Structural Changes During Microvascular Rarefaction in Chronic Hypertension

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Previous physiological studies have suggested that loss of microvessels (anatomic rarefaction) occurs in the skeletal muscle microcirculation of rats with chronic hypertension. However, little is known of the exact structural changes that occur during the process of anatomic rarefaction. The purpose of this study was to examine the muscle at the ultrastructural level to search for evidence of microvessel degeneration that would correlate with the concept of anatomic rarefaction in chronic hypertension. Cremaster muscles were removed from normal rats and from rats with chronic reduced renal mass hypertension, which was produced by a 75% reduction in kidney mass followed by salt loading (4% NaCl chow with water ad libitum) for 4 weeks. The muscles were fixed and prepared for histological examination by light and electron microscopy. Atrophy and degeneration of both endothelial cells and vascular smooth muscle cells were observed in many arterioles of the hypertensive rats. Some arterioles of hypertensive rats were degenerated to such an extent that the original identity of the cells could not be determined. The hypertensive rats also exhibited degeneration of capillaries and extravasation and uptake of red blood cells into lymphatic vessels. In contrast, age-matched control rats exhibited normal histology. The results of this study support previous physiological evidence for anatomic rarefaction in the cremaster muscle of chronically hypertensive rats. (Hypertension 1990;15:922-928)

A number of studies have suggested that microvessel density is reduced in several rat models of hypertension, including spontaneously hypertensive rats,1-3 one-kidney, one clip renal hypertension,4 two-kidney, one clip renal hypertension,5 and reduced renal mass (RRM) hypertension,6 and in human hypertension.2-8 In early hypertension, arterioles appear to be actively closed, but still present (functional rarefaction).2-6 However, later in hypertension, arteriolar density is reduced even when the vascular bed is maximally dilated2-6; this finding suggests that microvessels are actually lost (anatomic rarefaction) during chronic hypertension.

Although many studies of microvessel rarefaction in hypertension have used intravital microscopy or injection methods to estimate microvessel density, there have been few histological studies of the microcirculation in chronic hypertension. The object of the present study was to look for evidence of cellular and subcellular changes that correlate with the phenomenon of anatomic rarefaction in chronic hypertension.

Methods

Renal Mass Reduction

Male Sprague-Dawley rats were subjected to a 75% reduction in renal mass by means of a two-stage surgical procedure as previously described.6 The animals were 6 weeks old and weighed 180-190 g at the time of the initial surgery. Three to 5 days after the final reduction in renal mass, the RRM rats were placed on high salt rat chow containing 4% NaCl (Dyets Inc., Bethlehem, Pennsylvania) for 4 weeks, and a group of normal, age-matched rats was maintained on standard Purina rat chow (0.8% NaCl) for the same period of time to verify that any 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

The cremaster muscles from the control and hypertensive rats were removed after measurement of carotid artery blood pressure under pentobarbital anesthesia. The muscles were fixed by superfusion with 2.5% cacodylate-buffered glutaraldehyde. Some
of the samples were fixed while the muscle was pinned out in the position used for intravital observations; others were fixed in the intact position surrounding the testes. In the latter case, the testes were extirpated after an initial 5-minute fixation period, and the intact cremaster muscle sacs were removed and immersed in fixative. All samples were postfixed in 1% OsO₄, embedded in Epon, and sectioned into 1-μm and ultrathin sections as described previously.9

Two different sampling procedures were used to analyze the muscle. In one, tissue blocks were taken from several randomly sampled regions of the muscle of one control and two hypertensive animals, without reference to the vascular pattern. In the remaining muscles, second- and third-order microvessels were identified under a dissection microscope; the presence of intraluminal erythrocytes was used as a guide. These larger arterioles and venules could then be specifically oriented as transverse or longitudinal sections during embedding. A minimum of six to eight such samples per muscle was examined by light (1-μm sections) and electron microscopy. Fourth-order arterioles and capillaries, which cannot be specifically dissected out, were selected from randomly sampled regions of the tissue that did not contain the larger vessels. At least 10 such fields per muscle were sectioned for light microscopy.

Results

Hemodynamic Parameters

As previously reported,6 salt loading produced a significant hypertension in the RRM rats. Mean arterial pressure averaged 104±2 (SEM) mm Hg in the normal age-matched control rats (n=3) and 175±9 (SEM) mm Hg in the RRM rats on the high salt diet (n=6).

Microvessel Structure in Normotensive Control Rats (Light Microscopy)

The 1-μm plastic sections of cremaster muscles from normotensive control rats revealed the expected distribution of microvessels. Second- and third-order arterioles and venules were located in a space between an inner and outer thin layer of muscle fibers. Large lymphatic vessels, which were usually collapsed, were generally located within the same region. Several smaller (presumably fourth-order) arterioles and venules were normally present in the sections. These were typically distributed among the muscle fibers, rather than between the two layers of muscle. Numerous capillaries were clearly visible in all the sections.

Microvessel Structure in Normotensive Control Rats (Ultrastructure)

Figure 1 shows electron micrographs of typical microvessels from a normotensive rat. The ultrastructure of microvessels in the normotensive rats was similar to that reported in other microcirculatory beds.10-11 Capillary endothelial cells were normal, with abundant vesicles and extensive junctional processes between cells. Pericytes typically formed a "cap" over part of the endothelial cells.

Structural Changes in Reduced Renal Mass Hypertensive Rats (Light Microscopy)

Similar to the normotensive control rats, 1-μm sections of tissue from RRM hypertensive animals typically had a large arteriole, venule, or both between the two layers of muscle. On a subjective basis, it appeared that 1-μm sections of cremaster muscles from the hypertensive rats had fewer morphologically distinguishable small arterioles and capillaries than those of normotensive control rats. Lymphatic vessels of the hypertensive rats were pronounced due to their distention. Many were enlarged and filled with erythrocytes. Oval masses of extravasated red blood cells were also found in some areas. Some regions of the muscle appeared to be edematous; this condition was indicated by an increased staining of the interstitium with toluidine blue.

Structural Changes in Reduced Renal Mass Hypertensive Rats (Ultrastructure)

Although many normal microvessels were observed in samples taken from the RRM rats, degenerating blood vessels were also observed in each of the hypertensive rats studied (Figures 2 and 3). Some microvessels were so severely degenerated that only portions of the endothelial cells remained and little, if any, smooth muscle could be recognized (Figure 2). In some cases, persisting basement membranes could be used to identify degenerated vessels as arterioles (Figure 3). In those vessels, smooth muscle cells were extremely atrophic, and the endothelium was attenuated and irregular. Vesicles were prevalent in most (but not all) intact endothelial cells associated with degenerating vessels. However, extravascular white cells (suggestive of an inflammatory response) were not found.

Many capillary endothelial cells in the hypertensive animals appeared to be undergoing regressive changes, which were evidenced by the dense heterochromatin in the nuclei and the overall electron density of the cells. However, many of these capillaries had vesicles that were similar to those seen in control rats. Some capillaries of the hypertensive rats were extremely attenuated and irregular in shape and had pyknotic nuclei. Cells were often detached from the basement membrane, and in some areas, basement membranes were amorphous or absent. The pericytes of these vessels appeared to be atrophic and were not closely associated with the capillaries (Figure 4).

In a few samples, extremely thin-walled vessels were observed, which could not be clearly identified as either capillaries, small venules, or small lymphatic vessels (Figure 5). These vessels lacked a discrete basement membrane but contained red blood cells.
FIGURE 1. Electron micrographs showing vessels from cremaster muscles of age-matched control rat. Top panel: Arteriole showing portions of two endothelial cells (E) and closely adjacent smooth muscle cells (M) of normal size. Original magnification, ×21,000. Middle panel: Venule showing portions of three endothelial cells (E) and two closely adjacent layers of thin smooth muscle cells (M). Original magnification, ×9,000. Bottom panel: Longitudinal section of a capillary containing an erythrocyte. Original magnification, ×14,500.
Figure 2. Electron micrographs showing extremely degenerated blood vessels from rats after 4 weeks of reduced renal mass hypertension. Identity of the vessels cannot be determined with certainty. Top panel: Endothelium (E) has degenerated and separated from basement membrane. Smooth muscle (M) is extremely atrophic. Basement membrane is discontinuous in some regions (arrows). Stasis is indicated by packing of vessel lumen with deformed erythrocytes. Original magnification, ×9,000. Bottom panel: Cells are extremely atrophic and are discontinuous along basement membranes. Endothelium and smooth muscle cannot be distinguished from each other. Cellular debris and collagen are present. Basement membrane is amorphous in some regions (arrows). Original magnification, ×21,000.
FIGURE 3. Electron micrographs showing deteriorating arterioles from rats after 4 weeks of reduced renal mass hypertension. Top panel: Medium-sized arteriole has attenuated, irregular endothelial cells (E) that almost entirely lack intact basement membranes. Smooth muscle layer (M) is extremely atrophic and irregular. Basement membranes persist; their presence indicates original smooth muscle compartment. Debris is present between smooth muscle layers. Platelets (P) and red blood cells are found in lumen and within compartments of vessel wall (arrows). Original magnification, ×14,500. Bottom panel: Small arteriole shows discontinuity and irregularity of endothelium (E) and disintegration of basement membranes (arrows). Smooth muscle is extremely attenuated, with partial disintegration of the basement membrane in the outer layer. Lumen contains red blood cells and platelets. Adjacent capillary also has extremely attenuated endothelium. Original magnification, ×15,500.
Discussion

A number of studies have indicated that functional and anatomic rarefaction of microvessels occurs in hypertension. Previous studies have demonstrated that rats with chronic RRM hypertension exhibit about a 15% reduction in total microvessel density relative to normotensive control rats. However, conventional techniques for demonstrating microvessel rarefaction cannot provide insight into the exact structural changes that occur in microvessels during the process of anatomic rarefaction. The main objective of the present study was to use histological techniques to obtain morphological evidence that anatomic rarefaction is occurring in the microcirculation of rats with chronic hypertension.

In these experiments, degenerating vessels were found in samples taken from all the hypertensive rats studied. Pronounced degeneration and regressive changes in both the smooth muscle cells and the endothelial cells of terminal arterioles were observed in two of the rats. Degenerative changes were also observed in the other RRM rats, although these were less widespread and were usually found as isolated cases, rather than as groups of vessels in the same region. Some of the severely degenerated arterioles or lymphatic vessels that we observed in the hypertensive rats contained erythrocytes. The latter vessels could appear quite similar to venules in studies that use intravital microscopy and may account for the presence of "unusually large or prominent venules and veins" that have been reported in some models of hypertension. In contrast to the RRM hypertensive rats, we found no evidence of degenerating vessels in the normotensive control rats.

Although tissue sample sizes for ultrastructural studies are necessarily limited, our results clearly demonstrate that structural deterioration of terminal vessels occurs in rats with RRM hypertension. These observations support previous physiological evidence that anatomic rarefaction exists in the microcirculation of animals with chronic hypertension. However, neither the sequelae of the changes nor the cause of the degeneration can be clearly determined from the present observations.

Many of the ultrastructural changes observed in this study are similar to the degenerative changes that occur after ischemic injury in muscle. However, in ischemically injured muscle, degenerated vessels are rapidly removed by macrophages during the inflammatory stage of the process, and many of the remaining basement membranes become revascular-
ized. In contrast, we noted little evidence of a phagocytic response in the hypertensive rats, even though degenerating blood vessels were present 4 weeks after the surgical treatment that had induced the rapid development of hypertension. Therefore, it is possible that microvascular degeneration occurs too gradually or progresses in a way that is insufficient to induce phagocytosis and a subsequent repair response. This could result in the rarefaction that was documented in the present study.

A structurally based increase in microvascular resistance resulting from anatomic rarefaction would be refractory to vasodilator therapy and could have important implications for the therapeutic management of hypertension and the maintenance of adequate tissue perfusion in hypertensive individuals. Therefore, it is crucial to obtain further insight into the sequelae of vessel degeneration in hypertension, to determine why some vessels are affected but others are not, and to elucidate the functional consequences of anatomic rarefaction in chronic hypertension.

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