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Autoregulation of Cerebral Blood Flow in the Preterm Fetal Lamb

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ABSTRACT. The purpose of the present study was to determine if autoregulation of cerebral blood flow (CBF) is present in the preterm fetal lamb and, if present, to measure the range of mean arterial blood pressure over which autoregulation exists. Thirty-seven measurements of CBF were made in seven preterm fetal lambs (118–122 days gestation) over a mean carotid arterial blood pressure (CBP) range of 18–90 mm Hg. CBF was measured by the radionuclide-labeled microsphere technique. CBP was altered by graduated inflation of balloons placed around the brachiocephalic trunk and the aortic isthmus. To eliminate the effects of reflex changes in heart rate, the carotid sinus and aortic nerve were ablated bilaterally. CBF was linearly related to mean CBP from 18–45 mm Hg, constant over a mean CBP of 45–80 mm Hg, and again linear from 80–90 mm Hg. Resting mean CBP (normotension) was 53.8 ± 1.9 mm Hg during the control period and 51.7 ± 0.8 mm Hg during the equilibration periods. This study demonstrates that although autoregulation of CBF is intact in the preterm fetal lamb, the range is narrowed compared to the term lamb and resting mean CBP lies close to the lower limit of autoregulation. (*Pediatr Res* 19: 159–161, 1985)

Abbreviation

CBF, cerebral blood flow

In adult animals (3, 5, 13) and man (1, 9, 10), autoregulation of CBF has been shown to be an important adaptive mechanism whereby blood flow to the brain is maintained nearly constant over a wide range of systemic blood pressure. Above or below the autoregulatory plateau, CBF varies directly with systemic blood pressure. While several investigators have demonstrated that autoregulation is present in the term (4, 8) and near term (11, 12) experimental animal, no systematic investigation of autoregulation of CBF has been performed in the newborn infant. Moreover, the functional maturity of the autoregulatory mechanism has not been delineated in either the preterm infant or the immature fetal animal.

The present study was designed to determine if autoregulation of CBF is present in the preterm fetal lamb, and, if present, to measure the range of mean arterial blood pressure over which autoregulation exists.

MATERIALS AND METHODS

Studies were performed on fetuses of seven mixed breed Western ewes with time-dated gestational periods of 118–122 days. The ewes were fasted for 24 h prior to operation, and low spinal analgesia was induced with 2 ml of 1% tetracaine hydrochloride. Sedation was achieved with ketamine in amounts of 100–150 mg given intravenously as needed.

Surgical procedures. With the use of aseptic techniques, polyvinyl catheters were inserted in the maternal pedal artery and vein and advanced to the descending aorta and abdominal inferior vena cava, respectively. The uterus was exposed through a midline abdominal incision and the fetal parts were identified. An incision through the uterine wall was made over a fetal hind limb and after local infiltration with 0.25% lidocaine hydrochloride, catheters were placed in a pedal artery and vein and advanced to the descending aorta and distal inferior vena cava respectively. The limb was returned to the uterine cavity and the uterine incision was sutured.

A second incision was made over the left lateral thorax. The left fetal forelimb was withdrawn from the uterine cavity and after local anesthesia was administered, a thoracotomy was done at the second or third intercostal space. After careful exposure of the main pulmonary artery, the ascending aorta, the aortic isthmus and the brachiocephalic trunk, silicone rubber occluding balloons were placed around the aortic isthmus and the brachiocephalic trunk. The incision was sutured, the forelimb was returned to the uterus and the fetal head was exteriorized through the same uterine incision. A wet towel or a saline filled surgical glove was placed over the snout to prevent air breathing. A skin incision was made from the angle of the jaw to 3–4 cm caudad. The submaxillary gland was excised and the digastric muscle was divided, leaving the hypoglossal nerve intact. Polyvinyl catheters were inserted into the lingual artery and a branch of the external jugular vein and advanced into the carotid artery and the superior vena cava, respectively.

To prevent reflex changes in heart rate with balloon inflation the carotid artery and aorta were denervated (7). Carotid artery and sinus denervation was accomplished by stripping the carotid artery of all nervous and connective tissue from approximately one cm caudal to the occipital artery to one cm rostral to the lingual artery. The vagosympathetic trunk was identified and the angle formed by the junction of the vagus nerve with the superior laryngeal nerve was exposed. The superior laryngeal nerve was retracted and the aortic nerve was located. The vagosympathetic sheath was dissected caudal to the nodose ganglion and the aortic nerve and the superior laryngeal nerve were sectioned near the nodose ganglion. The same procedure was accomplished on the contralateral side of the neck. The head was returned to the uterus, a catheter was placed in the amniotic cavity and the uterine incision was closed.

The catheters were filled with heparin and brought to a pouch sewn to the ewe's flank. On the day of surgery and again on the first postoperative day the ewe received penicillin G (2 million units) and kanamycin (800 mg), half intravenously and half into the amniotic cavity.

Physiological measurements. Fetal superior venal caval mean pressure and systolic, diastolic and mean carotid and distal aortic

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blood pressures were measured continuously by Statham P23Db pressure transducers and recorded on a Beckman direct writing recorder. Fetal vascular pressures were calculated with amniotic cavity pressure as zero reference. Fetal heart rate was measured with a cardiotachometer from the arterial pressure wave. Fetal distal aortic blood gases and pH were determined on a Radiometer blood gas analyzer with appropriate electrodes. Arterial hematocrit was measured with the microhematocrit method.

Fetal cerebral blood flow was measured by the radionuclide-labeled microsphere technique as described previously (2, 6, 14). Microspheres, 15 μm in diameter and labeled with [^{114m}In], [^{153}Gd], [^{57}Co], [^{51}Cr], [^{113}Sn], [^{85}Sr], [^{95}Nb], and [^{46}Sc] were used. Approximately 1–1.5 $\times$ 10^6 microspheres with a single label were injected into the inferior vena cava over a 30-s period and the catheter was flushed with 2 ml of saline. The reference blood sample was withdrawn with a Harvard pump from a carotid arterial catheter during and for 1.25 min after the microsphere injection at a rate of 3.6 ml/min. The microsphere injections were not associated with changes in heart rate or blood pressure.

After completion of the experiment, the fetus was autopsied to verify catheter placement and the brain was removed. Tissue and reference blood samples were analyzed for radioactivity with an automated well-type γ scintillation counter (Inotech-Tracer Analytic Systems). All specimens and reference blood samples contained a minimum of 400 microspheres for each of the radionuclides used. Cerebral blood flow (ml $\cdot$ 100 $g^{-1} \cdot min^{-1}$) was calculated by the following formula:

$$CBF = \frac{RBF \times C_{cerebrum}}{C_{arterial}}$$

Where RBF is withdrawal rate of the reference arterial blood sample (ml $\cdot$ min^{-1}), $C_{cerebrum}$ is counts per 100 g of cerebral tissue, and $C_{arterial}$ is the total counts in the reference arterial blood sample.

Experimental procedures. Studies were performed on the first postoperative day. The experiment was designed to expose each fetal lamb to a wide range of carotid artery pressures in a random fashion. An increase in carotid arterial pressure was accomplished by graduated inflation of the occluding balloon around the aortic isthmus. Since the brachiocephalic trunk is the origin of both carotid arteries in the sheep, inflation of the occluding balloon around the brachiocephalic trunk resulted in a decrease in carotid arterial pressure.

On the day of the study, the ewe was brought to the laboratory and kept in a study cage. A 1-h baseline recording was obtained at the beginning of the study. One of the occluding balloons was then inflated to obtain a desired carotid arterial pressure. Forty-five to sixty seconds after achieving a given pressure, microspheres were injected to measure cerebral blood flow. The balloon was deflated slowly after the measurement and a 30-min equilibration period was allowed before performing another inflation. Fetal distal aortic blood gases were obtained at the end of the 1-h baseline period, and at the beginning of each 30-min equilibration period, *i.e.* immediately after each balloon deflation. After the withdrawal of 15% of the estimated blood volume, an isovolemic amount of maternal blood was given.

The unpaired Student's *t* test and linear regression were used to analyze the data.

RESULTS

The mean value for distal aortic blood gas parameters, mean carotid arterial pressure and mean heart rate for the seven control and 30 equilibration periods (Table 1) are within the range we have observed in chronically catheterized fetal lambs. There were no significant differences between these variables for the two periods. Mean hematocrit dropped from an initial value of 39.6 + 4.9% to a low of 35.2 ± 5.4% ($p > 0.05$).

Thirty-seven measurements of cerebral blood flow were determined; 15 measurements were obtained during inflation of the brachiocephalic balloon, 18 during inflation of the aortic balloon, and four without inflation. The number of microsphere injections per animal was four in two, five in one, and six in four. Changes in CBF in response to alterations in carotid arterial pressure are depicted in Figure 1. CBF was linearly related to mean carotid arterial pressure over the pressure range of 18–45 mm Hg. At a mean pressure of 18 mm Hg, CBF was 15 ml $\cdot$ 100 $g^{-1} \cdot min^{-1}$ whereas CBF was 80 ml $\cdot$ 100 $g^{-1} \cdot min^{-1}$ at a mean pressure of 45 mm Hg. CBF was constant at 75–86 ml $\cdot$ 100$g^{-1} \cdot min^{-1}$ from 45–80 mm Hg mean carotid arterial pressure ($r = 0.284$, $p > 0.50$). Above a mean carotid arterial pressure of 80 mm Hg, CBF was again linearly related to mean carotid artery pressure.

Table 1. *Fetal measurements during the control and equilibration periods*

Variable	Control period (7)	Equilibration period (30)
MCBP* (mm Hg)	53.8 ± 1.9†	51.7 ± 0.8
Heart rate	179.8 ± 3.4	174.8 ± 1.6
pH	7.39 ± 0.01	7.37 ± 0.01
Pa_{O_2} (torr)	22.2 ± 0.4	22.5 ± 0.5
Pa_{CO_2} (torr)	42.3 ± 2.1	41.9 ± 0.5

* Mean carotid artery blood pressure.

† All values are expressed as mean ± SEM.

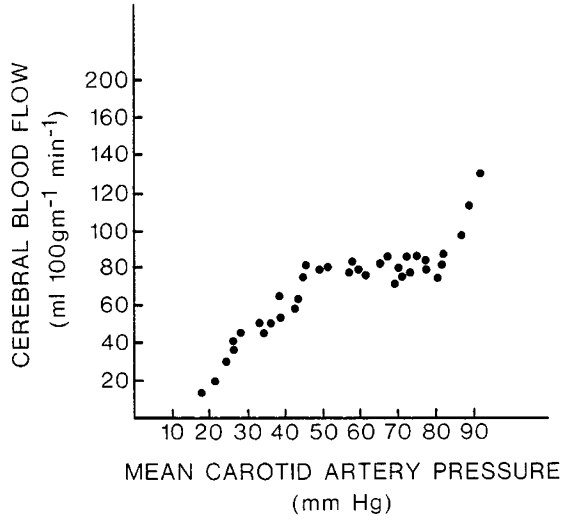

Fig. 1. CBF autoregulation in the preterm fetal lamb.

DISCUSSION

Autoregulation of cerebral blood flow and the lower limit of the autoregulatory plateau have been documented in the newborn animal (4, 8, 12). Purves and James (12) reported that gray matter blood flow was independent of mean arterial blood pressure over the range of 43–93 mm Hg for newborn lambs. In the newborn piglet, Laptook *et al.* (8) observed that cerebral blood flow was unaltered from 50–105 mm Hg mean arterial blood pressure. Hernandez *et al.* (4) determined that the limits of cerebral blood flow autoregulation in the newborn dog are 27 and 97 mm Hg. While it is evident from these studies that the lower limit of autoregulation varies among species, a constant observation was that resting mean arterial blood pressure (*i.e.* normotension) was 30–40 mm Hg above the lower limit of the autoregulatory plateau. Moreover, Hernandez *et al.* (4) demonstrated that, in the newborn dog, resting mean arterial blood pressure lies midway between the upper and lower limits of

cerebral blood flow autoregulation. Thus, in the newborn animal, cerebral blood flow autoregulation acts as a buffer which protects the brain from acute fluctuations in systemic blood pressure above or below the resting level. Because cerebral blood flow autoregulation has not been studied in the human newborn, it is not known if the human brain is afforded similar protection.

Studies relating to the development of the autoregulatory mechanism have been limited to near term experimental animals (12, 16). Using an acutely exteriorized fetal lamb model, Purves and James (12) found that, in the near term fetal lamb of 135–145 days gestation, cerebral blood flow autoregulation was intact over a mean arterial blood pressure range of 43–90 mm Hg, a range nearly identical to that observed in the newborn lamb. However, resting mean arterial blood pressure in the near term fetal lamb was only 25 mm Hg above the lower limit of the autoregulatory plateau compared to 40 mm Hg in the newborn lamb. Recently Tweed *et al.* (16) have documented CBF autoregulation within the range of 40–70 mm Hg mean arterial blood pressure in a chronically prepared near term fetal sheep model (130–140 days gestation). Although the lower limit of autoregulation observed was identical to that reported by Purves and James (12), the resting mean arterial blood pressure value was only 15 mm Hg above the lower limit of autoregulation.

In the present study we have demonstrated that CBF autoregulation is already developed in the preterm fetal lamb. Furthermore we have delineated that the limits of autoregulation in the preterm fetal lamb are 45–80 mm Hg mean arterial blood pressure. Since the lower limit of autoregulation that we observed is comparable to that noted in the near term (12, 16) and term lamb (12), it would appear that the critical mean arterial blood pressure necessary for adequate cerebral blood flow, does not vary with gestational age. Conversely, the upper limit of autoregulation in the preterm fetal lamb falls within the autoregulatory plateau of the more mature lamb. From this data, we conclude that the range of autoregulation is narrowed in the preterm lamb.

An additional observation in the present study was that resting mean arterial blood pressure in the physiologically stable preterm fetal lamb lies approximately 10 mm Hg above the lower limit of cerebral blood flow autoregulation. In view of the findings of Purves and James (12) and the observation of Tweed *et al.* (16), our data indicate that, with decreasing gestational age, resting mean arterial blood pressure approaches the lower limit of the autoregulatory plateau.

Previous investigators of cerebral blood flow autoregulation in the near term and term laboratory animal effected changes in systemic blood pressure by either manipulation of blood volume (8, 12, 16) or the administration of pharmacological agents (4, 12). The limitations of these techniques include the inability to control 1) repeated discrete increases or decreases in blood pressure, 2) changes in heart rate, 3) alterations in cerebral blood flow related to acute anemia or polycythemia, and 4) the impact of pharmacological agents on cerebral blood vessels. In order to measure CBF over a wide range of arterial pressure without eliciting reflex changes in heart rate, we denervated the carotid sinus and aorta. In a previous study from this laboratory Itskovitz and Rudolph (7) demonstrated that sinoaortic-denervation of the preterm fetal lamb does not alter resting heart rate or blood pressure. Moreover, by measuring CBF in a preterm fetal lamb who was subjected to the same surgical procedures as the sinoaortic-denervated lambs, we demonstrated that sinoaortic-denervation has no effect on CBF within the autoregulatory range (75–86 ml·100 g^{-1} min^{-1} *versus* 75–80 ml·100g^{-1} min^{-1}). Because any attempt to alter cerebral artery pressure more than 5 mm Hg in the nondenervated fetal lamb caused marked changes in heart rate, the effect, if any, of sinoaortic-denervation on the limits of CBF autoregulation could not be ascertained. Nonetheless, published data indicate that the autonomic nervous system does not appear to have a major role in the control of cerebral circulation (15).

This study indicates that although autoregulation of CBF is intact in the immature fetal lamb, the range is narrowed compared to the term lamb and resting mean arterial blood pressure lies close to the lower limit of autoregulation. Based on these findings we speculate that the fetal brain, even under normal physiological conditions *in utero*, is not protected against acute fluctuations in blood pressure, particularly hypotension.

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