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Alkalosis Attenuates Hypoxic Pulmonary Vasoconstriction in Neonatal Lambs

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ABSTRACT. Hyperventilation (respiratory alkalosis) is an important treatment for persistent pulmonary hypertension in neonates. The precise way that hyperventilation attenuates hypoxic pulmonary vasoconstriction is unclear. We studied the effect of alkalosis on hypoxia-induced pulmonary vasoconstriction in 13 acutely instrumented, pentobarbital anesthetized, neonatal lambs. We specifically examined the relative effects of a metabolic alkalosis *versus* a respiratory alkalosis on hypoxic pulmonary vasoconstriction and compared these results to the control response to hypoxia without alkalosis. Hypoxic pulmonary vasoconstriction was significantly milder whenever the animal was alkalotic, regardless of whether the alkalosis was respiratory or metabolic. Thus, the elevated pH_a rather than decreased $Paco_2$ during hyperventilation appears to be the major factor in moderating the response of the pulmonary vessels to acute hypoxia in this neonatal lamb model. (*Pediatr Res* 19: 1268–1271, 1985)

Abbreviations

$\Delta Aapo_2$, difference between alveolar and arterial PO_2
$[H^+]$, hydrogen ion concentration
Pas, systemic arterial pressure
Ppa, pulmonary arterial pressure
Pla, left atrial pressure
Pcv, central venous pressure
$\dot{Q}pa$, pulmonary artery flow
RA, respiratory alkalosis
MA, metabolic alkalosis
HS, hypertonic saline
PVR, pulmonary vascular resistance
SVR, systemic vascular resistance

The effect of alkalosis on the neonatal pulmonary vascular response to acute hypoxia is presently unclear. In adult animal models, an alkalotic state attenuates hypoxic pulmonary vasoconstriction, and this effect is fully or largely due to changes in hydrogen ion concentration $[H^+]$ rather than to changes in $Paco_2$ (1–4). Many physiologic responses in neonatal animals, including pulmonary vascular responses, are both qualitatively and quantitatively different from those seen in mature animals, necessitating the separate testing of hypotheses in young animals (5).

Evidence from human neonates with the syndrome of persistent pulmonary hypertension of the newborn (an illness complex characterized by pulmonary artery hypertension and hypoxemia secondary to extrapulmonary right-to-left shunting) shows that respiratory alkalosis produced by mechanical hyperventilation causes a reduction in pulmonary artery pressure (6, 7). It is uncertain, however, whether this effect is mediated through changes in $[H^+]$ or $Paco_2$ since both were altered simultaneously.

We used a neonatal lamb model to test the hypothesis that alkalosis attenuates hypoxic pulmonary vasoconstriction predominantly through changes in $[H^+]$ not through changes in $Paco_2$.

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METHODS

We studied 13 newborn lambs less than 1 wk of age with a mean weight of 4.70 ± 1.27 (SD) kg. The lambs were anesthetized with 20 mg/kg of intravenous sodium pentobarbital and additional smaller doses of approximately 5 mg/kg were given during the experiment to maintain adequate sedation. One percent xylocaine infiltration was used before all cutaneous incisions. A cuffed, 3 mm endotracheal tube was placed by tracheostomy and connected to a pressure-limited, time-cycled infant respirator (Baby Bird). Rectal temperature was continuously monitored with a thermistor probe (Yellow Springs Instruments), and a waterproof heating pad was used to maintain a core temperature of 39–40° C. Femoral artery and vein catheters were inserted and advanced into the descending aorta and inferior vena cava, respectively.

A left thoracotomy was performed with removal of the fourth rib. The ductus arteriosus was ligated and a 10 mm precalibrated, electromagnetic flow transducer (Biotronix Laboratories) was placed around the main pulmonary artery. A 3.5 French catheter was inserted through a small incision in the left atrial appendage and secured with a circumferential suture. An 18 gauge pressure catheter (Deseret Pharmaceuticals) was positioned in the main pulmonary artery and was held in place with a pursestring suture. This catheter caused no interference with flowmeter readings.

Pas, Ppa, Pla, and Pcv, all referenced to midchest, were measured with Statham P23-1D pressure transducers and were recorded along with $\dot{Q}pa$ on physiologic polygraphs (2 Gould/Brush 2400 Series).

Initial ventilator settings were a peak inspiratory pressure limit of 24–25 cm H_2O, inspiratory time of 0.7–0.8 s, and rate of 12 breaths per minute. Rate and inspired oxygen concentration were altered (see protocol below) as demanded by analysis of arterial blood gases (Instrumentation Laboratories pH/Blood Gas Analyzer 213).

After instrumentation and stabilization, a five-step protocol was used to study each animal. The acid-base status was carefully controlled throughout the experiment to allow separate analysis of the pulmonary circulation's response to severe, acute hypoxia in the presence of normal acid-base balance (control 1), acute RA, or acute MA. Table 1 shows the arterial blood gas requirements for each step of the protocol. Once these acid-base require-

Table 1. *Acid-base requirements for each step of the study*

Protocol steps	pHa	$Paco_2$ (mm Hg)
Control 1	7.40 ± 0.05	40 ± 5
RA	>7.50	≤ 25
MA	>7.50	40 ± 5
Control 2	7.40 ± 0.05	40 ± 5
HS	7.40 ± 0.05	40 ± 5

ments were satisfied, and the pressure and flow tracings were stable, we collected data during normoxemia (Pao_2 > 80 mm Hg) and then recorded the animal's physiologic responses to acute hypoxia. This sequence of events was repeated for each of the five steps.

Acute hypoxia was created in each experiment by a 1-min inhalation of 5% oxygen–95% nitrogen mixture at the same ventilator settings. Arterial blood gases, drawn at the end of the minute of hypoxia, were checked periodically during the study to be certain that the degree of hypoxemia was similar for each experimental situation.

Step 1 (control 1) measured the control response to hypoxia. Step 2 measured the response to hypoxia during RA which was produced by increasing ventilator frequency and keeping peak inspiratory pressure and inspiratory time constant. Step 3 measured the response to hypoxia during MA which was produced by the slow intravenous infusion of 1 M $NaHCO_3$ solution in an amount calculated to raise pHa to >7.50. Step 4 remeasured the control response (control 2) to verify that no changes in the animal's responsiveness to the hypoxic challenge had occurred secondary to the interventions of steps 2 and 3. Return to control pHa was accomplished by slowly infusing a 1 M solution of lactic acid. Step 5 measured the animal's response to hypoxia after the infusion of HS to be certain that any measured changes in pulmonary or systemic vascular reactivity were not simply incidently related to intravascular volume expansion or the infusion of a solution of high osmolality. A 1 M NaCl solution, equal in volume to the $NaHCO_3$ solution administered in step 3, was infused over 15 min. Once the animal's pressure and flow tracings stabilized, the vascular responses to hypoxia were recorded as in previous steps.

In order to avoid ventilator artifact, only vascular pressures and pulmonary artery flow data recorded during the expiratory phase were used for analysis. Mean arterial pressures ($\bar{P}pa$, $\bar{P}as$) and pulmonary and systemic vascular resistances were calculated using standard formulae. The animal's maximum response to the hypoxic challenge was compared to the pressure and flow values recorded during the stable period of normoxemia immediately prior to the initiation of each hypoxic challenge. The differences (Δ = hypoxic − normoxic value) in mean values for each physiologic parameter of interest ($\bar{P}pa$, PVR, etc.) were calculated and tabulated to display the results for all 13 animals for each of the five steps of the protocol. The resulting difference matrices were subjected to two-way analysis of variance as described by Snedecor and Cochran (8). Comparisons between the control 1 mean value and each experimental intervention mean value were made using Dunnett's test to adjust the value of t required for significance when multiple treatments are compared with a control value (9). The results were considered significant when the one-tailed value of p was <0.05. A one-tailed test was used since the *a priori* hypothesis stated that alkalosis attenuated hypoxic pulmonary vasoconstriction. Figures were produced which allow the reader to visually compare the results (10). The uncertainty interval associated with Dunnett's test for each data set was calculated and displayed as a window plot with the mean value occupying the interval midpoint. The control hypoxic response of step 1 is cross-hatched and intervals that do not overlap it are significantly different at the $p < 0.05$ level of confidence. Ninety-five percent confidence limits about zero change were also calculated using Dunnett's test to compare all results to the hypothetical mean change of zero and these confidence limits are shown as dashed lines above and below the solid line representing zero change. Mean values falling outside the dashed lines are different from zero at the $p < 0.05$ level of confidence.

RESULTS

At the beginning of experimentation, the baseline normoxemic values (mean ± SD) for the 13 animals were $\bar{P}pa$ = 17 ± 5 mm Hg, $\dot{Q}pa$ = 0.82 ± 0.28 liter/min, $\bar{P}la$ = 2.3 ± 0.9 mm Hg, PVR = 20 ± 8 mm Hg·liter^{-1}·min^{-1}, $\bar{P}as$ = 67 ± 10 mm Hg, $\bar{P}cv$ = 2.4 ± 1.6 mm Hg, and SVR = 86 ± 26 mm Hg·liter^{-1}·min^{-1}. Baseline normoxemic values were rechecked at the beginning of each step in the protocol. There were no differences in these baseline values throughout the experiment for the parameters, $\bar{P}pa$, $\bar{P}cv$, $\dot{Q}pa$, PVR, and SVR. There was a small but significant increase in $\bar{P}la$ at step 3 (2.3 *versus* 3.6 mm Hg, $p < 0.05$) and step 4 (2.3 *versus* 3.5 mm Hg, $p < 0.05$). $\bar{P}as$ was elevated above the baseline normoxemia value of step 1 only in step 5 (67 *versus* 73 mm Hg, $p < 0.025$).

Figure 1 shows the values for pHa and $Paco_2$ during each experimental step. Only during the intentional mechanical hyperventilation of step 2 was $Paco_2$ different from control 1. As expected, pHa was elevated above control 1 in steps 2 (RA) and 3 (MA).

During alveolar hypoxia, the Pao_2 fell from a mean of 109 ± 15 mm Hg to a mean of 17 ± 5 mm Hg ($p < 0.001$, by paired t test). Hypoxia caused a brisk, reproducible increase in $\bar{P}pa$ and PVR in the control group; the former rose 65% (Δ mean = 11 mm Hg) and the latter 85% (Δ mean = 17 mm Hg·liter^{-1}·min^{-1}). Figure 2 shows that the increase in $\bar{P}pa$ and PVR during hypoxia was different from zero change at each step in the experiment.

Respiratory alkalosis blunted the hypoxia-induced rise in both $\bar{P}pa$ and PVR. Metabolic alkalosis also markedly blunted the hypoxia-induced rise in PVR, but the decrease in $\bar{P}pa$ did not

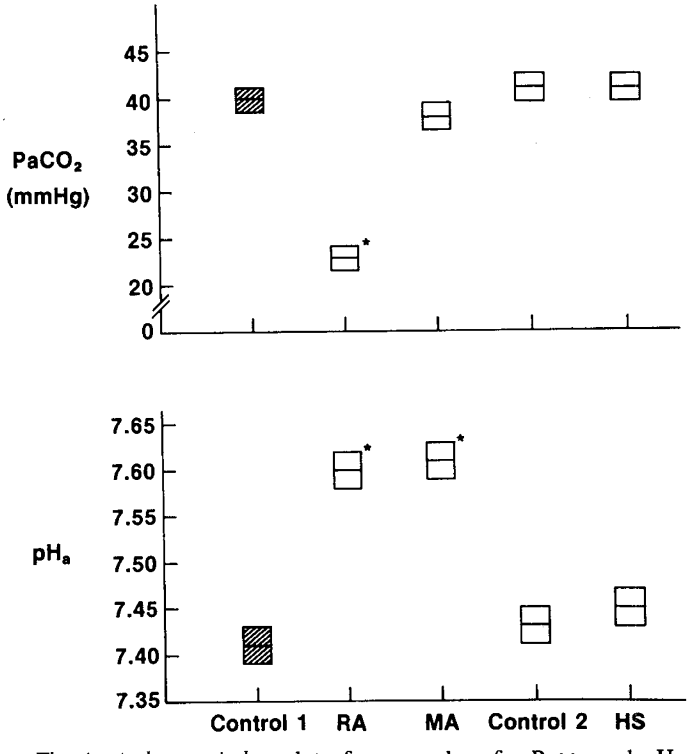

Fig. 1. *Andrews window* plot of mean values for $Paco_2$ and pHa during each protocol step. *Middle of window* is mean; *top* and *bottom* are uncertainty limits. *Windows* that do not overlap control 1 window *(cross-hatched area)* are different at $p \leq 0.05$ and are marked with an asterisk.

quite reach statistical significance. The hypertonic saline solution infused during step 5 produced no changes in the hypoxia-induced rise in $\bar{P}pa$ or PVR.

Figure 3 shows the changes in the systemic circulation occurring with hypoxia. Both $\bar{P}as$ and SVR fell in response to the acute hypoxic challenge during the control 1 period and responses were similar when rechecked in step 4. In steps 2, 3, and 5, $\bar{P}as$ could not be distinguished from the control 1 response, nor could it be distinguished from zero change. ΔSVR in steps 2, 3, and 5 were similar to the control response to hypoxia and all were different from zero change.

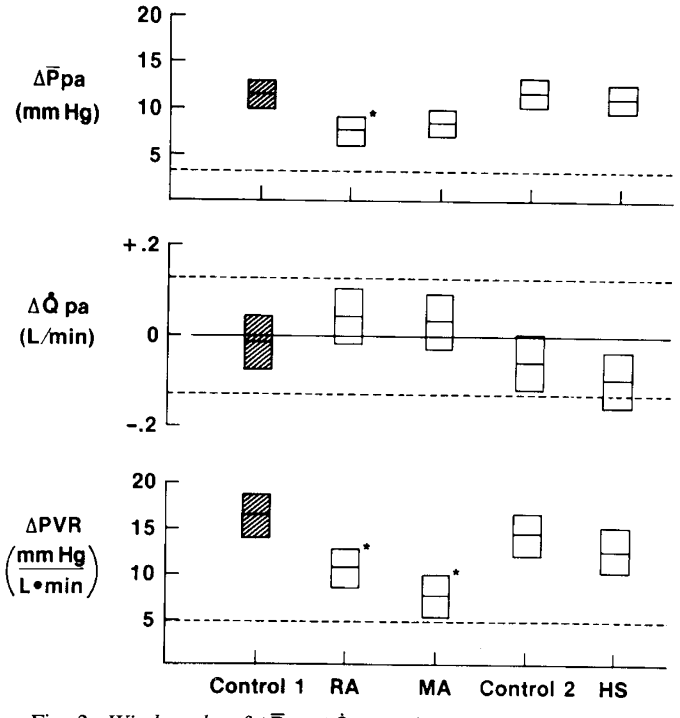

Fig. 2. *Window plot* of $\Delta\bar{P}pa$, $\Delta\dot{Q}pa$, and ΔPVR with acute hypoxia. Windows that do not overlap control 1 window (*cross-hatched area*) are different at $p \leq 0.05$ and are marked with an *asterisk*. *Dashed lines* are confidence limits about zero. Means lying beyond these limits are different from zero at $p \leq 0.05$.

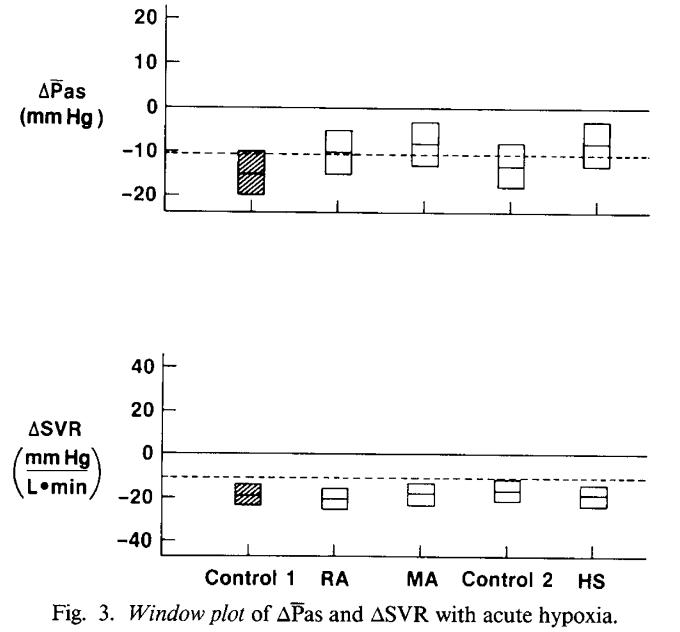

Fig. 3. *Window plot* of $\Delta\bar{P}as$ and ΔSVR with acute hypoxia.

DISCUSSION

The purpose of this experiment was to better define the pulmonary circulation's response to alkalosis in the hypoxic neonatal animal and to determine the relative roles of altered $[H^+]$ and $Paco_2$ in this response. We show that the increase in pulmonary vascular resistance caused by acute hypoxia was blunted by alkalosis, regardless of the reason for the alkalosis. Thus, the decrease in $[H^+]$ appears to be the major determinant of this attenuated response, not a change in the $Paco_2$.

Other investigators have examined the effects of alkalosis on the pulmonary circulation, but the evidence is contradictory. Silove *et al.* (11) found that alkalosis failed to modify the highly reactive hypoxic pulmonary vasoconstrictor response of newborn calves. In contrast, the study of Rudolph and Yuan (12) of neonatal calves showed that pulmonary vascular resistance was elevated minimally or not at all by a $Pao_2 < 40$ mm Hg when the pHa was above 7.3.

Studies in human neonates with persistent pulmonary hypertension of the newborn show that respiratory alkalosis reduces pulmonary artery pressure. Peckham and Fox (6) studied 10 neonates with persistent pulmonary hypertension by monitoring their pulmonary artery pressures prior to and during hyperventilation. A decrease in $Paco_2$ from a mean of 46.9 to 28.6 mm Hg and an increase in pHa from a mean of 7.43 to 7.60 caused a significant drop in pulmonary artery pressure (92 *versus* 50 mm Hg, $p < 0.001$) with a corresponding decrease in $\Delta AaPo_2$ (596 *versus* 450 mm Hg, $p < 0.001$) (6). Drummond *et al.* (7) studied the effect of hyperventilation on pulmonary artery pressure in five term neonates with pulmonary hypertension. Respiratory alkalosis (pH > 7.55) caused a significant decrease in the ratio of mean pulmonary artery pressure to systemic artery pressure and caused a decrease in right-to-left shunting as measured indirectly by a reduced $\Delta AaPo_2$ (7). Unfortunately, inferences about the relative roles of $[H^+]$ and $Paco_2$ cannot be made since both were altered simultaneously.

Experiments with adult animals provide more consistent data and support the conclusion of this experiment. Lloyd (1) used an excised lobe of dog lung with controlled perfusion and ventilation to show that decreasing $[H^+]$ with either THAM or $NaHCO_3$ reduced the hypoxic constrictor response produced by a Pao_2 of 45 mm Hg. This response was graded and could be completely eliminated by raising perfusate pH 0.2 units. Viles and Shepherd (3) used isolated, adult cat lungs and intact, adult, anesthetized cats to show that alkalosis (pH > 7.5) attenuated or eliminated hypoxic pulmonary vasoconstriction. They also demonstrated in the isolated lung preparation that an increased $Paco_2$, independent of its effect on hydrogen ion concentration, acts as a weak pulmonary vasodilator (4). This independent effect of carbon dioxide on pulmonary vascular tone was not substantiated by the work of Shapiro *et al.* (2) who found no consistent relationship between changes in $Paco_2$ and pulmonary vascular resistance when $[H^+]$ was kept constant in intact adult dogs.

While the information on the responses of mature animals allows us to conclude that alkalosis modifies hypoxic pulmonary vasoconstriction and that the modification is mediated fully or largely through changes in $[H^+]$, it is important to separately define the neonatal response since pulmonary vascular responsiveness in the immediate postnatal period often differs from that of older animals. In the neonatal period, vascular smooth muscle is increased, baseline pulmonary vascular resistance is higher, and the response to hypoxia is exaggerated. Qualitatively different responses to vasoactive agents also exist. For example, type 1 histamine (H_1) receptors in the newborn lamb mediate pulmonary vasodilation whereas H_1 receptors in adult animals mediate pulmonary vasoconstriction (13). Another example is the response to prostaglandin D_2 where infusion of prostaglandin D_2 in fetal goats (14) or hypoxic neonatal lambs (15) causes

pulmonary vasodilation, whereas prostaglandin D_2 infusion in the adult animal causes pulmonary vasoconstriction (14).

Johnson *et al.* (16) showed that the rapid administration of a neutral hypertonic fluid, independent of its effects on pH, blunts hypoxic pulmonary vasoconstriction. It is possible that any alteration in hypoxic vasoconstriction seen following the administration of the hypertonic $NaHCO_3$ solution was due to increased blood osmolality or blood volume, rather than to changes in acid-base status. We addressed this question by observing the effects of a hypertonic saline infusion on hypoxic vascular responses. We could demonstrate no independent effect of altered osmolality or volume on hypoxia-induced pulmonary vasoconstriction.

This study was conducted in an acutely operated, anesthetized preparation. Each of these factors may affect baseline cardiovascular parameters as well as the magnitude of response to stimuli. Yet, we found hypoxic pulmonary responses to be brisk and reproducible (15). This model actually fits the clinical corollary quite well since the infant with persistent pulmonary hypertension is acutely ill and most often mechanically ventilated, sedated, and paralyzed with neuromuscular blocking agents. Unfortunately, there is no stable animal model that mimics the changes in pulmonary vascular smooth muscle seen in the newborn with pulmonary hypertension. Acute hypoxia superimposed on a normal perinatal circulation may be only a distant parallel to the clinical state.

In this study exposure to the hypoxic challenge was brief. Silove and Grover (17) chronicled the development of the hypoxic response in the neonatal animal and noted that the response was not fully developed for 2–3 min. They observed, however, that during the early phase of the response to hypoxia (first minute) pulmonary blood flow remained relatively constant and that calculated pulmonary resistance increased primarily as a result of the rise in vascular pressure. Further increases (beyond 1 min) in PVR resulted predominantly from a fall in blood flow. They, therefore, stated that the most appropriate time to assess the pulmonary circulation's response to hypoxia was during that first minute. They believed that the early increase in resistance in the absence of changes in flow most likely represented changes in vessel tone. We also found the pulmonary circulation's response to hypoxia to be largely complete at 1 min and that further increases in PVR resulted primarily from depressed flow. We chose severe hypoxia as the stimulus since it was important to demonstrate whether a potential modifier such as alkalosis could alter the intense stimulus response seen at extreme hypoxia. The effect of alkalosis on less dramatic, sustained hypoxia is not answered in this study and is the subject of an ongoing investigation.

In conclusion, this study shows that the increase in pulmonary vascular resistance seen when neonatal animals become acutely hypoxic is blunted by alkalosis regardless of whether the alkalosis is metabolic or respiratory. We therefore conclude that the major determinant of this modification is the decrease in $[H^+]$.

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