Effect of Chronic Hypertension and Sympathetic Denervation on Wall/Lumen Ratio of Cerebral Vessels

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SUMMARY The purposes of this study were to determine whether 1) cerebral vessels undergo hypertrophy during chronic hypertension and 2) sympathetic nerves contribute to cerebral vascular changes in chronic hypertension. Morphometric studies were undertaken in stroke-prone spontaneously hypertensive rats (SP-SHR) and normotensive Wistar-Kyoto (WKY) rats. Unilateral superior cervical ganglionectomy was performed in the SP-SHR at 8 weeks of age. When the rats were approximately 13 months old, they were killed and the brain was fixed with formalin at a perfusion pressure of 80% of the rat's systolic pressure. Wall/lumen ratio was measured in approximately 1200 arteries and arterioles. In parenchymal, but not pial, cerebral vessels there was pronounced vascular hypertrophy in SP-SHR: wall/lumen ratio was 0.08 in WKY and 0.14 in SP-SHR (p < 0.05). Sympathetic denervation attenuated the development of vascular hypertrophy in SP-SHR: wall/lumen ratio was 0.14 in the innervated parenchymal vessels, and 0.10 in denervated vessels (p < 0.05). We conclude that cerebral vessels undergo hypertrophy in stroke-prone SHR and speculate that vascular hypertrophy may protect cerebral vessels by reducing wall stress in chronic hypertension. Sympathetic nerves appear to exert a trophic effect on cerebral vascular muscle in chronic hypertension.

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Key Words • stroke-prone SHR • morphometric study • vascular hypertrophy

FOLKOW et al.1 have emphasized the concept that an increase in wall/lumen ratio may be an important factor in the pathogenesis and maintenance of hypertension. Vascular hypertrophy appears to be of physiologic importance both in limiting blood flow during maximal vasodilatation and in increasing vascular responsiveness to constrictor stimuli. Vascular hypertrophy, however, also may serve a protective function, by attenuating increases in capillary pressure during hypertension.2 This protective effect could be especially important in cerebral vessels in which sudden increases in arterial pressure produce disruption of the blood-brain barrier.3,4

Morphometric studies have demonstrated hypertrophy of arteries in most,5-9 but not all,10 vascular beds in renal and spontaneous hypertension. One might expect that small arteries in the brain, like vessels in most other organs, would undergo hypertrophy during chronic hypertension. On the other hand, large arteries that supply the brain appear to contribute importantly to cerebral vascular resistance11-13 and to minimize increases in pressure in distal vessels during acute hypertension.14 Thus, if small cerebral arteries are protected from increases in pressure by increases in resistance in upstream vessels, small cerebral arteries might not undergo hypertrophy during chronic hypertension.

The first goal of this study was to determine whether cerebral arteries and arterioles undergo hypertrophy during chronic hypertension. Specifically, we determined wall/lumen ratio in cerebral vessels of stroke-prone SHR and normotensive Wistar-Kyoto (WKY) rats.

Our second goal was to determine whether sympathetic nerves contribute to cerebral vascular changes in chronic hypertension. Bevan16 and Bevan and Tsuru17 have proposed that sympathetic nerves exert a "trophic" effect and contribute to the normal development of arteries. Thus, sympathetic nerves also might contribute to vascular hypertrophy. To test this hypothesis, we performed unilateral sympathetic denervation in young SHR and compared wall/lumen ratio in cerebral vessels of the innervated and denervated hemispheres.
Methods

We studied eight stroke-prone spontaneously hypertensive rats (SHR) and eight normotensive Wistar-Kyoto (WKY) rats. When the SHR were 3 weeks old, the superior cervical ganglion was removed on one side (left ganglion in 4, right in 4). Ganglionectomy produced ptosis and enophthalmos on the ipsilateral side in all rats. Age-matched WKY rats were used in this study; both hemispheres were innervated. All rats were fed standard rat chow. When the rats were approximately 1 year old, systolic arterial pressure (SBP) was measured using an electrophygmonanometer (ITT, Inc.). The SBP was measured 3 times, and an average value was determined; it was 235 ± 10 (mean ± SE) mm Hg in SHR and 142 ± 4 mm Hg in WKY.

At the time of study, the SHR were 13.2 ± 3 months old and weighed 268 ± 20 g. The WKY were 13.8 ± 1.0 months old and weighed 274 ± 9 g. The rats were anesthetized with pentobarbital (50 mg/kg i.p.). A PE50 catheter was inserted into the abdominal aorta and advanced above the renal arteries to monitor pressure during perfusion fixation. Heparin (1000 units i.v.) was injected, and about 2 minutes later the rats were killed with KCl i.v. The sternum was split, and a stiff 18-gauge cannula was inserted quickly through the left ventricle into the ascending aorta. An incision was made in the right atrium to allow drainage of perfusate. The upper-body was perfused with 10% buffered formalin for 30 minutes. Perfusion was nonpulsatile from a pressurized reservoir. Perfusion pressure in each rat was maintained at 80% of its SBP, measured while the rat was unanesthetized; perfusion (fixation) pressure was 189 ± 8 mm Hg in SHR and 113 ± 2 in WKY.

Following perfusion, the brain was removed. The cerebrum was separated into right and left halves by longitudinal section. Blocks were taken in a plane parallel to the cortical surface to obtain cross sections of as many parenchymal vessels as possible. Blocks were then embedded in paraffin, cut at 6 μm thickness, and stained by the trichrome method. Approximately 1200 arteries and arterioles were measured. Measurements obtained were external diameter of the vessel and wall thickness; three measurements of wall thickness were made, and an average value was calculated. Vascular lumen was calculated by subtracting wall thickness ×2 from the external diameter. All measurements were made by light microscopy using an ocular ruler that was calibrated with a stage micrometer. Slides were coded so that the measurements were made "blind." Only arteries and arterioles that were cut in cross section were measured.

Vessel measurements were divided into paired categories: SHR and WKY, right and left, parenchymal and pial vessels. Statistical analysis comparing the two hemispheres was made by paired t tests; WKY and SHR were compared by unpaired t tests. All values were combined to obtain one value for each animal in each category.

Results

Effects of Hypertension

In pial vessels, wall/lumen ratio was similar in WKY and in the innervated hemisphere of SHR (fig. 1). In parenchymal arteries and arterioles, however, wall/lumen ratio was 73% larger (p < 0.05) in the innervated hemisphere of SHR than in WKY (fig. 2).

The average external diameter of all vessels measured in the innervated hemisphere was 34 μm in SHR and 45 μm in WKY (p < 0.05). If small resistance vessels have a larger wall/lumen ratio, the difference in wall/lumen ratio in SHR and WKY could be related in part to a greater fraction of smaller vessels in SHR. It was important, therefore, to determine whether the group differences in wall/lumen...
ratio were simply a function of differences in vessel diameter. We divided parenchymal vessels into those with outer diameter \( \leq 35 \) and \( >35 \) \( \mu m \), calculated regression lines for WKY, SHR-innervated, and SHR-denervated hemispheres, and compared the slope of the regression lines by analysis of variance (fig. 3). In vessels smaller than \( 35 \mu m \), the slope was different \( (p < 0.05) \) in SHR-innervated and WKY. In vessels larger than \( 35 \mu m \), the slope was similar in SHR-innervated and WKY. The intercept, however, was greater in SHR-innervated than in WKY \( (p < 0.05) \).

Thus, at the same outside diameter, the wall/lumen ratio was larger in SHR-innervated than in WKY. This effect appears to be most pronounced in parenchymal vessels smaller than \( 35 \mu m \), but also evident in vessels larger than \( 35 \mu m \). Vessels in SHR appeared to contain increased amounts of medial smooth muscle, as demonstrated by the trichrome stain. Increased amounts of collagen were not detected, but cannot be excluded.

**Effects of Sympathetic Denervation in SHR**

In pial vessels, wall/lumen ratio was not significantly different in innervated and denervated hemispheres of SHR (fig. 1). In parenchymal vessels, however, wall/lumen ratio was \( 36\% \) larger \( (p < 0.05) \) in the innervated hemisphere (fig. 2). The external diameter in the innervated hemisphere \( (34 \mu m) \) was not significantly different \( (p > 0.05) \) from the mean diameter in the denervated hemisphere \( (38 \mu m) \).

In vessels smaller than \( 35 \mu m \), the slope of the relationship between vessel diameter and wall/lumen ratio was different \( (p < 0.05) \) in innervated and denervated hemispheres (fig. 3). The slopes and intercepts were similar in the two hemispheres in vessels larger than \( 35 \mu m \).

**Discussion**

The major new findings in this study are as follows. First, cerebral vessels of adult stroke-prone SHR are hypertrophied, as manifested by an increase in wall/lumen ratio. This phenomenon was most pronounced in parenchymal arterioles \( (<35 \mu m \) diameter), less pronounced in parenchymal arteries \( (35-85 \mu m \) diameters), and was not detected in pial arterioles or arteries. Second, sympathetic nerves appear to affect development of cerebral vascular hypertrophy. Superior cervical ganglionectomy in young stroke-prone SHR greatly attenuated the development of cerebral vascular hypertrophy.

In this discussion we will consider: 1) methods of measuring the wall/lumen ratio; 2) vascular hypertrophy in chronic hypertension; 3) role of sympathetic nerves; and 4) implications of this study.

**Methods: Measurement of Wall/Lumen Ratio**

The brain was perfused at 80% of the rat’s SBP, which had been measured when the rat was unanesthetized. Bunag, in table 1, demonstrated that mean pressure is \( 74\% \) of systolic pressure in normotensive rats and SHR. Folkow has suggested that morphometric comparisons of hypertensive and normotensive vessels at an animals’ “ordinary” pressure may be misleading, and the vessels of normotensive rats and SHR should be fixed at the same pressure. It is not clear to us whether it is preferable to fix vessels from WKY and SHR at their “ordinary” pressure, as we did, or at the same pressure, as recommended by Folkow. We should emphasize, however, that neither of our major conclusions would have been altered if we had fixed the vessels from the two groups at the same pressure. If the SHR had been fixed at normotensive pressure, the wall/lumen ratio would have been even larger in SHR than WKY because SHR vessels would have distended less, and the difference between the innervated and denervated hemispheres almost certainly would still have been apparent.

It is possible that an inherent characteristic of SHR vessels (their greater contractility) could provide an alternate explanation for our findings and those of all other investigators who study vascular dimensions after fixation. If the aldehyde fixative were to contract SHR vessels more than WKY vessels, the relationship between vessel diameter and wall thickness would be shifted. Specifically, if SHR and WKY vessels of equivalent wall mass and diameter were exposed to fixative and the SHR vessels became smaller than WKY vessels, it would be possible to conclude that SHR vessels are thicker than WKY vessels at any given diameter. Findings such as a greater number of vascular muscle layers in SHR than in WKY, however, support the view that vessels in SHR may undergo true hypertrophy. Nevertheless, systematic studies on the influence of fixation on caliber of cerebral and other vessels need to be performed.
Vascular Hypertrophy in Chronic Hypertension

Russell\textsuperscript{18} demonstrated an elevated wall/lumen ratio in small, but not large, cerebral arteries of hypertensive humans by postmortem examination. Johansson and Nordborg\textsuperscript{26} reported an increase in wall/lumen ratio of cerebral arteries of 6.5 month-old SHR.

The present study differs from the studies by Russell\textsuperscript{18} and Johansson and Nordborg\textsuperscript{26} in several important ways: our rats were perfuse-fixed at the animals' in vivo pressure, stroke-prone rats were studied, and the morphometric method was different. Nevertheless, the conclusions concerning increases in wall/lumen ratio were similar in the three studies. It appears that the degree of hypertrophy in cerebral vessels of SHR is pronounced in small intraparenchymal arteries.

We did not examine effects of hypertension as systematically in large cerebral arteries as in small vessels. Our findings, however, suggest that the most pronounced cerebral vascular hypertrophy may occur in very small arteries and arterioles. Vascular hypertrophy was modest in parenchymal arteries larger than 35 $\mu$m in SHR. Furthermore, the pronounced hypertrophy of small vessels in SHR suggests that hypertrophy of large arteries, if it occurred, was not sufficient to protect the distal vessels. It is of interest that Wei et al.,\textsuperscript{21} who studied vessels 30-450 $\mu$m in diameter, have calculated that wall stress is greater in small cerebral arteries than in larger vessels. Thus, it appears that hypertrophy in chronic hypertension may occur in vessels that normally have the greatest wall stress.

Role of Sympathetic Nerves

Bevan\textsuperscript{13} and Bevan and Tsuru\textsuperscript{14} have proposed that sympathetic nerves exert a trophic effect on developing vascular muscle. They found that, after denervation of the ear artery in normotensive rabbits, the weight and medial thickness of the vessel were reduced. Sympathetic nerves also appear to exert a trophic influence on rat portal vein, apparently through an effect on membrane excitability.\textsuperscript{22} In a preliminary report, Bevan and Bevan\textsuperscript{23} have suggested that sympathetic nerves also affect the development of cerebral arteries in normotensive rabbits.

Our finding in this study that sympathetic denervation attenuates the development of vascular hypertrophy is, to our knowledge, the first demonstration of this effect in chronic hypertension. Edvinsson and O'Connor\textsuperscript{24} have suggested that not all intraparenchymal cerebral vessels receive sympathetic innervation. They found that, in the cerebral cortex, between 30% (occipital cortex) and 80% (parietal cortex) of arterioles are innervated. We have not attempted to determine whether there is corresponding regional heterogeneity in cerebral vascular hypertrophy.

We are unable to explain the finding that attenuation of vascular hypertrophy by denervation was evident in parenchymal vessels but not pial arteries. The absence of detectable effect in pial arteries probably is not the result of less innervation of pial vessels; all pial arteries appear to be innervated.\textsuperscript{25} It is not clear whether this differential effect between pial and parenchymal vessels is related to differences in degree of denervation, reinnervation, vascular stress, or other factors.

Implications

During acute, severe hypertension there is an increase in cerebral blood flow and disruption of the blood-brain barrier to albumin.\textsuperscript{16, 26} Because SHR appear to be resistant to disruption of the blood-brain barrier during acute increases in pressure,\textsuperscript{27} long-term adaptive mechanisms must protect cerebral vessels. This study provides direct, morphometric evidence that cerebral vessels of stroke-prone SHR are hypertrophic when compared with age-matched WKY. If we speculate that vascular hypertrophy protects the microcirculation against disruption of the blood-brain barrier during hypertension, one might predict that the cerebral microcirculation (because of the sensitivity of the blood-brain barrier to disruption by hypertension) would require substantial arteriolar hypertrophy. In fact, the degree of cerebral arteriolar hypertrophy appears to be more profound than that found in other vascular beds exposed to chronic hypertension.

Cerebral hemorrhage in hypertensive humans\textsuperscript{28} and rats\textsuperscript{29} almost always occurs in the parenchyma. Our finding, that wall/lumen ratio of stroke-prone SHR is increased in parenchymal but not pial arteries, but hemorrhage nevertheless occurs in parenchymal vessels of hypertensive patients and rats, suggests that vascular stress remains elevated in parenchymal vessels despite hypertrophy or that hypertrophy may not be fully protective against hemorrhage. Cerebral vascular hypertrophy may be protective against disruption of the blood-brain barrier and against hemorrhage early in the course of hypertension, but if long-term medial hypertrophy leads to fibrosis of the vessels,\textsuperscript{19} the protective effect may be lost.

Recent studies suggest that sympathetic nerves may play an important protective role during acute increases in arterial pressure by limiting increases in cerebral blood flow\textsuperscript{30} and disruption of the blood-brain barrier.\textsuperscript{24} Our present study suggests that sympathetic nerves also may play an important protective role during chronic hypertension, by contributing to cerebral vascular hypertrophy. These studies extend the concept that sympathetic nerves may exert a trophic influence on vessels\textsuperscript{13, 14, 24} with the demonstration that denervation attenuates the development of vascular hypertrophy in chronic hypertension.

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