Arteriolar Changes in Developing and Chronic Stages of Two-Kidney, One Clip Hypertension

Zenpei Ono, Russell L. Prewitt, and D. Lowell Stacy

Arteriolar internal and external diameters in the cremaster muscle of two-kidney, one clip hypertensive rats (2K1C) were measured in vivo with video microscopy, both before and after the topical application of adenosine (10⁻⁴ M). Arteriolar density was determined by stereologic techniques. Mean arterial blood pressure was significantly elevated in the 2K1C rats, rising to 186±6 mm Hg by 8 weeks compared with 113±4 mm Hg in controls. Lumen of larger arterioles showed a structural reduction at 2 weeks of hypertension and remained at the same level through 8 weeks, while arterioles of control rats showed a progressive increase in diameter with age (101±6 μm in 2K1C vs. 158±8 μm in controls at 8 weeks after operation). Wall-to-lumen ratios of larger arterioles were significantly increased at 2, 4, and 8 weeks of hypertension, but cross-sectional wall area was significantly reduced at 8 weeks. Medial hypertrophy was not evident at any stage of hypertension. Arteriolar rarefaction of smaller arterioles was functional at 2 weeks and structural at 8 weeks of hypertension. Vascular tone of the smaller arterioles was elevated in the developing and chronic stages of hypertension. At 2 weeks of hypertension when the structural reduction in diameters of larger arterioles was progressing, the increased vasoconstriction and functional rarefaction may have contributed to the elevated resistance. At 8 weeks, the marked diameter reductions of larger arterioles (36% in first-order arterioles and 25% in second-order arterioles) account for most of the increased resistance to flow. (Hypertension 1989; 14:36-43)

Arterioles have an important role in elevating vascular resistance in hypertension through increased vasoconstriction, structural alterations, and rarefaction. In two-kidney, one clip hypertensive rats (2K1C), the renin-angiotensin system plays an essential role in developing and maintaining hypertension. Increased activity of the sympathetic nervous system and vascular action of vasopressin are also involved. Therefore, strong vasoconstriction is anticipated in this model of hypertension. In the initial stage of 2K1C hypertension, Meininger et al found small arteriolar tone in the cremaster muscle to be increased, whereas larger arteriolar diameters were unchanged or even enlarged. After 4 weeks of hypertension, larger arteriolar diameters were significantly reduced but small arteriolar diameters were not different from those of control rats. However, these studies did not clarify whether the reduced lumen sizes in the larger arterioles were structural or functional. The purpose of the present study was to investigate the functional and structural changes of all four orders of arterioles of the cremaster muscle, from 2 to 8 weeks of 2K1C hypertension, and to determine if any structural reductions in lumen size are related to vascular wall hypertrophy.

Materials and Methods

Production of Hypertension

Male Wistar rats (Charles River Laboratory, Inc, Wilmington, Massachusetts) were anesthetized with ketamine hydrochloride (60 mg/kg i.p.) and xylazine (10 mg/kg i.p.) at the age of 6-7 weeks. Body weight was 177±3 g in the control group and 177±4 g in the hypertensive group. Through a midline laparotomy, a silver clip with a gap of 0.20 mm was placed on the left renal artery. Control rats were treated in the same manner as the hypertensive rats, except no clip was applied. Penicillin G (25,000 units i.m.) was injected after the surgery. The rats were individually caged, fed Purina rat chow and water ad libitum, and maintained on a 12-hour light/
dark cycle until their use in experiments 2, 4, or 8 weeks later.

Cremaster Preparation for Acute Experiments

Rats were anesthetized with 600 mg/kg i.p. urethane and 120 mg/kg i.p. α-chloralose. This dose was reduced by 20% for both control and hypertensive rats for 8 weeks because of increased sensitivity resulting in bradycardia and hypotension. The trachea was intubated and the left carotid artery was cannulated for the measurement of mean blood pressure and heart rate. The right cremaster muscle was prepared according to the technique described by Baez. During the preparation, warmed Tis-U-Sol (Travenol, Bridgeport, New Jersey) solution with CaCl2 (2.5 mM) was continuously dripped on the muscle. The core temperature of the rat was maintained at 37°C with a heating mat. The exposed cremaster muscle was spread over a Plexiglas pedestal, covered with polyvinyl film, and maintained at 34–35°C with warm circulating water through the base of the pedestal. The preparation was placed on the stage of a Zeiss (Thornwood, New York) microscope equipped with UD20 and UD40 objectives and transilluminated through a long working-distance condenser by a 100-W halogen lamp with a heat absorption filter in the light path. The preparation was allowed to equilibrate 30–60 minutes before microvascular observations were begun. During the equilibration period, cremaster arterioles were classified as first- (1A), second- (2A), third- (3A), and fourth- (4A) order on the basis of their branching pattern. For the in vivo measurement, vessels were selected in the central portion of the muscle because the vessels in the periphery lose tone due to operative insults. Two to five arterioles were selected for 2A, 3A, and 4A measurements. Cremaster arterioles were examined by closed-circuit television microscopy with a Vista Model 308 image splitter (Ramona, California), a Panasonic camera (Secaucus, New Jersey), and a Panasonic Model WV-5470 video monitor. With this equipment, the final magnification on the video monitor was ×1,250, and the resolution with the monitor was x 1,250, and the resolution with the monitor was x 30 in four separate areas of the muscle. The density of the arterioles (mm/mm²) was calculated from 4ITN/2L, where N is the number of intersections and L is the total length of the grid with respect to the microcirculation.

Microfil Preparation

After the intravital microscopic measurements were made, the rats were heparinized (1 unit/g body wt). A midline incision was performed, and the right common iliac artery was cannulated through the abdominal aorta with PE 190 tubing. The cremaster muscle was perfused with saline containing adenosine, verapamil, and sodium nitroprusside (all 10⁻⁴ M) and 58.7 g/l polyvinylpyrolidone (PVP) for several minutes, followed by 10% buffered formalin containing 58.7 g/l PVP for 5–7 minutes at the individual mean blood pressure of the rat. After perfusion fixation, cremaster arterioles were filled with Microfil MV 122 (Boulder, Colorado) through the right common iliac artery catheter. The cremaster preparation was allowed to sit for several hours until the Microfil had polymerized. The muscle was then cleared with increasing concentrations (50%, 75%, 85%, and 100%) of glycerin in water. Arteriolar density was determined by stereologic methods. Briefly, the intersections of the 1As and 2As with an eyepiece grid in a stereo zoom dissecting scope were counted at ×7 power, where the grid covered most of the muscle. The 3As, 4As, and 5As were counted at ×30 in four separate areas of the muscle. The density of the arterioles (mm/mm²) was calculated from πN/2L, where N is the number of intersections and L is the total length of the grid with respect to the microcirculation.

Statistical Analysis

Values are given as mean±SEM. Multiple data were analyzed by two-way analysis of variance (ANOVA). Then, multiple comparisons were made with Duncan’s new multiple range test. Differences were considered statistically significant at p level of <0.05.

Results

Table 1 shows blood pressure, heart rate, and body weight for 2K1C hypertensive rats and their
Table 1. Physiological Data for Control and Two-Kidney, One-Clip Hypertensive Rats at 2, 4, and 8 Weeks After Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Control (n=8)</th>
<th>2K1C (n=8)</th>
<th>Control (n=8)</th>
<th>2K1C (n=8)</th>
<th>Control (n=8)</th>
<th>2K1C (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>98±1</td>
<td>161±5*</td>
<td>96±4</td>
<td>162±8*</td>
<td>113±4</td>
<td>186±6*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>388±9</td>
<td>402±10</td>
<td>379±13</td>
<td>391±20</td>
<td>371±10</td>
<td>401±8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>263±8</td>
<td>266±9</td>
<td>308±5</td>
<td>316±12</td>
<td>441±16</td>
<td>372±9*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. 2K1C, two-kidney, one-clip hypertension.
*Different from control rats, p<0.05.

Hypertension was significantly elevated in hypertensive rats at all three stages of hypertension; heart rate was not different between the two groups, and body weight was significantly decreased in hypertensive rats at 8 weeks of hypertension. Figure 1 shows resting inside diameters of each arteriolar order in 2K1C and control rats. Inside diameters in hypertensive rats were significantly smaller than those in control rats except for 4As at 2 and 8 weeks. Figure 2 shows maximally dilated diameters of each arteriolar order. 1As in hypertensive rats were significantly smaller at all three stages of hypertension. 2As in hypertensive rats were significantly smaller at 4 and 8 weeks, whereas 3As and 4As were not different at any stage of hypertension. In summary, the structural reductions of 1A diameters occurred within 2 weeks of hypertension, followed by reductions of 2As between 2 and 4 weeks.

Figure 3 shows arteriolar tone of each order. All 3As and 4As in hypertensive rats showed higher tone, except 4As at 8 weeks. No significant differences were found among larger arterioles, although 2As tended to show higher tone in hypertensive rats than in control rats. Wall-to-lumen ratios (Figure 4) of 1As in 2K1C hypertensive rats were significantly higher at all three stages of hypertension than in their respective controls. These increases were not accompanied by concomitant increases in cross-sectional wall area. Rather, cross-sectional wall areas (Figure 5) of 1As and 2As in hypertensive rats were significantly less at 8 weeks than in control rats. Neither wall-to-lumen ratios nor cross-sectional wall areas of 3As and 4As showed any difference between hypertensive and normotensive rats at any stage of hypertension. Therefore, structural reductions of diameters were confined to the larger arterioles and were observed without medial hypertrophy.

Figure 6 shows the number of 4As on a 3A before and after the application of 10^{-4} M adenosine. The number of 4As opened to flow was significantly lower in 2K1C hypertensive rats than in control rats before the application of adenosine at all three stages. The number of 4As after the application of adenosine was not significantly different in 2K1C hypertensive rats and control rats at 2 weeks and 4 weeks. However, at 8 weeks, the number of 4As after the application of adenosine was mildly but significantly reduced in 2K1C hypertensive rats (2K1C, 6.5±0.4; control, 8.3±0.3; p<0.05). Figure 7 shows the arteriolar density for 1As and 2As and 3As and 4As in 2K1C and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Comparisons are by two-way analysis of variance followed by Duncan's new multiple range test.
for 3As, 4As, and 5As as length per unit area of cremaster muscle at the three stages after renal artery clipping. Arteriolar density for 1As and 2As was not different at any stage. Arteriolar density for 3As, 4As, and 5As was not different at either 2 (2K1C, 2.60±0.08 mm/mm²; control, 2.69±0.15 mm/mm²) or 4 weeks (2K1C, 2.53±0.08 mm/mm²; control, 2.97±0.17 mm/mm²). However, arteriolar density was significantly different at 8 weeks (2K1C, 2.31±0.14 mm/mm²; control, 3.04±0.18 mm/mm²).

FIGURE 2. Bar charts of relaxed inside diameters of first- through fourth-order arterioles after topical applications of adenosine $10^{-4} M$ in the cremaster muscle of two-kidney, one clip (2K1C) and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Comparisons are by two-way analysis of variance followed by Duncan’s new multiple range test.

FIGURE 3. Bar charts of arteriolar tone of first- through fourth-order arterioles in cremaster muscle of two-kidney, one clip (2K1C) and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Tone is expressed as 100×(relaxed inside diameter—resting inside diameter)/relaxed inside diameter. Comparisons are by two-way analysis of variance followed by Duncan’s new multiple range test.

Discussion

The major findings in the present study are 1) increased tone of smaller arterioles in 2K1C hypertensive rats throughout 8 weeks of hypertension, 2) structural reductions of large arteriolar diameters without medial hypertrophy, and 3) mild arteriolar rarefaction that was functional early in hypertension and structural at the later stages. As Laragh et al11 proposed, strong vasoconstriction is anticipated in 2K1C Goldblatt hypertensive rats. However, this enhanced vasoconstriction is not necessarily observed along the entire vascular bed. Meininger et al12 showed that tone in 3As and 4As increased in the first 3 hours of 2K1C hypertension, whereas 2A diameters were unchanged and 1A diameters increased. As shown in Figure 3, tone remained elevated in the small arterioles at 2 weeks and was never elevated in the larger arterioles. Tone in 3As remained elevated at 8 weeks of 2K1C hypertension, whereas in a previous study of 1K1C hypertension, tone in 3As and 4As decreased to control levels at 8 weeks.16 Functional rarefaction, the increased number of closed arterioles in the 2K1C rats (Figure 6), is also an indication of enhanced tone. Again, functional rarefaction was significant early, but at 8 weeks only structural rarefaction remained.

Several mechanisms may lead to the elevation of vascular tone in hypertension: autoregulatory mechanisms, increased neurohumoral factors, and increased reactivity to vasoconstrictors. In addition, the mechanisms responsible for increased tone appear to be different at different stages of hypertension. Autoregulatory mechanisms are important in the initial stage of hypertension, but with time they adapt to higher arterial pressure and cease enhancing vascular tone. The renal renin-angiotensin system plays an essential role, particu-
larly at the early stage of 2K1C hypertension—mainly because of its direct vasoconstrictor effect. Increased vascular renin-angiotensin activity may have some role in the increased tone at the later stage of 2K1C hypertension when plasma renin activity is no longer elevated. Vasopressin contributes to the elevated blood pressure in 2K1C hypertension because of its vasoconstrictor effect. Sympathetic nervous activity is increased through central and peripheral mechanisms. Impairment of endothelium-dependent relaxation may also be involved in the increased vascular tone.

The present experiments were carried out using chloralose-urethane anesthetics. Joshua et al. reported that arterioles exhibited little tone in both hypertensive and normotensive rats and suggest that much of the active tone of arterioles is abolished by chloralose-urethane anesthesia; this was not the case in the present experiments. Small arterioles exhibited good tone in both normotensive and hypertensive rats, which may be due to differences in rat species (Sprague-Dawley vs. Wistar), experimental models (1K1C vs. 2K1C), stages used (tissue bath vs. covered preparation), or doses of anesthetics (600 mg/kg urethane and 120 mg/kg α-chloralose in the present experiments vs. 800 and 60 mg/kg, respectively, in those of Joshua et al). Opposite to the distribution of vascular tone, structural reductions of diameters were observed only in the larger arterioles (Figure 2). The reduction of diameters first occurred in 1As within 2 weeks and then in 2As between 2 and 4 weeks; this progression of structural reduction of arteriolar diameters from 1A to 2A is in accordance with previous studies on 1K1C rats. As shown in Figure 2, diameters of 1As in 2K1C rats remained at

**FIGURE 4.** Bar charts of wall-to-lumen (W/L) ratios of first- through fourth-order arterioles in cremaster muscle of two-kidney, one clip (2K1C) and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Comparisons are by two-way analysis of variance followed by Duncan's new multiple range test.

**FIGURE 5.** Bar charts of cross-sectional wall areas (CSWA) of first- through fourth-order arterioles in cremaster muscle of two-kidney, one clip (2K1C) and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Comparisons are by two-way analysis of variance followed by Duncan's new multiple range test.
Ono et al  Microvascular Changes in Renovascular Hypertension 41

Figure 6. Plot of the number of fourth-order (4A) arterioles on a third-order (3A) arteriole before and after topical application of 10^(-4) M adenosine in two-kidney, one clip (2K1C) and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Comparisons are by two-way analysis of variance followed by Duncan’s new multiple range test.

the same level throughout, but the difference in diameters does not seem to be due to a mere failure of growth. Relaxed diameters of 1As are ~130 μm at 6-7 weeks (the age when clips are put on renal arteries) and 106 μm at 1 week of hypertension (unpublished observations). Therefore, structural diameter reductions occur early after renal artery stenosis.

Although the reductions of diameters were accompanied by increased wall-to-lumen ratios in 1As of hypertensive rats, cross-sectional wall areas of 1As and 2As were never increased and were significantly reduced at 8 weeks of hypertension. According to Folkow et al,3 increased wall tension is the stimulus for vascular medial hypertrophy that encroaches on the lumen, with the possible assistance of neurohumoral factors.28,29 Medial hypertrophy in hypertension is well documented in aorta or other conduit arteries.28-32 These arteries have little tone and are fully exposed to the hypertensive pressures, so an increase in wall thickness is necessary to keep wall stress constant.

In the microcirculation, however, the results vary with the model of hypertension. Harper33 showed vascular hypertrophy in the cerebral microcirculation of spontaneously hypertensive rats (SHR) and renovascular hypertensive rats. Bohlen et al34 showed no vascular hypertrophy or lumen reduction in skeletal muscle microcirculation in SHR. Hashimoto et al35 reported no significant increase in cross-sectional wall area in cremaster muscle microcirculation of 1K1C hypertensive rats.

Because neurohumoral factors are elevated in the 2K1C hypertensive rats, a marked vascular hypertrophy in the microcirculation would be expected. In the initial stage of 2K1C hypertension, constriction of the smaller arterioles increases blood pressure, whereas the diameter of larger arterioles remains the same or increases.12 Although micro-pressure data are lacking, one can expect that wall tension in the smaller arterioles is not increased because of their strong vasoconstriction, whereas tension must be increased in the larger arterioles. As to the neurohumoral factors, the distribution of receptors or innervation density should not be a problem. The recent study by Fleming et al35 showed that there are angiotensin II receptors on larger arterioles of rat cremaster muscle. Sympathetic innervation is dense in the small arteries and larger arterioles,36 and α- and β-adrenergic receptors are found in larger arterioles.37 The α-adrenergic receptor is involved in hypertrophy of cultured neonatal rat heart cells.38 The increase in tension along with

Figure 7. Bar chart of arteriolar density of cremaster muscle of two-kidney, one clip (2K1C) and control rats at 2, 4, and 8 weeks (W) after renal artery stenosis. Vessels were perfusion fixed in the dilated state and filled with Microfil. Comparisons are by two-way analysis of variance followed by Duncan’s new multiple range test.
the increased neurohumoral factors should cause vascular hypertrophy in larger arterioles.

However, as shown above, hypertrophy did not occur in the microvasculature. Therefore, other factors must also be involved in the alteration of the arteriolar wall in hypertension. At present, the factors are unknown but some speculation is possible. One possible mechanism is the relation between blood flow and vascular diameter. A reduction in flow can cause a decrease in the internal diameter of the rabbit carotid artery as early as 24 hours and a reduction in wall thickness within 1 month. Enhanced vasoconstriction and functional rarefaction in smaller arterioles could reduce blood flow to the cremaster muscle in the earlier stage of 2K1C hypertension. If this happens, the lumen could be structurally reduced by the same mechanism present in the rabbit carotid artery and the wall tension would be normalized; the net effect is the reduction of diameters without hypertrophy. Regardless, the exact mechanism for a reduction in diameter without vascular hypertrophy remains to be clarified.

As shown in Figure 7, mild rarefaction was observed to be functional at 2 weeks and structural at 8 weeks of hypertension. This reduction of vascular density occurred only in the smaller arterioles (3As, 4As, and 5As), as shown in Figure 6. The degree of rarefaction was mild compared with that found in 1K1C hypertensive rats but close to that observed in coarctation hypertensive rats. Rarefaction was reported in skeletal muscle of SHR and 1K1C renovascular hypertensive rats. The mechanisms proposed as a cause of rarefaction are intense neurohumoral vasoconstriction imposed on the resistance vessels or a long-term structural autoregulation. Pressures in 2As and 3As of 2K1C renovascular hypertensive rats are not different from those in normotensive rats at 4 weeks of hypertension. Marked reduction in diameters of the upstream arterioles protect the downstream vessels from elevated luminal pressure; this may mean that rarefaction happens to some extent because of neurohumoral factors in 2K1C hypertensive rats.

Increased vasoconstriction and functional rarefaction may contribute to the elevated resistance of the cremaster muscle microcirculation during the early stages of 2K1C Goldblatt hypertension, while the structural reductions in diameter of larger arterioles are progressing. However, at 8 weeks, the marked diameter reductions of larger arterioles account for most of the increased resistance to flow. The diameter reductions are not accompanied by medial hypertrophy, and the mechanism for these reductions remains to be clarified.

Acknowledgments
We thank Clark Effaw for his excellent technical help and Ms. Shanna Richardson for her expert secretarial skills.

References
40. Langille BL, O'Donnell F: Role of endothelium in flow-induced changes in arterial diameter (abstract). Fed Proc 1984;43:973

KEY WORDS • microcirculation • renovascular hypertension • hypertrophy • vasoconstriction • rarefaction
Arteriolar changes in developing and chronic stages of two-kidney, one clip hypertension.
Z Ono, R L Prewitt and D L Stacy

Hypertension. 1989;14:36-43
doi: 10.1161/01.HYP.14.1.36
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/14/1/36