Impaired Endothelium-Dependent Relaxations in Rabbits Subjected to Aortic Coarctation Hypertension

MARK J. S. MILLER, ALDO PINTO, AND KEVIN M. MULLANE

SUMMARY Rabbits were rendered hypertensive by suprarenal coarctation of the abdominal aorta. Seven days later, endothelium-dependent and endothelium-independent vascular relaxations were examined in vascular rings taken from hypertensive (thoracic aorta, carotid artery) and normotensive (abdominal aorta) regions. Relaxation of phenylephrine-contracted rings in response to endothelium-dependent agonists (acetylcholine, A23187) was impaired, compared with that in sham-operated and intact controls, in regions exposed to the elevated blood pressure (i.e., above the coarctation). Responses to acetylcholine and A23187 in the abdominal aorta, below the coarctation, were not altered. The diminished endothelium-dependent responses in the thoracic aorta were not affected by pretreatment with the cyclooxygenase inhibitor indomethacin. In contrast to acetylcholine and A23187, responses to the endothelium-independent agonist nitroprusside were not attenuated in vessels from hypertensive regions, indicating that the defect occurred in the endothelium. The EC50 for acetylcholine-induced relaxations of thoracic aorta correlated significantly with mean arterial pressure above the coarctation, indicating that the extent to which endothelium-dependent relaxation is impaired is in proportion to the degree of blood pressure elevation. This study suggests that the diminished relaxations by endothelium-dependent agonists is a local response to the elevation of blood pressure and is not due to a circulating factor. (Hypertension 10: 164—170, 1987)

KEY WORDS • endothelium • vascular relaxation • hypertension • endothelium-dependent relaxing factor • acetylcholine • nitroprusside

THE seminal observation by Furchgott and Zawadzki1 that the presence of the endothelium is essential for the relaxant effects of acetylcholine precipitated a deluge of studies demonstrating mediation of the vascular effects of a variety of autacoids by the endothelium, in a host of different blood vessels in vitro (see References 2-4 for review). Removal of the endothelial layer could ablate the relaxant properties of agents such as adenosine 5'-diphosphate or substance P or convert them to weak contractions, as observed with acetylcholine or thrombin, as well as enhance the contractile activity of agonists such as serotonin and norepinephrine.5,7 The loss of the endothelial lining of blood vessels is a rare event in vivo. More common are changes in endothelial cell morphology and function that accompany a variety of cardiovascular diseases8,9 and may be linked to alterations in responsiveness of the vessel to vasodilator and vasoconstrictor agents.

One to 2 weeks after induction of deoxycorticosterone acetate (DOCA)-salt hypertension there is an increase in endothelial cell number and altered morphology.8 In focal areas the endothelial cells frequently increase in height, assuming a cuboidal or columnar shape.10 These morphological changes are accompanied by functional aberrations, for example, an increase in vascular permeability.8,12 There is a decrease in the endothelium-dependent relaxations induced by acetylcholine or A23187 in aortic rings from genetically hypertensive rats of the New Zealand strain13 and in one-kidney, one clip and DOCA-salt models of experimental hypertension.14 Vessels from spontaneously hypertensive rats (SHR) display similar defects, al-
though they are less apparent than those in the New Zealand strain.15

It is not clear if any impairment in vasodilator responsiveness can be attributed to local, hypertension-induced endothelial impairment or to alterations in circulating mediators or factors elaborated by organs such as the kidney or brain, which can produce generalized changes in systemic vascular reactivity.16 Accordingly, we investigated the endothelium-dependent relaxations to acetylcholine and A23187 in blood vessels taken from rabbits with coarctation-induced hypertension. This model of hypertension has distinct advantages in that the responsiveness of blood vessels above the coarctation that are exposed to elevated blood pressure can be compared with that of segments of vessels below the coarctation where blood pressure does not exceed normotensive levels. By this means, changes in reactivity as a result of the local hypertension can be differentiated from general changes in overall responsiveness that would be expected to occur in response to circulating factors. This study indicates that the diminished relaxations by endothelium-dependent agonists occur only in segments of vessels taken from above the coarctation (i.e., hypertensive regions).

Materials and Methods

Suprarenal Aortic Coarctation Hypertension

Hypertension was induced according to the methods of Bevan et al.17 Male New Zealand white rabbits (weight, 2.5–2.8 kg; H.A.R.E., Hewitt, NJ, USA) were anesthetized intramuscularly with xylazine (2 mg/kg), ketamine hydrochloride (75 mg/kg), and acepromazine (0.7 mg/kg), and a midline laparotomy was performed. The aorta between the celiac and superior mesenteric arteries was cleared from surrounding adipose tissue, and a silver screw clamp was positioned. The aorta was constricted to a standard level (75%), reducing blood pressure distal to the clip to less than 30 mm Hg. The incision was closed layer by layer, and the screw clamp was positioned. The aorta was constricted to a standard level (75%), reducing blood pressure distal to the clip to less than 30 mm Hg. The incision was closed layer by layer, and the screw clamp was positioned. A maximum of four rings were used from each vessel. As such, four rings were equilibrated for 90 minutes in Krebs bicarbonate solution (pH = 7.4), which was aerated continuously with 95% O2/5% CO2. The composition of the Krebs bicarbonate solution was (mM) NaCl, 118; KCl, 4.74; CaCl2, 2.52; KH2PO4, 1.19; MgSO4·7H2O, 1.19; NaHCO3, 5.67; and dextrose, 5.54. The solution was changed at 15-minute intervals during equilibration, and the tension was maintained at 1.5 g. This procedure was found to produce optimal conditions for reproducible isometric force development. Changes in force were measured using Grass Model FT03C force transducers (Quincy, MA, USA) coupled to a Hewlett-Packard 775BA recorder (Palo Alto, CA, USA).

Concentration-response curves to all agonists were obtained by the cumulative addition of the drug to the organ bath, the concentration being increased only after the maximal response to the preceding concentration was attained. Phenytoin, 10–4 to 10–6 M, was used to contract vascular rings before examination of relaxant responses. In those experiments examining the effects of cyclooxygenase inhibition, 10–6 M indomethacin was added to the organ bath 15 minutes before the first dose of agonist to be tested.

Data Analysis

Vascular relaxation responses in the experimental groups were compared by the concentration of agonist (acetylcholine or A23187) required to elicit a vascular relaxation that was 50% of the maximal relaxation obtained with that agonist (this value is designated as the EC50). Maximal vascular relaxations expressed as a percentage of the phenylephrine-induced contraction were also compared. The data were analyzed by one-way analysis of variance and Student’s t test for paired and unpaired observations. A p value of less than 0.05 was considered significant.

Endothelial Integrity

The presence or loss of endothelium was tested by the ability of acetylcholine and A23187 to elicit relaxations as well as a direct silver nitrate staining at the end of the experiment in selected tissues, using the technique described by Spokas et al.18 Briefly, rings were cut to form strips, laid flat in petri dishes on paper towelling moistened with 5% dextrose. The tissues were immersed on 0.25% AgNO3 for 18 minutes under towelling moistened with 5% dextrose. This procedure was found to produce optimal conditions for reproducible isometric force development. Changes in force were measured using Grass Model FT03C force transducers (Quincy, MA, USA) coupled to a Hewlett-Packard 775BA recorder (Palo Alto, CA, USA).

Concentration-response curves to all agonists were obtained by the cumulative addition of the drug to the organ bath, the concentration being increased only after the maximal response to the preceding concentration was attained. Phenytoin, 10–4 to 10–6 M, was used to contract vascular rings before examination of relaxant responses. In those experiments examining the effects of cyclooxygenase inhibition, 10–6 M indomethacin was added to the organ bath 15 minutes before the first dose of agonist to be tested.

Data Analysis

Vascular relaxation responses in the experimental groups were compared by the concentration of agonist (acetylcholine or A23187) required to elicit a vascular relaxation that was 50% of the maximal relaxation obtained with that agonist (this value is designated as the EC50). Maximal vascular relaxations expressed as a percentage of the phenylephrine-induced contraction were also compared. The data were analyzed by one-way analysis of variance and Student’s t test for paired and unpaired observations. A p value of less than 0.05 was considered significant.

Endothelial Integrity

The presence or loss of endothelium was tested by the ability of acetylcholine and A23187 to elicit relaxations as well as a direct silver nitrate staining at the end of the experiment in selected tissues, using the technique described by Spokas et al.18 Briefly, rings were cut to form strips, laid flat in petri dishes on paper towelling moistened with 5% dextrose. The tissues were immersed on 0.25% AgNO3 for 18 minutes under a 60-W lamp, then gently washed five times with 5% dextrose. The tissues were immersed on 0.25% AgNO3 for 18 minutes under a 60-W lamp, then gently washed five times with 5% dextrose. The procedures were followed by reduction in a bath of bromide salt (3% cobalt and 1% ammonium) under the same 60-W light for 10 minutes. The strips were then fixed for 45 minutes in a bath of 10% neutral buffered formalin, followed by five successive washes in phosphate-buffered saline. The strips were dehydrated in ethanol and xylene and mounted for light microscopic examination of the luminal surface.
Results

Hemodynamic Profile

Seven days after SRAC, mean arterial pressure above the coarctation was significantly elevated above the preoperative value (97 ± 4 vs 67 ± 3 mm Hg, respectively; n = 9, p < 0.01). On the other hand, blood pressure below the coarctation remained normotensive (68 ± 3 mm Hg; n = 6) as did sham-operated animals (64 ± 4 mm Hg; n = 9).

Phenylephrine-Induced Contractions of the Aorta

Rings of thoracic and abdominal aorta were contracted to a similar level of force in both SRAC (2.7 ± 0.2 and 2.8 ± 0.1 g for thoracic and abdominal rings, respectively) and sham-operated groups (2.8 ± 0.1 and 3.3 ± 0.2 g for thoracic and abdominal rings, respectively). Concentrations of phenylephrine required to produce this degree of force were generally higher in thoracic (3.7 × 10^{-7} to 1.9 × 10^{-6} M) than in abdominal rings (2.2 × 10^{-7} to 5.1 × 10^{-7} M), a difference that was significant (p < 0.02) for both SRAC and sham-operated groups.

Acetylcholine-Induced Relaxations of the Aorta

Acetylcholine administration caused a dose-dependent relaxation of phenylephrine-precontracted rings of thoracic and abdominal aorta in all three treatment groups (intact controls, sham-operated and SRAC rabbits; Figure 1). In rings of thoracic aorta, acetylcholine-induced relaxations were superimposable for the normotensive control and sham-operated groups, whereas the relaxant response to acetylcholine in SRAC was attenuated (see Figure 1). Specifically, low doses of acetylcholine (5 × 10^{-8} M) failed to evoke a relaxation, with a significant increase in the EC_{50} and reduction in the maximal response in SRAC rabbits when compared with both normotensive control groups (Table 1).

Despite a depression of acetylcholine-induced relaxation of the thoracic aorta in the SRAC group, responses of all three groups were identical in the abdominal aorta (see Figure 1). Thus, acetylcholine responses in normotensive regions of the hypertensive SRAC animals were identical to those in normotensive controls, whereas regions exposed to an elevated blood pressure displayed an impaired endothelium-dependent vascular relaxation. These regional differences in SRAC rabbits are exemplified by the significant differences in EC_{50} and maximal relaxation between thoracic and abdominal segments of the aorta (see Table 1), which were not apparent in control or sham-operated animals.

A relationship appears to exist between the degree of hypertension and impaired endothelium-dependent relaxation; the EC_{50} for acetylcholine-induced relaxations in the thoracic aorta was well correlated (r = 0.814, p = 0.001) with absolute mean arterial pressure in conscious sham-operated and SRAC animals. This finding suggests that the extent to which endothelium-dependent vasodilation is impaired following SRAC is related to the degree of blood pressure elevation.

Nitroprusside-Induced Relaxations of the Aorta

Nitroprusside-induced vascular relaxations, unlike those of acetylcholine, are endothelium-independent, although responses to both agents are accompanied by an increase in cyclic guanosine 3',5'-monophosphate (cGMP) levels in vascular smooth muscle; direct measurements of cGMP were not done in this study. Relaxant responses of phenylephrine-precontracted rings to nitroprusside, used as an index of cGMP-mediated vascular responsiveness, were not impaired by aortic coarctation (Figure 2). There was no difference between SRAC and control animals in the maximal relaxant responses of thoracic and abdominal aortic rings. Further, the EC_{50} values were indistinguishable in the thoracic aorta, although a significantly lower value was observed in the abdominal aorta of SRAC animals (p = 0.003). Results for sham-operated animals were identical to those for controls (data not shown). These results indicate that cGMP-mediated vascular relaxation was not attenuated by SRAC and suggest that the impaired acetylcholine responses in thoracic aorta result from a diminished formation or release, or both, of an endothelium-derived relaxing factor (EDRF).
**TABLE 1. Comparison of EC₅₀ Values and Maximal Relaxations Induced by Acetylcholine in Aortic Rings from Control, Sham-Operated, and Suprarenal Aortic Coarctation Rabbits**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Maximal relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thoracic aorta</strong></td>
<td></td>
</tr>
<tr>
<td>Control (n = 19)</td>
<td>6.7±0.7</td>
</tr>
<tr>
<td>Sham (n = 21)</td>
<td>7.3±0.5*</td>
</tr>
<tr>
<td>SRAC (n = 41)</td>
<td>21.6±2.66§</td>
</tr>
<tr>
<td><strong>Abdominal aorta</strong></td>
<td></td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>4.6±0.6†</td>
</tr>
<tr>
<td>Sham (n = 13)</td>
<td>6.1±2.4**</td>
</tr>
<tr>
<td>SRAC (n = 16)</td>
<td>7.2±3.0¶</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n = number of rings. Sham = sham-operated; SRAC = suprarenal aortic coarctation.

* p = 0.68, † p = 0.47, ‡ p < 0.001, § p = 0.63, compared with thoracic aorta control values.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Maximal relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thoracic aorta</strong></td>
<td></td>
</tr>
<tr>
<td>Sham (n = 14)</td>
<td>8.5±0.8</td>
</tr>
<tr>
<td>SRAC (n = 25)</td>
<td>16.6±3.0*</td>
</tr>
<tr>
<td><strong>Abdominal aorta</strong></td>
<td></td>
</tr>
<tr>
<td>Sham (n = 6)</td>
<td>17.4±7.3</td>
</tr>
<tr>
<td>SRAC (n = 10)</td>
<td>14.0±6.4‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n = number of rings. Sham = sham-operated; SRAC = suprarenal aortic coarctation.

* p = 0.05, † p = 0.001, ‡ p = 0.68, § p = 0.44, compared with respective sham operation values.

**A23187-Induced Relaxations of the Aorta**

As the impaired endothelium-dependent vascular relaxation responses to acetylcholine in hypertensive regions may have been due to a reduced number or affinity of muscarinic receptors, a receptor-independent agonist for EDRF release, A23187, was studied. Results were similar to those seen for acetylcholine, with a reduction in the A23187-induced maximal relaxation of the thoracic but not abdominal aorta of SRAC rabbits and a greater EC₅₀ for A23187-induced relaxations in the thoracic but not abdominal aorta when compared with those in sham-operated controls (Table 2).

**Relaxation of the Carotid Artery by Acetylcholine and Nitroprusside**

To determine if the altered endothelium-dependent responses in thoracic aorta of SRAC rabbits were indicative of events in all vascular beds exposed to the elevated mean arterial pressure, rings of the carotid artery were prepared from SRAC and sham-operated animals. Acetylcholine-induced relaxation of the carotid artery was also impaired by SRAC, with a significant reduction in the maximal relaxant response (p = 0.007) and an increase in the EC₅₀ (p < 0.001; Figure 3). Relaxations to a direct-acting agent, nitroprusside, were augmented in carotid arteries of SRAC rabbits (p = 0.66 for EC₅₀, p = 0.025 for maximum response; see Figure 3). The increased sensitivity of the carotid artery to cGMP-mediated vascular relaxation in the SRAC may explain why the diminution of endothelium-dependent responses in this preparation was not as dramatic as those in the thoracic aorta, where nitroprusside-induced vascular relaxation was not affected by SRAC (see Figure 1).

**Effects of Indomethacin on Acetylcholine and A23187 Responses**

Although prostaglandins may be excluded as mediators of endothelium-dependent relaxations to acetylcholine and A23187 (the rabbit aorta is insensitive to vasodilator prostaglandins), both these agents release arachidonic acid and stimulate prostaglandin formation. Further, a contractile product of arachidonic acid metabolism by cyclooxygenase products may mediate the diminished endothelium-dependent relaxations in the SHR. The effects of indomethacin on...
Morphological Assessment of the Endothelium

Selected rings of aorta were stained with silver nitrate to examine the status of the endothelium at the conclusion of some experimental protocols. Light microscopic analysis did not reveal a loss of endothelial cells in hypertensive or normotensive regions, and cell-to-cell borders were well preserved, indicating that the morphology of endothelium was not altered at this level of analysis in all preparations.

Discussion

This study demonstrates a selective impairment of endothelium-dependent relaxations in rings of vessels taken from above a suprarenal aortic coarctation, which has resulted in hypertension, but not from segments below the constriction, which are still subject to normotensive pressures. In contrast, relaxations induced by the direct-acting agent nitroprusside were not diminished in vessels above the coarctation. Despite a selective reduction in responses to agonists that require the presence of the endothelium, no endothelial denudation could be observed to account for this attenuation. Rather, these results suggest that there is an impaired production or release (or both) of the EDRF that mediates the response. This interpretation is further supported by the finding that responses to both acetylcholine and A23187 were diminished. As A23187 does not act through a surface receptor, a reduction in muscarinic receptor number or affinity cannot be invoked to explain the diminished relaxant effect. Moreover, EDRF-induced relaxations are thought to be mediated through activation of a soluble guanylate cyclase and increased production of cGMP in the vascular smooth muscle.21 Vascular guanylate cyclase can also be stimulated by the NO radical formed from nitrates and nitrites, which accounts for their vasorelaxant properties.22 Responses to nitroprusside were not diminished in coarctation-induced hypertension, indicating that the impaired relaxation is not the result of a generalized defect in cGMP-dependent mechanisms. Rather, these results suggest that the defect is in the transduction mechanism coupling the endothelium to the vascular guanylate cyclase system — the role played by EDRF.

Rabbits subjected to aortic coarctation did not show an elevation in blood pressure above the site of con-

TABLE 3. Influence of Indomethacin (1 μM) on EC50 Values and Maximal Relaxations Induced by Acetylcholine and A23187 in Rings of Thoracic Aorta

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Acetylcholine</th>
<th>A23187</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC50 (M)</td>
<td>Maximal relaxation (%)</td>
</tr>
<tr>
<td>Control</td>
<td>21.6 ± 2.6</td>
<td>61.1 ± 2.4</td>
</tr>
<tr>
<td>Indo</td>
<td>35.8 ± 2.0*</td>
<td>49.3 ± 3.5†</td>
</tr>
<tr>
<td>Sham operation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.3 ± 0.5</td>
<td>93.4 ± 2.3</td>
</tr>
<tr>
<td>Indo</td>
<td>24.5 ± 0.6</td>
<td>80.2 ± 4.2†</td>
</tr>
</tbody>
</table>

Values are means ± SEM. SRAC = suprarenal aortic coarctation; Indo = indomethacin.

*p < 0.01, †p < 0.05, compared with control values.
striction until Days 4 to 5 after operation (results not shown). As the rabbits were studied on Day 7, the defect in endothelium-dependent responses is an early event and one that relates to the degree of hypertension. Some early morphological changes in blood vessels of hypertensive rabbits have been described, including smooth muscle proliferation and increases in wall thickness, that are suggested to parallel the rise in arterial pressure.\(^\text{24-26}\) Although there is some question whether the early increase in DNA synthesis reflects cellular proliferation or polyplody,\(^\text{27,28}\) if an alteration in smooth muscle structure or number solely contributed to the changes in vasoactivity, a similar alteration in the response to nitroprusside would be expected. Models of hypertension in rats (genetic and experimental) have demonstrated a shift in the dose-response curve to the right but no change in maximal response to direct-acting agents such as nitroprusside,\(^\text{13,14}\) an effect not evident in our rabbit model. This difference may reflect either the species or the duration of blood pressure elevation and may suggest that the initial defect is at the level of the endothelium, with a subsequent alteration in vascular smooth muscle as hypertension persists. Changes in endothelial cell morphology have been described in hypertension that are reversible with antihypertensive therapy,\(^\text{10}\) as are the attenuation of endothelium-dependent relaxations.\(^\text{14}\) Other functional aberrations are associated with these changes in endothelial cell morphology, including enhanced permeability.\(^\text{11,12}\) Thus, hypertension is associated with a variety of morphological and functional changes of the endothelium that may be interrelated and together contribute to cardiovascular disease. Although in the present experiments light microscopy did not reveal endothelial changes, electron microscopic analysis might have revealed alterations similar to those previously reported. However, our goal was to demonstrate that the attenuation of endothelium-dependent relaxations is not accompanied by a loss of endothelial cells.

Our studies do not permit any conclusion as to whether the defect in endothelium-dependent responses contributes to, or is merely a result of, the elevated blood pressure. Analysis of temporal changes in vascular reactivity associated with the development of hypertension would determine if these changes precede or follow the increase in blood pressure. An advantage of the aortic coarctation model is that each animal acts as its own control, with "normal" vessels below the coarctation. Future studies will address this cause-effect issue. However, if the loss of endothelium-dependent responses is merely a result of the increased blood pressure, a uniform defect may be expected in all models of hypertension, which does not appear to be the case. Moreover, the supposition that the events described in these large vessels are also reflected in the microcirculation, in particular the precapillary arterioles, may not be valid. To date, there is a paucity of studies on endothelial dependency in the microcirculation, due largely to problems of removing the endothelium without damaging subendothelial layers.

Previous studies have described nonuniform decreases in endothelium-dependent responses in various models of hypertension in the rat.\(^\text{13-15}\) Further, Konishi and Su\(^\text{19}\) demonstrated that maximal relaxation in response to acetylcholine in thoracic aorta was impaired; however, responses in the femoral artery were greater in the SHR compared with those in control Wistar-Kyoto rats (WKY). Therefore, different vascular beds may respond differently to the effects of hypertension, although the validity of the WKY as a control for the SHR may be a factor. In our study, the hypertension-induced defect in endothelium-dependent relaxations appeared to be a generalized phenomenon as the carotid artery and thoracic aorta gave similar results. Nevertheless, the impaired endothelium-dependent vascular relaxations observed in SRAC hypertension may have resulted from an interaction of elevated blood pressure and additional, yet unidentified, factors. Other components that may participate in this phenomenon include rheological variations and local regulatory factors, whose combined influence may account for the regional variations in EDRF release or effects throughout the vasculature under basal conditions and the alterations seen in hypertension.\(^\text{30,31}\)

Recently, Luscher and Vanhouette\(^\text{15}\) suggested that these diminished responses in the SHR were not due to a reduction in EDRF formation or release but rather to enhanced formation of an endothelium-derived contractile factor, which could be corrected with indomethacin. In our study, indomethacin did not reverse the endothelial impairment. Species differences, the model of hypertension, or duration of the disease state may explain this difference. We have also observed augmented indomethacin-sensitive endothelium-dependent contractions to arachidonic acid in the aortas of rabbits with coarctation hypertension.\(^\text{32}\) Thus, although there is an increase in the capacity to form this contractile factor, it does not appear to modulate the endothelium-dependent relaxant responses in this model.

In summary, aortic coarctation increased blood pressure above the constriction by Day 7, while blood pressure below the constriction remained normotensive. Segments of blood vessels taken from above the coarctation showed a selective impairment in response to endothelium-dependent agonists, but not to the direct-acting nitroprusside. These aberrations were localized to vessels from the hypertensive region and were not observed in segments of vessels below the coarctation. Consequently, these results suggest that a reduction in the formation or release of EDRF may contribute to the elevated arterial blood pressure in this model.

Acknowledgments

The authors thank Helen McNeill and Lisa Cohn for technical assistance, Pam Blank and Gayle Price for manuscript preparation, and Sallie McGiff for editorial assistance.

References

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-376


15. Luscher TF, Vanhouotte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 1986;8:344–348


Impaired endothelium-dependent relaxations in rabbits subjected to aortic coarctation hypertension.

M J Miller, A Pinto and K M Mullane

_Hypertension._ 1987;10:164-170
doi: 10.1161/01.HYP.10.2.164

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/10/2/164

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/