The Morphometry of Consecutive Segments in Cerebral Arteries of Normotensive and Spontaneously Hypertensive Rats

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SUMMARY The media cross-sectional area, the media thickness, the internal radius and the ratio between media thickness and internal radius were determined in consecutive sections of extraparenchymal cerebral arteries of 7- and 12-month-old normotensive and spontaneously hypertensive rats. The study included intracranial pial and basal arteries as well as extracranial cervical arteries. In the chronically hypertensive rats the media to radius ratio was consistently higher than in normotensive rats over the entire calibre spectrum investigated (radius 5–400 μm). The increase of the ratio in the extracranial arteries of the hypertensive rats was exclusively due to a thicker media. In the basal intracranial arteries the increase of ratio was due to a thicker media and/or a smaller internal radius in 7- and 12-month-old rats with moderate hypertension (mean arterial pressure, MAP 171 ± 8 and 177 ± 7 mm Hg respectively). In 7-month-old rats with severe hypertension (MAP 204 ± 11 mm Hg) the increase of ratio was mainly due to a smaller internal radius. The observed structural alterations are likely to be of hemodynamic importance.

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The Japanese strain of spontaneously hypertensive rats (SHR) and its stroke-prone substrain (SHRSP) are extensively used in experimental hypertension research. Hemodynamic studies indicate similarities to primary hypertension in man. Compared to normotensive rats the cerebrovascular resistance is enhanced. Thus, in most conscious SHRSP with markedly increased blood pressure the cerebral blood flow is not altered. SHR are also prone to develop blood-brain barrier dysfunction in response to an abrupt rise in blood pressure than normotensive rats, i.e. the microvessels are better protected against overdistension.

The heightened cerebrovascular resistance in SHR may be functional and/or structural. To what extent the various calibre segments of the cerebral arterial tree are involved is debated. Intravital studies on the main cortical surface branches of the middle cerebral artery, monitored through a closed cranial window by the aid of a multichannel videoangiometer, have demonstrated significantly smaller luminal diameters in SHR compared to normotensive Wistar Kyoto rats (WKY) at rest as well as during dilatation. This is in agreement with an earlier study indicating a higher resistance in the relaxed cerebrovascular bed in SHR than in WKY.

In the smallest pial and intraparenchymal arteries of SHR and SHRSP, morphometric measurements have shown increased media or wall thickness to radius ratios. Little is known about more proximal parts of the cerebral vasculature in SHR and SHRSP, although it has been reported that the diameters of the arteries in the circle of Willis do not differ significantly from controls. In the present study we investigated the entire calibre spectrum of the extraparenchymal cerebral arteries of WKY, SHR and SHRSP at standardized dilatation. Changes in media cross-sectional area, media thickness, internal radius and the media to radius ratio were related to vessel caliber and degree of chronic hypertension.

Material and Methods

The study was performed on the cerebral vasculature of 7-month-old male rats: 11 WKY, 10 SHR and 10 SHRSP, with body weights (mean ± SD) 385 ± 19, 364 ± 13 and 285 ± 18 g (WKY-SHR: p < 0.01, WKY-SHRSP: p < 0.05). Twelve-month-old female rats were also investigated: 8 WKY, 254 ± 25 g and 9 SHR, 233 ± 18 g (WKY-SHR: n.s.). The mean arterial pressure (MAP) was measured with indwelling aortic catheters in the conscious, unrestrained rats. A majority of the rats were also subjected to hemodynamic studies in connection with bilateral carotid ligation.

The animals were killed with an overdose anesthetic and the skull was carefully opened over the cerebellum and the olfactory bulbs. After fixation by immersion in 4% buffered formaldehyde, the brain was removed and specimens were collected from specified sites of the vertebral arteries, the basilar artery, the posterior communicating arteries, the proximal part of the posterior cerebral arteries and the middle cerebral arteries (see Greene and fig. 1). Specimens were also collected from the extracranial parts of the internal carotid arteries immediately proximal to the pterygopalatine artery and from the intracranial portion of the same vessel at the posterior margin of the pituitary gland. In the 7-month-old rats pial arteries were also investigated. Pial arteries were measured on coronal sections through both frontal lobes at a constant distance from the frontal poles. In this part of the study another 5 WKY, 4 SHR and 4 SHRSP were added.

After dehydration the specimens were embedded in Sorval medium. Two to three μm thick cross sections...
of the arteries were stained with a modified van Gieson method for demonstration of elastin. Calibrated microphotographs of cross-sectioned arteries were projected and drawn at large magnification. Measurements of the drawings were made according to a modification of a method described by Furuyama,\textsuperscript{21} which was developed by research engineer Lars Stage (Institute of Physiology, University of Göteborg). If the length of the inner media contour (i.e. the internal elastic membrane) and the media cross-sectional area are known, one may calculate the media thickness and internal radius for a standardized condition assuming a perfectly smooth and circular internal elastic membrane (fig. 2). A digitizer and a computer were used to measure the length of the internal elastic membrane as well as the areas inside this membrane and inside the outer contour of the media. The digitizer has a cursor which is moved manually along the contours to be measured. The position of the cursor is transferred from the digitizer to the computer as the $x$ and $y$ coordinates of the cursor at that point. The length between two points is

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{The basal cerebral arteries of the rat. Cross-sectional sites of the present study are marked with bars. ICI = intracranial part of the internal carotid artery; MCA = middle cerebral artery; PCE = posterior cerebral artery; PCO = posterior communicating artery; BA = basilar artery; VA = vertebral artery.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{a) Schematic drawing of an arterial cross section after fixation by immersion. $L =$ length of internal elastic membrane; $A =$ media cross-sectional area. b) The same artery in a standardized condition, implying a perfectly smooth and circular internal elastic membrane. $r =$ internal radius; $M =$ media thickness.}
\end{figure}
calculated as the square root of the sum of the squared coordinate changes. The total length of the contour is then calculated as the sum of length between points. Area from one point to the next is calculated as the product between the x difference and the mean y value. This gives the area of a figure with corners in the two points and their projections on the x axes. The total area is then calculated as the sum of all areas between the points. Contour length of a closed line and the enclosed area calculated in this way is independent of contour location in the coordinate system.

The arteries were consequently compared in a calculated, standardized condition which assumed a perfectly circular internal elastic membrane. For paired arteries the mean value of the bilateral measurements was calculated. Since only technically perfect cross sections were accepted, a few specimens had to be omitted from the investigation. Hence the number of measurements varies between different arterial segments within one and the same category of animals. Because of great technical difficulties encountered in the preparation of perfect cross sections of the thin posterior communicating arteries, the values in this part of the investigation do, for some animals, represent one side only. Differences in radius, media cross-sectional area, media thickness and media to radius ratio were evaluated using Wilcoxon’s rank sum test. The values are given as mean ± standard deviation (SD).

**Results**

**7-Month-Old Rats**

MAP (mm Hg) was 121 ± 6 (WKY), 171 ± 8 (SHR) and 204 ± 11 (SHRSP) (SHR-WKY: \( p < 0.001 \), SHRSP-WKY: \( p < 0.001 \), SHR-SHRSP: \( p < 0.001 \)).

**Internal Radius**

The radius of the extracranial part of the internal carotid artery was 359 ± 20 \( \mu \)m (SHRSP), 343 ± 14 \( \mu \)m (SHR) and 320 ± 16 \( \mu \)m (WKY). There was a significant increase in SHRSP \( (p < 0.01) \) as well as SHR \( (p < 0.05) \) but no difference between these two groups. The radius of the intracranial part of this artery was reduced in SHR but not significantly changed in SHRSP when compared to WKY (fig. 3).

The radius was significantly reduced in the vertebral, basilar and middle cerebral arteries of SHRSP compared to WKY. In SHR the radius was reduced in vertebral arteries whereas it was increased in the posterior cerebral arteries. The radii of SHRSP were significantly smaller than those of SHR in vertebral, basilar, middle cerebral, posterior cerebral and posterior communicating arteries (fig. 3).

**Media Cross-Sectional Area**

In the extracranial part of the internal carotid artery the media cross sectional area was 160 ± 19 \( \mu \)m² (SHRSP), 151 ± 8 (SHR) and 105 ± 7 (WKY) \( \times 10^4 \) \( \mu \)m². The area was significantly increased in SHR and SHRSP \( (p < 0.001) \), whereas there was no significant difference between these two groups. In the intracranial part neither SHRSP nor SHR showed any significant difference from WKY. However, the media cross sectional area of SHRSP was greater than that of SHR (fig. 3).

The media area was not increased in vertebral, basilar, middle cerebral, posterior cerebral or posterior communicating arteries of SHRSP compared to WKY. Instead it was reduced in the vertebral arteries of SHRSP. In contrast all these vessels except for the vertebral arteries of SHR showed significantly increased values compared to WKY. In the basilar, middle cerebral, posterior cerebral and posterior communicating arteries the media cross-sectional area was also greater in SHR than in SHRSP (fig. 3).

**Media Thickness**

The media thickness was 65 ± 5 \( \mu \)m (SHRSP), 64 ± 3 \( \mu \)m (SHR) and 49 ± 4 \( \mu \)m (WKY) in the extracranial part of the internal carotid arteries. It was significantly increased in SHRSP and SHR \( (p < 0.001) \), whereas no significant difference was seen between the hypertensive strains. In the intracranial segment the media thickness of SHRSP and SHR did not differ significantly from that of WKY controls.

The media thickness of the vertebral arteries was not significantly changed in the hypertensive rats. In the basilar arteries it was 22 ± 2 \( \mu \)m in SHRSP and 31 ± 2 \( \mu \)m in SHR compared to 20 ± 1 \( \mu \)m in WKY \( (p < 0.05 \) and \( p < 0.001 \)). In the middle cerebral arteries the media thickness was 21 ± 2 \( \mu \)m (SHRSP), 22 ± 4 \( \mu \)m (SHR) and 18 ± 2 \( \mu \)m (WKY) \( (p < 0.05 \) and \( p < 0.01 \)). In the posterior cerebral arteries no difference was noticed between SHRSP and WKY, whereas SHR showed significantly higher values than the controls \( (32 ± 5 \mu m \) and \( 23 ± 3 \mu m \) respectively; \( p < 0.001 \)). Neither was there any significant deviation in the posterior communicating arteries of SHRSP. In this segment the media thickness of SHR was 18 ± 2 \( \mu \)m and that of WKY 15 ± 1 \( \mu \)m \( (p < 0.05) \).

**Media Thickness/Internal Radius Ratio**

The media to radius ratio of the extracranial part of the internal carotid artery was 0.18 ± 0.01 (SHRSP), 0.19 ± 0.02 (SHR) and 0.15 ± 0.01 (WKY). It was significantly increased in SHRSP \( (p < 0.01) \) as well as in SHR \( (p < 0.001) \), but there was no significant difference between these two categories. The ratio of the intracranial part of this vessel was significantly increased only in SHR (fig. 3).

The media to radius ratio was increased in vertebral, basilar, middle cerebral, posterior cerebral and posterior communicating arteries of SHRSP as well as in vertebral, basilar and middle cerebral arteries of SHR. Furthermore, the quotient was significantly greater in vertebral arteries of SHRSP than in those of SHR (fig. 3).

The media to radius ratio of small \((r < 20 \mu m)\) and medium sized \((r 20-49 \mu m)\) pial arteries was significantly greater in SHRSP than in WKY \( (p < 0.05; p < 0.01) \). The quotient was also increased in small \((r < 20\)
**Figure 3.** Internal radii, media cross-sectional areas and media thickness to radius ratios of intracranial arteries in 7-month-old WKY (stars), SHR (black dots) and SHRSP (circles). Mean values ± standard deviation. Figures in the top diagram show the number of animals. For explanation of abbreviations see figure 1.
μm) arteries of SHR compared with WKY (p < 0.05) (fig. 4).

12-Month-Old Rats

MAP was 115 ± 7 (WKY) and 177 ± 7 mm Hg (SHR) (p < 0.001).

Internal Radius

The radius of the extracranial part of the internal carotid artery was 338 ± 25 μm (SHR) and 316 ± 18 μm (WKY). It was not significantly altered in the extra- or intracranial parts of the internal carotid arteries of SHR, whereas it was reduced in vertebral, middle cerebral and posterior cerebral arteries compared to WKY (fig. 4).

Media Cross-Sectional Area

The media areas of the extracranial part of the internal carotid arteries were 149 ± 13 (SHR) and 114 ± 9 (WKY) x 10³ μm² (p < 0.01). A significant increase was seen also in basilar and posterior cerebral arteries of SHR compared to WKY (fig. 5).

Media Thickness

The media thickness was 64 ± 7 μm (SHR) and 53 ± 3 μm (WKY) in the extracranial part of the internal carotid arteries (p < 0.05). No significant difference in media thickness was noticed in the intracranial segment of the same vessels.

The media thickness of the vertebral arteries of SHR and WKY did not differ significantly. It was 27 ± 3 μm (SHR) and 20 ± 2 μm (WKY) in the basilar arteries (p < 0.001) and 24 ± 2 μm (SHR) and 16 ± 3 μm (WKY) in the middle cerebral arteries (p < 0.05).

In the posterior cerebral arteries, the media thickness was 27 ± 2 μm in SHR and 21 ± 3 μm in WKY (p < 0.01). No significant difference was noticed between the posterior communicating arteries.

Media Thickness/Internal Radius Ratio

The media to radius ratio of the extracranial part of the internal carotid artery was 0.19 ± 0.03 (SHR) and 0.17 ± 0.01 (WKY) (n.s.). Significantly increased quotients were noticed in the intracranial part of the same vessel as well as in basilar, middle cerebral, posterior cerebral and posterior communicating arteries of SHR (fig. 5).

Discussion

The gradient between the intraluminal pressure and the perivascular tissue pressure determines the tension per unit wall layer (T) at a defined luminal radius (r) and wall thickness (w) according to Frank’s modification of Laplace’s law (fig. 6). A heightened intraluminal pressure (Pi) is immediately counteracted by increased myogenic activity in the resistance vessels which results in a decrease of (r) and normalization of (T). This acute functional autoregulation which, within certain limits, keeps the cerebral blood flow constant irrespective of fluctuations in blood pressure and protects the microvessels from undue pressure elevations and overdistension, has its chronic structural analogy. This structural autoregulation is accomplished by an increase of the ratio between the media thickness and the luminal radius in chronic hypertension. The increase of resistance induced by vascular smooth muscle shortening is exaggerated by a heightened media to radius ratio resulting in a steeper resistance curve and a higher maximal contractile strength. An additional decrease of the internal radius measured at maximal vasodilatation will further exaggerate this response and enhance minimal resistance to flow. There is evidence that the hypertensive structural modification may differ with the arterial bed studied. To what extent the various calibre segments of the arterial tree are involved is debated.
Measurements at specified sites in the arterial tree allow a comparison of luminal and wall dimensions as separate factors, which is essential for the evaluation of the hemodynamic effects of an altered wall to lumen ratio. In the intracranial basal arteries, where measurements were made at specified sites, the media to radius ratio was significantly increased at most sites in the hypertensive rats. In the seven- and in the twelve-month-old rats with moderate hypertension, the increase of the quotient was due to an increase of media.
thickness and/or a decrease of the radius, whereas it was mainly due to a decrease of internal radius in the seven-month-old rats with severe hypertension. As discussed above, these structural alterations should be of hemodynamic importance. The morphometric results are consistent with the hemodynamic effect of bilateral carotid artery ligation in the same animals. After ligation, the relative and absolute decrease of carotid back pressure was more pronounced in hypertensive than in normotensive rats and larger in SHRSP than in SHR. 19

The small pial arteries, measured in the seven-month-old rats only, were for technical reasons not compared at specified sites. The media to lumen ratio was enhanced in agreement with earlier studies using the same technique. 15, 25 The hemodynamic consequences of the enhanced ratio are difficult to evaluate in the absence of exact comparison of internal radius between hypertensive and normotensive rats. However, the radius of cortical surface arteries have been compared at specified sites with other techniques in-cluding intravital microscopy, 12 measurements on un-fixed pial arteries in vitro 26 and on vascular casts. 27 All these studies have shown significantly smaller internal radius in SHR and SHRSP than in WKY. No studies have indicated a larger radius in the hypertensive rats.

The body weight of spontaneously hypertensive rats is often reduced. In our study, SHRSP had considerably lower body weights than WKY. The media to radius ratio is not considered to correlate to body weight; however, the weight modifies the absolute dimensions of lumen and media. 28 The subnormal values of the internal radii and the normal or subnormal media cross-sectional areas of the intracranial arteries in SHRSP might thus, to some extent, be due to a reduced body weight. If this had been the major determining factor for the arterial dimensions of SHRSP, a more uniform alteration of the media area and the radius would, however, have been expected in the different vessel segments of these animals. Furthermore, a reduction of the internal radius was noticed in intracranial arterial segments of twelve-month-old SHR with body weights comparable to the controls. Chronic hyperten-sion seems therefore to be a major determinant not only for the increased media thickness but also for the decreased internal radius found in the hypertensive rats.

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References

\[ T = \frac{(P_i - P_e) r}{W} \]

Layer: \( P_e \) = perivascular tissue pressure; \( P_i \) = intraluminal pressure; \( r \) = luminal radius; \( w \) = wall thickness; \( P_i - P_e \) = perfusion pressure. cf. discussion.

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Figure 6. \( T = \frac{(P_i - P_e) r}{W} \) tension per unit vessel wall

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\[ T = \frac{(P_i - P_e) r}{W} \]
MORPHOMETRIC STUDIES on cerebral arteries ex vivo have demonstrated an increased media thickness and vessel wall to lumen ratio with or without a concomitant reduction of the internal radius in hypertensive man.1,2 As well as in spontaneously hypertensive rats.3-4 The increase of resistance induced by vascular smooth muscle shortening is augmented by a heightened media to radius ratio — for every degree of smooth muscle shortening a larger muscle mass has to be pulled into the lumen — resulting in a steeper resistance curve and a higher maximal contractile strength.5 The arterial wall tension increases with the radius and decreases with the wall thickness, hence the altered vascular geometry would enable the resistance vessels to withstand a higher intraluminal pressure than corresponding vessels in normotensive rats when relaxed as well as at any degree of vascular tone. Studies on the blood-brain barrier and cerebral blood flow support this hypothesis.5-11 However, intravital microscopy of cremasteric and mesenteric arteries indicate that, in vivo, the diameters of small arteries may be the same or even larger in hypertensive than normotensive rats.12-13 A decrease in number of arterioles per unit tissue rather than a change in arterial diameter could account for the increased peripheral resistance in hypertension.14 This situation would, however, not explain the increased capacity to autoregulate and the protection of the blood-brain barrier in hypertensive rats mentioned above.

To determine whether there is an in vivo difference in pial arterial diameter between normotensive and hypertensive rats in the resting state and/or when dilated, we have measured the diameter of the main para-sagittal cortical surface arteries through a closed cranial window during resting conditions as well as during hypercapnic vasodilatation. The diameter of cortical surface veins was also measured since functional or structural alterations may occur also in veins in hypertensive animals. Data for arteries have been briefly presented earlier.15

Materials and Methods
Six spontaneously hypertensive rats (SHR)6 and six Wistar-Kyoto rats (WKY), six months of age, were anesthetized with pentobarbital (30 mg/kg i.p.), tracheotomized and mechanically ventilated with air. Catheters were inserted into the aorta from a femoral artery for continuous recording of mean arterial pres-
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