Postnatal changes in pulmonary vein responses to endothelin-1 in the normal and chronically hypoxic lung

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Schindler MB, Hislop AA, Haworth SG. Postnatal changes in pulmonary vein responses to endothelin-1 in the normal and chronically hypoxic lung. Am J Physiol Lung Cell Mol Physiol 292: L1273–L1279, 2007. First published January 26, 2007; doi:10.1152/ajplung.00173.2006.—The response of pulmonary arteries to endothelin-1 (ET-1) changes with age in normal pigs and is abnormal in pulmonary hypertension. The purpose of this study was to determine if the same is true of the pulmonary veins. We studied the wall structure and functional response to ET-1 in pulmonary veins from normal pigs from fetal life to adulthood and from pigs subjected to chronic hypobaric hypoxia either from birth for 3 days or from 3 to 6 days of age. In isolated normal veins, the contractile response decreased by 40% between late fetal life and 14 days of age with a concomitant twofold increase in endothelium-dependent relaxant response. The ETA antagonist BQ-123 reduced the contractile response significantly more in newborn than older animals, whereas the ET-B antagonist BQ-788 had no effect in fetal animals and maximally increased contraction at 14 days of age. Hypoxic exposure significantly increased pulmonary vein smooth muscle area and contractile response to ET-1. The relaxation response was impaired following hypoxic exposure from birth but not from 3 to 6 days of age. The ETA antagonist BQ-123 decreased contractile and increased dilator responses significantly more than in age-matched controls. Thus pulmonary veins show age-related changes similar to those seen in the pulmonary arteries with a decrease in ETA-mediated contractile and increase in ET-B-mediated relaxant response with age. Contractile response was also increased in hypoxia as in the arteries. This study suggests that pulmonary veins are involved in postnatal adaptation and the pathogenesis of pulmonary hypertension.

A NUMBER OF ANIMAL AND HUMAN studies suggest a role for endothelin-1 (ET-1) in adaptation to extrauterine life (8, 21) and in the pathogenesis of pulmonary hypertension (18). Endo et al. (8) found plasma ET-1 concentrations to be threefold higher at birth compared with 5 or 30 days in healthy human neonates, and similar changes have been described in piglets (21). ET-1 blood levels are elevated in patients with pulmonary hypertension and decrease following resolution in persistent pulmonary hypertension of the newborn (24), pulmonary hypertension due to congenital heart disease (18), and pulmonary hypertension due to acute lung injury (19).

Experimental studies have shown that the contractile response to ET-1 decreases with increasing age in rabbit pulmonary arteries (7). The effect of age on the pharmacological responses of the porcine pulmonary veins to ET-1 has not been reported; however, as the threshold for ET-1-induced contraction is lower in pulmonary veins than in pulmonary arteries in the fetal lamb (29) and human (4), it is likely that the veins may also exhibit developmental changes in response to ET-1.

In hypoxia-induced pulmonary hypertension in piglets, we (25) have previously found that the pulmonary arterial contractile response to ET-1 is increased and pulmonary arterial smooth muscle area is also increased. Parallel morphometric changes, with an increase in intimal, muscle, and adventitial thickness, occur in the human pulmonary veins and arteries (28). These changes are most obvious in patients with pulmonary arterial hypertension, fibrosing mediastinitis, and mitral stenosis (5, 28). Similarly, pulmonary veins from newborn piglets exposed to chronic hypoxia had a decreased relaxant response to acetylcholine, as is seen in the pulmonary arteries (1), again suggesting that the pulmonary veins may be involved in the pathogenesis of pulmonary hypertension and thus may also exhibit changes in response to ET-1.

To further evaluate the role of pulmonary veins in postnatal adaptation and in the pathogenesis of pulmonary hypertension, the structure of the vein wall and the responses to ET-1 at baseline tone and at elevated tone were studied in isolated porcine pulmonary vein rings from fetal life to adult life and in pulmonary veins from animals with hypoxia-induced pulmonary hypertension. The newborn piglet has been used extensively to study adaptation of the pulmonary circulation to extrauterine life (13) in the normal and pulmonary hypertensive (14) animal.

METHODS

Pulmonary veins were studied from a total of 61 Large White pigs, which included fetal pigs (5 days preterm), 2-h-, 3-day-, 6-day-, and 14-day-old animals and adult pigs (n = 6 at each age group). The fetal pigs were obtained by humanely killing the mother and then removing the piglets before the onset of breathing. Two-hour, 3-, 6-, and 14-day-old piglets were delivered normally at term and left with the mother until killed with an overdose of pentobarbital sodium. Fetal animals were killed in the same way. Normal adult tissue was obtained from a local abattoir.

To study the effect of pulmonary hypertension immediately after birth, newborn piglets (2-h-old) were placed in a hypobaric chamber (50.8-kPa, FIO2 0.096) and cared for in a hypoxic environment for 3 days. A second group of normal 3-day-old piglets were placed in the hypobaric chamber for 3 days to examine the effect of the hypoxia after a period of normal postnatal adaptation. The animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH- Publication No. 80-23, Revised 1996). The study was approved by the Animal Ethics Committee of the Institute of Child Health. The animals exposed to hypoxia were cyanosed on emerging from the chamber. In all of the hypoxic animals, the ventricular heart weight

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Fig. 1. Bar graph of smooth muscle area (in \(\mu m^2/\mu m\) luminal length) of the pulmonary veins, showing no change with age. Smooth muscle area was increased in hypoxic pulmonary hypertension. *\(P = 0.03\) when birth to 3-day-old hypoxic pulmonary hypertension animals were compared with 3-day-old controls; #\(P = 0.01\) when 3- to 6-day-old hypoxic pulmonary hypertension animals were compared with 6-day-old controls. Number of animals studied is shown in Table 1. Values are means ± SE.

Pulmonary vein wall structure. Morphometric analysis showed that the pulmonary vein smooth muscle area related to lumen perimeter did not change with age, but it was increased by 30–47% in both groups of pulmonary hypertensive animals (\(P = 0.01\) and 0.03 in 0–3 days and 3–6 days hypoxia, respectively; Fig. 1).

Response to ET-1 in veins from normal animals. There was a concentration-dependent increase in tone in response to ET-1 in veins from all animals. The \(EC_{50}\) for ET-1 was unaffected by 30–47% in both groups of pulmonary hypertensive animals (\(P < 0.01\); Figs. 2 and 3). At low concentrations of ET-1 (10^{-11}–10^{-9} M), a predominantly relaxant response was seen. At intermediate doses (10^{-8.5}–10^{-8} M),
there was a biphasic response to ET-1 with an initial small relaxant response, which was followed by a contraction. At higher concentrations of ET-1 (10^{-8.5}–10^{-7.5} M), only a contractile response was seen in all animals. The fetal and newborn veins showed no relaxant response in 29% of rings, and when a relaxant response was present, it was 40% less than that seen in the 3- and 6-day-old animals (P = 0.01; Table 1). At higher concentrations (10^{-8.5}–10^{-7.5} M), a predominantly contractile response
was again seen with the fetal tissue producing a greater contractile response than the adult tissue (Fig. 4).

In the presence of the ETA receptor antagonist BQ-123, the ET-1 contractile response from baseline tone was reduced significantly in pulmonary veins from the fetal (45 \%/H11008\%), and newborn (42 \%/H11009\%) animals (P<0.01). BQ-123 had less effect in vessels from 14-day-old (11 \%/H1104\%) and adult (21 \%/H1106\%) animals, where the reduction was not statistically significant (Fig. 2). The ET-B receptor antagonist BQ-788 significantly increased the contractile response at all ages except in veins from fetal pigs, where the contractile response was unchanged (Fig. 2). The largest percentage increase in contractile response to BQ-788 was seen in veins from the 6- and 14-day-old animals. The effect of endothelial removal on ET-1 contractile response was similar to the response seen with BQ-788, with loss of the relaxant response and increased contractile response, but the increase in contractile response only reached statistical significance in the 6- and 14-day-old animals (Table 2). L-NAME also significantly increased ET-1 contractile response in the 6- and 14-day-old animals (Table 2).

This suggests that BQ-788 was predominantly acting on endothelial ET-B receptors, reducing the relaxation response while the main contractile effect of ET-1 was via the ETA receptor.

In the precontracted rings, the ETA antagonist BQ-123 significantly decreased the contractile response only in the fetal and newborn veins (P<0.03; Fig. 4) and increased the ET-1-induced relaxation response when all age groups were analyzed together (P=0.01); however, it did not significantly increase the relaxation response in any individual age group (Table 1, Fig. 5). In the fetal and newborn groups, the addition of BQ-123 allowed a relaxation response in all animals, even when it had been absent in 29% of control rings from these age groups.

The ET-B receptor antagonist BQ-788 increased the contractile response only in older animals as in the vessels studied at baseline. It completely inhibited the ET-1-induced relaxation, confirming that the relaxation was ET-B receptor-mediated. The relaxant response of the pulmonary veins was also completely abolished following the addition of L-NAME and removal of the endothelium in all age groups, suggesting that the relaxation was due to release of nitric oxide from the endothelium (Table 1).

The effect of hypoxic pulmonary hypertension on the response to ET-1. The baseline ET-1 contractile response of the pulmonary veins from animals exposed to hypoxia was greater than that of age-matched normoxic controls (Fig. 4). The addition of BQ-123 (●) had a larger effect in the fetal and newborn animals. The effect of the addition of BQ-788 (▲) was greatest in the 14-day-old animals; *P<0.05 compared with baseline ET-1 response (○). Number of animals studied is shown in Table 1. Values are means ± SE.

### Table 1. Maximum relaxation response to endothelin-1 in pulmonary veins from normal and pulmonary hypertensive animals

<table>
<thead>
<tr>
<th></th>
<th>Maximum Relaxation, % Precontraction</th>
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<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Fetal</td>
<td></td>
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<tr>
<td>Newborn</td>
<td></td>
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<tr>
<td>3-day-old</td>
<td></td>
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<tr>
<td>6-day-old</td>
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<tr>
<td>14-day-old</td>
<td></td>
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<tr>
<td>Adult</td>
<td></td>
</tr>
<tr>
<td>0–3-day-old</td>
<td></td>
</tr>
<tr>
<td>3–6-day-old</td>
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</table>

Values are means ± SE. n, number of animals; E−, vein rings in which the endothelium had been removed. *Significantly different from 3-day-old animals; †significantly different from age-matched 3-day-old normoxic controls; §significantly different from endothelin-1 maximum relaxation without BQ-123.
The ET-B receptor antagonist BQ-788 did not alter pulmonary vein ET-1 contractile response in all the hypoxic animals to a significantly greater extent than was seen in the age-matched controls (Fig. 3). In those subjected to hypoxia from birth, BQ-123 reduced ET-1-induced contractile response by 70 ± 10% compared with a reduction of 49 ± 5% in the 3-day-old control animals (P = 0.05). In the animals who were hypoxic from 3 to 6 days of age, BQ-123 reduced ET-1-induced contraction by 53 ± 16% compared with only 11 ± 3% in the 6-day-old control animals (P = 0.04).

The addition of the ET_A receptor antagonist BQ-123 reduced ET-1-induced contractile responses in all the hypoxic animals to a significantly greater extent than was seen in the age-matched controls (Fig. 3). In those subjected to hypoxia from birth, BQ-123 reduced ET-1-induced contractile response by 70 ± 10% compared with a reduction of 49 ± 5% in the 3-day-old control animals (P = 0.05). In the animals who were hypoxic from 3 to 6 days of age, BQ-123 reduced ET-1-induced contraction by 53 ± 16% compared with only 11 ± 3% in the 6-day-old control animals (P = 0.04).

The ET-B receptor antagonist BQ-788 did not alter pulmonary vein ET-1 contractile response from animals who were hypoxic from birth, unlike the veins from 3-day-old control animals where BQ-788 significantly increased the contractile response by 63 ± 12% (P = 0.005; Fig. 3). By contrast, BQ-788 significantly increased ET-1-induced contractile response both in animals exposed to hypoxia from 3 to 6 days of age and in the 6-day-old controls (P = 0.005; Fig. 3).

In the precontracted rings, the ET-1-induced relaxation was increased 2.5-fold by the addition of the ET_A receptor antagonist BQ-123 (P < 0.01) in the animals exposed to hypoxia from birth. A relaxation was seen even when no relaxation response was seen without BQ-123. By contrast, BQ-123 did not significantly alter the relaxation response in the animals exposed to hypoxia from 3 to 6 days or in the 3- and 6-day-old control animals (Table 1, Fig. 5). The ET-1-induced relaxation in the hypoxic animals was completely abolished by BQ-788, L-NAME, and removal of the endothelium.

**DISCUSSION**

Previous studies on the pig lung by our group have shown age- (16) and pulmonary hypertension-related (23, 25) changes in the ET-1 responsiveness and receptor binding to pulmonary vessels.

<table>
<thead>
<tr>
<th>Pulmonary Vein</th>
<th>n</th>
<th>E+, g/mg Tissue</th>
<th>E−, g/mg Tissue</th>
<th>+l-NAME, g/mg Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal</td>
<td>6</td>
<td>0.81 ± 0.09</td>
<td>0.79 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td>8</td>
<td>0.87 ± 0.18</td>
<td>0.81 ± 0.19</td>
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</tr>
<tr>
<td>3-day-old</td>
<td>8</td>
<td>0.82 ± 0.11</td>
<td>0.89 ± 0.14</td>
<td>0.98 ± 0.15</td>
</tr>
<tr>
<td>6-day-old</td>
<td>7</td>
<td>0.61 ± 0.06</td>
<td>0.78 ± 0.10*</td>
<td>0.94 ± 0.16*</td>
</tr>
<tr>
<td>14-day-old</td>
<td>9</td>
<td>0.48 ± 0.05</td>
<td>0.86 ± 0.11*</td>
<td>0.82 ± 0.14*</td>
</tr>
<tr>
<td>Adult</td>
<td>8</td>
<td>0.28 ± 0.04</td>
<td>0.39 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>0–3-day-old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7</td>
<td>1.29 ± 0.12</td>
<td>1.21 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>3–6-day-old</td>
<td>6</td>
<td>0.98 ± 0.06</td>
<td>1.17 ± 0.12</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. n, number of animals; E−, vein rings in which the endothelium had been removed. *Significantly different from endothelium intact (E+) pulmonary vein rings.

Fig. 5. Graph showing only the relaxation responses to ET-1 in pulmonary vein rings from animals exposed to hypoxia from birth for 3 days (top). An impaired relaxation response was seen in the hypoxic pulmonary hypertension animals (●) compared with the 3-day-old controls (○). The addition of BQ-123 significantly improved the ET-1 relaxation response in the hypoxic animals (●) but not in the 3-day-old controls (○). Bottom: similar relaxation-only concentration-response curves for ET-1 in pulmonary vein rings from animals exposed to hypoxia from 3 to 6 days of age, showing relaxation response is unchanged in the hypoxic pulmonary hypertension animals (●) compared with the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●). The addition of BQ-123 did not significantly change the ET-1 relaxation response in the hypoxic animals (●) or in the 6-day-old controls (●).
arteries. The present paper reports the response to ET-1 in porcine isolated pulmonary vein rings during adaptation to extraterine life and in hypoxic pulmonary hypertension. In the normal, we found a postnatal reduction in the contractile response to ET-1 via ET\textsubscript{A} receptors and a concomitant postnatal increase in ET-B receptor-mediated relaxation. These changes are similar to those found in the pulmonary arteries of rabbits, where contractile response to ET-1 decreased and response to the ET-B agonist sarafotoxin S6c increased between birth and 7 days of age (7). In the human lung, expression of the ET-B receptor was lower in early gestation than soon after birth (20). Norepinephrine-induced contractile responses were also significantly higher in the fetal compared with 14-day-old and adult porcine pulmonary veins and arteries (26). Thus the veins may be important in maintaining high pulmonary vascular resistance in utero and in the reduction of resistance after birth.

We found no age-related difference in pulmonary vein smooth muscle area in our study to explain the change in responsiveness. No significant difference in percentage of medial thickness of the pulmonary veins with age was found in newborn and 1-month-old lambs (22), and in human lungs, the amount of pulmonary vein muscle was similar in a stillborn-term infant and 3- and 10-year-old children (15).

The greater contractile response seen at higher concentrations of ET-1 in the fetal and newborn veins was probably associated with the presence of a relatively larger number of ET\textsubscript{A} receptors since the ET\textsubscript{A} receptor antagonist BQ-123 caused a greater decrease in contraction in these animals than in 14-day-old and adult animals. Binding studies have shown that the proportion of ET\textsubscript{A} receptors in the pulmonary veins decreases with age (16). Conversely, the ET-B receptor antagonist BQ-788 had no effect on contraction in the fetal animals and had a maximum effect in the 14-day-old animals, suggesting that the influence of the ET-B receptor increased with age. The ET-B antagonist had a similar effect on contraction as removal of the endothelium and the addition of L-NAME in the 6- and 14-day-old veins, suggesting that ET-B receptors on the endothelium played a significant role in the response of these veins.

The pulmonary vein relaxation, mediated via the ET-B receptor, was seen at lower concentrations of ET-1 both at basal and elevated tone and was due to nitric oxide release from the endothelium. Arrigoni and colleagues (1) observed that the relaxation in response to acetylcholine, which also occurs by release of nitric oxide from the endothelium, was greater in 14-day-old porcine pulmonary veins compared with fetal veins. Similarly, Gao and Raj (9) found that acetylcholine is 10-fold more potent in relaxing pulmonary veins of newborn than fetal lambs. The postnatal increase in relaxation response we observed may thus be due to an increase in available nitric oxide from the endothelium after birth (2) as well as the increase in ET-B receptor density previously described (16, 23).

In this study, we report that during the development of postnatal hypoxia-induced pulmonary hypertension in piglets there is an increase in the amount of muscle in the vein wall and an increased contractile response to ET-1, as is seen in hypertensive pulmonary arteries (14, 25). Dingemans and Wagenvoort (6) demonstrated an increase in muscle in both pulmonary veins and arteries in adult rats following exposure to chronic hypoxia. Thickening or muscularization of small pulmonary veins in addition to the pulmonary arteries has been reported in human patients with pulmonary hypertension associated with chronic bronchitis, emphysema, high altitude (28), and ventricular septal defect (12).

The increased ET-1-induced contractile response in the pulmonary veins following hypoxic exposure in our animals was probably due to an increase in ET\textsubscript{A} receptors as was seen in the pulmonary arteries (25). The ET\textsubscript{A} receptor antagonist BQ-123 reduced the ET-1-induced contractile response in the hypoxia-exposed animals by a significantly greater extent than was seen in the age-matched controls. Noguchi et al. (23) found that, in piglets exposed to hypoxia from birth, plasma ET-1 was increased for age, and there was an increase in the density of ET-1 and ET\textsubscript{A} receptor binding in pulmonary veins with no change in ET-B receptors. Similarly, immunoreactivity and mRNA expression of Big ET-1 and ET\textsubscript{A} receptors was significantly increased in adult rat pulmonary veins after exposure to hypoxia (27). This combined data suggests that pulmonary veins may be involved in the pathogenesis of pulmonary hypertension in animals and in humans. As the ET\textsubscript{A} receptor antagonist BQ-123 reduced ET-1-induced contractile response and increased the relaxation response in our hypoxic veins, a selective ET\textsubscript{A} antagonist may be a useful therapy to reduce the effects of pulmonary hypertension.

We found ET-1-induced pulmonary venous relaxation was significantly impaired and remained at the fetal and newborn level in animals exposed to chronic hypoxia from birth. Arrigoni et al. (1) found that acetylcholine-induced relaxation was also significantly reduced in porcine pulmonary veins and arteries following hypoxia from birth for 3 days. In our study, the presence of an ET\textsubscript{A} receptor antagonist returned relaxation in the pulmonary vein to a near normal level for age. Thus the reduced relaxation we observed was partly due to the increase in ET\textsubscript{A}-mediated contraction and partly a failure in the development of endothelium-dependant relaxation.

Relaxation to ET-1 was normal for age in animals exposed to hypoxia after a 3-day period of postnatal adaptation. The relaxation occurred, as in the normal, via ET-B receptors and release of nitric oxide from the endothelium. Similarly, in piglets exposed to hypoxia from 24 h of age for 3 or 14 days, pulmonary vein endothelial and inducible nitric oxide synthase (NOS) was unaltered by hypoxic pulmonary hypertension (3). ET-B receptor binding density was not altered by chronic hypoxia for this period of time (23), and exposure to hypoxia for a longer period (days 3–14) did not affect the pulmonary venous relaxation response to norepinephrine (26). This suggests that hypoxia, from birth, may delay the normal maturaion of nitric oxide-mediated pulmonary vein relaxation. However, once the normal postnatal increase in pulmonary vein relaxation has occurred, the relaxation appears to be largely unaffected by hypoxic exposure. This suggests that the nitric oxide pathway in venous endothelial cells is not as dysfunctional as in the pulmonary arterial endothelial cells, where after exposure to hypoxia for 3 to 6 days, the relaxation response to ET-1 was significantly reduced (10) or absent (25). The increase in contraction is also less severe in veins than arteries, where hypoxic exposure from 0 to 3 days increased the contractile response of the pulmonary arteries threefold (25). The thinner wall and more consistent presence of NOS in the veins may explain this difference (17).
In conclusion, as has been reported for the pulmonary arteries, there is a postnatal decrease in ET-1-induced contractile response and an increase in ET-1 dilator response in the pulmonary veins. Following the development of hypoxic pulmonary hypertension, pulmonary vein smooth muscle area and ET-1-induced contractile response were increased, and the relaxation was impaired if hypoxic exposure occurred immediately after birth but was less affected than has been reported in the pulmonary arteries. This suggests that the pulmonary veins are important in postnatal adaptation and reduction of pulmonary vascular resistance and are involved in the pathogenesis of pulmonary hypertension.

GRANTS
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REFERENCES