Development of intrinsic tone in isolated pulmonary arterioles

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Liu, Qiang, and J. T. Sylvester. Development of intrinsic tone in isolated pulmonary arterioles. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L805–L813, 1999.—In isolated porcine pulmonary arterioles with endothelium, intraluminal diameter measured at a transmural pressure of 20 mmHg decreased spontaneously from 233 ± 11 to 171 ± 12 µm in 135 min. This intrinsic constriction was not prevented by indomethacin, tetraethylammonium, or superoxide dismutase. Indomethacin plus N\(^{\text{6}}\)-nitro-L-arginine methyl ester caused initial constriction and BQ-123 or BQ-123 plus BQ-788 caused initial dilation, but these treatments did not prevent subsequent progressive constriction. In pulmonary arterioles with endothelium exposed to calcium-free conditions and pulmonary arterioles without endothelium, the intraluminal diameter measured at a transmural pressure of 20 mmHg was constant at 239 ± 16 and 174 ± 7 µm, respectively. Thus the spontaneous development of tone in isolated pulmonary arterioles required extracellular calcium and resulted from 1) time-independent smooth muscle contraction caused by mechanisms intrinsic to smooth muscle and 2) time-dependent constriction caused by decreasing activity of endothelium-derived relaxing factors other than nitric oxide, vasodilator prostaglandins, and hyperpolarizing factors acting on calcium-dependent potassium channels or increasing activity of endothelium-derived contracting factors other than endothelin-1, vasoconstrictor prostaglandins, and superoxide anions. Further investigation is indicated to identify these unknown mechanisms and determine their role in pulmonary vasoreactivity.

vasodilator prostaglandins; endothelin-1; superoxide anions; extracellular calcium concentration; pig

VASOMOTOR RESPONSES having the greatest impact on pulmonary vascular resistance and ventilation-perfusion matching are thought to occur in small, distal “resistance” pulmonary arteries rather than in large, proximal “conduit” arteries; therefore, isolated small pulmonary arteries are being used with increasing frequency to study the mechanisms of pulmonary vasoreactivity, and the characteristics of these vessels are becoming better appreciated (14, 22, 23, 27, 28). Recent studies (24, 25, 27) in isolated cat and pig pulmonary arteries 200–600 µm in diameter revealed that these resistance vessels developed intrinsic tone, a phenomenon that is not typical of larger conduit pulmonary arteries. Because baseline tone and the manner in which it is generated can exert profound effects on vasomotor responses (1, 3, 20, 33), the mechanisms by which isolated small pulmonary arteries develop intrinsic tone could be important determinants of vasoreactivity in this tissue.

In general, development of intrinsic tone could involve mechanisms intrinsic to vascular smooth muscle, endothelium, or both. For example, tone could develop because a progressive decrease in the activity of endothelium-derived relaxing factors (EDRFs) unmasked a contraction generated by mechanisms intrinsic to smooth muscle. Alternatively, a progressive increase in the activity of endothelium-derived contracting factors (EDCFs) could cause otherwise quiescent smooth muscle to contract. In isolated systemic arteries where intrinsic tone develops routinely and has long been used as a criterion of preparation acceptability (19, 21, 34), only a few studies have been reported, and these yielded inconsistent results. In coronary arteries, Rubanyi (32) found that development of intrinsic tone depended on the endothelium and elevation of transmural pressure (P\(\text{tm}\)), whereas Cohen et al. (6) found that intrinsic tone was attenuated by the endothelium. In cerebral arterioles, intrinsic tone was decreased by reductions in P\(\text{tm}\) and increased by inhibitors of nitric oxide (NO) synthase (NOS) (19, 34). To our knowledge, the only experiments investigating the mechanisms of intrinsic tone in small pulmonary arteries were those of Madden et al. (27), who demonstrated a requirement for extracellular calcium.

We performed the present study to determine the magnitude and mechanisms of intrinsic tone in porcine pulmonary arteries < 300 µm in diameter. Our results demonstrate that intrinsic constriction in these vessels was time dependent and quantitatively significant, achieving ~35% of maximum possible vasoconstriction at 135 min under control conditions and ~70% of maximum vasoconstriction when modulation by NO and vasodilator prostaglandins was blocked. In addition, our results confirm the dependence of this response on extracellular calcium and suggest that the initial spontaneous tone was generated by mechanisms intrinsic to smooth muscle and modulated by endothelium-derived NO and endothelin (ET)-1, whereas subsequent progressive tone development was due to altered activity of endothelium-derived vasoactive factors other than NO, prostaglandins, hyperpolarizing factors acting on calcium-dependent potassium channels, ET-1, or superoxide anions (O\(_2^\cdot\)). Further investigation is warranted to identify these unknown mechanisms and determine their role in pulmonary vasoreactivity.
METHODS

Preparation of isolated pulmonary arterioles. All protocols and procedures were approved by the Animal Care and Use Committee in accordance with National Institutes of Health guidelines. Male pigs (20–25 kg) were anesthetized with ketamine (30 mg/kg im) followed by pentobarbital sodium (12.5 mg/kg iv). After exsanguination via the femoral arteries, the lungs were rapidly removed and placed in ice-cold Krebs-Ringer bicarbonate 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, pulmonary arteries ~100–150 µm in diameter and 1 mm long were isolated from seventh-generation branches of intrapulmonary arteries. The vessels were placed in a microvascular chamber, cannulated at one end with a glass micropipette, and secured with 12-0 nylon monofilament suture. Krebs-Ringer bicarbonate solution was infused slowly through the pipette until the arteriole was completely filled. The other end of the vessel was then cannulated with a second micropipette prefilled with Krebs-Ringer bicarbonate solution. Both cannulas were connected to a reservoir that could be raised or lowered to control Ptm. Ptm was measured with a pressure transducer positioned at the level of the vessel lumen. In the microvascular chamber, the vessels were superfused with Krebs-Ringer bicarbonate solution (total volume 100 ml) gassed with 16% O2-5% CO2-balance N2 (pH 7.35–7.45), maintained at 37°C, and recirculated except as noted in Experimental protocols. 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 intraluminal diameter (ID) was continuously measured with a video dimension analyzer (Living Systems Instrumentation, Burlington, VT). The ID and Ptm were recorded with a recorder (Gould, Cleveland, OH). In some vessels, endothelial cells were disrupted by gently rubbing the intraluminal surface with a steel wire (70 µm in diameter). These vessels were then perfused with 2 ml of air bubbles followed by 2 ml of Krebs-Ringer bicarbonate solution (perfusion pressure < 5 mmHg) before being mounted in the microvascular chamber.

Experimental protocols. Initially, isolated pulmonary arterioles were allowed to equilibrate in the microvascular chamber for 20 min at a Ptm of 5 mmHg. Ptm was then increased to 20 mmHg in 5-mmHg steps at 5- to 7-min intervals and thereafter held constant. Measurements of the ID at Ptm of 20 mmHg (ID20) began 5 min after the Ptm was increased to 20 mmHg (time 0) and continued until the end of the experiment. This Ptm was chosen because it approximated the normal mean pulmonary arterial pressure in the pig (37). To assess viability, the vessels were exposed to KCl (60 mM) at 20 min and the thromboxane A2 agonist U-46619 (10-6 M) followed by ACh (10-6 M) at 50 min. In each case, the agonists were washed out of the microvascular chamber by 20 min of nonrecirculating superfusion with fresh Krebs-Ringer bicarbonate solution after the responses had stabilized (5–10 min). Thus, during the first 75 min of the experiment, the superfusate was recirculated for 35 min and not recirculated for 40 min. In most arterioles, KCl was administered again at the end of the experiment. In some untreated control arterioles with (Endo+; n = 6) and without (Endo−; n = 6) endothelium, KCl and U-46619-ACh were administered only at the end of the experiment. Control Endo+ (n = 19) and Endo− (n = 8) arterioles, which received no other treatment, were considered acceptable if 1) the contractile responses (decreased ID20) to KCl and U-46619 were ≥30% of the immediately preceding control ID20 and 2) the dilator responses (increased ID20) to ACh were ≥50% (Endo+ vessels) or ≤0% (Endo− vessels) of the U-46619 response.

RESULTS

Table 1 shows the average responses to KCl and U-46619-ACh administered at the beginning of the experiment to test vessel viability. KCl (60 mM) elicited vigorous contractile responses in Endo+ and Endo− arterioles but not in Endo+ arterioles exposed to calcium-free conditions. During exposure to U-46619, ACh (10-6 M) caused vasodilation in all groups except the Endo− arterioles and Endo+ arterioles exposed to L-NAME or calcium-free conditions. When viability testing was delayed until the end of the experiment,
KCl and U-46619 caused contraction in Endo+ and Endo− arterioles, but ACh reversed the U-46619-induced contraction only in Endo+ arterioles (data not shown).

As shown in Fig. 1, ID20 in Endo+ arterioles decreased progressively and spontaneously from 233 ± 11 µm at time 0 to 171 ± 12 µm at the end of the experiment (135 min). This intrinsic constriction did not occur in arterioles exposed to calcium-free conditions in which ID20 remained at its baseline value of 238 ± 10 µm. If this value represented the maximum (fully relaxed) ID20 in these vessels and the minimum (fully constricted) ID20 value was ~35 µm (Table 1), intrinsic vasoconstriction achieved ~35% of maximum possible vasoconstriction at 135 min.

Although ID20 at time 0 was the same in untreated Endo+ arterioles and Endo+ arterioles exposed to calcium-free conditions, it was markedly diminished in Endo− vessels (169 ± 21 µm). This constriction of Endo− arterioles, which began immediately after endothelial denudation under the dissecting microscope, remained constant throughout the experiment. As a result, ID20 was the same in Endo− and Endo+ vessels at 135 min. Similar results were obtained in Endo+ and Endo− arterioles in which viability testing with KCl and U-46619-ACh was delayed until the end of the experiment (Fig. 2).

Indomethacin (10−5 M), an inhibitor of cyclooxygenase (9, 38), did not alter the constrictor responses to KCl or U-46619, the dilator responses to ACh, or the development of intrinsic tone (Table 1, Fig. 3). Treatment with both indomethacin and L-NAME (3 × 10−5 M), an inhibitor of NOS (2, 11), abolished the vasodilator response to ACh (Table 1) and decreased ID20 at time 0 but did not prevent further intrinsic vasoconstriction (Fig. 3). In these vessels, ID20 decreased from 194 ± 17 µm at 0 min to 99 ± 21 µm at 135 min. When 10−4 M L-arginine, a substrate for NOS (11), was included as pretreatment with indomethacin and L-NAME, vasodi-

### Table 1. Changes in pulmonary arteriolar ID20 in response to KCl, U-46619, and ACh at beginning of experiment

<table>
<thead>
<tr>
<th>Endothelium</th>
<th>Treatment</th>
<th>Control</th>
<th>60 mM</th>
<th>Control</th>
<th>10−5 M</th>
<th>10−5 M + ACh (10−4 M)</th>
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<tbody>
<tr>
<td>Intact</td>
<td>None</td>
<td>207 ± 11</td>
<td>50 ± 7†</td>
<td>178 ± 13</td>
<td>99 ± 10†</td>
<td>198 ± 13‡</td>
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<tr>
<td>Denuded</td>
<td>None</td>
<td>154 ± 15</td>
<td>42 ± 7†</td>
<td>165 ± 18</td>
<td>78 ± 17†</td>
<td>73 ± 17</td>
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<tr>
<td>Intact</td>
<td>Indomethacin</td>
<td>215 ± 21</td>
<td>84 ± 16†</td>
<td>201 ± 20</td>
<td>108 ± 11†</td>
<td>208 ± 14</td>
</tr>
<tr>
<td>Denuded</td>
<td>Indomethacin</td>
<td>118 ± 30*</td>
<td>64 ± 12†</td>
<td>149 ± 18</td>
<td>92 ± 12†</td>
<td>88 ± 14</td>
</tr>
<tr>
<td>Intact</td>
<td>Indomethacin+ L-NAME</td>
<td>148 ± 25</td>
<td>56 ± 16†</td>
<td>118 ± 27</td>
<td>74 ± 27†</td>
<td>81 ± 33</td>
</tr>
<tr>
<td>Intact</td>
<td>Indomethacin+ L-NAME + L-arginine</td>
<td>231 ± 14</td>
<td>98 ± 20†</td>
<td>221 ± 15</td>
<td>129 ± 10†</td>
<td>203 ± 8‡</td>
</tr>
<tr>
<td>Intact</td>
<td>L-Arginine</td>
<td>268 ± 29</td>
<td>86 ± 26†</td>
<td>266 ± 25*</td>
<td>162 ± 14†</td>
<td>260 ± 24‡</td>
</tr>
<tr>
<td>Intact</td>
<td>Tetraethylammonium</td>
<td>177 ± 17</td>
<td>87 ± 8‡</td>
<td>162 ± 16</td>
<td>90 ± 11†</td>
<td>187 ± 27‡</td>
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<tr>
<td>Intact</td>
<td>Charybdotoxin</td>
<td>193 ± 18</td>
<td>56 ± 15*</td>
<td>184 ± 17</td>
<td>82 ± 21‡</td>
<td>162 ± 37‡</td>
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<tr>
<td>Intact</td>
<td>BQ-123</td>
<td>258 ± 25</td>
<td>51 ± 12†</td>
<td>217 ± 26</td>
<td>106 ± 20†</td>
<td>216 ± 26‡</td>
</tr>
<tr>
<td>Intact</td>
<td>BQ-788</td>
<td>237 ± 24</td>
<td>59 ± 16†</td>
<td>194 ± 20</td>
<td>72 ± 14‡</td>
<td>223 ± 12‡</td>
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<tr>
<td>Intact</td>
<td>BQ-123 + BQ-788</td>
<td>288 ± 14*</td>
<td>81 ± 17†</td>
<td>226 ± 14</td>
<td>123 ± 13†</td>
<td>259 ± 12‡</td>
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<tr>
<td>Intact</td>
<td>Superoxide dismutase</td>
<td>258 ± 20</td>
<td>35 ± 7†</td>
<td>189 ± 19</td>
<td>79 ± 13†</td>
<td>230 ± 10‡</td>
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<tr>
<td>Intact</td>
<td>Ca2+ free</td>
<td>237 ± 6</td>
<td>236 ± 8</td>
<td>233 ± 5</td>
<td>190 ± 18‡</td>
<td>220 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE. ID20, intraluminal diameter at transmural pressure of 20 mmHg; L-NAME, N⁶-nitro-L-arginine methyl ester. Significantly different (P < 0.05): †for control value vs. intact endothelium control value; ‡compared with control value; ‡compared with value for U-46619.

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**Fig. 1.** Time course of intraluminal diameter at transmural pressure of 20 mmHg (ID20) in isolated pulmonary arterioles with (E+; n = 19) and without (E−; n = 8) endothelium. Arterioles exposed to Ca2+-free conditions (n = 6) were superfused with Ca2+-free physiological salt solution containing 10−3 M EGTA. Data are means ± SE.

**Fig. 2.** Time course of ID20 in isolated E+ and E− pulmonary arterioles (n = 6/group) in which viability testing with KCl and U-46619-ACh was delayed until end of experiment. Data are means ± SE.
In Endo+ arterioles treated with L-arginine alone, ACh caused vasodilation (Table 1) and development of intrinsic tone was abolished (Fig. 4B). TEA, a nonspecific potassium-channel antagonist (8, 17), did not alter intrinsic vasoconstriction (Fig. 5A). CTX, a specific inhibitor of calcium-dependent potassium channels (8, 17), did not alter ID$_{20}$ at 0 min, but it enhanced intrinsic tone at 0–20 min and inhibited intrinsic tone at 70–135 min (Fig. 5A). Neither agent altered the vasoconstrictor response to KCl at the beginning of the experiment (Table 1); however, CTX had abolished this response by the end of the experiment (Fig. 5B).

Figure 6A directly compares Endo+ arterioles pretreated with indomethacin plus L-NAME with Endo− arterioles. Development of intrinsic tone was greater in Endo+ arterioles treated with indomethacin plus L-NAME. Figure 6B shows the effects of delayed administration of L-NAME (75 min) in Endo+ and Endo− arterioles pretreated with indomethacin. L-NAME caused contraction in Endo+ arterioles but had no effect in Endo− arterioles.

As shown in Fig. 7, ET-1 (10$^{-11}$ to 10$^{-8}$ M) caused a concentration-dependent contraction in Endo+ arterioles, which was blocked by the ETA-receptor antagonist BQ-123 (3 × 10$^{-6}$ M) but not by the ET$_B$-receptor antagonist BQ-788 (3 × 10$^{-6}$ M). In arterioles treated with both BQ-123 and BQ-788, high concentrations of ET-1 caused a slight vasoconstriction, but this contractile response was still markedly reduced compared with that in control Endo+ arterioles.

Figure 8 shows the effects of ET-1-receptor antagonists on the time course of ID$_{20}$. BQ-123 (3 × 10$^{-6}$ M) increased ID$_{20}$ at 0 min (262 ± 23 µm); however, it did not alter subsequent intrinsic vasoconstriction (ID$_{20}$ = 225 ± 28 µm at 135 min). BQ-788 (3 × 10$^{-6}$ M) did not alter ID$_{20}$ at 0 min (256 ± 15 µm) but increased subsequent intrinsic vasoconstriction (ID$_{20}$ = 134 ± 21 µm at 135 min). Treatment with BQ-123 and BQ-788...
together increased both ID$_{20}$ at time 0 (284 ± 15 µm) and subsequent constriction (ID$_{20}$ = 178 ± 14 µm at 135 min).

SOD (150 U/ml) did not affect the time course of ID$_{20}$ (Fig. 9).

**DISCUSSION**

Control Endo+ pulmonary arterioles constricted in response to KCl and U-46619 and dilated in response to ACh after precontraction with U-46619 (Table 1). Arterioles subjected to endothelial denudation maintained vigorous constrictor responses to KCl and U-46619 but did not exhibit dilator responses to ACh. These results indicate that contractile function and receptor-linked signal transduction were intact in both Endo+ and Endo− arterioles, whereas endothelial function was intact in only Endo+ arterioles.

As shown in Fig. 1, Endo+ arterioles exhibited progressive constriction, with ID$_{20}$ decreasing from 233 ± 11.0 µm at 0 min to 170 ± 12.0 µm at 135 min. Most of this constriction occurred during the first 75 min of the experiment. Because superfusate was not recirculated for 40 of these 75 min, the decrease in ID$_{20}$ is unlikely to have been caused by a time-dependent change in superfusate composition, such as increasing...
osmolality due to evaporation. Rather, it must have resulted from factors or mechanisms intrinsic to the vessel.

The time course of ID_{20} in Endo+ arterioles differed markedly from that in Endo− arterioles, which were already constricted at 0 min (ID_{20} = 169 ± 21.4 µm) and did not exhibit a significant change in ID_{20} thereafter. Constriction of Endo− arterioles at time 0 is more likely to have resulted from endothelial removal than vascular smooth muscle damage due to the denudation procedure because ID_{20} decreased rapidly in response to KCl and U-46619 and returned promptly to baseline on washout of these agonists, indicating normal smooth muscle function. Furthermore, the differences between Endo+ and Endo− arterioles were present when viability testing was delayed until the end of the experiment (Fig. 2), indicating that they were not the result of transient exposures to KCl, U-46619, or ACh. We conclude that the progressive development of tone seen in control Endo+ pulmonary arterioles was the product of two influences: 1) smooth muscle contraction already established at time 0 by mechanisms intrinsic to smooth muscle and 2) endothelial antagonism of smooth muscle contraction, which was present at time 0 and progressively diminished thereafter.

In Endo+ arterioles exposed to calcium-free conditions, vasoconstrictor responses to KCl were absent (Table 1). This finding is consistent with a previous work (5) that indicated that KCl causes smooth muscle contraction primarily via membrane depolarization and calcium influx through voltage-dependent calcium channels. In contrast, vasoconstriction induced by U-46619, although diminished, was still present in these arterioles (Table 1). This finding is consistent with previous results (7) that suggested that vasoconstrictor responses to U-46619 utilize calcium released from intracellular stores. As shown in Fig. 1, Endo+ arterioles exposed to calcium-free conditions remained dilated throughout the experiment (ID_{20} = 240 ± 16.1 µm), confirming previous studies (13, 15, 27) in both pulmonary and systemic arteries. These results indicate that the smooth muscle or endothelial mechanisms responsible for intrinsic constriction in Endo+ pulmonary arterioles required extracellular calcium.

Studies of the mechanisms responsible for intrinsic tone in systemic arteries have not been conclusive. Some (6, 30) suggested that constriction was caused by mechanisms intrinsic to smooth muscle, whereas others (19, 31) concluded that endothelial mechanisms played a major role. Because short periods of decreased P_{tm} reduced intrinsic tone in cerebral arteries (34), the mechanisms responsible for intrinsic constriction could be similar to those mediating myogenic responses (29). For example, depolarization and calcium influx in smooth muscle due to stretch-induced activation of ion channels (12, 29) could explain the constriction we observed at time 0 in Endo− arterioles. Alternatively, stretch could act on endothelium, altering the release of EDRFs such as NO and vasodilator prostaglandins or EDCF s such as ET, vasoconstrictor prostaglandins, and superoxide anions (12, 18, 29, 32). To evaluate the roles of EDRFs and EDCF s, we determined the effects of 1) indomethacin, an inhibitor of cyclooxygenase, which catalyzes production of prostaglandins from arachidonic acid; 2) L-NAME, an inhibitor of NOS, which catalyzes production of NO from L-arginine; 3) TEA and CTX, antagonists of calcium-dependent potassium channels, which may mediate the vasodilating effects of endothelium-derived hyperpolarizing factors (EDHFs); 4) BQ-123 and BQ-788, antagonists of ET\textsubscript{A} and ET\textsubscript{B} receptors, respectively; and 5) SOD, which catabolizes superoxide anions. As shown in Fig. 3, indomethacin did not alter development of intrinsic tone in pulmonary arterioles. This cyclooxygenase inhibitor has been shown to block prostaglandin production and contractile responses to arachidonic acid in systemic vascular tissue at concentrations less than or equal to that used in the present study (10^{-3} M) (9, 35, 38). Furthermore, indomethacin did not alter either the contractile responses to KCl and U-46619 or the dilator responses to ACh (Table 1), indicating the absence of nonspecific effects. We conclude that prostaglandins did not mediate intrinsic constriction in isolated pulmonary arterioles.

To evaluate the role of the EDRF NO, we determined the effects of L-NAME, an inhibitor of NOS (2, 11). As shown in Table 1, ACh caused a marked dilation in pulmonary arterioles treated with indomethacin but not in arterioles treated with indomethacin plus L-NAME. ACh-induced vasodilation was restored when L-arginine was added to indomethacin plus L-NAME. Vasoconstrictor responses to KCl and U-46619 were present in all three groups. These results indicate that L-NAME inhibited NO synthesis and did not impair contractile function.

The effects of L-NAME on the time course of ID_{20} are shown in Fig. 3. At 0 min, Endo+ arterioles treated with L-NAME plus indomethacin were markedly constricted compared with both untreated and indomethacin-treated Endo+ arterioles; however, L-NAME did not prevent the subsequent progressive decline in ID_{20}. These results indicate that although NO modulated vasomotor tone at time 0, a decrease in NO-mediated relaxation did not cause the progressive vasoconstriction that occurred from 0 to 135 min.

The addition of L-arginine (10^{-4} M) to indomethacin plus L-NAME (Fig. 4A) or treatment with L-arginine alone (Fig. 4B) abolished intrinsic tone. These results suggest that NO production in these vessels was limited by L-arginine concentration and that enhancement of NO production could overcome the mechanisms responsible for intrinsic constriction.

EDHF is an unknown vasodilator that may act by opening calcium-dependent potassium channels in smooth muscle (8); however, 5 mM TEA, which blocks these channels and the effects of EDHF (8, 17), did not alter intrinsic vasoconstriction in pulmonary arterioles (Fig. 5A). CTX, a more specific antagonist of calcium-dependent potassium channels (8), did not inhibit intrinsic constriction (Fig. 5A), but unlike TEA, it also abolished the vasoconstrictor responses to KCl at the end of the experiment (Fig. 5B). These results suggest...
that the effects of CTX were due to nonspecific toxicity. Thus our data provide no evidence that intrinsic vasoconstriction was caused by decreasing activity of an EDHF acting on calcium-dependent potassium channels.

As illustrated in Fig. 6A, intrinsic constriction was more severe in Endo+- arterioles treated with L-NAME plus indomethacin than in untreated Endo− arterioles. This difference could be due to smooth muscle vasodilator mechanisms in Endo− arterioles or unopposed EDCF activity in Endo+ arterioles treated with L-NAME plus indomethacin. To assess the role of smooth muscle type II NOS, which may have been induced by cytokines or other factors (36, 39), we administered L-NAME at 75 min to Endo− and Endo+ arterioles pretreated with indomethacin (Fig. 6B). L-NAME had no effect in Endo− arterioles but caused a marked constriction in Endo+ arterioles. These results indicate that NOS was absent or inactive in smooth muscle.

It seems more likely that the differences shown in Fig. 6A were due to increasing EDCF activity. The magnitude of tone generated by these contractile factors was impressive. Assuming again that arterioles exposed to calcium-free conditions were maximally relaxed (Fig. 1) and that fully constricted arterioles had an ID_{20} of ~35 μm (Table 1), intrinsic vasoconstriction in the absence of modulation by NO and vasodilator prostaglandins (Fig. 6A) achieved ~70% of the maximum potential constriction at 135 min. The EDHFs involved could be ET or superoxide anions (18, 26) but not vasconstrictor prostaglandins. To evaluate these possibilities, we studied arterioles exposed to ET antagonists or SOD.

The ET_{A}-receptor antagonist BQ-123 abolished the constrictor responses to ET-1, whereas the ET_{B}-receptor antagonist BQ-788 (3 × 10^{-6} M) had no effect (Fig. 7). When these antagonists were administered together, constriction to ET-1 was still inhibited, but the vessels were more contracted than after BQ-123 alone. These results indicate that ET-1-induced constriction of porcine pulmonary arterioles was mediated by ET_{A} rather than ET_{B} receptors. In contrast, previous results (16) in rat intrapulmonary arterioles suggested that ET-1-induced vasoconstriction was mediated by both ET_{A} and ET_{B} receptors. This difference may be due to species-dependent differences in receptor density (10). Indeed, in the presence of BQ-123, ET-1 caused vasoconstriction in pig pulmonary arterioles (Fig. 7), suggesting that activation of ET_{B} receptors mediated vasoconstriction, perhaps by increasing release of NO from the endothelium as previously suggested (16).

BQ-123 alone or in combination with BQ-788 increased ID_{20} at 0 min, suggesting that ET_{A} receptor-mediated constriction contributed to the initial vasoconstrictor tone; however, neither BQ-123 nor BQ-788 prevented subsequent progressive constriction (Fig. 8), indicating that this response was not due to activation of ET_{A} or ET_{B} receptors. Indeed, BQ-788 accelerated the decline in ID_{20}, suggesting that ET_{B} receptors exerted a vasodilatory influence that modulated progressive constriction.

At concentrations of 100–200 U/ml, SOD eliminated or markedly reduced release of superoxide anions from vascular tissues (4, 40). In preliminary studies of isolated porcine coronary arterioles exposed to anoxia-reoxygenation, we found that SOD (150 U/ml) caused a 70% decrease in superoxide anion release as assessed by lucigenin-enhanced chemiluminescence (data not shown). However, this concentration of SOD had no effect on the time course of ID_{20} in pulmonary arterioles (Fig. 9), indicating that intrinsic tone was not due to superoxide anions. These results do not eliminate the possibility that intrinsic constriction was mediated by H_{2}O_{2}, the product of SOD-induced catabolism of superoxide anions; however, in this case, SOD would be expected to enhance intrinsic constriction, which did not occur.

The development of intrinsic tone in pig pulmonary arterioles was similar to that described by Madden et al. (27) in cannulated cat pulmonary arterioles 200–600 μm in diameter. The ID measured at a P_{tm} of 10 mmHg decreased spontaneously by 23.9 ± 4.4% over 60–90 min. In contrast, Leach et al. (23) were unable to demonstrate intrinsic tone in rat pulmonary arterioles of similar size. In this study, resting isometric tension, adjusted to be equivalent to a P_{tm} of 30 mmHg, was not altered by exposure to calcium-free conditions or several vasodilator agents. This disparity may be due to differences in species, preparation, experimental conditions, or other factors. For example, it is likely that rat arterioles 200 μm in diameter were obtained from more proximal loci in the pulmonary vasculature than in pig or cat arterioles of the same diameter. Endothelium-dependent vasodilation was reduced or absent in rat pulmonary arterioles (23), whereas Endo+ arterioles that did not dilate at least 50% in response to 10^{-6} M ACh were excluded in our study. The higher resting P_{tm} and the isometric conditions used in the rat arterioles may have activated mechanisms not operative at lower constant levels of P_{tm}. Such considerations also lead to important questions about the role of intrinsic pulmonary arteriolar tone in the intact animal. Further investigation will be necessary to obtain answers.

In summary, our results demonstrate that development of intrinsic tone in isolated pulmonary arterioles was dependent on time and extracellular calcium concentration. Initially, tone was generated by mechanisms intrinsic to vascular smooth muscle and modulated by endothelium-derived NO and ET-1 but not by prostaglandins; however, subsequent progressive constriction could not be explained by these mechanisms. Rather, our results indicate that progressive development of intrinsic tone was caused by 1) decreased activity of an EDRF other than NO, vasodilator prostaglandins, and EDHFs acting via calcium-dependent potassium channels or 2) increased activity of an EDCF other than vasconstrictor prostaglandins, ET-1, or superoxide anions. Additional research is indicated to determine the identity of the unknown mechanisms responsible for intrinsic tone, their role in pulmonary
vasoreactivity, and whether they are unique to pulmonary vessels.

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