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Age-Dependent Effects of Indomethacin on Hypoxic Vasoconstriction in Neonatal Lamb Lungs

JOHN B. GORDON, MARY L. TOD, RANDALL C. WETZEL, MARY L. McGEADY, N. FRANKLIN ADKINSON, JR., AND J. T. SYLVESTER

Departments of Anesthesiology/Critical Care Medicine and Medicine, The Johns Hopkins Medical Institutions at the Francis Scott Key Medical Center, Baltimore, Maryland 21224

ABSTRACT. Although smooth muscle is abundant in the pulmonary vessels of young animals at birth, it is not clear if these vessels respond more vigorously to hypoxia than the less muscular vessels of older neonates. To determine the effect of age on the pulmonary vascular response to hypoxia during the neonatal period in a single species, we measured the steady-state stimulus-response relationship between inspired oxygen tension (200, 50, 30 and 0 mm Hg) and pulmonary artery pressure-flow curves in isolated blood perfused lungs from 2- to 4- and 12- to 14-day-old lambs. Hypoxic vasoconstriction was attenuated in the younger newborns at an inspired oxygen tension of 50 mm Hg, but not at the other oxygen tensions. To determine if this age-related difference was due to differences in modulation of hypoxic vasoconstriction by cyclooxygenase products, we assayed the metabolite of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$ in the perfusate and determined the effects of indomethacin (40 μg/ml) on the hypoxic stimulus-response relationship. There was no age-related difference in perfusate concentration of 6-keto-prostaglandin $F_{1\alpha}$ at any oxygen tension. However, indomethacin reversed the age-dependent attenuation of hypoxic vasoconstriction at inspired oxygen tension = 50 mm Hg such that in indomethacin-treated lungs pulmonary vasomotor tone was higher in 2- to 4-day-old lungs than in 12- to 14-day-old lungs. This marked enhancement of hypoxic reactivity by indomethacin in the younger lambs suggests that in isolated neonatal lamb lungs cyclooxygenase products exerted a vasodilatory modulation of hypoxic vasoconstriction that decreased with age. (*Pediatr Res* 23: 580–584, 1988)

Abbreviations

6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$
PVR, pulmonary vascular resistance
HPV, hypoxic pulmonary vasoconstriction
PG, prostaglandin
Ppa, pulmonary arterial pressure
PGI_2, prostacyclin
LT, leukotriene
P/Q̇ curves, pressure-flow curves
PiO_2, inspired oxygen tension
Q̇, constant flow

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Correspondence Dr. John B. Gordon, Room D-983, The Montreal Children's Hospital, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3.
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PVR decreases dramatically when ventilation is initiated at birth (1–7). This is likely a consequence of mechanical distention of the lung (1, 2), increased oxygen tension (1, 2), and release of dilator PG (2–6). Subsequent decreases in normoxic PVR have been well documented in the growing piglet (7) and in other species (2, 8, 9). In contrast, age-related changes in HPV are less clear. It has been suggested that the extent of arteriolar muscularization determines the vigor of HPV (10–12). In this case, the newborn animal with its highly muscularized vessels (2, 7, 9, 10) should have a more vigorous hypoxic response than the older animal. Although some studies show an age-dependent decrease in HPV (8, 13), others report an increase with age (10, 14, 15).

One possible explanation for these apparently conflicting results is that the vigor of HPV may be influenced by factors other than vascular muscularization. For instance, there could be age-dependent differences in the modulation of HPV by pulmonary vasodilators. Although vasodilators such as PGI_2 are released during HPV in both young neonates and mature animals (16, 17), the action and metabolism of various PG may vary with age (18, 19). For example, the vasodilator PGE_2 (20) is more slowly metabolized in the neonate (18). PGD_2 is a pulmonary vasodilator in neonatal lambs, but a vasoconstrictor in older animals (21–23). Such differences may be partially responsible for the variable effects of cyclooxygenase inhibition on the developing pulmonary vasculature. In the intact pig, meclofenamate increased normoxic PVR at 5–12 days of age, but had no effect between 13 and 88 days of age (24). Despite this age-related difference, meclofenamate did not alter HPV in either age group and there was no effect of age on HPV (24). In goats, indomethacin-enhanced normoxic PVR in perfused left lower lobes of both premature and mature newborns; however, in this species HPV was potentiated in both age groups (25). In isolated perfused lungs from 2- to 3-month-old lambs, however, there was no effect of indomethacin on steady-state pulmonary pressures during graded hypoxia (26, 27).

The purpose of our study was 2-fold: first to determine if the pulmonary vascular response to hypoxia changed during the neonatal period in a single species, the sheep, and second, to determine if HPV in this species was modulated by dilator PG in an age-dependent fashion.

METHODS

Pulmonary vasomotor responses to four levels of PiO_2 were determined in isolated lungs of 2- to 4- (n = 12) and 12- to 14- (n = 12) day-old lambs perfused *in situ*. In half of the lungs, indomethacin (40 μg/ml perfusate) was added to the reservoir before perfusion was initiated. This amount of indomethacin has previously been shown to inhibit cyclooxygenase activity (26, 27).

This preparation was only slightly modified from that used previously for the study of older animals (26–28). Briefly, lambs were anesthetized with ketamine (50 mg/kg, intramuscular) and supplemental oxygen was provided by a loose-fitting mask. Femoral venous and arterial catheters were inserted and a tracheotomy performed. Pancuronium bromide (0.1 mg/kg, intravenous) was administered and ventilation with a warm, humidified hyperoxic gas mixture was initiated with an animal ventilator (Harvard, model 613) at a tidal volume of 15 ml/kg, rate of 10/min and end-expiratory pressure of 4 mm Hg. Heparin (3000–5000 U, intravenous) was administered and the lamb was exsanguinated rapidly via the femoral artery catheter. As exsanguination progressed, the gas mixture was adjusted to 5.4% CO_2, 28% O_2, and balance N_2. A median sternotomy was performed and the ductus arteriosus ligated in all animals. When exsanguination was completed (about 5 min), the left atrium and pulmonary artery were cannulated and perfusion was initiated with a mixture of autologous blood and 3% Dextran-70 in Ringer's lactate (hematocrit 16–20%). The time of pulmonary ischemia was 12–17 min.

The perfusate was pumped (Sarns, model 3500) from a reservoir at a $\dot{Q}$ of 50 ml/kg·min (all weights refer to body weight) through a heat exchanger (Travenol Miniprime Pediatric), bubble trap/filter, and flow probe (Carolina Medical Instruments, model EP300A) into the pulmonary artery. Perfusate drained from the left atrium to the reservoir that was held below the level of the lungs to ensure subatmospheric pulmonary venous pressure. During perfusion, we noted retrograde flow of the perfusate from the pulmonary circulation to the aorta, presumably via the bronchial artery. This anastomotic flow was returned to the reservoir via the femoral artery catheter previously inserted into the thoracic aorta for exsanguination of the lamb.

Ppa, left atrial, and tracheal pressures (Statham transducer models P50 and PM5E), as well as flow (Carolina Medical Instruments Flowmeter, model 501), and inspired O_2 and CO_2 tensions (Beckman OM11, LB2) were constantly measured and recorded (Grass Polygraph, model 7). At the beginning of perfusion, and at each PiO_2 during the experiment, perfusate blood gases (Radiometer BMS3 MK2), hematocrit, and glucose concentrations (Dextrostix) were determined. The pH was maintained in a normal range by the addition of 1 M $NaHCO_3$. Glucose concentration was maintained between 90 and 130 mg/100 ml by addition of 50% glucose in water.

After a 40-min stabilization period (PiO_2 = 200 mm Hg, $PiCO_2$ = 38 mm Hg), the first hypoxic challenge was begun. To avoid time or order-dependent effects, each preparation within a group was exposed to a different order of hypoxic challenges (PiO_2 = 50, 30, and 0 mm Hg) with a return to PiO_2 = 200 mm Hg after each hypoxic challenge. After a 10-min stabilization period at each PiO_2, $P/\dot{Q}$ curves were determined. The ventilator was turned off at end expiration (tracheal pressure = 4 mm Hg), a stand-pipe in the system was opened and filled by increasing flow to 150 ml/kg·min. After Ppa had stabilized (about 10 s), the pump was turned off and the stand-pipe allowed to empty across the pulmonary vasculature while Ppa and $\dot{Q}$ were recorded on an X-Y plotter (Hewlett Packard, model 7044A). The stand-pipe was then closed, ventilation and perfusion resumed, and the lungs hyperinflated. These $P/\dot{Q}$ curves were measured every 5 min until two consecutive curves fell within 1 mm Hg of each other at $\dot{Q}$ = 50 ml/kg·min (Ppa_{50}), defining the steady-state response to that PiO_2. The next PiO_2 was then administered and the process repeated until all six exposures were completed.

At each PiO_2 the Ppa at flow = 10, 30, 50, 70, 90, and 110 ml/kg·min were determined from the $P/\dot{Q}$ recording obtained when the steady-state response had occurred. This permitted determination of mean $P/\dot{Q}$ curves at each PiO_2. The curves measured during the three exposures to an O_2 tension of 200 mm Hg were averaged in each preparation. The Ppa at flows = 0, 130, and 150 ml/kg·min were not included because of the noise of the pulmonary artery signal immediately after stopping the pump (flow = 150 and 130 ml/kg·min) and the difficulty in determining the exact pressure at zero flow.

At 40 min and after each steady-state response had occurred, a sample of perfusate (4.5 ml) was collected for PG analysis in tubes containing 5 g of indomethacin and 7.2 mg of EDTA. After centrifugation, the supernatant was stored at −16° C until radioimmunoassay for 6-keto-$PGF_{1\alpha}$ was performed as previously described (26, 27).

All results are expressed as means ± 1 SE. The differences in effects of graded hypoxia on the $P/\dot{Q}$ curves and on concentrations of 6-keto-$PGF_{1\alpha}$ among groups were compared by split-plot design analysis of variance. Other characteristics of each group were compared by one-way analysis of variance (29). Differences were considered significant when $p \leq 0.05$.

RESULTS

There were no differences between groups in terms of mean hematocrit (16–20%), mean perfusate temperature (37.8–38.5° C), tidal volume (15 ml/kg body weight), ventilator rate (10/min), peak or end-expiratory tracheal pressures (10–12 and 4 mm Hg, respectively) or duration of the study (190–212 min). The mean weight of the older lambs (8.0 ± 0.7 kg) was greater than that of the younger (5.0 ± 0.05 kg). Perfusate PO_2, PCO_2, and pH did not differ between groups at any PiO_2 (Table 1).

The $P/\dot{Q}$ relationships measured at different inspired O_2 tensions in the 2- to 4- and 12- to 14-day-old lambs are shown in Figure 1. Three-way analysis of variance (age, PiO_2, and $\dot{Q}$) of these data revealed a significant effect of age ($p < 0.01$) on the stimulus-response relationship between PiO_2 and the $P/\dot{Q}$ curves. To facilitate comparisons between ages, we quantified the response at each PiO_2 by measuring pulmonary artery pressure at a flow of 50 ml/kg·min (Ppa_{50}). Figure 2 shows the stimulus-response relationship between PiO_2 and Ppa_{50} measured directly from individual $P/\dot{Q}$ curves in both age groups. In both groups, mean Ppa_{50} increased as the PiO_2 was decreased from 200 to 30 mm Hg. Further decreases in PiO_2 below 30 mm Hg caused the Ppa_{50} to fall. This biphasic curve is similar to that reported in the pig, ferret, cat, rabbit, and older lambs (27, 28). The only difference between the two age groups was observed at a PiO_2 of 50 mm Hg, where the response was less in younger lambs.

The $P/\dot{Q}$ curves measured at each PiO_2 in lungs treated with indomethacin were also analyzed by three-way analysis of variance. As in the control lungs, there was a significant effect of age ($p < 0.05$) on the stimulus-response relationship between PiO_2

Table 1. *Perfusate gases at each PiO_2 (mean ± SE)**

Age (day)	PO_2 at PiO_2 (mm Hg)				pH (mean)	PCO_2 (mean)
	200	50	30	0		
2–4	138.2 ± 2.5	44.0 ± 1.7	27.7 ± 2.2	3.3 ± 2.4	7.39 ± 0.01	28.8 ± 1.1
2–4 (indo)	134.7 ± 5.1	41.5 ± 2.4	25.0 ± 1.1	4.2 ± 2.0	7.39 ± 0.004	28.9 ± 0.7
12–14	122.3 ± 7.2	37.7 ± 1.9	22.2 ± 1.3	1.8 ± 1.8	7.39 ± 0.01	29.1 ± 1.0
12–14 (indo)	139.5 ± 6.5	42.5 ± 2.1	25.0 ± 1.1	4.6 ± 2.2	7.38 ± 0.01	28.6 ± 0.7

* The perfusate PO_2 at each PiO_2 as well as mean pH and PCO_2 are shown for each group. Only the effects of PO_2 were significant within groups. There were no differences between groups.

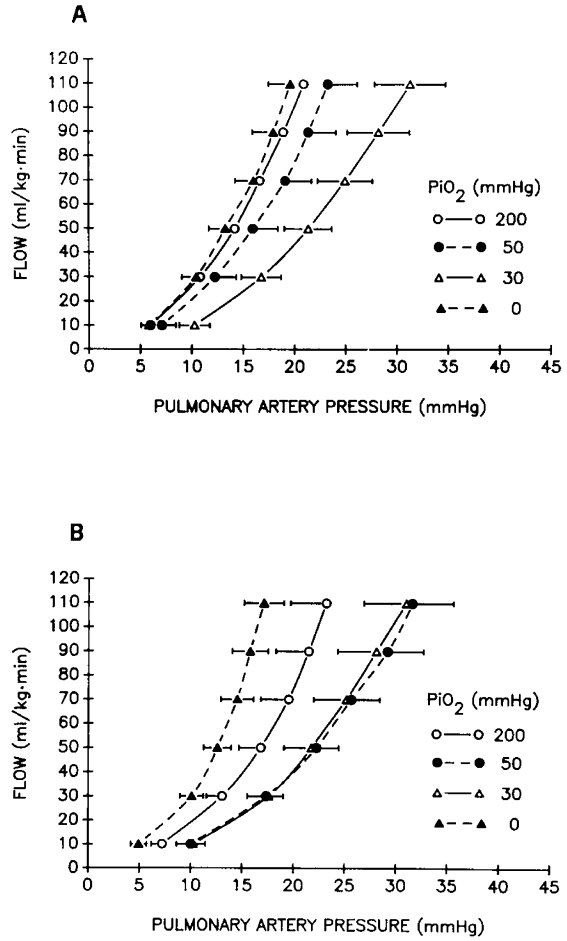

Fig. 1. Effects of age on P/Q̇ curves in response to different levels of PiO_2. P/Q̇ curves for control lungs from 2- to 4-day-old (*A*) and 12- to 14-day-old (*B*) lambs are shown at four different inspired O_2 tensions (PiO_2 = 200, 50, 30, 0 mm Hg).

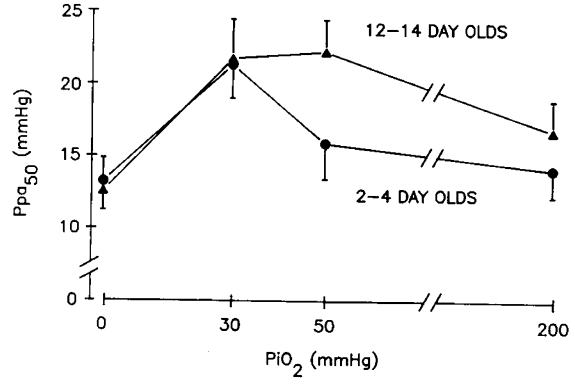

Fig. 2. Stimulus-response relationships between PiO_2 and Ppa_{50}. Mean stimulus-response relationships for 2- to 4- and 12- to 14-day-old lambs are shown.

and the P/Q̇ curves. To determine whether there was an age-dependent effect of indomethacin on hypoxic vasoconstriction, we compared P/Q̇ curves from control and treated lungs at each PiO_2 (Fig. 3). At PiO_2 = 200 mm Hg (Fig. 3*A*), there were no differences due to age or indomethacin. At PiO_2 = 50 mm Hg (Fig. 3*B*), indomethacin had no effect in 12- to 14-day-old lambs, but markedly enhanced vasoconstriction (*i.e.* shifted the P/Q̇ curve to higher pressures) in the 2- to 4-day-old lambs. This marked effect reversed the influence of age: vasoconstriction was enhanced by increasing age in control lungs but diminished by increasing age in indomethacin-treated lungs. This striking reversal of the effects of age by indomethacin was not observed at other levels of O_2. At PiO_2 = 30 mm Hg (Fig. 3*C*), there was no effect of age; however, indomethacin enhanced vasoconstriction by equal amounts in each age group. At PiO_2 = 0 mm Hg (Fig. 3*D*), both indomethacin and age independently altered the P/Q̇ curves but the analysis of variance revealed that the effects of indomethacin were not altered by age.

The mean perfusate concentrations of 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, were not different in the control lungs of 2- to 4- as compared to 12- to 14-day-old sheep (Table 2). Furthermore, there was no significant change in concentration of 6-keto-$PGF_{1\alpha}$ with hypoxia in either age group. The addition of indomethacin markedly reduced the concentration at both ages ($p < 0.001$), indicating effective cyclooxygenase inhibition.For example, the concentrations of 6-keto-$PGF_{1\alpha}$ measured at the end of perfusion of indomethacin-treated lungs were 0.9 ± 0.1 and 1.3 ± 0.2 ng/ml at 2–4 and 12–14 days of age, respectively.

DISCUSSION

The developmental aspects of HPV have been studied using a variety of species, ages, and methods. These studies have yielded differing results (8, 10, 13–15, 24). Rendas *et al.* (10) described an increase in hypoxic reactivity in intact piglets between 2–4 wk and 6 months and correlated this with peripheral extension of arterial smooth muscle to the acinar level. Owen-Thomas and Reeves (14) reported an increase in HPV between 1 and 9–11 days in isolated pump-perfused lungs of rabbits. Recently Fike and Hansen (15) have extended this finding to show an increase in HPV between 3–8 and 10–14 days in isolated rabbit lungs. In contrast, Reeves and Leathers (8) observed a decrease in hypoxic pulmonary artery pressure in intact growing calves. By measuring the diversion of flow from the hypoxic to the normoxic lung in sheep, Custer and Hales (13) concluded that HPV was more vigorous at 3–21 days than at 2–3 yr of age. Finally, Redding *et al.* (24) found no developmental differences in HPV in intact piglets at 5–12 and 13–88 days of age. Herein we found a significant effect of age on the relationship between PiO_2 and the pulmonary vascular P/Q̇ curve (Fig. 1). This difference was observed at PiO_2 = 50 mm Hg where the hypoxic vasoconstrictor response in lungs from 2- to 4-day-old lambs was less than that of lungs from 12- to 14-day-old lambs (Fig. 2). Thus, our results suggest that there is an increase in hypoxic reactivity with age, in agreement with those reported in isolated rabbit lungs (14, 15).

There are several potential reasons for the apparent differences between our results and those of others who did not find that HPV increased with age. Clearly, the extrapulmonary neuroendocrine modulation of pulmonary vasomotor tone in intact animals could explain the difference between intact and isolated preparations such as ours. Differences in species may also be important. For example, comparable developmental stages occur at different ages in different species (7). The level of oxygen tension used as a stimulus for HPV has varied. As demonstrated by our results, we could have reached a different conclusion had we studied but one level of PiO_2; *e.g.* 30 mm Hg, where no differences were found. Finally, we measured the resistive characteristics of the pulmonary vasculature by P/Q̇ curves generated over a wide range of flows when the response to hypoxia had attained a steady-state. Relative to other studies, this approach may have eliminated potential inaccuracies resulting from insufficient time to allow a complete hypoxic response to develop, as well as from using simply the ratio of pressure to flow or pressure at constant flow to quantify the pulmonary vascular response (30).

In many species medial muscle thickness of pulmonary arterioles is greatest immediately postnatally (7, 31, 32). The medial

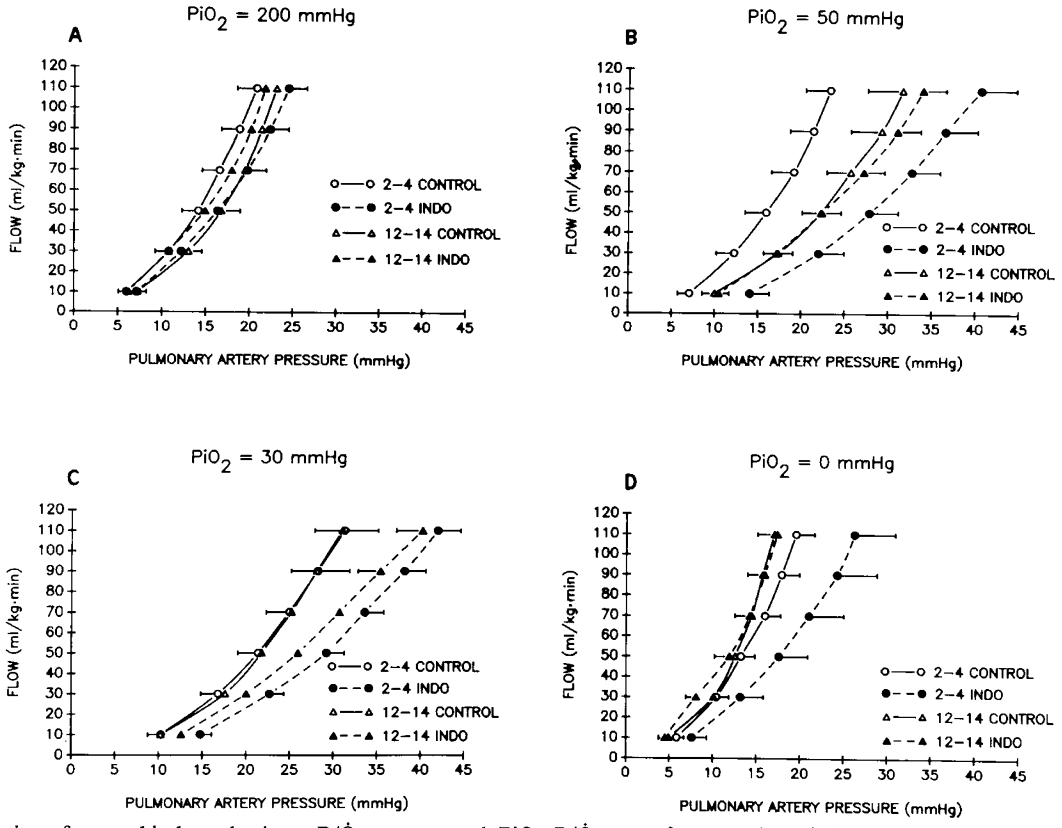

Fig. 3. Interaction of age and indomethacin on P/Q̇ curves at each PiO_2. P/Q̇ curves for control and indomethacin-treated lungs at both ages are

Table 2. *Perfusate concentration of 6-keto-$PGF_{1\alpha}$ (ng/ml) (mean ± SE)*

	PiO_2 (mm Hg)			
Age (day)	200	50	30	0
2–4	15.3 ± 4.4	19.1 ± 6.2	16.6 ± 5.4	14.3 ± 6.2
12–14	18.0 ± 5.8	14.6 ± 5.5	22.4 ± 8.9	16.0 ± 8.8

* The values at each PiO_2 are shown for control groups at each age.

muscularization of these arterioles has been shown to involute with age. Inasmuch as vascular responsiveness to hypoxia among species has been related to the degree of vascular muscularization (11), this suggests that HPV should be greater in younger animals. If the pulmonary vasculature of sheep undergoes the same involution seen in other species, then some mechanism other than muscularization is required to explain why HPV was depressed in the isolated lungs of our younger lambs.

Many studies suggest that dilator PG may contribute to the normally low PVR occurring during normoxia and may modulate HPV (6, 16, 17, 24, 25, 33–35). Potent prostanoid vasodilators, which contribute to the decrease in PVR after birth (3–6), modulate the hypoxic pressor response in older animals of several species (17, 33–35). Tyler *et al.* (25) observed potentiation of HPV by indomethacin in isolated perfused left lower lobes of perinatal goats. This potentiation may have been greater in lungs of premature newborns than in lungs of mature newborns (25). In contrast, Redding *et al.* (24) did not observe an age-related difference in HPV in intact piglets either before or after meclofenamate. However, they found that meclofenamate significantly increased normoxic PVR in piglets 5–12 days old, but had no effect in older piglets. Our results showed that indomethacin markedly diminished perfusate 6-keto-$PGF_{1\alpha}$ levels, indicating effective blockade of cyclooxygenase, and enhanced HPV at PiO_2 = 30 and 50 mm Hg (Fig. 3 *B* and *C*); however, indomethacin altered the effect of age only at a PiO_2 of 50 mm Hg. At this PiO_2, indomethacin reversed the age-related increase in HPV seen in control lungs, such that in indomethacin-treated lungs the P/Q̇ curve in 2- to 4-day-old lambs was shifted to the right of the curve in older lambs (Fig. 3*B*). These results suggest that at a PiO_2 of 50 mm Hg dilator PG modulated HPV to a greater extent in younger lambs.

Inasmuch as prostacyclin is a potent pulmonary vasodilator reported to modulate the hypoxic response in other species (17, 33–35), we assayed perfusate samples for 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2. The concentrations of 6-keto-$PGF_{1\alpha}$ were not different in the two age groups at any level of O_2 (Table 2). Despite this, prostacyclin could still be responsible for an age-dependent effect of indomethacin if the response of the pulmonary vasculature to PGI_2 varied with age in a fashion analagous to responses to arachidonic acid (36) and $PGF_{2\alpha}$ (37). At comparable doses of arachidonic acid, newborn lambs showed a greater increase in PVR than in ventilaged fetal lambs (36). Also, mature newborn goats were more sensitive to $PGF_{2\alpha}$ than premature newborn goats (37).

Another possible mechanism to explain the age-dependent effects of indomethacin is the formation of a dilator PG other than PGI_2. Both PGE_2 and PGD_2 cause pulmonary vasodilation in the newborn lamb (20, 22, 23). In addition, pulmonary PGE_2 catabolism is low at birth in lambs, but rapidly increases with age (18). Age-dependent differences in the concentration of either of these agents may have contributed to our results. Alternatively, there may have been age-related effects of indomethacin not due to inhibition of cyclooxygenase. For example, indomethacin may divert arachidonic acid to the lipoxygenase pathway leading to the production of potent vasoconstrictor LT (38). Although LTD_4 is a potent pulmonary vasoconstrictor in newborn lambs (39), this explanation seems unlikely because *1*) cyclooxygenase inhibition blunted the constrictor response to LTD_4 in adult sheep (40) and *2*) indomethacin inhibited the pulmonary pressor response to arachidonic acid in adult sheep (41) and perinatal

lambs (36). Other nonspecific effects of indomethacin are possible (42, 43). However, as indomethacin is 90% protein bound, the free indomethacin concentration in the perfusate was estimated to be 3–5 μg/ml. At this concentration, indomethacin is thought to act relatively specifically as a cyclooxygenase inhibitor (42, 43).

In summary, we found that the pulmonary vascular response to an inspired oxygen tension of 50 mm Hg, but not 30 or 0 mm Hg, increased with age in isolated lungs from neonatal lambs. This age-dependent effect was reversed by indomethacin. These data suggest that at PiO_2 = 50 mm Hg dilator prostaglandins modulated HPV to a greater extent in 2- to 4-day-old lambs than in 12- to 14-day-old lambs.

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