Arteriolar Reactivity to Pressure Stimuli in Hamsters with Renal Hypertension

D. Lowell Stacy, William L. Joyner, and Joseph P. Gilmore

SUMMARY The responses to alterations in extravascular pressure were studied in five orders of arterioles in the cheek pouch of normotensive and renal hypertensive hamsters. Renal hypertension was induced by bilateral compression of both kidneys using figure-of-eight ligatures. Ten to 16 days later, hamsters were anesthetized with pentobarbital (6.0 mg/100 g body weight) and a Plexiglas chamber was positioned in the cheek pouch. Chamber pressure, or extravascular pressure, was increased and decreased by ± 10, 20, and 40 mm Hg, and arteriolar diameters were monitored continuously. The responses at −20 mm Hg and the slope of the linear portion of the chamber pressure-diameter curve (arteriolar gains) were compared between groups for each branching order of arteriole. Arteriolar responses at one chamber pressure and the arteriolar gains were enhanced in third and fourth order arterioles of the renal hypertensive group compared with the normotensive group, and the responses of these small arterioles were greater than those of larger arterioles in both groups. Control diameters of second and third order arterioles were significantly smaller in the renal hypertensive group, while the diameters after adenosine were not different. These results suggest that the enhanced responses of small arterioles in the renal hypertensive group were not related to structural alterations but may be related to an increased reactivity of smooth muscles in these small arterioles to volume expansion, thus a pressure stimulus. (Hypertension 10: 82-92, 1987)

KEY WORDS • renal hypertension • microcirculation • arteriolar diameter • autoregulation • arteriolar tone • medial area • transmural pressure

STRUCTURAL and functional alterations of blood vessels are associated with human essential, renal, and genetic hypertension. The relative contribution of arteriolar vasoconstriction, medial hypertrophy, and rarefaction to altered peripheral resistance associated with microvessels in spontaneous and renal hypertension varies according to the vascular bed studied and the progression of the hypertension. Small arterioles contribute markedly to the local control of blood flow/resistance in individual tissues because of their intrinsic tone, enhanced reactivity to vasoactive agents, and reactivity to nerve stimulation. The relative contribution of small arterioles to peripheral resistance in vascular beds has been demonstrated by the large pressure gradient across the microcirculation, especially with renal hypertension. In various animals with renal hypertension, an increased reactivity of small arterioles has been observed routinely; also, in renal hypertension in the rat, sympathetic nerve activity was elevated. Thus, most studies have concentrated on the neural or humoral control of vascular tone without considering the role that autoregulation may play in elevating peripheral resistance in hypertension. Recently, Meininger et al., using the cremaster muscle in the rat, demonstrated that autoregulation of small arterioles may contribute to the elevated resistance in the rat after acute renal artery stenosis. However, Alson et al. found that autoregulation did not contribute to the elevated resistance of the perfused hindlimb in the spontaneously hypertensive rat, as determined by the slope of the perfusion pressure-flow curves or the lumen diameters of small arterioles in the cremaster muscle, or both. Studies of autoregulation in microvessels have been conducted by lowering arteriolar pressure, raising venous pressure, and altering extravascular pressure. Altering extravascular pressure has the rela-
tive advantage of selectively altering transmural pressure without markedly altering perfusion pressure. With the use of this technique, arterioles in the hamster cheek pouch, \(^{23}\) rat cerebral cortex, \(^{24}\) and bat wing \(^{23}\) demonstrated active arteriolar responses (i.e., the diameters of the vessels were altered in a manner appropriate for regulation of blood flow with changes in extravascular pressure or transmural pressure). These arterioles constricted when transmural pressure was increased and dilated when it was decreased.

Recently, myogenic responses of isolated arterioles and small arteries have been studied to determine their vascular smooth muscle sensitivity. \(^{26,27}\) These isolated vessels responded very weakly to changes in transmural pressure, possibly because of their relatively large size \(^{26}\) or poor vascular tone. The vascular tone of arterioles has been shown to be dependent on both dynamic and static transmural pressure \(^{28}\) and on flow in isolated vessels. \(^{29}\) In addition, the interstitial matrix surrounding small vessels can influence smooth muscle mechanics. \(^{30}\) For these reasons, arteriolar responses to pressure stimuli were studied in vivo with formalin, pulsatile pressure, vasoactive substances, nutrients, and interstitial matrix. The aims of this study were to describe and compare the arteriolar reactivity of small blood vessels to alterations in transmural pressure and to compare vascular geometry and tone in relation to branching sequence in renal hypertensive (RHT) and normotensive (NT) hamsters.

**Materials and Methods**

**Induction of Renal Hypertension**

To induce renal hypertension (two-kidney, two figure-8), female hamsters (SASCO, Omaha, NE, USA) weighing 140 to 180 g were anesthetized with pentobarbital (6.0 mg/100 g body weight intraperitoneally). Each kidney was exposed separately through a dorsal flank incision, and bilateral figure-of-eight ligatures (No. 2 silk) were applied to each kidney. \(^{11}\) Care was taken to avoid damaging the renal pelvis and adrenal glands while maintaining compression of the renal parenchyma. The sham-operated group consisting of weight-matched hamsters were handled in a similar manner except the ligatures were not tied.

All hamsters were allowed to recover on a heating pad and were then returned to hanging cages and given access to food and water ad libitum. No renal surgery or any other type of manipulation was performed on the NT group. Systolic blood pressure was measured in hindlimbs by the plethysmographic method using a pneumatic pulse transducer and spaghmomanometer. \(^{11}\) On the day of the experiment, three systolic pressure measurements in each hindlimb were averaged and, if the mean value was at least 150 mm Hg, the hamster was considered a possible candidate for renal hypertension. Only when the mean arterial pressure averaged at least 120 mm Hg for the entire microvascular experiment, as measured directly by cannulation of the femoral artery, was the hamster classified as hypertensive and thus included in the analysis of the data.

**Microvascular Preparation**

To observe the vessels in the cheek pouch, a Plexiglas chamber was placed in the cheek pouch (Figure 1). This was accomplished by positioning a baseplate that was supported by a holder into the cheek pouch; then an incision was made in the skin over the baseplate, and the skin was retracted back around the baseplate, uncovering the cheek pouch membrane. Two small holes of about 1 mm in diameter were cut in the cheek pouch tissue to allow the screws of the baseplate to pass through the tissue. This procedure was done with a dissecting microscope to minimize damage to the microvessels, and the incisions were made far enough apart to avoid stretching the cheek pouch tissue. The upper chamber was placed on top of the baseplate, and hex nuts were used to attach the screws and baseplate to the upper chamber. To remove any further tension on the cheek pouch, the skin from the incision made to expose the cheek pouch was supported by five bent 18-gauge hypodermic needles attached to the upper chamber.

Once the cheek pouch tissue was exposed, it was kept moist continuously with Ringer's bicarbonate solution. The hamster then was moved to a heated microscope stage where the avascular layer of the cheek pouch was dissected carefully away while the Ringer's bicarbonate solution bathed the cheek pouch. The composition of the Ringer's bicarbonate solution (37°C, pH 7.4), which was gassed with 95% N\(_2\), 5% CO\(_2\), was (mM) NaCl, 132; KCl, 4.7; CaCl\(_2\), 1.5; MgSO\(_4\), 1.2; and NaHCO\(_3\), 25. The top plate of the entire chamber was sealed to the upper chamber by six hex nuts, and the degree of seal around the cheek pouch was varied by turning the two hex nuts that brought the baseplate into apposition with the cheek pouch and the silicon rubber layer of the upper chamber (see Figure 1, inset). When the velocity of red blood cells in the venules appeared to be reduced slightly, the screws were loosened until blood velocity in the venules resumed, thus sealing the chamber without any apparent effects on microvascular hemodynamics or vascular tone.

The preparation was transilluminated by a Zeiss 100-W mercury lamp (Collins Microscope, Overland Park, KS, USA) attached to a fiberoptic light rod that was placed into the cheek pouch under the baseplate (see Figure 1, inset). The optical system consisted of a Zeiss microscope with a Leitz 10× or 6.3× objective (Rockleigh, NJ, USA) connected by a Zeiss Optivar (1.0-2.0×) magnifier and a Zeiss image rotator to a closed-circuit television system containing a videotape recorder (Collins Microscope), image shearing monitor (Instruments for Physiology and Medicine, San Diego, CA, USA), and a video camera (Cohu, San Diego, CA, USA).

**Classification of Branching Orders for Arterioles**

The chamber was positioned in the right cheek pouch so that the largest arteriole entered the area of the chamber at about the 11-o'clock position; this arte-
The arteriole was classified as the first order arteriole. The next branch, which was usually smaller in diameter by about 50%, was classified as the second order arteriole. Succeeding arteriolar branches were studied only if they were also about 50% of the diameter of the parent vessel. Often, a large arteriole would branch into two vessels that were either not much smaller than the parent vessel or less than 30% of its diameter; these were not considered true branches. First and second order arterioles that formed arcades were not used in this study. If the arteriolar branching order of an arteriole was not clear, it was standardized by counting backward from the fifth order arterioles that directly fed the capillaries or by comparing the relaxed diameters after the application of adenosine. This classification scheme produced five orders of arterioles.

Alterations of Chamber Pressure

Chamber pressure was altered by establishing a closed system for the cheek pouch; this was accomplished by closing the stopcock (labeled V1 in Figure 2) to the Ringer's solution (A in Figure 2), thus opening the chamber to the pressure transducer (B in Figure 2). Then, the outflow port of the chamber (2 in Figure 2) was opened to the reservoir (D in Figure 2) containing the syringe (F in Figure 2) used to alter chamber pressure by opening the stopcock (V2 in Figure 2). Finally, the outflow port (3 in Figure 2) was clamped, encompassing the cheek pouch in a closed system. The syringe (F in Figure 2) connected to a pressure-dampening reservoir was used to set the pressure in the closed chamber to a predetermined level by manual operation of the syringe with continual monitoring of the chamber pressure.23

To study the responses of arterioles to changes in transmural pressure, a series of positive and negative pressures were applied to the outside of the cheek pouch, thus each arteriole. Initially, pressure in the chamber was decreased by 10 mm Hg for 1 minute, then it was returned to and held at atmospheric pressure for 1 minute, and subsequently it was increased by 10 mm Hg for 1 minute (Figure 3, upper panel). Then, chamber pressure was returned to atmospheric pressure for about 30 seconds; followed by 2 minutes of suffusion with the Ringer's solution. This procedure was repeated for ±20 and ±40 mm Hg. In most instances, the arteriole in question was allowed to return to its control diameter between the series of positive and negative changes in chamber pressure. Davis et al.23 showed that arterioles responded maximally to a change in chamber pressure of ±20 mm Hg within 30 seconds and returned to control values within 1 minute after the chamber pressure was returned to atmospheric pressure.

Diameters and Responses of Arterioles and the Calculation of Medial Area and Media Lumen Ratio

Arteriolar responses to changes in chamber pressure were determined by continuously recording the lumen diameter of the selected arteriole on videotape before and during each step-change in chamber pressure. These measurements in representative third order arterioles of a NT and RHT hamster are shown in Figure 3. The response of the arterioles is expressed as the experimental diameter (De) divided by the control diameter (Dc). The control diameter was taken as the internal diameter of the arteriole just before the chamber pressure was altered, and the experimental diameter was taken at the point where the maximum change in diameter (De) occurred during the pressure stimulus. External diameters also were measured before the pressure stimulus to calculate the media/lumen ratio and medial area. The media thickness, often called the wall thickness, included the media and intima. Medial cross-sectional area was calculated as the area of the circle bounded by the external diameter minus the area of the circle bounded by the lumen diameter. Media/lumen ratio was calculated as the medial thickness divided by the lumen diameter.

Calculation of Arteriolar Gain

The responses of arterioles to alterations in transmural pressure were analyzed by two parameters: the midrange responses to chamber pressure (−20 mm Hg) and the slope of the chamber pressure-diameter relation. This slope was termed the arteriolar gain (Gd) for a particular arteriole, and it is a measure of the magnitude of arteriolar reactivity over the range of
Figure 2. Schematic of the system used to alter chamber pressure and provide the cheek pouch of the hamster with the suffusion solution: Reservoir of Ringer's bicarbonate (A); pressure transducer used to measure chamber pressure (B); Plexiglas chamber and holder (C); reservoir filled with Ringer's bicarbonate and some air to dampen the changes in extravascular pressure (D); beaker used to collect the suffusion solution outflow (E); glass syringe used to alter chamber pressure (F); valve used to stop the suffusion solution flow (V1); valve used to open the chamber to the reservoir and glass syringe (V2); inflow port (1); port used to alter chamber pressure (2); outflow port (3).

Figure 3. Representative record illustrating the measurement of the diameter (D) in a third order arteriole from a normotensive (NT) and a renal hypertensive (RHT) hamster during alterations in chamber pressure (Pc). The black bar represents the time that the suffusion solution was open to the chamber. Mean arterial pressure (MAP) was recorded continuously.

Figure 4. Calculation of arteriolar gain (G_d) from the linear portion of the relative diameter (D_e/D_c)—chamber pressure relation in the same third order arteriole from the normotensive (NT) and renal hypertensive (RHT) group. D_e/D_c = experimental diameter/control diameter.

alterations in transmural pressure, where the subscript d denotes that gain refers to the control of lumen diameter.

To illustrate how arteriolar gain was calculated, the response of the same third order arteriole in a NT and RHT hamster shown in Figure 3 is shown in Figure 4. The chamber pressure-diameter curves were linear, or the responses reached a plateau, thus the curves were sigmoid-shaped. For those curves that were sigmoid-shaped, second order polynomial regression analysis of each half of the curve provided the best fit of the data points from a chamber pressure of 0 to +40 mm Hg or from 0 to −40 mm Hg. This analysis was used to find the chamber pressure where the maximum response occurred. To determine the point on the curve where the slope is zero, the first derivative of the polynomial equation was found and set equal to zero. The data points within these maximum responses described the linear portion of the curve, and the experiment points encompassed by these maxima were used for linear regression analysis. For example, in the third order arteriole of the NT group (see Figure 4), a second order polynomial regression of the responses from 0 to +40 mm Hg chamber pressure produced no maximum response, whereas the responses from 0 to −40 mm Hg produced a minimum response at −33 mm Hg. Therefore, the responses at +40, +20, +10, −10, −20 mm Hg, and the control value were used for the
linear regression; the resulting slope was 0.0086. Then, this slope was multiplied by 100 to depict the gain (0.86). The units for this arteriolar gain (G_d) are the percentage of change in diameter per millimeter of mercury and are the same units as those gains calculated from studies of isolated vessels,26 except the sign is the opposite. For the third order arteriole in the RHT hamster, a maximum point was found at 34 mm Hg and a minimum at —48 mm Hg (see Figure 4). Thus, all the data points except the one at 40 mm Hg were used for the linear regression. This resultant slope (G_d) was 1.6. Therefore, the larger the value for the arteriolar gain, the larger the response. Also, if chamber pressure is decreased (increasing transmural pressure) and the arteriole constricts, or if the chamber pressure is increased (decreasing transmural pressure) and the arteriole dilates, the value is positive.

Maximal Dilation of Arterioles in Response to Adenosine

To test vascular tone and to compare the vasodilated diameters between NT and RHT groups for a given arteriolar order, several drops of adenosine (10^{-3} M) were applied topically to cheek pouch arterioles. Since the volume of the chamber was about 1.0 ml, the final concentration of adenosine was 10^{-4} M. Maximal dilation of arterioles in the cheek pouch was produced with this dose of adenosine with no effect on blood pressure measured in the femoral artery. Vessels with good tone showed their greatest dilation after about 30 seconds: large arterioles dilated 20 to 30%, and small arterioles dilated 60 to 80%. Those hamsters with poor arteriolar tone, as indicated by a maximum dilation of less than 10% for large arterioles (first and second orders) or less than 20% for small arterioles (third, fourth, and fifth orders), were not used in the analysis of the results.

Experimental Protocol

On the day of the microvascular experiment, 10 to 16 days after operation, hamsters in each group, NT, sham-operated, and RHT, were anesthetized with pentobarbital (6.0 mg/100 g i.p.). Supplemental doses (1.5 mg/100 g i.p.) were given as needed. Systolic blood pressure was measured using the indirect technique, a catheter (PE-205) was placed in the trachea for ventilation, and a second catheter (PE-10) was placed in the femoral artery for direct measurement of arterial blood pressure. The signal from the transducer connected to the arterial catheter was averaged electronically on a Grass 79B polygraph (Quincy, MA, USA), while the blood pressure waveform was observed on an oscilloscope to ensure a high fidelity recording.

Arterioles often were dilated after operation; thus, 30 minutes was required before beginning the microvascular experiments. One criterion for continuing the experiment was that small arterioles (third and fourth orders) regained their vascular tone, as indicated by constricting to about 50% of their dilated state. Furthermore, preparations showing poor red blood cell velocity in any of the microvessels, venous stasis, petechiae, or a fall in blood pressure were not used.

Once these criteria were satisfied, an arteriole to be studied was selected randomly and the internal and external diameters were measured. A series of alterations in chamber pressure were applied, as previously described. Usually, only one arteriole from each branching order in a given hamster was studied; however, not all of the five orders were used in every preparation because the optical resolution of the internal diameter was not adequate. In some hamsters, more than one arteriole of a particular order was studied, and in these instances the measurements were averaged. At the end of the experiment, adenosine was applied topically to the cheek pouch to measure the vasodilated diameters and to test the vascular tone defined as the percentage of increase in diameter.

Mean arterial blood pressure was sampled every 15 to 20 minutes during the entire experiment and was averaged to give the mean blood pressure for that hamster. The length of the experiment, including the preparation of the hamster and microvascular observations, was usually 2 to 3 hours.

Plasma Volume, Blood Volume, and Hematocrit

In another series of hamsters, plasma volumes were measured by an indicator dilution method using fluorescein isothiocyanate dextran (FITC dextran 150) with a mean molecular weight of 150,000. The femoral artery and vein were cannulated with PE-10 tubing, and the trachea was cannulated with PE-205 tubing. Then, 90 μl of a 5% solution of FITC dextran was injected into the femoral vein with a Hamilton syringe, and blood samples were taken 2 to 3 minutes and 8 to 12 minutes later from the arterial catheter. After 4 to 5 drops of blood were cleared from the arterial catheter, two microhematocrit tubes were filled with arterial blood. The blood samples were centrifuged for 3 minutes, and the hematocrit was measured. The tube was scored and broken at the red blood cell/plasma interface, then various dilutions of the plasma were made (1/1000, 1/10,000, and 1/100,000). Fluorescein intensity was determined in an Aminco Bowman spectrophotofluorometer (American Instrument, Silver Springs, MD, USA); with an excitation wavelength of 490 nm and emission wavelength of 510 nm. Serial dilutions of 5% FITC 150 dextran solutions were used as standards, and linear regressions were performed on the concentration of the standard solutions versus the percentage of transmission. These regressions were linear over the range of 5 to 400 ng/ml of FITC dextran 150, and the plasma samples were diluted to fall within this range.

Plasma volume was calculated based on the quantity of FITC dextran 150 injected divided by its final plasma concentration. This procedure assumes the FITC dextran was uniformly distributed throughout the blood and remained in the intravascular space. Previous studies have shown that the FITC dextran molecule is stable and that 90% of FITC dextran 70 remains in the circulation 30 minutes after intravenous infusion into rabbits. Blank samples also were measured to determine the background level of transmission, and it was subtracted from the 5% FITC dextran transmission.
to derive the standard curves. Blood volume was calculated as the plasma volume $\times 100/(100 -$ hematocrit).

Statistical Analysis
Comparisons of arteriolar responses at $-20$ mm Hg and the arteriolar gains were made between the NT and RHT groups by unpaired $t$ tests. The arteriolar gains of the five branching orders were compared within the NT and RHT hamsters by analysis of variance and the Newman-Keuls multiple-range test. $t$ tests also were used to compare vascular dimensions and plasma and blood volumes between the NT and RHT groups. All tests were considered significant at the 0.05 level.

Results

Body Weight and Arterial Blood Pressure
In the eight RHT hamsters, arterial blood pressure measured either directly or indirectly was elevated significantly as compared with the 15 NT hamsters (Table 1). These measurements were completed 10 to 16 days after the induction of renal hypertension. Since the arterial blood pressures were not significantly different between the hamsters in the sham-operated group ($n = 2$) and the NT group ($n = 13$), all of the data were pooled. Hamsters in the RHT group lost weight (see Table 1) during the interval of this study, while the sham-operated hamsters maintained their weight.

Arteriolar Responses
The responses of a representative arteriole, third order, in a NT and a RHT hamster to a stimulus of $\pm 10$, $\pm 20$, and $\pm 40$ mm Hg change in chamber pressure are shown in Figure 3. When the chamber pressure or extravascular pressure was reduced, transmural pressure (intravascular $-$ extravascular pressure) was increased, and when chamber pressure was increased, transmural pressure was decreased. In both the NT and RHT hamsters, when chamber pressure was reduced, the diameter of these arterioles decreased rapidly and the maximum responses were reached within 30 seconds. The arterioles remained constricted during the duration of the stimulus (60 seconds). When chamber pressure was returned to atmospheric pressure, the diameter of the vessels returned to the control values. The opposite response was observed when chamber pressure was increased (i.e., the arterioles dilated). As the magnitude of the change in chamber pressure was increased, the magnitude of the vascular response increased. The change in the diameter of the arteriole in the RHT hamsters to each step-change in chamber pressure also appeared to be greater than that observed in the arteriole from the NT hamster. The response ($De/Dc$) of these same arterioles (third order) in the NT and RHT hamsters in relation to each change in chamber pressure is shown in Figure 4. With the use of the analysis described previously for these two arterioles, a gain of 0.8 and 1.6 was determined for the NT and RHT hamsters, respectively.

In some experiments, nitroprusside ($10^{-4} M$) was added to the suffusion solution and the response of the arterioles to changes in chamber pressure was tested again. The response was reversed completely in all arterioles tested: all arterioles dilated in response to a decrease in chamber pressure, and a decrease in diameter was observed with an increase in chamber pressure. Thus, they responded passively.

The arteriolar responses ($De/Dc$) at each chamber pressure are shown for the five orders of arterioles in the NT and RHT groups in Figure 5. All responses are depicted as means with their standard error. The responses were graded with respect to chamber pressure, and they were essentially equal for positive and negative chamber pressures. Because the responses reached a plateau in third, fourth, and fifth order arterioles at about $\pm 40$ mm Hg chamber pressure, comparisons of the responses at one point ($-20$ mm Hg) were used as well as the calculation of the regulatory gain ($G_\text{r}$) to unmask the relation of arteriolar reactivity in the NT and RHT hamsters.

In comparing the arteriolar responses at a constant chamber pressure ($-20$ mm Hg) for the NT versus the RHT hamsters, the responses of third and fourth order arterioles were significantly elevated in the RHT hamsters (Table 2). These same observations were confirmed when the data for each arteriole was expressed and compared as arteriolar gains (Figure 6). The arteriolar gains of the third and fourth order arterioles were significantly greater in the RHT hamsters as compared with the NT hamsters. The arteriolar gains within the NT hamsters were greater for the fifth order arterioles, whereas in the RHT hamsters, the third, fourth, and fifth order arterioles were more reactive than either the first or the second order arterioles.

Vascular Dimensions and Tone

Morphometric characterization and vascular tone for all orders of arterioles in each group of hamsters are shown in Table 3. The control lumen diameters ($Dc$) of second and third order arterioles in the RHT hamsters were reduced significantly as compared with the NT

<table>
<thead>
<tr>
<th>Table 1. Arterial Blood Pressure and Body Weight in Normotensive and Renal Hypertensive Hamsters</th>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Normotensive ($n = 15$)</td>
</tr>
<tr>
<td>Hypertensive ($n = 8$)</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SEM. Indirect measurement of systolic blood pressure was done using the plethysmographic technique. S = systolic; D = diastolic; M = mean.

*p < 0.05, compared with values in normotensive hamsters.
hamsters. However, the vasodilated \((10^{-4} \text{ M adenosine})\) diameters \((D_v)\) were the same in both groups. The vascular tone, as shown by their dilation after the application of adenosine of first, second, and third order arterioles, was elevated significantly in the RHT group. The media/lumen ratios were significantly greater in second and fourth order arterioles of the RHT as compared with the NT group, while there were no differences in the medial areas of any arterioles between the NT and RHT groups.

**Plasma Volume, Blood Volume, and Hematocrit**

No difference was found in the calculated plasma volumes taken at an average of 3.4 minutes (6.2 ml) and 9.5 minutes (6.0 ml) after injection in the normotensive hamsters. Thus, these values were average for each hamster. Plasma volume and blood volumes were higher in the RHT than in the NT hamsters (Table 4). The increase in plasma volume found in the RHT group resulted in a hematocrit of 43%, and the measured value was 42%; therefore, the significant decrease in hematocrit was due to an increase in plasma volume and not to a decline in packed cell volume.

**Discussion**

**Arteriolar Reactivity**

These studies demonstrated that in the cheek pouch of the hamster small arterioles are more responsive to changes in transmural pressure than large arterioles, and this responsiveness is enhanced with renal hypertension. It has been shown that small arterioles of the rat cremaster muscle are more responsive to reductions in arterial pressure\(^9\) and that the initial vasoconstriction in the rat cremaster with acute renal hypertension is due partially to autoregulation.\(^{17}\) Until now, however, the responsiveness of arterioles in renal hypertension to graded pressure stimuli has not, to our knowl-

**TABLE 2. Responses of Arterioles in Normotensive and Renal Hypertensive Hamsters at a Constant Chamber Pressure (-20 mm Hg)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Response of arterioles ((D_e/D_c))</th>
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<tbody>
<tr>
<td></td>
<td>1A</td>
</tr>
<tr>
<td>Normotensive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
</tr>
<tr>
<td>Hypertensive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. The number of arterioles is shown in parentheses. \(D_e/D_c\) = experimental diameter/control diameter. 1A–5A = first through fifth order arterioles.

\(*p < 0.05\), compared with values in normotensive hamsters.
edge, been studied in vivo. Thus, arteriolar reactivity to changes in transmural pressure is augmented in small arterioles in renovascular hypertension.

Myogenic responses may be elicited by changes in wall tension or stress as a result of changes in transmural pressure, and in experiments where perfusion pressure is altered, metabolic mechanisms also contribute to the autoregulatory response. Thus, the technique employed in the present study was designed to minimize the metabolic component of the autoregulatory response (i.e., the use of the chamber to alter extravascular pressure). However, even though blood flow was not measured in this study, it is related directly to diameter to the fourth power of the radius in individual arterioles and to the third power in a vascular network; therefore, any significantly different responses of the diameters of the arterioles to changes in transmural pressure should result in greater changes in arteriolar flow. In addition, a given change in extravascular pressure stimulus is relatively less for an arteriole in the hypertensive as compared with the NT hamster since intravascular pressures have been reported to be higher in hamsters with two-kidney, two figure-8 hypertension. Thus, the greater reactivity of these small arterioles in renal hypertension could be magnified.

![Figure 6](image)

**Figure 6.** Arteriolar gain ($G_d$) for five orders of arterioles in the normotensive (NT) and renal hypertensive (RHT) groups. All values are means ± SEM. Asterisk indicates significant difference ($p<0.05$) compared with values for the NT group.

### TABLE 3. Vascular Dimensions and Tone of Arterioles in Normotensive and Renal Hypertensive Hamsters

<table>
<thead>
<tr>
<th>Group</th>
<th>DC (μm)</th>
<th>DV (μm)</th>
<th>ΔD (%)</th>
<th>M/L</th>
<th>MA (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive 1A</td>
<td>89 ± 6 (10)</td>
<td>108 ± 7 (7)</td>
<td>19 ± 3 (7)</td>
<td>0.01 ± 0.01 (7)</td>
<td>2863 ± 233 (7)</td>
</tr>
<tr>
<td>2A</td>
<td>49 ± 5 (9)</td>
<td>58 ± 6 (6)</td>
<td>28 ± 5 (6)</td>
<td>0.13 ± 0.03 (8)</td>
<td>1093 ± 190 (8)</td>
</tr>
<tr>
<td>3A</td>
<td>23 ± 2 (11)</td>
<td>31 ± 2 (8)</td>
<td>41 ± 11 (8)</td>
<td>0.22 ± 0.03 (10)</td>
<td>398 ± 47 (10)</td>
</tr>
<tr>
<td>4A</td>
<td>11 ± 1 (10)</td>
<td>16 ± 1 (7)</td>
<td>53 ± 10 (7)</td>
<td>0.30 ± 0.02 (9)</td>
<td>157 ± 22 (9)</td>
</tr>
<tr>
<td>5A</td>
<td>7 ± 1 (7)</td>
<td>11 ± 1 (5)</td>
<td>56 ± 14 (5)</td>
<td>0.36 ± 0.06 (7)</td>
<td>79 ± 16 (7)</td>
</tr>
<tr>
<td>Hypertensive 1A</td>
<td>82 ± 3 (6)</td>
<td>106 ± 3 (6)</td>
<td>28 ± 3* (6)</td>
<td>0.12 ± 0.01 (6)</td>
<td>2661 ± 240 (6)</td>
</tr>
<tr>
<td>2A</td>
<td>38 ± 2* (7)</td>
<td>60 ± 1 (7)</td>
<td>62 ± 9* (7)</td>
<td>0.19 ± 0.03* (7)</td>
<td>993 ± 141 (7)</td>
</tr>
<tr>
<td>3A</td>
<td>19 ± 1* (8)</td>
<td>29 ± 2 (8)</td>
<td>60 ± 6* (8)</td>
<td>0.27 ± 0.04 (8)</td>
<td>340 ± 15 (8)</td>
</tr>
<tr>
<td>4A</td>
<td>11 ± 1 (7)</td>
<td>17 ± 2 (7)</td>
<td>55 ± 7 (7)</td>
<td>0.38 ± 0.03* (7)</td>
<td>203 ± 22 (7)</td>
</tr>
<tr>
<td>5A</td>
<td>7 ± 1 (4)</td>
<td>13 ± 2 (4)</td>
<td>79 ± 25 (4)</td>
<td>0.39 ± 0.02 (4)</td>
<td>90 ± 14 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. The number of arterioles is shown in parentheses.

DC = control diameter; DV = relaxed diameter after adenosine (10$^{-4}$ M); ΔD = increase in diameter after topical application of 10$^{-4}$ M adenosine; M/L = media/lumen ratio; MA = medial area; 1A-5A = first through fifth order arterioles.

*p<0.05, compared with values in normotensive hamsters.
Further if the data were analyzed in terms of transmural pressure and flow in the small arterioles. Therefore, in renal hypertension, myogenic autoregulation of small arterioles may contribute to the development of the elevated blood pressure.

An increase in the reactivity of these small arterioles may be due to changes in the sensitivity of these arterioles to vasoactive stimuli, or there may be a mechanical explanation. Various studies have shown that the sensitivity of small arterioles to vasoconstrictor agents (e.g., norepinephrine and angiotensin) is augmented in renal hypertension, but there is no evidence that alterations in arteriolar reactivity are related to arteriolar mechanics (radius-stress relation) in hypertension. However, arterioles have been shown to have an optimal wall stress in response to norepinephrine, and small arterioles operate at that point in normotensive states, whereas large arteries operate past this point. This finding may explain, in part, the differential reactivity to vasoactive stimuli in segmental arterioles in normotensive as well as in hypertensive states. For example, in the RHT group, the third and fourth order arterioles may be operating closer to this point of optimum wall stress than are those same arterioles in the NT group; thus, upon activation, the result is an enhanced reactivity. With hypertension, the increase in arteriolar pressure, if not occurring with an equivalent decrease in radius/wall thickness, will increase wall stress and reactivity; thus causing a shift toward the point of optimal stress. This sequence assumes that the arteriolar of a NT hamster operates on the ascending limb of the length-stress curve and that with hypertension the optimum point of wall stress is not exceeded. In addition, the arterioles of both the NT and hypertensive groups must exhibit good vascular tone, since maximal vasodilation with adenosine in the cheek pouch has been shown to nearly double wall tension in third and fourth order arterioles, with little change in tension of first and second order arterioles. In the present experiment, the resting diameters of the larger arterioles was decreased in the RHT hamsters while the vasodilator tone was increased. Thus, the optimum point of stress may not have been altered in these arterioles; therefore, the altered reactivity of the third order arteriole may be due to a change in the sensitivity of the vascular smooth muscle to vasoactive stimuli. However, this scenario is not consistent for the results obtained in the small arteriole (fourth order), that is, no change in either the resting diameter or vasodilator tone, but an increased arteriolar gain to changes in transmural pressure. Therefore, there may be a shift of the optimal stress for the arterioles toward the smaller vessels and thus, an increased mechanical advantage.

In most microvascular studies of renal hypertension, either some or most of the arteriolar orders are constricted. Autoregulatory responses may contribute to the vasoconstriction of arterioles seen in animals with renal hypertension, since these responses are enhanced with developing renal hypertension and exist after acute renal artery stenosis. Third order arterioles of the RHT group were constricted and demonstrated greater responses to chamber pressure than did those of the NT group. However, fourth order arterioles of the RHT group were not constricted, although they exhibited enhanced responses to pressure. Constriction of second and third order arterioles may have normalized microvascular pressure or wall stress, or both, in fourth order arterioles, thus removing the stimulus for autoregulation.

Metabolic or flow-dependent mechanisms could also contribute to changes in vascular tone and control diameters in renal hypertension. Increasing chamber pressure may have also increased arteriolar pressure and thus decreased perfusion pressure. Davis et al. found that increases in chamber pressure result in less of a decrease in transmural pressure and diminished responses compared with equivalent decreases in chamber pressure. This response may be due to venous collapse and an increase in intravascular pressure. In this study, the responses to positive and negative chamber pressure were equal. This finding could be explained by a larger contribution of flow-dependent mechanisms and less of a contribution of pressure-dependent mechanisms when chamber pressure was increased.

**Autoregulation in Hypertension**

Renal dysfunction that leads to fluid retention can increase plasma volume, venous return, and cardiac output; this sequence could lead to overperfusion of the various tissues and constriction of the small arterioles or "whole body" autoregulation. Plasma volume does not necessarily have to be elevated to increase cardiac filling and output. For example, an increased venous tone or decreased venous compliance could enhance venous return, which could lead to an early increase in cardiac output and thus be an important factor in the genesis of renal hypertension.

In general, one-kidney, one clip hypertension in the rat is thought to be a low renin, volume-dependent type of renal hypertension. Plasma renin may be high initially but usually returns to normal levels. Plasma and blood volumes have been found to be high in rats with moderate, but not severe, hypertension. With two-kidney, two clip renal hypertension in rats, plasma or blood volumes have been reported to rise transiently, but, in general, do not change with either moderate or severe hypertension. In the present study, these two-kidney, two figure-8 hypertensive hamsters, like the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 5)</th>
<th>Hypertensive (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>122 ± 8</td>
<td>112 ± 5</td>
</tr>
<tr>
<td>Plasma volume (ml/100 g)</td>
<td>4.4 ± 0.2</td>
<td>6.3 ± 0.6*</td>
</tr>
<tr>
<td>Blood volume (ml/100 g)</td>
<td>8.3 ± 0.4</td>
<td>10.8 ± 0.8*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46.6 ± 0.8</td>
<td>42.0 ± 1.6*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.05, compared with values in normotensive hamsters.
one-kidney rat, had mild hypertension and were volume-expanded 10 to 16 days after renal compression. The relation between volume expansion and arteriolar reactivity has not been established; however, there may be a link between the elevated blood volume and the release of a natriuretic compound that could alter vascular tone or mechanics, or both, and thus, alter reactivity to pressure. Therefore, the increased blood volume in renal hypertension may have resulted in arteriolar autoregulation and vasoconstriction through a change in the mechanical properties or the sensitivity of the vascular smooth muscle to vasoactive stimuli.

Furthermore, generalized autoregulation could contribute to renal hypertension as the result of a nonuniform increase in peripheral resistance during acute renal hypertension. Meininger et al. point out that autoregulation of arterioles contributes to the rise in peripheral resistance in acute renal hypertension without the necessity of volume expansion, which presumably does not occur immediately after unilateral renal artery constriction. However, it still remains to be shown that these acute autoregulatory responses can be maintained or that they contribute to the development of renal hypertension. This point is accentuated further by the studies of Alson et al., in which they concluded that the autoregulatory response mechanisms were not affected by the developing or hypertensive state in spontaneously hypertensive rats; this conclusion is consistent with a structural basis for the maintenance of an elevated peripheral resistance. However, the present study suggests that enhanced autoregulatory responses still may contribute to the elevated peripheral resistance after the initiation of renal hypertension.

Geometric and Structural Alterations

Hypertrophy of vascular smooth muscle will cause an increase in the media/lumen ratio that will give the vessel a mechanical advantage and an increase in reactivity to various stimuli. In the present study, this ratio was elevated in the second and fourth order arterioles of the RHT hamster, but the medial area was not changed. Thus, the greater reactivity of third order arterioles cannot be explained by an increased media/lumen ratio, whereas this increased ratio could explain the increased reactivity of the fourth order arterioles. However, the media/lumen ratio also was greater in second order arterioles of the RHT group, and the reactivity of these arterioles was identical. Thus, neither the media/lumen ratio nor the medial cross-sectional area appeared to be related to the altered reactivity of arterioles at this stage of renal hypertension. This conclusion does not rule out the possibility that changes in the media/lumen ratio or vascular hypertrophy could affect arteriolar reactivity with chronic renal hypertension or that the decreased diameters of the larger arterioles may initiate vascular hypertrophy, which would maintain the elevated blood pressures.

In conclusion, small arterioles of the NT and RHT groups were more reactive to pressure stimuli and this reactivity was greater in hamsters with renal hypertension. This enhanced reactivity could contribute to the maintenance of elevated peripheral resistance in this early stage of renal hypertension. This increased reactivity of small arterioles was not related to structural alterations, either to the media/lumen ratio or to the medial cross-sectional area; however, this increased reactivity occurred at a time when plasma volume was elevated.

Acknowledgments

The authors express their appreciation to Glenda Sharpe, Tim Grinbergs, and Denise Raenisch for technical assistance; to Ruth D. Cozette for preparation of the manuscript; and to Cindy Norton for administrative assistance. Dr. Russell L. Prewitt, Jr., provided pertinent suggestions.

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Hypertension. 1987;10:82-92
doi: 10.1161/01.HYP.10.1.82

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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