Developmental changes in endothelium-dependent pulmonary vasodilatation in pigs

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1 We compared *in vitro* endothelium-dependent vasorelaxant responses to acetylcholine (ACh) and the endothelium-independent vasodilator response to sodium nitroprusside (SNP) in prostaglandin F_{2α} (PGF_{2α})-precontracted muscular pulmonary arteries (PA) from pigs aged 5 min to 2 h (neonatal), 3–10 days, 3–8 weeks and adults.

2 In the pulmonary artery (PA) rings from neonatal animals, the vasodilator response to ACh was negligible. However, responses to ACh were present in all PA rings from older animals, being greatest at 3–10 days and then decreasing with age ($P < 0.001$, ANOVA). ACh (30 μ M) induced a $1 \pm 1\%$, $92 \pm 9\%$, $62 \pm 5\%$ and $51 \pm 6\%$ reduction of the PGF_{2α}-generated tension in neonatal, 3–10 days, 3–8 weeks and adult groups, respectively.

3 The relaxant response to SNP was present in the PA rings from all age groups and increased with age ($P < 0.001$, ANOVA). SNP (1 μ M)-induced relaxation was $55 \pm 9\%$, $73 \pm 7\%$, $97 \pm 5\%$ and $93 \pm 6\%$ in neonatal, 3–10 days, 3–8 week and adult groups, respectively.

4 Removal of the vascular endothelium abolished the relaxant response to ACh but had no effect on the response to SNP in any groups.

5 N^G-monomethyl-L-arginine (30 μ M), a nitric oxide synthesis inhibitor, inhibited the response to ACh but not to SNP. The lipoxygenase inhibitor, nordihydroguaiaretic acid, had no significant effect on responses to ACh or SNP in any group.

6 These findings suggest that the nitric oxide pathway may not play a part in dilating the pig pulmonary arteries at birth, but may be important during the transitional period of establishing a stable post-natal pulmonary circulation. The increase in response to SNP with age parallels the increase in smooth muscle cell myofilaments to which it may be related.

Keywords: Endothelium-derived relaxing factor; pulmonary artery; endothelium; vascular development

Introduction

Since Furchgott & Zawadzki (1980) first demonstrated that the vasodilator action of acetylcholine (ACh) was dependent on the presence of an intact endothelium, the vascular endothelium has proved to play a crucial role in mediating the dilator response to a variety of stimuli in the systemic and pulmonary circulations (Furchgott, 1984; Greenberg *et al.*, 1987). In response to these stimuli, endothelial cells release a labile endothelium-derived relaxing factor (EDRF), which causes vascular smooth muscle relaxation (Furchgott & Vanhoutte, 1989). EDRF has been identified as nitric oxide (NO) or a related compound (Palmer *et al.*, 1987; 1988; Moncada *et al.*, 1989; Ignarro, 1989), and inhibition of NO synthesis by compounds such as N^G-monomethyl-L-arginine (L-NMMA) inhibits the vasodilator response to ACh in both systemic (Amezcuca *et al.*, 1988; Rees *et al.*, 1989a) and pulmonary circulations (Davidson & Eldermersdash, 1990; Crawley *et al.*, 1990; Liu *et al.*, 1991). L-NMMA induces dose-dependent increase in mean systemic blood pressure (Rees *et al.*, 1989b; Gardiner *et al.*, 1990) and decreases regional blood flow *in vivo* (Gardiner *et al.*, 1990).

The pulmonary circulation undergoes significant changes, both structurally (Haworth & Hislop, 1981; Hall & Haworth, 1986; 1987), and functionally (Su *et al.*, 1977; Davidson, 1987; Dunn *et al.*, 1989; Teitel *et al.*, 1990), at birth. The mechanisms responsible for this pulmonary adaptation are incompletely understood. Vasodilators such as prostacyclin (Leffler *et al.*, 1978; Leffler & Hessler, 1981; Davidson, 1988)

have been implicated. Endothelium-derived NO may also play a role in the sheep (Abman *et al.*, 1990), but not in the rabbit (Zubrow *et al.*, 1989). The role of the vascular endothelium in regulating or modulating perinatal pulmonary adaptation and reactivity has not been studied in detail.

EDRF activity has been shown to be present in pulmonary vessels at 1–3 days in guinea-pigs (Davidson & Eldermersdash, 1990) but is absent or greatly reduced in foetal pulmonary arteries of rabbit and sheep (Zubrow *et al.*, 1989; Abman *et al.*, 1991). These results suggest that, in addition to a developmental change after birth (Abman *et al.*, 1991; Zellers & Vanhoutte, 1991), the endothelium-dependent response may change rapidly at birth with the dramatic pulmonary haemodynamic changes.

We have, therefore, examined the endothelium-mediated dilator response to ACh and the endothelium-independent dilator responses to sodium nitroprusside (SNP) in the pulmonary arteries from pigs immediately after birth (aged 5 min to 2 h: neonatal), compared those with the responses in the pulmonary arteries from 3–10 days, 3–8 weeks and young adults, in order to characterize further the developmental change in the endothelium-dependent response. To study the mechanism(s) involved, we used L-NMMA to inhibit NO synthesis and indomethacin to inhibit the cyclooxygenase production. Lipoxygenase products may play a role in mediating endothelium-dependent relaxation in rabbit aorta (Singer & Peach, 1983; Forstermann & Neufang, 1984; Forstermann *et al.*, 1986), therefore, we also studied the effect of nordihydroguaiaretic acid (NDGA, a lipoxygenase inhibitor) on the endothelium-dependent relaxation in these vessels.

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Methods

Twenty-five large white pigs were killed by exsanguination. Immediately after death the heart and lungs were removed and pulmonary arteries were carefully dissected. The arteries were put in oxygenated Krebs-Henseleit (K-H) solution kept at 4°C and transported to the laboratory for use. The transport time and conditions were standardized for all groups. Intrapulmonary arteries were examined in 4 groups of pigs aged less than 2 h (neonatal, $n = 6$), 3–10 days ($n = 6$), 3–8 weeks ($n = 8$) and 6–9 months (adult, $n = 5$). In all animals the distal 2/3 of both lower lobe pulmonary arteries were cleaned of surrounding tissues and cut into rings of 2.5 or 3.5 mm in length depending upon the size of the lung. A total of 4 rings (neonatal and 3–10 day groups) or 6 rings (3–8 week and adult groups) were studied in each animal. In each animal at least one ring was a control and two rings were for L-NMMA or NDGA treatment. In the remaining ring(s), vascular endothelium was removed by gently rubbing the intimal surface with a steel probe or a piece of fine sand paper.

Organ chamber studies

Vessel rings from pigs aged less than 10 days were positioned in 2 ml organ baths by a pair of stainless steel wires inserted horizontally in the lumen of the vessel rings. One wire was fixed and the other attached to a force transducer (FT. 03 Grass, Quincy, MA, U.S.A.). Rings from large pigs were fixed vertically between two hooks in 5 ml organ chambers. One hook was fixed and the other attached to a force transducer. The organ chambers contained K-H solution composed of: NaCl 118, KCl 5.9, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 2.5, NaH_2PO_4 1.2, glucose 5.6 and NaHCO_3 25.5 mM. They were maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2 . Indomethacin (10 μM) was present in all experiments. The optimal resting tension of each vessel was determined initially by increasing resting tension gradually in 200 mg increments and challenging repeatedly with 40 mM KCl, until the response to KCl was not further increased. The optimal resting tension for each vessel was calculated from the tension-response curve (see Results section). The vessel ring was allowed to equilibrate at its optimal resting tension for 30 min and a concentration-response curve to prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) was obtained. Each individual concentration-response curve to $\text{PGF}_{2\alpha}$ was fitted and the EC_{50} value estimated by a computer programme (Graph PAD InPlot, GraphPAD software, San Diego, CA, U.S.A.). After a further 1 h equilibration, relaxation studies were started. In each animal one ring was incubated with 30 μM L-NMMA and one with 50 μM NDGA for 20 min before precontraction with $\text{PGF}_{2\alpha}$. All vessel rings were precontracted with the EC_{50} concentration of $\text{PGF}_{2\alpha}$ and after a stable contraction had been achieved, cumulative concentration-response curves to ACh and then SNP were obtained. At the end of each experiment the vessel rings were fixed with 2.5% glutaraldehyde for histological examination.

Drugs

The following drugs were used: prostaglandin $\text{F}_{2\alpha}$ (Upjohn Ltd, Crawley, Sussex), acetylcholine chloride, sodium nitroprusside, indomethacin, nordihydroguaiaretic acid, L-N^G-monomethyl arginine (Sigma, Poole, Dorset). All drugs were freshly prepared on the day of experiment.

Calculation and analysis of results

Contractions were measured from the vertical distance between the baseline and the stable tone induced by $\text{PGF}_{2\alpha}$ and are presented in absolute tension (mg). Relaxations were measured from a common reference line denoting the stable contractions elicited by EC_{50} concentrations of $\text{PGF}_{2\alpha}$ before adding ACh or SNP. The vertical distance between this reference line and the largest downward deflection in the tracing at each concentration of ACh or SNP was recorded and expressed as the percentage of the $\text{PGF}_{2\alpha}$ -induced contraction. The mean values at each concentration point were used to compare the relaxant response to ACh and then SNP in vessel rings of different ages, and the same vessels in the presence and absence of an intact endothelium, L-NMMA, or NDGA. All values in the text are presented as mean $\pm$ s.e.means. n refers to the numbers of animals from which the PA rings were taken. Results were compared by using paired Student's t test or one-way analysis of variance followed by Bonferroni corrected t test for stepwise comparison, when multiple comparison was made (Wallenstein *et al.*, 1981). P values < 0.05 were considered to be significant.

Morphological examination

Vascular rings were processed for electronmicroscopic examination. One μm sections were cut and stained with toluidine blue. From these it was possible to confirm the presence or absence of endothelial cells. Transmission electronmicrographs of endothelial cells were also studied.

Results

The optimal resting tension increased significantly with maturation and was 933 ± 10 ($n = 6$ animals), 1060 ± 40 ($n = 6$ animals), 1138 ± 11 ($n = 8$ animals) and 1124 ± 35 ($n = 5$ animals) mg for neonatal, 3–10 day, 3–8 week and adult groups respectively ($P < 0.03$ between any two adjacent groups, except between 3–8 week and adult group). $\text{PGF}_{2\alpha}$ -generated contraction also increased significantly with age in rings with and without endothelium (Table 1). Light microscope examination confirmed that an intact endothelial lining was consistently present in unrubbed vessel rings and absent in rubbed vessel rings (Figure 1). Examination by transmission electron microscopy showed that the endothelial morphological appearances were identical among all age groups.

Table 1 Prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$)-induced tensions (mg, pre-acetylcholine) in developing and adult pig pulmonary arteries

	n	Neonatal	n	3–10 day	n	3–8 week	n	Adult
E ⁺	6	544 $\pm$ 52*	6	761 $\pm$ 155†	8	1238 $\pm$ 141	5	1641 $\pm$ 310
E [−]	6	516 $\pm$ 105*	6	796 $\pm$ 182†	8	1223 $\pm$ 170	5	1884 $\pm$ 309
L-NMMA	6	540 $\pm$ 102*	6	870 $\pm$ 144†	8	1255 $\pm$ 160	5	2009 $\pm$ 271
NDGA	6	442 $\pm$ 102*	6	720 $\pm$ 107†	8	965 $\pm$ 154	5	1525 $\pm$ 238

EC_{50} concentration of $\text{PGF}_{2\alpha}$ -induced contractions are shown.

E⁺: endothelium-intact rings; E[−]: endothelium-denuded rings. L-NMMA or NDGA: endothelium intact rings pretreated with L-N^G-monomethyl arginine (30 μM) or nordihydroguaiaretic acid (50 μM).

* $P < 0.01$, compared with 3–8 week and adult groups.

† $P < 0.02$, compared with adult group.

Data show means $\pm$ s.e.mean.

Figure 1 Photomicrographs from two sections of adjacent rings of pulmonary artery from a neonatal pig (5 min after delivery), showing the presence of an intact endothelial lining on the unrubbed ring (a) and the absence of the endothelium on the rubbed ring (b). Toluidine blue stain, magnification $\times 1000$.

Endothelium-dependent relaxation

There was no significant difference in $\text{PGF}_{2\alpha}$ (EC_{50} concentration)-induced contractions in vessel rings with or without endothelium at any age (Table 1). ACh had little relaxant effect on the endothelium intact vessel rings in the neonatal group (Figure 2a). The maximal relaxation was of $2.3 \pm 2.3\%$ (at $100 \mu\text{M}$, $n = 6$) reduction of the $\text{PGF}_{2\alpha}$ -induced contraction. ACh induced a concentration-dependent relaxation in the endothelium intact precontracted vessel rings from pigs aged 3 days onwards (Figure 2b-d). This relaxation was greatest at 3–10 days, less at 3–8 weeks and still less in adult group (Figure 4a). The relaxant responses to ACh were abolished by removal of endothelium in all age groups (Figure 2a-d). Higher concentrations of ACh (above $30 \mu\text{M}$) caused a further contraction in the precontracted neonatal vessel rings with or without endothelium. In rings from older animals, higher concentrations of ACh produced a contraction of the already dilated vessel rings. The precontracted endothelium intact neonatal PA rings which relax poorly in response to ACh, had little relaxant response to bradykinin. The maximal relaxation at $1 \mu\text{M}$ was 9.7 ± 3.7 ($n = 2$) of the $\text{PGF}_{2\alpha}$ -induced contraction.

The tensions generated by a second administration of $\text{PGF}_{2\alpha}$ (EC_{50} concentration) before adding SNP were not significantly different in the endothelium intact or denuded rings in any age group (Table 2). In all age groups, SNP induced concentration-dependent relaxation of the $\text{PGF}_{2\alpha}$ precontracted vessel rings, which was unaffected by endothelial denudation (Figure 3a-d). The vasorelaxant response to SNP increased significantly with age (Figure 4b).

Effects of L-NMMA and NDGA

The tensions induced by EC_{50} concentration of $\text{PGF}_{2\alpha}$ in the endothelium-intact vessel rings in the absence and presence of $30 \mu\text{M}$ L-NMMA or $50 \mu\text{M}$ NDGA before the administration of ACh or SNP are shown in Table 1 and Table 2. Neither

Figure 2 The vasorelaxant response to acetylcholine (ACh) in the precontracted pulmonary artery rings from neonatal (a), 3–10 days (b), 3–8 week (c) and adult (d) pigs. Relaxations were expressed as a percentage of the EC_{50} concentration of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$)-induced contractions which are shown in Table 1. (○) Rings with endothelium; (●) rings without endothelium; means $\pm$ s.e.mean (vertical bars) of 5–8 animals.

Figure 3 The vasorelaxant response to sodium nitroprusside (SNP) in precontracted pulmonary artery rings from neonatal (a), 3–10 days (b), 3–8 week (c) and adult (d) pigs. Relaxations were expressed as a percentage of the EC_{50} concentration of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$)-induced contractions which are shown in Table 2. (Δ) Rings with endothelium and (▲) rings without endothelium; means $\pm$ s.e.mean (vertical bars) of 5–7 animals.

Table 2 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-elicited contractions (mg, pre-sodium nitroprusside) in developing and adult pig pulmonary arteries

	n	Neonatal	n	3-10 day	n	3-8 week	n	Adult
E ⁺	6	544 ± 77*	6	862 ± 126†	7	1342 ± 187	5	1535 ± 318
E ⁻	6	504 ± 73*	6	857 ± 155†	7	1329 ± 119	5	2047 ± 513
L-NMMA	6	663 ± 111*	6	886 ± 152†	7	1427 ± 293	5	1954 ± 540
NDGA	5	587 ± 49*	5	923 ± 152†	6	1404 ± 245	5	1439 ± 100

Symbols and abbreviations used here are the same as in Table 1.

L-NMMA nor NDGA had a significant effect on the $PGF_{2\alpha}$ -induced contraction in any age group. L-NMMA had no effect on the baseline tension in the neonatal group, but increased the baseline tension by 149 ± 49 ($n = 6$), 137 ± 51 ($n = 8$) and 197 ± 96 ($n = 5$) mg in 3-10 day, 3-8 week and adult groups respectively ($P < 0.05$, compared with control rings in each group). L-NMMA incubation significantly

Figure 4 Comparison of the endothelium-dependent relaxant responses to acetylcholine (ACh) (a) and of the endothelium-independent response to sodium nitroprusside (SNP) (b) in the endothelium-intact, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-precontracted, pulmonary artery rings from neonatal (○, ●), 3-10 days (□, ■), 3-8 weeks (Δ, ▲) and adult (◇, ◆) pigs. Relaxations were expressed as a percentage of EC_{50} concentration of $PGF_{2\alpha}$ -generated tensions which are shown in Tables 1 and 2. * $P < 0.05$, compared with other 3 groups; ** $P < 0.05$, compared with neonatal and adult groups; # $P < 0.05$, compared with 3-8 week and adult groups; ## $P < 0.05$, compared with 3-8 week group; means ± s.e.mean (vertical bars) of 5-8 animals.

Figure 5 Effects of L-N^G-monomethyl arginine (L-NMMA, 30 μM) and nordihydroguaiaretic acid (NDGA, 50 μM) on acetylcholine (ACh)-induced relaxation in the endothelium intact prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-precontracted pulmonary artery rings from 3-10 day (a), 3-8 week (b) and adult (c) pigs. Relaxations were expressed as percentage of EC_{50} concentration of $PGF_{2\alpha}$ -induced contractions shown in Table 1. (○) Control rings; (●) in the presence of L-NMMA and (Δ) in the presence of nordihydroguaiaretic acid. * $P < 0.05$, compared with control rings; means ± s.e.mean (bars) of 5-8 animals.

Figure 6 Effects of N^G-monomethyl-L-arginine (L-NMMA, 30 μ M) and nordihydroguaiaretic acid (NDGA, 50 μ M) on sodium nitroprusside (SNP)-induced relaxation in the endothelium intact prostaglandin F_{2α} (PGF_{2α})-precontracted pulmonary artery rings from 3–10 day (a), 3–8 week (b) and adult (c). Relaxations were expressed as percentage of EC₅₀ concentration of PGF_{2α}-elicited contractions shown in Table 2. (Δ) Control rings; (▲) in the presence of L-NMMA and (■) in the presence of NDGA; means $\pm$ s.e. mean (bars) of 5–6 animals.

reduced the relaxation induced by ACh (Figure 5a–c). The inhibition of ACh induced relaxation by L-NMMA was greater at 3–10 days than at 3–8 weeks and adults. L-NMMA had no effect on SNP-induced relaxations (Figure 6a–c). NDGA had no detectable effect on the baseline tension and did not affect either ACh (Figure 5a–c) or SNP (Figure 6a–c)-induced relaxations in the endothelium-intact vessel rings of any group.

Discussion

Our results demonstrated a two phase change in the endothelium-dependent response to ACh in the pig pulmonary

arteries. The response to ACh is negligible at birth, but develops rapidly after birth with a maximal effect at 3–10 days, and then declines gradually towards adulthood. This confirms findings of other investigators who showed absent, or greatly reduced, endothelium-dependent dilator response to ACh in foetal pulmonary arteries of rabbit and sheep (Zubrow *et al.*, 1989; Abman *et al.*, 1991). The vasodilator response is present by 1–3 days in isolated pulmonary arteries of guinea-pig (Davidson & Eldermerdash, 1990) and pig (Zellers & Vanhoutte, 1991). Our results extend the initial observation that showed a maturational change in the endothelium-dependent response to ACh in the perinatal pulmonary arteries (Abman *et al.*, 1991; Zellers & Vanhoutte, 1991).

Dilatation of small pulmonary arteries and reduction in pulmonary artery pressure occur minutes after birth, with continuing decrease in pulmonary artery pressure and resistance for the first 24 h in the pig (Haworth & Hislop, 1981). In normal full-term lambs, pulmonary arterial blood pressure falls to near adult levels within 2–4 h (Heymann & Soifer, 1989). After the initial rapid fall in pulmonary vascular resistance and pulmonary arterial blood pressure, there is a slow progressive fall, reaching adult levels within 2–6 weeks after birth (Heymann & Soifer, 1989; Haworth & Hislop, 1981). A previous study (Zellers & Vanhoutte, 1991) showed a greater dilator response to ACh and bradykinin in 10 day pig pulmonary arteries compared with pulmonary artery rings from 3 day old pigs, indicating that endothelium-dependent relaxation mediated by ACh and bradykinin may play a role in the slow reduction of pulmonary artery pressure. The time course for the change in the endothelium-dependent response to ACh in the early stages of adaptation remains unclear. We therefore compared the endothelium-dependent relaxant response to ACh in the pulmonary arteries from pigs aged 5 min to 2 h, 3–10 days, 3–8 weeks and adults. The virtual absence of an endothelium-dependent relaxant response to ACh in the pig pulmonary artery rings 5 min to 2 h after birth indicates that the endothelium-dependent response develops after birth. Thus endothelium-dependent relaxation does not appear to play a part in the initial pulmonary vasodilatation which occurs at birth in the pig. However, the greater response to ACh at 3–10 days of age suggests that EDRF may play an important role in the transitional phase of adaptation to extra-uterine life.

There appears to be a difference in the EDRF activity *in vitro* and *in vivo* in the foetal and neonatal pulmonary vessels. ACh dilates late-gestation foetal sheep pulmonary arterioles *in vivo*. Infusion of nitro-L-arginine, an inhibitor of NO synthesis, inhibits both basal and stimulated EDRF activities, and attenuates the rise in pulmonary blood flow at delivery (Abman *et al.*, 1990). On the other hand, the same authors have reported a greatly reduced basal and stimulated EDRF activity in foetal PA rings compared with newborn and adult in the same specie *in vitro* (Abman *et al.*, 1991). EDRF production is absent in the foetal rabbit PA rings (Zubrow *et al.*, 1989). Conditions *in vivo* and *in vitro* differ significantly. Other factors, such as species-dependent variation and difference in the responses to these vasodilators between large conductive and small resistance vessels may also contribute to this discrepancy. Nevertheless, all the *in vitro* results indicate that EDRF activity in the foetal and neonatal pulmonary arteries is less than in pulmonary arteries of other age groups. The relevance of these *in vitro* results to *in vivo* control of pulmonary vascular tone needs to be further investigated.

EDRF has been characterized as NO or a related substance (Palmer *et al.*, 1987; 1988; Moncada *et al.*, 1989; Ignarro, 1989). L-NMMA is a selective inhibitor of NO synthesis (Moncada *et al.*, 1989; Johns *et al.*, 1990). Our results showed that L-NMMA treatment markedly reduced the relaxant response to ACh, which strongly suggests that the ACh-induced vasorelaxant response in the immature pulmonary arteries (in animals over 3 days) is mediated predominantly by endothelium-derived NO, as in the adult

pulmonary arteries. The much greater relaxation in 3–10 day pulmonary arteries than in the 3–8 week and adult pulmonary arteries in response to ACh indicates that either more EDRF is released from pulmonary vascular endothelium or that pulmonary vascular smooth muscle cells of 3–10 day animals are more sensitive to EDRF than those in 3–8 week and adult vessels. The latter possibility is unlikely, since the vasodilator response to SNP which relaxes pulmonary vessels via the same intracellular mechanism as NO, is less in the younger animals. Moreover, in the presence of L-NMMA the response to ACh was similar at all ages, indicating that L-NMMA had a greater inhibitory effect on 3–10 day animals. This result suggests that more NO is produced in young animals. Another possibility is that 3–10 day pulmonary arteries have more endothelial muscarinic receptors or alternatively these receptors have a higher affinity for ACh. The latter possibility is unlikely, since the maturation-dependent change in the response to ACh is very similar to the response to bradykinin and A23187, a non-receptor-mediated, endothelium-dependent vasodilator, in pulmonary arteries from pigs aged 3 to 30 days (Zellers & Vanhoutte, 1991). Moreover, we found that neonatal PA rings had little relaxant response to both ACh and bradykinin. It is unlikely that the endothelium-smooth muscle barrier is more accessible for NO in 3–10 days PA rings than that of other age groups, since our transmission electronmicrographs showed no morphological difference in the endothelium layer among these four groups of PA rings. Zellers & Vanhoutte's (1991) report of an increase in the response to ACh and BK up to 10 days is in agreement with our results, which showed that the ACh-induced relaxation peaked at 3–10 days.

The lack of a significant vasodilator response to ACh in neonatal pulmonary arteries cannot be explained by a defect in vascular smooth muscle relaxation, because the relaxant response to SNP was present in these vessels. Other investigators have found that the response to SNP was similar in foetal and adult pulmonary arteries, although the relaxant response to ACh was absent or greatly reduced in the foetal vessels (Zubrow *et al.*, 1989; Abman *et al.*, 1991). Several possibilities, such as lack of endothelial muscarinic receptor or receptor coupling mechanism, or an inability of endothelial cells to synthesize EDRF could explain the failure to respond to ACh in the neonatal pulmonary artery. Other factors such as the availability of L-arginine and a defect of signal transduction may also contribute to the lack of response to ACh in the neonatal pulmonary artery. All these possibilities remain to be explored. The fact that L-NMMA had no effect on the baseline tension in the neonatal pulmonary arteries suggests a lack of EDRF activity in these vessels. Abman *et al.* (1991) have also shown that foetal sheep pulmonary artery rings were virtually unresponsive to increasing concentrations of haemoglobin, whereas haemoglobin induced a concentration-related increase in the vascular tensions in 1–4 week and adult pulmonary arteries.

In the present study the possible effect of the cyclo-oxygenase products on the endothelium-dependent relaxation to ACh was prevented by use of indomethacin. Although ACh-induced EDRF is not a cyclo-oxygenase product, ACh can stimulate prostacyclin (PGI₂) release from vascular endothelial cells (Fedderson *et al.*, 1986). PGI₂ is involved in the adjustment of the pulmonary circulation at birth (Leffler & Hessler, 1981; Davidson, 1988). With respect to the lipoxygenase products, the failure of NDGA to inhibit ACh-induced relaxation in the precontracted endothelium intact vessel rings indicates that lipoxygenase products were not involved in mediating the ACh relaxation in the immature pulmonary arteries. NDGA has been shown to inhibit ACh- or methacholine-induced relaxation in the rabbit aorta (Singer & Peach, 1983; Forstermann & Neufang, 1984; Forstermann *et al.*, 1986). These differences could be explained by region and/or species-dependent variation.

The present functional studies have shown that both the PGF_{2α}-generated tension and the vasorelaxant response to SNP increased progressively with age. These results are in agreement with the earlier report showing a maturational change in the vasorelaxant response to SNP in guinea-pig aorta (Balaraman *et al.*, 1990), but inconsistent with others who reported that the vasorelaxant response to SNP is similar within these age ranges in sheep and pig (Abman *et al.*, 1991; Zellers & Vanhoutte, 1991). Our findings are compatible with our previous morphological studies showing that the number of contractile myofilaments in pig pulmonary artery smooth muscle increased with age during the developmental period (Hall & Haworth, 1987). A similar increase in myofilaments has also been described in man (Allen & Haworth, 1988). Developmental changes have also been found in pulmonary arterial innervation (Wharton *et al.*, 1988) and in the responses to other vasoactive agents such as histamine, noradrenaline and 5-hydroxytryptamine (Newman *et al.*, 1979; Dunn *et al.*, 1989). All these observations suggest that the response to many therapeutic agents may be different in neonates from that seen in adult life.

In summary, our results show a two phase change in the endothelium-dependent response to ACh in the immature pig pulmonary artery, being absent at birth, reaching a maximum at 3–10 days and then declining with age. The vasorelaxant response to SNP increases progressively with age. These results suggest that the NO pathway may not play a role in the earlier pulmonary vasodilatation which occurs at birth, but be particularly important in the transitional pulmonary adaptation process to establish a stable pulmonary circulation after birth.

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