Transient Hypertension Directly Impairs Endothelium-Dependent Vasodilation of the Human Microvasculature

Oscar A. Paniagua, Melissa B. Bryant, Julio A. Panza

Abstract—Hypertension is associated with decreased endothelium-dependent vasodilation. However, whether endothelial dysfunction is a cause or a consequence of elevated blood pressure is unknown. Therefore, to determine whether hypertension can directly induce endothelial dysfunction, we investigated the effect of increases in intra-arterial pressure on endothelium-dependent vasodilation of the human microvasculature. Small arteries (internal diameter 202±75 μm) were isolated from gluteal fat biopsies in 12 healthy normotensive subjects (8 men and 4 women; age, 46±10 years). Arteries were cannulated and perfused in chambers oxygenated at 37°C. Endothelium-dependent and -independent responses to acetylcholine (Ach; 10⁻⁹ to 10⁻⁹ mol/L) and sodium nitroprusside (SNP; 10⁻⁹ to 10⁻⁴ mol/L), respectively, were obtained after incubating the vessel with incremental intravascular pressures of 50, 80, and 120 mm Hg for 60 minutes each. The response to Ach was also obtained in different arteries after 3 consecutive incubation periods at 50 mm Hg. Arterial internal diameter was measured directly from amplified digital images. A significant reduction in the vasodilator response to Ach was observed with increases in intravascular pressure (mean vasodilation, 62%, 49%, and 26% at 50, 80, and 120 mm Hg, respectively; P<0.001). In contrast, the response to SNP showed a nonsignificant trend toward greater vasodilation with increases in pressure (mean vasodilation, 40%, 52%, and 57% at 50, 80, and 120 mm Hg, respectively; P=0.10). There was no difference in the consecutive dose-response curves to Ach obtained at the same intravascular pressure (mean vasodilation: 48%, 46%, and 49%; P=0.61). Transient increases in intravascular pressure significantly depress endothelium-dependent vasodilation in human resistance arteries. These findings suggest that elevated blood pressure per se may cause endothelial dysfunction in humans and have implications for the pathophysiology of endothelial dysfunction in hypertension. (Hypertension. 2000;36:941-944.)

Key Words: endothelium ■ hypertension, essential ■ blood vessels

Several previous studies have demonstrated that essential hypertension is associated with impaired endothelium-dependent vascular relaxation.¹⁻³ However, whether this is a primary or secondary abnormality is unknown. Certain previous investigations have suggested that endothelial dysfunction is a primary defect in essential hypertension, present even before the clinical documentation of elevated blood pressure. Thus, normotensive offspring of hypertensive patients were found to have depressed endothelium-dependent vasodilation compared with normotensive offspring of normotensive parents.⁴ Furthermore, the same authors have reported that the bioactivity of nitric oxide (NO) may be reduced in normotensive individuals with a family history of hypertension.⁵

On the other hand, certain observations suggest that endothelial dysfunction may occur as a consequence rather than a cause of elevated blood pressure. In particular, several animal models of induced hypertension, including suprarenal coarctation of the abdominal aorta in rabbits,⁶ salt-induced hypertension in Dahl salt-sensitive rats,⁷ and pressure increases in cat cerebral⁸ and dog coronary arteries,⁹ have all shown a selective impairment of endothelium-dependent vasodilation after elevations in blood pressure.

Because essential hypertension is a heterogeneous and probably multifactorial process, the possibility that endothelial dysfunction may develop as a consequence of elevated blood pressure may have important implications. In particular, the induction of endothelial dysfunction by hypertension may create a vicious cycle that contributes to increased vascular resistance and enhanced propensity to the development of atherosclerosis regardless of the initial cause of the hypertensive process. However, whether arterial hypertension can directly induce endothelial dysfunction in humans has not been determined. Therefore, the purpose of this study was to investigate the effect of increases in intra-arterial pressure on endothelium-dependent vasodilation of small arteries obtained from normotensive individuals.

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From the Cardiology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md.
Correspondence to Dr Julio A. Panza, National Institutes of Health, 10 Center Dr, MSC 1650, Bldg 10, Room 7B-15, Bethesda, MD 20892-1650.
E-mail panzaj@nih.gov
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Methods

Study Subjects
Twelve healthy subjects (8 men and 4 women) were recruited for this study. Mean age was 46 ± 10 years. Each subject was screened by clinical history, physical examination, ECG, chest radiograph, and routine chemical analyses. None had evidence of present or past hypertension, hyperlipidemia, or any other cardiovascular disease, or any other systemic condition. Their blood pressure measurements and chemistry results at the time of screening are included in the Table. None of the study participants was taking any medication at the time of the study. In particular, they were asked to refrain from vitamin supplements for 4 weeks, aspirin for 2 weeks, and smoking and caffeine for at least 24 hours before the study. The study protocol was approved by the National Heart, Lung, and Blood Institute Institutional Review Board, and all participants gave written informed consent.

Subcutaneous Biopsies and In Vitro Procedures
In each subject, biopsy specimens of skin and subcutaneous tissue (~0.5 cm wide × 1.2 cm long × 1.5 cm deep) were taken from the gluteal region under local anesthesia with 2% lidocaine. The specimens were immediately placed in cold physiological saline solution (PSS) and transported to a laboratory where subcutaneous arteries (internal diameter 202 ± 75 μm) were dissected under a light microscope. After removal of surrounding adipose and connective tissue, a segment of the artery (length ~3 mm) was transferred to a 15-mL vessel chamber (Living System Instrumentation) containing cold PSS and 2 glass micropipettes used to perfuse the artery.\(^9\) The proximal end of the artery was slipped onto the proximal cannula and secured with a knot of microsurgery thread. The residual blood in the artery was gently flushed and the distal end was then slipped onto the distal cannula and similarly secured. Approximately 2 mm of the arterial segment lay between the cannulas. The axial length of the vessel segment was set by carefully modifying the position of the proximal cannula to eliminate any warping or buckling and avoiding excessive longitudinal stretch.

Once the artery was mounted, the chamber was transferred to the stage of an inverted microscope and connected to a reservoir containing PSS with the following composition (in mmol/L): 119.0 NaCl, 4.7 KCl, 1.76 CaCl\(_2\), 1.17 MgSO\(_4\), 5.5 glucose, 17 NaHCO\(_3\), 1.17 KH\(_2\)PO\(_4\), and 0.026 K-EDTA. From the reservoir, the vessel chamber was continuously perfused at a rate of 40 mL/min with PSS equilibrated with a gas mixture of 95% O\(_2\) and 5% CO\(_2\). The pH was maintained at 7.4 and the temperature at 37°C by means of a water thermal regulator. Intravascular pressure was sensed by a proximally placed transducer connected to a pressure servo pump (Living System Instrumentation). Pressure was maintained at 20 mm Hg during the mounting procedure and raised to 50 mm Hg thereafter. The artery was then checked for leaks and left for 1 hour under no-flow conditions for equilibration before commencing with the experiments.

**Experimental Protocol**

An attempt was made in each biopsy sample to dissect 2 arterial segments to perform the study and the control experiments. In the study experiment, the vessel response was investigated after incubation with increasing concentrations of intravascular pressure (see below). In the control experiments, endothelium-dependent vasodilation was assessed repeatedly, following the same procedures as in the study experiment, except that the intravascular pressure was maintained constant. The study and the control experiments were conducted on 2 consecutive days in random order.

All experiments were performed under no-flow conditions, and drugs were added to the suffusion solution. After the equilibration period, vessels were exposed to PSS with high K\(^+\) content (composition in mmol/L: 78.6 NaCl, 60 KCl, 2.5 CaCl\(_2\), 1.17 MgSO\(_4\), 17 NaHCO\(_3\), 1.17 KH\(_2\)PO\(_4\), 5.5 glucose, and 0.026 K-EDTA). Arteries that did not constrict to >50% of the basal internal diameter were considered not viable and discarded.

In the study experiments, the vasodilator response to acetylcholine (Ach [Sigma]; 10\(^{-5}\) to 10\(^{-7}\) mol/L) and sodium nitroprusside (SNP [Sigma]; 10\(^{-5}\) to 10\(^{-7}\) mol/L) were assessed after preconstriction with noradrenaline (NE [Sigma]; 10\(^{-2}\) mol/L). The response to Ach and SNP were obtained in random sequence and separated by a washout and equilibration period of ≥20 minutes. The first dose-response curves to Ach and SNP were performed with an intravascular pressure of 50 mm Hg. After the last washout, the pressure was raised to 80 mm Hg and vessels were incubated for 60 minutes. The applied pressure was then decreased to the original 50 mm Hg and cumulative dose-response determinations to the same agents mentioned above were assessed. The intravascular pressure was then raised to 120 mm Hg for another 60 minutes, and subsequently, after decreasing the pressure to 50 mm Hg, dose-responses determinations were assessed again. Microscopic examination (maximum power ×100) did not reveal the formation of endothelial lesions (such as blebs or endothelial cell dislodgment) after incubation with increased intravascular pressure. At the conclusion of each experiment, the suffusion solution was changed to a Ca\(^{2+}\)-free solution containing 1 mol/L EDTA. Vessels were incubated for 10 minutes to reach maximal passive diameter at 50 mm Hg.

Control experiments were performed to ascertain that any changes observed in the response to Ach in the study experiments were not related to spontaneous decay in endothelium-mediated vasodilation with time. To this end, 3 consecutive dose-response curves to Ach (separated by washout and equilibration periods as described above) were obtained while the intravascular pressure was maintained at 50 mm Hg during the 60-minute incubation period.

**Analysis of Vascular Responses**

Vascular responses were assessed by measuring the internal diameter of the arterial segment at the end of each stage of the experimental protocol. To this end, images of the blood vessel were continuously captured by a video camera mounted on an inverted microscope and projected on a TV monitor. After the vessel reached a stable diameter in response to each concentration of a vasodepressor (usually within 2 to 3 minutes), 10 seconds of in vivo images displayed on the monitor were recorded on videotape. Images were subsequently digitized and analyzed off-line with a commercially available system (Eastman Kodak). At each stage of the protocol, the internal diameter of the arterial segment was measured using electronic calipers. If vasomotion was present, the maximum and minimum diameters were measured and the average was used for calculations.

**Statistical Analysis**

For each dose-response curve, data are expressed as percent of the NE-induced constriction for each vessel by the formula Vascular response (%) = [(diameter before NE − current diameter)/(diameter before NE − diameter after NE)] × 100. Dose-response curves were compared by means of 2-way ANOVA for repeated measurements with the Student-Newman-Keuls method used for pairwise multiple comparisons. A value of \(P<0.05\) was considered to indicate statistical significance.

**Results**

At least 1 arterial segment was isolated from the biopsy sample in each of the 12 study subjects. In 11 of these

**Blood Pressure and Chemistry Values of 12 Study Subjects**

<table>
<thead>
<tr>
<th>Blood pressure (systolic/diastolic), mm Hg</th>
<th>126±14/77±9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>172±17</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>48±15</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>102±21</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>94±54</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>94±12</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>15±6</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.9±0.2</td>
</tr>
</tbody>
</table>

**Results**

At least 1 arterial segment was isolated from the biopsy sample in each of the 12 study subjects. In 11 of these
The findings of this study demonstrate that short-term increases in intra-arterial pressure directly and significantly depress endothelium-dependent vasodilation of small resistance arteries from normotensive subjects. Thus, transient elevations in the intra-luminal hydrostatic pressure, to which microvessels obtained from subcutaneous biopsies were subjected, produced a selective impairment in the vasodilator response to acetylcholine. In contrast, the response to sodium nitroprusside was not reduced, indicating that the increases in intravascular pressure did not affect the ability of vascular smooth muscle to relax in response to nitrovasodilators. Of note, the magnitude of the impairment in endothelium-dependent responses was directly proportional to the applied intravascular pressure, with progressive reduction in the vasodilator effect of acetylcholine as the incubating pressure was raised from 50 to 80 and finally 120 mm Hg. Importantly, the latter values were selected to mimic measurements of mean blood pressure commonly recorded in normotensive and hypertensive subjects, respectively, and thus underscore the clinical relevance of our study findings.

To ascertain that the observed changes in the response to acetylcholine were not related to a potential spontaneous decay in endothelium-dependent vasodilation over time, we performed control experiments with repeated assessment of vascular responses by using the same intravascular pressure (50 mm Hg) during the incubation period. The results of these experiments showed that, if the intravascular pressure is not increased, the ability of the blood vessel to dilate in response to acetylcholine is maintained during the time course of our studies. These observations, therefore, further emphasize the specificity of the relation between intra-arterial pressure and endothelium-dependent vascular relaxation. Moreover, that this relation is not merely due to mechanical interference of increased intravascular tension with the blood vessel response to pharmacological agents was ruled out in our study by maintaining the same intravascular pressure during the performance of the dose-response curves and by the demonstration that the increases in intravascular pressure did not affect the response to sodium nitroprusside. Finally, it is unlikely that our findings can be explained by anatomic disruption of the endothelium caused by increased intravascular pressure because optical microscopy did not reveal the appearance of blebs or other lesions compatible with anatomic injury of endothelial cells.

The present study findings have pathophysiological and clinical implications. First, our results provide a mechanistic link to explain, at least partly, why hypertension is associated with endothelial dysfunction. The concept that impaired endothelial vasodilator function may be secondary to hypertension does not contradict previous studies suggesting that
decreased nitric oxide activity is a primary phenomenon in the hypertensive process. In fact, because it is unlikely that all hypertensive patients share the same pathophysiology, our findings expand the association between hypertension and endothelial dysfunction to patients in whom reduced nitric oxide activity may not play a primary role in the process leading to elevated blood pressure. Second, because impaired endothelial function is, in turn, associated with increased platelet aggregability, lipid oxidation, and macrophage migration, the demonstration that increased intravascular pressure can directly affect endothelial function may explain why hypertension is a risk factor for the development of atherosclerosis, regardless of the initial causative mechanism. Furthermore, these observations could also provide a mechanistic explanation for the development of vascular hypertrophy in response to hypertension because endothelial dysfunction is associated with the release of mitogenic factors and smooth muscle proliferation from the endothelium. The structural abnormalities developed by vascular hypertrophy can play an important role in the perpetuation of the hypertensive process, as postulated by Folkow. Thus, the present observations suggest the presence and mechanism of a vicious cycle in which hypertension begets hypertension through the impairment of endothelial function.

It must be acknowledged that our study does not identify the precise mechanism by which increased intravascular pressure induces endothelial dysfunction. It is possible that hypertension itself stimulates the production and release of oxygen free radicals, which, in turn, can cause endothelial dysfunction through scavenging and destruction of nitric oxide. In fact, this possibility has been demonstrated in animal models of induced hypertension. Thus, Wei et al. have shown that superoxide released in response to high intra-arteriolar pressure also reduces nitric oxide–mediated shear stress in gracilis muscle endothelial cells from Wistar rats. At the same time, this mechanism is consistent with the previous demonstration of reduced nitric oxide activity in hypertensive humans and its improvement with antioxidant agents.

It must also be noted that our results do not allow us to determine the time course of endothelial dysfunction induced by increases in intravascular pressure. Our observations indicate that the impairment in the response to endothelium-dependent agents develops shortly after the increase in pressure; however, we cannot ascertain whether this is a temporary or permanent phenomenon. Previous studies from our and other laboratories have shown that clinically effective antihypertensive therapy does not improve the impaired endothelium-dependent vasodilation of hypertensive patients. Whether this is a result of permanent and irreversible endothelial damage or is indicative of a primary endothelial abnormality in these patients that cannot be corrected with medical treatment remains to be determined.

In conclusion, the present study demonstrates that increases in intravascular pressure impair endothelium-dependent relaxation of the microvasculature in normotensive humans. These findings suggest that elevated blood pressure per se may cause endothelial dysfunction in humans and have implications for the pathophysiology of endothelial dysfunction in hypertension.

References
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