Brain Blood Flow and O₂ Delivery during Hemorrhagic Hypotension in the Piglet

ABBOT R. LAPTOOK, BARBARA S. STONESTREET, AND WILLIAM OH⁽²⁷⁾

Department of Pediatrics, Women and Infants Hospital of Rhode Island, and The Program in Medicine, Brown University, Providence, Rhode Island, USA

Summary

We have previously demonstrated regional differences in brain blood flow reduction when hemorrhagic hypotension was induced in spontaneously breathing newborn piglets. Both the mean arterial blood pressure and PaCO₂ correlated with the changes in brain blood flow. To determine if changes in brain blood flow during hypotension can be attributed to alterations of mean arterial blood pressure alone, eight newborn piglets were studied under conditions of controlled ventilation. Regional brain blood flow was measured by microspheres and the blood pressure was varied by phlebotomy. After baseline determinations, three measurements of blood flow were performed during hypotension. Hypotension produced a significant reduction in blood brain flow confirming the independent effect of mean arterial blood pressure in autoregulation of cerebral circulation. Significantly different regional blood flow responses occurred in response to hypotension ($P < 0.05$). The % reduction from control blood flow was greatest to the cerebrum, less to the cerebellum, and least to the brainstem. Furthermore, the blood flow was consistently decreased in the cerebral hemispheres during the entire exposure to hypotension (mean $\pm$ S.E.: $-36 \pm 10\%$, $-18 \pm 11\%$, and $-33 \pm 11\%$ change from control) whereas the brainstem demonstrated an initial reduction and then an increase in blood flow (mean $\pm$ S.E.: $-14 \pm 12\%$, $+16 \pm 15\%$, and $+5.5 \pm 13\%$ change from control). The cerebellar responses were intermediate between those of the cerebrum and brainstem. O₂ delivery was significantly decreased to all brain regions during hypotension, maintaining the same rank order of the regions as when blood flow reductions occurred.

Speculation

Newborn piglets subjected to profound hypotension demonstrate regional differences in the reduction of brain blood flow. Cranial window studies of the microvasculature in adult cats clearly demonstrate that precapillary vessels in the brain do not respond in an identical manner to hypotension but rather react in a size dependent fashion. The observed differences in brain regional blood flow are possibly a manifestation of the relative composition (with respect to vessel size) of the regional parenchymal arterial beds.

Numerous studies have been performed in adult animal models examining the response of brain blood flow to hemorrhagic hypotension. It has been established that autoregulatory responses maintain brain perfusion pressure over a range of systemic arterial pressure and that below a "critical" level of the latter, brain blood flow will be significantly reduced (5, 16). In the newborn piglet, we have demonstrated a similar autoregulatory phenomenon when hypotension was induced by phlebotomy (15); however, in these experiments, the piglets were allowed to breathe spontaneously thus producing hypocapnea (as a result of tachypnea) when hemorrhage was induced. Because hypocapnea is known to result in a significant reduction in brain blood flow, it was not clear whether the brain hypoperfusion was a direct result of hypotension

and/or hypocapnea. The first objective of the current study is to demonstrate the effect of hypotension on brain blood flow by maintaining a constant PaCO₂ with controlled ventilation.

In adult animals during hypotension regional blood flow responses reveal conflicting results. Using the microsphere technique in adult mongrel dogs (22) and cynomolgus monkeys (6) after induced hypotension (via hemorrhage in the former and pharmacologic means in the latter) no differences in regional blood flow to the brain were detected. In contrast, during hemorrhagic hypotension in adult dogs (18), blood flow (microspheres) was preferentially maintained to the medulla. In the piglet (15) we have shown that during hemorrhagic hypotension, regional differences in brain blood flow reduction did occur. The reduction was most pronounced in the cerebrum, less in the cerebellum, and least in the brainstem. The presence of hypotension and hypocapnea made it uncertain as to which was responsible for the regional differences in reduced perfusion. Therefore, the second objective of the present study is to determine if regional differences in brain blood flow reduction and oxygen delivery exist when the mean arterial blood pressure is altered during hemorrhagic hypotension under conditions of controlled ventilation.

MATERIALS AND METHODS

Eight piglets were studied at a mean $\pm$ S.E. age of 5 ± 0.4 days and weight of 1.8 ± 0.1 kg. Premedication consisted of a single dose of ketamine (5 mg/kg) administered intramuscularly. After infiltration of the pretracheal neck tissue with xylocaine (1%), a 3.0-mm cuffed endotracheal tube was placed via tracheostomy and the piglet was ventilated (Harvard Apparatus Rodent respirator, Model 681) with 70% nitrous oxide and 30% oxygen. To achieve an initial PaCO₂ of approximately 35 mmHg, tidal volumes of 10 ml/kg body weight and rates of 30-43 cycles/min were used. A 3½ french vascular catheter (Argyle, St. Louis, MO) was placed in the left ventricle via the right axillary artery. Polyethylene catheters (Deseret Pharmaceutical Co., Sandy, UT) were placed into the left axillary artery, abdominal aorta (via the femoral artery), and the inferior vena cava (via the femoral vein).

An infant radiant warmer was used to maintain the piglet's body temperature between 38-39°C. Mean arterial blood pressure (MABP) was continuously monitored using a Bentley Trantec pressure transducer and recorded on a Hewlett-Packard Polygraph (7700 series). Arterial PaO₂, PaCO₂, pH, and base excess were measured using a Corning 165 blood gas analyzer. Arterial O₂ content was measured with a Lex-O₂-Con (Lexington Instruments, Waltham, MA). Arterial hematocrit was measured with the microhematocrit method.

Microspheres (15 μ , New England Nuclear) labeled with [⁴⁶Sc], [⁹⁵Nb], [⁶⁷Co], [¹¹³Sn], and [¹⁰³Ru] were utilized to measure brain blood flow according to the technique of Heymann, *et al* (10). Approximately 5×10^5 microspheres suspended in 2.0 ml of 20% dextran were injected into the left ventricle for each measurement. The microspheres were infused over a 30-sec period and the catheter was flushed with 2.0 ml of saline. Starting 15 sec before

the microsphere injection blood was withdrawn from the left axillary artery at 1.03 ml/min for 2 min into glass syringes with a Harvard pump. More than 400 microspheres were found in each sample of brain tissue and reference blood.

After catheter placement a stabilization period of 1 h followed. Nitrous oxide and oxygen were administered throughout stabilization and the experimental protocol. An arterial blood gas was measured at 30 min of stabilization to ensure appropriate respirator settings. After stabilization, a control measure of brain blood flow was performed (time 0 min). The piglets were then paralyzed to achieve controlled ventilation by slowly infusing 0.3 mg/kg of tubocurarine chloride (Eli Lilly and Co.) over 15 min via the venous catheter. No alterations of mean arterial blood pressure occurred during or after tubocurarine. A second determination of brain blood flow was performed (time 20 min) to assess the effect of tubocurarine. Hypotension was subsequently induced by phlebotomy via the venous catheter until the MABP was <50 mmHg. The third, fourth, and fifth microsphere injections (time 30, 37, and 44 min) were then performed in consecutive fashion at approximately 7-min intervals (range 5–9 min). Except in one piglet, additional phlebotomy was performed in between the last three blood flow measurements because the MABP tended to increase above 50 mmHg. The mean $\pm$ S.E. % of total blood volume removed at study completion [based upon an estimated blood volume of 90 cc/kg (17)] was $50 \pm 3\%$ (range 41–61). Alterations in the frequency of respirator cycling maintained a constant PaCO_2 . At the time of each blood flow determination an arterial blood gas, hematocrit, and O_2 content were obtained.

At study termination the animals were sacrificed by injecting potassium chloride into the left ventricle. Autopsy included verification of proper catheter placement and removal of the brain, separating it from the spinal cord at the first cervical vertebrae. The brain was weighed before and after fixation (10% formalin) and was cut into specified sections. All sections of brain tissue and reference bloods were counted in a Packard Auto Scintillation Spectrometer. The calculated original wet weight of tissue was used to compute brain blood flow (BBF) by the following formula: $\text{BBF} = C_B \times 100 \text{ RBF}/C_R$ where BBF is brain blood flow in $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g brain}$; C_B is counts per g of brain tissue; RBF is reference blood flow ($\text{ml} \cdot \text{min}^{-1}$); and C_R is the total counts in the reference arterial blood.

Statistical analysis was performed with a one-way analysis of variance with repeated measures for each of the arterial blood gas parameters, O_2 content, and hematocrit. A polynomial regression was used to analyze nonlinear relationships. A three-way analysis of variance (SAS Statistical Package, General Linear Model Program) was used to analyze the regional blood flow over the course of the study. Significant interactions were localized by a Newman-Keuls multiple comparison procedure. Statistical significance was designated at a level of $P < 0.05$.

RESULTS

Table 1 presents the arterial blood gas, hematocrit, O_2 content, and MABP data. The pH remained stable until the fourth and fifth microsphere injections (37 and 44 min) at which time de-

creases occurred secondary to the induced hypotension. The PaO_2 and PaCO_2 remained unchanged throughout the course of the study. As expected, the pattern of change in base excess mirrored the reduction of arterial pH. Both the arterial hematocrit and oxygen content were unchanged until phlebotomy resulted in progressive decrements in both parameters. The MABP demonstrate comparable degrees of hypotension during the last three microsphere injections.

Figure 1 shows the relationship between total brain blood flow (BBF) and mean arterial blood pressure (MABP) when the PaCO_2 is maintained constant. The data demonstrates an autoregulatory relationship between BBF and MABP and are fitted by a polynomial regression line. Also included is the polynomial regression line from our previous study (15) where the piglets were hypocapneic. Both regression lines indicate that the BBF begins to fall at similar values of MABP (approximately 40–50 mmHg). Due to the wide variability in BBF, we grouped values of the latter at MABP greater than and less than 50 mmHg. Mean BBF at MABP greater than 50 mmHg is $119 \pm 7.8 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}$, which differs ($P = 0.005$, unpaired t test) from the mean BBF of $82.4 \pm 9.5 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}$ at MABP less than 50 mmHg.

Figure 2 plots the values of regional blood flow, oxygen delivery, and MABP. In the top panel, the control cerebral blood flow (time 0 min) is lower than the brainstem and cerebellar blood flow. After tubocurarine administration (time 20 min) no changes are present in regional blood flow. After reduction of MABP by phlebotomy, significant reductions in blood flow occur to each region at 30 min. Cerebral blood flow remains low throughout hypotension but brainstem blood flow increases to values higher than control (time 37 min) and then equal to control (time 44 min)

Fig. 1. Brain blood flow at various levels of mean arterial blood pressure in normocapneic piglets. Polynomial regression lines are shown for the normocapneic current data and the hypocapneic data previously reported (15).

Table 1. Arterial blood gas parameters, hematocrit, O_2 content, and mean arterial blood pressure at each blood flow determination

	Time (min)				
	0	20	30	37	44
pH	7.41 ± 0.02	7.42 ± 0.02	7.41 ± 0.01	7.29 ± 0.02^2	7.23 ± 0.04^2
PaO_2 (mmHg)	114.9 ± 6.2	123.3 ± 4.2	122.4 ± 7.3	115.7 ± 9.4	127.0 ± 6.2
PaCO_2 (mmHg)	34.3 ± 0.8	35.3 ± 1.2	34.1 ± 1.7	37.1 ± 1.6	35.4 ± 1.1
Base excess (mEq/liar)	-1.4 ± 1.3	-0.4 ± 1.3	-1.7 ± 0.7	-7.4 ± 1.0^2	-11.2 ± 1.9^2
Hematocrit (%)	25.1 ± 1.6	23.9 ± 1.7	20.1 ± 1.5^2	16.8 ± 1.5^2	13.9 ± 1.1^2
O_2 content (volume %)	10.0 ± 0.8	9.6 ± 0.8	8.2 ± 0.7^2	6.3 ± 0.6^2	5.3 ± 0.5^2
Mean arterial blood pressure (mmHg)	88 ± 4	90 ± 5	39 ± 3^2	40 ± 5^2	36 ± 2^2

¹ Mean $\pm$ S.E.

² $P < 0.05$ as compared to control.

Fig. 2. Absolute values of blood flow and oxygen delivery to the brain and its various regions during hypotension. Mean $\pm$ S.E., * $P < 0.05$ versus control; † $P < 0.05$ versus brainstem and cerebellum; ‡ $P < 0.05$ when all three regions are compared with each other.

at the study termination. The cerebellar blood flow falls between the two extremes of the cerebrum and brainstem. In the middle panel are the derived regional O₂ delivery (regional blood flow $\times$ arterial O₂ content). The O₂ delivery to the three regions were unchanged when tubocurarine was given; however, significant reductions from control occur in all three regions throughout hypotension. The same regional hierarchy is present for O₂ delivery as was found for blood flow. The MABP at each blood flow determination confirmed the hypotension induced by phlebotomy.

Because regional differences in blood flow exist at control conditions, we examined the regional responses during the study by analyzing the % change from control in Figure 3. After tubocurarine was administered (time 20 min) no significant change occurred. Phlebotomy-induced hypotension resulted in significant differences among the three regions during the total duration of hypotension. The cerebral hemispheres demonstrated the greatest % change from control blood flow which consistently showed reduction from control. This is contrasted by the brainstem which displayed the least change from control blood flow and a reduction occurred only initially during hypotension. The pattern of change in the cerebellum was interposed between the cerebrum and the brainstem.

DISCUSSION

The anesthetic and muscle relaxant used to maintain a constant PaCO₂ deserve comment. Nitrous oxide and oxygen was used because of its mild cerebrovascular effects when studied in adult humans. The latter include an unaltered cerebral blood flow, a mild depression of cerebral oxygen consumption ($\sim 15\%$) (26) and no impairment of autoregulation (23). The effects of tubocurarine have been investigated in adult humans (25) and resulted in no alteration of cerebral blood flow; however, in dogs (24) a 40% increase in cerebral blood flow (assessed by internal carotid flow probes) was found after tubocurarine was administered. In this study we demonstrated no significant difference in brain blood flow after tubocurarine administration confirming the findings in

man and eliminating this factor in interpreting the effects of hypotension of BBF.

One of the purposes of this study was to demonstrate the independent effect of hypotension on the reduction of BBF. We have successfully shown this phenomenon. In comparing the current data with our previous work (15) in which the PaCO₂ fell along with the MABP, we showed that the reduction in BBF was similar in both normocapnic and hypocapnic states. Our results agree with those of Hernandez, *et al.* (9) who have demonstrated that the newborn dog's cerebral vascular responsiveness to CO₂ is lower than that reported for adult dogs. The lack of an additive effect of PaCO₂ on the reduction of brain blood flow during hypotension suggests that hypocapnea may not produce an adult-like effect on neonatal cerebral circulation. It is clear that further investigation of hypocapnea in relation to perfusion pressure is needed because of the clinical relevance of hyperventilation as a treatment modality in persistent pulmonary hypertension of the newborn. (3)

The present study also substantiates that differences in reduction of regional blood flow exist in the piglet when hemorrhagic hypotension is induced. The reduction in blood flow was most prominent in the cerebral hemispheres, less in the cerebellum, and least in the brainstem. Furthermore, the cerebral hemispheres consistently demonstrate significant reductions from control blood flow during hypotension whereas the brainstem blood flow is reduced significantly from control values only after initial induction of hypotension. Subsequent determinations of brainstem blood flow during hypotension are significantly higher (37 min) and equal to (44 min) control blood flow. The apparent rebound in regional BBF may be related to the development of a metabolic acidosis following induced hypotension. Bucciarelli and Eitzman (2), using radioactive-labeled microspheres to study newborn pre-term and term goats, have demonstrated increases in BBF after lactic acid infusions. Furthermore, Powell, *et al.* (20) using the iodoantipyrine technique to study newborn puppies have dem-

REGIONAL BRAIN BLOOD FLOW PERCENT CHANGE FROM CONTROL

Fig. 3. Regional blood flow expressed as % change from control. Mean $\pm$ S.E., * $P < 0.05$ versus control; ‡ $P < 0.05$ when all three regions are compared to each other.

onstrated that a lactic acid infusion results in increases in BBF with disproportionate increments to brainstem structures. Other newborn animals [puppies (7) and lambs (21)] demonstrate appropriate autoregulatory responses of the brain vasculature; however, because of the methods employed (isotope washout) no further information can be obtained with respect to regional blood flow.

The calculated O₂ delivery demonstrated regional differences during hypotension similar to the blood flow responses; however, because of the lowered O₂ content after phlebotomy, the O₂ delivery is decreased to all regions throughout hypotension. We did not measure cerebral O₂ consumption in this study; however, the latter remains unchanged in both adult humans (4) and dogs (19) during hemorrhagic hypotension. If tissue O₂ consumption is to remain unaltered during hypotension, then a compensatory mechanism other than preserving O₂ delivery must be present. This contrasts with states such as hypoxic hypoxia (11) or anemic hypoxia, (1) where BBF increases so that O₂ delivery and consumption is maintained constant. These changes are reflected in the reciprocal relationship between arterial O₂ content and brain blood flow (12). We speculate that the extraction of O₂ must increase for the cerebral O₂ consumption to remain unchanged during hypotension.

The etiology of the regional differences in blood flow reduction after hemorrhagic hypotension is unknown. Differential regional blood flow responses to such an insult is probably consistent with local tissue regulatory mechanisms.¹³ The observations of Kontos, *et al.* (14) concerning the quantitation of precapillary pial vessel response to alterations of blood pressure in cats may offer a speculation with respect to the newborn piglet. These investigators found that changes in arterial caliber in response to blood pressure changes were size dependent. Vessels of small diameter contributed to blood flow regulation at lower blood pressures whereas alterations of the size of larger arterial vessels occurred with minor changes in blood pressure. The response to the pial vasculature provides evidence that all blood vessels do not respond in identical fashion to a particular stimulus. The regional blood flows measured using microspheres reflects the total response of all parenchymal blood vessels. Certainly then, one possibility for the observed regional differences in newborn piglets would be the composition of the parenchymal vascular bed with respect to vessel size. An alternative explanation includes the sympathetic nervous system's control of the cerebral vasculature. Hernandez, *et al.* (8) have documented a disproportionate increase in blood flow to the brainstem of newborn puppies during hypotension and asphyxia suggesting that differential innervation density of preparenchymal resistance vessels may be responsible for these findings.

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- Requests for reprints should be addressed to: Dr. William Oh, 50 Maude Street, Providence, RI 02908-9976.
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