Structural Alterations of Microvascular Smooth Muscle Cells in Reduced Renal Mass Hypertension

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Loss of microvessels (anatomic rarefaction) occurs in chronic reduced renal mass (RRM) hypertension and is mediated via structural degeneration of vascular smooth muscle (VSM) and endothelial cells. The purpose of the present study was to determine if structural changes occur in VSM cells of the microvessels that remain in the tissue of rats with chronic RRM hypertension. Samples of cremaster muscles were taken from normotensive control rats and rats with acute (3–7 days) and chronic (14–28 days) RRM hypertension (75% reduction in kidney mass with 4% NaCl loading). The samples were fixed in situ and processed for light and electron microscopy. Ultrastructural morphology of VSM cells in terminal arterioles of control animals was normal. Although VSM morphology in many microvessels of RRM hypertensive rats was also normal, some vessels exhibited structural changes that were not present in arterioles of the normotensive animals. The most striking change was the appearance of more extensive dense bodies anchoring the contractile filaments around the outer membrane of the cells. Extreme vasoconstriction was observed in some arterioles of RRM rats as long as 2 weeks after salt loading. Focal areas of VSM cell proliferation were evident. Many of the changes occurring in RRM were detected as early as 1 week after the onset of hypertension. These observations suggest that renal mass reduction–salt loading hypertension is associated with early structural and functional changes in the VSM cells. (Hypertension 1991;17:902–908)

It is well recognized that chronic hypertension is associated with structural changes in blood vessels. The most striking change of vascular structure in hypertension is the thickening of the vascular wall that occurs in the aorta and other large arteries. This wall thickening may involve either hypertrophy or hyperplasia of the vascular smooth muscle cells.1–4 Structural changes of small arteries and arterioles (e.g., wall thickening, increases in wall-to-lumen ratio, and structural narrowing of the lumen) have been reported in some studies of hypertensive animals5–8 but not others.9–11 Finally, other studies suggest that arterioles and capillaries are actually lost (anatomic rarefaction) during chronic hypertension.3,6,9,12–14 Theoretical studies suggest that the latter phenomenon may have significant effects on vascular resistance and tissue perfusion in hypertension.15

A recent study of the structural basis for anatomic rarefaction in the cremaster muscle of rats with reduced renal mass (RRM) hypertension has demonstrated that some microvessels are irreversibly degenerated or severely atrophied after 4–6 weeks.16 Although some arterioles and capillaries exhibit degenerative changes and others are apparently lost in chronic hypertension, many microvessels remain in the tissue and appear to be normal when viewed via intravital microscopy.13 The purpose of the present study was to evaluate the structure of microvascular smooth muscle cells in persisting arterioles of rats with various durations of RRM hypertension to determine if cellular changes occur in smooth muscle of vessels that have not undergone rarefaction.

Methods

Renal Mass Reduction

Male Sprague-Dawley rats were subjected to a 75% reduction in renal mass via a two-stage surgical procedure as previously described.13 The animals were 6 weeks old and weighed 180–190 g at the time of surgery.
of the initial surgery. Three to 5 days after the final reduction in renal mass, RRM rats were placed on high salt rat chow containing 4% NaCl (Dyets Inc., Bethlehem, Pa.) for as long as 4 weeks. Cremaster muscles were studied after 3 days (four animals), 1 week (four animals), 2 weeks (five animals), or 4 weeks (seven animals). Seven age-matched controls were placed on standard (0.8% NaCl) rat chow and studied after 4 weeks to verify that structural changes in RRM rats were the result of the renal mass reduction–salt loading procedure. All animals were allowed to drink water ad libitum.

**Electron Microscopic Studies**

Cremaster muscles from control and hypertensive rats were removed after measurement of the carotid blood pressure. The muscles were fixed by superfusion with 2.5% cacodylate-buffered glutaraldehyde. Three controls and two 4-week RRM samples were fixed while the muscle was pinned in the position used for intravital observations. The other muscles were fixed in the intact position surrounding the testes. After an initial 5-minute fixation period, the testes were removed, and the intact muscle sacs were removed and immersed in fixative. Tissue blocks were taken from randomly sampled regions throughout the muscles. To ensure optimal orientation of most of the samples, approximately half of the samples from each muscle were taken by cutting a thin strip around terminal blood-containing vessels. By removing elongated blocks from such strips, the samples could be oriented during embedding to yield transverse sections through the vessels. To remove any bias that might be encountered by observing only blood-perfused vessels, remaining samples were taken from areas that did not contain visible terminal vessels. A minimum of eight blocks per sample were postfixed in 1% OsO₄, embedded in Epon, sectioned into 1-μm sections, and stained with toluidine blue. Selected ultrathin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy as described previously.

**Blood Pressures**

As previously reported, placement of RRM rats on a high salt diet resulted in a significant elevation of mean arterial pressure. Mean arterial pressure in the groups from which the samples were taken averaged 103 ± 6 mm Hg for age-matched controls, 151 ± 9 mm Hg for 3-day RRM, 139 ± 6 mm Hg for 1-week RRM, 161 ± 6 mm Hg for 2-week RRM, and 162 ± 8 mm Hg for 4-week RRM.

**Microvessel Structure in Normotensive Controls**

Sections obtained from the majority of the control (and experimental) samples contained a transversely sectioned large arteriole with an accompanying venule. These large arterioles were approximately 150 μm in outer diameter and were located in the fascia between the two perpendicularly oriented layers of the cremaster muscle. Large arterioles were characterized by one or two layers of circularly or spirally oriented smooth muscle cells, with a distinct inner elastic lamina separating the tunica media from the endothelium. Two or three unmyelinated nerves were invariably found in the adventitial layer of these arterioles.

The most terminally located arterioles were among the muscle fibers in each of the two layers of the cremaster muscle. These arterioles consisted of a single layer of circularly arranged smooth muscle cells. The vessels had a reduced or fenestrated elastic lamina, and most showed no evidence of innervation. As shown in Figure 1, the ultrastructure of the smooth muscle cells in vessels from controls was similar to that previously described by others and will not be further described here.

**Microvessel Structure in Reduced Renal Mass Rats**

The ultrastructure of most of the nondegenerating arterioles appeared to be normal. However, a number of structural alterations were apparent in some vessels of RRM rats that were not observed in control animals. Both large and small arterioles exhibited euchromatous nuclei and abundant ribosomes and mitochondria, suggesting highly active metabolism in the vascular smooth muscle cells.

The most striking change, evident within 1 week after placing the rats on the high salt diet, involved the organization of the dense bodies associated with the contractile filaments. In contrast to the small, regularly spaced dense bodies found in control animals, dense bodies in vascular muscle cells of several arterioles in RRM rats formed extensive, dense mats that were preferentially located on the outer circumference of the cell (Figure 1). Similar extensive dense bodies were associated with cell–cell junctions between vascular muscle cells. These modified dense bodies were most noticeable in the smallest arterioles, where a single smooth muscle cell typically formed most of the circumference of the muscle layer in the plane of section.

Some arterioles containing heterogeneously staining types of vascular smooth muscle cells were noted (Figure 2). The vascular muscle cells forming the wall were irregular in size, shape, and electron density when observed in longitudinal sections. Cells having cytoplasm with a lighter electron density typically had less-dense contractile filaments and a general appearance that was consistent with an “activated” state like that found in proliferating vascular smooth muscle cells. Two examples of completely undifferentiated or dedifferentiated vascular smooth muscle cells lacking basement membranes were found (Figure 2). We interpret these as putative proliferating cells.

Some small arterioles of RRM exhibited a substantial constriction that resulted in almost complete closure of the vessel by endothelial cells pushed into the lumen (Figure 3). This constriction was observed in several arterioles as early as 3 days after initiation of the high salt diet and was still...
Figure 1. Photomicrographs of smooth muscle in wall of arterioles from normotensive control rat (panel a), 1-week renal mass reduction rat (panel b), and 2-week renal mass reduction rat (panel c). L, lumen; E, endothelial cells. *Elastic lamina. Arrows point to dense bodies in vascular smooth muscle cell. Bar, 1 μm.
FIGURE 2. Photomicrograph of longitudinally sectioned arteriole from a 2-week reduced renal mass rat. Panel a: Vascular smooth muscle cells (1, 2, and 3) show variation in the electron density of their cytoplasm. Panel b: Higher magnification of two vascular smooth muscle cells in another region of same arteriole shows outer basement membrane (black arrows) but no basement membrane surrounding adjacent undifferentiated, putatively proliferating cells (white arrows). L, lumen; A, adventitia. Bar, 1 μm.

evident as late as 2 weeks. However, no closed arterioles were found at 4 weeks. The smooth muscle cells in many small arterioles also appeared to have alterations in their contractile filaments and dense bodies that were similar to those observed in the larger arterioles.

In one fortuitous section from a 2-week RRM (Figure 4), it was possible to view the point at which a small arteriole branched from a larger arteriole. Although the larger arteriole was normal, the branch was obstructed by an aggregation of smooth muscle cells of varying electron densities that protruded into the lumen. At this site, the underlying endothelium appeared to be damaged relative to the other regions. The lumen of the small arteriole near the aggregation of vascular smooth muscle cells was filled with red blood cells, suggesting stasis. Downstream from this area, the lumen was devoid of blood cells, and endothelial cells projected into the lumen.
FIGURE 3. Photomicrograph shows two arterioles from a 2-week reduced renal mass rat are completely constricted, closing off their lumens (arrows). M, vascular smooth muscle; E, endothelial cell; N, nerve. Bar, 1 μm.

Discussion

Rats with chronic RRM hypertension exhibit a significant reduction in total microvessel density (anatomic rarefaction) relative to normotensive controls. Anatomic rarefaction in this form of hypertension is mediated by degenerative structural changes in microvessels. In many cases, vascular muscle cells of the degenerating vessels are extremely atrophic or nearly absent. However, a substantial number of arterioles remain in the tissue of the hypertensive animals and appear to be normal when viewed by intravital microscopy. It is unknown whether these remaining vessels exhibit structural changes that may affect their functional characteristics or provide insight into the processes underlying vascular remodeling in hypertension.

The results of the present study suggest that constriction or obstruction of arterioles may contribute to anatomic rarefaction in RRM hypertension. Complete closure of small arterioles in RRM rats was observed as early as 3 days and as late as 2 weeks into the experimental period, but it was not found in the 4-week group. The constriction of arterioles observed in the present study is consistent with the results of microcirculatory studies that show active closure (functional rarefaction) of arterioles in the early stages of RRM and other models of hypertension and supports the hypothesis that active closure of arterioles may eventually lead to anatomic rarefaction. In addition, a fortuitous section enabled us to propose an additional mechanism for anatomic rarefaction in RRM rats (i.e., that smooth muscle cells proliferate focally at sites of apparent endothelial injury, ultimately closing the vessel). The prolonged ischemia resulting from active closure or focal obstruction of arterioles would be expected to lead to the cellular atrophy and degeneration that we previously described in some cremasteric microvessels of rats with chronic (4-week) RRM hypertension.

Another distinctive change that we observed in arterioles of the RRM rats was the appearance of more prominent and extensive dense bodies for anchoring the contractile filaments at the periphery of the smooth muscle cells. The development of a more extensive network of dense bodies in vascular muscle cells of the hypertensive animals could reflect an alteration of the contractile apparatus of the cell that may help the vessels to maintain a relatively constant level of wall stress, thereby reducing the stimulus for medial hypertrophy.

In the present study, we made no quantitative measurements of vessel wall dimensions because the cremaster muscle was fixed by superfusion in situ to allow the presence and relative distribution of blood cells to be used as a guide in selecting the vessels and interpreting the in vivo conditions. However, our qualitative observations suggest that hypertrophy of vascular smooth muscle in microvessels is not an...
over response to RRM hypertension. This conclusion is consistent with the results of several previous studies in other forms of hypertension.\textsuperscript{5,7,9-11} We did, however, find evidence that focal hyperplasia of vascular smooth muscle cells may occur. In one case, the proliferation appeared to be associated with endothelial cell injury, as reported in the acute phase of other types of hypertension.\textsuperscript{4} Additional studies are needed to determine whether vascular muscle proliferation is a significant response in RRM hypertension and
whether it is related to cell injury, humoral factors, or elevations in vascular wall tension.

Acknowledgments

The technical assistance of Laura Watson Morris, Loan Dang, and Rosalie Zamiatowski is gratefully acknowledged.

References


Key Words: microcirculation • renovascular hypertension • arterioles • capillaries • reduced renal mass hypertension • histology • vascular smooth muscle • rarefaction
Structural alterations of microvascular smooth muscle cells in reduced renal mass hypertensive.
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Hypertension. 1991;17:902-908
doi: 10.1161/01.HYP.17.6.902

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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