Hypoxic constriction of porcine distal pulmonary arteries: endothelium and endothelin dependence

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Liu, Q., J. S. K. Sham, L. A. Shimoda, and J. T. Sylvester. Hypoxic constriction of porcine distal pulmonary arteries: endothelium and endothelin dependence. Am J Physiol Lung Cell Mol Physiol 280: L856–L865, 2001.—To determine the role of endothelium in hypoxic pulmonary vasoconstriction (HPV), we measured vasomotor responses to hypoxia in isolated seventh-generation porcine pulmonary arteries < 300 μm in diameter with (E+) and without endothelium. In E+ pulmonary arteries, hypoxia decreased the vascular intraluminal diameter measured at a constant transmural pressure. These constrictions were complete in 30–40 min; maximum at PO2 of 2 mmHg; half-maximal at PO2 of 40 mmHg; blocked by exposure to Ca2+-free conditions, nifedipine, or ryanodine; and absent in E− pulmonary arteries of similar size. Hypoxic constrictions were unaltered by indomethacin, enhanced by indomethacin plus L-arginine methyl ester, abolished by BQ-123 or endothelial denudation, and restored in endothelium-denuded pulmonary arteries pretreated with 10−9 M endothelin-1 (ET-1). Given previous demonstrations that hypoxia caused contractions in isolated pulmonary arterial myocytes and that ET-1 receptor antagonists inhibited HPV in intact animals, our results suggest that full in vivo expression of HPV requires basal release of ET-1 from the endothelium to facilitate mechanisms of hypoxic reactivity in pulmonary arterial smooth muscle.

vascular smooth muscle; internal diameter; calcium; acetylcholine; U-46619; potassium chloride

OVER THE LAST DECADE, two fundamentally different hypotheses about the mechanisms of hypoxic pulmonary vasoconstriction (HPV) have emerged. According to one hypothesis (73), hypoxia inhibits voltage-dependent potassium (Kv) channels in plasma membranes of pulmonary arterial myocytes, leading to depolarization, Ca2+ influx through voltage-dependent Ca2+ channels, and contraction. According to the other (50), hypoxia acts on endothelium to stimulate production and release of the potent vasoconstrictor peptide endothelin (ET)-1, which mediates HPV by activating ET A receptors on smooth muscle. Both hypotheses have considerable experimental support. On one hand, hypoxia inhibited Kv currents, increased membrane potential and intracellular Ca2+ concentration ([Ca2+]i), and caused contraction in isolated pulmonary arterial smooth muscle cells (7, 8, 15, 42, 47, 53, 54, 56, 70, 82). On the other, ET-1 receptor antagonists blocked HPV in intact animals (23, 24, 50, 71, 78).

The major site of HPV is thought to be small distal pulmonary arteries (1, 21, 59). In myocytes isolated from distal pulmonary arteries of the pig, we found that hypoxia inhibited Kv channels, increased [Ca2+]i, and decreased cell length; however, these effects were quite small (58). If the cells were pretreated with a low concentration of ET-1 (10−10 M), which itself had no effect on cell length or [Ca2+]i, the magnitude of hypoxic contraction increased eightfold. Because ET-1 receptor antagonists inhibited HPV in intact animals (23, 24, 50, 71, 78), these results suggested that full expression of HPV in vivo might require release of ET-1 from the endothelium to facilitate mechanisms of hypoxic reactivity in pulmonary arterial smooth muscle.

The purpose of the present study was to test this possibility. First, we characterized the time course, PO2 dependence, and Ca2+ dependence of hypoxic constriction in small [intraluminal diameter (ID) < 300 μm] distal porcine pulmonary arteries and determined whether hypoxic vasoconstriction occurred in bronchial arteries of similar size. Next, we determined whether hypoxic constriction in pulmonary arteries was endothelium dependent and performed experiments to clarify the mechanism of this dependence.

METHODS

Preparation of isolated arteries. Male pigs (20–25 kg) were anesthetized with ketamine (30 mg/kg im) followed by pentobarbital sodium (12.5 mg/kg iv). After the animal was exsanguinated from the femoral arteries, the lungs were rapidly removed and placed in ice-cold Krebs-Ringer bicarbonate (KRB) solution containing (in mM) 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 25.0 NaHCO3, and 11.1 glucose. Under a dissecting microscope, 1-mm segments of pulmonary arteries (OD 100–150 μm) were isolated from seventh-generation branches in the left upper lobe. Bronchial arteries of similar size were isolated from the outer surface of segmental bronchi. The vessels were placed in a chamber filled with KRB solution, cannulated at one end with a glass

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micropipette, and secured with 12-0 nylon monofilament suture. KRB solution was infused slowly through the pipette until the artery was completely filled. In some pulmonary arteries, endothelial cells were disrupted by gently rubbing the intraluminal surface with a steel wire (diameter 70 μm). These vessels were then perfused with 2 ml of air bubbles followed by 2 ml of KRB solution (perfusion pressure < 5 mmHg). The other end of the vessel was cannulated with a second micropipette filled with KRB solution. Both cannulas were connected to a reservoir that was adjusted to set the transmural pressure (Ptm) to the desired value. Ptm was measured with a pressure transducer positioned at the level of the vessel lumen. The chamber was covered, and the vessels were superfused at 20 ml/min with recirculating KRB solution (total volume 50 ml) gassed with 16% O2-5% CO2-79% N2 and maintained at 37°C. The same gas mixture flowed over the surface of the superfusate in the covered chamber. To measure the vascular ID, the chamber was placed on the stage of an inverted microscope (Nikon TMS-F) connected to a video camera (Panasonic CCTV camera). The vascular image was projected onto a video monitor, and the ID was determined with a video dimension analyzer (Living Systems Instrumentation, Burlington, VT). ID and Ptm were recorded continuously (Gould, Cleveland, OH).

Experimental protocols. Initially, isolated arteries were allowed to equilibrate for 20 min at a Ptm of 10 mmHg. Ptm was then increased to 20 (pulmonary arteries) or 40 (bronchial arteries) mmHg and held constant. To test viability, the vessels were exposed to 4% O2 (60 mM) and then to the thromboxane A2 agonist U-46619 (10^-8 M) followed by ace
tylycholine (ACh; 10^-6 M). After the responses had stabilized (5–10 min), the agonists were washed out of the chamber by 15–20 min of nonrecirculating superfusion with fresh KRB solution. Vessels in which the ID decreased <30% after KCl and U-46619 were excluded. Endothelium-intact (E+) vessels were excluded if ACh reversed the U-46619 contraction by <50%. These criteria were not applied to E+ pulmonary arteries subjected to Ca2+-free conditions or nifedipine (see below). Endothelium-denuded (E−) vessels were excluded if ACh caused any vasodilatation. In some preparations, viability tests were repeated at the end of the experiment.

To expose E+ pulmonary arteries to hypoxia, the O2 concentration of the gas mixture bubbling the reservoir and flowing over the superfusate surface in the vascular chamber was decreased from 16 to 7 (n = 7), 4 (n = 27), or 0% (n = 6) for 30 or 60 min. After hypoxic exposure, O2 concentration was returned to 16%. Arteries continuously exposed to 16% O2 served as time-matched controls (n = 36). Bronchial arteries were exposed to 4% (n = 9) or 16% (n = 5) O2 in a similar manner. Responses were quantified as changes in ID (∆ID) from the values at the onset of exposure. To determine the degree of hypoxia at the outer surface of the vessels, we measured O2 tension with a microelectrode (Microelectrodes, Londonderry, NH) positioned at the level of the vessel in the chamber. In other experiments, we used a blood gas analyzer (Synthesis 15, Instrumentation Laboratory, Lexington, MA) to measure pH and PO2 in superfusate samples obtained anaerobically from the chamber.

To evaluate the role of Ca2+ in HPV, we determined the effects of hypoxia (4% O2 for 30 min) in untreated E+ pulmonary arteries and E+ pulmonary arteries exposed to 1) Ca2+-free conditions (Ca2+-free KRB solution containing 10^-3 M EGTA; n = 5), 2) the L-type Ca2+ channel antagonist nifedipine (10^-6 M; n = 7), or 3) the sarcoplasmic reticulum (SR) Ca2+ channel agonist ryanodine (10^-5 M; n = 5). Ca2+-free conditions and nifedipine were instituted before viability was tested to document effects on responses to KCl, U-46619, and ACh. Ryanodine was given after viability testing, and its effects were documented by a bolus injection of caffeine into the reservoir (final superfusate concentration 10 mM). The caffeine was then washed out by 15 min of nonrecirculating superfusion with KRB solution containing 10^-5 M ryanodine.

To evaluate the role of the endothelium in HPV, we measured the responses to 4% O2 in 1) E− pulmonary arteries (n = 15); 2) E+ pulmonary arteries treated with the cyclooxygenase inhibitor indomethacin (Indo; 10^-6 M; n = 5), indomethacin plus the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine-methyl ester (L-NAME; 3 × 10^-5 M; n = 12), or the ETa receptor antagonist BQ-123 (3 × 10^-6 M; n = 6); and 3) E− pulmonary arteries pretreated with a low superfusate concentration of ET-1 (10^-10 M; n = 13). Responses were compared with those of time-matched control vessels not exposed to hypoxia but otherwise treated similarly (n = 17, 5, 6, and 7, respectively). Exposure to Indo began with superfusion. L-NAME, BQ-123, and ET-1 were given 20–30 min before hypoxia.

Drugs. ACh, Indo, and L-NAME were purchased from Sigma (St. Louis, MO); BQ-123 and ET-1 were from American Peptide (Sunnyvale, CA); U-46619 was from Cayman Chemical (Ann Arbor, MI); and nifedipine, caffeine, and ryanodine were from Calbiochem (La Jolla, CA). Stock solutions of ACh, L-NAME, ET-1, and caffeine were prepared each day in deionized water and stored at 4°C until used. Indo was dissolved each day in an aqueous solution of NaHCO3 (2.35 × 10^-2 M). Stock solutions of BQ-123 and nifedipine in ethanol and ryanodine in dimethyl sulfoxide were stored at −60°C and diluted with deionized water before use. Concentrations are expressed as final molar concentrations in the superfusate.

Data analysis. t-Tests and one-, two- or three-factor analysis of variance were used for statistical analysis of the data, taking repeated measures into account when appropriate. When analysis of variance yielded a significant F-ratio, the least significant difference was calculated to permit pairwise comparison among means or Dunnett’s test was used to compare means to a control value. P values ≤ 0.05 were taken to indicate significance. Values are means ± SE. On each experimental day, two vessels from the same animal were subjected to different protocols; therefore, n is the number of both animals and vessels in each group.

RESULTS

Viability tests. Under normoxic conditions at the beginning of the experiment, the ID measured at a Ptm of 20 mmHg (ID0) was greater in E+ than in E− pulmonary arteries (Table 1). KCl and U-46619 caused vigorous constriction in both E+ and E− arteries. ACh reversed constriction induced by U-46619 in E+ arteries but had no effect in E− arteries. Responses of E+ bronchial arteries to KCl, U-46619, and ACh were similar to those of E+ pulmonary arteries.

Viability tests were repeated under normoxic conditions at the end of the experiment in 18 E+ pulmonary arteries exposed to 4% O2 for 30 min and 13 E+ pulmonary arteries exposed to 16% O2 throughout the experiment (Fig. 1). By the end of the experiment, vasoconstrictor responses to KCl decreased 74% in arteries exposed to hypoxia but only 26% in arteries not exposed to hypoxia. Vasoconstrictor responses to U-46619 did not change in either group. Vasodilator responses to ACh decreased 78% in arteries exposed to...
hypoxia but were unaltered in arteries not exposed to hypoxia. The effects of hypoxic exposure on vasoconstrictor responses to KCl and U-46619 in E− pulmonary arteries (data not shown) were similar to those in E+ arteries.

Responses of pulmonary and bronchial arteries to hypoxia. Decreasing the O2 concentration from 16 to 7, 4, or 0% for 30 min caused P O2 in the chamber superfusate to decrease rapidly from 114 ± 1 to 49 ± 1, 29 ± 1, and 2 ± 3 mmHg, respectively (Fig. 2A). On reoxygenation with 16% O2, P O2 increased rapidly, reaching 113 ± 2 mmHg at 60 min. When the O2 concentration was maintained at 16% throughout the experiment, P O2 averaged 116 ± 2 mmHg. PCO2 and pH were constant at 33 ± 0.2 mmHg and 7.40 ± 0.003, respectively.

As previously observed (38), ID20 in normoxic E+ pulmonary arteries decreased gradually due to development of intrinsic tone [change in ID20 (ΔID20) = −13 ± 7 μm at 30 min; Fig. 2A]. Compared with these vessels, arteries exposed to 4 and 0% O2 developed vigorous constriction (ΔID20 at 30 min = −57 ± 10 and −62 ± 8 μm, respectively; P < 0.0001), whereas arteries exposed to 7% O2 did not (ΔID20 at 30 min = −23 ± 10 μm; P = 0.999). The relationship between mean ΔID20 (expressed as a fraction of maximum ΔID20) and mean P O2 at 30 min appeared to be sigmoid (Fig. 2B).

Using least squares iteration to fit these data to the Hill equation (r² = 0.999996), we estimated that hypoxic vasoconstriction was half-maximal at a P O2 of 40 mmHg. After 30 min of reoxygenation (Fig. 2A), ΔID20 in arteries exposed to 7 and 4% O2 (−23 ± 10 and −23 ± 10 μm, respectively) returned to the value observed in arteries exposed to 16% O2 throughout the experiment (−25 ± 10 μm); however, arteries exposed to 0% O2 continued to constrict (ΔID20 = −78 ± 17 μm after 30 min of reoxygenation).

Although 4% O2 elicited vigorous reversible vasoconstriction in pulmonary arteries (P < 0.0001), it had no effect on vasomotor tone in bronchial arteries (P = 0.95; Fig. 3).

Ca2+ dependence of HPV. Vasoconstrictor responses to KCl were virtually abolished in pulmonary arteries subjected to Ca2+-free conditions or nifedipine (ΔID20 = −9 ± 6 and −7 ± 2% of control ID20, respectively, compared with −69 ± 2% in untreated arteries; P < 0.0001; Table 1). Vasoconstrictor responses to U-46619 were also reduced but less severely (ΔID20 = −26 ± 5 and −18 ± 4% of control ID20, respectively, compared with −46 ± 2% in untreated arteries; P < 0.0001; Table 1). Vasoconstrictor responses to ACh were not altered (ΔID20 = 91 ± 14 and 74 ± 16% of U-46619-induced constrictions, respectively, compared with

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**Table 1. Responses of pulmonary and bronchial arteries with and without endothelium to KCl, U-46619, and ACh**

<table>
<thead>
<tr>
<th></th>
<th>Responses to KCl</th>
<th>Responses to U-46619 and ACh</th>
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<tr>
<td></td>
<td>ID, μm</td>
<td>ΔID, %</td>
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<tr>
<td>Pulmonary arteries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E+</td>
<td>227 ± 7</td>
<td>−69 ± 2</td>
</tr>
<tr>
<td>E−</td>
<td>177 ± 12†</td>
<td>−63 ± 3</td>
</tr>
<tr>
<td>Bronchial arteries</td>
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<tr>
<td>E+</td>
<td>215 ± 16</td>
<td>−76 ± 3</td>
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Values are means ± SE. Intraluminal diameter (ID) was measured at a transmural pressure of 20 mmHg for pulmonary arteries and 40 mmHg for bronchial arteries. Responses to KCl (60 mM) and U-46619 (10^{-8} M) are percent of control value. Responses to U-46619 plus ACh (10^{-6} M) are percent of response to U-46619. ΔID, change in ID; E+, with endothelium; E−, without endothelium. Significantly different (P < 0.05) from: *control; †E+ pulmonary arteries; ‡U-46619.
Caffeine did not alter the vasomotor tone in arteries treated with ryanodine ($\Delta ID_{20} = 11 \pm 7\%$ in untreated arteries; $P > 0.05$; Table 1). As shown in Fig. 4, exposure to $\text{Ca}^{2+}$-free conditions, nifedipine, or ryanodine eliminated the vasoconstrictor responses to $4\% \text{O}_2$ ($\Delta ID_{20} = -4 \pm 5$ and $-27 \pm 27 \mu m$, respectively) but was decreased in arteries exposed to $\text{Ca}^{2+}$-free conditions ($\Delta ID_{20} = 26 \pm 11 \mu m$). Baseline $ID_{20}$ measured before hypoxic exposure in E+ arteries subjected to $\text{Ca}^{2+}$-free conditions (223 $\pm$ 9 $\mu m$) or ryanodine (196 $\pm$ 17 $\mu m$) did not differ from the baseline $ID_{20}$ in untreated E+ arteries (193 $\pm$ 7 $\mu m$); however, baseline $ID_{20}$ in nifedipine-treated arteries was increased (252 $\pm$ 21 $\mu m$; $P < 0.05$).

Endothelium and endothelin dependence of HPV. As shown in Fig. 5, $ID_{20}$ in E+ pulmonary arteries exposed to $4\% \text{O}_2$ for 60 min decreased relative to $ID_{20}$ in normoxic E+ arteries ($\Delta ID_{20} at 60 \text{ min} = -71 \pm 14$ and $112 \pm 5\%$ in untreated arteries; $P > 0.05$; Table 1). Caffeine did not alter the vasomotor tone in arteries treated with ryanodine ($\Delta ID_{20} = 11 \pm 7\%$; $P = 0.13$). As shown in Fig. 4, exposure to $\text{Ca}^{2+}$-free conditions, nifedipine, or ryanodine eliminated the vasoconstrictor responses to $4\% \text{O}_2$ ($\Delta ID_{20}$ at 30 min $= 9 \pm 4$, $-6 \pm 4$ and $-2 \pm 12 \mu m$, respectively, compared with $-57 \pm 10 \mu m$ in untreated arteries; $P < 0.0001$). After 30 min of reoxygenation ($16\% \text{O}_2$), the vasomotor tone in arteries treated with nifedipine or ryanodine ($\Delta ID_{20} = -4 \pm 5$ and $-27 \pm 27 \mu m$, respectively) was not different from that in untreated arteries ($\Delta ID_{20} = -23 \pm 18 \mu m$) but was decreased in arteries exposed to $\text{Ca}^{2+}$-free conditions ($\Delta ID_{20} = 26 \pm 11 \mu m$). Baseline $ID_{20}$ measured before hypoxic exposure in E+ arteries subjected to $\text{Ca}^{2+}$-free conditions (223 $\pm$ 9 $\mu m$) or ryanodine (196 $\pm$ 17 $\mu m$) did not differ from the baseline $ID_{20}$ in untreated E+ arteries (193 $\pm$ 7 $\mu m$); however, baseline $ID_{20}$ in nifedipine-treated arteries was increased (252 $\pm$ 21 $\mu m$; $P < 0.05$).

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In E− pulmonary arteries, this hypoxic vasoconstriction was abolished (ΔID20 at 60 min = −4 ± 10 and −6 ± 14 μm in normoxic and hypoxic E− arteries, respectively; P = 0.999). Compared with untreated E+ arteries, ID20 measured before hypoxic exposure in untreated E− arteries was decreased (162 ± 9 μm; P < 0.01).

To determine which endothelium-derived factors were responsible for the endothelium dependence of HPV, we exposed E+ pulmonary arteries to 4% O2 for 30 min after treatment with Indo, Indo plus L-NAME, or BQ-123 (Fig. 6). Compared with untreated arteries (ΔID20 at 30 min = −44 ± 8 μm), hypoxic vasoconstriction was unaltered in arteries treated with Indo (ΔID20 at 30 min = −40 ± 10 μm; P = 0.9), enhanced in arteries treated with Indo plus L-NAME (ΔID20 at 30 min = −124 ± 6 μm; P < 0.0001), and abolished in arteries treated with BQ-123 (ΔID20 at 30 min = −3 ± 4 μm; P = 0.0006). Baseline ID20 measured before hypoxic exposure averaged 198 ± 20, 168 ± 5, and 196 ± 23 μm in E+ arteries treated with Indo, Indo plus L-NAME, or BQ-123, respectively. These values did not differ from baseline ID20 in untreated E+ arteries (193 ± 7 μm).

To further test the role of ET-1 in hypoxic pulmonary vasoconstriction, we “primed” E− pulmonary arteries by exposing them to a low concentration of ET-1 (10−10 M) for 20–30 min before hypoxia (4% O2 for 30 min; n = 13) or continued normoxia (16% O2; n = 7). Responses in these vessels were compared with those of untreated normoxic (n = 17) and hypoxic (n = 15) E− arteries (Fig. 7). During normoxia, ET-1 priming decreased ID20 18 ± 4% compared with a 4 ± 2% increase over a similar time period in unprimed E− arteries (P < 0.0001). Hypoxia did not alter the vasomotor tone in unprimed E− arteries (ΔID20 at 30 min = −1 ± 5 and −9 ± 5 μm in normoxic and hypoxic arteries, respectively; P = 0.14); however, hypoxic vasoconstriction...
was restored in E− arteries primed with ET-1 (ΔID20 at 30 min = −2 ± 9 and −39 ± 8 μm in normoxic and hypoxic arteries, respectively; P < 0.0001).

**DISCUSSION**

HPV has been thoroughly studied in intact animals and isolated lungs. These preparations provide consistent and physiologically relevant responses but impose significant limitations on mechanistic investigation. More reduced preparations permit more precise intervention but may yield inconsistent responses of uncertain relevance (63). In the present study, we used small distal porcine pulmonary arteries to determine whether HPV was endothelium dependent. We chose the pig as our experimental animal because HPV is vigorous in this species (9, 62) and porcine lungs are large enough to permit dissection of small distal pulmonary arteries. We studied seventh-generation pulmonary arteries < 300 μm in diameter because small distal pulmonary arteries are thought to be the major site of HPV (1, 21, 59). We measured changes in vasomotor tone as changes in ID at a constant Pm because HPV normally occurs locally and does not alter pulmonary arterial pressure and because this measurement may more accurately reflect properties of in vivo arteries in which contraction is neither isometric nor isotonic (17).

As shown in Fig. 2, physiological decreases in PO2 caused vigorous sustained vasoconstriction in our preparation. Relative to the gradual vasoconstriction caused during normoxia by intrinsic tone (38, 40), hypoxic vasoconstriction began 5–10 min after the onset of exposure and appeared to be complete within 30–40 min (Figs. 2A and 5). Maximum HPV decreased ID20 62 μm or ~40% of the constrictor response to 60 mM KCl (Fig. 2A, Table 1). HPV was half-maximal at a PO2 of 40 mmHg (Fig. 2B). In isolated and intact lungs, HPV occurred somewhat more rapidly, requiring 10–15 min to achieve initial plateau responses, which were half-maximal at O2 tensions of 30–60 mmHg (3, 9, 61, 62). In isolated ferret lungs, maximum HPV was 38% of the maximum vasoconstrictor response to KCl (16). Thus, in terms of magnitude, PO2 dependence, and temporal characteristics, hypoxic vasoconstriction in our isolated arteries was similar to that in intact and isolated lungs.

Although severe hypoxia caused sustained vasoconstriction in arteries (Fig. 2, 0% O2), it caused vasoconstriction followed by vasodilation in isolated lungs (62). Vasodilation to severe hypoxia was probably caused by deterioration of the vascular smooth muscle energy state, activation of ATP-dependent K+(KATP) channels, hyperpolarization, decreased Ca2+ influx through voltage-dependent Ca2+ channels, and relaxation (33, 76, 77). This sequence may have not have occurred in our arteries because unlike in isolated lungs perfused at a constant flow, intraluminal pressures (and, therefore, Pm) did not increase during HPV, resulting in a lower workload on pulmonary arterial smooth muscle and better maintenance of smooth muscle energy state and tone during severe hypoxia.

Vasoconstriction elicited by severe hypoxia (0% O2) was not reversible in isolated arteries (Fig. 2). Indeed, reoxygenation after 0% O2 caused further constriction. Even in arteries exposed to 4% O2, where HPV was reversible, transient constriction was observed on reoxygenation. The mechanism of this constriction remains to be determined; however, because it occurred with reoxygenation, it may have been mediated by reactive oxygen species (27, 79). Injury due to reactive oxygen species may explain depression of the vasoconstrictor responses to KCl and the vasodilator responses to ACh seen after reoxygenation (Fig. 1). Because vasoconstrictor responses to U-46619 (and, therefore, contractility) were unchanged, these results suggest that voltage-dependent Ca2+ channels and NO responsiveness in smooth muscle and/or ACh-induced NO production in endothelium were dysfunctional after reoxygenation. Further studies are needed to confirm these results and determine the underlying mechanisms. Because of the possibility of reoxygenation injury, we utilized single exposures to 4% O2 to elicit HPV in subsequent experiments.

In more proximal pulmonary arteries, hypoxic responses were typically triphasic (early transient contraction followed by relaxation and late sustained contraction) and did not occur unless the arteries were subjected to 0% O2 after some special treatment such as precontraction with vasoactive agonists or prolonged exposure to moderate hypoxia (22, 30, 32, 63). In small (OD < 300 μm) distal pulmonary arteries of the cat, increases in isometric tension caused by decreases in PO2 from >400 to 250, 150, 100, 50, or 20 mmHg were transient, and the peaks of these contractions were half-maximal at a PO2 of 100 mmHg (41). These differences may be attributable to species, arterial size or locus, or method of measurement. Because increases in vasomotor tone were measured as increases in isometric tension, deterioration of energy state may have contributed to hypoxic relaxation in proximal pulmonary arteries (33) and the transience of hypoxic constriction in small distal pulmonary arteries (41). We are unaware of previous attempts to assess hypoxic responses in pulmonary arteries from measurements of ID at a constant Pm.

HPV in intact animals and isolated lungs and hypoxia-induced increases in [Ca2+]i in pulmonary arterial myocytes were inhibited by L-type Ca2+ channel antagonists (2, 8, 28, 45, 67) and potentiated by agonists of these channels (8, 44, 66), indicating that HPV required influx of extracellular Ca2+ through sarcoplasmic L-type Ca2+ channels. Our findings that Ca2+-free conditions or nifedipine prevented HPV in isolated pulmonary arteries (Fig. 4) support this view and demonstrate further similarity between the hypoxic responses of our preparation and those of intact and isolated lungs. It has been suggested that inhibition of HPV by Ca2+ channel antagonists could be due to nonspecific decreases in baseline tone (55). Because baseline ID20 in nifedipine-treated E+ arteries was
higher than in untreated E+ arteries, this possibility cannot be ruled out.

Some investigators reported that depletion of Ca\(^{2+}\) stores in the SR with ryanodine and caffeine, which activate SR ryanodine receptors, or thapsigargin and cyclopiazonic acid, which inhibit SR Ca\(^{2+}\) uptake, inhibited hypoxia-induced increases in myocyte \([\text{Ca}^{2+}]_i\) (15, 56, 70). Our observations that ryanodine blocked HPV in isolated pulmonary arteries are consistent with these findings and support the proposal that HPV is triggered by release of SR Ca\(^{2+}\), which then acts on sarcolemmal ion channels to cause depolarization and Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels (15, 52).

Alternatively, HPV could be mediated by an initial influx of Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels, leading to Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR and sustained elevation of \([\text{Ca}^{2+}]_i\) (8). Further work is required to evaluate these possibilities.

Although hypoxia caused vigorous vasoconstriction in pulmonary arteries, it did not alter the ID in bronchial arteries (Fig. 3). These results are consistent with previous studies (6, 48) of systemic arteries in which hypoxia had no effect or caused vasodilation. We used bronchial arteries because these vessels could be isolated from the same organ and studied in the same manner as pulmonary arteries, thereby minimizing the influence of methodological factors on our results. The only methodological difference was \(P_{\text{tm}}\), which was higher in bronchial arteries to reflect in vivo conditions; however, it is unlikely that the higher \(P_{\text{tm}}\) prevented hypoxic constriction in bronchial arteries because these vessels constricted vigorously to KCl and U-46619 (Table 1). It is more likely that the hypoxic responses of pulmonary and bronchial arteries were different due to intrinsic differences in reactivity.

The similarities in temporal characteristics, \(P_{\text{O}_2}\) dependence, and Ca\(^{2+}\) dependence of hypoxic vasoconstriction between small porcine pulmonary arteries and isolated and intact lungs and the absence of this response in bronchial arteries suggested that studies in small porcine pulmonary arteries could help to clarify the mechanisms of in vivo HPV; therefore, we used this preparation to determine if HPV was endothelium dependent.

As shown in Fig. 5, endothelial denudation abolished HPV in these vessels. This effect was not due to denudation-induced injury to vascular smooth muscle because E− arteries constricted vigorously to KCl and U-46619 (Table 1). Neither was it due to decreased baseline vasomotor tone (55) because \(ID_{20}\) before hypoxic exposure was smaller than in E+ arteries, suggesting that baseline tone had increased. Rather, it must have been due to elimination of some endothelial influence on vascular smooth muscle. Endothelium can release relaxing factors, such as NO and prostacyclin, and contracting factors, such as endothelin and thromboxane; therefore, HPV could be due to a hypoxia-induced decrease in relaxing factor activity or a hypoxia-induced increase in contracting factor activity. Alternatively, basal release of endothelial factors might facilitate the mechanisms of HPV intrinsic to vascular smooth muscle. To evaluate these possibilities, we determined how Indo, l-NAME, and BQ-123 altered HPV in E+ arteries and whether ET-1 restored HPV in E− arteries.

Liu and Sylvester (38) have shown that l-NAME (3 × 10\(^{-5}\) M) blocked the vasodilator responses to ACh in this preparation, indicating inhibition of NO synthase, and that BQ-123 (3 × 10\(^{-6}\) M) blocked the vasconstrictor responses to ET-1, indicating inhibition of ETA receptors. Measurement of prostaglandins D\(_2\), E\(_2\), and F\(_{2\alpha}\), 6-keto prostaglandin F\(_{1\alpha}\), and thromboxane B\(_2\) production by gas chromatography/mass spectrometry indicated that Indo (10\(^{-6}\) M) blocked cyclooxygenase in isolated proximal porcine pulmonary arterial rings (Shimoda T, Sylvester J, Undem B, and Hubbard W, unpublished data). Indo (10\(^{-5}\) M), l-NAME (3 × 10\(^{-5}\) M), or BQ-123 (3 × 10\(^{-6}\) M) did not alter the vasoconstrictor responses to KCl or U-46619 in small porcine pulmonary arteries (38). Finally, \(ID_{20}\) measured before hypoxic exposure was not different among untreated E+ arteries and E+ arteries treated with Indo, Indo plus l-NAME, or BQ-123, suggesting similar baseline vasomotor tone. These results indicate that our pharmacological interventions had the intended effects on the endothelium and did not have unintended nonspecific effects on vascular smooth muscle.

As shown in Fig. 6, HPV was unaltered by Indo, enhanced by Indo plus l-NAME, and abolished by BQ-123. Many studies in isolated and intact lungs (16, 19, 34, 39, 68, 72, 74) have demonstrated enhancement of HPV by inhibitors of cyclooxygenase and NO synthase alone or in combination. Like our results, these data indicate that cyclooxygenase products and NO modulated but did not mediate HPV. In contrast, antagonists of ETA receptors blocked HPV in intact and isolated lungs (11, 14, 23, 24, 50, 57, 71, 78) and acute hypoxia increased ET-1 production in intact and isolated lungs and cultured endothelium (20, 25, 29, 60, 64). These data are consistent with the effects of BQ-123 shown in Fig. 6 and suggest that ET-1 mediated HPV.

Other observations question this possibility. ET-induced pulmonary vasoconstriction, unlike HPV, reversed very slowly (4, 43), perhaps due to prolonged receptor binding (75). ET-1 caused transient vasodilation during HPV, perhaps by activating K\(_{\text{ATP}}\) channels in vascular smooth muscle or ET\(_B\) receptors and NO production in endothelium (10, 18, 20, 35–37, 80). Some investigators (11, 46, 51) were unable to demonstrate that hypoxia increased pulmonary ET-1 production. Others (20, 64) found that hypoxia-induced increases in ET-1 concentration occurred much later than or did not correlate with HPV. Thus the role of ET-1 in HPV may be more subtle than direct concentration-dependent activation of smooth muscle contraction.

To reconcile these discrepancies as well as the observations that isolated pulmonary arterial myocytes contract to hypoxia (42, 47, 58), we hypothesized that the basal production of ET-1 by the endothelium allowed...
full expression of HPV in vivo by facilitating mechanisms of hypoxic reactivity in vascular smooth muscle. To test this hypothesis, we determined whether exposure to a low concentration of ET-1 (10^{-10} \text{ M}) would restore HPV in E-\text{ pulmonary arteries. We used 10^{-10} M because Liu and Sylvester (38) previously found that this concentration was at the threshold for contraction in these vessels. As shown in Fig. 7, ET-1 priming almost completely restored HPV in E-\text{ arteries (AID}_{20} at 30 \text{ min} = -39 \pm 8 \ \text{ HU} or \ -70\% \text{ of the response to } 4\% \text{ O}_2 \text{ in untreated E-\text{ arteries}. These results are supportive of our hypothesis and consistent with previous observations by Sham et al. (58) in freshly isolated smooth muscle cells from these vessels in which ET-1 priming potentiated an otherwise small but significant hypoxic contraction nearly eightfold. ET-1 could facilitate HPV in several ways. In pulmonary arterial myocytes, ET-1 priming potentiated hypoxic contraction but did not alter hypoxia-induced increases in \left[ \text{Ca}^{2+} \right]_i, suggesting an increase in myofilament Ca^{2+} sensitivity (58). ET-1 is known to have this effect in both systemic and pulmonary arterial smooth muscle (13, 49), and increased Ca^{2+} sensitivity has been proposed to contribute to hypoxic responses in precontracted rat pulmonary arteries (54, 72). Other possibilities include an increased open probability of \text{K}_\text{ATP} channels (26) and a depolarized resting membrane potential (69). The latter could make sarcoplasmic \text{K}_\text{ATP} channels more susceptible to hypoxic inactivation, thought by some investigators to be an important early step in HPV (73). It was recently suggested that ET-1 might facilitate HPV by preventing hypoxic activation of \text{K}_\text{ATP} channels in pulmonary vascular smooth muscle (57); however, glibenclamide, an \text{K}_\text{ATP} antagonist, did not alter HPV in isolated ferret lungs exposed to moderate hypoxia (76). Further studies are needed to evaluate these possibilities. It is possible that agonists other than ET-1 could facilitate HPV. This might explain why ET-1 receptor antagonists sometimes did not inhibit HPV in intact animals (81), isolated lungs (65), or pulmonary arteries (12, 31). In support of this possibility, Sato et al. (57) found that ETA receptor antagonists blocked HPV in intact and isolated rat lungs but not in isolated lungs costimulated with angiotensin II. Such data emphasize that our results should not be extended to other preparations, conditions, or species without experimental confirmation of applicability. Nevertheless, it is interesting to note that the inhibitory effect of ETA receptor antagonists on HPV in vivo has been reasonably consistent across species (5, 14, 23, 24, 50, 57, 71, 78), suggesting that ET-1 may play a pivotal physiological role.

REFERENCES

HYPOTHETICAL CONSTRICTION IN PULMONARY ARTERIES


