Connection of Smooth Muscle Cells to Elastic Lamellae in Aorta of Spontaneously Hypertensive Rats

Yvonnick Bezie, Patrick Lacolley, Stéphane Laurent, Giorgio Gabella

Abstract—We have recently demonstrated that in large arteries of spontaneously hypertensive rats (SHR), there is no increase of stiffness despite the increase in wall thickness, a sign of mechanical adaptation of the arterial wall to the higher level of stress. Because the dense plaques of smooth muscle are a major site of anchorage between the muscle cells and extracellular matrix, we determined by electron microscopy the distribution of dense plaques and their connections to elastic lamellae in the abdominal aorta of 1-year-old SHR and control Wistar rats. In vivo echo-tracking measurement of aortic distensibility and elastic modulus indicates a reduction of arterial stiffness in SHR compared with Wistar rats when they are studied over a common range of blood pressure. The media thickness to body weight ratio was higher in SHR than in Wistar rats. In the media, the percentage of sectional area occupied by extracellular matrix was not different between Wistar rats and SHR. The average number of dense plaques per muscle cell was not different between Wistar rats and SHR. However, the percentage of cell surface occupied by dense plaques was increased in SHR, and the percentage of cell surface connected to the elastic lamellae was twice as high in SHR compared with Wistar rats (9.4 ± 1.5% versus 3.8 ± 1.1%). These results suggest that the elastin network plays a major role in the mechanical adaptation of the arterial wall in SHR, not through variations of its total amount but through variations of the extent of anchorage to the muscle cells. (Hypertension. 1998;32:166-169.)

Key Words: aorta ■ muscle, smooth ■ rats, inbred SHR ■ elastin

Until recently, it was generally accepted that hypertension produces an increase in large artery stiffness.1–4 However, our group and others have shown that arterial stiffness is not increased in hypertensive patients or in spontaneously hypertensive rats (SHR) despite wall hypertrophy.5–9 A recent study has shown that the aortic wall of SHR and Wistar rats has similar mechanical properties despite the fact that in SHR the wall is submitted to a higher level of stress.10 This finding suggests that sustained hypertension is associated with a restructuring of the arterial wall that produces the mechanical adaptation of the arterial wall.

Electron microscopy has shown that the membrane-associated dense plaques of muscle cells are a major site of anchorage of contractile apparatus to extracellular matrix in arteries.11,12 The mechanical link between muscle cells and elastic lamellae provided by the dense plaques may play an important role in regulating contractile and elastic tension in mechanically stressed vessels. We hypothesized that the mechanical adaptation of the arterial wall to hypertension involves an increase in the links between muscle cells and elastic lamellae. Thus, our objective was to measure the dense plaques and their connections to elastic lamellae in a large elastic artery, the abdominal aorta, in normal Wistar rats and SHR.

Methods

Aortic Mechanical Parameters

We simultaneously recorded arterial diameter and blood pressure at the abdominal aorta in pentobarbital anesthetized rats to determine the pressure-diameter and pressure-distensibility curves, as previously described.5,6,13–15 The incremental elastic modulus (E inc) was calculated from in vivo distensibility and in vitro measurement of wall cross-sectional area after fixation at mean arterial pressure (MAP). Arterial diameter measurement was obtained using an ultrasonic echo-tracking device (NIUS-01, Asulab SA).

In Situ Perfusion and Fixation for Microscopy

Segments of abdominal aorta were obtained from 1-year-old male Wistar rats (n=3) and SHR (n=3) weighing 632±36 g and 408±32 g, respectively. The abdominal aorta was fixed in situ at MAP by perfusion via the heart using a fixative consisting of 4% glutaraldehyde and 1% paraformaldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.4).16 The specimens were then postfixed in 1% osmium tetroxide in cacodylate buffer for 1 hour, washed in several changes of distilled water, and immersed in a saturated solution of uranyl acetate for 1 hour. They were then dehydrated in ethanol and epoxy-propane and placed in araldite for infiltration. Semithin sections (0.5 μm) were cut either transversely to the length of the vessel (for area and shape of the lumen and for area and thickness of the media) or longitudinally, ie, parallel to the length of the vessel and through its middle (for thickness of the wall and for study of muscle cells and stroma). These sections were stained with toluidine blue and observed with a Zeiss photomicroscope with phase-contrast...
optics. Ultrathin sections (100 nm) were cut longitudinally, collected on copper grids, stained with uranyl acetate and lead citrate, and viewed in a Philips 400 electron microscope. For quantification, photographic montages were made, reconstructing the full thickness of the wall for a length of 150 to 400 µm at a magnification of ×3700. Dense plaques were identified by the presence of electron-dense areas on the cytoplasmic side of the cell membrane. Measurements were made using a video imaging technique (Quancoul) to calculate the transverse sectional area of nucleated cell profiles, the cell surface to cell perimeter ratio, the percentage of extracellular space in the media, the number and lengths of dense plaques, and the proportion of dense plaques connected to elastic lamellae.

Results

Arterial Mechanics

Table 1 shows the mechanical parameters of abdominal aorta in SHR and Wistar rats. At MAP, which reflects the operational pressure level, distensibility was lower and Einc higher in SHR than in Wistar rats. The distensibility-pressure curve of SHR was shifted to the right compared with that of Wistar rats (data not shown), indicating that arterial distensibility was not decreased in SHR for a given level of blood pressure. Thus, the lower value of aortic distensibility at fixed at MAP was increased in SHR compared with Wistar rats (42% versus 34% of total dense plaques per cell was not different between strains studied. The cell profiles presented deep invaginations, laminar or fingerlike (in the latter case, they often appeared isolated from the cell surface) (Figure). The cell membrane at the cell surface proper and in the invaginations was in contact with basal lamina and extracellular materials. For the present purposes, we recognized two configurations, namely, the cell membrane formed 2 distinct domains: (1) some areas of the membrane were in contact with a basal lamina, and immediately beyond, with microfibrils and collagen fibrils, and (2) other areas of the membrane were in contact with elastic fibers and elastic lamellae. Elastic fibers and elastic lamellae were connected with each other, and a precise separation was difficult. However, it was still possible to distinguish, within the elastin-adhesion domains of the membrane, areas of direct contact with elastic lamellae and areas of contact with elastic fibers, and we considered for quantification only those in direct contact with elastic lamellae.

Distribution of Membrane-Associated Dense Plaques

Dense plaques were studied in longitudinal sections of the vessels. All the muscle cells of the media had prominent dense plaques encrusting the cell membrane and projecting deep into the cell. Smooth muscle cell to elastic lamellae connections were defined as elastin expansions that spanned obliquely from the elastic laminae to the surface of the smooth muscle cell, where they attached in a region occupied by membrane-associated dense plaque (Figure). The number of total dense plaques per cell was not different between Wistar rats and SHR (12.0 ± 0.4 versus 11.3 ± 0.4). However, the percentage of cell surface occupied by dense plaques was increased in SHR compared with Wistar rats (42 ± 3% versus 34 ± 2%). The percentage of dense bands connected to elastic laminae was increased 2-fold in SHR compared with that in Wistar rats (9.4 ± 1.5% versus 3.8 ± 1.1%; Table 2).

### TABLE 1. Individual Values of Mechanical Parameters of Abdominal Aorta in 1-Year-Old SHR and Control Wistar Rats

<table>
<thead>
<tr>
<th></th>
<th>Weight, g</th>
<th>MAP, mm Hg</th>
<th>Dd, mm</th>
<th>Dist at MAP, mm Hg⁻¹ · 10⁻³</th>
<th>Wall Stress, kPa</th>
<th>Einc, kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>590</td>
<td>82</td>
<td>1.68</td>
<td>3.15</td>
<td>103</td>
<td>727</td>
</tr>
<tr>
<td>W2</td>
<td>650</td>
<td>84</td>
<td>1.32</td>
<td>3.70</td>
<td>116</td>
<td>666</td>
</tr>
<tr>
<td>W3</td>
<td>655</td>
<td>94</td>
<td>1.50</td>
<td>3.32</td>
<td>76</td>
<td>487</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>632±36</td>
<td>87±4</td>
<td>1.50±0.10</td>
<td>3.39±0.16</td>
<td>98±12</td>
<td>627±72</td>
</tr>
<tr>
<td>SHR (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>445</td>
<td>166</td>
<td>1.50</td>
<td>1.92</td>
<td>302</td>
<td>1633</td>
</tr>
<tr>
<td>S2</td>
<td>395</td>
<td>181</td>
<td>1.56</td>
<td>2.33</td>
<td>438</td>
<td>1736</td>
</tr>
<tr>
<td>S3</td>
<td>385</td>
<td>169</td>
<td>1.52</td>
<td>1.41</td>
<td>231</td>
<td>1735</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>408±32</td>
<td>172±5</td>
<td>1.53±0.02</td>
<td>1.87±0.27</td>
<td>324±61</td>
<td>1701±34</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; Dd, diastolic diameter; Dist, distensibility; and Einc, incremental elastic modulus.
The aim of the present study was to correlate mechanical properties and ultrastructure of the abdominal aorta in hypertensive and control rats. The main finding is a marked increase in connections of muscle cell to elastic lamellae in hypertension.

As we recently showed, the incremental elastic modulus of the aortic wall material (E\textsubscript{inc}), determined for a given level of blood pressure, was not significantly increased in SHR compared with Wistar rats. This indicates that the arterial stiffness is not increased in SHR. However, the circumferential wall stress is higher in SHR than in Wistar rats. Under these conditions, an important issue is to determine how the hypertensive artery is mechanically adapted despite the higher level of stress. Considering that weight of SHR was lower than that of Wistar rats, the increases in arterial thickness associated with cellular hypertrophy and extracellular matrix accumulation were only observed after normalization by body weight. We previously found no difference between SHR and Wistar rats concerning elastin and collagen densities. Because these compounds play a major role in the mechanical properties of the arterial wall, we hypothesized

**Table 2. Distribution of Membrane-Associated Dense Plaques and Their Connections to Elastic Lamellae in 1-Year-Old SHR and Control Wistar Rats**

<table>
<thead>
<tr>
<th>Rats</th>
<th>Cells Analyzed Per Rat, n</th>
<th>Dense Plaques Per Cell, n</th>
<th>Cell Surface Occupied by Dense Plaque (CS-DP), %</th>
<th>CS-DP Connected to Elastic Lamellae, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>12</td>
<td>11.2±2.0</td>
<td>38±4</td>
<td>2.0±1.1</td>
</tr>
<tr>
<td>W2</td>
<td>11</td>
<td>12.6±1.4</td>
<td>35±3</td>
<td>3.5±1.9</td>
</tr>
<tr>
<td>W3</td>
<td>11</td>
<td>12.3±1.2</td>
<td>30±2</td>
<td>5.8±2.7</td>
</tr>
<tr>
<td>Mean</td>
<td>...</td>
<td>12.0±0.4</td>
<td>34±2</td>
<td>3.8±1.1</td>
</tr>
<tr>
<td>SHR (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>11</td>
<td>11.4±1.1</td>
<td>39±3</td>
<td>8.2±2.2</td>
</tr>
<tr>
<td>S2</td>
<td>11</td>
<td>10.5±1.2</td>
<td>49±8</td>
<td>12.3±4.3</td>
</tr>
<tr>
<td>S3</td>
<td>9</td>
<td>11.9±1.4</td>
<td>39±5</td>
<td>7.6±4.1</td>
</tr>
<tr>
<td>Mean</td>
<td>...</td>
<td>11.3±0.4</td>
<td>42±3</td>
<td>9.4±1.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
that hypertension induces some structural reorganization of the media, namely, changes in the muscle to stroma connections, leading to mechanical adaptation. Clark and Glagov, among others, have shown that in the aorta of rabbit and pig, membrane-associated dense bodies represent the mechanical attachment site between elastic fibers and contractile apparatus. These focal attachments are stable and have great mechanical strength because they remain unchanged after hyperdistension of the vessel. Here we have shown that while some structural parameters are unchanged in the hypertensive abdominal aorta (eg, density of elastin and collagen, number of cells, and number of dense plaques per cell), there is a significant increase in the percentage of the muscle cell surface that is occupied by dense plaques. In contrast to our results, a study on renovascular hypertension (2-kidney, 1-clip) showed a reduction of the density of cell to elastic fiber contacts in hypertensive compared with normotensive rats. This finding, however, is not in disagreement with our hypothesis because in this model, carotid stiffness was not adapted but increased compared with that in normotensive animals. Thus, we would conclude that the number of cell to elastic connections within the aortic media is related to arterial wall stiffness. Another study of hypertensive rats, which reported a decrease of membrane dense bodies, is not directly comparable with ours because it was carried out on mesenteric arteries.

The rise in blood pressure causes an increase in wall stress and an enhancement of the stretch to which the stroma of the wall is subjected. The stress is discharged via the dense apparatus and cytoskeleton. Among the latter changes, we suggest that it also modifies the anchorage site between elastic fibers and contractile apparatus. This ultrastructural change alters the distribution of the wall stress onto the cell membrane, the cytoskeleton, and the wall is related to arterial wall stiffness. Another study of hypertensive rats, which reported a decrease of membrane dense bodies, is not directly comparable with ours because it was carried out on mesenteric arteries.

In conclusion, the present study shows a novel organization of the aortic media of SHR, characterized by changes in smooth muscle cell–elastic lamellae connections, which may be a means for preserving mechanical integrity of the arterial wall at elevated arterial pressures. Ultrastructural characterization of cell-matrix interactions could provide new insights into the mechanisms by which genetic hypertension autoregulates the mechanical properties of the arterial wall.

Acknowledgments

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References

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