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Phenobarbital and Cerebral Blood Flow during Hypotension in Newborn Pigs[1]

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ABSTRACT. Phenobarbital sodium (PhS) has been used in anticonvulsant concentrations in premature newborns in attempts to prevent peri- and intraventricular hemorrhages (PIVH). Its effectiveness in preventing PIVH in clinical situations is still uncertain; however, PhS has reduced PIVH after hypertension in newborn beagles, and it has lowered cerebral blood flow (CBF) during hypertension in newborn beagles and piglets. We hypothesized that PhS might reduce CBF during systemic hypotension. Twelve control and 12 PhS-treated piglets (1 to 2 d old) were used for microsphere determinations of CBF during *1*) steady state; *2*) 30 min after PhS (treatment group) or saline infusion (controls); and *3* and *4*) during two levels of graded hypotension. Mean arterial blood pressure (MABP) was 61 ± 13 (SD) mm Hg (controls) and 57 ± 13 (SD) mm Hg (PhS) during steady state. Thirty min after the PhS or saline infusion, MABP and CBF remained unchanged in both groups. CBF during hypotension at MABP of 41 ± 5 (SD) mm Hg was significantly higher in controls than was CBF at MABP of 39 ± 6 (SD) mm Hg in the PhS-treated group ($p = 0.044$); CBF in the two groups during the second hypotensive phase was not significantly different. However, LOWESS regression suggested that the CBF from the controls dropped as the arterial pressure decreased to less than 37 mm Hg, whereas PhS treatment lowered CBF during hemorrhagic hypotension compared with controls at blood pressures greater than 37 mm Hg but did not lower CBF further at lower systemic blood pressures. This suggests that PhS would not, by itself, cause ischemia at blood pressures near the lower limit of autoregulation. (*Pediatr Res* 33: 598–602, 1993)

Abbreviations

CBF, cerebral blood flow
MABP, mean arterial blood pressure
PhS, phenobarbital sodium
PIVH, periventricular and intraventricular hemorrhage
SS, steady state
PG, prostaglandin
LOWESS, locally weighted regression and smoothing scatter plots
TX, thromboxane

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Some clinical studies have reported that the incidence and/or severity of PIVH in preterm infants decreases when anticonvulsant dosages of PhS are given either antenatally or early in the postnatal period (1–5). Other studies have questioned the effectiveness of PhS in preventing PIVH (6–10). It has been shown that PhS in anticonvulsant concentrations lowers CBF during hypertension in newborn beagles and pigs, which may explain its protective effect against PIVH (11, 12). In addition, the incidence of PIVH in PhS-treated newborn beagles after hypertensive insult has been reduced (13). Because of the frequency with which newborns are subjected to hypotensive stress and the question of whether the blood flow–lowering effect of PhS might potentiate cerebral oligemia or ischemia, we determined the effects of PhS upon CBF during graded hemorrhagic hypotension in the newborn piglet.

MATERIALS AND METHODS

Approval for this study was obtained from the Baylor College of Medicine Animal Care and Use Committee (NIH Assurance no. A3823–01). One- to 2-d-old cross-bred newborn piglets were obtained from a local breeder. Twelve animals served as controls, and 12 animals were PhS-treated.

Surgical procedures were performed under local Xylocaine (Astra Pharmaceutical Products, Inc., Westborough, MA) and i.v. α-chloralose (50 mg/kg) anesthesia (Alpha-Chloralose, Sigma Chemical Co., St. Louis, MO) after Ketamine (Ketaset, Aveco Co., Fort Dodge, IA: 200 mg) and Xylazine (Anased, Lloyd Laboratories, Shenandoah, IA, 20 mg) were given in combination intramuscularly (0.5 mL/kg). Body temperature was monitored by rectal probe and was kept at 38 to 39°C (normothermia) by heating pad. Polyethylene catheters (Intramedic, Clay Adams, Parsippany, NJ) were placed in the abdominal aorta via the femoral arteries for monitoring arterial blood pressure, for blood sampling, and for obtaining the microsphere reference samples. A catheter was also placed in the left cardiac ventricle via the left carotid artery. Each animal was artificially ventilated by Harvard small-animal respirator (Harvard Apparatus, Millis, MA) after tracheostomy.

CBF was quantitated by the radioactive microsphere method described previously by Goddard-Finegold *et al.* (11). CBF values were quantitated by the infusion of radioactive microspheres (15-μm size) using the radiolabels ^{141}Ce, ^{51}Cr, ^{103}Ru, and ^{95}Nb (Medical Surgical Division, 3M Corp., St. Paul, MN, and New England

Nuclear Corp., Dupont, Boston, MA). Reference arterial 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 the experimental protocol, the animals were killed by intracardiac infusion of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL). The brain was extracted immediately, and areas were separated for isotope counting. Sections from medulla, pons, midbrain, cerebellum, thalamus, white matter, and cortex were weighed, placed into scintillation tubes, and counted after correction for cross-channel spillover by a Compugamma 1282 gamma counter (Wallac; Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). Flow values were calculated by the following equation:

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

Total CBF values were calculated using total brain weights and total brain counts.

Experimental design. CBF was determined during SS, 30 min after PhS (Phenobarbital, Goldline Co., Ft. Lauderdale, FL) infusion (PhS-treated group) or saline infusion (controls), and twice during graded hypotension. The first SS CBF and second CBF were determined at normal MABP. Arterial blood gases (0.4-mL samples, Corning 170 pH/Blood Gas Analyzer, Corning Inc., Medfield, MA), Hb (BMS Cynox-1 Digital Hemoglobinometer, Cynox Corp., Clearwater, FL), and hematocrit were determined at each blood flow quantitation. PhS was infused at a dose of 20 mg/kg over 5 min intraarterially immediately after the SS CBF determination. Serum levels of PhS were obtained 30 min after its administration; because the drug was administered just after the first microsphere infusion, serum levels were obtained just before the second microsphere infusion. The same volume was infused and withdrawn in control animals. The amount of blood withdrawn for each reference sample was reinfused using lightly heparinized sibling donor blood after each CBF determination in both groups. After the second CBF determination, blood was withdrawn into a heparinized reservoir system for the induction of hemorrhagic hypotension. The third and fourth blood flow determinations were done at two stable successive hypotensive phases, each of which lasted 20 min.

Analysis of PhS concentrations in serum. The serum concentration of PhS were measured 30 min after administration of the drug using an enzyme immunoassay (EMIT, SYVA, Palo Alto, CA) by the Texas Children's Hospital Chemistry Laboratories.

Statistical analyses. Analysis of the within-group variations in physiologic parameters and flow values at each treatment time was made using analysis of variance and Bonferroni *t* tests when necessary. Analysis of between-group differences at each treatment time was performed using unpaired *t* tests. The data were further analyzed using the BMDP5V programs (Biomedical Data Program, 5V) for unbalanced repeated-measures designs. This program was used because it allows for the presence of missing data in a repeated-measures experiment and allows for deviations from the standard repeated-measures variance assumptions. Both within- and between-group comparisons were made with this method. To examine the relationship of CBF to blood pressure, the method of LOWESS smoothing was used to generate separate curves for the treated piglets and controls (14). Based on these curves, separate regressions were performed using the random effects model for longitudinal data as developed by Laid and Ware (15). The possibility that the slope of the regression changed at 37 mm Hg was tested using this method.

RESULTS

Physiologic data (MABP, pH, P_{O_2}, P_{CO_2}, and Hb) for PhS-treated and control animals are presented in Table 1. No animals were hypoxic, and there were no significant changes in O_2 or CO_2 tensions within or between the two groups. MABP at 30 min after infusion of PhS or saline was not significantly different from SS in either group. MABP was significantly decreased during hypotension compared with SS in both groups ($p < 0.01$). MABP of the two groups during each of the four CBF determinations did not show significant differences. PhS serum concentrations were 1016 ± 172 μmol/L (23.6 ± 4 μg/mL) in the treated animals.

The sequential changes of CBF in both groups are presented in Figure 1. CBF during the four measurements were 58 ± 13, 57 ± 13, 47 ± 12, and 29 ± 12 mL · min^{-1} · 100 g^{-1} in controls, and 54 ± 15, 47 ± 12, 37 ± 11, and 36 ± 14 mL · min^{-1} · 100 g^{-1} in the PhS-treated group. There were no significant differences in CBF between the two groups during SS, 30 min after PhS or saline infusion (controls), and hypotension 2; however, the CBF in controls was significantly higher than that of the PhS-treated group during hypotension 1 ($p = 0.044$).

In the PhS-treated group, significant CBF decreases compared with SS were observed during hypotension at MABP of 39 ± 6 mm Hg ($p < 0.01$) and 29 ± 6 mm Hg ($p < 0.01$). In contrast, in controls, CBF did not differ significantly from SS during hypotension at a MABP of 41 ± 5 mm Hg, and only decreased significantly at 31 ± 4 mm Hg ($p < 0.01$). These results were confirmed using BMDP5V. Analysis of regional CBF demonstrated that decreases occurred in all regions during moderate hypotension in the PhS-treated group, but decreases occurred only in the white matter and cerebral cortex in the controls during the first hypotensive phase (Fig. 2).

Results of statistical analysis. Using the BMDP5V program, the PhS (treated) and control (untreated) groups were compared. As the presence of missing data are allowed by this method, all animals contributed to the analysis. It was found that for both blood pressure and CBF there was evidence that the correlation among the treatment periods was neither constant nor patterned, so the unstructured covariance matrices were used in the analysis. The models fit were in the "classic" repeated measures form, with a treatment period effect, treatment effect, and period by treatment interactions. Significant period effects indicate that the quantity being measured changes with time; significant treatment effects indicate differences in the groups overall, and significant period by treatment interactions indicate that the two groups do not respond in the same way at each of the treatment times in which measurements are made. For the blood pressure measurements, the period effect was extremely significant, with a χ^2 of 144.65 (3 *df*, $p < 0.001$). This reflects the substantial changes in blood pressure introduced at hypotension 1 and hypotension 2 in both groups. For the CBF measurements, the treatment period (time) effect was found to be extremely significant, with a χ^2 of 56.37 (3 *df*, $p < 0.001$); and the χ^2 for the period by treatment interaction was significant at the $p < 0.022$ level, with a value of 9.61 (3 *df*).

The LOWESS lines indicated that the CBF was fairly constant across all the observed blood pressures in the PhS-treated group but that it fell rather sharply in the control group for pressures less than 37 mm Hg (Fig. 3). Using the random effects method for longitudinal data, a regression line was fit in the control animals only for the data at blood pressures less than 37 mm Hg and one for data at blood pressures greater than 37 mm Hg. No statistically significant difference in the slopes was found ($\chi^2 = 1.76$, 1 *df*, $p < 0.185$). In a separate analysis, using both control and treated animals, and fitting one line for the range of blood pressure data for each group, a nearly significant interaction of blood pressure and treatment group was found ($p < 0.088$), with the difference indicating lower CBF at the lower blood pressures in the control group.

DISCUSSION

PhS has been used in anticonvulsant concentrations in some nurseries in attempts to prevent PIVH and has also been the

Table 1. *Physiologic variables**

	SS	30 min	Hypo 1	Hypo 2
Controls (n = 12)				
MABP (mm Hg)	61 ± 13	65 ± 14	41 ± 5†	31 ± 4†
P_{CO_2} (kPa)	4.5 ± 0.3	4.5 ± 0.03	4.9 ± 0.8	4.4 ± 0.5
P_{O_2} (kPa)	12.4 ± 3.2	11.7 ± 3.1	10.3 ± 2.4	11.7 ± 4.0
pH	7.44 ± 0.07	7.44 ± 0.07	7.46 ± 0.05	7.41 ± 0.05
Hb (g/L)	91 ± 17	93 ± 16	88 ± 21	84 ± 18
PhS-treated (n = 12)				
MABP (mm Hg)	57 ± 13	53 ± 20	39 ± 6†	29 ± 6†
P_{CO_2} (kPa)	4.5 ± 0.3	4.7 ± 0.3	4.7 ± 0.5	4.4 ± 0.4
P_{O_2} (kPa)	13.7 ± 1.9	12.7 ± 2.7	11.2 ± 2.0	11.3 ± 2.5
pH	7.43 ± 0.07	7.41 ± 0.06	7.43 ± 0.07	7.43 ± 0.08
Hb (g/L)	88 ± 19	87 ± 12	89 ± 18	88 ± 19

* All values are means ± SD. 30 min, 30 min after PhS or saline infusion; hypo 1, first hypotensive phase; hypo 2, second hypotensive phase.
† $p < 0.01$ compared with SS value.

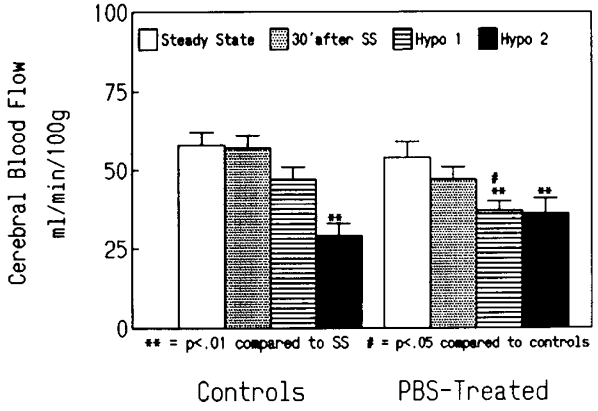

Fig. 1. Total CBF in controls and PhS-treated animals. All values are ±SD. *30' after SS*, 30 min after SS; *hypo 1*, first hypotensive phase; *hypo 2*, second hypotensive phase.

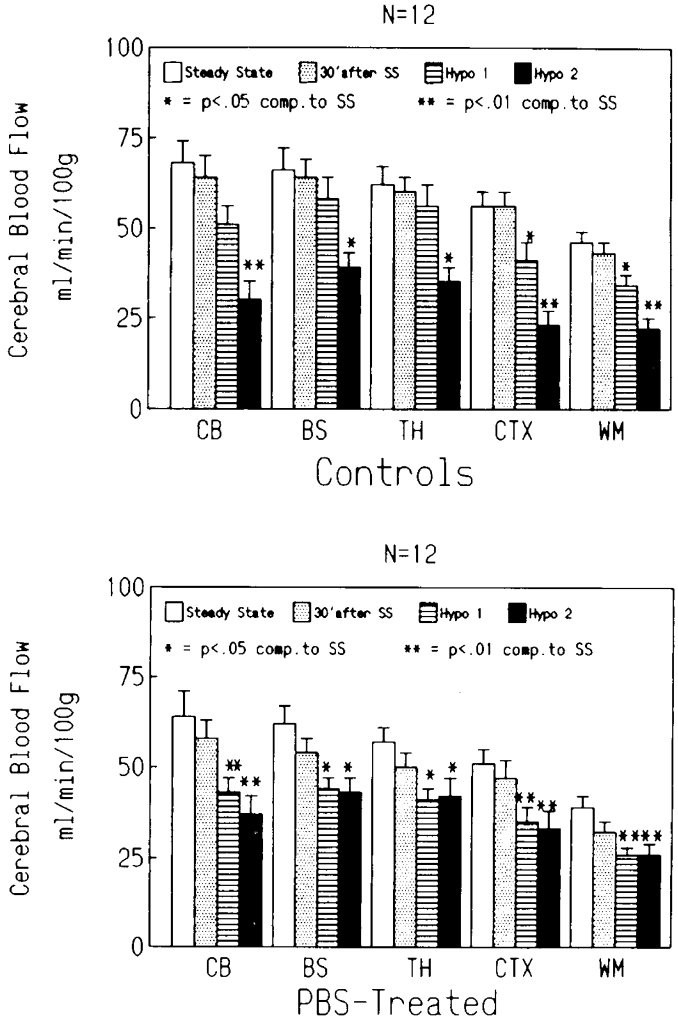

Fig. 2. Regional CBF in controls and PhS-treated animals. All values are ±SD. *30' after SS*, 30 min after SS; *hypo 1*, first hypotensive phase; *hypo 2*, second hypotensive phase; *CB*, cerebellum; *BS*, brainstem; *TH*, thalamus; *CTX*, cortex; *WM*, white matter.

drug of choice in the treatment of neonatal seizures. However, its routine use in the first postnatal week in premature infants raises the question of potential risks related to PhS-induced hypotension and/or the possibility of reduction of blood flow to the immature brain. PIVH is rarely an isolated abnormality in the premature infant brain. As many as 92% of premature infants with PIVH at autopsy have been reported to have ischemic lesions as well (16), and, in the clinical situation, hypotension may occur frequently in premature newborns. Although the pathophysiology of brain ischemia that occurs with PIVH is unclear at present and may be multifactorial (including secondary mechanisms triggered by the hemorrhage itself), we feel that we should be certain that physician-initiated pharmacologic interventions do not make ischemia worse. Thus, our study was designed to test the effect of PhS on CBF during hypotension in newborn pigs.

Our results showed that significant CBF decreases compared with SS were observed during hypotension at MABP of 39 ± 6 mm Hg and 29 ± 6 mm Hg in the PhS-treated piglets. However, CBF were unchanged during hypotension at MABP of 41 ± 5 mm Hg and only decreased significantly at MABP of 31 ± 4 mm Hg in controls. The reduction of CBF due to anticonvulsant levels of PhS has been reported previously in hypertensive newborn beagles (11) and in newborn pigs with seizures (17). PhS has also attenuated CBF during hypertension in newborn piglets, and that effect has persisted during 5 min of hypoxia without hypercapnia (12).

Our analyses indicate that whereas the manipulation to lower blood pressure produced significant changes, these changes were not different between the two groups. Although CBF changed compared with baseline in both the PhS-treated and control animals, there was no overall difference between the CBF in the treated and control animals until the final treatment period, during which the CBF in the treated animals was higher than

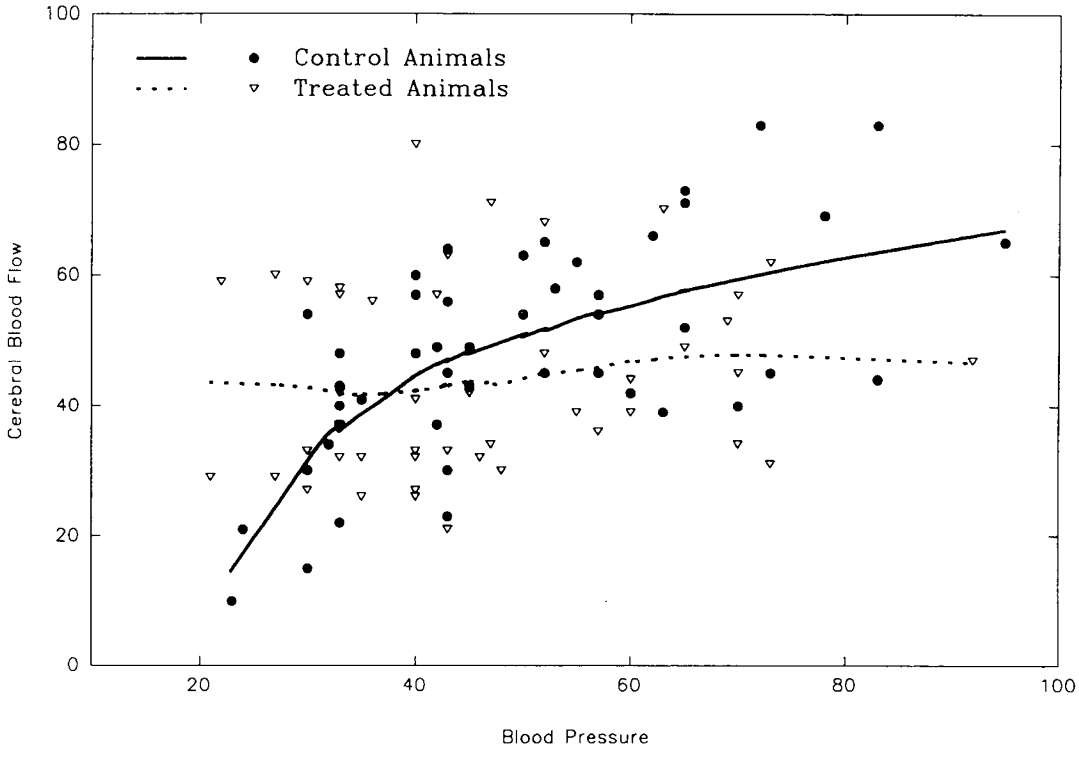

Fig. 3. LOWESS lines for CBF in PhS-treated (▽) and control (●) piglets. The data intersect clearly at the blood pressure of 37 mm Hg.

that in the controls. This difference is supported by the significant interaction of period with treatment from the BMDP5V analysis.

Analysis of the current data suggests that CBF began to fall when the MABP fell below 37 mm Hg in the control group and that, whereas the CBF values of the PhS-treated group were lower than those of the controls at MABP greater than 40 mm Hg, they were actually greater than those of the controls when MABP were lower than 37 mm Hg. The lack of significance in the difference in slope above and below the 37-mm Hg value is not surprising, as only eight observations fell into this region.

These findings suggest that, rather than simply lowering CBF during hemorrhagic hypotension, PhS may lower it during modest hypotension and maintain it at that level during moderate hypotension. The mechanism of these actions has not been determined. Laudignon *et al.* (18) suggested in their study of newborn piglets under stress that PhS potentiates the vasoconstrictor effect of catecholamines. There is also evidence in newborn pigs that sympathetic nerve stimulation or administration of norepinephrine constricts pial arteries (19) and that sympathetic nerve stimulation reduces CBF (20). Other hypotheses include a direct action of PhS on vascular smooth muscle (21), a decrease in metabolic rate (thus producing a decrease in CO_2 production and H^+ content in the perivascular space, leading to a reduction of vasodilatation) (17) or an effect on intracellular movement of calcium ions in vascular smooth muscle (22).

These possible explanations do not give insight into the action of PhS during hypotension, however. The prostanoid synthesis inhibitor, ibuprofen, has been shown to widen the autoregulatory range of newborn piglets during hypotension and hypertension and, yet, not to lower resting CBF (23). Ibuprofen's widening of the autoregulatory range has been associated with significant decreases in concentrations of PGE and PGF_{2a} in arterial and sagittal sinus blood and with virtually undetectable TXB_2 in sagittal sinus blood in piglets during both hypotension and hypertenion. However, in vehicle-treated control piglets, PGE increased dramatically during both hypotension and hypertension, and PGF_{2a}, 6-keto-PGF_{1a}, and TXB_2 increased significantly only during hypotension (23). The authors' hypothesis was that the TX concentrations may play an important role in setting the lower limit of autoregulation, whereas the PGE, PGF, and PGI concentrations might play a role in setting the upper limit of autoregulation. The interactions of PhS, TX, and PG have not been determined in the newborn piglet, however, and certainly should be questions for further study.

In summary, PhS in anticonvulsant concentrations reduced CBF during modest hypotension (blood pressure > 37 mm Hg) but seemed to allow CBF to be maintained at lower blood pressures (blood pressure < 37 mm Hg > 22 mm Hg). Although this finding should be further corroborated, it suggests that PhS does not by itself cause ischemia at blood pressures near the lower limit of autoregulation.

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