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Increasing Ventilation Pressure Increases Cortical Subarachnoid Cerebrospinal Fluid Prostanoids in Newborn Pigs

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ABSTRACT. This study examines the responses of pial arterioles and venules to increased mean airway pressure ($\bar{P}_{aw}$) in newborn pigs. We further characterized the changes in cortical subarachnoid cerebrospinal fluid prostanoids with increased $\bar{P}_{aw}$, both before and after cyclooxygenase inhibition with indomethacin. Eight chloralose anesthetized newborn pigs were equipped with closed cranial windows and ventilated with a conventional infant pressure-cycled respirator. Increasing $\bar{P}_{aw}$ from 3.2 ± 0.3 cm water to 14.3 ± 0.6 cm water did not change pial arteriole or venule diameters. Cerebrospinal fluid prostanoids (6-keto-$PGF_{1\alpha}$, TxB_2, PGE_2, and $PGF_{2\alpha}$), however, were increased reversibly (3- to 5-fold) by increasing $\bar{P}_{aw}$. After indomethacin (5 mg/kg, intravenous) pial arterioles constricted approximately 15% with increased $\bar{P}_{aw}$. These results suggest that increasing ventilation pressure increases brain prostanoid production. Prostanoids appear to inhibit vasoconstriction and may be important in maintaining cerebral blood flow during the stress of mechanical ventilation. (*Pediatr Res* 22: 647–650, 1987)

Abbreviations

$\bar{P}_{aw}$, mean airway pressure
CSF, cerebral spinal fluid
im, intramuscular
iv, intravenous
PIP, peak inspiratory pressure
PEEP, peak end expiratory pressure
PG, prostaglandin
TxB_2, thromboxane B_2
PVH/IVH, periventricular/intraventricular hemorrhage

Positive pressure ventilation commonly is used in neonatal intensive care units. We have noted previously that cerebral perfusion is maintained during positive pressure ventilation of newborn pigs despite significant reduction of cardiac output (1). In addition we have found that locally produced prostanoids are an important factor in the maintenance of cerebral blood flow during hemorrhagic hypotension (2) and in the increase in cerebral blood flow during asphyxia (3) in the neonatal pig. These two observations led to the hypothesis that prostanoids also may play a role in the maintenance of cerebral perfusion during positive pressure ventilation. The objective of the present study is to investigate the role of locally produced prostanoids in controlling microcerebral vascular tone during periods of increased airway pressure.

The use of the closed cranial window technique allows direct visualization of pial arterioles and veins and provides the opportunity to sample cortical subarachnoid CSF which can be used to measure cerebral prostanoid synthesis (4). In the present study we used the cranial window to observe the response to pial arterioles and venules (50–200 μm) to positive pressure ventilation. Further, we quantified the effect of positive pressure ventilation in the levels of prostanoids in subarachnoid CSF samples taken from the cranial window.

METHODS

Eight piglets (0.6–1.6 kg, age 1–5 days) were anesthetized with ketamine hydrochloride (33 mg/kg, im) and acepromazine (3.3 mg/kg, im) and then maintained on α-chloralose (50 mg/kg initially followed by 5 mg/kg/h, iv). Catheters were placed into a femoral vein and artery. The venous catheter allowed for drug administration while the arterial catheter was used for continuous blood pressure monitoring and for withdrawal of arterial blood gas and pH samples. Blood pressure was determined using a Statham pressure transducer and a Gould recorder. The trachea was cannulated with a 3.0 mm (id) straight endotracheal tube and tied in place to prevent air leak. The animals were ventilated with an infant pressure respirator (Bourns BP 200, Bourns Life Systems, Riverside, CA), and the proximal airway pressure was monitored continuously. The animals were ventilated with room air with initial ventilatory parameters of 20–30 breaths per minute, an inspiratory time of 0.5 s, a PIP of 12–15 cm water, and a PEEP of zero. Body temperature was maintained at 37–38° C with a water-circulating heating pad.

The scalp was removed, and a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura and arachnoid membranes were cut without touching the brain, and all cut edges were reflected over the bone so that the subarachnoid space was not exposed to damaged tissue. A stainless steel and glass cranial window was placed in the hole and cemented into place with dental acrylic. The space under the window was filled with artificial CSF (220 mg KCl, 132 mg $MgCl_2$, 221 mg $CaCl_2$, 7710 mg NaCl, 402 mg urea, 665 mg dextrose, 2066 mg $NaHCO_3$ per liter, pH = 7.33; PCO_2 = 46 mm Hg; PO_2 = 43 mm Hg) through needles incorporated into the sides of the window. The volume of fluid directly under the window is approximately 500 μl and was continuous with the subarachnoid space. After implantation of the window, at least 30 min was allowed before experimentation was begun.

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Pial vessels were observed with a Wild M75 trinocular stereomicroscope, a television camera (model VC-65SL, Dage-MTI, Michigan City, IN) mounted on the microscope, and a video monitor (model CT 1930V, Panasonic Corp., Secaucus, NJ). Vessel diameter was measured with a video microscaler (Model VPA 1000, For-A-Corp, Los Angeles, CA).

Protocol. The CSF under the cranial window was replaced, and pial arteriole and venule diameters were measured during an initial control period of five minutes. During this period the animals were ventilated at a low mean airway pressure ($\overline{P_{aw}}$ = 3.2 ± 0.3 cm water). At the end of this 5-min control period 300 μl of CSF from under the window was collected and frozen for prostanoid analysis and the CSF under the window replaced. Airway pressure then was increased by increasing both the PIP (to 18–20 cm water) and the PEEP (to 8–10 cm water) thus maintaining a constant tidal volume and PCO_2. The $\overline{P_{aw}}$ at this time was 14.3 ± 0.6 cm water. This pressure was selected from previous experience which has shown that this airway pressure would decrease cardiac output without altering arterial blood pressure (1). Furthermore, we know from this same previous study that sagittal sinus pressure would be increased at this $\overline{P_{aw}}$ thus forcing the cerebral circulation to accommodate to an increase in venous pressure. During this 5-min period the response of the pial vessels was recorded. At the end of five minutes a second sample of CSF was taken and the ventilation returned to the control settings ($\overline{P_{aw}}$ = 3.2 ± 0.3 cm water). During a third period (control 2) the vessels were measured again. At the end of this 5-min period a third CSF sample was taken. We then administered indomethacin trihydrate (5 mg/kg, iv). The dose of indomethacin used has been shown previously to cross the blood-brain barrier in sufficient quantity to inhibit the formation of cyclooxygenase metabolites (5) on the cerebral surface. During the ensuing 30- to 45-min period the CSF under the window was flushed several times. The protocol then was repeated at the same three airway pressures (control 1:increased $\overline{P_{aw}}$:control 2). No CSF samples were collected after indomethacin as previous experience (4) has shown that CSF prostanoids are undetectable after this dose of indomethacin.

Sagittal sinus pressure. Four piglets of similar size and age were prepared and ventilated as above except that a sagittal sinus catheter was placed instead of a cranial window. A midline scalp incision was made and a 23-gauge teflon catheter was inserted into the sagittal sinus. Sagittal sinus pressure as well as proximal airway pressure were then monitored in the same manner as arterial blood pressure. Airway pressure then was increased in a gradual stepwise fashion from a $\overline{P_{aw}}$ of 3 cm water to a $\overline{P_{aw}}$ of 40 cm water.

Time control animals. Four additional animals were prepared as described above and pressure ventilated for 4 h. At hourly intervals the airway pressure was increased. Pial arteriolar and venule measurements were recorded to insure that responses of the vessels were consistent.

Prostanoid analysis. CSF samples were analyzed for 6-keto-$PGF_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), TxB_2, PGE_2, and $PGF_{2\alpha}$ using radioimmunoassay methods previously described (4). Antibodies to 6-keto-$PGF_{1\alpha}$, TxB_2, PGE_2, and $PGF_{2\alpha}$ were produced in rabbits immunized with prostanoids coupled to thyroglobulin using the mixed anhydride method. Cross-reactivity of all the prostanoids with other eicosanoids tested was less than 1%. The assays were performed in gelatin-Tris buffer using the appropriate tritiated prostanoid. After 24 h of incubation at 4° C, the free fraction was separated from that bound to antibody by adsorbing the unbound ligand on activated charcoal. Data were handled by computer with determination of second order regression of tracer bound to antibody against unlabeled prostanoid by method of least squares. All unknowns were assayed at three dilutions; the unknown dilution curves and the standard curve had to be parallel before the results were used. Values are reported in pg/ml.

Statistical analyses. Pial arteriole and venule diameters, systemic arterial pressure, and prostanoid levels were analyzed using repeated measures analysis of variance. If the F value was significant, the Student-Newman-Keuls test was performed. A level of $p < 0.05$ was considered significant in all statistical tests. Values are reported as means ± SEM of raw values.

RESULTS

Increasing ventilation pressure had no effect on pial arteriole or venule diameters prior to treatment with indomethacin (Table 1). Table 1 also lists the airway pressures, mean arterial blood pressures, pH, and blood gases recorded during this study.

Figure 1 shows the prostanoid values from this study. The levels at control 1 represent basal prostanoid levels and demonstrate that cortical CSF prostanoids are present without stimulating their production. The hydrolysis product of prostacyclin (6-keto-$PGF_{1\alpha}$), PGE_2, TxB_2, and $PGF_{2\alpha}$ all increased significantly when $\overline{P_{aw}}$ was increased. When $\overline{P_{aw}}$ was reduced (control 2) prostanoid levels returned to near baseline.

After the administration of indomethacin pial arterioles constricted 15% when $\overline{P_{aw}}$ was increased while the venule diameters were unchanged (Table I). When the $\overline{P_{aw}}$ was returned to its original value, the vessels returned to their original diameter.

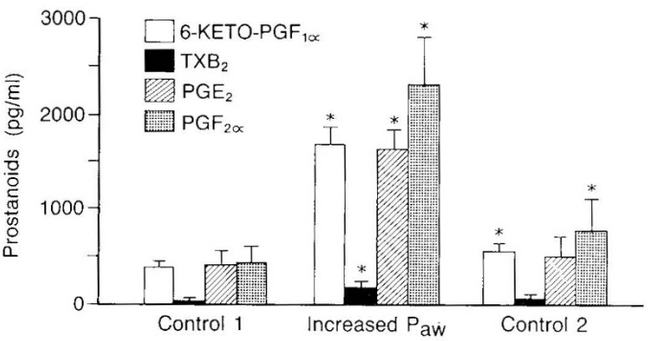

Fig. 1. Effects of increased $\overline{P_{aw}}$ on cortical subarachnoid CSF prostanoids. Levels of 6-keto-$PGF_{1\alpha}$, TXB_2, PGE_2, and $PGF_{2\alpha}$ are given in pg/ml (mean ± SEM) (*$p < 0.05$ compared to control 1).

Table 1. *Pial arteriole and venule diameters, $\overline{Paw}$, mean arterial blood pressure (BP), and blood Gas/pH [mean ± SEM (n = 8)]*

	Before indomethacin			After indomethacin		
	Control 1	Increased $\overline{P_{aw}}$	Control 2	Control 1	Increased $\overline{P_{aw}}$	Control 2
Arteriole diameter (μm)	168 ± 11	174 ± 14	169 ± 11	174 ± 14	148 ± 13*	175 ± 14
Venule diameter (μm)	192 ± 12	190 ± 14	188 ± 13	192 ± 15	187 ± 9	192 ± 14
$\overline{P_{aw}}$ (cm H_2O)	3.2 ± 0.3	14.3 ± 0.6*	3.2 ± 0.3	3.1 ± 0.3	14.1 ± 0.3*	3.1 ± 0.3
BP (mm Hg)	56 ± 8	56 ± 9	56 ± 9	53 ± 6	53 ± 8	52 ± 6
pH	7.48 ± 0.12	7.46 ± 0.18	7.48 ± 0.11	7.46 ± 0.14	7.49 ± 0.20	7.47 ± 0.18
PCO_2 (mm Hg)	28 ± 2	27 ± 3	27 ± 3	28 ± 3	29 ± 4	27 ± 4
PO_2 (mm Hg)	82 ± 4	88 ± 3	86 ± 3	85 ± 3	89 ± 2	87 ± 4

* Significantly different when compared to corresponding control 1 (Student-Newman-Keuls $p < 0.05$).

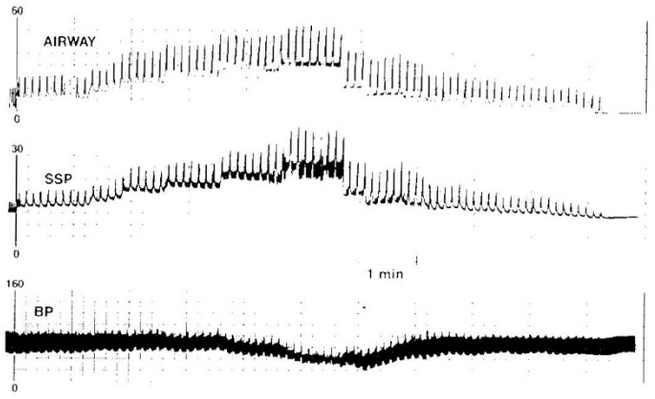

Fig. 2. Recordings of proximal airway pressure (*AIRWAY*), sagittal sinus pressure (*SSP*), and arterial blood pressure (*BP*) recorded while $\bar{P}_{aw}$ was increased from 10 to 38 cm water. Sagittal sinus pressure increases directly with airway pressure. Arterial blood pressure is affected beginning at a $\bar{P}_{aw}$ of approximately 35 cm water.

Blood pressure as well as arterial blood gases during these three periods were unchanged.

In all four animals prepared with sagittal sinus catheters, increasing airway pressure was reflected directly in sagittal sinus pressure (Fig. 2). Baseline mean sagittal sinus pressure was 3 ± 1.0 mm Hg with minimal ventilation pressure ($\bar{P}_{aw}$ = 3 ± 0.2 cm water) and increased to mean of 21 ± 1.5 mm Hg when $\bar{P}_{aw}$ was 40 cm water. Arterial blood pressure was not affected until the $\bar{P}_{aw}$ was 36 ± 2 cm water.

The time control animals maintained vessel diameters constant when $\bar{P}_{aw}$ was increased. For example, pial arterioles were 165 ± 8, 167 ± 3, and 168 ± 4 μm for control 1, increased $\bar{P}_{aw}$, and control 2 during the first increase in $\bar{P}_{aw}$. On subsequent increases in $\bar{P}_{aw}$ arteriolar diameters were 167 ± 6, 168 ± 8, and 170 ± 8 μm for control 1, increased $\bar{P}_{aw}$, and control 2. No indomethacin was used in the time control animals.

DISCUSSION

The results of the present study indicate that when ventilation pressure is increased, cortical subarachnoid CSF prostanoids increase to attenuate vasoconstriction.

Prostanoids play an important role in perinatal cerebrovascular physiology (6). Specifically, evidence from our own laboratory suggests that prostanoids are involved in regulating perinatal cerebral blood flow during periods of asphyxia and hypotension. In newborn pigs inhibition of cyclooxygenase blocks the cerebral hyperemia associated with asphyxia (3). When a similar animal preparation is subjected to hemorrhagic hypotension, CSF prostanoids increase except when the animals are treated with indomethacin. Indomethacin inhibits prostanoid production and brain blood flow falls (2).

Increasing ventilation pressure in newborn pigs decreases cardiac output and increases sagittal sinus pressure prior to decreasing systemic arterial pressure (1). Despite these cardiovascular effects, cerebral blood flow is maintained. The results of the present study suggest that the prostanoid system contributes to the maintenance of cerebral perfusion when the cardiovascular system is stressed by pressure ventilation, as with asphyxiated and hypotensive newborn pigs. Elevation of airway pressure causes cortical subarachnoid CSF prostanoids to increase. When prostanoid synthesis is inhibited, pial arterioles constrict in response to elevated $\bar{P}_{aw}$. Thus, prostanoids appear to inhibit vasoconstriction during pressure ventilation.

The stress of mechanical pressure ventilation causes vasoconstriction in most vascular beds. Increased cerebral prostanoid synthesis during increased airway pressure appears to attenuate cerebral vasoconstriction thereby aiding in maintenance of cerebral blood flow. As both vasodilator and vasoconstrictor prostanoids increase, we assume from the arteriolar response after indomethacin that dilator prostanoids (6-keto-$PGF_{1\alpha}$ and PGE_2) play a dominant role. Furthermore, it is possible that the constrictor prostanoids are produced secondarily to a general prostanoid synthesis stimulation. Such a pressor response followed by cerebral prostanoid production could occur via cerebral adrenergic nervous stimulation or via circulating catecholamines. We have found that both α-adrenergic stimulation and topical norepinephrine constrict piglet cerebral arterioles (7), while topical norepinephrine causes dilator prostanoids to be released into the cortical subarachnoid CSF (8).

We cannot explain why the diameter of pial venules did not increase when intrathoracic pressure was increased. It may be that the increased venous pressure does not reach distally to pial venules and that venous engorgement occurs only in the larger vessels of the brain.

A clear association exists between pressure ventilation and neonatal PVH/IVH (9) with more severe respiratory illness having a higher incidence of PVH/IVH. The role that increased intrathoracic pressure and thus increasing venous pressure play in PVH/IVH has been discussed (9), and it appears that restricted venous return may contribute to PVH/IVH. Furthermore, involvement of prostanoids in the development of PVH/IVH has been suggested. Some authors (10) report inhibiting prostanoid synthesis with indomethacin prevents PVH/IVH, while others (11) have failed ito show such an advantage. Although none of these studies examined cerebral blood flow during indomethacin therapy, other investigators have shown in both newborn animals (3) and humans (12) that indomethacin decreases cerebral blood flow. Although a link between mechanical ventilation and PVH/IVH appears possible, no previous work has demonstrated an association between mechanical ventilation and cerebral prostanoid production.

The closed cranial window technique used in this study allows both direct observation of pial vessels and sampling of the cortical subarachnoid CSF that bathes the observed blood vessels. Prostanoids in cortical subarachnoid fluid appear to have originated from the cortical surfaces because CSF from the cisterna magna has much lower prostanoid concentrations under control conditions (4). Possible sources of these prostanoids include cortical vessels, autonomic nerves associated with these vessels, and/or brain parenchyma. We speculate from the evidence available from other tissues that 6-keto-$PGF_{1\alpha}$ and TxB_2 are from the vessels while PGE_2 is from the nervous tissue (13–15). In the present experiment several possibilities exist for this local prostanoid production. In particular, it seems possible that when airway pressure is increased thus increasing venous pressure, the source of the measured prostanoids is the venous system. The major increase in venous pressure is probably transmitted to the sagittal sinus, and it is possible that this is the main site of prostanoid synthesis.

In summary, we have shown that increasing airway pressure using a conventional pressure-limited infant ventilator increases cerebral cortical production of prostanoids while not affecting pial arteriole or venule diameter. When prostanoid production is inhibited with indomethacin, pial arterioles constrict when $\bar{P}_{aw}$ is increased. This evidence further supports the impact that mechanical ventilation can have on the peripheral vascular system and also supports the important role that prostanoids play in the regulation of the cerebral circulation of stressed neonates.

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