Brain Vasoactive Effects of Phenobarbital during Hypertension and Hypoxia in Newborn Pigs

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ABSTRACT. Phenobarbital in anticonvulsant concentrations has been shown to lower cerebral blood flow (CBF) during hypertension and to reduce the incidence of intraventricular hemorrhage in newborn beagles after hypertensive insult. We proposed that hypoxic dilatation of brain blood vessels might alter the effect of phenobarbital (PBS) on blood flow during hypertension. Thus, in 14 control and nine PBS-treated 1- to 2-d-old newborn piglets, the radioactive microsphere technique was used to determine CBF during 1) steady state (SS), 2) hypertension (HT), and 3) HT plus hypoxia of 5 min duration. In seven controls and in four PBS-treated piglets, CBF was also determined during recovery from hypoxia. PBS was infused after SS in a 20-mg/kg dose, and serum levels were obtained 30 min later. Blood pressures were not significantly different between groups when compared during SS, HT, hypoxia, and recovery. Similarly, pH, PO_2 , and PCO_2 were not significantly different between groups when compared during normoxia and hypoxia, and hematocrits were maintained by transfusions after reference sample withdrawals. CBF in control animals increased significantly during HT and remained significantly higher than SS values throughout the 5 min of hypoxia and into the recovery period. In PBS-treated piglets, however, there was no significant increase in CBF during HT. Blood flows also stayed at SS levels during HT plus 5 min hypoxia and recovery from hypoxia. Thus, in newborn piglets, PBS lowered CBF during HT. Furthermore, this blood flow lowering effect persisted during hypoxia, preventing compensatory increases in CBF that might have otherwise occurred. Although this may explain why PBS might be useful in preventing hemorrhages that result during hyperemia, further studies will determine if PBS pretreatment results in reduced oxygen delivery to brain tissues during hypoxia. (*Pediatr Res* 32: 103-106, 1992)

Abbreviations

CBF, cerebral blood flow
HT, hypertension
HTHY, hypoxia during hypertension
PBS, phenobarbital sodium
RE, recovery
SS, steady state

Anticonvulsant concentrations of PBS that do not reduce SS CBF have been shown to reduce CBF during HT and to reduce the incidence of peri- and intraventricular hemorrhages in the newborn beagle (1, 2). The same concentrations of PBS have been shown to reduce CBF during systemic hypotension without altering SS CBF in newborn piglets (3). PBS has also been given just before parturition (4, 5), or on the 1st d and during the 1st wk of extrauterine life, to prevent cerebral hemorrhages in immature newborn humans (6-10); however, the prophylactic effect has been inconsistent. PBS may prevent hemorrhages by reducing blood pressure peaks in some infants with inadequate autoregulatory mechanisms (11, 12), thereby secondarily reducing the pressure-passive cerebral hyperemia, and/or it may reduce CBF by primary vasoactive or metabolic phenomena (13). In the newborn pup, the latter seem to predominate, because during hypertension PBS has been shown to decrease CBF without altering the blood pressure response to phenylephrine (1, 14).

If another cerebral vascular response were to intervene or to be present before the PBS loading dose, the PBS effect might not be as prominent. We hypothesized that hypoxia, which usually provokes a rapid vasodilatory response, might reduce the effect of PBS. Because many immature infants are already asphyxiated to some extent and, therefore, hypoxic or posthypoxic when PBS is given, this might explain some of the inconsistent responses to this therapy. Therefore, in this study we evaluated the CBF responses to HT with and without hypoxia in newborn control piglets and in piglets treated with anticonvulsant concentrations of PBS.

MATERIALS AND METHODS

Piglets were chosen for this study because despite their similarity in brain maturation to the near-term human newborn a number of their cerebral hemodynamic responses have already been shown to be similar to those of the less mature, newborn beagle model of intraventricular hemorrhage (1, 3). This study was approved by the Baylor College of Medicine Animal Protocol Review Committee of the Animal Care and Use Committee (National Institutes of Health Assurance No. A3823-01). Cross-bred 1- to 2-d-old piglets were obtained from a local breeder and were brought to the laboratory on the day of the experiment. The piglets were weighed and warmed to maintain normothermic rectal temperatures of 38.0-38.5°C. Each piglet was initially anesthetized with a mixture of ketamine/xylazine, 200:20 mg, intramuscularly, 0.45 mL/kg (Ketaset, Aveco Co., Fort Dodge, IA, and Anased, Lloyd Laboratories, Shenandoah, IA). Tracheostomy was then performed, and each animal was ventilated by Harvard small animal volume ventilator (Harvard Apparatus, Millis, MA). α -Chloralose was administered (50 mg/kg; 30 mg/kg maintenance per h) (Sigma Chemical Co., St. Louis, MO) after a polyethylene catheter (Intramedic, Clay Adams, Parsippany, NJ) was placed into the femoral vein. One femoral artery catheter was attached to a Statham strain gauge (Gould Instru-

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ments, Cleveland, OH) and Grass polygraph recorder (Grass Medical Instruments, Quincy, MA) for continuous arterial pressure monitoring. Another femoral artery catheter was threaded into the abdominal aorta to obtain reference samples. A PE50 cannula was directed into the left cardiac ventricle from the left common carotid artery; the position was ascertained by the pressure recording. This cannula was used for microsphere infusions. Arterial blood gas values were determined on 0.4-mL samples by a Corning 170 analyzer (Corning, Inc., Medfield, MA).

Measurements of blood flows. The basis of the radioactive microsphere technique and our adaptations of it have been published previously (1, 14) (see also Appendix). In this study, the radiolabels ^{141}Ce , ^{51}Cr , ^{103}Ru , and ^{95}Nb (all 15 μm size) (3M Corp., St. Paul, MN, and New England Nuclear Corp., Dupont, Boston, MA) were used. Reference samples were obtained using a Sage Instruments model 351 withdrawal pump (Orion Research Inc., Boston, MA) at a speed of 1.5 mL/min over 1 min and 10 s. At the end of each experimental protocol, the animals were euthanized by infusion of 100 mg of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL). The brain was extracted immediately, and areas were separated for isotope counting. Sections from cortex and thalamus, white matter, brain stem, and cerebellum were weighed and counted with corrections for cross-channel spillover by a Compugamma 1282 gamma counter (Wallac, Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). Blood flow values were calculated in per 100-g equivalents by the following formula:

$$\text{mL} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1} = \frac{1 \text{ min} \cdot \text{counts} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1} \text{ of brain}}{\text{counts} \cdot \text{min}^{-1} \text{ in 1 mL of reference blood}}$$

Total CBF values were calculated using the sum of all brain weights and the total brain counts.

Statistical analyses. The control or experimental status of each animal was determined by random drawing after the first microsphere infusion. Within-group variations in physiologic parameters and CBF values for SS, HT, and HTHY were analyzed using the repeated measures analysis of variance, followed by *t* tests with the Bonferroni correction when within-group variations were significant. (Because there were missing values for the RE period in both groups, differences between the values in this time period and those for SS, HT, and HTHY were analyzed using the one-way analysis of variance followed by the Bonferroni *t* test when necessary). Between-group differences were assessed by the one-way analysis of variance followed by two-tailed *t* tests when the between-group differences were significant.

Analysis of PBS concentrations. PBS was measured in serum 30 min after its administration by EMIT, SYVA (Palo Alto, CA) enzyme immunoassay, as reported previously (1). The choice of the 30-min period after PBS infusion is based upon its short equilibration phase; a relatively constant serum concentration is achieved 30 min after i.v. infusion (15).

Experimental design. The controls consisted of 14 newborn piglets whose CBF were quantitated in SS (defined by normal blood pressure, heart rate, arterial blood gases, and body temperature) during HT induced by i.v. phenylephrine hydrochloride (0.3 mg/kg), during persisting HTHY induced by having the piglets breathe a ventilatory gas mixture of 50% nitrogen and the remainder air (*i.e.* approximately 10% oxygen), and in seven piglets, during RE. Some piglets required 30% oxygen after hypoxia to achieve normal oxygen tensions when ventilatory rates were adjusted to maintain SS PCO_2 . A 1-mL volume of saline was infused intraarterially over 5 min after the SS infusion of microspheres to simulate the PBS infusion in experimental piglets; similarly, a 1-mL volume of blood was withdrawn from the arterial line 30 min after the first microsphere infusion to simulate the withdrawal for PBS levels in experimental animals. The second blood flow determination was made 35 min after the first, 3–5 min after the onset of phenylephrine-induced HT.

Hypoxia was then imposed, and the third blood flow determination was made 5 min later. Arterial blood gas determinations were made before each blood flow, and sibling donor blood was given to replace reference samples and to maintain hematocrits in each piglet. A blood flow determination was made during the posthypoxia recovery period in seven of the 14 controls 10 min after restitution of normal arterial blood gas values.

This same experimental design was carried out in nine piglets given 20 mg/kg PBS by intraarterial infusion over a 5-min period just after the first microsphere infusion. (Intraarterial infusions were made because the arterial line was more reliable than the underlying venous line and because no differences had been previously observed in blood pressure responses to PBS when given by i.v. or intraarterial routes.) The line was flushed carefully with saline after the infusion. Thirty min after this infusion, blood was withdrawn from the arterial line for PBS levels. In the PBS-treated animals, four of nine also had blood flows quantitated during the recovery period. Blood flows were not quantitated 30 min after the PBS infusion because previous studies in newborn dogs and in newborn piglets have shown that CBF under those conditions does not change from SS values (1, 3).

RESULTS

Physiologic variables. Control and PBS-treated piglets were normoxic until the hypoxia-hypertensive period, and the animals were normocarbic, with no significant differences in PCO_2 within or between groups throughout the experiments (Table 1). pH changed variably during the hypoxic period, but rapidly returned to baseline. Mean pH were not significantly different between the groups or within groups at any point. Systemic arterial blood pressures in both groups were significantly elevated after phenylephrine was infused and remained elevated throughout the hypoxic and recovery periods (Table 1). Hematocrits ranged between 0.26 and 0.311 (mean 0.295), and PBS levels in treated piglets were $1107 \pm 474 \mu\text{mol/L}$ (SD). Farm-bred piglets in our experience have all had SS hematocrits averaging 0.28–0.301. The levels of PBS in the piglets have all been within the range considered therapeutic for seizure prevention (646–1722 $\mu\text{mol/L}$).

Total and regional CBF. Total CBF were not significantly different during SS when the values from the two groups were compared. When compared to SS, total CBF in the controls were significantly elevated during HT, HTHY, and RE (Fig. 1). In PBS-treated piglets, total CBF at all measurement periods were not significantly different from SS values (Fig. 1).

These findings were mirrored in the regional CBF, inasmuch as all regions except white matter showed significant increases in flow during HT, HTHY, and RE in controls, and in the white matter flows increased significantly during HT and HTHY but not during RE (Fig. 2). In PBS-treated piglets, there were no

Table 1. Physiologic variables*

	SS	HT	HTHY	RE
Controls				
BP (mm Hg)	57 ± 3	$97 \pm 3^\dagger$	$80 \pm 5^\dagger$	$76 \pm 6^\ddagger$
PCO_2 (kPa)	4.4 ± 0.1	4.0 ± 0.1	4.5 ± 0.2	4.8 ± 3
PO_2 (kPa)	12.3 ± 0.9	15.1 ± 0.2	$4.3 \pm 2^\dagger$	18.4 ± 3.1
PBS-treated				
BP (mm Hg)	51 ± 3	$103 \pm 4^\dagger$	$78 \pm 9^\dagger$	$85 \pm 4^\dagger$
PCO_2 (kPa)	4.4 ± 0.1	4.7 ± 0.2	4.3 ± 0.4	4.3 ± 0.5
PO_2 (kPa)	11.9 ± 1.5	16.5 ± 2.5	$4.3 \pm 0.3^\dagger$	16.7 ± 2.1

* PBS given immediately after SS microsphere infusion; levels at 30 min: $1107 \pm 474 \mu\text{mol/L}$ (SD). All hematocrits between 0.26 and 0.31. Values shown are means $\pm$ SEM; $n = 14$ for controls except for RE ($n = 7$). PBS-treated: $n = 9$ ($n = 4$ for RE).

$^\dagger p < 0.01$ compared to SS.

$^\ddagger p < 0.05$ compared to SS (analysis of variance for repeated measures, two-tailed and Bonferroni *t* tests).

Fig. 1. Total CBF for controls ($n = 14$) and PBS-treated ($n = 9$) newborn piglets during hypertension and hypoxia are shown. The values are means $\pm$ SEM.

Fig. 2. Regional CBF for the control and PBS-treated animals are shown. Values are means $\pm$ SEM. *, $p < 0.05$ compared to SS value; **, $p < 0.01$ compared to SS value; #, $p < 0.05$ compared to HTHY value; +, HT vs HTHY, $p < 0.05$.

significant increases in flow in any of the regions during HT, HTHY, or RE (Fig. 2).

Percent changes in CBF reflected the changes in mean flow values when SS/HT, SS/HTHY, and SS/RE for each group were calculated. The percentage of increase in tCBF during HTHY was significantly greater for the controls than it was for the PBS-treated animals (the mean increase for controls was 111%, for PBS-treated animals 35%).

DISCUSSION

The use of anticonvulsant doses (20 mg/kg) of PBS as prophylaxis against peri- and intraventricular hemorrhages in premature newborn infants remains a source of controversy (6–10). Clinical studies of its postnatal use have shown varying results without clear evidence of efficacy, although reports of the antenatal use of PBS suggest that preterm infants whose mothers receive 10 mg/kg PBS before delivery have significantly lower frequencies of grades 3 and 4 hemorrhages after birth (4, 5).

The rationale for PBS use in preterm infants has been 2-fold.

First, phenobarbital is a sedating agent and has been shown to reduce blood pressure peaks in preterm infants (11, 12). The supposition has been that the reduction of blood pressure peaks may reduce CBF surges that occur during those peaks, either because the peaks supersede the upper limit of autoregulation or because some infants' cerebral circulations are already pressure-passive. Second, barbiturates in varying doses have been shown to have some neuroprotective effects and possibly to protect other cellular elements (endothelial cells) by oxygen radical scavenging activity (16–19). It is not certain that PBS in anticonvulsant doses is neuroprotective, but it has been shown to reduce the incidence of HT-induced intraventricular hemorrhages in one animal study (2). In addition, it has been shown to reduce CBF during experimentally induced hypertension in newborn dogs (1) and after bicuculline-induced seizures in newborn pigs (20), but not to reduce the incidence of intraventricular hemorrhages after severe asphyxial insult in newborn dogs (21). It is well known that asphyxia and the physiologic components of asphyxia cause significant changes in vascular reactivity and CBF (22, 23). Thus, we have hypothesized that the effectiveness of an agent such as PBS might depend upon the status of the brain's vasculature at the time of its administration.

Because hypoxia is a major component of an asphyxial insult, and because an initial cerebrovascular response to hypoxia is rapid dilatation, we proposed that pretreatment with PBS might not have a blood flow lowering effect during HT and hypoxia, which might explain some of the inconsistent results from clinical studies of preterm, frequently posthypoxic, newborn infants.

However, we found that during short-term HTHY, PBS pretreatment continued to be effective in preventing cerebral hyperemia. We do not know if this effect would have persisted for longer lasting hypoxia or for hypoxia in conjunction with hypercarbia. What was evident, however, was that PBS caused significant lowering of CBF during HT without lowering the systemic arterial blood pressure, inasmuch as blood pressures during phenylephrine-induced HT were the same in both the controls and the PBS-treated animals [the mean systemic arterial pressures in both the control and experimental animals during HT were above the upper limit of autoregulation for the newborn piglet (24)]. It has been reported that barbiturates will increase α -adrenoreceptor-mediated vasoconstriction in arterial smooth muscles under certain circumstances (25). Thus, pretreatment with PBS may cause a degree of cerebral vasoconstriction that can moderate blood flow pressure-passivity after systemic blood pressure is increased.

It remains to be determined whether the hyperemia-sparing effect of PBS has secondary metabolic consequences because the vasodilatation occurring during hypoxia most certainly results in compensatory increases in oxygen delivery when oxygen supply is low. Might pretreatment with PBS cause an increase in tissue oxygen debt? The answer to this depends upon whether PBS in this dose also causes a decrease in the cerebral metabolic rate, so that the need for oxygen delivery is decreased. A study of PBS at doses of 50–250 mg/kg in young rats showed that there were dose-dependent reductions in CBF, increases in cerebral vascular resistance, and decreases in cerebral metabolic rate; however, PBS at 20 mg/kg doses was not evaluated (13). In another study, no changes in cerebral oxygen consumption were documented 5 min after infusion of 20 mg/kg PBS in newborn piglets (26); however, it can be argued that the equilibration time for phenobarbital is 30 min (15), and thus maximum tissue distribution of the drug had not yet occurred.

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APPENDIX

The labeled microsphere technique for blood flow determinations. Isotopically labeled 15- μ m microspheres are injected into the left cardiac ventricle and are trapped in the microcirculation. For the moment at which they are infused, they represent the proportion of cardiac output going to a particular organ. This proportion can be calculated by comparing the counts in the tissue to counts in a reference arterial blood sample drawn at the time of the microsphere infusion. Assumptions of the method are that all of the microspheres going to an organ are trapped in the capillaries, that their flow characteristics are similar to those of red blood cells, that good mixing is achieved by left ventricular injections, and that the spheres do not alter flow or other rheologic characteristics (27). In addition, the accuracy of the method can be reduced when the number of spheres in each sample of tissue falls below 400 (27). In small animals, we have found that infusions of spheres into the left atrium are fraught with error because of easy penetration of the pulmonary veins with resultant infusion of the spheres into the lungs. We have obtained consistent reference sample counts using left ventricular infusions of the spheres, and we have found that cannulation of the left ventricle via the left carotid artery is easily accomplished without compromise of blood flow to either brain hemisphere. Four injections of about 500 000-750 000 microspheres each are tolerated in the newborn piglets without hemodynamic changes or compromise of the coronary arteries. The microspheres are prepared for injection by ultrasonification, vortexing, and mixing with 0.05% Tween-80. When they are to be injected, they are drawn into a plastic 1-mL syringe and injected over 10 s into the left ventricular catheter. This is followed immediately by a saline flush. The injection commences just after a reference arterial blood sample has begun to enter a 5-mL syringe from the catheter in the abdominal aorta. A Sage (Orion Research Inc.) withdrawal pump is used to deliver the reference sample. Arterial blood (2.2 mL) is pulled by the withdrawal pump over a 1-min, 10-s time interval. This timing is chosen to cover a complete circulation time in the newborn piglet. The reference sample is hemolyzed and separated into three counting tubes. Sibling donor blood that has been lightly heparinized is transfused into the piglet to replace the reference blood sample. After all experimental procedures are completed and all four sets of microspheres have been infused, the animal is killed with i.v. Nembutal. The brain is quickly removed and sectioned, each section is weighed and placed into a counting tube, and the tubes are analyzed by a multichannel, Wallac Compugamma counter. Reference samples, background, and standards are also counted, and all counts are corrected for cross-channel spillover. Flows are calculated by the equation described earlier in this article.