Responses of Small Intrapulmonary Arteries to Vasoactive Compounds in the Fetal and Neonatal Lamb: Norepinephrine, Epinephrine, Serotonin, and Potassium Chloride

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ABSTRACT. Despite considerable study, the mechanisms responsible for the transition of the pulmonary circulation from the fetal to newborn life remain obscure. We compared the responses of third and fourth generation pulmonary arteries to norepinephrine, epinephrine, serotonin, and KCl from lambs 7 d preterm and 1, 7, and 21 d of age to assess differences between age and third and fourth generations of the pulmonary arterial tree during the transitional period. Preterm vessels were significantly smaller in internal diameter than all other aged vessels for both generations. Fourth generation vessel response to KCl increased with age (94 ± 15 mg/mm^2, preterm; 259 ± 31 mg/mm^2, 21 d). Third generation vessel response to KCl did not change with age (135 ± 15 mg/mm^2, preterm; 158 ± 18 mg/mm^2, 21 d). There were no differences in maximum response to norepinephrine and epinephrine between ages; however, third generation vessel response to these compounds was significantly greater (30–60% of maximum KCl response) than fourth generation vessel response (0–10%). Third and fourth generation vessels had the same maximum response to serotonin regardless of age or generation. Third generation vessels were significantly reduced in sensitivity (log molar concentration which produced 50% of the maximum response—EC$_{50}$) to norepinephrine at d 1 (5.89 ± 0.12 log molar) and 7 (5.90 ± 0.21) compared to preterm (6.48 ± 0.10) and 21 d of age ($6.50 \pm .03$). Both third and fourth generation vessels were less sensitive to serotonin at d 1 (5.04 ± 0.17, third; 5.61 ± 0.10, fourth) compared to d 7 (5.77 ± 0.13, third; 6.36 ± 0.12, fourth) and 21 d (5.82 ± 0.24, third; 6.50 ± 0.15, fourth). A similar nonsignificant trend occurred in third generation vessels to epinephrine at d 1 and 7 compared to vessels at preterm and 21 d of age. Greater than 75% of fourth generation vessels did not respond to norepinephrine and epinephrine. The small intrapulmonary arteries of the neonate are undergoing dynamic changes in physiologic response which are dependent upon age and generation. (*Pediatr Res* 25:360–363, 1989)

Abbreviations

NE, norepinephrine
EPI, epinephrine
5HT, 5-hydroxytryptamine, serotonin

At birth, the neonatal lung is still undergoing maturation and development: multiplication of intraalveolar vessels, remodeling of vessels under 250 μm, and extension of smooth muscle into the alveolar region occur (1–8). Physiologically, the lungs adapt to extrauterine life with a progressive decrease in pulmonary vascular resistance and increase in blood flow to perform the vital function of gas exchange. However, despite considerable study, the mechanisms involved in the neonatal transitional circulation remain obscure.

A wealth of information exists regarding the contractility and reactivity of large diameter vessels (>1 mm) in the neonatal lung (9–11). However, arteries with a diameter greater than 500 μm are unimportant in controlling resistance (12). Furthermore, because of tremendous regional variation in both structure and function throughout the vasculature, extrapolation of data from larger vessels to those less than 500 μm is inappropriate (11, 13–18). An assessment of blood vessel response at levels potentially important in controlling pulmonary vascular resistance and flow may further clarify the mechanisms of the transitional circulation.

In this study we examined the reactivity and contractility of third generation pulmonary vessels (530–1000 μm, internal diameter) and fourth generation pulmonary vessels (175–330 μm, internal diameter) to the vasoactive compounds NE, EPI, 5HT, and KCl in the lamb at 1 wk preterm and 1, 7, and 21 d of age, over a period of adaptation to extrauterine life.

MATERIALS AND METHODS

Fetal and neonatal purebred lambs were used. Preterm lambs of approximately 136–140 d of gestation were delivered by cesarean section and a saline-filled bag was placed over the nose to prevent breathing. Lambs of 1, 7, and 21 d of age were delivered by natural birth without intervention. For all ages, a euthanizing intracardiac dose (45 mg/kg) of sodium pentobarbital (Elkins-Sinn, Inc.) was injected. In preterm animals, the cord was then clamped and cut. The lungs and heart were rapidly removed en bloc via a median sternotomy. The ductus arteriosus was not manipulated in any of the animals. The pulmonary vascular bed was injected with cold (4°C), oxygenated (95% O$_2$-5% CO$_2$) Krebs bicarbonate solution (composition in mM: NaCl 118.3, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 25.0, CaCl$_2$ 2.5, and glucose 11.1) through an incision in the right ventricle. The main pulmonary artery was dissected free and cut longitudinally down the right and left branches to the base of both lungs and down third generation arteries. Blocks of lung tissue containing third and fourth generation arteries were excised, placed in cold Krebs solution, and segments were dissected free and cleaned of surrounding parenchyma. Vessel segments were

threaded onto two 40-μm stainless steel wires (California Fine Wire Co.) and secured to mounting supports. The mounting supports were attached on one side to a displacement micrometer (Starret) and on the other side to a force transducer (Kistler-Morse DSC-6BE4-110, Kistler Instrument Corp., Amherst, NY) to record isometric tension. Responses were amplified (Beckman RB Dynograph, Beckman Instruments Inc., Fullerton, CA) and recorded on a 2-channel rectilinear chart recorder (Kipp & Zonen Model BD 41, Bohemia, NY).

The vessels were equilibrated for 90 min while temperature was brought up to 37°C. At the end of the equilibration period, a length-tension curve was performed by stretching in increments and stimulating the vessels with isoosmotic 125 mM KCl solution until an optimal contractile response was obtained. Vessel diameters were measured via the displacement micrometers and circumferences were calculated (19).

Standard cumulative half-log dose response curves were generated to NE (Winthrop-Breon) EPI (Parke, Davis & Co., Detroit, MI) and 5HT (Sigma Chemical Co., St. Louis, MO) by the method of Van Rossum (20). Fresh stock solutions of all drugs were made and used within 8 h.

Responses to KCl were expressed as mg/mm² (vessel circumference × length). As we did not know whether blood vessels of different sizes had the same amount and arrangement of smooth muscle, the constrictor responses were normalized in terms of the maximum response to KCl (16) where:

Response (%) = mg/mm² constrictor/mg/mm² KCl × 100

The sensitivity to each compound was assessed by determining the concentration which produced 50% of the maximal response (EC$_{50}$) extrapolated from a plot of log concentration *versus* percentage of the maximal response. The EC$_{50}$ responses are expressed as a negative logarithm ($-$log molar EC$_{50}$). Two-way ANOVA and Tukey's HSD test were used to compare internal diameters and response to KCl. Repeated measures (dose) 2-way ANOVA and Tukey's HSD test were used to compare differences of main treatment effects.

RESULTS

Internal diameter. Table 1 shows the internal diameters in micrometers of third and fourth generation vessels as a function of age. Third generation vessels were significantly larger than fourth generation vessels in internal diameter ($p < 0.0001$). Preterm vessels were significantly smaller than vessels at all other ages ($p < 0.005$). In addition, a significant statistical interaction occurred between age and generation ($p < 0.05$).

Response to KCl. The response of third and fourth generation vessels to KCl are shown as a function of age (Table 2). Day 21 response was significantly greater than preterm vessel response ($p < 0.005$). Third and fourth generation vessels were similar in their response to KCl. A statistically significant interaction was determined between age and generation ($p < 0.01$).

Maximum response to vasoactive compounds. Figures 1 through 3 show the responses of third and fourth generation blood vessels expressed as a percentage of the KCl response. Third generation vessels had a significantly greater maximal response to NE ($p < 0.0001$) and EPI ($p < 0.0005$) than fourth generation vessels at all ages (Fig. 1 *A* and *B* and Fig. 2 *A* and *B*). No differences were detected between ages. Third generation vessels were not different between ages or generation to 5HT (Fig. 3 *A* and *B*).

Sensitivity to vasoactive compounds. The $-$log molar EC$_{50}$ values to EPI, NE, and 5HT are shown in Table 3. Because of lack of responsiveness (>75%) to NE and EPI in fourth generation vessels, EC$_{50}$ values were not calculated. Third generation vessels were significantly less sensitive to NE at d 1 and 7 compared to preterm and d 21 vessels ($p < 0.005$). This same trend, although not significant, also occurred at d 1 and 7 to EPI. Both third and fourth generation vessels were significantly

Table 1. *Internal diameters (in μm) (mean $\pm$ SEM) of third and fourth generation blood vessels*

| | Age | | | |
Generation	Preterm* ($n = 5$)	D 1 ($n = 12$)	D 7 ($n = 11$)	D 21 ($n = 11$)
Third†	530 ± 40	880 ± 70	965 ± 110	1000 ± 60
Fourth	175 ± 15	330 ± 40	275 ± 25	245 ± 20

* Preterm vessels significantly smaller than all other ages ($p < 0.01$).

† Third generation vessels significantly larger than fourth generation vessels at all ages ($p < 0.001$).

Table 2. *Response (in mg/mm²) (mean $\pm$ SEM) of third and fourth generation vessels to 125 mM KCl*

| | Age | | | |
Generation	Preterm* ($n = 5$)	D 1 ($n = 12$)	D 7 ($n = 11$)	D 21 ($n = 11$)
Third	136 ± 15	189 ± 32	103 ± 12	158 ± 18
Fourth	94 ± 14	165 ± 22	183 ± 16	259 ± 31

* Preterm vessels significantly different from 21 d vessels, fourth generation ($p < 0.005$).
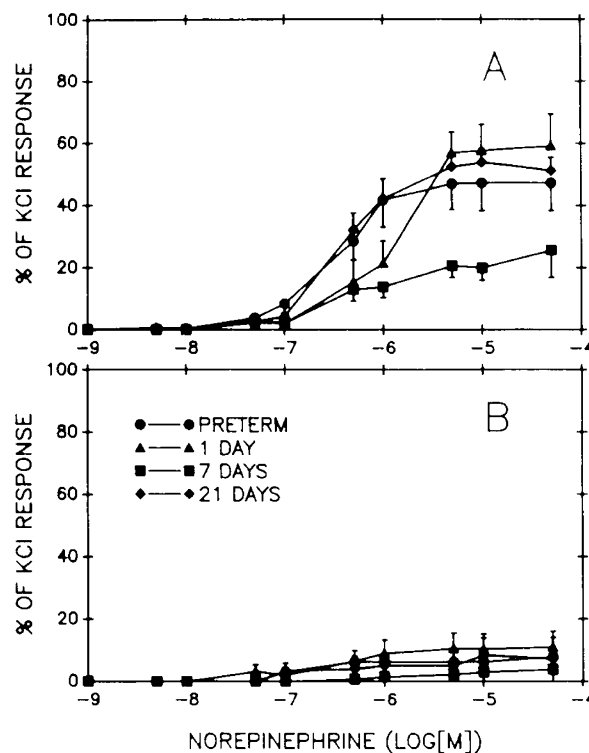

Fig. 1. Responses of third (A) and fourth (B) generation vessels at preterm and d 1, 7, and 21 to NE normalized as a function of the KCl response.

less sensitive to 5HT at d 1 compared to d 7 and 21 ($p < 0.0001$). In addition, fourth generation vessels were significantly more sensitive to 5HT than third generation vessels ($p < 0.0001$). Preterm vessel response to 5HT was not performed.

DISCUSSION

This work has demonstrated that third and fourth generation pulmonary arteries of the neonatal lamb differ in their responsiveness to the vasoactive compounds KCl, NE, EPI, and 5HT *in vitro*. These differences suggest that for a complete understanding of the transitional circulation it is necessary to assess re-
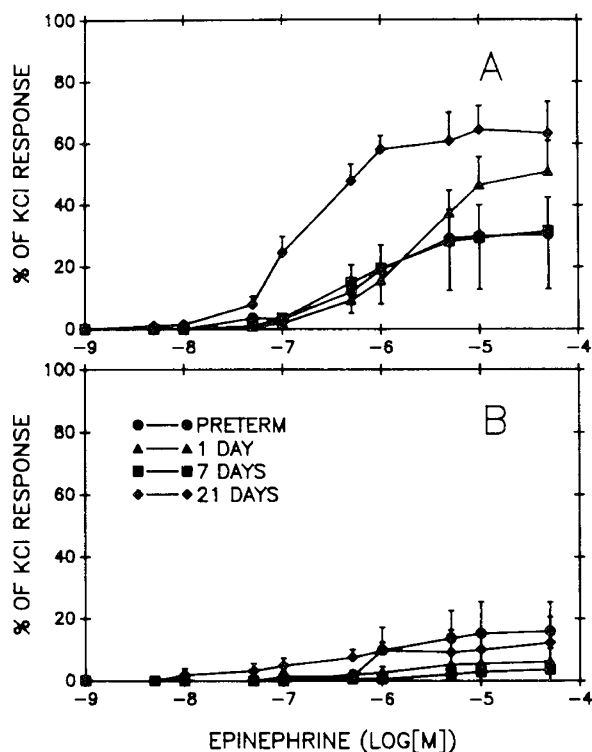
Fig. 2. Responses of third (*A*) and fourth (*B*) generation vessels at preterm and d 1, 7, and 21 to EPI normalized as a function of the KCl response.
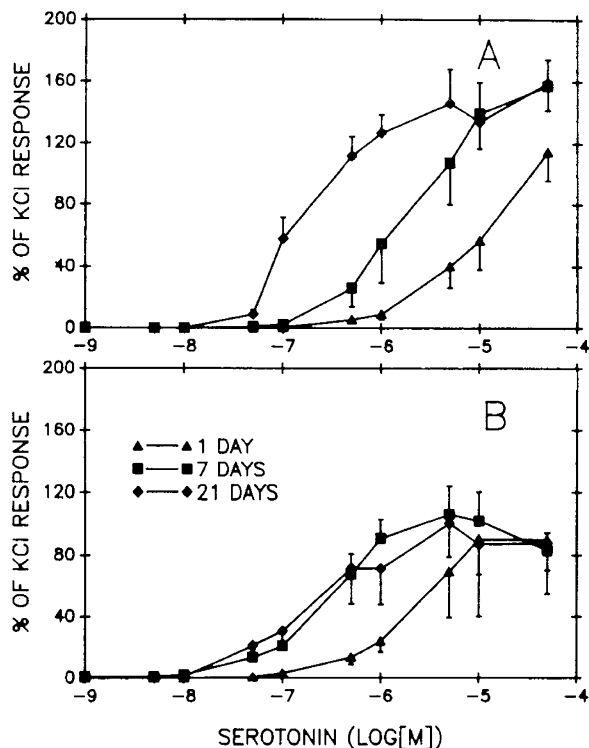

Fig. 3. Responses of third (*A*) and fourth (*B*) generation vessels at d 1, 7, and 21 to serotonin normalized as a function of the KCl response.

sponses at vessels more important in controlling pulmonary resistance and flow.

The significantly smaller internal diameters of preterm vessels compared to all other ages is consistent with the drop in pulmonary vascular resistance at birth. We do not feel that growth

Table 3. *—Log molar EC_{50} values (mean ± SEM) for third and fourth generation vessels to NE, EPI, and 5HT*

Drug	Generation	Age			
		Preterm ($n = 4$)	D 1 ($n = 8$)	D 7 ($n = 6$)	D 21 ($n = 6$)
NE	Third	6.48 ± .10	5.89 ± .12*	5.90 ± .21*	6.50 ± .03
	Fourth				
EPI	Third	6.20 ± .03	5.77 ± .11	5.64 ± .24	6.20 ± .25
	Fourth				
5HT	Third		5.04 ± .17†	5.77 ± .13	5.82 ± .24
	Fourth‡		5.61 ± .10	6.36 ± .12	6.50 ± .15

* D 1 and 7 vessels significantly less sensitive to NE than preterm and d 21 vessels ($p < 0.005$).

† D 1 vessels significantly less sensitive to 5HT than d 7 and 21 aged vessels ($p < 0.0001$).

‡ Fourth generation vessels significantly more sensitive to 5HT than third generation vessels at all ages ($p < 0.0001$).

alone can account for these diameter differences. Pulmonary vascular remodeling occurs throughout the the postnatal ages tested, yet d 1, 7, and 21 vessels were not different in internal diameter. Both lung expansion and alveolar oxygenation play a role in decreasing the pulmonary vascular resistance at birth; however, the relative contribution of these factors is an area of some debate (21–24). In this study, fetal and neonatal vessels were removed and placed in Krebs solution perfused with 95% oxygen. Also, the fetal lung never underwent lung expansion. Thus, while oxygen may be an important mediator of pulmonary vasodilation at birth, it may not be an initiating or sustaining factor. In addition, oxygen may require an intact pulmonary bed to mediate the vasodilatory process at birth.

A statistically significant interaction was encountered for internal diameter between age and generation. A comparison of third and fourth generation vessel internal diameter shows that these vessels do not change in a parallel fashion with age. The third generation vessel internal diameter progressively increases with age. The fourth generation vessel internal diameter increases at 1 d of age and then progressively decreases at d 7 and 21. This may reflect the dramatic remodeling of the small vessels and extension of vascular smooth muscle (1, 8).

Although the muscular wall thickness was not obtained in this study, the response to KCl, which is mediated through its effect on the membrane potential (25) and is therefore receptor independent, indicates the integrity and mass of smooth muscle present. Third generation arteries did not show any changes in KCl response over time, suggesting that the smooth muscle at this generation did not undergo detectable functional alteration throughout the time period tested. However, fourth generation vessel response to KCl continued to increase over the time period tested, indicating smooth muscle extension or maturation at this generation. Certainly the muscular coat at this generation is not thinning and therefore would not account for the diameter increases that occurred at birth.

Third generation maximal response was similar between ages to NE and EPI and between ages and generation to 5HT. Thus, there are no changes in receptor quantity in these vessels (10, 20). However, there is a relative paucity of receptors to EPI and NE in fourth generation vessels compared to third generation vessels as indicated by the significantly smaller maximal response to these compounds. Decreases in adrenergic activity with decreases in vessel diameter have also been reported in the rabbit lung (26). The significance of this finding was not discussed, but may reflect the relative lack of vasomotor tone in the adult lung (13).

Unlike maximal contractile response, the sensitivity of vessels to EPI and NE decreased at 1 and 7 d in third generation vessels. Also, both third and fourth generation vessels were least sensitive to 5HT at 1 d of age. The decreased sensitivity at these ages may

be due to the degree of neuronal and extraneuronal uptake (14). Furthermore, this decrease in sensitivity may be an important mechanism in the neonate to adjust to the stress of birth. During the birth process, high levels of catecholamines are released into the circulation (27, 28). The pulmonary vascular bed may exhibit selective desensitization of the third generation vessels and a paucity of adrenergic receptors of the fourth generation vessels. Thus, the dramatic drop in pulmonary vascular resistance necessary at birth is maintained, despite the presence of high circulating vasoconstrictor agents.

A high concentration of oxygen (95%), a potent pulmonary vasodilator, was perfused throughout our studies. Oxygen may selectivity exert effects that either decrease or eliminate the responses to NE and EPI. This possibility and the possibility of increased adrenergic uptake require further study.

Serotonin produced a marked vasoconstrictor response that exceeded the maximal response to KCl in both third and fourth generation vessels. Its ability to produce a marked vasoconstrictor response in the absence of effects to EPI and NE of the fourth generation vessels, indicates that the serotonin response was not mediated by α receptors. Work using specific serotonergic agonists and antagonists would be helpful in clarifying this response.

In conclusion, the neonatal pulmonary vascular bed is undergoing dynamic changes characterized by a developing vascular smooth muscle effector system of the fourth generation vessels and by changes in agonist affinity of both third and fourth generation vessels. These changes are dependent upon age and generation. This study verifies that third and fourth generation vessels are quite different in their patterns of responsiveness. Thus, responsiveness of large arteries does not reflect the reactivity of arteries and arterioles important in controlling pulmonary vascular hemodynamics of the fetus and newborn.

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