The Loss of Endothelium-Dependent Vascular Relaxation in Hypertension

WARREN LOCKETTE, YUJI OTSUKA, AND OSCAR CARRETERO

SUMMARY We investigated endothelium-dependent relaxation in rat aortae, using three models of experimental hypertension: deoxycorticosterone and salt; one-kidney, one clip renovascular hypertension; and coarctation. Isolated aortae were contracted with phenylephrine, and relaxation was subsequently induced with acetylcholine or calcium ionophore A23187. Blood vessels denuded of endothelium did not relax in response to acetylcholine or A23187. Blood vessels from animals with high blood pressure had decreased relaxation responses to acetylcholine and A23187, and also to the endothelium-independent vasodilator sodium nitroprusside. Unlike acetylcholine and A23187, however, nitroprusside completely relaxed the blood vessels from the hypertensive animals, though the sensitivity to nitroprusside was much lower in these vessels. Subsequent reversal of hypertension caused a return of endothelium-dependent relaxation. Loss of endothelium-dependent relaxation occurs readily in the aortae with the development of hypertension; this phenomenon appears to be related to elevated pressure. (Hypertension 8 [Suppl II]: II-61-II-66, 1986)

KEY WORDS • endothelium-derived relaxing factor • aortae • experimental hypertension • rats • blood vessels

ALTERATIONS in vascular responsiveness to various endogenous vasodilators and vasoconstrictors are found in hypertension. Depending upon the vascular bed studied, the model of experimental hypertension, and the vasodilator used, blood vessels may have an increased, decreased, or normal response to agents that induce vascular relaxation. Many vasodilators act on the vascular endothelial cells to release a substance that will relax the vascular smooth muscle.1,2 This endothelium-derived relaxing factor is released by endothelial cells from large and small arteries in response to a number of vasodilators, including acetylcholine, histamine, bradykinin, adenosine, vasopressin, and arachidonic acid. Unfortunately, most studies investigating vascular relaxation in hypertension were done before the physiological importance of the vascular endothelium in determining vascular tone was known, and therefore the integrity of the vascular endothelium was not verified in many of these earlier studies. Also, it has been reported that endothelium-dependent relaxation is attenuated in the genetically hypertensive rat in response to adenosine, acetylcholine, and the calcium ionophore A23187.3 We attempted to determine whether endothelium-dependent relaxation is generally reduced in hypertension and, if so, whether such changes are due to the increased blood pressure.

Methods

Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI, USA) weighing 250 to 300 g were made hypertensive in one of three ways: 1) implantation of deoxycorticosterone acetate (DOCA), 200 mg/kg, in a Silastic matrix subcutaneously and supplementation of normal rat chow (Ralston Purina, St. Louis, MO, USA) with 0.9% NaCl/0.2% KCl drinking water; 2) placement of a silver clip (0.23-mm gap) around the renal artery of the remaining kidney in uninephrectomized rats; or 3) placement of a ligature around the aorta between the renal arteries, resulting in coarctation.4 Blood pressure was measured with tail cuff plethysmography 4 weeks after the operative intervention in the DOCA hypertensive rats and the one-kidney one clip (1K1C) renovascular hypertensive rats. In the animals with coarctation-induced hypertension blood pressure was measured directly from carot-
id catheterization under light anesthesia 2 weeks after the coarctation procedure. To study the effects of blood pressure normalization on endothelium-dependent relaxation, we lowered the pressure in some of the DOCA hypertensive rats after 1 month of elevated pressure by placing these animals on a diet of low sodium rat chow (Purina) and regular tap water. Similarly, a subpopulation of the 1K1C renovascular hypertensive rats had their elevated blood pressure treated by removal of the silver clip after 1 month.

Four to 6 weeks after their initial surgery, the animals were killed, and the thoracic aortae were excised and cleaned of connective tissue. Helical (0.3 × 1.5 cm) strips were cut, with special care taken to avoid harm to the endothelium. The strips were suspended in a standard muscle bath chamber in physiologic salt solution of the following composition (in mM/L): NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.17; CaCl₂·2H₂O, 1.6; NaHCO₃, 14.9; dextrose, 5.5; and CaNa ethylenediaminetetraacetic acid, 0.03. The strips were maintained at 37°C and aerated with 95% O₂, 5% CO₂. The strips were equilibrated for 1 hour with a resting tension of 1500 mg for strips from the normotensive, DOCA, and renovascular hypertensive rats. Preliminary studies indicated that 2400 mg of tension was needed to achieve maximal contractile force in aortae from the coarctation rats. Phenylephrine (Neo-Synephrine, Winthrop), acetylcholine (Miochol, Cooper Vision), and sodium nitroprusside (Nitropride, Abbott) were obtained from the Henry Ford Hospital Pharmacy. A23187 was obtained from Calbiochem, and DOCA was obtained from Sigma.

Since hypertension may induce different contractile responses to phenylephrine in various experimental groups of rats, and since the degree of endothelium-dependent relaxation may be a function of the magnitude of the contractile response of the tissue, a complete dose-response relationship to phenylephrine was determined for each strip. The dose of phenylephrine that caused a 50% maximal contractile response (ED₅₀) was used to contract the isolated blood vessels. Acetylcholine, A23187, or sodium nitroprusside was then added in a cumulative fashion, and the relaxation of the phenylephrine-contracted vessels was expressed as a percentage of the contractile response of the strip to an ED₅₀ dose of phenylephrine (Figure 1). In some strips, the endothelium was removed by gently rubbing the luminal surface with a cotton swab. These vessels contracted with phenylephrine, but no relaxation was observed in response to the addition of acetylcholine or A23187 (see Figure 1). To determine whether cyclooxygenase inhibition had any effect on endothelium-dependent relaxation, we studied acetylcholine-induced relaxation in some of the isolated strips from the DOCA hypertensive rats before and after the addition of 10⁻⁵ M indomethacin in 0.1% ethanol.

Statistical analysis was performed with the MIDAS computer program package of the University of Michigan.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Relaxation responses of isolated aortae precontracted with a dose of phenylephrine that gave a 50% maximal contractile response (ED₅₀ dose). The vasodilator was added in cumulative fashion, and the relaxation responses were expressed as a percentage of the contractile response of the strips to the ED₅₀ dose of phenylephrine. The lower tracing shows a typical response to acetylcholine or ionophore A23187 when the endothelium was denuded.
gan. For comparison of the six groups (the normotensive controls, DOCA rats, 1K1C renovascular hypertensive rats, coarctation rats, DOCA low sodium rats, and 1K1C unclipped rats), analysis of variance for each dose of each drug was done. If the test of the assumption of equality of variance was significant, the nonparametric Kruskal-Wallis test was used. A Bonferroni adjustment was performed when necessary for multiple testing. Adjusted logit transformations were done to compare the ED50 and ED90 values of the phenylephrine-induced contractions in the normotensive and hypertensive aortae, with and without endothelium.

Results

Within 4 weeks, all experimental groups of animals had significant elevations in blood pressure, as compared with the control animals: DOCA rats, 206 ± 8 mm Hg (SEM); 1K1C renovascular hypertensive rats, 217 ± 8 mm Hg; coarctation hypertensive rats, 256 ± 15 mm Hg; and control rats, 125 ± 5 mm Hg. The coarctation rats were studied 14 days after surgery, since they developed the most severe form of hypertension and had a high mortality rate. Placement of the DOCA rats on a low sodium diet for 2 weeks lowered their blood pressure to 146 ± 7 mm Hg; similarly, unclipping of the renal arteries in the 1K1C renovascular hypertensive rats lowered their blood pressure to 121 ± 3 mm Hg by the end of 2 weeks (Figure 2).

Although there were no significant differences in the ED50 for the phenylephrine responses in the control aortae with (5.75 × 10⁻⁸ M) and without (5.87 × 10⁻⁸ M) endothelium, the DOCA hypertensive rats had a higher ED50 (7.94 × 10⁻⁸ M) in vessels with intact endothelium than in those with denuded endothelium (3.68 × 10⁻⁸ M). Though these differences were not significant at the ED90 level (p = 0.1), they were clearly significant at lower doses (i.e., as compared with ED50 values, p < 0.05).

Aortae from the DOCA, 1K1C, and coarctation hypertensive rats had significantly decreased relaxation responses to the muscarinic receptor agonist acetylcholine (Figure 3). At a dose of 10⁻⁷ M, acetylcholine induced 77% relaxation of aortic strips from the normotensive rats, which had been contracted to 50% of the maximal tension possible with phenylephrine (i.e., the ED50, for phenylephrine). In contrast, maximal relaxation was 24% in aortae from the coarctation hypertensive rats. Similarly, a 10⁻⁷ M concentration of the calcium ionophore A23187 induced 52% relaxation in the control rat aorta, as compared with a maximal relaxation of 10% of the phenylephrine-induced contraction in the 1K1C rat aortae. A further 10-fold increase in the A23187 concentration actually caused a contractile response in some of the aortae from the hypertensive animals, though the control aortae relaxed maximally to 62% at 10⁻⁶ M, the highest concentration used (Figure 4).

A reduction in the relaxation response to acetylcholine and A23187 might have been secondary to a decrease in the synthesis of the endothelium-derived relaxing factor or the reduction might have been due to a decreased sensitivity to the relaxing factor in blood vessels from the hypertensive rats. However, these vessels also showed a decreased sensitivity to the endothelium-independent vasodilator sodium nitroprusside. Despite this reduction in sensitivity, the vessels from these animals could be completely relaxed (Figure 5).

Having determined that loss of endothelium-dependent relaxation was consistent in three models of hypertension, we next attempted to see whether this phenomenon was truly a result of elevated blood pressure. After the DOCA-treated rats had been placed on a low sodium diet, there was a marked reduction in blood pressure. Within 2 weeks, aortae from these animals had relaxation responses to acetylcholine that were not significantly different from the values in normotensive rats (Figure 6). Similarly, when the blood pressure in the 1K1C renovascular hypertensive rats was lowered by unclipping the renal artery, blood pressure fell to normal levels, and acetylcholine-induced relaxation returned to control responses (see Figure 6). The aortae from these unclipped renal hypertensive rats also demonstrated relaxation responses to A23187 that were

![Figure 2. Blood pressure measurements with tail plethysmography, in awake animals, or directly from a carotid cannula, in lightly anesthetized animals. DOCA plus salt, clipping of the remaining renal artery in uninephrectomized rats (IKIC), or placement of an aortic ligature caused a significant elevation of blood pressure within 2 to 4 weeks, as compared with animals that did not undergo an operative intervention. DOCA plus a low sodium diet and unclipping of the renal artery significantly lowered blood pressure toward normal values in the DOCA high salt and 1K1C renovascular hypertensive animals, respectively. *p < 0.05, compared with blood pressure in controls.](image-url)
Figure 3. Relaxation responses to the endothelial-dependent muscarinic agonist acetylcholine. Aortae from the hypertensive animals had significantly decreased relaxation responses, as compared with aortae from the normotensive control rats. The strips were preconstricted with an ED_{50} dose of phenylephrine, and the magnitude of the relaxation response is expressed as a percentage of the contraction in response to phenylephrine. IKIC = one-kidney, one clip.

Figure 4. Relaxation responses to the calcium ionophore A23187. Responses were reduced in the vessels from the hypertensive animals, as compared with control aortae. IKIC = one-kidney, one clip.

Figure 5. Relaxation responses to sodium nitroprusside. The dose-response curves were significantly shifted to the right in aortae from rats with three forms of hypertension. All vessels relaxed completely at higher doses of sodium nitroprusside. IKIC = one-kidney, one clip.

Figure 6. Relaxation responses to acetylcholine after reversal of hypertension. The endothelium-dependent relaxation response was restored after placement of DOCA rats on a low salt diet and after unclipping of the renal artery in one-kidney, one clip (IKIC) rats.

Similar to the responses of control rat aortae (data not shown). However, aortae from the DOCA hypertensive rats that were placed on the low sodium diet did not have complete normalization of relaxation responses to A23187. The blood pressure in the DOCA hypertensive rats on a low sodium diet fell but still remained significantly higher than the pressure in the control animals (see Figure 2).
Discussion

We have shown that endothelium-dependent relaxation is markedly attenuated in three different forms of hypertension. Since the mechanisms involved in the pathogenesis of these forms are different (e.g., we have studied a high-renin and a low-renin form of hypertension), it is most likely that the loss of endothelium-dependent relaxation is a result of elevated blood pressure. Further evidence is provided by the return of endothelium-dependent relaxation in aortae from hypertensive animals whose blood pressure is lowered.

It has been reported that acetylcholine induced endothelium-dependent relaxation in aortae from genetically (spontaneously) hypertensive rats and that aortae from these rats relaxed significantly less than aortae from matched, normotensive, Wistar-Kyoto controls. However, the femoral arteries of the spontaneously hypertensive rats were found to relax more in response to acetylcholine than femoral arteries from the normotensive rats. In addition, these investigators studied adenosine-induced vascular relaxation responses and demonstrated that the role of the endothelium in vascular relaxation is dependent upon the vascular bed studied and the vasodilator used.3

Winquist et al. have demonstrated a decrease in endothelium-dependent relaxation induced by acetylcholine and A23187 in aortae from the New Zealand strain of genetically hypertensive rats.5 They also found a decreased sensitivity to nitroprusside in the aortae from the New Zealand rats, though there was no change in the maximal relaxation that could be induced by nitroprusside in aortae from the New Zealand rats as compared with aortae from normotensive controls.5 These studies support our finding that elevations in blood pressure affect endothelium-dependent vascular relaxation; however, the mechanism underlying these changes is not known.

The magnitude of endothelium-dependent relaxation is a function of the preexisting contractile state of the blood vessel (i.e., the greater the contractile tension on a blood vessel, the smaller the magnitude of the endothelium-dependent relaxation response). The loss of endothelial relaxation has been reported to result in an increased sensitivity to phenylephrine in isolated vessels.6 We cannot explain our different findings in the vessels from the normotensive control rats. However, another group reported no change in the sensitivity to phenylephrine after the removal of endothelium from canine vessels.9

We have previously shown that aortae from DOCA hypertensive rats and from 1K1C renal hypertensive rats were more sensitive to the cyclooxygenase-dependent, vasoconstricting action of arachidonic acid.8 If we were stimulating endogenous arachidonic acid release under our experimental conditions and making this substrate available to the cyclooxygenase, it is possible that the potent vasoconstricting effects of arachidonic acid in the blood vessels hindered endothelium-induced relaxation. Endothelium-derived relaxing factor may be a metabolite of arachidonic acid, or it may be generated as a by-product of arachidonic acid metabolism; its exact nature is disputable.10, 11 However, the addition of indomethacin, in a dose that we have previously shown inhibits the vasoconstricting action of arachidonic acid in aortae from hypertensive animals, did not change endothelium-dependent relaxation in the vessels from the DOCA hypertensive animals. Before the addition of indomethacin, these vessels had a maximum acetylcholine-induced relaxation response of 10 ± 3%; the same response was observed in this set of animals after indomethacin treatment (n = 4).

The loss of endothelium-dependent relaxation in hypertension may be the result of changes in the synthesis, release, or metabolism of endothelium-derived relaxing factor from the endothelial cell, brought on by changes in the endothelium in hypertension. It has been shown that there is a remodeling of the rat aortic endothelial layer during experimental hypertension, with changes in the endothelial cell replication rate, cell density, and surface morphology in rats with aortic coarctation or DOCA hypertension.12 Alternatively, changes in endothelium-dependent relaxation may result from changes in the vascular smooth muscle response to endothelium-derived relaxation factor. The level of cyclic guanosine 3',5'-monophosphate (cGMP) in vascular smooth muscle and endothelial cells has been reported to rise in response to endothelium-dependent vasodilators.13 Perhaps the loss of endothelium-dependent relaxation in vascular smooth muscle in hypertension is a result of perturbations in cGMP.

In summary, there is a loss of endothelium-dependent relaxation in blood vessels from animals with different models of hypertension; the physiological importance of this loss is unknown. The integrity of the vascular endothelium is clearly important for the maintenance of normal vascular function. Aberrations in the endothelium cell may contribute to the development of hypertension or other disease states that manifest altered vascular reactivity. Fortunately, endothelium-dependent relaxation returns with normalization of blood pressure — an observation with important implications for the treatment of hypertension, if endothelium-derived relaxing factor does prove to have a role in the maintenance of normal blood pressure or the pathogenesis of hypertension or other disease states involving altered vascular reactivity.

Acknowledgment

Steven Krumm provided technical assistance.

References

The loss of endothelium-dependent vascular relaxation in hypertension.
W Lockette, Y Otsuka and O Carretero

Hypertension. 1986;8:II61
doi: 10.1161/01.HYP.8.6_Pt_2.II61

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/8/6_Pt_2/II61