Small Artery Resistance Increases During the Development of Renal Hypertension

John D. Imig and Gary L. Anderson

Vascular pressures were measured in the principal (A1) arteriole and in upstream small arteries of the rat cremaster muscle to investigate vascular resistance changes associated with one-kidney, one clip Goldblatt hypertension. Pressure measurements were made at a proximal and distal site of each vessel using a servonull micropipette system. Vessel diameters were measured using video microscopy. A1 arteriole and external spermatic artery diameters were both decreased after 2 and 4 weeks of hypertension. Mean arterial pressure was elevated after 2 weeks of hypertension (106±4 mm Hg versus 140±5 mm Hg). Likewise, vascular pressures were elevated at every site: pudic-epigastric artery (36%), external spermatic artery (47%), and A1 arteriole (38%). The pressure drop along the external spermatic artery was increased (87%) after 2 weeks of hypertension. Mean arterial pressure was further elevated from 2-4 weeks of hypertension (106±4 mm Hg versus 162±7 mm Hg) but only the proximal pudic-epigastric artery pressure was further elevated during this time from 2 to 4 weeks (131±5 mm Hg versus 147±7 mm Hg) of hypertension development. This was associated with an increased pressure drop (87%) along the artery compared with the situation at 2 weeks. These data indicate that small arteries upstream from the microcirculation contribute significantly to the increase in vascular resistance during hypertension. In addition, these data indicate that the increases in small artery resistance do not develop uniformly throughout all vessel branches.

R enovascular hypertension is the result of a progressive increase in total peripheral resistance.1 Microvascular alterations in one-kidney, one clip (1K1C) hypertensive rats appear to occur in at least two distinct stages. There is an early stage characterized by vasoconstriction of larger arterioles2 followed by a later stage where large arteriole vasoconstriction is replaced by structural alterations.3 Although microcirculatory alterations contribute to the increase of organ vascular resistance during hypertension, pressure measurements made in the cremaster muscle,4 as well as other vascular beds,5-8 indicate that as much as 30–50% of the increase in organ vascular resistance occurs in the large arterioles and small arteries proximal to the true microcirculation. Borders and Granger,9 through calculations of power dissipation by microvessels in the cremaster muscle, concluded that the large arterioles had a greater effect on increased microcirculatory power dissipation than any other microvascular segment during hypertension. The vascular resistance change that occurs during hypertension in the small arteries that lie between the iliac artery and the cremaster muscle microcirculation is not known. Therefore, the objective of the present study was to assess the contribution of the large feeder arteriole (A1) and the upstream small arteries to the increased pressure drop across the cremaster muscle vasculature after 2 and 4 weeks of 1K1C hypertension. Vascular pressures at various sites were measured with a servonull pressure system, and vascular diameters were measured by using video microscopy. The major finding is that small arteries do contribute significantly to the increased pressure drop and that the small arteries show progressive changes in this hypertensive model that are not uniform at all vessel segments.

Methods

Production of Hypertension

Male Sprague-Dawley rats (75–100 g) were anesthetized with a 15:2 mixture of 37.5 mg/ml ketamine and 5 mg/ml xylazine (0.10 ml/100 g body wt). 1K1C hypertension was produced by removing the left kidney and placing a silver clip with a 200 μm gap on the right renal artery. Acute experiments were performed 2 or 4 weeks after surgical induction of hypertension and on age-matched normotensive controls.
Circulatory Preparation

Rats were anesthetized by an intraperitoneal injection (100 mg/kg) of Inactin (BYK Gulden Konstanz, FRG). A rectal probe was inserted to monitor core body temperature, which was maintained at 36.5±1°C by means of an animal heating pad and heating lamp. The rats were tracheostomized, and the right carotid artery was cannulated to permit measurement of mean arterial pressure and heart rate.

The cremaster muscle and upstream feeder vessels were then prepared for observation as previously described.10 To prepare the cremaster muscle, the scrotum was incised along its ventral surface to expose the right cremaster and enclosed testis. The intact cremaster muscle was carefully separated from its connection to the scrotum. The rat was then placed on a Plexiglas board so that the hind legs straddled a raised pedestal bath with an optical window. The muscle was slit along its ventral surface using a thermal cautery and was spread flat with sutures (5–0 silk) over the optical window. After preparing the cremaster muscle, an incision was made through the skin and muscle of the abdominal wall beginning at the inguinal canal and extending anteriorly to the level of the pelvis. To expose the cremaster feeder vessels, the skin and abdominal muscle were reflected back and secured by placing sutures at the edges of the incised tissue and then fastening them to the Plexiglas board. Fat and connective tissue surrounding the small arteries and veins supplying the cremaster muscle were dissected to clearly expose the vessels for microscopic observations. During the entire surgical procedure the cremaster muscle and exposed inguinal area were kept moist by frequent irrigation with warmed (35°C) Krebs solution.

During acute experiments, a Krebs bicarbonate solution was continuously suffused over the cremaster muscle and the tissues containing the upstream feeder vessels. The Krebs solution was composed of (mM) NaCl 113, NaHCO3 25, dextrose 11.6, KCl 4.7, CaCl2 2H2O 2.6, MgSO4 7H2O 1.2, and KH2PO4 1.2, which had an osmolality of 285 mosm/kg H2O. Temperature of the Krebs solution was maintained at 35±0.5°C. The solution was bubbled with N2 and CO2 to maintain a relatively constant pH (7.35–7.45), Pco2 (40–50 mm Hg), and PO2 (30–40 mm Hg).

The cremaster microcirculation and upstream feeder vessels were viewed by using a trinocular microscope (Olympus Vanox, Japan) equipped with a ×10 objective lens. The A1 arteriole was transilluminated through the optical window, whereas the feed vessels were epi-illuminated by a fiber-optic light pipe surrounding the objective lens. Transillumination of the cremaster muscle microcirculation allowed measurement of A1 arteriole lumen diameter, while epi-illumination of feeder vessels allowed measurement of external vascular diameter. The images were recorded on videotape with a closed circuit television system using a RCA Vidicon camera, Panasonic AG-1210 videocassette recorder, and a Hitachi 12-in. black and white video monitor. The final magnification viewed on the screen was ×500.

Vascular Pressure Measurements

Vascular pressure measurements were made using a servonull micropipette pressure system (model 3, Instrumentation of Physiology and Medicine, Inc., San Diego), which was calibrated with a mercury manometer. Pressure measurements were obtained with glass micropipettes made from glass capillary tubing (1 mm o.d.; filled, World Precision Instruments, Inc., New Haven, Conn.). Micropipettes were sharpened to an outside diameter of 1–2 μm and were filled with a filtered 2 M NaCl solution by immersing them in a reservoir. The pipettes were sharpened by lowering their tip onto a rotating surface of fine glass sanding paper, which was covered with a thin layer of 2 M NaCl solution.

To puncture a vessel for pressure measurement, the micropipettes were inserted into vessels using a micromanipulator (MO-103, Narishige USA, Inc., New York).

Experimental Protocol

Five sites were selected for intravascular pressure measurements beginning at the level of the A1 arteriole. Measurements were then made at each successive upstream site (Figure 1). Paired measurements of all successive vascular sites were obtained in each rat. For an individual experiment to be accepted in our study, it was necessary to obtain successful pressure measurements from all five vascular sites. Before the vascular pressure measurement, the diameter of the vessel was recorded during a 1-minute control period. The vessel was then punctured with a micropipette, and a pressure recording (minimum 30 seconds) was obtained from the vessel site. On playback of videotape, measurements of diameter were taken at 30-second intervals, and the average for a 3-minute period was recorded as vessel diameter. Pressure measurements were discarded if platelet aggregation at the pipette tip obstructed the vessel lumen, or if micropuncture of the vessel resulted in a significant and persistent diameter change.

Data Analysis

The data for this study are expressed as the mean±SEM. Two-way analysis of variance was used to determine significant differences in vascular diameters and pressures among groups. One-way analysis of variance was used to determine differences in mean arterial pressure, heart rate, body weight, and vascular pressure drops among groups. For multiple comparisons among groups, the Tukey method of modified t statistic was applied after analysis of variance (p<0.05 was considered significant).11
Results

Mean Arterial Pressure, Heart Rate, and Body Weights

Mean arterial pressure was elevated (Table 1) at both 2 (24% increase) and 4 (54% increase) weeks after the induction of hypertension. Heart rate was not different among any of the groups. Body weights of the hypertensive group were not different from the age-matched normotensive group.

Table 1. Mean Arterial Pressure, Heart Rate, and Body Weight

<table>
<thead>
<tr>
<th>Animal group</th>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Body wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-week NT</td>
<td>7</td>
<td>106±4</td>
<td>354±20</td>
<td>185±11</td>
</tr>
<tr>
<td>Two-week 1K1C</td>
<td>6</td>
<td>140±5*</td>
<td>391±19</td>
<td>177±11</td>
</tr>
<tr>
<td>Four-week NT</td>
<td>6</td>
<td>105±4</td>
<td>358±14</td>
<td>228±6†</td>
</tr>
<tr>
<td>Four-week 1K1C</td>
<td>6</td>
<td>162±7†</td>
<td>344±13</td>
<td>229±7†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of rats; MAP, mean arterial pressure; HR, heart rate; NT, normotensive rats; 1K1C, one-kidney, one clip hypertensive rats.

Table 2. Diameters and Pressures of Vascular Sites

<table>
<thead>
<tr>
<th>Vessel type</th>
<th>Group</th>
<th>Diameter (μm)</th>
<th>Pressure (mm Hg)</th>
<th>μP MAP ×100</th>
<th>Diameter (μm)</th>
<th>Pressure (mm Hg)</th>
<th>μP MAP ×100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal PEA</td>
<td>NT</td>
<td>256±55</td>
<td>96±4</td>
<td>89±3</td>
<td>257±6</td>
<td>93±5</td>
<td>89±3</td>
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<tr>
<td></td>
<td>1K1C</td>
<td>240±13</td>
<td>131±5*</td>
<td>91±2</td>
<td>279±22†</td>
<td>147±7*†</td>
<td>90±3</td>
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<tr>
<td>Distal PEA</td>
<td>NT</td>
<td>230±6</td>
<td>81±4</td>
<td>76±3</td>
<td>245±8</td>
<td>72±2</td>
<td>70±4</td>
</tr>
<tr>
<td></td>
<td>1K1C</td>
<td>214±9</td>
<td>109±6*</td>
<td>77±3</td>
<td>261±10†</td>
<td>105±3*†</td>
<td>64±3†</td>
</tr>
<tr>
<td>Proximal ESA</td>
<td>NT</td>
<td>130±6</td>
<td>73±3</td>
<td>67±2</td>
<td>136±7</td>
<td>59±4</td>
<td>57±4</td>
</tr>
<tr>
<td></td>
<td>1K1C</td>
<td>108±9*</td>
<td>107±7*</td>
<td>76±4</td>
<td>120±3*</td>
<td>98±4*</td>
<td>59±2†</td>
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<tr>
<td>Proximal A1</td>
<td>NT</td>
<td>97±5</td>
<td>58±4</td>
<td>56±3</td>
<td>101±4</td>
<td>50±1</td>
<td>48±1</td>
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<tr>
<td></td>
<td>1K1C</td>
<td>82±6*</td>
<td>79±5*</td>
<td>58±3</td>
<td>94±5*</td>
<td>75±2*</td>
<td>48±3†</td>
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<tr>
<td>Distal A1</td>
<td>NT</td>
<td>98±5</td>
<td>51±3</td>
<td>49±3</td>
<td>103±5</td>
<td>48±2</td>
<td>46±2</td>
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<tr>
<td></td>
<td>1K1C</td>
<td>78±6*</td>
<td>71±3*</td>
<td>52±2</td>
<td>88±4*</td>
<td>67±2*</td>
<td>42±2†</td>
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</tbody>
</table>

Values are mean±SEM. μP, microvascular pressure; MAP, mean arterial pressure; PEA, pudic-epigastric artery; ESA, external spermatic artery; A1, large first-order arteriole; NT, normotensive rats; 1K1C, one-kidney, one clip hypertensive rats.

*Significantly different from age-matched normotensive group (p<0.05).
†Significantly different from 2-week hypertensive group (p<0.05).
‡Significantly different from 2-week normotensive group (p<0.05).
proximal pudic-epigastric artery pressure was significantly elevated at 4 weeks compared with 2 weeks of hypertension. All other vascular pressures remained elevated but not further increased as the hypertension progressed from the second to the fourth week of development.

Normalized pressure is the percent of mean arterial pressure transmitted to a vessel site. A decrease in normalized pressure in the hypertensive rats would indicate that the increase in mean arterial pressure was not proportionally transmitted to that vessel site. The normalized pressure at all sites downstream from the proximal pudic-epigastric artery were significantly decreased after 4 weeks of hypertension compared with the 2-week hypertensives.

**Pressure Drop Across Vessel Levels**

The pressure drop along the external spermatic artery (site 3–4) also was increased (87%) after 2 weeks of hypertension (Figure 2). This increased pressure drop was maintained at the same level after 4 weeks of hypertension. An increased pressure drop along a vessel segment, which has no branch points between the locations of pressure measurement, indicates an increased vascular resistance. In contrast, the pressure drop along the pudic-epigastric artery (site 1–2) was not increased after 2 weeks of hypertension but by 4 weeks was greatly increased (105%).

**Discussion**

We measured vascular pressures and diameters to determine what involvement small arteries proximal to the cremaster muscle microcirculation might have on the increase in vascular resistance during the development of hypertension. Two weeks after the induction of hypertension, the pressure drop along the external spermatic artery increased (Figure 2); this contributed to the rise in cremaster muscle resistance. The pudic-epigastric artery pressure drop was increased after 4 weeks of hypertension; this blunted any further rise in A1 feeder pressure within the cremaster muscle. Thus, small arteries proximal to the cremaster muscle microcirculation contribute to the changes in overall cremaster muscle vascular resistance. The vascular alterations in the small arteries appear to involve different vessels at various times during the development of hypertension.

**Vascular Diameters**

The A1 arteriole and external spermatic artery had smaller diameters after 2 and 4 weeks of hypertension (Table 2). Because the A1 arteriole is the microvascular extension of the external spermatic artery, a prolonged decrease in vascular diameter that causes an increase in resistance may contribute to the increase in mean arterial pressure, but this also prevents the increase in pressure from being fully transmitted to the downstream small arterioles and capillaries. In the early phase (2 weeks) of hypertension, 59% of the increase in mean arterial pressure was transmitted down to the distal A1 arteriole. Whereas, after 4 weeks, only 35% of the increase in mean arterial pressure was transmitted down to the distal A1 arteriole.

Our findings of a reduced A1 lumen diameter agree with previous studies of renovascular hypertension.2-4,12,13 Joshua et al? found reduced cremaster muscle A1 arteriole diameters after 2 and 4 weeks of 1K1C hypertension. Structural reductions of the cremaster muscle A1 arteriole have been observed as early as 2 weeks after clipping in two-kidney, one clip hypertension.12 The changes in A1 arteriole diameter observed in the present study could be in part due to structural changes within the vascular wall, particularly at 4 weeks. However, it is not possible to determine from our data the relative contributions of functional and structural events to the observed changes in diameter.

Although the pressure drop across the pudic-epigastric artery increased after 4 weeks, no change in vascular diameter was observed. The pudic-epigastric artery was observed by epi-illumination, and only external vascular diameters could be determined; therefore, changes in luminal diameter...
could not be determined. Stacy and Prewitt\(^3\) histology evaluated fully dilated cremaster feeder arteries 12 weeks after the induction of 1K1C hypertension and found no change in luminal diameter and no smooth muscle hypertrophy. This suggests that the increase in pudic-epigastric artery resistance was not due to structural changes and could have been due to active vasoconstriction.

**Vascular Pressures**

The pressure distribution of a vascular bed can be used to identify vascular segments, which contribute to increased vascular resistance during hypertension. In the present study, after 2 weeks of 1K1C hypertension, mean arterial pressure rose 36 mm Hg, the A1 pressure rose 20 mm Hg, and the pressure drop along the external spermatic artery increased by 13 mm Hg compared with the normotensive rats. These data suggest that vascular resistance increased in the external spermatic artery. The effect of this rise in small artery vascular resistance was to prevent microvascular pressures from rising to the same degree as mean arterial pressure. In the later stage (4 weeks) of hypertension, the pressure drop along the pudic-epigastric artery increased by 21 mm Hg, and at the same time mean arterial pressure rose 22 mm Hg. Thus, the pudic-epigastric artery exhibited an increased resistance that minimized any further increase of pressure in downstream vessels. It was the external spermatic artery resistance that increased during the first 2 weeks, and it was the pudic-epigastric artery resistance just upstream that increased during the next 2 weeks of hypertension development.

Past studies of the pressure distribution during hypertension have concentrated on the microcirculation. The studies performed in the adult spontaneously hypertensive rat have shown that small arterioles contribute only 10–15% of the 50% increase in vascular resistance of the organs tested.\(^1\)\(^4\) Therefore, the large arterioles and small arteries must account for the remaining 35–40% of the increase in resistance. Other models\(^4\)\(^7\) of hypertension also show only a small contribution by arterioles to the increase in organ vascular resistance and a large contribution from vessels that are proximal to the microcirculation.

The contribution of a vascular segment to the total pressure drop across the vascular bed can identify segments involved in increasing the organ vascular resistance. Previous studies have demonstrated that large venular pressures in renal hypertension are unchanged\(^4\)\(^7\) and that an increased pressure drop across an organ vasculature occurs at the small arteries and arterioles. Therefore, assuming pudic-epigastric vein pressure (5 mm Hg)\(^10\) does not change during hypertension, the contribution of various vascular segments to the increased pressure drop across the cremaster muscle can be assessed.

The present study showed that after 2 weeks of hypertension, the external spermatic artery had an increased resistance and accounted for 38% of the increased pressure drop across the cremaster muscle. At the same time, the pudic-epigastric artery accounted for 21% and vessels distal to the A1 arteriole accounted for 38% of the increased pressure drop. In contrast, after 4 weeks of hypertension the pudic-epigastric artery accounted for the majority (37%) of the increased pressure drop. The external spermatic artery accounted for 25%, the A1 arteriole accounted for 11%, and vessels distal to the A1 accounted for only 26% of the increased pressure drop. Thus, the pudic-epigastric artery accounted for 90% of the increased pressure drop that occurred between 2 and 4 weeks of hypertension development. Therefore, vessels upstream from the cremaster muscle A1 arteriole accounted for 60% after 2 weeks and 74% after 4 weeks of the increased pressure drop that occurs during hypertension. This confirms a previous study,\(^8\) which suggested that the vessels upstream from the cremaster A1 arteriole contribute to the increase in vascular resistance.

The results of the present study demonstrate that the development and maintenance of hypertension is not the result of a generalized increase in vascular resistance. Instead, there is a varying degree of involvement of the small arteries during the development of hypertension.

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**References**


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