreasing blood pressure (8) | | | | Increasing blood pressure (7) | | | |
|---|-------------------------------|-----------------|------------|---------|-------------------------------|-----------------|------------|---------|
| | Control | Hypotension | Change (%) | P value | Control | Hypertension | Change (%) | P value |
| rCBF ($ml \cdot g^{-1} \cdot min^{-1}$) | | | | | | | | |
| Brain stem rCBF | 2.27 ± 0.28 | 1.41 ± 0.19 | -38 | <0.02 | 1.77 ± 0.31 | 2.85 ± 0.21 | +61 | 0.05 |
| Cerebellum rCBF | 1.69 ± 0.11 | 1.18 ± 0.18 | -30 | <0.02 | 1.55 ± 0.25 | 2.05 ± 0.20 | +32 | NS |
| Basal ganglia rCBF | 2.26 ± 0.26 | 1.28 ± 0.20 | -43 | <0.01 | 1.65 ± 0.29 | 2.85 ± 0.26 | +73 | <0.02 |
| Cerebral hemispheres rCBF | 1.36 ± 0.12 | 0.80 ± 0.13 | -41 | <0.001 | 1.02 ± 0.16 | 1.56 ± 0.15 | +53 | <0.05 |
| MABP (torr) | 66.3 ± 4.5 | 45.8 ± 3.2 | -31 | <0.01 | 52.3 ± 3.8 | 72.9 ± 5.3 | +39 | <0.001 |
| PaO_2 (torr) | 16.0 ± 0.6 | 20.1 ± 1.3 | +4.1 | <0.02 | 16.4 ± 1.0 | 15.0 ± 0.8 | | NS |
| O_2 Saturation (%) | 44.5 ± 3.0 | 51.4 ± 4.2 | | NS | 45.3 ± 3.2 | 38.6 ± 4.8 | | NS |
| $PaCO_2$ (torr) | 38.2 ± 2.0 | 42.0 ± 2.3 | +3.8 | NS | 38.7 ± 1.3 | 41.9 ± 2.7 | | NS |
| pH | 7.36 ± 0.01 | 7.28 ± 0.03 | -0.08 | <0.05 | 7.35 ± 0.04 | 7.33 ± 0.03 | | NS |

¹ Abbreviations as for Table 1. Statistical comparison by the *t* test for paired samples. Paired measurements of all variables were compared before and after blood withdrawal and before and after reinfusion. In most cases, the measurements made during hypotension served as the controls for the measurements made during hypertension. All values are expressed as mean \pm S.D.M.

capnia prevails, as in the present study, no difference is seen in autoregulation to decreased or increased BP within the range of 40–70 torr.

Although in our animals PCO_2 increased during hypotension, probably as a result of decreased fetal placental perfusion, the increase was the same in normoxic and hypoxic animals and therefore could not account for the observed differences in rCBF response. During hypertension PCO_2 was unchanged in normoxic animals but rose by an average of 3.2 torr in the hypoxic group. The CO_2 responsiveness of CBF in fetal animals has been measured (16). This may explain why the % increase in rCBF in the hypoxic group was out of proportion compared to the % increase in MABP.

Impaired autoregulation of CBF in sick newborn infants has been inferred by the studies of Lou *et al.* (11) and Milligan (14). They correlated measurements of CBF and MABP in stressed infants and concluded that CBF was pressure dependent. It can be reasonably assumed, therefore, that the observations we have made in fetal lambs may have some relevance to the human newborn. Because the type of controlled experiment possible with an animal model is impossible to reproduce in a clinical setting, validation of this assumption will be difficult; however if a theoretical model, based on animal experimentation, is utilized to guide clinical management, the applicability of the model can at least be tested (10).

These studies indicate that autoregulation of CBF in the fetal lamb is intact under ideal physiologic conditions, but is abolished during moderate hypoxia. Because the degree of hypoxia corresponds to that observed during mild stress or even normal delivery (17) we can predict that CBF is likely to be pressure passive in most newborns.

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