Histology of Subcutaneous Small Arteries From Patients With Essential Hypertension

Niels Korsgaard, Christian Aalkjær, Anthony M. Heagerty, Ashley S. Izzard, Michael J. Mulvany

The purpose of the present study was to determine the cellular basis for the increased ratio of media thickness to lumen diameter (media-lumen ratio) consistently found in the peripheral resistance arteries from patients with essential hypertension using an unbiased stereological principle (the "disector"). Segments of subcutaneous resistance arteries (approximately 200 μm internal diameter) were isolated from gluteal biopsies of skin and subcutaneous fat taken from 16 untreated patients with essential hypertension and 16 age- and sex-matched normotensive control subjects. Measured under standardized conditions (ie, relaxed and under controlled mechanical conditions) on an isometric myograph, vessels from hypertensive patients had a significant (P<.05) reduction in lumen diameter and an increase in media-lumen ratio (P<.05) compared with vessels from normotensive control subjects. These changes were not associated with alterations in the estimated media volume per segment length. After these measurements had been made, the arteries were fixed, serial sectioned, and stained. The volume fraction of smooth muscle cells within the media was estimated by point counting on photomicrographs of the vessels. Using the disector principle, we determined the numerical density (number per unit volume) of smooth muscle cells within the media of each vessel and calculated the average smooth muscle cell volume (1775±122 [mean±SEM] and 1532±112 μm³, hypertensive and normotensive, respectively, P>.05) on the basis of these measurements. Furthermore, by a combination of the myograph measurements and the histological estimates, the number of smooth muscle cells per unit segment length (4.61±0.55 and 5.81±0.57 μm⁻¹, hypertensive and normotensive, respectively, P>.05) also was calculated. These results suggest that the reduction in lumen diameter and the increase in media-lumen ratio observed in essential hypertension are brought about primarily by a rearrangement of smooth muscle cells within the medial layers of the arterial wall. The nonsignificant increase in individual cell size would indicate that myocyte hypertrophy may occur in the circulation at this level but is less important than remodeling in human hypertension. (Hypertension. 1993;22:523-526.)

KEY WORDS • hypertension, essential • arteries • muscle, smooth, vascular • hypertrophy • hyperplasia

Established human essential hypertension is characterized by an increase in total peripheral resistance,1 and hemodynamically it has been shown that this is present at maximal vasodilatation.1,2 This indicates that the increased resistance is structurally determined rather than attributable to functional changes in the vasculature. Histological investigations on autopsy material support this demonstration by a decreased lumen diameter3 and an increased ratio of media thickness to lumen diameter (media-lumen ratio)4,5 in arteries from essential hypertensive patients. In these investigations the structural changes were mainly confined to small arteries that are known to participate actively in the physiological regulation of blood pressure.3 With the use of isolated vessels from biopsies of subcutaneous fat, it has become possible to investigate such small arteries in vitro under standardized conditions. It has been confirmed that hypertension is associated with an increased media-lumen ratio, whereas smooth muscle cell function in these vessels is either unchanged or even depressed.5,6 However, the cellular basis for the increased media-lumen ratio is unknown. In spontaneously hypertensive rats,8 the increased media-lumen ratio is associated with an increased number of smooth muscle cells per vessel segment length, perhaps suggesting hyperplasia, whereas the increased media-lumen ratio in one-kidney, one clip Goldblatt hypertensive rats is associated with smooth muscle hypertrophy.9 In contrast, neither cellular hypertrophy nor hyperplasia is found in resistance arteries taken from mouse Ren-2 renin gene transgenic rats.10 This indicates that in these rats the increased media-lumen is caused by what has recently been termed remodeling,11 defined as a rearrangement of an unchanged number of identical smooth muscle cells within a greater number of layers around a smaller lumen. Therefore, to clarify the cellular basis for the increased media-lumen ratio in essential hypertension, we have compared the cellular morphology in isolated subcutaneous resistance arteries taken from 16 untreated patients with essential hyper-
tension with the morphology of 16 age- and sex-matched normotensive control subjects using an unbiased counting rule (the "disector").

**Methods**

Sixteen untreated patients (10 men and 6 women) with essential hypertension and without any signs of kidney disease were recruited from the outpatient clinic of the Department of Medicine, Leicester, UK. All subjects had a supine blood pressure greater than 140/95 mm Hg measured at least three times with a random-zero sphygmomanometer. Sixteen age- and sex-matched healthy normotensive control subjects were recruited through an advertisement placed in a local newspaper. All participants were informed of the nature of the experiment and gave their consent in accord with the requirements of the local ethics committee.

Biopsies, approximately 0.5 x 0.5 x 1.5 cm, of skin and subcutaneous tissue were taken from the gluteal region of each individual under local anesthesia (3 to 5 mL 2% lidocaine hydrochloride). Segments of artery, approximately 4-mm long, were dissected from the biopsies. From 11 biopsies, two vessel segments were dissected out, whereas in 21 biopsies, only one vessel segment was found. Where two segments were found, the mean of the measurements made in those vessels was used for further calculation.

The vessel segments were mounted as ring preparations in a myograph by threading them on two 40-μm steel wires attached to a force transducer and a micrometer, respectively. Details of the myograph measurements have been described elsewhere. Briefly, the arteries were held completely relaxed in physiological salt solution (PSS). The media cross-sectional area was determined using a microscope with a x40 water immersion lens and an ocular micrometer. The vessel was set to internal circumference, L₁, corresponding to 90% of the internal circumference, which the vessel would have had when relaxed and subjected to a transmural pressure of 100 mm Hg. Normalized lumen diameter, l₂, was taken as L₁/π. The corresponding media thickness, mᵢ, was calculated from the media cross section, which was assumed to be constant.

When the myograph measurements were completed, the bathing solution was changed to calcium-free PSS for 10 minutes to prevent a vasoconstrictive effect of the fixative. With the arteries still mounted on the wires, the solution was changed to fixative, 2.5% buffered glutaraldehyde. The vessels were dismounted, washed in PSS, preembedded in agar to maintain orientation, and finally embedded in 2-hydroxyethyl methacrylate (Historesin, LKB, Stockholm, Sweden). In each artery, from a point approximately halfway between where the mounting wires had been, a series of three to five 3-μm serial sections parallel to the vessel axis was made on a precision microtome (Supercut 2065, Reichert-Jung, FRG). All sections were placed on glass slides, coded, stained with Giemsa stain, and mounted in Eukitt (Merck, Darmstadt, FRG) under glass cover.

Unbiased estimates of smooth muscle cell number within the arteries were determined using the disector principle. Successive sections were placed under two specially equipped microscopes (BH2, Olympus, Tokyo, with x 100 oil immersion lenses, Planochromat, 100 W halogen lamp), projecting the images of the sections side by side on to a table top at a total magnification of x1650. The number of nuclei present in the first section but not in the second together with the number of nuclei present in the second but not in the first were counted in 10 nonoverlapping areas per vessel. Assuming that each smooth muscle cell contained one and only one nucleus, the mean number of smooth muscle cells per unit volume of media (the cell numerical density, Nᵥ) could be calculated by dividing half the total number of nuclear ends by the total disector volume. Disector volume was equal to the known counting area multiplied by the section thickness (hₛ, 3±0.05 μm), estimated by measuring steps in a plastic block after stepwise sectioning. The volume fraction of media containing smooth muscle cells (Vᵥ) was determined by point counting on micrographs (magnification x1450) made of the first section (Agfapan ASA 64, Agfa-Gevaert, FRG, on AH-2, Vanox, Olympus, Tokyo). Mean cell volume (vᵥ) was then equal to Vᵥ/Nᵥ. The number of cells per unit vessel length was equal to the cell numerical density (Nᵥ) multiplied by the media cross-sectional area determined on the myograph.

Results are shown as mean ± SEM. Initially, statistical evaluation was done with a two-way analysis of variance testing for variation among groups (blood pressure) and within groups (sex). No changes were found in any of the investigated parameters according to sex, and further statistical testing was therefore done only between the hypertensive and normotensive groups. Significance of differences between hypertensive and normotensive individuals was assessed by an unpaired, double-sided Student's t test. Probability levels less than 5% were considered significant.

**Results**

Characteristics of patients are summarized in Table 1. Both lying and standing diastolic pressures were increased by approximately 40%, and systolic pressure was raised by approximately 30% in the hypertensive patients compared with control subjects.

The morphological results are summarized in Table 2. Increased blood pressure was associated with a 17% reduction in arterial lumen diameter (P<.05) and a 31% increase in media-lumen ratio of the resistance arteries from the essential hypertensive patients compared with control subjects (P<.05). An increased media-lumen

**Table 1. Characteristics of Untreated Essential Hypertensive Patients and Age- and Sex-Matched Normotensive Control Subjects**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypertensives (n=16)</th>
<th>Normotensives (n=16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.2±2.7</td>
<td>48.8±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>LDP, mm Hg</td>
<td>104±5</td>
<td>72±3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LSP, mm Hg</td>
<td>171±7</td>
<td>130±6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SDP, mm Hg</td>
<td>109±4</td>
<td>79±4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SSP, mm Hg</td>
<td>168±7</td>
<td>128±6</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

LDP indicates lying diastolic pressure; LSP, lying systolic pressure; SDP, standing diastolic pressure; and SSP, standing systolic pressure. Significance levels are by two-tailed Student's t test. Values are mean±SEM.
A hallmark of established hypertension is, together with a structurally determined reduction in lumen diameter, an increased media-lumen ratio in small subcutaneous resistance arteries of patients with untreated essential hypertension, which apparently is independent of whether the hypertension is primary or secondary. An increased media-lumen ratio has often been taken as an indicator of vascular growth. However, our myograph measurements did not reveal signs of growth, because media volume per segment length was normal; indeed, it tended to be reduced. This conclusion is supported by our findings of a significantly reduced outer diameter of the vessels in hypertensive patients. The histometric results (Table 2) also suggested a lack of growth, because these showed that there was no significant evidence of cellular hypertrophy or hyperplasia or any difference in the volume fraction of smooth muscle cells within the media. Therefore, taken together, these results indicate that the increased media-lumen ratio found in subcutaneous resistance vessels from patients with untreated essential hypertension could be accounted for primarily by a rearrangement (remodeling) of smooth muscle cells around a smaller lumen.

**Discussion**

The main findings of the present investigation are that the reduced lumen diameter and increased media-lumen ratio found in small subcutaneous resistance arteries of patients with untreated essential hypertension are not associated with smooth muscle cell hyperplasia or hypertrophy but were caused by a reorientation of the same number of smooth muscle cells within the media of hypertensive vessels compared with vessels obtained from normotensive control subjects.

A hallmark of established hypertension is, together with a structurally determined reduction in lumen diameter, an increased media-lumen ratio in resistance arteries, which apparently is independent of whether the hypertension is primary or secondary. An increased media-lumen ratio has often erroneously been taken to be synonymous with growth. However, as has been pointed out before, this may not be the case. Recent histological examinations of isolated subcutaneous resistance vessels investigated under standardized conditions have suggested that any vascular growth that occurs is small and therefore at most plays a minor role in determining vascular media-lumen ratio in essential hypertension. These findings are supported by the present study in which the media volume per segment length (media cross-sectional area) was unchanged in essential hypertension, even showing a slight tendency for reduction in spite of a significant 31% increase in media-lumen ratio. An increase in the media-lumen ratio without growth could, of course, be a consequence of a decrease in vascular distensibility. However, in rats with primary hypertension, studies have indicated that the distensibility in small arteries is not decreased, whereas more recent investigations have shown that this is also true for small arteries from essential hypertensive patients (M.J. Mulvany, personal communication). Therefore, the results of these investigations taken together indicate that vascular growth is not a major prerequisite for changes in media-lumen ratio. In other words, it seems that the increased media-lumen ratio observed in resistance arteries in essential hypertension is mainly caused by a rearrangement of an unchanged amount of material within the tunica media, resulting in a reduced lumen diameter (remodeling).

An important question concerning this apparent remodeling is whether the constituents of the wall are also identical. Thus, remodeling implies not only that the amount of material is the same but that both the amount of interstitial tissue and the smooth muscle cell volume in vessels of hypertensive patients and control subjects are identical. The present study, which to the best of our knowledge is the first to determine the cellular morphology in resistance arteries from untreated patients with essential hypertension, indicates that this is the case. Thus, both the volume fraction of smooth muscle cells in the media (75%) and smooth muscle cell volume were the same in the resistance arteries of the hypertensive patients and control subjects. Therefore, the results suggest that the remodeling indicated in a variety of investigations from morphological measurements is due to a rearrangement of cells within the same amount of extracellular matrix.

There are important caveats concerning the above conclusion. First, there is the question of sampling. If the vascular architecture of hypertensive and normotensive individuals differs, then a systematic sampling error may be introduced, even though the operator concerned is unaware of whether hypertensive or normotensive samples are being examined. However, our data confirm the results of Short, who circumvented the sampling problem by analyzing all arteries found in perfusion-fixed mesenteric vascular bed and ranking them accord-
ing to measured lumen diameter. Second, the data have been obtained from only one vascular bed that is readily accessible. Presently, it is not clear if this is representative. Third, there is substantial variance in the data of each individual investigation. Indeed, the nonsignificant increase in cell size might suggest that smooth muscle cell hypertrophy does play a role, albeit minor, in essential hypertension. Fourth, the extent to which the altered structure measured under in vitro conditions reflects the in vivo situation remains to be determined. Nevertheless, despite these caveats, the available evidence points toward remodeling as being the major factor in accounting for the altered structure of small arteries in essential hypertension.

This conclusion echoes the results of similar investigations concerning genetic hypertensive rats. In both spontaneously hypertensive rats and transgenic hypertensive rats containing the mouse Ren-2 gene, smooth muscle cell volume in mesenteric resistance arteries was normal compared with controls. However, in one-kidney, one clip Goldblatt hypertensive rats and in rats made hypertensive by infusion of subpressor doses of angiotensin II, smooth muscle cell volume in mesenteric resistance arteries was increased (unpublished observations) compared with controls. Taken together, these results suggest that resistance artery structure in genetic hypertension may be determined primarily by the manner in which otherwise normal smooth muscle cells are arranged; by contrast, the altered resistance artery structure in induced hypertension appears to be associated with hypertrophy of the smooth muscle cells. From the therapeutic point of view, these findings imply that if antihypertensive therapy in essential hypertension is to normalize vascular structure, the aim should be not so much to inhibit growth but to facilitate an appropriate rearrangement of the vascular smooth muscle cells within the extracellular matrix.

Acknowledgments

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References

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