In Vitro Perfusion Studies of Human Resistance Artery Function in Essential Hypertension

Brendan J. Falloon, Anthony M. Heagerty

Abstract To simulate in vivo conditions as closely as possible to in vitro conditions, we examined the morphological and functional characteristics of isolated human subcutaneous small arteries from 17 essential hypertensive patients and 14 normotensive control subjects using a perfusion myograph. Vessel segments were cannulated and exposed to conditions of constant flow and pressure. The ratio of media thickness to lumen diameter in arteries from hypertensive patients increased significantly. With the endothelium intact, sensitivity to extraluminally applied norepinephrine was not different, and this was not affected by inhibition of neuronal amine uptake with cocaine. After removal of the endothelium, sensitivity to norepinephrine was augmented in normotensive vessels to a greater extent than in hypertensive vessels. Endothelium-dependent relaxation to acetylcholine was significantly reduced in arteries from hypertensive patients, but endothelium-independent relaxation to sodium nitroprusside was not different from that observed in vessels from normotensive control subjects. These data demonstrate that sensitivity to exogenous norepinephrine is not different in essential hypertension but that there is defective endothelium-dependent dilatation, suggesting a contributory role for endothelial dysfunction in human essential hypertension. (Hypertension. 1994;24:16-23.)

Key Words • hypertension, essential • vascular resistance • perfusion • endothelium • norepinephrine • acetylcholine • nitroprusside

Established essential hypertension is characterized by an increase in peripheral vascular resistance when cardiac output is normal.1 A number of experimental techniques have suggested that much of the increased resistance to blood flow in small resistance arteries may be ascribed to changes in the vascular wall architecture.2 In addition, in vivo studies of human forearm blood flow have reported functional changes in the hypertensive circulation that have included evidence of reduced endothelium-dependent dilatation.3-6 However, few in vitro studies have examined the pharmacologic and morphological properties of resistance arteries from essential hypertensive and normotensive individuals. Recently, similar agonist sensitivity of small arteries isolated from subcutaneous biopsies has been demonstrated in patients with untreated moderate to severe essential hypertension.7 This study used the wire myograph technique,8,9 which is essentially a ring preparation. Therefore, the objective of the present study was to assess agonist sensitivity and morphological parameters of isolated subcutaneous resistance arteries from essential hypertensive patients in vitro using a perfusion myograph.10 This type of preparation allows vessels to experience conditions that approximate those present in vivo.11

Methods

Study Population

Seventeen patients, 11 of whom were males, were recruited for the study. Each patient had been diagnosed as having essential hypertension after secondary causes had been excluded. The mean diastolic blood pressure of each patient was greater than 95 mm Hg after at least three measurements with a random-zero sphygmomanometer. None of them had a history of diabetes, hyperlipidemia, peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis. Fourteen normotensive subjects recruited from the public via advertisements in a local newspaper were selected as a control group. Histories, physical examination, and routine chemical analyses showed that the control subjects had no evidence of present or past hypertension, cardiovascular disease, or any other systemic condition. None of the individuals in either group had been on antihypertensive treatment. All participants gave written informed consent for minor surgery to be performed. The study was approved by the Local District Ethics Committee.

Biopsy Procedure

Gluteal subcutaneous biopsies12 were performed on all individuals. Tissue was obtained by infiltrating skin and subcutaneous tissue with 2% plain lignocaine (4 to 5 mL) and taking an elliptical piece of skin and adherent gluteal fat (dimensions approximately 2.5 cm long, 1 cm wide, 1.5 cm deep). The wound was then sutured using 5-0 vicryl and 4-0 prolene. After excision, the tissue was placed immediately into ice-cold physiological saline solution (PSS) and, where possible, a 3- to 4-mm segment of artery was carefully dissected and cleaned of all adherent extraneous adipose tissue under a dissecting microscope. The proximal end of the artery was identified by following the arterial branching, when present, and marked by a diagonal cut for maintenance of directional flow when the vessel was mounted in the preparation.

Drugs and Solutions

All experiments involving isolated subcutaneous resistance arteries were performed using PSS of the following composition (mmol/L): NaCl 119, KCl 4.7, CaCl2 2.5, MgSO4 1.17, NaHCO3 25, KH2PO4 1.18, EDTA 0.026, and glucose 5.5. Potassium-PSS was PSS with an equimolar replacement of NaCl with KCl to give a final potassium concentration of 125

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mmol/L. NEK was potassium-PSS containing $10^{-2}$ mol/L norepinephrine. Cocaine-PSS had $3 \times 10^{-4}$ mol/L cocaine added to the PSS. In the norepinephrine concentration-response experiments, norepinephrine was added to PSS or cocaine-PSS in the concentrations indicated. In the acetylcholine concentration-response experiments, acetylcholine was added to PSS in the concentrations indicated. All solutions were bubbled with 95% O$_2$/5% CO$_2$ to give a pH of 7.4 at 37°C. Norepinephrine [(±)-arterenol hydrochloride], acetylcholine chloride, sodium nitroprusside, and cocaine hydrochloride were purchased from Sigma Chemical Co.

**Experimental Setup**

After dissection, the arterial segment was transferred to a vessel flow chamber. Two glass resistance-matched microcannulas were used to perfuse the artery (Fig 1). The distal cannula was fixed while the proximal cannula could be positioned as appropriate. The procedure for mounting an artery in the flow chamber has been previously described. Briefly, the proximal end of the artery was slipped onto the proximal cannula and any residual blood gently flushed. The distal end of the artery was then slipped onto the distal cannula, and both ends of the artery were secured to the microcannulas with glue (Superbonder 495, Industrial Fasteners) to obtain a watertight seal. The axial length was set by viewing the vessel under a microscope and carefully adjusting its length by positioning the proximal cannula to eliminate any warping, taking care not to stretch or compress the vessel longitudinally. The temperature in the flow chamber was controlled by a circular heating coil and monitored continuously by a thermistor probe (Ellab) placed through the chamber wall into the bath. The flow chamber was placed onto the stage of an upright microscope (Nikon Labophot, Seescan) with a video camera attached to the viewing tube and connected to an imaging system (Seescan) that allows arterial images to be stored for subsequent measurement of arterial dimensions. The temperature of the vessel environment was raised to 37°C. Vascular flow was initiated using a peristaltic pump (Flowgen). Preheated and pregassed PSS was passed at a constant rate through a short length of relatively noncompliant insulated polyethylene tubing attached to the proximal cannula. A transducer attached upstream from the proximal cannula allowed measurement of perfusion pressure. Transmural pressure was produced by a PSS column connected downstream from the distal cannula. Attachment of a syringe to the PSS column allowed for pressure adjustment. After transmural pressure and flow had been established, the vessel was checked for leaks, which were identified by a drop in the level of the PSS column and/or a reduction in the preset intraluminal pressure. In the absence of leaks the vessel was equilibrated for at least 1 hour. During the equilibration period the intraluminal pressure of the vessel was raised to 70 and 120 mm Hg and the proximal cannula gradually adjusted until the artery was unbuckled and the walls were parallel. After equilibration, an optically clean portion of the magnified vessel was chosen, usually close to the midpoint, for morphological measurements. These were done at 30 mm Hg. Therefore, the vessel was under a slight longitudinal stretch. Media thickness and lumen diameter were measured with the artery at its resting lumen diameter in perfusate and superfusate in the absence of any agonist. These parameters were measured at six points along the length of the clean portion of the vessel, and the mean value was calculated. The perfusion flow rate throughout the experiments was 30 μL/min, and the transmural pressure was 30 mm Hg (4.0 kPa). This pressure was found to be optimal for constriction of subcutaneous resistance arteries from both hypertensive patients and normotensive subjects by testing repeated responses to potassium-PSS at various intraluminal pressures (Fig 2).

**Vascular Reactivity**

**Arterial Viability**

After the equilibration period the viability of vessels was evaluated using a standard start procedure. Vessels were activated twice with an extraluminal application of NEK and then with $10^{-4}$ mol/L norepinephrine in PSS, and then again with NEK. An artery was considered viable if the contraction

![Fig 1](image1.png)

**Fig 1.** Diagram shows experimental setup used to study isolated pressurized and perfused subcutaneous resistance arteries. An artery is glued onto two glass microcannulas, the proximal and distal cannulas, in an organ chamber. Flow is initiated at the proximal cannula and leaves the system via the distal cannula where a physiological saline solution column effects transmural pressure. Perfusion pressure is measured by a transducer connected to the proximal cannula. Glue is omitted for clarity.

![Fig 2](image2.png)

**Fig 2.** Bar graphs show contractions in response to potassium-physiological saline solution in subcutaneous resistance arteries with intact endothelium from normotensive subjects (top) and hypertensive patients (bottom) in the absence of cocaine. Maximal contractions occurred at a perfusion pressure of 30 mm Hg for both sets of vessels. For normotensive vessels, responses tended to decrease in the higher pressure range; for hypertensive vessels, contractions were similar over a larger pressure range. n=6.
was greater than 50% of its resting lumen diameter with NEK. Any artery that did not fulfill this criterion was discarded. A steady contraction was maintained for 1 minute for each activation. Between each stimulation, the vessel was perfused with PSS and allowed to reach its resting diameter. The maximum contraction was produced by either the first or second application of NEK, and the greater response was taken as the maximum. Of the 17 hypertensive vessels and 14 normotensive vessels mounted in the perfusion system, 2 hypertensive vessels and 1 normotensive vessel did not fulfill the viability criteria because of leaks or poor NEK response. This gave a rejection rate for hypertensive and normotensive vessels of 12% and 7%, respectively. Of the 15 viable hypertensive vessels and 13 viable normotensive vessels, 3 from each group had preliminary pressure-response relations determined only. The remaining 12 hypertensive vessels and 10 normotensive vessels had full protocols carried out, which included 3 vessels from each group also being subjected to pressure-response relations at the beginning of the protocol.

**Endothelium Removal**

Endothelium removal was accomplished by passing an air bubble down the lumen of the vessel. The flow rate was 200 μL/min, and the size of the bubble was approximately 2 μL. This procedure was carried out three times. Therefore, the length of time that air was present in the vessel lumen was less than 2 seconds. This indicates the difficulty of maintaining endothelial function in this type of preparation. Endothelium removal did not affect the resting lumen diameter and contraction to NEK. The absence or presence of the endothelium was verified by the response to the endothelium-dependent dilator acetylcholine (10⁻⁴ mol/L).

**Pharmacology**

After measurement of morphological parameters, concentration-response relations were obtained for each artery by cumulative application of norepinephrine in concentrations of 6.25×10⁻⁸ through 10⁻⁴ mol/L in PSS and in cocaine-PSS in both the presence and absence of the endothelium. Endothelium-dependent and endothelium-independent relaxations were investigated in the absence of cocaine using cumulative application of acetylcholine and sodium nitroprusside, respectively, in concentrations from 10⁻⁹ through 10⁻⁴ mol/L. In arteries maximally preconstricted with norepinephrine (10⁻⁴ mol/L), endothelium-independent relaxation was investigated after endothelium removal to determine the direct effect of nitric oxide on the smooth muscle cells. Acetylcholine and sodium nitroprusside relaxations were generated in the same tissues as the norepinephrine curves. In all cases each concentration was applied for 2 minutes, the response for each concentration being taken as the maximal contraction or relaxation before application of the new concentration. Concentration changes were effected by draining the flow chamber and refilling with a new solution containing the required concentration. Between each concentration-response curve, vessels were allowed to reach their resting diameters and to equilibrate for 15 minutes in PSS or cocaine-PSS as appropriate. Before norepinephrine in cocaine-PSS concentration-response relations were performed, equilibration was achieved by intraluminal perfusion and extraluminal superfusion of vessels with cocaine-PSS for 15 minutes. In all concentration-response relations, application of drugs was effected extraluminally.

**Nomenclature**

Contractile responses to norepinephrine alone and norepinephrine plus cocaine were calculated as a percentage of the maximal response obtained under each condition as described by the expression

\[
\% \text{ Maximal Contraction} = \frac{(L_{D_{MN}} - L_{D_{NE}})}{(L_{D_{MN}} - L_{D_{NEma}})} \times 100
\]

where \(L_{D_{MN}}\) is the resting lumen diameter in the absence of any agonist, \(L_{D_{NEma}}\) is the lumen diameter achieved by a particular concentration of norepinephrine applied to the vessel, and \(L_{D_{NEma}}\) is the smallest lumen diameter induced by norepinephrine. In addition, contractions were calculated as the maximal percentage decrease in lumen diameter that could be induced by a particular agonist and calculated from the expression

\[
\% \text{ Relaxation} = \frac{(L_{D_{AS}} - L_{D_{NEma}})}{(L_{D_{MN}} - L_{D_{NEma}})} \times 100
\]

where \(L_{D_{AS}}\) is the lumen diameter achieved by a particular concentration of acetylcholine or sodium nitroprusside, \(L_{D_{NEma}}\) is the lumen diameter achieved by preconstriction with norepinephrine in the absence of acetylcholine or sodium nitroprusside, and \(L_{D_{MN}}\) is the resting lumen diameter when the artery is fully relaxed. Norepinephrine sensitivities are expressed as \(pD_2\) values, which were calculated as \(-\log_{10} E_{D_2}\) (moles per liter).

**Statistical Analyses**

For comparison of relaxation responses to acetylcholine and sodium nitroprusside and contractile responses to norepinephrine, ANOVA was used followed by the least significant difference test. Student’s unpaired \(t\) test was used to compare \(pD_2\) values, morphological measurements, and demographic data. Statistical significance was set at the 5% level. Data are given as mean±SEM.

**Results**

Table 1 shows the demographic details of the hypertensive patients and normotensive control subjects used in this study. Both systolic and diastolic blood pressures were significantly greater in hypertensive patients than in normotensive control subjects. Both age and height of the hypertensive patients were not different from normotensive control subjects. Body weight, though greater in hypertensive patients, was not significantly different from normotensive control subjects (\(P = .07\)).

**Small Artery Morphology**

Morphology measurements were carried out with arteries at their resting lumen diameters. In subsets of arteries from both groups there was no difference in the resting
TABLE 2. Characteristics of Human Gluteal Subcutaneous Resistance Arteries

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypertensive (n=17)</th>
<th>Normotensive (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media thickness, μm</td>
<td>31.5±1.5*</td>
<td>25.2±1.0</td>
</tr>
<tr>
<td>Lumen diameter, μm</td>
<td>289.3±12.4</td>
<td>316.0±15.3</td>
</tr>
<tr>
<td>Media-lumen ratio, %</td>
<td>11.0±0.5*</td>
<td>8.1±0.4</td>
</tr>
<tr>
<td>Media CSA, μm²</td>
<td>32 128±2458</td>
<td>27 327±2002</td>
</tr>
</tbody>
</table>

CSA indicates cross-sectional area.
*P<.01, †P<.001, hypertensive vs normotensive.

lumen diameter and the lumen diameter when the arteries were fully relaxed (normotensive: 310.5±21.1 and 311.4±20.8 μm in the presence and absence of calcium, respectively; hypertensive: 298.7±19.5 and 299.3±19.5 μm in the presence and absence of calcium, respectively; n=6 for both groups). Therefore, vessels from both groups did not develop myogenic tone. There were significant differences in vascular morphology between the two groups (Table 2). There was a highly significant increase in media thickness, whereas mean lumen diameter was not significantly decreased in arteries from hypertensive patients. The hemodynamically important parameter of the ratio of media thickness to lumen diameter was significantly increased in vessels from hypertensive patients. Media cross-sectional area was not significantly different between the two groups. The remodeling and growth indexes were 65% and 18%, respectively. These were calculated from previously defined equations.15

Contractile Responses

Potassium-PSS-Mediated Contractions

At a perfusion pressure of 15 mm Hg, potassium-PSS evoked a contraction of 50.3±4.4% of the baseline diameter of normotensive vessels. With increasing intraluminal pressure the contractile response increased to a maximum of 59.8±5.6% at 30 mm Hg. Above 30 mm Hg the contractions declined to a minimum of 23.2±3.8% at 120 mm Hg. Compared with normotensive vessels the contractile responses of hypertensive arteries were not influenced by intraluminal pressure. Contractions were similar over a larger pressure range, but the maximal response (57.5±4.6%) tended to be at 30 mm Hg. These data suggest a difference in the pressure-diameter response curves for normotensive and hypertensive vessels, with a flatter pressure-diameter response in hypertensive vessels.

Norepinephrine-Mediated Contractions

In vessels with intact endothelium, norepinephrine produced a concentration-dependent constriction. No time-dependent shift in norepinephrine sensitivity was found in arteries from either group, and the contractions evoked by extraluminal application of norepinephrine were comparable in hypertensive and normotensive vessels in the absence of cocaine (Fig 3). The maximal response did not differ between the groups (62.9±4.3% and 66.4±3.6% decrease in intraluminal diameter for hypertensive and normotensive vessels, respectively). Table 3 shows the sensitivities to norepinephrine expressed as pD₃ values. The addition of cocaine to inhibit neuronal amine uptake had no effect on the concentration-response curve from either group (Fig 4). After endothelium removal in the absence of cocaine, the contractions evoked by norepinephrine were augmented to a greater extent in vessels from normotensive subjects at the lower concentration range, and the maximal response to norepinephrine was augmented in both groups (Fig 5). Endothelium removal did not affect the resting lumen diameters in either group (normotensive: 331.2±24.3 and 326.0±23.1 μm, endothelium intact and removed, respectively; hypertensive: 318.5±20.8 and 320.0±21.0 μm, endothelium intact and removed, respectively). Similarly, the contractions evoked by NEK before and after endothelium removal did not differ either in vessels from normotensive subjects (74.4±2.1% and 75.6±2.1% decrease in intraluminal diameter, endothelium intact and removed, respectively) or
TABLE 3. pD₂ Values for Sensitivity to Norepinephrine in Human Subcutaneous Resistance Arteries

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Hypertensive (n=12)</th>
<th>Normotensive (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE+END-COC</td>
<td>6.47±0.12</td>
<td>6.38±0.08</td>
</tr>
<tr>
<td>NE+END+COC</td>
<td>6.46±0.09</td>
<td>6.31±0.07</td>
</tr>
<tr>
<td>NE-END-COC</td>
<td>6.52±0.11</td>
<td>6.65±0.11</td>
</tr>
<tr>
<td>NE-END+COC</td>
<td>6.43±0.12</td>
<td>6.69±0.08</td>
</tr>
</tbody>
</table>

NE indicates norepinephrine; COC, cocaine; and END, endothelium.

in arteries from hypertensive patients (72.2±1.9% and 73.4±2.0%, endothelium intact and removed, respectively).

The change in norepinephrine pD₂ (ΔpD₂E), calculated as pD₂ endothelium intact minus pD₂ endothelium removed in the absence of cocaine, was −0.27±0.10 in arteries from normotensive subjects and −0.05±0.06 in arteries from hypertensive patients (P=.08 for normotensive ΔpD₂E versus hypertensive ΔpD₂E). The change in norepinephrine sensitivity in endothelium-intact arteries in the presence of cocaine (ΔpD₂C), calculated as pD₂ in the absence of cocaine minus pD₂ in the presence of cocaine, was 0.07±0.33 in arteries from normotensive subjects and 0.04±0.15 in arteries from hypertensive subjects.

Cocaine had no effect on the norepinephrine concentration-response curves in vessels from normotensive subjects or hypertensive patients after endothelium removal (data not shown).

Vascular Relaxation

Acetylcholine produced a concentration-dependent relaxation of subcutaneous resistance arteries preconstricted with norepinephrine. At higher concentrations (10⁻⁵ and 10⁻⁴ mol/L), arteries from hypertensive patients displayed attenuated relaxation. The maximal relaxation response was greater in arteries from normotensive subjects than those from hypertensive patients (91.5±1.4% and 66.4±5.0%, respectively, at a concentration of 10⁻⁴ mol/L; P<.001), and dilatation induced by acetylcholine was abolished after the passage of an air bubble down the lumen of vessels from both groups, providing functional evidence of endothelium removal (Fig 6).

In the absence of the endothelium, extraluminal application of sodium nitroprusside produced a concentration-dependent relaxation of resistance artery segments preconstricted with norepinephrine from both hypertensive patients and normotensive control subjects. The responses were comparable between the two groups (Fig 6). The maximal relaxation response in vessels from normotensive subjects was 74.2±4.4% and from hypertensive patients was 78.1±3.2%.

Fig 4. Line graphs show concentration-response relations for extraluminal application of norepinephrine (NE) in arteries with intact endothelium in the presence and absence of cocaine. Top, Arteries from normotensive subjects (n=10); bottom, arteries from hypertensive patients (n=12). Cocaine had no effect on the response of each group of arteries.

Fig 5. Graphs show effects of endothelium removal on contractile responses to extraluminal norepinephrine (NE) in the absence of cocaine. Top, Concentration-response relation of normotensive vs hypertensive arteries. Response was significantly augmented in normotensive compared with hypertensive arteries at lower concentrations. *P<.05, **P<.01; n=10 for normotensive subjects and n=12 for hypertensive patients. Bottom, Maximal responses to norepinephrine. Responses to norepinephrine were augmented in both groups after endothelium removal. *P<.01, endothelium intact vs endothelium removed.
Discussion

This was the first study of the contractile and dilator responses of human resistance arteries using pressurized and perfused arterial segments obtained from hypertensive patients and normotensive control subjects. The function of these arteries has been compared under in vitro conditions that achieve for the most part those that exist in vivo. The application of all drugs to vessels was achieved by extraluminal application via the adventitial surface. This was done to mimic the in vivo situation in which endogenous transmitter release is effected from sympathetic nerve endings on the adventitia-media border.

In this study there was no difference between the groups in age, height, and weight although there were five more male essential hypertensive patients than normotensive control subjects. Therefore, it is unlikely that various factors relating to the demographic data could have affected the results.

The thickness of the arterial wall is a particularly important parameter in hypertension because vascular hypertrophy (thickening of the medial layer) is considered to be the adaptive hallmark of the disease, resulting in medial encroachment on the lumen. The structural measurements produced are similar to those previously reported for subcutaneous resistance arteries mounted on a myograph. In each case, both a greater media thickness and a greater ratio of media thickness to lumen diameter have been demonstrated. In addition, in the present study we found no difference in media cross-sectional area between hypertensive and normotensive vessels. An increased media thickness may be brought about by the rearrangement of existing material around a smaller lumen without change in media cross-sectional area or increase in growth—a process called remodeling. Remodeling is distinct from growth but can occur in conjunction with it. In practice, both processes may be involved in the hypertensive process. This increase in the media-lumen ratio is largely a consequence of remodeling rather than medial growth because the remodeling and growth indexes as defined previously were 65% and 18%, respectively.

Although it is perhaps speculative to extend these results to the rest of the resistance vasculature, the vessels used in the present study are considered to be of resistance size, and because we found an increase in the media thickness in vessels obtained from patients with established essential hypertension, these vessels may play a role in the etiology of the disease.

A potential problem when obtaining arteries from subcutaneous biopsies is the consequence of infiltrating the subcutaneous tissue with the anesthetic. It is not possible to determine whether the use of lignocaine affected the function of the vessels. However, the arteries were incubated in PSS for approximately 2 hours followed by both perfusion and superfusion for 1 hour before the experimental procedure was begun. Also, lignocaine has a short half-life, and its effects wear off in patients within 2 to 3 hours. Therefore, it is unlikely that any trace of the anesthetic remained in the vessels that might have influenced the results.

In essential hypertension there is evidence of an increased pressor response to vasoconstrictors but no increase in sensitivity. In our study we found no difference in sensitivity between hypertensive and normotensive vessels to the vasoconstrictor norepinephrine. This is in agreement with other studies that have looked for alterations in vascular sensitivity in essential hypertension. Earlier plethysmographic studies in essential hypertensive patients and in vitro studies of arteries have shown little or no change in excitation-contraction coupling properties although these arteries were too large to contribute to the peripheral vascular resistance. Furthermore, more recent in vitro studies using wire-mounted arteries from human glutal subcutaneous biopsies in the absence of flow have shown...
unaltered or decreased sensitivity to various agonists such as norepinephrine, serotonin, and vasopressin. Indeed, in the present study the arterial smooth muscle sensitivity to norepinephrine appears to be decreased because after removal of the dilator influence of the endothelium, the constrictor response to norepinephrine is suppressed in arteries from essential hypertensive patients. Thus, for the most part, there appears to be general agreement regarding the lack of evidence for an enhanced sensitivity to vascular agonists in essential hypertension. However, an increase in sensitivity to norepinephrine has been reported for the extramural artery of the gall bladder from females with mild hypertension and in gluteal subcutaneous resistance arteries from essential hypertensive patients despite a lack of detectable medial hypertrophy. The $pD_2$ values for norepinephrine sensitivity in our study are similar to those found previously. In our experience cannulated and pressurized rat mesenteric arteries are more sensitive than those mounted on wires (unpublished data). However, we have no data on human vessels using the two methods.

There is considerable evidence supporting the role of the sympathetic nervous system in the development and maintenance of hypertension, and neuronal amine uptake appears to be greater in the spontaneously hypertensive rat. This increase in uptake has also been documented for essential hypertension using the myograph. In the present study we found that inhibition of neuronal amine uptake with cocaine in both hypertensive and normotensive vessels produced no change in sensitivity to norepinephrine in vessels from either group. Therefore, the reduced norepinephrine sensitivity in decidualized arteries from hypertensive patients is not a consequence of an increased neuronal amine uptake. Indeed, in studies in which the neuromuscular junction has been assessed in essential hypertension by measurements of the rate at which $[^3H]$norepinephrine is removed from the plasma, there is evidence of an impairment in the neuronal uptake of norepinephrine. The reason for this lack of effect of cocaine is currently difficult to explain. In other experiments in our laboratory we have found that cocaine in cannulated and pressurized vessels has no effect on the concentration-response relation of norepinephrine in rat mesenteric vessels from both spontaneously hypertensive and normotensive Wistar-Kyoto rats, whereas on the wire myograph the concentration-response curves are shifted to the left (unpublished data). This may be due to the difference in the properties of the vessels set up under the two conditions. Pressurized vessels experience both longitudinal and axial stretch, whereas isometrically contracted, wire-mounted vessels experience the latter only. Also, it is possible that the structural geometry of the vascular smooth muscle cells, which must differ in the two systems, alters their functional properties. Therefore, the role of neuronal amine uptake on norepinephrine sensitivity remains controversial.

Arteries from both normotensive subjects and hypertensive patients exhibited a concentration-dependent relaxation to extraluminal acetylcholine. In the lower concentration range, responses between the two groups were similar. However, at the higher concentrations the response was significantly attenuated in vessels from hypertensive patients. This impairment of endothelium-dependent relaxation has been reported in human in vivo studies and is of particular importance in established hypertension because attenuated endothelium-dependent relaxation may have a role in the elevated peripheral resistance by maintaining or facilitating contraction. The impaired response in hypertensive vessels could be caused by the thicker media, resulting in an increase in the diffusion pathway for endothelial cell vasodilators to reach the endothelium. However, impairment of relaxation was most marked at the higher acetylcholine concentrations. If limited diffusion were the cause of impaired relaxation, then it might be expected that the low concentrations of acetylcholine would be associated with reduced relaxation. As relaxation to sodium nitroprusside did not differ between hypertensive and normotensive vessels, this would indicate that there is no difference in the smooth muscle response to nitric oxide, now thought to be the identity of endothelium-derived relaxing factor. Furthermore, endothelium removal enhanced the constrictor response to norepinephrine to a much greater extent in normotensive vessels, suggesting an impairment in the endothelial ability to limit norepinephrine-induced tone in arteries from essential hypertensive patients. Several possible explanations exist for an impaired net dilator response, and these have been addressed previously.

The type of preparation used in this study is a relatively new and much improved technique compared with wire-mounted preparations for the in vitro investigation of the functional and morphological properties of small arteries. With this technique the vessels experience a true transmural pressure that maintains the vessel in a more physiological shape. The integrity of the endothelium remains intact during the mounting procedure, and vessels experience the influence of fluid flow across the luminal surface. In addition, drugs may be added to vessels via the luminal or abluminal surfaces, and vascular reactivity is determined in a more physiological manner, with measurement of active changes in vessel diameter rather than changes in active tension in the vessel wall.

Therefore, the more appropriate technique of cannulated and pressurized arteries provides further evidence for the altered structure and function of small arteries in essential hypertension. In particular, the finding of apparent endothelial dysfunction is of importance. Although this finding in itself is not novel, it is significant because it provides important confirmation of previous observations obtained in vivo.

In summary, the main findings of this study are that in the presence of flow subcutaneous resistance arteries from patients with established essential hypertension exhibit norepinephrine sensitivity that is comparable to that from arteries of normotensive subjects in the presence of functional endothelium and is unaffected by inhibition of neuronal amine uptake. In addition, hypertensive vessels are characterized by a depressed endothelium-dependent dilator response and reduced endothelium-mediated inhibition of vasoconstriction. Therefore, the data suggest a potential pathogenic role for endothelial dysfunction contributing to the increased peripheral resistance in essential hypertensive vascular disease.

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B J Falloon and A M Heagerty

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