Role of the Vascular Endothelium in Regulating the Response of Small Arteries of the Dog Kidney to Transmural Pressure Elevation and Reduced Po2

Hanna Eskinder, David R. Harder, and Julian H. Lombard

The goal of this study was to determine the role of the vascular endothelium in regulating the response of small renal arteries to increased transmural pressure and reduced Po2. Canine small renal arteries (496±32 μm) were isolated, cannulated with micropipettes, and mounted in an in vitro chamber that allowed transmural pressure, tissue bath Po2, and vessel lumen Po2 to be controlled. In intact arteries, elevation of transmural pressure caused contractile activation and a progressive depolarization (0.17±0.09 mV/mm Hg pressure increase) of the vascular smooth muscle cells. Endothelial damage by air perfusion caused a 13–14% reduction in resting diameter and an 18±3 mV depolarization of the vascular smooth muscle, but eliminated the progressive contraction and depolarization that occurred in intact vessels in response to transmural pressure elevation. Reduction of either tissue bath O2 concentration or vessel lumen Po2 significantly enhanced norepinephrine-induced contractions of the arteries. Endothelial damage prevented the enhancement of norepinephrine-induced contractions by reduced Po2 in the vessels. The results of this study suggest that the vascular endothelium regulates both myogenic contraction and the enhancement of norepinephrine-induced contractions by reduced Po2 in small arteries of the dog kidney. (Circulation Research 1990;66:1427–1435)

In 1902, Bayliss1 proposed that changes in active vascular smooth muscle tone could be mediated via an intrinsic reaction of blood vessels to changes in the amount of stretch on the vascular wall. This hypothesis was subsequently challenged by von Anrep,2 who hypothesized that the changes in vessel tone observed by Bayliss resulted from the action of “asphyxial products” on the blood vessel, rather than a direct effect of pressure or stretch on the vascular wall.

Since the original experiments of Bayliss and von Anrep, numerous studies have demonstrated that changes in both transmural pressure3–7 and local oxygen availability8–14 can affect contractile force in individual blood vessels. Although myogenic contraction in response to transmural pressure elevation appears to be confined to small arterioles in some vascular beds3, recent studies of isolated vessels have demonstrated that myogenic contractile responses to increased transmural pressure occur in larger arteries of the cerebral,4,5,15,16 renal,6 and skeletal muscle17 vascular beds. However, the exact mechanisms of myogenic activation and the direct effects of reduced Po2 on active tone in small arteries have not been completely characterized.

The development of techniques for the study of isolated, cannulated segments of very small arteries18,19 has allowed the regulation of contractile force in these vessels to be studied in the complete absence of parenchymal cell influences and under conditions of circumferential stress that are similar to those encountered in situ. These techniques are also well suited for studies of the role of the vascular endothelium in regulating contractile force in small arteries. Studies of vessel responses to changes in transmural pressure and oxygen availability before and after impairment of endothelial function can provide valuable information concerning the regulation of contractile force in these vessels, because several studies have recently suggested that vessel responses to both increased transmural pressure15,20,21 and reduced oxygen availability8,10,14 may be mediated via effects on the vascular endothelium.

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The object of the present study was to investigate the role of the vascular endothelium in regulating the response of canine small renal arteries to increased transmural pressure and reduced Po2. In this study, we tested three hypotheses: 1) myogenic activation of small arteries of the dog kidney in response to transmural pressure elevation is mediated via the vascular endothelium; 2) reductions in Po2 can directly modulate active contractile force in small renal arteries, independent of parenchymal cell influences; and 3) the effects of reduced Po2 on small arteries of the dog kidney are mediated via the vascular endothelium. The results of these experiments suggest that 1) the vascular endothelium either mediates or indirectly modulates the myogenic contractile activation and vascular smooth muscle depolarization that occurs in small arteries of the dog kidney in response to transmural pressure elevation, and 2) low Po2 significantly enhances norepinephrine-induced contractions of these vessels via an effect on the vascular endothelium.

Materials and Methods

General Procedures

Kidneys were removed from mongrel dogs (9–16 kg) of either sex that had been anesthetized with sodium pentobarbital (35 mg/kg i.v.) before tissue samples for these and other protocols were obtained. The kidneys were immediately placed in physiological salt solution and transported to the laboratory for isolation of small arteries. Segments of small arteries were gently dissected from the parenchyma and cleaned of adhering connective tissue.

In our initial experiments (designated as single-cannulated vessel experiments), the proximal end of the selected vessel was cannulated with a 100–200-μm diameter tapered glass micropipette and tied in place with 10-0 (22-μm) nylon suture. The micropipette generally extended about 0.5 mm into the vessel lumen, allowing a 2–4-mm segment of vessel to be pressurized. After the proximal end of the vessel was cannulated, any blood remaining in the lumen was removed by gentle flushing with physiological salt solution. The distal end of the artery was then tied off with 10-0 suture and fixed in a pair of plastic jaws. The cannulating micropipette was connected to a sealed reservoir system containing physiological salt solution that had been equilibrated with a 93% O2–7% CO2 gas mixture. This reservoir system allowed transmural pressure in the vessel segment to be precisely controlled. After the artery was cannulated and fixed in the plastic jaws, any branches were tied off, and the artery was checked for possible leaks by determining if pressure in the vessel decreased after the initial pressurization. A micrometer connected to the micropipette mounting block was then used to lengthen the vessel in order to remove any buckling at the control equilibration pressure (100 mm Hg).

In a subsequent series of experiments (double-cannulated vessel experiments), both ends of the vessel were cannulated with micropipettes. The double cannula system could serve as either a flow-through system (when the outflow cannula was open) or a single-ended system (when the outflow cannula was closed). Use of the double-cannulated preparation allowed the luminal Po2 to be controlled independently of the tissue bath Po2 (see below) and allowed the endothelium to be removed or damaged by air perfusion without untying and subsequent religation of the vessel. This allowed the length of the vessel to be maintained at a constant value and avoided any changes in the mechanical state that could occur in single-cannulated arteries after being untied, perfused with air, and subsequently religated.

During the course of the experiment, the arteries were continuously superfused (7 ml/min) with physiological salt solution that had the following constituents in millimolar concentrations: Na+ 143.6, K+ 4.5, Ca2+ 2.3, Mg2+ 0.7, H2PO4 1.2, HCO3 22.8, Cl− 124.2, glucose 5.5, and HEPES 4.6. The pH of the superfusion solution in the vessel chamber was 7.35, and the temperature of the superfusate was maintained at 37°C via a circulating, water-jacketed bath.

In the single-cannulated vessel experiments, superfusion solution Po2 was controlled by equilibrating the supply reservoir and the vessel chamber with gas mixtures containing 93% O2, 10% CO2 (one experiment), or 0% O2 with 7% CO2 and the balance N2. Vessel chamber Po2 was monitored with an oxygen electrode (Microelectrodes, Inc, Londonderry, New Hampshire) and closely corresponded to the oxygen content of the gas equilibration tank. In the double-cannulated vessel experiments, the external superfusion solution was kept equilibrated with the high-oxygen gas mixture (93% O2–7% CO2) for the entire experiment, while the lumen of the vessel was continuously perfused with physiological salt solution that was equilibrated with either 93% O2–7% CO2 or 0% O2–7% CO2–93% N2.

Diameter Measurements

The arteries were observed through a Carl Zeiss Model DRC stereomicroscope (Thornwood, New York), fitted with a television camera (model KP-130 AU, Hitachi Denshi America, Woodbury, New York) connected to an RCA Model TC 115 video monitor (Lancaster, Pennsylvania) and a Quasar Model VH 5151YW VHS video recorder (Franklin Park, Illinois). Total magnification of the system was ×250. Vessel diameters were measured throughout the experiment with a movable reference line system operated via a modified Colorado Model 321 video analyzer (Boulder, Colorado).

Electrical Measurements

Vascular smooth muscle transmembrane potentials were measured with glass microelectrodes filled with 3 M KCl and connected to a high impedance biological amplifier (Dagan Cell Explorer, Dagan
Instruments, Minneapolis, Minnesota). The electrodes used in these experiments had impedances of 30–60 MΩ, estimated tip sizes of 0.1–0.2 μm, and tip potentials of <3 mV. Criteria for a successful impalement included an abrupt drop in potential to a steady value for 6–10 seconds and an abrupt return to the original baseline when the electrode left the cell. Because of the difficulty of maintaining impalements, reported values for transmembrane potential represent multiple impalements in each vessel segment.

Endothelial Damage

The effects of endothelial damage or removal on pressure-dependent myogenic responses or norepinephrine-induced contractions of the arteries at high and low Po2 were determined in two different series of experiments. In the initial series of experiments, the effects of endothelial impairment were tested in vessels that were cannulated with single micropipettes. In those experiments, control responses were obtained in the presence of the vascular endothelium. The end of the vessel opposite the micropipette was then freed from the plastic jaws and untied. The endothelium was damaged by perfusing the vessel lumen with a 2-ml bolus of air, and the vessel lumen was refilled with physiological salt solution. The open end of the artery was then religated with 10-0 suture and secured in the plastic jaws. After the artery was remounted in the chamber, the effectiveness of endothelial removal was assessed by contracting the vessel with norepinephrine and verifying the lack of an acetylcholine-induced dilation (see below). When the dilator response to acetylcholine was effectively eliminated, the artery was again equilibrated for 1 hour at 100 mm Hg before the experimental protocols (transmural pressure elevation or norepinephrine superfusion at high and low Po2) were repeated.

In the second series of experiments, control responses to transmural pressure elevation or norepinephrine superfusion were obtained in double-cannulated arteries. The endothelium was then removed or damaged by perfusing the vessel lumen with an air bolus through the two micropipettes. This procedure allowed us to impair endothelial function without untying the artery and changing its length and mechanical state. After air perfusion, the lumen of the vessel was refilled with physiological salt solution, and the effectiveness of endothelial removal or damage was verified by testing for acetylcholine-induced dilation as described below. If the arteries failed to dilate in response to acetylcholine, vessel responses to transmural pressure elevation or norepinephrine superfusion at high and low Po2 were repeated. In all cases, results obtained with double-cannulated vessels were similar to those obtained with single-cannulated vessels.

The effectiveness of endothelial removal or damage was assessed pharmacologically by measuring the amount of dilation that occurred in response to 1 μM acetylcholine superfusion in arteries that had been previously contracted with 1 μM norepinephrine.

<table>
<thead>
<tr>
<th>TABLE 1. Effect of Reduced Superfusion Solution Po2 on Contraction of Small Renal Arteries in Response to 1 μM Norepinephrine Before and After Endothelial Damage</th>
</tr>
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<tbody>
<tr>
<td>Superfusate O2 concentration</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>+Endothelium (n=6)</td>
</tr>
<tr>
<td>Control diameter (μm)</td>
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<tr>
<td>Diameter with 1 μM norepinephrine (μm)</td>
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<tr>
<td>Diameter change (μm)</td>
</tr>
<tr>
<td>−Endothelium (n=6)</td>
</tr>
<tr>
<td>Control diameter (μm)</td>
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<tr>
<td>Diameter with 1 μM norepinephrine (μm)</td>
</tr>
<tr>
<td>Diameter change (μm)</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. *Significantly greater (p<0.05) than diameter change during 93% O2 superfusion (paired t test).

Before endothelial removal, acetylcholine caused vessel diameters to increase by 146±34 μm in the single-cannulated arteries and 236±45 μm in the double-cannulated arteries. After endothelial damage, mean diameter changes (±SEM) in response to acetylcholine were 8±7 μm and −2±4 μm in single- and double-cannulated vessels, respectively.

In most cases, a single air bolus completely eliminated the dilation of the vessels in response to acetylcholine. However, in a few vessels, an additional air perfusion had to be performed to completely eliminate the endothelium-dependent dilator response to acetylcholine. In either case, air perfusion had no significant effect on vessel contraction in response to 1.0 or 0.1 μM norepinephrine (Tables 1 and 2, “Norepinephrine Responses”) or 30 mM K+, indicating that this procedure effectively removes endothelial influences with minimal damage to the vascular smooth muscle cells of these arteries.

Myogenic Responses

In our initial series of experiments, single-cannulated vessels were equilibrated for 1 hour under 93% O2 superfusion at a transmural pressure of 100 mm Hg. The equilibration pressure of 100 mm Hg was chosen to approximate the normal pressure in the artery and would apply if there was no pressure gradient between the renal artery and interlobular artery in the dog.22 In these experiments, myogenic responses and norepinephrine-induced contractions (see “Norepinephrine Responses”) were quite consistent at the 100 mm Hg equilibration pressure. However, it is important to note that pressure relations along the renal vascular tree may be different in other species, such as the rat.23 Any such differences in perfusion pressure could be important in evaluating vessel responses to excitatory stimuli at different branching orders of the renal circulation.
TABLE 2. Effect of Reduced Luminal Po2 on Contraction of Small Renal Arteries in Response to 0.1 μM Norepinephrine Before and After Endothelial Damage

<table>
<thead>
<tr>
<th>Condition</th>
<th>Luminal 02 concentration</th>
<th>93% O2</th>
<th>0% O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Endothelium (n=11)</td>
<td>Control diameter (μm)</td>
<td>529±69</td>
<td>411±36</td>
</tr>
<tr>
<td></td>
<td>Diameter with 0.1 μM norepinephrine (μm)</td>
<td>516±50</td>
<td>356±37</td>
</tr>
<tr>
<td></td>
<td>Diameter change (μm)</td>
<td>-44±7</td>
<td>-86±11*</td>
</tr>
<tr>
<td>-Endothelium (n=5)</td>
<td>Control diameter (μm)</td>
<td>381±42</td>
<td>372±33</td>
</tr>
<tr>
<td></td>
<td>Diameter with 0.1 μM norepinephrine (μm)</td>
<td>332±39</td>
<td>327±32</td>
</tr>
<tr>
<td></td>
<td>Diameter change (μm)</td>
<td>-50±11</td>
<td>-45±9</td>
</tr>
<tr>
<td></td>
<td>Diameter with 1 μM norepinephrine (μm)</td>
<td>166±32</td>
<td>172±19</td>
</tr>
<tr>
<td></td>
<td>Diameter change (μm)</td>
<td>-198±10†</td>
<td>-200±20†</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM.
*Significantly greater (p<0.05) than diameter change during 93% O2 perfusion.
†Significantly greater (p<0.05) than response to 0.1 μM norepinephrine.

After the equilibration period, the pressure in the system was released and vessel diameters (or transmembrane potentials) were measured as transmural pressure was increased in 20 mm Hg increments between 0 and 160 mm Hg. All measurements were made at least 5 minutes after transmural pressure was changed to the next level. At the end of the experiment, the vessel was returned to its equilibration pressure (100 mm Hg) and treated with a calcium-free relaxing solution containing 20 mM Mg2+ and 1 mM EGTA. After 5–10 minutes of incubation in the relaxing solution (equilibrated with 93% O2–7% CO2), a passive pressure diameter curve was obtained over the same pressure range previously studied in physiological salt solution. The latter curve was used to assess the amount of active tone in the artery at each level of transmural pressure during the control myogenic activation curve.

The effect of endothelial damage on myogenic activation of the arteries was tested by equilibrating intact vessels for 1 hour at 100 mm Hg transmural pressure during 93% O2 superfusion and then measuring vessel diameters or vascular smooth muscle transmembrane potentials as transmural pressure was increased as described above. After the initial myogenic activation protocol was completed in the intact vessel, the endothelium was removed or damaged by perfusing the lumen of the artery with an air bolus; we used one of the two protocols (single- or double-cannulated vessels) described in “General Procedures.” The myogenic protocol was then repeated in the absence of endothelial influences. After pressure-diameter curves were obtained in vessels with endothelial damage, the arteries were superfused with calcium-free relaxing solution to obtain the passive pressure-diameter curve in the maximally relaxed vessels.

Norepinephrine Responses

In our initial experiments, the effect of endothelial removal on the contraction of single-cannulated vessels in response to 1 μM norepinephrine was determined during 93% O2 and 0% O2 superfusion. In those experiments, vessel chamber Po2 was reduced, but no attempt was made to equilibrate the luminal perfusate with the low-oxygen gas mixture. In a subsequent series of experiments, the amplitude of vasoconstriction in response to 0.1 μM norepinephrine superfusion was measured before and after reduction of luminal Po2 in double-cannulated vessels. In the latter experiments, the external solution in the vessel chamber was continuously equilibrated with the high-oxygen (93% O2–7% CO2) gas mixture to establish that the potentiation of norepinephrine-induced contractions by reduced Po2 was mediated via specific effects on the vascular endothelium.

As described in detail in “Results,” removal or damage of the endothelium caused a significant contraction of the small renal arteries used in these experiments. Therefore, it was important to verify that the lack of potentiation of norepinephrine-induced contractions by reduced Po2 after endothelial damage in our single-cannulated vessel experiments did not occur because the vessels were maximally contracted due to the combined effects of endothelial removal and a high dose of norepinephrine. To eliminate this possibility, a lower contraction of norepinephrine (0.1 μM) was used in the double-cannulated vessel experiments. Because 0.1 μM norepinephrine produced far less contraction of the arteries before and after endothelial damage than the 1.0 μM norepinephrine superfusion used in the single-cannulated vessel experiments, any failure of reduced Po2 to enhance norepinephrine-induced contractions of double-cannulated vessels with impaired endothelial function could not be attributed to a lack of reserve capacity for vasoconstriction in the vessels.

In both series of experiments, the effects of reduced Po2 on norepinephrine-induced contractions were measured at a transmural pressure of 100 mm Hg in arteries with an intact endothelium. The effect of reduced Po2 was then measured in arteries in which the endothelium had been intentionally damaged by the air perfusion procedure described in “Endothelial Damage.” In a few experiments, the effect of reduced Po2 on norepinephrine-induced contractions was also tested in arteries that exhibited a strong constriction in response to norepinephrine but failed to dilate in response to acetylcholine superfusion during the initial test for integrity of the vascular endothelium. The latter arteries, which had been stored overnight, were used because fresh vessels were not available.
Data Analysis

The effect of endothelial removal on pressure-induced myogenic activation was analyzed via a two-way analysis of variance for repeated measures followed by a Duncan’s multiple range test to determine significant differences between mean values for different treatment groups at specific transmural pressures. Differences between mean values obtained before and after various treatments (e.g., endothelial removal or low-oxygen superfusion) in the same vessels were analyzed via a paired Student’s t test, whereas differences between any two means obtained from different vessels were analyzed via an unpaired Student’s t test. In the electrophysiology experiments, the correlation between vascular smooth muscle transmembrane potential and intraluminal pressure was analyzed via standard regression analysis techniques. All data were expressed as mean±SEM. A value of p<0.05 was considered to be statistically significant.

Results

Myogenic Activation in Small Renal Arteries

Figure 1 shows typical responses of single- and double-cannulated small renal arteries to progressive increases in transmural pressure before and during superfusion with calcium-free relaxing solution. During superfusion with normal physiological salt solution, vessel diameters increased passively as transmural pressure was elevated in the lower part of the pressure range (0–60 mm Hg). However, as transmural pressure was increased beyond approximately 60 mm Hg, the arteries either maintained their diameter or constricted as myogenic activation began to oppose the tendency for diameters to increase in response to transmural pressure elevation. Superfusion with calcium-free relaxing solution caused the arteries to dilate and become passive, that is, to exhibit large increases in diameter for each increment in transmural pressure between 0 and 160 mm Hg. Vessel responses to transmural pressure elevation were similar in single- and double-cannulated vessels.

Elevation of transmural pressure also caused a progressive depolarization of the smooth muscle cells in small renal arteries with an intact endothelium (Figure 2). In these experiments, vascular smooth muscle transmembrane potential was significantly correlated with transmural pressure (r=0.88, p<0.05). The slope of the relation was 0.17±0.09 mV depolarization/mm Hg pressure increase.

Effect of Endothelial Damage on Resting Tone and Myogenic Activation

Impairment of endothelial function by air perfusion caused a significant depolarization and contraction of the vascular smooth muscle at the equilibration pressure of 100 mm Hg. After removal or damage of the endothelium, vascular smooth muscle transmembrane potentials depolarized by 18±3 mV (n=5), and arterial diameters decreased by 13±6%
In experiments with single-cannulated vessels \( (n=5) \) and \( 14\pm3\% \) in experiments with double-cannulated vessels \( (n=5) \).

Although impairment of endothelial function increased resting tone in the arteries, it eliminated myogenic contractile activation of the vessels in response to increases in transmural pressure. After endothelial damage, arterial diameters increased with each step of elevation in transmural pressure, rather than remaining constant or contracting as in vessels with an intact endothelium (Figure 3). Arterial responses to transmural pressure elevation before and after endothelial damage were similar in both the single-cannulated (Figure 3, upper panel) and double-cannulated (Figure 3, lower panel) vessels.

Endothelial damage also eliminated the depolarization of the vessels in response to transmural pressure elevation (Figure 4). In the latter experiments, vascular smooth muscle transmembrane potential did not change significantly \( (r=0.17, \ p>0.05) \) as transmural pressure was increased from 0 through 160 mm Hg after impairment of endothelial function by air perfusion.

**Effect of Reduced \( \text{PO}_2 \) on Norepinephrine-Induced Contractions Before and After Endothelial Damage**

The effect of reduced superfusion solution \( \text{PO}_2 \) on norepinephrine-induced contractions of single-cannulated arteries with and without impaired endothelial function is summarized in Table 1. In those experiments, reduction of superfusion solution \( \text{PO}_2 \) significantly enhanced vessel contraction in response to 1 \( \mu \text{M} \) norepinephrine in vessels with an intact endothelium. Norepinephrine-induced contractions were not enhanced by reduced superfusate \( \text{PO}_2 \) in vessels in which the endothelium had been removed or damaged via air perfusion, or in vessels that failed to respond to acetylcholine without intentional damage to the endothelium.

Table 2 summarizes the effects of luminal perfusion with reduced \( \text{PO}_2 \) solution on the contraction of double-cannulated vessels in response to 0.1 \( \mu \text{M} \) norepinephrine before and after endothelial damage. The lower concentration of norepinephrine used in these experiments produced substantially less contraction of the arteries before and after endothelial damage than the 1.0 \( \mu \text{M} \) norepinephrine solution used in our initial experiments with single-cannulated vessels (described above). Even though external (superfusion solution) \( \text{PO}_2 \) was maintained at a high value throughout the experiments, perfusion of the vessel lumen with reduced \( \text{PO}_2 \) solution still enhanced norepinephrine-induced contractions of the arteries when the endothelium was intact. However, the enhancement of norepinephrine-induced contractions of the vessels by reduced \( \text{PO}_2 \) was eliminated...
when the endothelium was removed or damaged by air perfusion.

Finally, to determine whether the potentiation of norepinephrine-induced contractions might occur at hypoxic (rather than anoxic) PO₂ levels, we performed one experiment in which the response of a single-cannulated artery to 0.1 μM norepinephrine was tested when the tissue bath was equilibrated with 10% O₂ as well as 0% O₂. In that experiment, 10% O₂ produced an intermediate (relative to 0% O₂) and reversible enhancement of the norepinephrine-induced contraction of the vessel (Figure 5).

Discussion

The present experiments and other studies⁶ demonstrate that isolated small arteries of the dog kidney exhibit myogenic activation and vascular smooth muscle depolarization in response to increases in transmural pressure. This myogenic activation and vascular muscle depolarization are similar to the calcium-dependent depolarization and contraction that occur in response to transmural pressure elevation in the cat middle cerebral artery.⁵ As in previous studies of cerebral and renal arteries, superfusion of the vessel with calcium-free relaxing solution eliminated active tone in the arteries and caused vessel diameters to increase passively in response to increases in transmural pressure (Figure 1).

Recent studies suggest that the endothelium may be important in mediating the contractile responses of blood vessels to different excitatory stimuli, including stretch, transmural pressure elevation,⁵,¹⁵,²⁰,²¹ and the enhancement of agonist-induced contractions by reduced PO₂.⁶,¹⁴ Although there is evidence that stretch-induced contraction of some blood vessels is independent of the vascular endothelium,⁶ myogenic responses in a number of other vessels (e.g., cat middle cerebral artery,¹⁵ canine basilar artery,²⁵ and dog carotid artery²⁹) all appear to be endothelium dependent. However, the role of the endothelium in mediating myogenic responses of small renal arteries has not been investigated to date.

The present study indicates that pressure-dependent myogenic activation in small arteries of the dog kidney is either directly mediated or indirectly modulated by the vascular endothelium. After removal of the endothelium in these experiments, vessel diameters progressively increased as transmural pressure was elevated, and the depolarization of the vascular smooth muscle cells in response to the increases in transmural pressure was eliminated.

Although removal or damage of the endothelium eliminated the progressive myogenic activation of the arteries in response to transmural pressure elevation, it did not eliminate active tone in the vessels, because they depolarized and contracted in response to impairment of endothelial function per se. The increase in active vascular smooth muscle tone after endothelial damage in these experiments and the results of cascade perfusion experiments utilizing arterial segments from other vascular beds²⁷–²⁹ are both consistent with the hypothesis that there is a basal production and spontaneous release of endothelium-derived relaxing substance(s) in some blood vessels, including small arteries of the dog kidney.

The increase in resting tone in the vessels and the contraction of the arteries in response to norepinephrine superfusion after endothelial removal (Tables 1 and 2) indicate that the loss of myogenic activation in these vessels after air perfusion does not result from severe damage to the vascular smooth muscle cells or a nonspecific inhibition of the ability of the vessels to contract. It is also important to note that although endothelial damage per se led to a decrease in the resting diameter of the vessels, the lack of a myogenic response in arteries with impaired endothelial function did not reflect an inability of the vessels to contract further from their resting diameter, because arteries with impaired endothelial function exhibited substantial additional contraction in response to norepinephrine superfusion (Tables 1 and 2).

The loss of pressure-induced myogenic activation that we observed after endothelial damage in the present experiments suggests that myogenic activation of small renal arteries may be directly mediated via the vascular endothelium (probably through the release of an endothelium-derived contracting factor). This hypothesis is supported by recent studies²¹
that suggest that transmural pressure elevation releases a transferable contractile factor from cerebral arteries that have an intact endothelium, but not from vessels from which the endothelium has been removed.

On the other hand, it is also possible that the depolarization and contraction of the arteries after removal of the endothelium indirectly inhibits the electrical and mechanical responses of the vessels to transmural pressure elevation. Such an indirect effect of endothelium removal could occur via several different mechanisms. For example, the depolarization that occurs in response to removal of the endothelium could prevent further depolarization in response to transmural pressure elevation and perhaps cause voltage inactivation of membrane channels involved in myogenic activation of the vascular smooth muscle. The contraction of the arteries after removal of the endothelium could also alter the mechanical signals (e.g., increases in wall stress) produced in the arterial wall during transmural pressure elevation. Finally, it is possible that the process of endothelial removal could cause subtle damage to specific muscular elements involved in myogenic activation without measurably inhibiting vessel contraction in response to 30 mM K+ or norepinephrine. Thus, although the vascular endothelium appears to be a key determinant of the myogenic response of small renal arteries to transmural pressure elevation, the question of whether it directly mediates or indirectly modulates myogenic activation of these vessels has yet to be resolved.

Another goal of this study was to investigate the effect of reduced oxygen availability on norepinephrine-induced contractions of small arteries of the dog kidney and to evaluate the role of the vascular endothelium in determining the response of these vessels to reduced PO2. It is important to obtain additional information concerning the response of small arteries to changes in oxygen availability, because some studies have suggested that arterioles that are isolated from their parenchymal cell environment exhibit changes in active tone in response to altered PO2 while other studies do not support the hypothesis that arterioles are intrinsically sensitive to changes in oxygen availability. Moreover, larger blood vessels exhibit substantial differences in their response to changes in PO2. For example, reduced PO2 inhibits myogenic activation of cat middle cerebral artery, spontaneous contractions of isolated portal veins, and agonist or potassium-induced contractions of some vessels, but potentiates the contraction of other vessels in response to vasoactive agonists.

The effects of decreased oxygen availability on contractile force in isolated blood vessels could be mediated via the action of reduced PO2 on either the vascular smooth muscle cells or the endothelial cells. The liberation of different chemical mediators from endothelial cells in response to changes in oxygen availability could explain the heterogeneity of vessel responses to reduced PO2. Although some investigators have suggested that the inhibitory and excitatory effects of reduced oxygen availability on vascular smooth muscle are mediated via the endothelium, others have concluded that vessel relaxation in response to reduced PO2 does not depend on the vascular endothelium.

In this study, reducing vessel chamber PO2 or selectively perfusing the vessel lumen with reduced PO2 solution while external (superfusate) PO2 was equilibrated with a high-oxygen gas mixture both caused a significant enhancement of norepinephrine-induced contractions of vessels with an intact endothelium. Although most of our experiments investigated the effects of very low PO2 levels on norepinephrine-induced contractions of the vessels, the intermediate (relative to 0% O2) and reversible increase in the amplitude of norepinephrine-induced contraction that occurred during 10% O2 superfusion (Figure 5) suggests that the enhancement of norepinephrine-induced contractions of the arteries by reduced PO2 does not occur solely under anoxic conditions, but may also occur during less severe reductions in PO2. The latter hypothesis is consistent with the results of Dhein et al. who reported that reduction of luminal PO2 from 700 mm Hg to 150 mm Hg in perfused rabbit aorta caused the liberation of an endothelium-derived contracting factor that depended on the phospholipase A2 and cyclooxygenase pathways for its synthesis or release.

In our experiments, the enhancement of norepinephrine-induced contractions of small renal arteries by reduced chamber or luminal PO2 was completely abolished by removal or damage of the vascular endothelium. The obligatory role of the endothelium in the enhancement of norepinephrine-induced contractions of small renal arteries by reduced PO2 is consistent with the results of previous studies of large vessels, where potentiation of agonist-induced contractions by reduced PO2 in canine femoral and coronary arteries also depends on the vascular endothelium.

We think that the endothelium-dependent enhancement of norepinephrine-induced contractions of small renal arteries by reduced PO2 that we observed in the present studies is probably due to the release of an endothelium-derived contracting factor, as suggested by previous studies of other vessels. However, because norepinephrine releases endothelium-derived relaxing factor (EDRF) via an action on α2-receptors in the endothelial cells and EDRF synthesis is inhibited by reduced PO2, the potentiation of norepinephrine-induced contractions by reduced PO2 could also reflect an inhibition of the synthesis or release of EDRF in response to reduced oxygen availability. According to the latter hypothesis, EDRF released by the action of norepinephrine on α2-receptors in the endothelial cells would exert an inhibitory influence on norepinephrine-induced contractions during high PO2 perfusion but not during low PO2 perfusion, when its synthesis would be inhibited.
In summary, the present experiments demonstrate that the myogenic activation of small renal arteries in response to transmural pressure elevation and the enhancement of norepinephrine-induced contractions of these vessels by reduced Po2 both depend on the presence of the vascular endothelium. Although the precise mechanisms by which the endothelium regulates vessel responses to transmural pressure elevation and reduced Po2 remain to be determined, our observations suggest the possibility that the vascular endothelial cells may be the sensors for both increased transmural pressure and reduced oxygen availability in small arteries of the dog kidney.

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