Evidence for Increased Media Thickness, Increased Neuronal Amine Uptake, and Depressed Excitation–Contraction Coupling in Isolated Resistance Vessels From Essential Hypertensives

Christian Aalkjær, Anthony M. Heagerty, Karin K. Petersen, John D. Swales, and Michael J. Mulvany

The functional and morphologic characteristics of isolated subcutaneous resistance vessels (about 170 μm i.d.) from 15 untreated subjects with essential hypertension and 15 matched controls were examined. The vessels from the hypertensives had a 29% increase in the media-thickness-to-lumen-diameter ratio. The maximal force development to noradrenaline (NA) expressed as active pressure (an estimate of the pressure the vessels could have contracted against in vivo) was 30% higher in vessels from the hypertensives, while active media stress (force per square unit of smooth muscle) and sensitivity to NA was not significantly different. Increased active pressure, as well as unaltered active media stress and sensitivity, was seen for vasopressin, serotonin, angiotensin II, and K+. There was, however, an enhanced leftward shift of the NA sensitivity with cocaine (an inhibitor of the neuronal amine pump) in vessels from the hypertensives [pD2( + cocaine) and pD2(− cocaine) were 0.185 ± 0.053 and 0.040 ± 0.044, hypertensives and normotensives, respectively, p < 0.05] suggesting an abnormality of presynaptic function in essential hypertension. Furthermore, the calcium sensitivity was depressed (pD2 was 4.197 ± 0.050 and 4.381 ± 0.068, hypertensives and normotensives, respectively, p < 0.05), and the rate of relaxation was faster (p < 0.05) in vessels from hypertensives, suggesting that excitation–contraction coupling might be depressed. The results suggest that the increased pressor response in essential hypertension can, to a large extent, be explained by altered vascular structure, while smooth muscle function is either unchanged or possibly depressed.

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The increased vascular reactivity seen in patients with established essential hypertension could be due to an altered vascular structure and/or to alteration in the excitation–contraction coupling (e–c coupling) of the vascular smooth muscle (VSM).1-2 Although increased wall-thickness-to-lumen-diameter ratio has previously been demonstrated by hemodynamic measurements3-4 and by histologic measurements on autopsy material,5-6 there is still some lack of quantitative information concerning the structure of the resistance vasculature in essential hypertension.1 Regarding the evidence for the increased reactivity being caused by an altered e–c coupling of the VSM, in the few studies where human material has been used, the results have been negative, although the arteries used were too large to have been of hemodynamic importance.5-10 Thus, the relative roles of altered vascular structure and e–c coupling in human essential hypertension are still not clarified. However, the roles can be clarified by in vitro studies of arteries small enough to contribute to the control of the peripheral resistance. Recently, it has become possible to study arteries that meet this criterion by using arteries from human subcutaneous biopsies taken during local anesthesia.11 Arteries (about 170 μm i.d.) from 15 patients with essential hypertension and 15 normotensive controls were used in this study.

Materials and Methods

Subjects

The 15 subjects (11 males) with essential hypertension were recruited from the outpatient clinic of the Department of Medicine, Leicester Royal Infirmary, Leicester, U.K. All subjects had a supine blood pressure of over 140/95, measured at least 3 times with a random zero sphygmomanometer, and had no evidence of kidney disease. The group had a mean supine blood pressure of 175 ± 6/104 ± 4 mm Hg (mean ± SEM), a mean standing blood pressure of 172 ± 7/115 ± 4 mm Hg, a mean age of 51.1 ± 3.2 years, and a mean weight of 79.4 ± 3.1 kg; none of the individuals had been or was on antihypertensive treatment. The 15 normotensive control subjects (11 males) were recruited from the public through advertisement in a local newspaper. They had a mean supine blood pressure of 132 ± 5/76 ± 2 mm Hg, a mean standing blood pressure of 178 ± 6/111 ± 3 mm Hg, and a mean age of 51.1 ± 3.2 years.
pressure of 130 ± 5/82 ± 3 mm Hg, a mean age of 52.6 ± 3.2 years, and a mean weight of 73.9 ± 3.1 kg. All participants were informed of the nature of the experiment and gave their consent in accordance with the requirements of the local ethical committee.

**Preparation**

Artery segments, about 2 mm long, were dissected from biopsies (about 0.5 x 0.5 x 1.5 cm) of skin and subcutaneous tissue taken under local anesthesia (3–5 ml 2% lidocaine hydrochloride) from the gluteal region. From each of 23 biopsies, 2 vessel segments were dissected out, while in 7 biopsies (5 from hypertensives, 2 from normotensives), only 1 vessel segment was found. For all parameters, only one value is given for each individual (where two vessel segments were found, the mean of the two is given). Vessel segments were mounted as ring preparations in a myograph by threading them on two 40-μm steel wires, which were attached to a force transducer and a micrometer, respectively.

**Protocol and Solutions**

The vessels were mounted in the myograph and then kept in a standard saline solution for 60 minutes at 37°C. The standard saline solution had the following composition (mM): NaCl 119, NaHCO3 25, KCl 4.7, KH2PO4 1.18, MgSO4 1.17, CaCl2 2.5, ethylenediaminetetraacetic acid (EDTA) 0.026, glucose 5.5, and pH 7.45 when gassed with 5% CO2-95% O2. After the rest period, the media thickness was measured using a light microscope. The vessel was then set to an internal circumference, L0, which was determined as described previously. In brief, the passive-tension–internal-circumference relation was determined. The circumference that the vessels would have had in vivo when relaxed and under a transmural pressure of 100 mm Hg was found (L100) using Laplace’s law (ΔP = ΔT/r, where ΔP is transmural pressure, ΔT is tension, and r is radius). L0 was then taken as 0.9 L100, and the normalized internal diameter, Ln, was taken as L/r. The normalized media thickness at the normalized diameter was determined assuming a constant media volume. In pilot experiments, the force development was found to be near maximal at this setting for both groups of vessels. The vessels were then stimulated using the following protocol: 1) three stimulations (2 minutes) with K-saline solution (standard saline with potassium chloride substituted for sodium chloride) and then one stimulation with K-saline solution containing 5 μM noradrenaline (NA) (Sigma Chemical Co., St. Louis, Mo.); 2) two cumulative NA dose-response determinations (from 0.04 to 5.0 μM, 2 minutes per concentration), the last one in the presence of 3 μM cocaine (in preliminary experiments, 3 μM of cocaine was found to give a maximal and reversible effect on the NA sensitivity); 3) cumulative dose-response determinations to vasopressin (arginine-vasopressin [ADH], Sigma, from 0.016 to 2.0 mU/ml), serotonin (serotonin creatinine sulphate, Sigma, from 0.01 to 5.0 μM), angiotensin II (ang II) (human sequence, Sigma, from 0.3 to 100.0 nM), and K+ (from 6 to 125 mM, potassium chloride substituted for sodium chloride); these dose-response determinations were made in random order; and 4) cumulative calcium dose-response determinations with NA activation as previously described.

**Calculations and Statistics**

The force development was expressed either as active pressure (Δp) on the basis of Laplace’s law (Δp = 2ΔT/10) where Δp is the pressure against which the vessels can contract and ΔT is increase in wall tension on stimulation, or as active media stress (Δp = ΔT/m) where m is the normalized media thickness. The sensitivity to agonists was determined as ED50 (Mu), except for ADH, which was given as ED50 (IU/ml) and expressed as pD2 = —log(ED50). Differences in pD2, media stress, and active pressure for stimulation with ADH, serotonin, ang II, and K+ were tested with a two-way analysis of variance as indicated in Table 1, and the effect of cocaine on the NA sensitivity was tested with a paired t test, while differences between other parameters were tested with a two-tailed Student’s t test (p < 0.05 was considered significant). The correlations were made using least-squares regression.

**Results**

Although there was no difference between the normalized internal diameters of the vessels from the two groups, the media thickness was increased by 22%, and the ratio of media-thickness-to-lumen-diameter ratio was increased by 29% in vessels from the hypertensives (Table 1). From Figure 1, it is seen that the increase in media thickness was most prominent in the larger vessels (about 200 μm diameter) while the vessels with a diameter of about 100 μm had no increase in media thickness.

The mechanical responses to NA are shown in Figure 2 and to a number of other agonists in Table 1. In all

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**FIGURE 1. Relation between normalized media thickness and normalized lumen diameter of subcutaneous resistance vessels from 15 patients with essential hypertension (•) and 15 normotensive controls (○). Correlations were made using least-squares regression, and correlation coefficients were 0.50 (p < 0.05) and — 0.15 (NS), hypertensives and normotensives, respectively. Furthermore, slopes of two lines (0.037 ± 0.017 and —0.005 ± 0.009, hypertensives [H] and normotensives [C], respectively) were significantly (p < 0.05) different.**
cases, there was an increased active pressure in the vessels from the hypertensives. It appeared, however, that there was no difference in either sensitivity to the vasoactive substances or active media stress (Figure 2 and Table 1).

To determine the calcium sensitivity of these vessels, calcium dose-response experiments were carried out with NA-stimulated vessels, and a decreased calcium sensitivity was found in the vessels from the hypertensives (Figure 3). Also, the rate of relaxation after a stimulation was evaluated, and a faster relaxation was found in vessels from the hypertensives (Figure 4). Possible presynaptic abnormalities were investigated using cocaine to inhibit presynaptic uptake of NA. The sensitivity to NA (pD$_2$) in the presence of cocaine was 6.512 ± 0.050 and 6.507 ± 0.067, hypertensives and normotensives, respectively, and this was not significantly different. Star indicates that maximal effective pressure was significantly (p < 0.05) increased in vessels from hypertensives using two-tailed Student's t test.

Furthermore, this cocaine-induced shift in NA sensitivity was significantly greater in vessels from the hypertensives (Figure 5), and it was significantly and positively correlated (r = 0.61, p < 0.05) with lumen diameter in vessels from the hypertensives.

**Discussion**

Dissecting small arteries from skin biopsies taken from subjects under local anesthesia makes it possible to investigate isolated small arteries from very well-defined groups of subjects. This is a great advantage compared with similar previous studies that have relied either on biopsy material obtained in connection with surgery on patients for various reasons or on autopsy material. The vessels were obtained from either patients who were in the established phase of essential hypertension or controls who were motivated volunteers from the public and were carefully matched for donor’s sex, age, and weight so there was little chance that the results were influenced by various confounding factors.

**Table 1. Characteristics of Isolated Subcutaneous Resistance Vessels**

<table>
<thead>
<tr>
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<th>Essential hypertensives</th>
<th>Controls</th>
<th>p</th>
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<tbody>
<tr>
<td>Lumen diameter (μm)</td>
<td>167 ± 10</td>
<td>180 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Media thickness (μm)</td>
<td>16.4 ± 0.7</td>
<td>13.4 ± 0.4</td>
<td>$\dagger$</td>
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<tr>
<td>Media volume/segment length (μm$^2$)</td>
<td>9.746 ± 819</td>
<td>8.277 ± 562</td>
<td>NS</td>
</tr>
<tr>
<td>Media thickness/lumen diameter (%)</td>
<td>10.29 ± 0.60</td>
<td>7.95 ± 0.58</td>
<td>$\dagger$</td>
</tr>
<tr>
<td>pD$_2$ (ADH)$\S$</td>
<td>3.636 ± 0.055</td>
<td>3.819 ± 0.061</td>
<td>$\dagger$</td>
</tr>
<tr>
<td>pD$_2$ (serotonin)$\S$</td>
<td>6.482 ± 0.181</td>
<td>6.709 ± 0.159</td>
<td>NS</td>
</tr>
<tr>
<td>pD$_2$ (angiotensin II)$\S$</td>
<td>7.873 ± 0.096</td>
<td>7.711 ± 0.081</td>
<td>NS</td>
</tr>
<tr>
<td>pD$_2$ (K$^+$)$\S$</td>
<td>1.447 ± 0.021</td>
<td>1.467 ± 0.023</td>
<td>NS</td>
</tr>
<tr>
<td>Media stress (ADH) (mN/mm$^2$)</td>
<td>141.3 ± 13.8</td>
<td>160.2 ± 16.7</td>
<td>$\dagger$</td>
</tr>
<tr>
<td>Media stress (serotonin) (mN/mm$^2$)</td>
<td>62.6 ± 13.9</td>
<td>93.6 ± 22.8</td>
<td>NS</td>
</tr>
<tr>
<td>Media stress (angiotensin II) (mN/mm$^2$)</td>
<td>89.5 ± 15.0</td>
<td>108.7 ± 20.8</td>
<td>NS</td>
</tr>
<tr>
<td>Media stress (K$^+$) (mN/mm$^2$)</td>
<td>104.9 ± 7.9</td>
<td>117.8 ± 14.0</td>
<td>NS</td>
</tr>
<tr>
<td>Active pressure (ADH) (mN/mm$^2$)</td>
<td>29.5 ± 2.1</td>
<td>23.9 ± 1.4</td>
<td>$\dagger$</td>
</tr>
<tr>
<td>Active pressure (serotonin) (mN/mm$^2$)</td>
<td>12.9 ± 3.1</td>
<td>12.2 ± 2.5</td>
<td>$\dagger$</td>
</tr>
<tr>
<td>Active pressure (angiotensin II) (mN/mm$^2$)</td>
<td>17.8 ± 2.7</td>
<td>14.5 ± 2.1</td>
<td>$\dagger$</td>
</tr>
<tr>
<td>Active pressure (K$^+$) (mN/mm$^2$)</td>
<td>20.5 ± 1.5</td>
<td>17.1 ± 1.6</td>
<td>$\dagger$</td>
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*p < 0.05, $p < 0.02, $p < 0.005, $\S$pD$_2$ = −log(ED$_{90}$). $\dagger$analysis of variance. ADH, arginine-vasopressin.
The arteries were taken from the subcutaneous bed, which like all vascular beds has a specialized function but, in addition, takes part in peripheral resistance control. It is, of course, difficult to predict the extent to which results obtained in these vessels are representative for the entire resistance vasculature. However, the fact that an increase was found in the media thickness of the vessels from the hypertensives strongly suggests that these vessels take part in the pathogenesis of hypertension. Another possible difficulty concerns the use of local anesthetic when removing the biopsies. For obvious reasons, we have not been able to exclude entirely the possibility that the local anesthetic has affected the functional characteristics of the vessels. However, because the vessels were held in saline solution for at least 3 hours before starting the functional studies, it is almost certain that the anesthetic had been completely washed out. Furthermore, as we have been most concerned with comparing vessels from hypertensives and normotensives, it is unlikely that the anesthetic would have affected the two sets of vessels differently.

The 29% increase in media-thickness-to-lumen-diameter ratio of the vessels from the hypertensives almost precisely matches the 36% increase in blood pressure. In the gluteal region, where the blood pressure in both groups will be about 30 mm Hg higher than at the heart level, the increase in blood pressure is only 28%, which makes the match even more precise. The structural alteration alone could, therefore, account for the increased peripheral resistance in the section of the vascular bed studied here. It seems, however, from Figure 1 that the more distal arteries have less structural changes, and to the extent that this

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Panel A: Recording of noradrenaline stimulated calcium dose-response curves of subcutaneous resistance vessels from normotensive control (169 μm i.d.) and from patient with essential hypertension (167 μm i.d.). Panel B: Scattergram of sensitivity to calcium expressed as $pD_2 = -\log ED_50(M)$. Bars show mean ± SEM, and star indicates significant ($p < 0.05$) difference between two groups using a two-tailed Student's $t$ test.
Figure 4. Panel A: Recordings of responses of force development to K-saline solution containing 5 μM noradrenaline (indicated by horizontal line), and relaxation following washout of artery (210 μm i.d.) from hypertensive subject (H) and artery (223 μm i.d.) from control subject (C). Panel B: Scattergram of relaxation after stimulation expressed as relative (%) decline in tone 1 minute after washout of activating solution. Bars show mean ± SEM, and star indicates significant (p < 0.05) difference between two groups using two-tailed Student's t test.

Figure 5. Scattergram of effect of cocaine on noradrenaline sensitivity of isolated resistance vessels from subjects with essential hypertension (H) and from normotensive controls (C). Sensitivity is expressed as \( pD_2(NA + coc) - pD_2(NA) \), where \( pD_2(NA + coc) \) and \( pD_2(NA) \) is \(-\log(ED_50)\) in presence and absence of 3 μM cocaine, respectively. Bars show mean ± SEM, and star indicates significant (p < 0.05) difference between two groups using two-tailed Student’s t test.

Reflects the situation within the individual, it is in accordance with the observations reported by Furuyama and Short. Since about 50% of the peripheral resistance probably lies distal to the 100-μm arteries, from these data it does not appear likely that an increased media-thickness-to-lumen-diameter ratio alone will explain the entire increase in peripheral resistance. Furthermore, it is interesting that the effect of cocaine on NA sensitivity was positively correlated with the lumen diameter, which is consistent with the possibility that the increased media thickness is most prominent in the vessels where the neurotrophic influence is most important.

The functional relevance of an increased media-thickness-to-lumen-diameter ratio as predicted by Folkow is fully supported by our results because the increased active pressure seen in vessels from the hypertensives could be explained on the basis of the altered structure alone since there was no increase in active media stress. This finding is in accordance with previous studies on larger arteries, where any increase in force development could also be accounted for by an altered structure. In addition, our finding of no significant difference in the sensitivity to various agonists is again in accordance with previous studies on larger arteries; although in one study, an increased NA sensitivity of the extramural artery of the gall bladder from females with mild hypertension was found, while it was depressed in similar vessels from males. Thus, there seems to be a reasonable agreement regarding the lack of evidence for an enhanced e-c coupling in isolated arteries from essential hypertensives when judged from dose-response determinations to various agonists, and this also suggests that the increased pressor response found in vivo can to a great extent be explained on a structural basis.

In further contrast to the notion that smooth muscle sensitivity should be increased in essential hypertension, a decreased sensitivity to Ca2+ and an increased rate of relaxation in vessels from the hypertensives was found. These two findings are consistent with the possibility that vascular smooth muscle e-c coupling is depressed in essential hypertension in the established phase, possibly as a consequence of the increased muscle mass. It is tempting to speculate that such alterations could account for the enhanced effectiveness of calcium antagonists as vasodilators in human essential hypertension.

There is much evidence for an alteration in the sympathetic system in essential hypertension. Our present finding of an increased effect of cocaine in the vessels from hypertensives, suggesting an increased neuronal uptake of NA, is in accordance with our findings in vessels from the SHR. However, in studies where the neuromuscular junction has been assessed in essential hypertension by measurements of the rate with which \(^{3}H\)-noradrenaline disappears from the plasma, there is evidence for a defect in the neuronal uptake of NA. The reason for this discrepancy is currently difficult to understand, but one possibility is that the plasma has an effect on the in vivo uptake, which is different in hypertensives and normotensives. Further investigations are clearly required to make more direct measurements on the activity of the
neuronal amine pump in essential hypertension.

In conclusion, the results suggest that the increased vascular reactivity in established essential hypertension can, to a large extent, be ascribed to structural alterations in the resistance vasculature. We found no evidence for an enhanced e-c coupling of the VSM, which is in accordance with the sparse information previously obtained by others using larger arteries. Indeed, there was some evidence for a depressed e-c coupling from the decreased calcium sensitivity and the increased rate of relaxation after a contraction. In addition, evidence for an increased effect of cocaine on NA sensitivity was found. Although the interpretation of these experiments requires further investigations, it does point to an involvement of the neuromuscular junction in the pathogenesis of hypertension. The results have therapeutic implications that require further exploration — antihypertensive therapy, which causes vascular structural regression as opposed to reduced VSM activity, may be a more logical and effective therapy for essential hypertension in man.

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**Key Words**: essential hypertension • resistance vessels • sympathetic nerves • calcium • noradrenaline
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