

# Porcine Pulmonary Artery and Bronchial Responses to Endothelin-1 and Norepinephrine on Recovery from Hypoxic Pulmonary Hypertension

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**ABSTRACT:** Many infants recovering from acute lung disease and pulmonary hypertension still have evidence of reactive airways disease at one year of age, suggesting longer-term airway effects. We hypothesized that parallel changes in smooth muscle would occur in airways and pulmonary arteries from animals with pulmonary hypertension and during normoxic recovery. Thus, two-hour-old piglets were subjected to 3 d chronic hypobaric hypoxia and 3-d-old piglets were subjected to 11 d hypoxia. Some animals were allowed to recover in room air for 3 or 6 d. The amount of smooth muscle and responses of isolated paired bronchial and pulmonary artery rings to endothelin-1 (ET-1) and norepinephrine were studied at the end of hypoxic exposure, on recovery and in age-matched control animals. In all hypoxia induced pulmonary hypertensive animals, smooth muscle area and ET-1 contractile response was increased in the pulmonary arteries and bronchi. Norepinephrine-induced relaxant response was impaired significantly in both bronchi and pulmonary arteries. After 3 d recovery, pulmonary arterial smooth muscle area decreased by 65%, and ET-1-induced contractile responses were normal for age. In the airways, ET-1 contractile response only normalized after six days and bronchial smooth muscle was still increased. After 6 d recovery pulmonary arterial norepinephrine-induced relaxant response had returned to normal, but bronchial response remained impaired. Thus during pulmonary hypertension, both bronchial and pulmonary arterial smooth muscle area and contractile responses are increased. On recovery, regression of bronchial structural and functional abnormalities is slower than in pulmonary arteries. (*Pediatr Res* 60: 71–76, 2006)

Anatomically, the pulmonary arteries and airways lie in close proximity and local mediator release may affect both bronchial and pulmonary vascular reactivity. Pulmonary hypertension in children with congenital heart disease is associated not only with an elevated pulmonary arterial vascular resistance and medial smooth muscle hypertrophy, but also with increased respiratory system resistance and bronchial smooth muscle hypertrophy (1).

Fifty percent of infants requiring Extra Corporeal Membrane Oxygenation (ECMO) for persistent pulmonary hypertension of the newborn still required pulmonary medications

after six months, despite ECMO utilizing a strategy of lung rest to prevent lung injury (2). Twenty-six percent of infants who participated in the UK randomized ECMO trial, many of whom had severe persistent pulmonary hypertension, still had evidence of reactive airways disease with cough and wheeze at one year of age, and 8% still required regular pulmonary medications (3). These changes could be due to the damaging effects of high pressure and the high FiO<sub>2</sub> used during the mechanical ventilation given before instituting ECMO (4), however pulmonary hypertension may have contributed to the end result by affecting both the pulmonary arteries and bronchi. The clinical findings also suggest that any bronchial changes, once present, are slow to resolve (2,3). Pulmonary hypertension can also have long-term pulmonary arterial sequelae. Human adults who had had perinatal pulmonary hypertension, showed a significantly greater mean increase in pulmonary artery pressure at high altitude than was seen in controls adults with no previous history of pulmonary hypertension (5). We hypothesize that there may be parallel changes in the pulmonary arteries and bronchi during pulmonary hypertension and on recovery. We have previously found that bombesin-immunostained pulmonary neuroendocrine cells, which also contain Endothelin-1 (ET-1) and norepinephrine, are increased during pulmonary hypertension in both the pulmonary arteries and bronchi in children with congenital heart disease (1). In man, ET-1 blood levels are elevated during pulmonary hypertension, and decrease following resolution in both persistent pulmonary hypertension of the newborn (6) and pulmonary hypertension due to congenital heart disease (7). Children with elevated pulmonary vascular resistance due to congenital heart disease or primary pulmonary hypertension also have elevated plasma norepinephrine levels (8). Thus evidence is accumulating that increased ET-1 and norepinephrine activity may contribute to the pathogenesis of pulmonary hypertension in children.

We therefore exposed newborn and 3-d-old piglets to chronic hypobaric hypoxia for three or 11 d to produce a

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**Abbreviations:** ET-1, endothelin-1; ET-B, endothelin-B receptor; ECMO, extracorporeal membrane oxygenation

raised pulmonary arterial pressure and then allowed some animals to recover in room air. The change in the amount of smooth muscle and the responses of isolated paired bronchial and pulmonary artery rings to ET-1 and norepinephrine were studied at the end of hypoxic exposure and after recovery to see if there are parallel changes in pulmonary arteries and bronchi.

## METHODS

To study the effect of pulmonary hypertension immediately after birth, newborn piglets (two hours old) were placed in a hypobaric chamber (50.8kPa,  $\text{FiO}_2$  0.096) and cared for in a hypoxic environment for three days (0–3 d hypoxia group) as previously described (9). A second group of normal three-day-old piglets were placed in the hypobaric chamber for eleven days (3–14 d hypoxia group) to examine the effect of onset of pulmonary hypertension after a period of post natal adaptation. Three and 14-d-old normoxic littermates were used as controls. Some piglets from both of the hypoxic pulmonary hypertension groups were allowed to recover in room air for 3 d. Six- or 17-d-old normoxic littermates were used as controls for these animals. Other piglets from the 3–14 d hypoxic pulmonary hypertension group were given a 6 d recovery period and 20-d-old littermates were used as controls (Table 1). The pigs received humane care in compliance with the "Guide for the Care and use of Laboratory Animals" published by the National Institutes of Health (Publication No. 80-23, Revised 1996). The study was approved by the Animal Ethics Committee of the Institute of Child Health.

The animals were killed with an overdose of pentobarbital sodium and the heart and lungs removed. The right ventricle (RV) was dissected free from the left ventricle plus septum (LV + S), weighed and the LV+S/RV ratio calculated.

To assess changes in the amount of bronchial and pulmonary arterial smooth muscle, lung tissue blocks were processed, cut and stained with smooth muscle alpha actin. Images of bronchi and accompanying pulmonary arteries (0.3–2 mm internal diameter) were captured with a Zeiss camera and then analyzed using OpenLab (10). Bronchial and pulmonary arterial smooth muscle area, lumen perimeter and diameter were measured. To standardize the smooth muscle mass for airway and vessel size, the smooth muscle area was divided by the measured lumen circumference. Four to nine pairs of bronchi and pulmonary arteries were measured in each animal. Only those airways and arteries cut in near-perfect cross-section were included for analysis (1).

To assess if there are parallel changes in bronchial and pulmonary arterial ET-1 and adreno-receptor pharmacological responses, intra-lobe pulmonary arteries (1–2 mm diameter) and adjacent bronchi (2–3 mm diameter) were dissected free and cut into rings 2–3 mm long. The rings were suspended in a 10 mL organ chamber and isometric tension was measured with a Grass FT 0.03 force transducer (Grass Instruments, Quincy, MA) and MacLab (AD Instruments Ltd., UK) as previously described (9). The organ chambers were filled with modified Krebs-Ringer solution, bubbled with 95%  $\text{O}_2$  – 5%  $\text{CO}_2$

at 37°C. The rings were stretched to their optimum resting tension and contracted with potassium chloride (KCl) 40 mM to assess their viability.

A cumulative contractile dose-response curve to ET-1 ( $10^{-10}$ – $10^{-7.5}$  M) was performed in the arteries and bronchi from baseline resting tension. A cumulative relaxant dose-response curve to ET-1 ( $10^{-10}$ – $10^{-8}$  M) and norepinephrine ( $10^{-8}$ – $10^{-4}$  M) was also performed in both pulmonary arteries and bronchi after the baseline tone had been elevated with U46619 (arteries) or carbachol (airways) to approximately 50% of the KCl 40 mM contraction. Some rings were pre-incubated with either the Endothelin-B receptor (ET-B) antagonist BQ788  $10^{-6}$  M or L-NAME  $10^{-4}$  M before the ET-1 relaxation.

The contractile response was expressed as developed tension (in grams/mg tissue), and the relaxation response was expressed as the percentage relaxation of the pre-constricted tension induced by U46619 or carbachol. The effect of hypoxic pulmonary hypertension and normoxic recovery on ET-1 and norepinephrine-induced responses of the arteries and bronchi were determined using a general linear model with repeated measures. The heart weight ratio, animal weight and smooth muscle area were analyzed using the *t*-test with Bonferroni correction, comparing the pulmonary hypertensive and recovery animals to the age-matched normoxic controls.

## RESULTS

The weight of the pulmonary hypertensive and recovery animals was not significantly different from that of age-matched controls (Table 1). The male-to-female ratio was similar in the pulmonary hypertensive, recovery and control groups.

The heart weight ratio of the 0–3 d hypoxia and 3–14 d hypoxia animals indicated significant right ventricular hypertrophy compared with three and 14-d-old controls and therefore the presence of pulmonary hypertension (Table 1). Following 0–3 d hypoxia and 3 d recovery, the mean heart weight ratio was greater than in hypertensive animals ( $p < 0.01$ ), but the ratio remained significantly lower than in 6 d control animals ( $p < 0.01$ ), indicating residual right ventricular hypertrophy (Table 1). Following 3–14 d hypoxia, the heart weight ratio became greater than that of the hypertensive animals after 6 d of recovery ( $p = 0.02$ ); however, it remained significantly lower than in the 20-d-old controls ( $p = 0.03$ ), again indicating residual right ventricular hypertrophy.

The pulmonary arterial and bronchial smooth muscle area related to lumen perimeter was increased two-fold in both groups of hypoxia-exposed animals,  $p < 0.001$  (Table 2). When regression analysis was performed on the data from all the paired bronchi and pulmonary arteries of 0–3 d hypoxic pulmonary hypertensive and 3 d control animals, there was a significant correlation between smooth muscle area in the bronchi and pulmonary arteries ( $r = 0.75$ ,  $p < 0.0001$ , Fig. 1). A similar correlation was found in the 3–14 d hypoxia group ( $r = 0.86$ ,  $p = 0.0001$ ).

In animals exposed to 0–3 d hypoxia there had been a significant reduction in the pulmonary arterial smooth muscle after 3 d recovery ( $p = 0.03$ ), but was still greater than in age-matched controls ( $p = 0.001$ ) being similar to the normal at 3 d (Table 2). The pulmonary arteries of the animals exposed to 3–14 d hypoxia and then allowed to recover did not show a significant reduction in muscle until after 6 d of recovery ( $p = 0.03$ ) and muscle was still greater than normal (Table 2).

The bronchial smooth muscle area of all animals exposed for 0–3 or 3–14 d hypoxia was still increased after 3 or 6 d recovery (Table 2).

**Table 1.** Number of animals, weight, sex and heart weight ratio

	<i>n</i>	Wt Kg	male/female	LV+S/RV
0–3 days hypoxia	9	1.3 ± 0.1	1:1.2	1.52 ± 0.1*
3-d-old control	9	1.6 ± 0.1	1.2:1	2.01 ± 0.1
0–3 hypoxia + 3d recovery	8	1.6 ± 0.2	1:1	1.96 ± 0.1†‡
6-d-old control	7	2.2 ± 0.3	1.3:1	2.58 ± 0.2
3–14 days hypoxia	7	3.0 ± 0.2	1:1.3	1.8 ± 0.1*
14-d-old control	7	4.1 ± 0.4	1:1.3	3.1 ± 0.1
3–14 hypoxia + 3d recovery	6	3.26 ± 0.2	1:1	2.0 ± 0.1‡
17-d-old control	6	3.9 ± 0.4	1:1	3.1 ± 0.1
3–14 hypoxia + 6d recovery	3	3.5 ± 0.1	1:2	2.3 ± 0.1†‡
20-d-old control	3	4.9 ± 1	1.5:1	3.2 ± 0.2

LV+S/RV, left ventricle + septal weight, divided by right ventricular weight.

\*  $p < 0.01$  when hypoxic pulmonary hypertensive animals compared to age-matched controls; †  $p < 0.01$  when recovery animals compared to hypoxia group; ‡  $p < 0.01$  when recovery animals compared to age-matched control animals, indicating that despite some recovery, there is still significant residual RV hypertrophy in the recovery animals.

*n*, number of animals.

Values are mean + SEM.

**Table 2.** Effect of hypoxia and recovery on bronchial and pulmonary arterial smooth muscle area

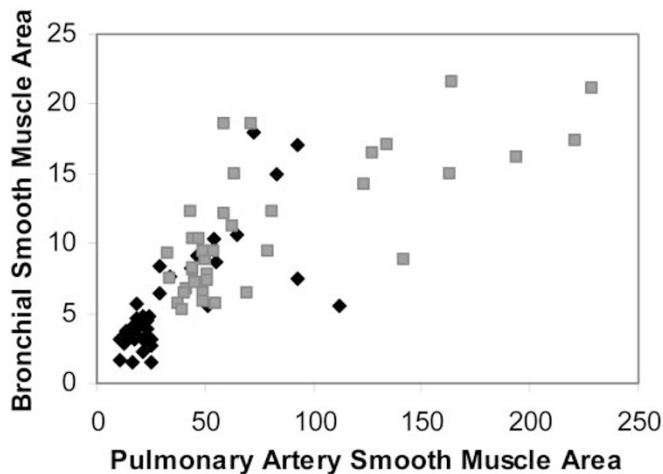
	Smooth muscle area ( $\mu\text{m}^2/\mu\text{m}$ luminal length)	
	Pulmonary artery	Bronchi
0–3 days hypoxia	76 $\pm$ 7*	11 $\pm$ 0.7*
3-d-old control	33 $\pm$ 4	6 $\pm$ 0.4
0–3 hypoxia + 3 d recovery	39 $\pm$ 6†‡	10 $\pm$ 0.9†
6-d-old control	22 $\pm$ 2	7 $\pm$ 0.8
3–14 days hypoxia	68 $\pm$ 9*	18 $\pm$ 1*
14-d-old control	33 $\pm$ 5	9 $\pm$ 0.9
3–14 hypoxia + 3 d recovery	51 $\pm$ 8†	20 $\pm$ 2†
17-d-old control	32 $\pm$ 6	10 $\pm$ 1
3–14 hypoxia + 6 d recovery	43 $\pm$ 8†‡	20 $\pm$ 2†
20-d-old control	29 $\pm$ 6	12 $\pm$ 1

Following hypoxia, pulmonary arterial and bronchial smooth muscle area increased two-fold compared to age-matched controls. During recovery, pulmonary arterial but not bronchial smooth muscle decreased significantly compared to the pulmonary hypertensive animals.

\*  $p < 0.001$  when hypoxic pulmonary hypertensive animals compared to age-matched controls; †  $p < 0.01$  when recovery animals compared to age-matched controls; ‡  $p < 0.03$  when recovery animals compared to hypoxia-exposed animals.

Number of animals studied is shown in Table 1.

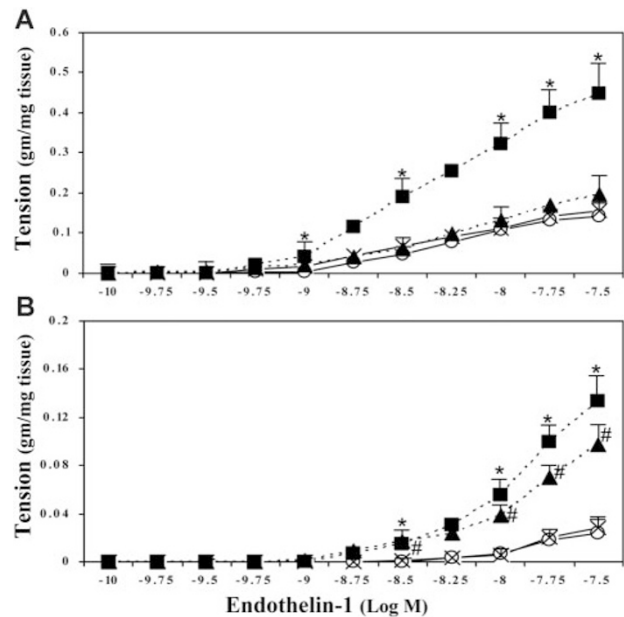
Values are mean  $\pm$  SEM.



**Figure 1.** Relationship between bronchial and pulmonary arterial smooth muscle area. Scatter plot of smooth muscle area ( $\mu\text{m}^2/\text{mm}$  luminal length) of the paired pulmonary arterial and bronchial rings from the 3 d control (black diamond) and the 0–3 d hypoxic pulmonary hypertensive (grey squares) animals. There was a significant correlation between bronchial and pulmonary arterial smooth muscle area  $r = 0.75$ ,  $p < 0.001$ .

**Organ chamber studies.** In both the pulmonary arteries and bronchi, the dose-dependent contractile response to ET-1 was increased three-fold in the 0–3 d hypoxic pulmonary hypertensive animals when compared with the 3 d control group,  $p < 0.008$  (Fig. 2). Following 3 d of recovery, the pulmonary arterial ET-1-induced contractile responses had decreased and were not significantly different from those of the 6 d control group (Fig. 2). By contrast, in the bronchi the ET-1-induced contractile responses had not decreased and were significantly greater than in the 6 d controls,  $p = 0.01$  (Fig. 2).

Following a period of postnatal adaptation and 3–14 d hypoxia, pulmonary arterial contractile response to ET-1 was



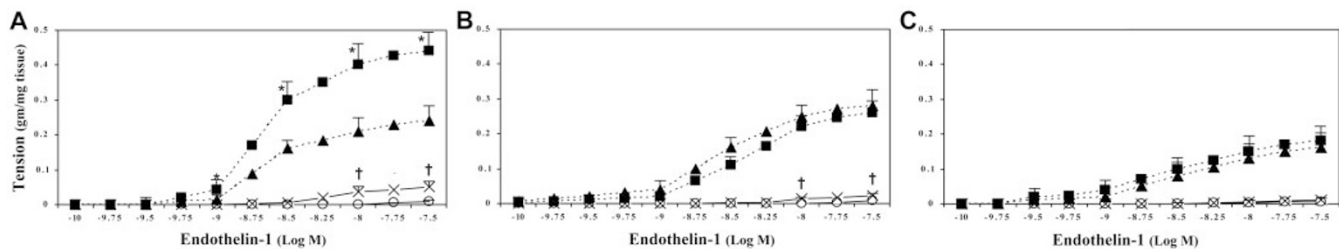
**Figure 2.** Contractile response curves for ET-1 after 3 d hypoxia and recovery. Contractile concentration-response curves for ET-1 in pulmonary arteries (A) and bronchial rings (B) in 0–3 d hypoxia (solid square), 3-d-old (cross), 0–3 hypoxia + 3 d recovery (solid triangle) and 6-d-old (open circle) pigs. The hypoxia-exposed tissue produced a greater contractile response compared with the 3-d-old controls (\*  $p < 0.05$ ). Following 3 d of recovery, the bronchial rings still had a significantly increased contractile response compared with the 6-d-old controls (#  $p < 0.05$ ). Number of animals studied is shown in Table 1. Values are mean  $\pm$  SEM.

increased two-fold, and the bronchial contractile response was increased five-fold,  $p < 0.01$  (Fig. 3). Following 3 d of recovery, the pulmonary arterial response had decreased and was not significantly different from the age-matched control, however the bronchial responses remained significantly elevated,  $p = 0.04$  (Fig. 3). After 6 d of recovery, the pulmonary arterial and bronchial contractile responses were similar to those of the age-matched controls (Fig. 3).

The ET-1-induced relaxant responses seen in the pulmonary arteries of control animals were absent in all hypoxia-exposed animals (Table 3). Following 0–3 d hypoxia and 3 d recovery, the relaxant response was not significantly different from that seen in the 3 or 6 d control animals (Table 3). However following 3–14 d hypoxia and 3 d recovery, the pulmonary arterial relaxant response remained significantly impaired compared with controls,  $p = 0.01$  (Table 3). After 6 d of recovery, the relaxant response was similar to that of 20-d-old controls (Table 3).

The relaxant response when present was completely inhibited by the ET-B receptor antagonist BQ788, and the nitric oxide synthase inhibitor, L-NAME, suggesting that the relaxation was due to release of nitric oxide *via* the ET-B receptor (Table 3). No ET-1-induced relaxation responses were seen in the bronchi.

Hypoxic pulmonary hypertension reduced the maximal norepinephrine-induced relaxant response in the pulmonary arteries 10-fold and 3-fold from the 0–3 d and 3–14 d hypoxic pulmonary hypertensive animals, respectively ( $p < 0.001$ ,  $p < 0.002$ ; Figs. 4 and 5).



**Figure 3.** Contractile response curves for ET-1 after 11 d hypoxia and recovery. End of Hypoxic exposure (A): Contractile concentration-response curves for ET-1 in 3–14 d hypoxia pulmonary artery rings (*solid square*) and bronchial rings (*cross*) compared with 14-d-old control pulmonary arteries (*solid triangle*) and bronchi (*open circle*), showing that the hypoxia-exposed tissue produced a two-fold greater contractile response compared with the controls. Three-day recovery (B): Following 3–14 hypoxia + 3 d recovery, the bronchial rings (*cross*) but not pulmonary arterial rings (*solid square*) still had a significantly increased contractile response compared with the 17 d pulmonary arterial (*solid triangle*) and bronchial (*open circle*) controls. Six-day recovery (C): Following 3–14 hypoxia + 6 d recovery, both the bronchial (*cross*) and the pulmonary arterial (*solid square*) contractile responses were similar to the 20 d pulmonary arterial (*solid triangle*) and bronchial (*open circle*) controls. \*  $p = 0.02$  when 3–14 d hypoxia pulmonary arteries compared with 14 d control; †  $p < 0.04$  when hypoxia-exposed and recovery bronchi compared with age-matched controls. Values are mean  $\pm$  SEM.

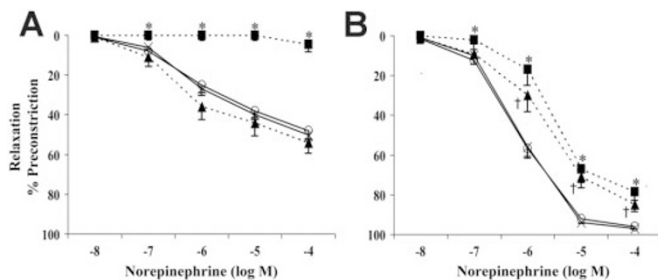
**Table 3.** Relaxation of pulmonary arteries to ET-1 and in the presence of BQ788 or L-NAME

	Endothelin-1		ET-1+BQ788	+L-NAME
	Maximum relaxation (% pre-contraction)	IC <sub>50</sub> (Log mol/l)	Maximum relaxation (% pre-contraction)	
0–3 days hypoxia	0*		0	0
3-d-old control	20.6 + 4	−9.4 + 0.2	0	0
0–3 hypoxia + 3 d recovery	30.4 + 6	−9.5 + 0.2	0	0
6-d-old control	15.1 + 3	−9.3 + 0.2	0	0
3–14 days hypoxia	0*		0	0
14-d-old control	26 + 5	−9.2 + 0.2	0	0
3–14 hypoxia + 3 d recovery	5 + 2†	−9.1 + 0.3	0	0
17-d-old control	25 + 5	−9.2 + 0.2	0	0
3–14 hypoxia + 6 d recovery	32 + 10	−9.1 + 0.2	0	0
20-d-old control	34 + 3	−9.0 + 0.2	0	0

Pulmonary hypertension, BQ788 and L-NAME completely inhibited the ET-1-induced relaxation. L-NAME, N<sup>w</sup>-nitro-L-arginine methyl ester; IC<sub>50</sub>, the ET-1 concentration producing half-maximal concentration.

\*  $p < 0.01$  when hypoxic pulmonary hypertensive animals compared to age-matched controls; †,  $p < 0.01$  when recovery animals compared to age-matched controls.

Values are mean + SEM.



**Figure 4.** Relaxation response curves for norepinephrine after 3 d hypoxia and recovery. Relaxation concentration-response curves for norepinephrine in pulmonary arteries (A) and bronchial rings (B) from 0–3 d hypoxia (*solid square*), 3-d-old control (*cross*), 0–3 hypoxia + 3 d recovery (*solid triangle*) and 6 d control (*open circle*) pigs. The hypoxia-exposed tissue produced an attenuated relaxation response compared with the 3 d controls (\*  $p < 0.01$ ). Following 3 d recovery, the bronchial rings still had a significantly impaired relaxation response compared with the 6 d controls (†  $p < 0.01$ ). Number of animals studied is shown in Table 1. Values are mean  $\pm$  SEM.

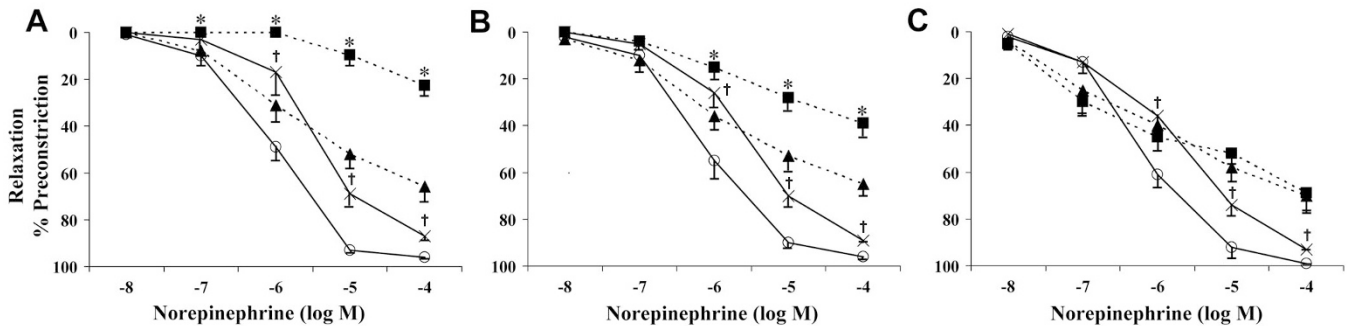
In the normal bronchi, norepinephrine-induced relaxation is much greater than in normal pulmonary arteries. The response was significantly reduced by 20% in 0–3 d hypoxia ( $p =$

0.005) and 10% in 3–14 d hypoxia groups ( $p = 0.01$ ; Figs. 4 and 5).

After 0–3 d hypoxia and 3 d recovery the pulmonary arterial norepinephrine-induced relaxant response was not significantly different from that in the 6-d-old control piglets (Fig. 4). By contrast, in the bronchi the norepinephrine-induced relaxation was still significantly impaired (Fig. 4). After 3–14 d hypoxia and 3 d recovery, both pulmonary arterial and bronchial norepinephrine relaxation responses remained significantly impaired compared with the control animals (Fig. 5). After 6 d recovery, the pulmonary arterial response was normal, but the bronchial response remained significantly impaired (Fig. 5).

## DISCUSSION

We hypothesized that exposure to chronic hypoxia from birth and from 3 d would lead to a parallel increase in the amount of airway and pulmonary arterial smooth muscle, parallel changes in reactivity to contractile and relaxant ago-



**Figure 5.** Relaxation response curves for norepinephrine after 11 d hypoxia and recovery. End of hypoxic exposure (A): At the end of 3–14 d hypoxic exposure, pulmonary artery rings (*solid square*) and bronchial rings (*cross*) produced a significantly attenuated relaxation response compared with the 14 d control pulmonary arteries (*solid triangle*) and bronchi (*open circle*). Three-day recovery (B): Following 3–14 hypoxia + 3 d recovery, the bronchial rings (*cross*) and pulmonary arterial rings (*solid square*) still had a significantly impaired relaxation response compared with the 17-d-old pulmonary arterial (*solid triangle*) and bronchial (*open circle*) controls. Six-day recovery (C): Following 3–14 hypoxia + 6 d recovery, the pulmonary arterial (*solid square*) relaxation response was similar to the 20 d pulmonary arteries (*solid triangle*) but the bronchial (*cross*) relaxation response was still significantly impaired compared with the 20 d bronchial (*open circle*) controls. \*  $p < 0.01$  when hypoxia-exposed and recovery pulmonary arteries compared with age-matched controls; †  $p < 0.045$  when hypoxia-exposed and recovery bronchi compared with age-matched controls. Values are mean  $\pm$  SEM.

nists and that the pattern of structural and functional recovery when animals were returned to room air would be similar. We demonstrated that although there were parallel changes in the airway and pulmonary artery during pulmonary hypertension, during recovery the regression of bronchial structural and functional abnormalities was slower than those of the arteries.

Exposure to hypoxia from birth and from 3 d led to pulmonary hypertension as judged by right ventricular hypertrophy and an increase in pulmonary artery smooth muscle area, as previously described (11). We also demonstrated an increase in the amount of muscle in the adjacent airways. On recovery in room air for 3 and 6 d, right ventricular and pulmonary arterial muscle regressed but was still excessive for age, as has previously been described (9,10), while there had been no regression of the bronchial smooth muscle.

Human infants and children with pulmonary hypertension are known to have an increase in both bronchial and pulmonary arterial smooth muscle in congenital heart disease (1) and bronchopulmonary dysplasia (12). Anatomically, the pulmonary arteries and the bronchi lie in close proximity to each other and it is conceivable that a mediator or neurotransmitter released locally, such as ET-1, might act on both vascular and bronchial smooth muscle to produce co-hypertrophy and co-constriction of bronchial and vascular smooth muscle. In human embryos, the innermost pulmonary arterial smooth muscle cells appear to derive from the bronchial smooth muscle cells of the adjacent airways from 38–98 d gestation (13). This similar embryological origin may also predispose the pulmonary arteries and bronchi to respond in a similar manner in pulmonary hypertension.

For the pharmacology experiments, we studied paired arteries and airways from intra-parenchymal division 3–4 pulmonary vasculature. Although in the adult lung, much of the resistance is in the microvascular segment, in the young human and in young rabbits, pulmonary vascular resistance has been shown to reside mainly in the arteries and veins, with a shift to the microvascular segment occurring with increasing age (14). Structural and pharmacological changes in response

to chronic hypoxia are similar in large muscular and resistance arteries (10,15).

In the present study, the ET-1-induced pulmonary arterial and bronchial contraction both increased at least two-fold during hypoxic exposure, and ET-B receptor mediated relaxation was absent in the pulmonary arteries. Using the same experimental model, Noguchi (16) found that in piglets exposed to hypoxia from birth, the plasma ET-1 was greater than normal, the ET-1 receptor binding increased in both pulmonary arteries and bronchi, and ET-B binding density decreased. Similarly, in lung biopsies of patients with pulmonary hypertension due to congenital heart disease, Lutz (17) found a higher Endothelin-A receptor density in pulmonary arteries and lung parenchyma and low ET-B receptor expression. In a porcine model of endotoxic shock bosentan, a dual ET receptor antagonist, completely abolished both the pulmonary hypertensive response and the increased airway resistance indicating that both the pulmonary arterial and airway changes were mediated by ET-1 (18). Similarly in human pulmonary artery rings taken at lobectomy, the responsiveness to ET-1 was increased in patients with reversible airways constriction detected on preoperative pulmonary function testing (19), again supporting the concept of co-constriction of bronchial and adjacent pulmonary arterial smooth muscle.

We have previously shown that the pulmonary arterial relaxation response to norepinephrine in piglets greater than 24 h of age was largely mediated by nitric oxide release from the endothelium and that following exposure to hypoxia from birth for 3 d, the relaxation response was absent or severely attenuated (20). We have now shown that norepinephrine-induced relaxation is also significantly decreased in the airways. Hislop (21) found that the normal postnatal increase in  $\beta$  adrenoceptor density was attenuated in both pulmonary arterial endothelium and bronchial epithelium in piglets exposed to hypoxia from birth for 3 d. Similarly lymphocyte  $\beta$  adrenoceptor density was attenuated by 27% and platelet  $\alpha$  adrenoceptor density was increased in children with congenital heart disease with left-to-right shunts and pulmonary

hypertension (22). This reduction of  $\beta$  adrenoceptors in pulmonary hypertension, in addition to impaired endothelial nitric oxide release (9), may explain the reduced adrenoceptor stimulated pulmonary arterial and bronchorelaxation we observed in our study.

Recovery in room air even for a comparatively short time led to changes in structure and function. In some instances recovery was rapid, as demonstrated by ET-1-induced nitric oxide release, while in others it was slower as in the reversal of right ventricular hypertrophy, and regression of bronchial structural and functional abnormalities. The implications of these findings are potentially clinically important, as some effects of pulmonary hypertension persist for long periods and may affect how patients react to subsequent illnesses and treatment. Schulz-Neick (23) found that persistence of a raised pulmonary vascular resistance following surgical repair of congenital heart defects resulted in significantly longer periods of postoperative mechanical ventilation.

In our study, pulmonary arterial smooth muscle had decreased after only 3 and 6 d recovery in normoxia, but bronchial smooth muscle mass was still similar to that seen at the end of hypoxic exposure. The lack of regression of bronchial smooth muscle was associated with persistence of the enhanced contractile response to ET-1 and the impaired relaxant response to norepinephrine. The clinical significance of these observations is uncertain but they may help explain the much higher prevalence of asthmatic symptoms and abnormal bronchial reactivity seen in six to 11-y-old survivors of neonatal meconium aspiration syndrome and pulmonary hypertension (24), although the respiratory management may also have influenced outcome in these patients (4). This suggests however that the bronchial abnormalities can persist for many years and further studies are required to examine the effects of longer periods of recovery.

In contrast to the bronchi, pulmonary arterial ET-1 mediated contraction recovered rapidly, having returned to levels similar to that in age-matched controls after 3 d of normoxic recovery in both pulmonary hypertension groups despite the muscle still being increased. In the same porcine model of neo-natal chronic hypoxia, the binding density of Endothelin-A receptors decreased and that of ET-B receptors increased to levels normal for age after 3 d recovery (16). Recovery of pulmonary arterial ET-1 and norepinephrine relaxation response was age-dependent. Recovery of relaxation response was rapid in the young piglets exposed to hypoxia from birth, being normal after 3 d. However, in the older piglets exposed to hypoxia for 3–14 d after a period of postnatal adaptation, the relaxation response was still impaired after 3 d, but was normal after 6 d of recovery. This may be due to either the age of the piglets or the longer duration of exposure to hypoxia.

In summary, we present evidence of co-hypertrophy and co-constriction of bronchial and vascular smooth muscle in an experimental model of neo-natal pulmonary hypertension. Arterial contractile and relaxant responses recovered rapidly,

with a slower recovery of the arterial wall structure and right ventricular hypertrophy. The amount of bronchial smooth muscle and its responsiveness recovered the least. These findings support and may help explain clinical reports of persistent compromise of pulmonary vascular and airway function in those who survive neo-natal pulmonary hypertension. Longer follow-up experimental studies are indicated.

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