Progressive Microvascular Alterations with the Development of Renovascular Hypertension

Irving G. Joshua, Ph.D., David L. Wiegem, Ph.D., Patrick D. Harris, Ph.D., and Frederick N. Miller, Ph.D.

SUMMARY  Norepinephrine-induced changes in diameters of first- (1A), second- (2A), and third-order (3A) arterioles in the exposed cremaster muscles of normotensive and renovascular hypertensive rats were quantitated via television microscopy. By 2 weeks following the surgery to induce hypertension, we found that 3A sensitivity to norepinephrine had increased and the 1As had chronically constricted. By 4 weeks, the constriction had progressed to include both 1A and 2A. Sensitivity was no longer increased in 3As and, in fact, sensitivity had decreased in 1As and 2As. The 1As and 2As could not be dilated with isoproterenol or nitroprusside; thus, the vessels appeared to have undergone a structural alteration. We suggest from these results that the early increased 3A sensitivity contributes to the initial development of hypertension. The larger arterioles then constrict to protect the downstream vessels from increased luminal pressure. As the hypertension develops, the constriction progresses to smaller arterioles in an attempt to maintain normal pressure in the capillaries (site of water exchange). The constricted arterioles contribute to increased total peripheral resistance, and with the constriction, there occurs a general decrease in vessel responsiveness.

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KEY WORDS  • microcirculation • arteriole • norepinephrine sensitivity • rats

We have reported that first-order cremasteric arterioles in one-kidney, one clip hypertensive rats have smaller diameters than comparable vessels in normotensive rats. These studies were done 4 weeks after the surgery to produce the hypertension. The decreased arteriole diameter could contribute to an increase in total peripheral resistance and, thus, to the elevation in arterial blood pressure. Others have, in fact, proposed that hypertrophy of the arteriolar smooth muscle with hypertension could result in reduced luminal diameters and, thus, could explain the increased total peripheral resistance. Still other workers have proposed that the increased total peripheral resistance is due to an increase in vascular reactivity to vasoconstrictor stimuli. For instance, Click et al., using the check pouch preparation in Grollman hypertensive hamsters, noted an increase in the sensitivity of small arterioles (30-50 μm) to both norepinephrine and angiotensin. In our previous study, however, we found a decrease, not an increase, in the first-order (90-110 μm) arteriole sensitivity to norepinephrine.

In this study, we observed microvascular responses of arterioles in the rat cremaster during both early and established stages of one-kidney, one clip Goldblatt hypertension. We undertook this study to determine if the development of renovascular hypertension is characterized by an early increase in arteriolar responsiveness. In addition, we attempted to determine if the reductions in microvascular diameters and vasoconstrictor responsiveness observed during an established stage of this disease are associated with a structural alteration in these vessels.

Methods

Animal Model

Male Sprague-Dawley rats were used as normotensive control (NT) animals and also for the surgical production of one-kidney, one clip (1K1C) Goldblatt hypertension. Our surgical procedure involved unilateral nephrectomy and contralateral renal artery restriction (outside diameter, 260 μm) at 3 to 4 weeks of age. These rats received a saline drinking solution (250 mM NaCl and 10 mM KCl) ad libitum beginning the day after surgery. The NT rats had no surgery and were given tap water to drink. At either 2 or 4 weeks after surgery, the mean arterial blood pressure of the 1K1C hypertensive rats was measured through a cannula in
the tail artery while the rats were lightly anesthetized with ether. Animals with a mean tail artery pressure greater than 110 mm Hg at 2 weeks or greater than 140 mm Hg at 4 weeks were considered hypertensive and were used in subsequent studies of the cremaster microvasculature. Approximately 50% of the rats survived our surgical procedure, and about 50% of these survivors demonstrated hypertension at the time of tail artery cannulation.

Experimental Preparation

Observations of the microcirculation of the cremaster muscle of hypertensive rats were made 2 to 3 days after tail artery cannulation. Normotensive rats were studied at the same ages. Animals (weight range of 140–206 g) were anesthetized with an intraperitoneal injection of urethane (800 mg/kg) and alpha-chloralose (60 mg/kg). The left femoral artery was cannulated to measure blood pressure, and heart rate was monitored via an electrocardiogram. Rectal temperature was maintained at 36°C by a heating pad placed under the animal.

In our preparation of the rat cremaster,10 the right testicle was exposed by an incision in the scrotum, and the cremaster muscle surrounding the testicle was incised ventrally. The cremaster, which still had intact innervation and circulation, was spread in a flat sheet with sutures over an optical port in a 60 ml tissue bath, which was filled with a modified Krebs solution. This Krebs solution contained 113 mM NaCl, 11.6 mM dextrose, 4.7 mM KCl, 1.2 mM MgSO4·7H2O, 1.2 mM KH2PO4, 2.6 mM CaCl2·2H2O, and 25 mM NaHCO3. The entire preparation was moved to the stage of a transilluminated compound microscope for observation. An image of the cremaster was displayed on a television monitor at approximately x 1500 magnification. In addition, the image was recorded on videotape for subsequent analysis. Bath PO2 (30–40 mm Hg), PCO2 (35–45 mm Hg), and pH (7.40 ± 0.05) were controlled by bubbling carbon dioxide and nitrogen gases through the cremaster bath. Bath pH was continuously monitored via an indwelling pH electrode, and bath gases were measured using an IL 213 Blood Gas Analyzer (Instrumentations Laboratory, Lexington, Massachusetts). Bath temperature was maintained at 34.5°C.

Before the start of the drug response studies, arterioles were identified on the basis of their branching pattern (Figure 1). First- (1A), second- (2A), and third-order (3A) arterioles were used in each experiment. Resting diameters were recorded for the main arteriole (1A) in the cremaster and for a second-order arteriole (2A) that branched from 1A. The 2A that supplied the anterolateral quarter of the right cremaster was selected for observation of the drug responses. It is our experience that this 2A is present in approximately the same position in over 90% of our cremaster preparations. The diameters were recorded for all of the arterioles (3A) that branched from this selected 2A, and the 3A with a diameter that was nearest the mean was selected for observation of the drug responses.

Experimental Protocol

In the first series of experiments, we obtained concentration-response curves for the effects of norepinephrine bitartrate (Sigma Chemical Company, St. Louis, Missouri) on the 1A arteriole and the selected 2A and 3A arterioles. In these studies, we used 1K1C hypertensive rats (n = 20) at either 2 or 4 weeks after induction of hypertension and age-matched NT rats (n = 16). We measured vessel diameters for all three vessels from the television monitor at 1-minute intervals throughout the experiment.

Figure 2 gives the protocol and a typical response for one arteriole in one norepinephrine experiment. Each experiment began with a 10-minute control period. The first dose of norepinephrine was then added to the cremaster bath to give a concentration of 10⁻⁴ M in the bath. After a 10-minute period for the measurement of the response to norepinephrine (change in vessel diameter), the bath was quickly drained and refilled with fresh Krebs solution and a higher concentration of norepinephrine. Earlier studies10 have demonstrated that a maximal response to any one concentration of norepinephrine occurs within 10 minutes. This procedure was continued for 10 to 12 concentrations of norepinephrine until a maximal response (that is, a minimal vessel diameter) was obtained for each of the observed arterioles. Responses to each dose of norepinephrine were obtained simultaneously for the three arteriolar levels in each animal.
The raw data for arteriolar diameters were smoothed with a three-point digital computer filter and were normalized by expressing each data point as a percentage of the average value during the control period that preceded the application of norepinephrine. The maximal responses for each dose were used to construct concentration response curves. The concentration (ED50) that produced 50% of the maximal constriction was graphically determined for each of the three arterioles in each animal experiment. These values were converted to pD2 values (pD2 = −log ED50) as an indication of sensitivity of the vessels to norepinephrine.

In the second series of studies, we observed the microvascular response to the beta-adrenergic receptor agonist isoproterenol (10⁻³ to 10⁻⁴ M) and to the non-specific vasodilator sodium nitroprusside (10⁻⁶ to 10⁻³ M). Responses to each drug for the three arteriolar levels (1A, 2A, and 3A) were recorded for a group of eight hypertensive rats at 4 weeks after surgery and for a group of six age-matched normotensive rats. A protocol similar to that described above for norepinephrine (a 10-minute control period followed by a series of 10-minute response periods) was used in these studies. The first concentration-response curve was followed by a 30-minute recovery period, with frequent bath changes of fresh Krebs solution to allow the arterioles to return to control diameters before the second curve was obtained.

In a final series of studies, six NT rats (263 ± 5 g, body weight) and five 1K1C hypertensive rats (257 ± 6 g, body weight) at 5 to 6 weeks after surgical induction of hypertension were sacrificed with an overdose of ether. The thoracic aorta (from the distal end of the aortic arch to the diaphragm) was measured in situ and removed. The aortic segments were incised longitudinally, blotted with tissue paper, and weighed (wet weight) to the nearest 0.01 mg. The heart and both adrenal glands were also removed, cleaned of connective tissue, and weighed. The tissues were oven-dried at 100°C for 24 hours and reweighed (dry weight). The difference between the "wet" and "dry" weights was considered to be water content and was expressed as a percentage of the tissue wet weight.

### Statistical Analysis

Group comparison t tests were used for comparison between hypertensive and normotensive data groups. Within-group comparisons were made using paired t tests. A statistical significance level of 0.05 was used for all tests. Data are presented as means ± standard error of the mean (X ± SEM).

#### Results

Table 1 shows that the mean arterial blood pressure during urethane-chloralose anesthesia was significantly higher for renovascular 1K1C hypertensive rats at the 2-week and 4-week stages of hypertension than for age-matched NT Sprague-Dawley control rats. Heart rate of the 1K1C rats at the 2-week and 4-week stages of hypertension was not significantly different (p < 0.05) from that of the age-matched NT rats.

Figure 3 illustrates the control lumenal diameters of 1A, 2A, and 3A arterioles of normotensive and hypertensive rats. Observations made at 2 weeks after hypertensive surgery indicate a significant reduction (24%) in control diameters of the 1A arteriole of 1K1C (85 ± 6 μm) compared to the 1A of NT (112 ± 8 μm). A comparison of diameters of the 2A and 3A arterioles for these groups showed no significant differences. At 4 weeks after surgery, 1A and 2A diameters of 1K1C arterioles were significantly smaller (36% and 22% respectively) than comparable vessels of NT. Diameters of 3As were not significantly different between groups. A comparison of control arteriolar diameter of NT at times corresponding to 2 and 4 weeks after hypertensive surgery indicated no significant differ-

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**TABLE 1. Blood Pressure and Heart Rate for Normotensive (NT) and One-Kidney, One Clip (1K1C) Hypertensive Rats at 2 and 4 Weeks after Surgical Induction of Goldblatt Hypertension**

<table>
<thead>
<tr>
<th></th>
<th>2 weeks postoperative</th>
<th>4 weeks postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT</td>
<td>1K1C</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(10)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>100±3</td>
<td>118±5*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>400±21</td>
<td>347±17</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Numbers in parentheses indicate the number of animals in each group. *p < 0.05.
Figure 3. Control diameters for arterioles of normotensive rats (open bars) and hypertensive rats (dotted bars) at 2 weeks (upper panel) and 4 weeks (lower panel) after surgery to induce hypertension. Values are means ± SEM. Numbers in parentheses indicate the number of arterioles observed for each vessel level. An asterisk indicates that the value for the hypertension group is significantly different (p < 0.05) from the value for its control normotensive group.

Figure 4. Maximal reduction in diameters of arterioles to high doses of norepinephrine expressed as a percentage of control diameters. The percentage of constriction (± SEM) is presented for normotensive rats (open bars) and hypertensive rats (dotted bars) at 2 and 4 weeks after surgery to induce hypertension. Numbers in parentheses indicate the number of arterioles observed for each vessel level.

Table 2. Water Content and Dry Weight of Aorta, Heart, and Adrenal Gland from One-Kidney, One Clip (1K1C) Goldblatt Hypertensive and Normotensive Rats

<table>
<thead>
<tr>
<th></th>
<th>Water content (%)</th>
<th>Dry tissue weight (mg/cm)</th>
<th>Dry tissue weight (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aorta</td>
<td>Heart</td>
<td>Adrenals</td>
</tr>
<tr>
<td>Normotensive (6)</td>
<td>61.5 ± 0.7</td>
<td>77.7 ± 0.2</td>
<td>68.6 ± 0.6</td>
</tr>
<tr>
<td>Hypertensive (5)</td>
<td>67.2 ± 0.5*</td>
<td>78.2 ± 0.2</td>
<td>69.0 ± 1.0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. Numbers in parentheses indicate the number of animals in each group. Aorta dry tissue weights are expressed as milligrams of tissue per centimeter of in situ tissue length. Heart and adrenal dry tissue weights are expressed as milligrams of tissue per kilogram of body weight.

*p < 0.05.
tions of nitroprusside and isoproterenol, for 1K1C (4 weeks) and age-matched NT. Figure 6 illustrates the maximal responses of the three levels of arterioles to nitroprusside and isoproterenol. These vasodilators caused very little dilation at the three arteriolar levels in 1K1C and age-matched NT rats. Thus, the 1A and 2A diameters for 1K1C continued to be significantly reduced in comparison to those of NT. Third-order arteriole diameters were not significantly different between 1K1C and NT rats even after the application of isoproterenol and nitroprusside.

Data for water content and dry weight for thoracic aortic segments, hearts, and adrenal glands are summarized in Table 2 for 1K1C (5-6 weeks) and age-matched NT rats. The water content of 1K1C thoracic aortic segments was increased in comparison to that of NT aortas, while the water content for hearts and adrenals of 1K1C was similar to the water content for the hearts and adrenals of NT. Dry tissue weights of all three tissues (aorta, heart, adrenal glands) were significantly elevated for hypertensive rats in comparison to normotensive rats.

**Discussion**

This study was designed to quantitate changes in microvascular diameters and reactivity to norepinephrine during the development of 1K1C hypertension. In general, we interpret the data from this study to indicate that an increase in the sensitivity of small arterioles (<50 µm) to endogenous vasoconstrictors could contribute to an initial increase in total peripheral resistance in this form of hypertension. As the hypertension progresses, there is a structural reduction in luminal diameters of larger arterioles. We hypothesize that these diameter reductions act to protect the downstream vessels from the increased systemic pressure.
but, at the same time, the larger arterioles actually contribute to the maintenance of increased peripheral resistance during the established phase of hypertension.

In the current study, an increased norepinephrine responsiveness was exhibited by the third-order arterioles during the early stage of renovascular hypertension (2 weeks post surgery). Collis and Alps,\textsuperscript{13} using a perfused mesentery preparation, have shown that mesentery arteries are also supersensitive to norepinephrine during an early stage of hypertension (2–3 weeks after induction of a Grollman form of hypertension) when no structural changes appear to have taken place. Click et al.\textsuperscript{8, 9} have observed the small arterioles (30–50 μm) in the microcirculation of the hamster cheek pouch during Grollman hypertension. Their studies indicated that there is an increased reactivity to norepinephrine and angiotensin in small arterioles of hamsters in an early stage (4–14 days post surgery) of hypertension development. Recently, Bohlen et al.\textsuperscript{14} have noted that denervation of the cremaster of SHR caused a marked increase (62%) in the number of third-order arterioles open to flow in comparison to the increase (22%) noted for arterioles of Wistar control rats. He has also postulated that these vessels that were closed to flow during the innervated state were hypersensitive to norepinephrine. Studies by Prewitt et al.\textsuperscript{15} also show an increase in the number of small arterioles that are actively closed to flow in one-kidney, one clip hypertension. At present, it appears that the major site of this early hyperresponsiveness in the microcirculation may be limited to arterioles with luminal diameters of 50 μm or less. This hyperresponsiveness could be an important contributor to the initial increase in total peripheral resistance which is seen with hypertension.

The reduction in arteriolar diameters appears to increase with the duration of hypertension and to progress from larger to smaller vessels. At 2 weeks after surgery, luminal diameters of first-order arterioles were decreased by approximately 24%, and at 4 weeks, 1A diameters were reduced by 36%. In addition, at 4 weeks, 2A diameters were reduced by 22%. Thus, the participation of these larger arterioles in the maintenance of an increased peripheral resistance increases markedly with duration of the disease. In fact, in this microcirculatory preparation, the early (2 weeks) increase in the norepinephrine sensitivity of third-order arterioles (< 50 μm) was not present during the later stage of hypertension (Figure 5). This suggests that an increased arteriolar sensitivity is not involved in the maintenance of increased peripheral resistance during the latter stages of this form of hypertension.

Arterioles of normotensive and hypertensive rats observed in our study exhibited very little resting vascular tone, as indicated by the response to isoproterenol and nitroprusside. Recent studies in our laboratory, utilizing a decerebrate rat preparation, suggest that much of the active tone for arterioles is abolished by urethane-chloralose anesthesia. Several other studies have indicated that various anesthetics have a predominantly depressant effect on vascular tone in the microcirculation.\textsuperscript{17, 18} Previous studies by us\textsuperscript{19} and others\textsuperscript{20} have demonstrated that a portion of the third-order arterioles in the rat cremaster has some degree of vascular tone remaining during urethane-chloralose anesthesia. In any population of third-order arterioles, these vessels tend to have the smaller diameters. Since our selection criteria involved choosing third-order arterioles with diameters nearest the overall mean, most of the 3As with substantial tone were not selected for our study.

Our data clearly indicate that arterioles of hypertensive rats in this study are significantly smaller than for normotensive rats (Figure 3). Increases in vascular sensitivity to endogenous constrictor agents and increased sympathetic activity of peripheral nerves have both been proposed as possible mechanisms of increased peripheral resistance with hypertension. Both of these mechanisms could have produced an active constriction in arterioles of hypertension rats to account for the significant reduction in vascular diameters. The absence of dilation for the arterioles of hypertensive rats (Figure 6) discounts the involvement of these two mechanisms and strongly indicates that the observed decrease in vascular diameters of hypertensive rats is due to a structural alteration. Data from in vitro studies with larger vessels and perfusion studies with renovascular forms of hypertension\textsuperscript{21, 22} have also suggested that vascular wall thickening at the arteriolar level of the circulation is involved in peripheral resistance changes during hypertension. In addition, Folkow\textsuperscript{2} has postulated that thickening of the vascular wall encroaches on the lumen of the arteriole. Due to marked striations and thickness of the cremaster tissue in our preparation, we were unable to make in vivo wall thickness measurements.

Our data from the aortas of 1K1C rats in this study indicate that the water content of vascular tissue increases in this renovascular hypertension model. This abnormality in water composition could reflect underlying defects in ion metabolism in vascular smooth muscle.\textsuperscript{21, 22} However, the observed increase in the dry weight of the aortas in our study suggests that there is either an increase in nonelastic components (e.g., collagen) of the vascular wall\textsuperscript{23} or smooth muscle hypertrophy.\textsuperscript{24} If we assume that the arteriolar walls were hypertrophied in our study and that this was due to an increase in vascular smooth muscle mass, one might expect to see an increase in reactivity to norepinephrine in these arterioles. In contrast, those vessels that exhibited decreased luminal diameters in the presence of vasodilators during the late stage of hypertension (1A and 2A arterioles) showed decreased reactivity to norepinephrine (Figure 5), suggesting an increase in nonelastic components in the arteriolar wall. Our earlier microvascular studies with this hypertension model have also shown a similar attenuation in the responses of first-order arterioles to elevations in extra-cellular calcium.\textsuperscript{23} Collectively, these data suggest that there is a general loss of vascular reactivity in the
larger arterioles of the microcirculation, possibly due to vascular wall thickening.

It is generally believed that structural changes associated with hypertension constitute a secondary vascular response to a pressure-induced increase in wall tension. Recent studies in our laboratory by Meininger et al. tend to support the hypothesis that increases in intraluminal pressures are responsible for initiating and/or propagating the observed reductions in arteriolar diameters. We found that micropressures in the first-order arterioles of 1K1C rats (4 weeks after surgery) were elevated at a time when the lumenal diameters of these vessels were reduced. However, the pressures in smaller arterioles and venule were normal.

In a deoxycorticosterone acetate-salt (DOCA-salt) model of hypertension, we found a significant increase in intraluminal pressure in first-, second-, and third-order arterioles and a corresponding decrease in the diameters of these vessels. These results, along with the data in our present work, suggest to us that the reduction of the lumenal diameters of upstream arterioles is a microvascular defense mechanism that acts to protect downstream exchange vessels from the increased pressures associated with hypertension. We also speculate that, with progressive increases in systemic pressure with the continued development of the hypertension, there is progressive involvement of smaller arterioles to maintain a normal microvascular pressure at the level of the exchange vessels. In some situations, this process could continue to progress through the smallest arterioles until the increased pressure finally reaches the capillaries. When this occurs, there would be little microvascular control of filtration at the capillary level and we might expect to see a relatively sudden onset of tissue edema, which is sometimes characteristic of severe hypertension.

In conclusion, the alterations that we have observed in this renovascular hypertensive model may not indicate changes that occur in the microcirculation of all experimental and human forms of this disease. Indeed, the structural alterations observed in the microcirculation of the spontaneously hypertensive rat appear to be quite different from those observed in our study. Our hypertensive model involved removal of one kidney, restriction of blood flow to the remaining kidney, and increased salt intake. Our observations from this hypertensive model probably represent structural and functional responses of the microcirculation to elevations in intraluminal pressures. These microvascular responses could, however, reflect one or more of the other factors that we employed to induce the hypertension.

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