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Alterations in Cerebrovascular Reactivity after Positive Pressure Ventilation

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ABSTRACT. Pressure ventilation of the newborn can adversely affect the cardiovascular system. Increasing airway pressure increases cerebral venous pressure, thus stressing brain vasculature. To test the hypothesis that cerebral venous distension caused by mechanical ventilation alters cerebral microvascular responses, we studied cerebrovascular responses before, during, and after positive pressure ventilation. Anesthetized newborn pigs were ventilated with a standard time-cycled, pressure-limited infant respirator. Pial arterioles were measured in response to hypercapnia, topical isoproterenol, and topical norepinephrine during control [mean airway pressure ($\overline{Paw}$) = 0.9 ± 0.05 kPa (4.8 ± 0.3 cm H_2O)] conditions, during 40–60 min of increased $\overline{Paw}$ [2.5 ± 0.2 kPa (13.9 ± 1.3 cm H_2O)], and when the $\overline{Paw}$ was lowered again. Pial arteriolar dilation in response to hypercapnia was not changed by increasing $\overline{Paw}$. Similarly, responses to isoproterenol and norepinephrine were unaltered during raised $\overline{Paw}$. However, a significant decrease in responses to topical isoproterenol and norepinephrine was observed after increased $\overline{Paw}$. These experiments show that specific prostanoid-independent cerebrovascular responses are altered subsequent to pressure ventilation, whereas prostanoid-dependent dilation to hypercapnia was not affected. These changes suggest that the newborn cerebral vasculature is affected by positive pressure ventilation, further raising the possibility that ventilation-induced alterations in microvascular responses could make the brain more vulnerable to added stresses after pressure ventilation. (*Pediatr Res* 32: 114–117, 1992)

Abbreviations

aCSF, artificial cerebral spinal fluid
CBF, cerebral blood flow
CSF, cerebral spinal fluid
Paw, mean airway pressure
LT, leukotriene
PVH/IVH, periventricular/intraventricular hemorrhage

The association between PVH/IVH and mechanical ventilation has been demonstrated by the prospective evaluation of 151 newborns with less than 35 wk gestation reported by Dykes *et al.* in 1980 (1). Further evidence suggesting that certain vascular factors that are influenced by respiratory mechanics contribute to PVH/IVH have also been reviewed by Volpe (2). Studies examining the mechanisms that control CBF in the neonate have shown that several vasoactive stimuli, including hypercapnia (3, 4), work through the generation of prostanoids, whereas other vasoactive substances such as isoproterenol (5) and norepinephrine (6) are not dependent on prostanoid synthesis. In addition, we have shown that the stress of increasing $\overline{Paw}$ is followed by cerebrovascular compensation that is modulated, in part, by vasodilator prostanoids (7–9).

This previous work has focused on cerebral hemodynamics during periods of increased $\overline{Paw}$. In contrast, we now seek to characterize vascular alterations that may persist as a result of exposure to high ventilation pressures. Our intent, therefore, with the present experiments was to examine both prostanoid-dependent and prostanoid-independent vascular responses before, during, and after mechanical ventilation at an increased $\overline{Paw}$. A further suggestion from these studies is that an altered cerebrovascular system would make the brain less likely to tolerate subsequent cardiovascular stress.

MATERIALS AND METHODS

All protocols were approved by the Animal Care and Use Committee of The University of Tennessee, Memphis.

Cranial window. Newborn pigs (1–4 d old) were anesthetized with ketamine hydrochloride (33 mg/kg intramuscularly) and acepromazine (3.3 mg/kg intramuscularly) and maintained on α-chloralose (50 mg/kg initially followed by 5 mg/kg/h i.v.). Catheters were placed into a femoral vein and artery. The venous catheter allowed for drug administration and the arterial catheter was used for continuous blood pressure monitoring and for withdrawal of arterial blood gas and pH samples. The trachea was intubated with a 3.0-mm (inside diameter) straight endotracheal tube, and the animals were ventilated with an infant time-cycled, pressure-limited respirator (Bourns BP 200, Bourns Life Systems, Riverside, CA). Proximal airway pressure was monitored continuously. The animals were ventilated with room air with initial ventilator settings of 20–30 breaths/min, an inspiratory time of 0.5 s, a peak inspiratory pressure of 2.2–2.7 kPa (12–15 cm) water, and a positive end expiratory pressure of zero. Body temperature was maintained at 37–38°C with a servo-controlled overhead radiant warmer.

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 periarachnoid 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 as previously described (7) through needles incorporated into the sides of the window. The volume of fluid directly under the window was approximately 500 μL and was contiguous with the periarachnoid space. After implantation of

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the window, at least 30 min were allowed before experimentation was begun.

Pial arterioles were observed with a Wild M38 dissecting microscope (Heerbrugg, Switzerland), a television camera (Digital model 5010, Panasonic Corporation, Secaucus, NJ) mounted on the microscope, and a video monitor (model CT 2081Y, Panasonic). Vessel diameter was measured with a video microscaler (model VPA 1000, For-A-Corp, Los Angeles, CA). Pial vessel diameters were measured at the same point (internal diameter) and on the same vessel before and after each intervention.

Cerebral surface CSF (300 μL) collections were made by placing a 1-mL syringe on an injection port of the cranial window. CSF was collected by slowly infusing aCSF into one side of the window and allowing the CSF under the window to drip freely into a collection tube on the opposite side.

Experimental protocol. The CSF under the window was replaced with aCSF, and a pial arteriole (50–150 μm) was measured during an initial control period of 10 min. During this period, the $\overline{Paw}$ was 0.9 ± 0.05 kPa (4.8 ± 0.3 cm H_2O). At the end of this initial control period, 300 μL of CSF from under the window were collected and frozen for prostanoid analysis. We then began a protocol that tests vascular reactivity. This consisted of first producing hypercapnia by ventilating the piglets with a 10% CO_2, 21% O_2, 69% N_2 gas mixture; second, topically applying isoproterenol (10^{-6} M); and third, topically applying norepinephrine (10^{-4} M). During each of these three challenges, pial vessels were observed for a 10-min period. The window was flushed with aCSF after each challenge and the arteriole allowed to recover between treatments. After hypercapnia and increased $\overline{Paw}$ only, a CSF sample was taken for prostanoid analysis. These challenges were preselected for CSF sampling because they are known to be dependent on prostanoid synthesis (3, 4, 7–9). Airway pressure was then increased by increasing both the peak inspiratory pressure and the positive end expiratory pressure to maintain a constant tidal volume. The $\overline{Paw}$ at this time was 2.5 ± 0.2 kPa (13.9 ± 1.3 cm H_2O). This pressure was selected from our previous experience, which had shown that this airway pressure would affect cardiac output and increase cerebral venous pressure without altering arterial blood pressure (7, 10). The same three tests of vascular reactivity were then used at this higher $\overline{Paw}$. The protocol at the higher $\overline{Paw}$ required 40–60 min, at which time the $\overline{Paw}$ was returned to its initial setting, and the three tests of vascular reactivity were repeated over the next 40–60 min.

Time control animals. Two additional animals were prepared as described above and ventilated at low $\overline{Paw}$ for a comparable amount of time. Challenges of vascular reactivity were repeated three times as described in the experimental group.

Prostanoid analysis. Prostanoids 6-keto-prostaglandin-$F_{1\alpha}$ and prostaglandin E_2 in cortical periarachnoid CSF were analyzed by RIA against an aCSF matrix as described previously (11). For these experiments, we elected to measure only 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin E_2, as these two prostanoids have the highest concentrations in CSF and generally reflect the overall prostanoid response. In addition, we have previously described the profile of prostanoid changes seen with hypercapnia (3) and increased $\overline{Paw}$ (7). All unknowns were processed at three dilutions, with parallelism between the unknown dilution curve and the standard curve required before the result was used. Sample dilutions allowed analysis of prostanoid concentrations between 100 and 50 000 pg/mL. Previously, using this assay, we demonstrated large proportional increases in prostanoids after topical application of arachidonic acid and >90% decreases in concentrations of all prostanoids examined in the cortical periarachnoid fluid after treatment with indomethacin (10 mg/kg i.v.) in basal conditions and when stimulated with exogenous arachidonic acid (11). Our antibodies cross-react minimally (<1%) with other prostanoids studied. Furthermore, target ligands are not displaced from the antibodies by arachidonic acid (20 μg/mL); 5hydroxyeicosatetraenoic acid or 15-hydroxyeicosatetraenoic acid (1 μg/mL); LTB_4, LTC_4, LTD_4, or LTE_4 (5 μg/mL); or lipoxin A_4 or lipoxin B_4 (10 ng/mL).

Statistical analyses. Pial arteriolar diameter, systemic arterial blood 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 mean ± SEM of raw values.

RESULTS

Mean arterial blood pressures, arterial blood gases, and pH before, during, and after increased $\overline{Paw}$ are shown in Table 1. These values were similar during the three parts of these experiments. Arterial blood gases and pH were changed, however, during hypercapnia, with P_{CO_2} increasing to 9.5 ± 0.7 kPa (71 ± 5.2 mm Hg) and pH dropping to 7.16 ± 0.06.

As expected, prostanoids increased in response to hypercapnia and increasing $\overline{Paw}$ (Table 2).

Table 3 lists the values for pial arterial diameters and the corresponding control values for these experiments. Before, during, and after the increased $\overline{Paw}$, hypercapnia significantly dilated pial arterioles. Before and during, but not after, increased $\overline{Paw}$, isoproterenol significantly dilated and norepinephrine significantly constricted pial arterioles. Increasing $\overline{Paw}$ had no effect on pial arteriolar diameter.

Figure 1 shows the percentages of change in pial arterial diameters seen before, during, and after ventilation with increased $\overline{Paw}$. Pial arteriolar responses were unchanged during increased $\overline{Paw}$. Vascular responses to isoproterenol and norepinephrine were markedly attenuated after pressure ventilation, whereas hypercapnic dilation was unchanged.

Vascular responses in the time control animals were the same as the before group shown in Figure 1. These responses were unchanged with time, with hypercapnia and isoproterenol continuing to dilate 42 ± 6% and 36 ± 8%, respectively, and norepinephrine continuing to constrict 28 ± 12%.

DISCUSSION

The present experiments demonstrate an alteration in two nonprostanoid-dependent pial arteriolar responses after positive pressure ventilation. This finding is in contrast to our previous work using the alternative insults of cerebral ischemia-reperfusion (6, 12) and superoxide anion generation on the cerebral surface (13), both of which selectively attenuate prostanoid-dependent responses. These findings become particularly important when compared to our previous experiments showing that prostanoids are important modulators of CBF during mechanical ventilation (7–9). The mechanism of vascular alterations seen after mechanical ventilation must therefore be different from other insults and not due to inhibition of prostanoid synthesis in response to appropriate stimuli. We must assume from these results that the mechanism and probably the vascular cell type (endothelial *versus* smooth muscle) involved in the abnormal responses after increased $\overline{Paw}$ are different from those altered after ischemia-reperfusion or activated oxygen insult.

Table 1. *Mean arterial blood pressure (BP), arterial blood gases, and pH before, during, and after increased $\overline{Paw}$**

	Before	During	After
BP (mm Hg)	74 ± 2.4	72 ± 3.3	71 ± 3.9
pH	7.43 ± 0.06	7.46 ± 0.07	7.42 ± 0.08
P_{CO_2} (kPa)	5.1 ± 0.3	5.6 ± 0.5	5.4 ± 0.6
(mm Hg)	38 ± 2.2	42 ± 3.6	40 ± 4.2
P_{O_2} (kPa)	11.1 ± 1.2	11.5 ± 1.3	11.3 ± 0.9
(mm Hg)	83 ± 8.6	86 ± 10.0	84 ± 7.2

* Values are mean ± SEM (n = 10).

Table 2. *CSF values (pg/mL) for 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and prostaglandin E_2 (PGE_2) before, during, and after increased $\overline{Paw}$**

	Before		During		After	
	6-keto-$PGF_{1\alpha}$	PGE_2	6-keto-$PGF_{1\alpha}$	PGE_2	6-keto-$PGF_{1\alpha}$	PGE_2
Control	457 ± 99	942 ± 180	2474 ± 304†	3320 ± 460†	1170 ± 306	1673 ± 483
CO_2	2073 ± 165†	3838 ± 256†	2952 ± 403†	3864 ± 380†	2970 ± 246†	3586 ± 480†

* Values are mean ± SEM. $n = 7$. CO_2, hypercapnia.
† Statistically significant change from "before" control value: $p < 0.05$.

Table 3. *Pial arterial diameters (μm) before, during, and after increased $\overline{Paw}$**

	Before	During	After
Control	116 ± 11	110 ± 11	111 ± 12
CO_2	169 ± 15†	149 ± 13†	143 ± 12†
Control	112 ± 11	112 ± 11	112 ± 13
ISO	154 ± 17†	137 ± 12†	120 ± 14
Control	116 ± 13	117 ± 12	116 ± 15
NE	88 ± 11†	92 ± 10†	110 ± 15

* Values are mean ± SEM. CO_2, hypercapnia; ISO, isoproterenol; and NE, norepinephrine.
† Statistically significant change from corresponding control: $p < 0.05$.

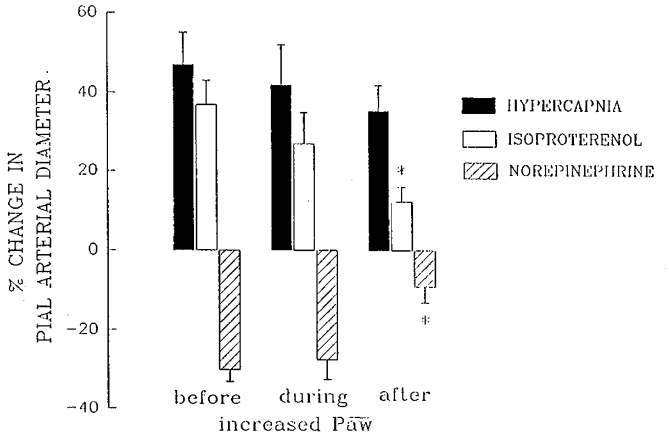

Fig. 1. Percentage of change in pial arterial diameter before, during, and after increased $\overline{Paw}$. Vascular reactivity tested with hypercapnia [P_{CO_2} = 9.5 ± 0.7 kPa (71 ± 5.2 mm Hg)], topical isoproterenol (10^{-6} M), and topical norepinephrine (10^{-4} M). Values are mean ± SEM. *, Statistically significant change from corresponding before value ($p < 0.05$); $n = 10$.

The relationship between cardiovascular hemodynamics and mechanical ventilation is an important consideration when ventilating neonates. Although cardiac output is less affected when respiratory compliance is decreased, increasing ventilation pressure can decrease cardiac output and adversely affect organ perfusion (10). The cerebral vasculature, however, appears to compensate for a drop in cardiac output by increasing vascular production of prostacyclin (9) to attenuate a yet unidentified circulating pressor substance (7).

The mechanism by which mechanical ventilation alters vascular function is unclear, and we can only speculate that distension of pial vessels exerts either pressure or stretch forces on the vessel (endothelium and/or smooth muscle). This speculation, however, suggests that the damage is selective to either the endothelium or the smooth muscle layer, which translates into a selective effect on prostanoid or nonprostanoid responses. The α- and β-receptors located on vascular smooth muscle account for constriction and dilation in response to norepinephrine and isoproterenol, respectively. In contrast, vasodilation to hypercapnia requires prostanoid synthesis by some as yet unspecified cell type, quite possibly endothelial. The altered responses to α- and β-agonists may suggest an effect on the vascular smooth muscle itself.

A delay in vascular alterations is also a finding that suggests that the mechanism of this vascular effect is complicated. Microvascular responses do not change during the increased $\overline{Paw}$. In these experiments, the duration of increased $\overline{Paw}$ was relatively short (40–60 min) and was not associated with a drop in arterial blood pressure. It appears that the combination of increasing (and perhaps distending or stretching the vessel) and then decreasing $\overline{Paw}$ (now attempting to recoil) is required to observe these vascular changes. An additional question that remains is the duration of such vascular changes.

Cerebral microvascular responses are important modulations of brain blood flow, especially during pathologic conditions. Changing the ability of the vessels to handle stresses could lead to major malfunctions during subsequent challenges. From our data we could suggest that short periods of aggressive mechanical ventilation (during resuscitation for example) could alter microvascular responses to certain stimuli but not to others. Responses to catecholamines and therefore the sympathetic nerves that are associated with cerebral vessels are clearly altered. Piglet pial arterioles respond to sympathetic stimulation (14) and appear to play an important role in attenuating hypertensive-induced increases in CBF in both adult (15) and newborn animals (16). The significance of sympathetic innervation of newborn cerebral vasculature is unclear, but responses to activation of such nerves may be altered by pressure ventilation. Alterations in vascular reactivity could change the ability of the vessels to respond appropriately to abrupt changes in arterial blood pressure, placing a neonate at further risk for PVH/IVH (17).

A possible clinical situation in which pulmonary compliance may suddenly change and thus cerebral venous pressure may increase is the administration of exogenous surfactant. Although not conclusive, some studies have reported an increase in PVH/IVH after surfactant therapy (18). From the present study, it is not difficult to suggest that a sudden change in pulmonary compliance would mean that more of the ventilation pressure would be transmitted to the brain vessels, which could lead to altered vascular responses. Subsequent cardiovascular stresses such as sudden increases in arterial blood pressure could further alter or rupture an already tenuous cerebral vascular bed.

In summary, these experiments demonstrate that cerebral microvascular responses to two nonprostanoid-dependent stimuli are attenuated by a relatively short period of positive pressure ventilation, whereas the response to hypercapnia, a prostanoid-dependent stimulus, is unchanged. Although the mechanisms of such alterations will require further consideration, the clinical implications with regard to the cerebral circulation's ability to handle subsequent stresses after mechanical ventilation must be questioned.

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