Developmental Effects of Hypoxia and Indomethacin on Distribution of Vascular Resistances in Lamb Lungs

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ABSTRACT. The influence of postnatal age on the distribution of vascular resistances was studied in *in situ*, isolated, blood perfused lungs of lambs aged 6–12 h, 2–4 d, 2 wk, and 1 mo. The pressure gradients across the upstream, downstream, and compliant middle segments were determined by an inflow-outflow occlusion technique during mild hyperoxia ($\text{FIO}_2 = 0.28$) and severe hypoxia ($\text{FIO}_2 = 0.04$) under control and indomethacin-treated conditions. The greatest response to hypoxia was seen in the middle segment. A lesser, but significant response was also seen in the upstream segment. Indomethacin accentuated the hypoxic response of the total circuit and the middle segment at all ages, and the upstream segment in the two younger age groups. An age-dependent increase in total hypoxic reactivity was seen in control lungs, but not in indomethacin-treated lungs. When the pressure gradient across the middle segment alone was considered, an age-dependent increase in hypoxic reactivity was seen in controls and a decrease was seen in indomethacin-treated lungs. In contrast there were no age-dependent changes in the hypoxic responses of the upstream or downstream segments. These data suggest that the predominant site of hypoxic pulmonary vasoconstriction and dilator prostaglandin activity lies within the middle gradient in newborn lamb lungs. Furthermore, the age-dependent changes in the hypoxic response of the middle gradient appear to be altered by the modulating influence of dilator prostaglandins. (*Pediatr Res* 26:325–329, 1989)

Abbreviations

HPV, hypoxic pulmonary vasoconstriction
PVR, pulmonary vascular resistance
PG, prostaglandin
Paw, tracheal pressure
Ppa, Pulmonary artery pressure
Pla, left atrial pressure
 FIO_2 , fractional inspired oxygen
 $\dot{Q}$, constant flow
LSD, least significant different test
 ΔPm , middle segment gradient
 ΔPt , total pressure gradient
 ΔPa , upstream gradient
 ΔPv , downstream gradient

It has long been established that normoxic PVR decreases rapidly after birth (1–3). In contrast, although some studies have shown that the vigor of HPV also decreases (3–5), others have indicated that HPV increases with age (6–11). In piglets the increase in hypoxic reactivity was positively correlated with the peripheral extension of arteriolar muscularization (9, 10) suggesting that arterioles might be the site of HPV. However, other newborn studies suggested that veins, not arterioles are the site of HPV (12–14). Moreover, in a previous study we found that dilator PG appeared to modulate HPV more in isolated lungs of 2- to 4-d than 2-wk-old lambs, suggesting that factors other than vascular muscularization may also influence the age-dependent changes in HPV (8).

In this study we examined the effects of hypoxia ($\text{FIO}_2 = 0.04$) on the distribution of pulmonary vascular resistances in isolated lamb lungs under control and indomethacin-treated conditions at four ages during the 1st mo of life. We used an inflow-outflow occlusion technique to partition the pulmonary vascular circuit into relatively noncompliant upstream and downstream segments and a compliant middle segment (11, 15–17). The goals of our study were: 1) to identify the functional site of HPV by determining the segment most responsive to hypoxia, 2) to determine which segment(s) responds to indomethacin, and 3) to determine whether the responses to hypoxia and/or indomethacin are age dependent.

MATERIALS AND METHODS

Preparation. The *in situ*, isolated, blood perfused lungs of 39 newborn lambs were studied using an isolated lung preparation and occlusion technique previously described (8, 11). Each lamb was anesthetized with ketamine (50 mg/kg intramuscularly) after which a femoral artery and vein were cannulated and a tracheostomy tube was inserted. The lamb was paralyzed (pancuronium bromide 0.1 mg/kg, intravenously) and ventilation initiated with a hyperoxic gas mixture. Heparin (3000–5000 U, intravenously) was administered via the femoral venous line and the lamb was rapidly exsanguinated. A median sternotomy was performed, the ductus arteriosus was ligated, and the pulmonary artery was cannulated via the right ventricular outflow tract. The left ventricle was excised and a large bore cannula was placed in the left atrium via the mitral valve. This was tightly secured just above the entry of the pulmonary veins into the atrium minimizing redundant atrial tissue below the cannula so that the contribution of the left atrium to pulmonary vascular compliance was negligible. The perfusion system was connected to the cannulae, the lungs were hyperinflated, and after removing all air from the perfusion system, flow was initiated.

The perfusate (autologous blood and 3% dextran in Ringer's lactate) was pumped (Fisher, Manostat, New York, NY) from a reservoir to a heat exchanger (Travenol, pediatric miniprime,

Received October 19, 1988; accepted June 5, 1989.

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Supported by the Quebec Heart Foundation and the McGill University/Montreal Children's Hospital Research Institute.

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Travenol Canada, Ville St. Laurent, Quebec, Canada), bubble trap/particulate filter, and a flow probe (Carolina, EP 300A Carolina Medical Electronics, King, NC) then to an occluder from which it could be directed either to the pulmonary artery or directly back to the reservoir. Perfusate that entered the pulmonary artery exited the lungs through the left atrium to the reservoir that could be raised or lowered to achieve the desired P_{la} . P_{pa} , P_{la} , and P_{aw} were zero referenced to the top of the lungs and constantly measured (Statham Gould P23id, Gould Inc. Medical Products Oxnard, CA). $\dot{Q}$ was measured (Carolina, model 501) and pressures and $\dot{Q}$ were constantly recorded (Grass Polygraph, model 701, Grass Inst. Co., Quincy, MA). After exsanguination, ventilation (Harvard model 613, Harvard Apparatus, S. Natick, MA) was established at 14–16.5 mL/kg (body wt) tidal volume, a rate of 10/min and end expiratory pressure of 3 mm Hg. The gas mixtures were either slightly hyperoxic ($FIO_2 = 0.28$, CO_2 5.5%, balance N_2) or severely hypoxic ($FIO_2 = 0.04$, CO_2 5.5%, balance N_2). This level of hypoxia has been found to cause a maximal pressor response in isolated lungs of lambs (8). Inspired FIO_2 was constantly monitored (Amitek O_2 analyzer, Amitek, Pittsburgh, PA). Perfusate hematocrit was between 15 and 17% and did not change during the experiment. Perfusate temperature was maintained between 38.5 and 39.5°C. Initial blood glucose concentrations were 80–120 mg/100 mL and were maintained at this level by the addition of 50% dextrose during the study. Perfusate gases were verified at each FIO_2 and pH was maintained between 7.33 and 7.45 by the addition of 1 N $NaHCO_3$.

Study groups. In all studies the distribution of vascular resistances was determined at $FIO_2 = 0.28$ and 0.04. A total of 26 studies was performed using control lungs from four age groups (6–12 h, $n = 5$; 2–4 d, $n = 6$; 12–16 d (2 wk), $n = 6$; 26–35 d (1 mo), $n = 9$). Twenty-six studies were also performed in indomethacin treated lungs from the four age groups (6–12 h, $n = 5$; 2–4 d, $n = 6$; 2 wk, $n = 7$; 1 mo, $n = 8$). In 13 of the 26 indomethacin studies, the drug was added at the beginning of perfusion whereas in the other 13 it was added after completing a control study (four at 6–12 h, three at 2–4 d, three at 2 wk, three at 1 mo). In all cases indomethacin (40 μ g/mL perfusate) was added to the reservoir. This dose had previously been shown to inhibit cyclooxygenase activity in this blood perfused preparation (8).

Protocol. After completing the surgical preparation (<17 min of absent pulmonary blood flow), ventilation with the hyperoxic gas mixture and perfusion at a $P_{la} < 0$ mm Hg were started. $\dot{Q}$ was gradually increased to 100 mL/kg (body wt)·min over 20–40 min and at 50–55 min the reservoir was raised so that $P_{la} = 5$ mm Hg. After a further stabilization period (~5 min), arterial then venous occlusions were performed as described below. The lungs were then ventilated with the hypoxic gas mixture and after 20–25 min (*i.e.* when P_{pa} was stable) the occlusions were repeated. In the 13 preparations in which indomethacin was added after control studies, an additional 20-min stabilization period was allowed before repeating hyperoxic and hypoxic occlusions as in the controls.

Occlusion technique. All occlusions were performed with the ventilator off at end expiration, so that P_{la} (5 mm Hg) was greater than P_{aw} (3 mm Hg) (*i.e.* zone III conditions). The arterial occlusions were accomplished by diverting the flow from the pulmonary artery to a bypass circuit. The occluder was a plexiglass mechanical slide valve that was well lubricated so that occlusions were essentially instantaneous (<50 ms). After $\dot{Q}$ at 100 mL/kg·min had been established and P_{pa} was stable, paper speed was increased to 50 mm/s and an inflow occlusion was performed. After ~3 s, the pulmonary artery pressure at $\dot{Q} = 0$ mL/kg min had reached a stable baseline (~5 mm Hg), and the perfusate was redirected through the lungs. Immediately after occlusion the P_{pa} dropped suddenly to a point P_{pa}' , after which an exponential decline in pressure occurred (Fig. 1). P_{pa}' was determined by extrapolating the initial part of the exponential

Fig. 1. Tracings of the inflow occlusions at $FIO_2 = 0.28$ and 0.04 and outflow occlusion at $FIO_2 = 0.28$. Hypoxia caused an increase in both P_{pa} and P_{pa}' , whereas P_{la} and P_{la}' were unchanged. Therefore ΔP_m showed the greatest increase while ΔP_a increased a little and ΔP_v was unchanged.

decline in pressure (*i.e.* about 300–800 ms after occlusion) back to time 0 using semilog paper as previously described (11, 15–17). The mean P_{pa} and P_{pa}' were determined after two or three occlusions had been performed to ascertain that the results were reproducible. Then, after a stable P_{pa} at $\dot{Q} = 100$ mL/kg·min was reestablished, the outflow cannula was suddenly occluded. The left atrial pressure rose suddenly to P_{la}' then more slowly (Fig. 1). P_{la}' was determined directly from the strip chart recording by finding the intercept of the first part of the slow rise and time = 0. Again, at least two occlusions were used and the mean P_{la} and P_{la}' determined at each FIO_2 .

Data analysis. After determining P_{pa} , P_{pa}' , P_{la}' , and P_{la} , at both high and low FIO_2 , the pressure gradients across each segment of the pulmonary circuit were calculated: $\Delta P_t = (P_{pa} - P_{la})$, $\Delta P_a = P_{pa} - P_{pa}'$, $\Delta P_v = P_{la}' - P_{la}$, and $\Delta P_m = \Delta P_t - (\Delta P_a + P_v)$. The effects of hypoxia and indomethacin on ΔP_t and each segment were determined by analysis of variance and LSD at each age. The effect of age on total hypoxic reactivity was determined by one-way analysis of variance and LSD for control and indomethacin-treated lungs. The effect of indomethacin on the change in hypoxic response with age was evaluated by three way analysis of variance and LSD. All results are expressed as means $\pm$ SE and were considered significant at $p \leq 0.05$.

RESULTS

The mean hematocrit (15.7–17.5%), peak P_{aw} (10–12.5 mm Hg), and perfusate temperature (38.6–39.1°C) did not differ among groups. The tidal volumes were slightly lower in the oldest lambs (14.2–14.5 mL/kg) when compared to the youngest lambs (15.4–16.4 mL/kg) ($p = 0.05$). The weight of the lambs increased with age, but were similar between groups at every age. Blood gas results are shown (Table 1).

Hypoxia caused a significant increase in the total pressure gradient (ΔP_t) in control lungs of 2-wk and 1-mo-old lambs and at all ages in the indomethacin-treated lungs (Table 2). The pressure gradients across each segment are shown in Figures 2. In the older controls and at all ages in indomethacin-treated lungs, both ΔP_a and ΔP_m increased with hypoxia (Fig. 2 A and B). However, the absolute value of ΔP_m was greater than ΔP_a during hypoxia, but less than ΔP_a at an FIO_2 of 0.28. Moreover, the relative contribution of ΔP_m to the total pressure gradient

Table 1. Blood gas results*

Age	pH	PCO ₂ controls	PO ₂ (H)	PO ₂ (L)
6-12 h	7.40 ± 0.02	31 ± 1.4	166 ± 5	30 ± 1.4
2-4 d	7.39 ± 0.05	35 ± 5.3	157 ± 18	30 ± 5.4
2 wk	7.38 ± 0.04	35 ± 1.0	151 ± 12	34 ± 6.8
1 mo	7.39 ± 0.03	33 ± 4.8	160 ± 19	31 ± 6.0
Indomethacin				
6-12 h	7.38 ± 0.04	33 ± 2.7	158 ± 8	28 ± 2.0
2-4 d	7.37 ± 0.04	35 ± 3.2	155 ± 9	32 ± 4.0
2 wk	7.39 ± 0.05	35 ± 1.0	154 ± 11	33 ± 8.8
1 mo	7.40 ± 0.03	32 ± 2.0	162 ± 13	30 ± 5.5

* There were no differences within or between ages in mean pH, mean PCO₂ or mean PO₂ (mm Hg) at each FIO₂ (H = 0.28, L = 0.04).

Table 2. Total pressure gradients (ΔP_t)

Age	Control		FIO ₂	Indomethacin	
	0.28	0.04		0.28	0.04
6-12 h	10.7 ± 2.0	14.3 ± 2.1		14.2 ± 2.5*	35.1 ± 5.3
2-4 d	12.0 ± 2.0	18.3 ± 2.9		18.7 ± 1.1*	37.4 ± 3.5
2 wk	11.9 ± 2.4*	20.0 ± 2.4		12.4 ± 1.4*	28.6 ± 1.6
1 mo	10.0 ± 1.3*	19.9 ± 2.3		10.8 ± 1.3*	29.2 ± 2.8

* Hypoxia increased ΔP_t (mm Hg) at the two older ages in control lungs and all ages in indomethacin treated lungs ($p < 0.05$). Indomethacin accentuated hypoxic ΔP_t at all ages ($p < 0.05$).

rose during hypoxia, whereas the contribution of ΔP_a to ΔP_t remained largely unchanged. Therefore, the predominant hypoxic response appeared to occur within ΔP_m . Hypoxia had no effect on ΔP_t or any segment in the 6- to 12-h-old control group (Table 2, Fig. 2). In the 2- to 4-d-old control group the response of ΔP_t and ΔP_m approached but did not reach statistical significance ($p = 0.07$). Hypoxia had no effect on ΔP_v in any group except the oldest where a physiologically small (but statistically significant) decrease in pressure gradient was seen in both control and indomethacin-treated lungs (Fig. 2C).

At all ages indomethacin caused a significant accentuation of ΔP_t during hypoxia (Table 2). This was due to a striking increase in the hypoxic responses of ΔP_m (Fig. 2B) at all ages ($p < 0.05$). In the two younger age groups an accentuation of the hypoxic responses of ΔP_a (Fig. 2A) was also seen ($p < 0.05$). Indomethacin had no effect on the responses of ΔP_a in older lambs or on the responses of ΔP_v at any age.

The effects of indomethacin on age-dependent changes in the hypoxic responses of each segment were determined by three-way analysis of variance (between factors: age and indomethacin; within factor: inspired oxygen tension). Indomethacin significantly altered the effect of age on the response of ΔP_m (Fig. 2B; $p = 0.05$, LSD = 3.7). In controls the response was greater in lungs of the 1-mo-old group than the 6- to 12-h group, whereas after indomethacin treatment the response was greater in the lungs of the 6- to 12-h-old than either the 2-wk or 1-mo-old groups. In contrast there was neither an age-dependent increase nor decrease in the responses of ΔP_a or ΔP_v under control or indomethacin-treated conditions (Figs. 2A and C).

When total hypoxic reactivity (defined as the change in total pressure gradient between an FIO₂ of 0.28 and 0.04) was considered, indomethacin did not reverse the effects of age. One way analysis of variance showed that in controls total hypoxic reactivity was greater in the 1-mo group (9.9 ± 1.3 mm Hg) compared with the 6- to 12-h group (3.6 ± 1.3 mm Hg) ($p = 0.05$, LSD = 5.9). However, the decrease in hypoxic reactivity over the four ages studied in indomethacin-treated lungs did not reach statistical significance.

The control lungs were all examined in a similar manner in

Fig. 2. Effects of hypoxia and indomethacin on ΔP_a (A), ΔP_m (B), and ΔP_v (C) at all ages. Hypoxia caused an increase in ΔP_a and ΔP_m in older controls and indomethacin-treated lungs of all ages and a decrease in ΔP_v in the oldest groups (* $p < 0.05$, all results are mean $\pm$ SE). In ΔP_m , the hypoxic response of controls was less in the 6- to 12-h than the 1-mo group (δ), but greater in the 6- to 12-h than either the 2-wk or 1 mo group after indomethacin (γ). Indomethacin accentuated the hypoxic response of ΔP_a in the 6- to 12-h and 2- to 4-d groups and of ΔP_m at all ages.

this study, but two approaches were used in the indomethacin groups. In 13 of 26 the indomethacin was added after a control study was performed whereas in the other 13 it was added at the beginning of the experiment. With both techniques, indomethacin significantly accentuated the hypoxic response (hypoxic ΔP_t controls = 18.5 ± 1.2 mm Hg, ΔP_t indomethacin at start = 35.9

± 2.7 mm Hg, ΔP_t indomethacin in the middle = 28.3 ± 1.4 mm Hg). Inasmuch as the pressures were not significantly higher in the group in which indomethacin was added at the start, compared with those in which it was added in the middle, we lumped all indomethacin-treated lungs together.

DISCUSSION

In our study we used an inflow-outflow occlusion technique to show that the pulmonary vascular response to hypoxia occurred predominantly in the middle segment in *in situ* isolated newborn lamb lungs. A smaller, but significant response also occurred in the upstream segment (Fig. 2). These data are consistent with similar studies in mature dogs (15) and pigs (16), as well as with a recent study in fetal and newborn lambs (11). The anatomic limits of the middle segment have not been established. Tod *et al.* (17) performed inflow-outflow occlusions after embolizing mature pig lungs with glass beads. They found that the upstream gradient did not increase despite embolization with beads as large as 2–3 mm in diameter suggesting that ΔP_m might include arterioles as large as 3 mm in diameter (17). However, Michel *et al.* (18) showed in dogs that pressures measured in small arterioles with 1.2-mm lumen catheters were greater than those defined as P_{pa} by the occlusion technique, suggesting that the upstream limit of ΔP_m is arterioles under 1.2 mm in diameter. Because the occlusion technique relies on differences in compliance between small vessels and larger veins to define the downstream limits of ΔP_m , small veins also likely reside within ΔP_m . Using small catheters in veins as they did on the arteriolar side, Michel *et al.* (18) showed that the veins within ΔP_m were also likely less than 1.2 mm in diameter. In a recent abstract, Hakim and Kelly (19) have reported that the limits of ΔP_m are arterioles and veins that are less than 0.9 mm in diameter by small catheter studies but larger than 60 μ m in diameter as determined by micropuncture (19). These studies suggest that small arterioles, veins, and capillaries all likely lie within ΔP_m . We are therefore unable to state which vessels are the anatomic effectors of HPV in the newborn lamb lungs studied here.

Others have made use of the changes in HPV which occur during the newborn period to try to define the anatomic site of HPV through various other techniques. However, there is little agreement among these studies. The vigor of HPV was positively correlated with peripheral extension of arteriolar muscularization in piglets aged 2–4 wk to 6 mo, supporting the hypothesis that small arterioles are the site of HPV (9, 10). Other evidence for this includes an angiographic study in mature cats showing that arterioles 300 μ m in diameter were the vessels most responsive to acute hypoxia (20). However, hypoxia was shown to increase lymph flow in newborn lambs as compared with older sheep suggesting that (at least in newborns) the site of HPV is on the venous side of the circuit, downstream from permeable capillaries (12). Hazinski and Kennedy (13) also supported a primary role for capillaries or veins in their study showing that hypoxia increased pulmonary capillary wedge pressure, but not left atrial pressure. The micropuncture technique has also been used in newborn animals. This technique involves determining the pressures within small (20–60 or 80 μ m diameter) arterioles and venules visualized on the pleural surface of the lung (21–23). Inasmuch as pulmonary artery and left atrial pressures are also measured, gradients between the pulmonary artery and small arterioles (upstream segment), small arterioles and small veins (microcirculation), and small veins and left atrium (downstream segment) can be identified. Using this technique Fike *et al.* (21) have shown that the upstream segment is the predominant site of HPV in newborn rabbits. However, Raj and Chen (23) showed that in lambs, the pressure gradients across both the upstream and downstream segments increased in response to hypoxia.

Although the micropuncture and lymph flow studies excluded a major role for the microcirculation in HPV in newborn lambs, neither technique identifies the size of vessels in the upstream or

downstream segments responsible for HPV. The segment we have defined as ΔP_m likely includes a portion of the upstream and downstream segments as defined by micropuncture. Furthermore, ΔP_m likely includes the small arterioles (<500 μ m diameter) which undergo vascular muscular involution during early development (1) and the venules downstream from the permeable capillaries (12). Identification of the anatomic site of HPV in the newborn lamb may be better accomplished in the future by combining micropuncture, occlusion, and other techniques such as small catheter pressure measurements with detailed morphologic data.

In our study, we also examined the effect of the cyclooxygenase inhibitor, indomethacin, on hypoxic reactivity and the distribution of vascular resistances during development. As seen in previous studies of isolated lungs of mature (24), newborn (25), and fetal animals (25), cyclooxygenase inhibition accentuated the hypoxic response at all ages (Table 2). In the 2-wk and 1-mo-old groups this was due solely to an increase in the hypoxic response of the middle segment (Fig. 2B). However, in the younger age groups, the hypoxic responses of both the upstream and middle segments were accentuated by indomethacin (Fig. 2A and B). This apparent change in site of dilator PG activity and the marked attenuation of HPV in isolated lungs of controls at 6–12 h and 2–4 d of age, confirmed our previous observation that dilator PG appear to have a greater effect on isolated lungs in younger than older newborn lambs.

Given the difficulties in determining and interpreting PG concentrations in serum (8), we did not measure PG concentrations in this study. Instead we assumed that the accentuation of HPV by indomethacin seen in our study was due to the inhibition of production of any, or all, potential dilator PG. These include PGI_2 which has been shown to increase in response to HPV in both newborn and mature animals (24, 26), as well as PGD_2 (27) and the PGE series (28) that cause vasodilation in young newborns. Whether the greater attenuation of HPV and the accentuation of ΔP_a as well as ΔP_m in lungs of younger lambs in our study were due to increased concentrations of specific dilator PG or altered vascular sensitivity to PG in the younger lambs remains to be addressed. It is also possible that indomethacin may have acted by a noncyclooxygenase-dependent mechanism. However, other cyclooxygenase inhibitors such as meclofenamate have been shown to accentuate HPV in isolated lungs (24). Furthermore, although it has been argued that cyclooxygenase inhibition might divert arachidonic acid to the leukotriene pathway resulting in increased production of vasoconstrictor leukotrienes (29); in both newborn lambs (30) and mature sheep (31) the increase in PVR normally seen after arachidonic acid administration was prevented by pretreatment with indomethacin, making this explanation unlikely.

In this study, the total hypoxic response and the hypoxic response of the middle segment increased between 6–12 h and 1 mo of age in control lungs (Fig. 2, Table 2). This was consistent with the age-dependent increase in HPV previously seen in isolated lungs of rabbits (6, 7) and lambs (8, 11). Indomethacin blocked the increase in total hypoxic reactivity in this study and the hypoxic response of ΔP_m (*i.e.* that segment most responsive to hypoxia) decreased between 6–12 h and 2 wk or 1 mo once the modulating influence of dilator PG was removed (Fig. 2, Table 2). This decrease in hypoxic response with age was similar to that seen in intact calves (3), pigs (4), and sheep (5). A possible explanation for this effect of cyclooxygenase inhibition is that in control isolated lungs the manipulation involved in the preparation caused an increase in PG release resulting in greater modulation of HPV than seen in intact animals. Indirect support for this idea includes the observation that rapid ventilatory rates (32) and rapid alteration in vascular pressures or flow rates (33) caused an increase in PGI_2 production. That isolated lungs may produce more dilator PG is also supported by the findings that meclofenamate did not accentuate HPV in intact piglets between 5 and 88 d of life (4) and in intact 2- to 3-wk-old lambs,

indomethacin had only a transient effect on hypoxic vasoactivity (34). Nonetheless, the manipulation of the lungs in our study was similar at all ages. Therefore, the greater attenuation of HPV in control isolated lungs of younger rather than older lambs likely represented a greater capacity to modulate HPV in the younger animals.

In summary, we have identified the compliant middle segment as the predominant site of HPV and the site most affected by indomethacin in isolated lungs of newborn lambs. The hypoxic response of this segment rose between 6–12 h and 1 mo of age in controls, but after the possibly confounding influence of dilator PG was removed, the response decreased with age. The upstream gradient showed a significant, but lesser, response to hypoxia, and in younger lambs the response was accentuated by indomethacin. However, there was neither an age-dependent increase nor decrease in the hypoxic responses of ΔP_a or ΔP_v . As discussed earlier the anatomic limits of the middle segment are not certain, thus we cannot know which vessels are responsible for HPV. However, we find it interesting to speculate that if arteriolar medial involution in the growing lamb is similar to that described in the piglet (1, 9), then the decrease in the hypoxic response of ΔP_m over the 1st mo of age seen after indomethacin administration would parallel the decrease in arteriolar muscularization. Such a finding would support the possibility that small arterioles play a major role in HPV. Future studies aimed at more directly identifying the anatomic site of HPV within ΔP_m and identifying the dilator PG modulating HPV during development must be undertaken.

Acknowledgments. The authors thank Ms. T. O'Riley, N. Corman, and S. Watters for technical help and Ms. S. Lapointe and J. de Foy for secretarial assistance. Thanks also to Mary Tod, Ph.D. for continued long distance collaboration.

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