Morphometric Study on Cerebral Vessels In Spontaneously Hypertensive Rats

CLAES NORDBORG, M.D. AND BARBRO B. JOHANSSON, M.D.

SUMMARY The ratio between media thickness and lumen radius was determined in cerebral arterial vessels of 15- and 200-day-old spontaneously hypertensive (SHR) and normotensive rats (Kyoto Wistar and local Wistar rats). A modification of Furuyama's morphometrical method was used. There was a statistically significant increase of media/radius ratio among medium size and large vessels in 15-day-old SHR. Furthermore, the media cross section area and lumen radius was increased in the internal carotid arteries of young SHR. These early vascular aberrations could be caused by the slight increase of blood pressure at this age or be due to other genetically determined mechanisms in SHR. In 200-day-old SHR a significantly increased media/radius ratio was seen in arterial vessels with a radius <80 um when compared to local Wistar rats but only in the smallest arterioles (r < 20 um) when compared to Kyoto Wistar rats. The present results offer a likely explanation for the increased cerebrovascular resistance during maximum vasodilatation in SHR.

Methods

Animals: 15 Days. Cerebral vessels were measured in non stroke-prone SHR and Wistar Kyoto rats (WKR), each group consisting of 4 males and 4 females. Internal carotid arteries were collected from a different population, comprising 7 sex- and weight matched rats of either kind. Since the controls of this last study had slightly lower body weight, they were compared with 13-day-old SHR.

Blood Pressure Measurements. In the 200-day-old rats mean arterial pressure (MAP) was measured in the aorta through a catheter from the femoral artery during diazepam anesthesia. In 15-day-old rats MAP was measured through a specially designed needle in the femoral artery. Initially, a small dose of methohexitol (Brietal®), was given intraperitoneally, whereupon the animals were maintained on N2O and O2 in the proportion of 2:1. For technical reasons, other 15-day-old animals were used than were used in the morphometric study. Each group consisted of 4 males and 4 females. The mean value of 6 recordings was calculated for each animal. Student's t-test was used for statistical evaluation. In the 15-day-old rats of the

INCREASED MEDIA THICKNESS in arteries of hypertensive individuals has generally been considered to imply an adaptation of arterial design to increased intraluminal pressure. It has been shown that arterial vessels of the rat adapt structurally during renal hypertension by increasing their media thickness. However, according to Friedman et al. a sustained hypertensive state does not necessarily result in hypertrophy of the arterial wall. Although the arterial media has been reported to be thicker during established hypertension in man and spontaneously hypertensive rats (SHR), there exists no morphological proof that the aberration in these cases implies an adaptation to increased intraluminal pressure. To study structural adaptation, information is needed not only about arterial morphometry during established hypertension, but also about arterial structure during early hypertension or a pre-hypertensive stage. In order to clarify the question of arterial structural adaptation in SHR an investigation of different vascular beds of 15- and 200-day-old SHR as well as normotensive controls was carried out. Preliminary results have previously been reported. The final results and statistical evaluation of cerebral arterial vessels and internal carotid arteries are presented in this paper.

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Table Heart weight (g)/body weight (g) in 15-day-old rats.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>WKR</td>
<td>SHR</td>
<td>WKR</td>
<td>SHR</td>
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<tr>
<td>0.0045</td>
<td>0.0060</td>
<td>0.0048</td>
<td>0.0062</td>
<td></td>
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<td>0.0052</td>
<td>0.0083</td>
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<tr>
<td>0.0054</td>
<td>0.0083</td>
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<td>0.0055</td>
<td>0.0082</td>
<td>0.0058</td>
<td>0.0089</td>
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p < 0.05  p < 0.05

**Figure 1.** Schematic drawing of artery during contraction (a) and during a hypothetical state (b) implying perfectly smooth internal elastic membrane. L: length of internal elastic membrane. A: media cross section area. r: lumen radius. M: media thickness.

Morphometric study heart weight/body weight ratio was determined instead of blood pressure measurements.

**Morphometric Method: Cerebral Vessels.** The brain was fixed in situ in 4% buffered formaldehyde after removal of the skull roof. After sectioning the material according to a defined procedure the specimens were dehydrated and embedded in Sorvall JB4 medium. Using a modified elastine-van Gieson method, 2.5 μm thick sections were stained. Calibrated microphotographs of cross-sectioned arteries were projected at large magnification and drawn by hand on paper. Measurements of the drawings were made with a digitizer, connected to a Hewlett-Packard calculator according to a method, developed by research engineer Lars Stage (Institute of Physiology, University of Göteborg). The total length of the internal elastic membrane was measured and the area of the media was determined planimetrically (fig. 1a). The internal radius and media thickness could then be calculated for a hypothetical state (fig. 1b), where the internal elastic membrane was perfectly smooth and circular. In the 15-day-old rats 256 (SHR) and 271 (WKR) vessels were measured. At 200 days 235-241 vessels were measured in each of the 3 categories. The media over radius quotients were determined within different radius intervals. To evaluate the difference in media/radius ratio between hypertensive and normotensive animals, Student's t-test was used.

**Carotid Arteries.** Two different sites were studied i.e. extracranially, immediately proximal to the pterygopalatine artery, and intracranially, at the posterior margin of the pituitary gland. After careful dissection, the vessels were prepared and measured in the same way as the cerebral specimens. Student's t-test was used for statistical evaluation.

**Results**

In the 200-day-old animals MAP was 150-185 mm Hg in SHR, 105-120 mm Hg in WKR and 100-115 mm Hg in LWR. The table shows the heart weight/body weight ratio of the 15-day-old rats included in the morphometric study. The separate investigation of MAP in 15-day-old rats revealed a significant difference in pressure at this age (SHR: 75 ± 4 mm Hg, WKR: 59 ± 3 mm Hg, p < 0.02).

The media/radius ratio was greater than normal in medium sized and large arterial vessels of 15-day-old SHR (50-79 μm: p < 0.05; >80 μm: p < 0.01). However, when comparing the female and male rats separately, there was a significant difference within the same radius intervals in females only (figs. 2, 3).

**Figure 2.** Media/radius ratios in cerebral arterial vessels of 15-day-old female rats.

**Figure 3.** Media/radius ratios in cerebral arterial vessels of 15-day-old male rats.
significantly greater media area in SHR extracranially as well as intracranially. Lumen radius of the internal carotid (fig. 5) was, however, significantly greater only in the extracranial part.

Only the smallest arterial vessels of 200-day-old SHR showed a significantly greater media/radius ratio than WKR of the same age (fig. 6). When compared with LWR, SHR had significantly greater media/radius ratios for vessels with a radius less than 80 μm. WKR had significantly greater media/radius ratio than LWR for arterial vessels with a radius less than 50 μm in spite of comparable MAP (fig. 6).

Discussion

A greater arterial wall thickness during hypertension has generally been considered to imply an adaptation of the vessel wall to an increased intraluminal pressure load. However, previous results from our laboratory as well as studies by Greenberg et al. on pulmonary arteries, portal and caval veins, suggest that non-pressure dependent morphometrical aberrations may occur in blood vessels of SHR. In the present investigation the media/radius ratio was significantly greater in medium size and large arteries of 15-day-old SHR. According to previous reports MAP is not significantly elevated in SHR during the first 4 weeks of life. However, our intra-arterial recordings on 15-day-old animals showed significantly higher blood pressure in SHR at this age. The early increase of media/radius ratio in SHR could thus theoretically be an adaptation to a slightly increased intraluminal pressure load. However, it seems possible that the greater media/radius ratio in young SHR, at least in part, is a non-pressure dependent aberration, e.g. caused by an increased trophic influence from the sympathetic nervous system. The medium size and large vessels of 200-day-old WKR were not significantly different from those of SHR. These vessels seem to “catch up” structurally later in life despite increased difference in MAP. This may either be due to a real media growth spurt in WKR or reflect a greater increase of radius in SHR than in WKR, which would cause a relative decrease of the media/radius quotients in the former group. The later possibility would be in accordance with Ross-Russel who reported an increased lumen radius in anterior and middle cerebral arteries in hypertensive man.

Only the smallest arterial vessels (r < 20 μm) of 200-day-old SHR had significantly greater media/radius ratio than WKR. Since no such difference was found in 15-day-old rats, it is suggested that the smallest vessels adapt to an increased pressure by increasing their wall thickness. However, the smallest vessels measured in 200-day-old animals may not yet have a complete internal elastic membrane at 15 days and would thus not be possible to measure with our method. The greater media/radius ratio in the smallest vessels of SHR at 200 days may be due to an addition of small arteries, which had thick walls even during earlier stages of development, but which could not be measured due to lack of a continuous internal elastic membrane.

The present result emphasizes the importance of choosing a control strain as adequate as possible for the experiment. Thus, when compared with LWR the SHR had greater media/radius ratios not only among the smallest arterial vessels, but in all groups with a radius less than 80 μm. The enhanced ratio in WKR compared to LWR in spite of comparable levels of MAP indicates that other genetic factors than intraluminal pressure may have influence on the morphology of arterial vessels. The fact that the media area was increased in both extracranial and intracranial portions of the internal carotid artery of 15-
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FIGURE 6. Media/radius ratios in 200-day-old male rats.

<table>
<thead>
<tr>
<th>Radius (μm)</th>
<th>SHR-WKR</th>
<th>p &lt; 0.01</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-LWR</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.02</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>WKR-LWR</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

day-old SHR, but the radius was increased in the extracranial part only, furthermore suggests that physical properties of the surrounding tissue may modify pressure-induced changes of the vascular design.

Several earlier studies indicate that arterial vessels during hypertension differ morphometrically from those of normotensive individuals. There are, however, considerable discrepancies in the literature as to the nature of this difference. Cook and Yates\(^*\) found medial hypertrophy in all artery sizes in kidney and brain during hypertension in man. According to Furuyama\(^4\) renal and mesenteric arterial vessels of hypertensive patients have an increased media/radius ratio, while the lumen radius of renal arteries is normal. Ross-Russell\(^7\) reported an increased lumen radius in middle and anterior cerebral arteries of hypertensive patients but a decreased lumen and increased wall thickness in small penetrating arteries of the brain. According to Short and Thomson\(^16\) the caliber of submucosal mesenteric arteries is reduced in man during hypertension without concomitant thickening of media or intima. In an in vitro investigation Mulvany\(^7\) found that mesenteric arteries of SHR had smaller lumen radius and thicker media than corresponding vessels of normotensive rats. Bohlen\(^18\) reported that arterioles and venules in the cremaster muscle of SHR had normal or larger than normal lumen radius in vivo.

The discrepancies related above may partly be due to differences in methods and choices of vascular beds. When perfusion of the vascular bed is performed in situ some investigators perfuse with the same pressure in hypertensives and in controls whereas others use higher pressure in hypertensives. There is evidence that the distensibility of hypertensive and normotensive vessels differ and whichever alternative is used it is difficult to evaluate the degree of distension obtained. The elegant method developed by Japanese investigators\(^*\), which was used in the present study with some modifications, seems to overcome this difficulty to a great extent. The absolute values obtained might differ from those in vivo due to fixation procedures, but this method probably gives the best postmortem material for comparison among the groups. The method requires the presence of an elastic membrane which excludes the smallest intracerebral precapillary vessels. Since we were able to measure a defined segment of arterial vessel only in the carotid arteries we can not say if the increased media/radius ratio in cerebral vessels is caused by a decreased radius and/or increased media thickness. However, the thicker media in the internal carotid arteries of young SHR makes it probable that media hypertrophy is present also in the cerebral vessels. An increased media to lumen ratio has been suggested from studies showing an enhanced resistance of the blood-brain barrier in SHR to abrupt pressure increase.\(^17-19\) According to hemodynamic studies cerebrovascular resistance during maximum vasodilatation is considerably higher in SHR than in WKR.\(^20\) The most likely explanation for this finding would be that structural changes in the resistance vessels of SHR cause encroachment on the lumen. Present morphometrical results support this theory.

The finding of differences between 15-day-old SHR and WKR suggests that structural vascular aberrations, whether adaptive or primary, may be of importance not only for the maintenance of established hypertension, but also for the development of hypertension in young SHR.

Acknowledgment

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