Newborn intrapulmonary veins are more reactive than arteries in normal and hypertensive piglets

F. I. ARRIGONI, A. A. HISLOP, S. G. HAWORTH, AND J. A. MITCHELL

Arrigoni, F. I., A. A. Hislop, S. G. Haworth, and J. A. Mitchell. Newborn intrapulmonary veins are more reactive than arteries in normal and hypertensive piglets. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L887–L892, 1999.—The reactivity of pulmonary veins during adaptation from pre- to postnatal life is not well characterized. With an in vitro organ bath technique, the responses to the contractile and relaxant agonists U-46619 (10^{-10} to 3 \times 10^{-6} M) and acetylcholine (10^{-9} to 10^{-4} M) were compared in adjacent conduit pulmonary vein and artery rings from 66 piglets aged 1 wk preterm to 14 days of postnatal life and from adult tissue.

Five additional piglets were made hypertensive by exposure to chronic hypoxia for 3 days after birth. Both arteries and veins showed smaller contractile and relaxant responses before birth than after. By 5 min after birth, the contraction by arteries and relaxation by veins had increased (P < 0.05). By 3 days of age, arterial relaxation increased, but in all animals, venous relaxation exceeded that in arteries (P < 0.05). Veins contracted more than arteries in animals aged 3–14 days. Neonatal hypoxia diminished the responses to both agonists in the veins (P < 0.05), whereas the response in the arteries remained similar to that in the normal newborn. We speculate that veins may be more important in postnatal adaptation than previously suggested.

METHODS

Lungs from normoxic animals. Isolated intrapulmonary arteries and veins were studied from healthy, nonbreathing fetal piglets (1 wk preterm), newborn animals (<5 min), piglets aged 24 h and 3, 6, and 14 days, and adult pigs. A total of 71 animals were studied, with at least 6 animals in each age group. Immature piglets were killed with an overdose of pentobarbital sodium (100 mg/kg). Tissue from adult pigs was obtained from a local abattoir. The lungs were removed immediately after death and transported to the laboratory in ice-cold Krebs-Henseleit solution within 2 h.

Lungs from hypoxic animals. Newborn pigs (n = 5) were placed in a hypobaric chamber for 3 days. The internal temperature was maintained at 29°C, and the air pressure was maintained at 50.8 kPa. The pressure was returned to normal while the piglets were cleaned and fed three times daily for a maximum of 20 min. The animals had a continuous supply of milk and were also tube fed three times daily. The animals placed in these chambers developed pulmonary hypertension with right ventricular hypertrophy and a systemic arterial oxygen saturation of 71 ± 5% (23). All animals received humane care in compliance with the British Home Office Regulations and with the Principles of Laboratory Animal Care formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892).

Preparation of tissue. The upper lobes of the lungs were removed for dissection of the axial intrapulmonary arteries and veins. Tissue from these animals was also used in other experiments within the department. Once adjacent vessels were cleared of connective tissue, rings each 2–3 mm in length were cut from the axial vessel after the first branch. These had a diameter of 1–2 mm in vessels from all the young of adjacent pulmonary arteries and veins in piglets from fetal to adult life. Because changes in endothelium-dependent relaxation are implicated in pulmonary adaptation, we also compared the relaxant responses of the arteries and veins to the agonist acetylcholine.

In a proportion of newborn infants, the adaptive mechanisms fail and pulmonary vascular resistance remains high, resulting in persistent pulmonary hypertension. Improved understanding of the pathogenesis of this disorder has important implications for its prevention and treatment (8). Exposing piglets to hypobaric hypoxia can mimic the clinical disorder of neonatal pulmonary hypertension (23). How hypoxia affects the responsiveness of pulmonary veins in this and other models of persistent pulmonary hypertension of the newborn has not been studied. Thus we assessed the contractile and relaxant responses of adjacent pulmonary arteries and veins from newborn piglets with hypoxia-induced pulmonary hypertension.

DURING FETAL LIFE, oxygen is derived from the placental circulation, and right ventricular output is mainly diverted away from the lungs through the ductus arteriosus to the aorta. This fetal diversion is facilitated by a high pulmonary vascular resistance, and at birth, when the lung replaces the placenta as the organ of gas exchange, adaptive processes lead to a decrease in pulmonary vascular resistance, leading to an increase in pulmonary blood flow.

Previous studies from our group (17, 23) and others (26) have shown that pulmonary arteries taken from piglets and sheep in the newborn period react differently to vasoconstrictor agents than adult vessels. Most studies to date have concentrated on the changes in relaxant responses in arteries taking place between birth and adulthood, with only a few observing the changes in contractile responses with age. Also, with few notable exceptions, the role that the pulmonary veins might play in adaptation has received relatively little attention (5, 21). The purpose of the present study was to compare the contractile responses
animals, and there was no direct correlation between an increase in diameter or the weight of these vessels and the age of the animals. In the adult animals, vessels from the same position were studied, and these measured 3–4 mm in diameter. Care was taken throughout the dissection to maintain an intact endothelial layer. Each ring of artery or vein was then mounted between two wires, one fixed and the other attached to an isometric force transducer, in 5-ml organ baths containing gassed (95% O₂-5% CO₂) and warmed (37°C) Krebs-Henseleit solution. The output was to a Grass model 7 polygraph. In preliminary experiments, after an equilibration period of 1 h, length-tension responses were determined after progressive passive and active (125 mM KCl) stimulation. From these studies, an optimum resting tension of 300 mg for veins was determined in all age groups except adult, where the optimum resting tension was 1 g (n = 4–5 animals/age group). For the arteries, 1 g of tension was found to be in the optimum range of resting tension in vessels from fetal through adult age groups (data not shown). For all further experiments, baseline tensions of 1 g for arteries from all age groups and adult veins and 300 mg for veins from all younger animals were applied progressively before the experiment was started.

Protocol for measurement of vascular reactivity. After equilibration, the vessels were stimulated to contract by adding a solution of 125 mM KCl. Once a stable contraction had been obtained, KC1 was washed out and the chambers were filled with Krebs-Henseleit solution. The reproducibility of contraction was confirmed by performing a second stimulation with KC1. After reequilibration in Krebs-Henseleit solution and adjustment of any drift from the baseline tension, cumulative concentration-response curves were constructed. Once a maximum response was achieved, we assessed the presence of an intact responsive endothelium by the cumulative addition of acetylcholine (10⁻⁶ to 3 x 10⁻⁴ M). At the end of each experiment, to test the integrity of the relaxant response, vessels were maximally dilated by the addition of papaverine (10⁻⁵ M) to the organ bath.

Materials. U-46619, acetylcholine, and papaverine (6,7-dimethoxy-1-teravatrylisoquinoline) were purchased from Sigma (Poole, UK). U-46619 was made up in ethanol, and dimethoxyl-1-veratrylisoquinoline) were purchased from Sigma. NaCl, 4.9 mM KCl, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 1.2 mM MgSO₄, 11.1 mM glucose, and 2.5 mM CaCl₂.

Data analysis. Data are expressed as an increase in tension above the resting tension, with any drift accounted for. Relaxation is expressed as percent decrease in tension from a maximum induced by U-46619 to a minimum induced by papaverine. Concentration-response curves were compared with two-way ANOVA (2-way repeated-measures test). E_max (effective maximum) for U-46619 and acetylcholine and EC₅₀ for U-46619 were compared with one-way ANOVA, with Bonferroni correction as a post hoc test. Comparison between arteries and veins was analyzed with an unpaired t-test (GraphPad Prism, GraphPad Software). Comparison between KC1 and U-46619 contractions was analyzed with a paired t-test. A P value of <0.05 was assumed to be significant.

RESULTS

Contractile responses of arteries and veins. Intrapulmonary arteries and veins from all age groups contracted with a single challenge of KCl (125 mM; Table 1) and with U-46619 (1 x 10⁻¹¹ to 3 x 10⁻⁶ M) in a concentration-dependent manner (Fig. 1). For each case, EC₅₀ and E_max values were calculated for U-46619 (Table 1). The maximum contractile responses to U-46619 were greater than those produced by KC1 at all ages. This difference was significant in arteries (P < 0.05; paired nonparametric t-test) from fetal, newborn, and 14-day-old animals and in veins from all but the fetal animals (Table 1).

We observed differences in the abilities of arteries and veins from different age groups to contract to U-46619 and KC1 (Table 1, Fig. 1). In response to both agonists, the smallest contraction was seen in the fetal preparations and the largest contraction occurred in the adult (Table 1). In the pulmonary artery rings, the concentration-response curve to U-46619 increased significantly (by two-way ANOVA) above the fetal level within 5 min of birth. The response then decreased so that by 3 days of age, it was similar to that observed in the fetus. By 6 days of age, the response was greater than the fetal level, and there was a further marked increase in the contractile response of the arteries between 14 days and adult life (P < 0.05 by two-way ANOVA). In response to KC1, arteries from fetal lungs contracted less effectively than vessels from any other age group (P < 0.05 compared with 14-day and adult lungs by one-way ANOVA; Table 1). Thus, for both agonists, birth was observed to cause large increases in the arterial contractile response.

Table 1. Contractile responses of arteries and veins to KCl and U-46619

<table>
<thead>
<tr>
<th>Age</th>
<th>125 mM KCl</th>
<th>E_max for U-46619</th>
<th>Log EC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arteries, mg</td>
<td>Veins, mg</td>
<td>Arteries, mg</td>
</tr>
<tr>
<td>Fetus</td>
<td>250±32</td>
<td>399±59*</td>
<td>424±32</td>
</tr>
<tr>
<td>Newborn</td>
<td>519±97</td>
<td>548±119</td>
<td>778±134†</td>
</tr>
<tr>
<td>24 Hour</td>
<td>439±80</td>
<td>558±146</td>
<td>568±105</td>
</tr>
<tr>
<td>3 Day</td>
<td>370±46</td>
<td>1,111±236*</td>
<td>470±75</td>
</tr>
<tr>
<td>6 Day</td>
<td>366±57</td>
<td>791±118*</td>
<td>544±87†</td>
</tr>
<tr>
<td>14 Day</td>
<td>543±61</td>
<td>1,209±128*</td>
<td>758±133†</td>
</tr>
<tr>
<td>Adult</td>
<td>2,149±659</td>
<td>1,022±298</td>
<td>2,611±937†</td>
</tr>
<tr>
<td>3-Day hypoxia</td>
<td>379±112</td>
<td>526±123</td>
<td>610±92</td>
</tr>
</tbody>
</table>

Values are means ± SE. E_max, effective maximum. Significant difference (P < 0.05): *between arteries and veins (by unpaired t-test); †compared with fetus (by 2-way ANOVA of curves); ‡compared with 3 day (by 1-way ANOVA with Bonferroni post hoc test); §compared with 3-day control (by 2-way ANOVA of curves).
Pulmonary veins also showed an increase in contractile response to U-46619 with age, with the fetal veins responding the least. The response then increased progressively until the third day of life when it was similar to that seen in the adult (P < 0.05 in comparison to fetal veins for all but newborn veins by two-way ANOVA; Fig. 1, Table 1). Arterial EC50 remained stable with age (log EC50 -6.1 to -6.9), but in the veins, sensitivity was significantly greater in 3-day veins than in the fetal or adult veins (P < 0.05 by one-way ANOVA). The sensitivity of pulmonary veins to U-46619 was greater than that of arteries at 3, 6, and 14 days of age (P < 0.05 by unpaired t-test; Table 1). In all age groups except adult, the ability of the veins to contract was either similar to or greater than that of arteries. Veins from 3-, 6-, and 14-day-old pigs contracted with significantly greater force than their age-matched adjacent arteries on stimulation with either U-46619 or KCl (P < 0.05 by unpaired t-test; Table 1).

Relaxant responses of arteries and veins. Acetylcholine did not produce 100% relaxation in either arteries or veins from any age group (Fig. 2). By contrast, papaverine (10^-5 M) caused full relaxant responses, returning the force generated by each ring to baseline. After maximum contractile responses were induced with U-46619, stimulation with acetylcholine produced no response in arteries from fetal or newborn piglets (P < 0.05 by one-sample t-test; Fig. 2A; also see Fig. 4A). Pulmonary arteries from all older animals relaxed in a concentration-dependent manner to acetylcholine, achieving a similar maximum relaxation that was never >40% of the maximum relaxation produced by papaverine (Fig. 2A). The maximum response was at a concentration of -4.5 to -5.5 log M in all cases and was not related to the age of the animals. Fetal arterial relaxation curves were significantly lower than those at 24 h; 3, 6, and 14 days; and adult (P < 0.05 by two-way ANOVA). Pulmonary veins from all age groups, including both fetal and newborn piglets, relaxed with acetylcholine in a concentration-dependent manner, with relaxation never exceeding 70–80% of the maximum and always >30% (Fig. 2B). Maximum relaxation was attained at concentrations between -4 and -6 log M and was not related to age. The maximum response was least in the fetal veins (P < 0.05 in comparison with 14-day animals by one-way ANOVA).

In all age groups studied except adult, the degree of maximum relaxation produced by the veins in response to acetylcholine was greater than in the adjacent arteries (P < 0.05 for all comparisons by unpaired t-test).

Contractile and relaxant responses in hypertensive piglets. In animals kept in hypobaric hypoxia for 3 days from birth, the pulmonary arteries responded to U-46619 and KCl in a fashion similar to the arteries from both fetal and 3-day-old age-matched control animals (Fig. 3, Table 1). However, for U-46619, the contractile response was less than that in newborn pulmonary arteries, the time at which hypoxia was started (P < 0.05 by two-way ANOVA). The response of pulmonary veins to U-46619 compared with that in 3-day age-matched control veins was
significantly reduced for the concentration-response curve (P < 0.05 by two-way ANOVA; Fig. 3B), but the EC_{50} was not significantly changed. The response after hypoxia was still greater than that found in veins from fetal and newborn animals. A similar pattern was seen in response to KCl (Table 1).

There was no relaxant response to acetylcholine in the hypertensive pulmonary arteries, unlike in the normal age-matched 3-day control animals (P < 0.05 by two-way ANOVA; Fig. 4A). Veins from pigs made hypoxic for 3 days after birth displayed reduced relaxant responses to acetylcholine compared with 3-day control veins (P < 0.05 by two-way ANOVA) and also compared with the relaxation produced in normal fetal and newborn veins (P < 0.05 by two-way ANOVA; Fig. 4B).

**DISCUSSION**

In this study, we have demonstrated changes with age in both the contractile and relaxant responses of adjacent conduit pulmonary arteries and veins. Although in the adult the arteries have a greater contractile response than the veins, during early development, the veins showed a greater ability to contract and relax in response to both U-46619 and KCl. A stable level of venous contraction was attained from 3 days of age onward, with a transient increase in sensitivity to U-46619 at this age. By contrast, the ability of arteries to contract varied with age, and at 3 days, both contractility and sensitivity to U-46619 were at their lowest. After birth, the relaxant response to acetylcholine in the veins was similar at all ages and greater than that in adjacent arteries, particularly in the fetus and newborn where minimal arterial relaxation was seen. In the hypertensive piglets aged 3 days, the contractile and relaxant responses were diminished in both veins and arteries.

**Contractile properties.** For most ages, the maximum force of contraction produced in both arteries and veins by U-46619 was greater than that produced by KCl. KCl stimulates vascular smooth muscle to contract by depolarizing cells, resulting in an influx of calcium to replace potassium (15). U-46619 induces contraction after activation of receptors linked to the inositol phosphate pathway, leading to release of calcium from intracellular stores. However, U-46619 has recently been shown to inhibit the actions of cGMP via the activation of protein kinase C in porcine pulmonary arteries (19). Thus U-46619 may cause contraction by two independent mechanisms, which may explain why it is more efficient than KCl as a contractile agent and also why it produced such relatively consistent levels of contraction in pulmonary arteries and veins at different ages. Despite the fact that the responses to KCl remained consistently less than those to U-46619, both showed a similar pattern of change with age. Given the differences in the mechanisms of action of the two agonists, the changes in response to U-46619 with age are not likely to be due solely to differences in the density or effectiveness of the thromboxane receptors.
For the pulmonary arteries, the smallest contractile responses to U-46619 and KCl were produced in the fetus. This may be related to the smaller amount of the contractile protein actin present in the pulmonary arterial smooth muscle cells as described in sheep (2). But within 5 min after birth, the contractile responses produced were greater than those seen at any other age except adult. A previous study from our group (16) showed that the contractile response of porcine pulmonary arteries to PGF₂α followed a similar pattern. In that study, fetal vessels were not investigated; however, the contractile responses in newborn porcine pulmonary arteries were greater than those in arteries from 3-day-old piglets, although less than those in adult vessels.

In the present study, the variation in response within the young animals was not due to an increase in the size of the pulmonary arteries in the upper lobe because there was little change in diameter or weight during this period but might be related to their arterial wall composition. Porcine pulmonary arteries show a significant decrease in pulmonary arterial smooth muscle cell contractile myofilament volume density between birth and 14 days of age, with the lowest density at 3 days of age (7). During the same period of time, there is a marked increase in connective tissue deposition associated with an increase in structural stiffness (6, 7). These morphological changes as well as an increased influence of relaxant factors such as nitric oxide (NO), known to increase at this time (23), could influence contractility in the postnatal period.

Adult veins were less contractile than adult arteries, but during early life, the contractile responses elicited were equal or greater than those of adjacent arteries. In the young piglets, the vein wall is particularly thin, as in human infants (10), containing little connective tissue relative to smooth muscle cell content. This might facilitate the response to contractile agonists. Adult veins are thicker and contain more connective tissue in both the porcine and human lungs. In the present study, the contractile response of veins to U-46619 increased between fetal life and 3 days, by which time it had reached the adult level. In isolated perfused newborn (0- to 4-day) lamb lungs, thromboxane induced an increase in pulmonary arterial pressure, and with the use of an occlusion technique, this change was found to be primarily produced by venoconstriction (25). In our study, at 3 days of age, the pulmonary veins had a greater responsiveness to U-46619 at a time when the pulmonary artery responses were at their lowest. This may indicate a rapid upregulation of thromboxane-receptor expression in the veins. Further investigations including receptor ligand binding studies are necessary to ascertain whether changes in response to U-46619 are due to changes in thromboxane-receptor density.

Relaxant properties. The vasodilator response to acetylcholine was absent in the pulmonary arteries from the fetal and newborn vessels precontracted with U-46619. When present in the vessels from all the older animals, it was never >40% of the maximum contraction. This was in contrast to the effect of papaverine, which was always able to fully reverse the U-46619-induced contraction in all preparations including fetal pulmonary arteries. In mature vessels, acetylcholine causes vasodilatation via the release of endothelium-derived NO, which activates soluble guanylate cyclase to form cGMP (14, 18). Papaverine causes vasodilatation by a different signal transduction pathway, the activation of adenylate cyclase to form cAMP. It would seem, therefore, that fetal and newborn pulmonary arteries lack some component of the NO-cGMP pathway. Previous studies on pulmonary arteries from newborn pigs precontracted with PGF₂α also showed that acetylcholine did not cause dilatation, whereas NO (23) and the NO donor sodium nitroprusside (17) did. However, newborn porcine pulmonary arteries contain more abundant endothelial NO synthase (eNOS) than adult arteries (12). Thus failure to dilate may be related to a smaller number and a lower sensitivity of muscarinic receptors (11). A recent study from our group (1) has failed to demonstrate eNOS activity in fetal porcine lung tissue despite the presence of immunoreactive protein. These observations could be explained by the presence of an NOS inhibitor such as N⁵, N⁷-dimethylarginine during fetal life (4, 24).

Arterial and venous relaxation at different ages. In contrast to the arteries, relaxation to acetylcholine in the veins produced similar, consistent responses at all ages, even in the fetus where the lowest level of relaxation was still greater than the largest arterial relaxation at 14 days of age. The expression of eNOS in piglet pulmonary veins is consistently high, whereas that in pulmonary arteries varies with age (12). In fetal sheep, higher levels of cGMP were produced in pulmonary veins than in pulmonary arteries stimulated with acetylcholine (5), and strongly positive immunostaining for soluble guanylate cyclase in pulmonary veins of near-term fetal sheep was associated with a greater sensitivity to NO than that in pulmonary arteries (3). Direct measurements have indicated that the pre- and postcapillary beds afford the greatest resistance to flow (20). But the present study on conduit veins suggests that the whole of the venous pathway, being so reactive, may also make a significant contribution to pulmonary vascular resistance in the neonatal period. Perhaps NO activity in veins may be more important in lowering pulmonary vascular resistance at birth than had previously been thought.

Effects of hypoxia. In piglets, exposure to chronic hypoxia from birth prevents the normal decrease in pulmonary vascular resistance. After 3 days, right to left shunting is still present across persistent fetal channels, and right ventricular hypertrophy and an increase in pulmonary arterial medial thickness are present (9, 23). This picture is associated with a reduction in pulmonary arterial eNOS (13) and a lack of development of endothelium-dependent relaxation in the pulmonary arteries after birth (23). Similar results
were found in the present study, but we also found that the relaxant response to pulmonary veins, normally present both before and after birth, was completely abolished. Fetal lambs with pulmonary hypertension produced by ligation of the ductus arteriosus showed impairment of pulmonary arterial smooth muscle cell relaxation at the level of soluble guanylate cyclase, without affecting the ability of the pulmonary veins to relax (22). In addition, we demonstrated retention of the capacity of the veins to contract to thromboxane after hypoxic exposure. However, although still contracting, the response in the chronically hypoxic veins at 3 days of age is less than that in normal age-matched control veins. In the adult rat, chronic hypoxia impairs the contractility of venous smooth muscle in isolated veins (27).

In conclusion, normal fetal pulmonary arteries respond relatively poorly to both contractile and relaxant stimuli and increase their responses immediately after birth. In contrast, the pulmonary veins contract and relax as well during late fetal life as they do immediately after birth. In the neonatal piglets with pulmonary hypertension, endothelium-dependent relaxation in the pulmonary veins, normally present at birth, was abolished, and both pulmonary arteries and veins contracted in response to agonist stimulation. Although the present studies were made on conduit vessels, they suggest that the veins may have the potential to play an active role in the adaptation to extrauterine life.

This work was supported by the British Heart Foundation. Address for reprint requests and other correspondence: A. A. Hislop, Developmental Vascular Biology and Pharmacology Unit, Institute of Child Health, 30 Guilford St., London WC1N 1EH, UK (E-mail: A.Hislop@ich.ucl.ac.uk).

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