Hemodynamic and Microvascular Responses in the Hindquarters during the Development of Renal Hypertension in Rats

Evidence for the Involvement of an Autoregulatory Component

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SUMMARY. Microvascular responses in the cremaster muscle were compared with changes in mean arterial pressure and hindquarters vascular resistance during the development (initial 3 hours) of two-kidney one-clip renal hypertension in rats, to examine the possibility that an autoregulatory mechanism may contribute to the development of hypertension. Rats were anesthetized with urethane and chloralose and implanted with Doppler flow probes on the renal artery and abdominal aorta. Acute hypertension was produced by inflation of a balloon occluder on the renal artery to reduce renal flow by 50%. The cremaster muscle was isolated with intact innervation and circulation for measurement of microvessel diameters. To determine whether increased pressure contributed to the changes in hindquarters vascular resistance or in microvascular diameters during acute hypertension, arterial pressure was prevented from increasing in the hindquarters region after renal artery stenosis by servo-controlled inflation of a balloon occluder around the sacral aorta to maintain hindquarters pressure at normotensive levels. In hypertensive rats with unprotected hindquarters, mean arterial pressure and hindquarters vascular resistance increased 26% and 20%, respectively, after renal artery stenosis. In comparison, hypertensive rats with protected hindquarters exhibited a similar increase in mean arterial pressure, but hindquarters vascular resistance was significantly reduced compared to that in hypertensive rats with unprotected hindquarters. In the cremaster microcirculation, vasoconstriction was observed only in the small third- (mean ± SEM: 29 ± 4 μm) and fourth- (12 ± 2 μm) order arterioles in rats with unprotected hindquarters. In general, microvessel diameters in rats with protected hindquarters that had lower hindquarters vascular resistance than those with unprotected hindquarters were larger for the second- (82 ± 5 μm), third-, and fourth-order arterioles, suggesting less vasoconstrictor tone. Our data indicate that the increase in hindquarters resistance during the development of renal hypertension is, in part, dependent on the presence of a pressure-dependent autoregulatory process. (Circ Res 55: 609-622, 1984)

THE development and maintenance of an increased arterial pressure in hypertension is consistently associated with the concurrent development and maintenance of an elevated total peripheral resistance (Haddy et al., 1968). This abnormally high resistance is produced and sustained by both functional (vasomotor) and structural changes in the blood vessels (Winquist et al., 1982). However, separation of these vascular changes according to mechanism, location, and time course has been difficult, probably due to their complex interactions. In this regard, delineation of the structural and functional alterations within the vasculature would help to distinguish those vascular changes that primarily cause pressure to increase from those reactions that are secondarily induced by the hypertensive process. Structural alterations are thought to be adaptive in nature and to evolve over time as hypertension progresses from early developmental to more established phases (Brecher et al., 1978; Folkow, 1978; Spector et al., 1978). In our study, the regional and microvascular components of resistance were investigated during the early development of renal hypertension. Thus, we were able to study the vaso-motor changes that alter peripheral resistance in the absence of superimposed structural alterations in the vasculature.

Autoregulation is one vasomotor response that has been implicated as having a primary role in the development of hypertension (Granger and Guyton, 1969; Coleman et al., 1971). However, the actual role it plays in hypertension remains a controversial issue for various reasons (Haddy and Overbeck, 1976; Davis, 1977; Ferrario and Page, 1978; Freeman et al., 1982). One reason for the continued controversy is the difficulty in experimentally eliminating those variables that would induce autoregulation, such as increased pressure and/or flow, in an intact
whole animal that is developing hypertension. A major goal of this study was to investigate the hypothesis that an autoregulatory mechanism may be one functional vascular process that contributes to the changes in hindquarters resistance and in the diameters of skeletal muscle arterioles during the development of two-kidney one-clip renal hypertension in the rat. To test this hypothesis, a method was devised to prevent arterial pressure from increasing in the hindquarters region during the onset of hypertension by controlling the inflation of a balloon occluder placed around the lower abdominal aorta. Using this technique, we were able to assess the impact of an elevated local pressure on the development of an increase in hindquarters vascular resistance during the early development of renal hypertension.

In our study, measurement techniques to examine microcirculatory (tissue level) and regional (organ level) vascular hemodynamics were combined within a single animal to localize the relevant sites of microvascular responses that would correlate with the regional change in resistance. Also, we used an acute model of hypertension to evaluate the very early hemodynamic events that immediately follow stenosis of the renal artery. Thus, regional and microvascular components of resistance were studied not only in the absence of structural alterations, but, also, within the time frame that local autoregulatory mechanisms are known to operate (Johnson, 1964). Collectively, these techniques permitted us to associate the changes in hindquarters resistance with specific sites of microvascular change in skeletal muscle during the early stage of renal hypertension and to determine whether an autoregulatory mechanism is important during the development of renal hypertension.

**Methods**

**General Animal Preparation**

In our study, seven- to eight-week-old, male, Sprague-Dawley rats (mean ± SEM: 215 ± 8 g) were anesthetized by an intraperitoneal injection of urethane (425 mg/kg) and chloralose (100 mg/kg) and were tracheostomized. Anesthetic supplements consisting of 10–20% of the initial anesthetic dosage were given when an animal demonstrated signs of arousal. Supplements were given by intraperitoneal injection at intervals not less than 30 minutes apart to prevent over-anesthetization of the rats. Mean arterial pressure was measured from the cannulated left carotid artery and heart rate was measured from ECG recordings. Rectal temperature of the rats was monitored with a temperature probe and maintained between 35.5°C and 37.5°C by a heating pad placed beneath the rat.

**Pulsed Doppler Flow Velocity and Measurement of Hindquarters Resistance**

The Doppler flow velocity measurement system utilized miniaturized flow probes that are composed of a single piezoelectric crystal (1 mm diameter) which emit a 20 MHz signal and alternately receive reflected sound waves from passing blood cells. The flow probes are approximately 2.5–4 mm long and 2 mm in cross-section, with lumen diameters that were matched to the artery on which the probes were used (Haywood et al., 1981). For measurement of hindquarters and renal blood flow velocity, probes were placed on isolated segments of the lower abdominal aorta and left renal artery, respectively. Flow probe cuffs were closed with silk suture to maintain the internal cuff diameter, and the probe leads were anchored with silk suture to surrounding muscle tissue to prevent probe movement and to maintain the orientation of the probe with respect to the vessel. Flow probe leads were exteriorized through the abdominal wall and connected to the Doppler flow meter. Signals from the flow probe were recorded as mean velocity in KHz Doppler shift. Hindquarters resistance was calculated by dividing mean arterial pressure by mean Doppler flow velocity in KHz.

**Production of Acute Renal Hypertension**

For production of acute renal hypertension, an inflatable vascular occluder cuff (Faber and Brody, 1983) was placed around the left renal artery upstream from the Doppler flow probe. The occluder cuff was inflated with a servo-controlled peristaltic pump which was set to reduce and maintain renal artery flow velocity at 50% of the initial control value. Control renal flow was determined during a 30-minute control period immediately preceding renal artery stenosis. The right kidney was not surgically disturbed during any of the procedures.

**Maintenance of Normal Arterial Pressure within the Hindquarters**

To prevent arterial pressure from increasing in the hindquarters region during the development of hypertension, an inflatable vascular occluder cuff was placed around the lower abdominal aorta downstream from the aortic Doppler flow probe. The occluder cuff was inflated with a servo-controlled peristaltic pump which was set to maintain local arterial pressure, as measured in the cannulated tail artery or femoral artery, at the control normotensive level. Control normotensive pressures were determined during a 30-minute control period prior to reduction of renal artery flow.

**Microcirculatory Preparation of the Cremaster Skeletal Muscle**

The cremaster muscle was prepared for observation with a technique previously described (Miller and Wiegmans, 1977). The right cremaster muscle was gently dissected free from the scrotal skin and testicle. At this point, the animal was positioned over a heating pad on a custom designed Plexiglas board so that the hind legs of the rat straddled a raised, 45-ml tissue bath chamber. The cremaster with intact innervation and circulation then was extended into the bath chamber and positioned to lay flat over an optical port in the bath chamber. The cremaster was anchored over the optical port with sutures placed in the margin of the muscle and secured to the chamber wall. The bath chamber was filled with warmed (34.5°C) Krebs solution consisting of 25.5 mM NaHCO3, 112.9 mM NaCl, 4.7 mM KCl, 2.00 mM CaCl2·2H2O, 1.19 mM MgSO4·7H2O, and 11.6 mM dextrose. Bath pH was controlled at 7.35 to 7.45, bath PO2 at 30 to 40 mm Hg, and bath PCO2 at 40–50 mm Hg by controlling the rate at which nitrogen and carbon dioxide were bubbled into the bath chamber. The entire preparation was placed on the
stage of a trinocular microscope, and the microcirculation was viewed using a video microscopy system.

**Measurement of Microvascular Diameter**

Lumen diameter (μm) measurements for selected arterioles and venules were made on-line with an imageshearing monitor. Vessels were classified for study on the basis of their branching pattern in the cremaster. The largest central arteriole in the cremaster was defined as the first-order arteriole or 1A. The largest venule, which paralleled the first-order arteriole, was designated as the first-order venule or 1V. Vessels that branched from the first-order arteriole and venule were defined as second-order arterioles and venules, 2A and 2V, respectively. Further branchings were numbered consecutively.

In a given microcirculation experiment, the fourth-order arteriole was selected as a branch from the selected third-order arteriole, and the selected third-order arteriole as a branch from the selected second-order arteriole. Venules were likewise selected branches that connected branches were connected in series. Selected third- and fourth-order arterioles always terminated in finer branches giving rise to capillaries.

**Experimental Protocols**

All experiments consisted of a 30-minute control period during which measurements were made at 5-minute intervals. After the 30 minutes of control recordings, renal artery stenosis was produced by inflating the balloon occluder to reduce renal artery flow velocity by 50%. Measurements were then continued for 3 hours at 5-minute intervals. After 3 hours of observations, the renal artery stenosis was removed and measurements were continued at 15-minute intervals throughout a 45-minute recovery period. Control groups of rats underwent similar surgical preparation and treatment, but did not undergo renal artery stenosis. In our hands, the sham-operated, control rats had baseline arterial pressures that were not different from the baseline pressures observed in normotensive rats from other studies that have not undergone handling of the kidney, indicating that the renal manipulations were not having an effect on resting arterial pressure.

In rats implanted with a balloon occluder around the abdominal aorta to control hindquarters arterial pressure, the control of hindquarters pressure was initiated at the time of renal artery stenosis. To study the microvascular changes in cremaster muscle of hypertensive rats with hindquarters normotension, we cannulated the femoral artery to monitor hindquarters arterial pressure. In another group of hypertensive rats, the changes in hindquarters resistance were studied during hindquarters normotension and were calculated using arterial pressure as measured in the cannulated tail artery. To control for the placement of an occluder on the aorta, a second group of hypertensive rats with unprotected hindquarters and a second sham normotensive group were implanted with vascular occluders around the aorta, but the occluders were not inflated.

In hypertensive rats with protected hindquarters, the effect of a step increase in hindquarters pressure on hindquarters resistance was studied by sudden deflation of the vascular occluder on the aorta. This procedure quickly increased hindquarters pressure to hypertensive levels. In several of these animals, the occluder was again inflated to return hindquarters pressure to normotensive levels so that we might examine the effect of reprotein.
TABLE 1
Mean Control Period Values for Macrocirculatory and Microcirculatory Variables Collected during a 30-Minute Period before Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Animal groups*</th>
<th>With cremaster</th>
<th>Without cremaster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT/NT (8)</td>
<td>HT/HT (14)</td>
</tr>
</tbody>
</table>

**Macrocirculation**

<table>
<thead>
<tr>
<th>Variables</th>
<th>NT/NT</th>
<th>HT/HT</th>
<th>NT/NT</th>
<th>HT/HT</th>
<th>NT/NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>86 ± 4</td>
<td>78 ± 2</td>
<td>85 ± 3</td>
<td>93 ± 5</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>394 ± 24</td>
<td>335 ± 15</td>
<td>376 ± 15</td>
<td>393 ± 26</td>
<td>415 ± 17</td>
</tr>
<tr>
<td>Aortic flow velocity (KHz shift)</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.6 ± 0.4</td>
<td>2.8 ± 0.9</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Hindquarter resistance (mm Hg/KHz)</td>
<td>35 ± 2</td>
<td>34 ± 4</td>
<td>36 ± 6</td>
<td>32 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>Renal flow velocity (KHz shift)</td>
<td>3.4 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.5 ± 0.2</td>
</tr>
</tbody>
</table>

**Microcirculation**

<table>
<thead>
<tr>
<th>Diameter (μm)</th>
<th>NT/NT</th>
<th>HT/HT</th>
<th>NT/NT</th>
<th>HT/HT</th>
<th>HT/NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>129 ± 4</td>
<td>139 ± 6</td>
<td>131 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>82 ± 7</td>
<td>82 ± 5</td>
<td>82 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>23 ± 2</td>
<td>29 ± 4</td>
<td>24 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4A</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3V</td>
<td>46 ± 4</td>
<td>48 ± 4</td>
<td>47 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2V</td>
<td>119 ± 15</td>
<td>116 ± 10</td>
<td>111 ± 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1V</td>
<td>215 ± 9</td>
<td>217 ± 9</td>
<td>217 ± 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* NT/NT designates the normotensive control rats and HT/HT the whole body hypertensive rats unprotected hindquarters. Hypertensive rats with protected hindquarters are designated HT/NT. The number of animals is given in parentheses.

† Values presented are mean ± SEM. Table values represent the means for the 30-minute control period which preceded renal artery stenosis. 1A, 2A, 3A, and 4A designate the first-, second-, third-, and fourth-order arterioles, respectively. IV, 2V, and 3V represent the first-, second-, and third-order venules, respectively. The diameter data for the 4A arterioles in each group represents the data from four rats.

‡ The number of 4A arterioles is 5 for NT/NT, 6 for HT/HT, and 4 for HT/NT.

FIGURE 1. This figure compares the changes in mean arterial pressure (top panel) and hindquarters resistance (middle panel) following reduction of renal artery flow velocity (bottom panel-dotted lines) in two-kidney one-clip hypertensive rats with sham normotensive control rats. Data are presented as the mean % of control ± SEM. The filled circles represent the hypertensive rats and the open circles designate the control normotensive rats. A statistically significant difference (P ≤ 0.05) between the two groups is indicated with an asterisk.
normotensive control rats (middle panel, Fig. 1; third panel, Fig. 2). Removal of the stenosis was followed by a return of vascular resistance to control values. By contrast, hindquarters resistance in hypertensive rats with protected hindquarters was significantly less than hindquarters vascular resistance in hypertensive rats with unprotected hindquarters throughout the period of renal artery stenosis (third panel, Fig. 2). Immediately after renal artery stenosis, hindquarters resistance in rats with hindquarters normotension decreased, compared to the control period and compared to the sham normotensive group (third panel, Fig. 2). This fall in hindquarters resistance was followed by a slow but significant increase in resistance over the remainder of the hypertensive period. During the recovery period, hindquarters resistance in rats with protected hindquarters was not different from resistance in rats with unprotected hindquarters (third panel, Fig. 2).

In hypertensive rats in which the balloon occluder on the aorta was inflated to maintain normal hindquarters arterial pressure, the hemodynamic effects...
of a step increase in hindquarters arterial pressure were examined by sudden deflation of the vascular occluder. Sudden deflation of the balloon occluder resulted in an immediate increase in hindquarters arterial pressure, as measured in the tail artery (second panel, Fig. 3). In the new steady state, carotid artery pressure was at or near the pre-deflation level (top panel, Fig. 3). In comparison, flow velocity in the aorta increased transiently to a peak 61% above the pre-release control velocity, followed by a return toward control to a steady state value 16% above pre-release aortic flow velocity (third panel, Fig. 3). After occluder deflation, hindquarters resistance fell transiently and then increased to 22% above the pre-release control resistance (bottom panel, Fig. 3).

In several animals, the reversibility of the effect of protection on hindquarters resistance was examined by again inflating the balloon occluder on the aorta to reprotect the hindquarters from the elevated pressure. The results of this reprotection are summarized in Figure 4. Reinflation of the occluder returned tail artery pressure to control normotensive values (second panel, Fig. 4) resulting in a significant reduction in flow velocity in the aorta (third panel, Fig. 4) and hindquarters vascular resistance (bottom panel, Fig. 4).

The combined groups’ mean ± SEM heart rate for the control period was 393 ± 12 beats/min (Table 1). Heart rate did not change significantly after renal artery stenosis for any of the groups.

**Microcirculatory Changes**

The mean 30-minute control period diameters for the arterioles and venules which were selected for study are summarized in Table 1. There were no differences in the 30-minute control period arteriole and venule diameters between the groups.

**Hypertensive Rats with Unprotected Hindquarters vs. Sham Normotensive Rats**

After renal artery stenosis, there was a gradual increase (9%) in the diameter for the first-order arterioles in hypertensive rats with unprotected hindquarters, compared to the sham normotensive rats (second panel, Fig. 5). Removal of the renal artery stenosis was followed by a return of first-order arteriole diameter to control values. In contrast, second-order arteriole diameters in the hypertensive rats were not significantly different from comparable arteriole diameters in sham normotensive rats (third panel, Fig. 5). During the initial 60 minutes after renal artery stenosis, third-order arterioles in the hypertensive rats decreased compared to third-order arterioles in sham normotensive rats (bottom panel, Fig. 5). This was followed by a gradual increase in the diameter of these arterioles to diameters similar to those observed in the sham normotensive rats. Fourth-order arterioles in the hypertensive rats responded comparably to the third-order arterioles (top panel, Fig. 6).

After stenosis of the renal artery, there were no detectable venule diameter differences between the hypertensive rats and the sham normotensive rats at any branch level (Fig. 7).

**Hypertensive Rats with Unprotected Hindquarters vs. Hypertensive Rats with Protected Hindquarters**

During the hypertensive period, the first-order arterioles in the hypertensive rats with protected hindquarters were not different from prestenosis control diameters (third panel, Fig. 8). Second-order arterioles in rats with protected hindquarters dilated during the hypertensive period, followed by a return toward the prestenotic control diameter after removal of the renal artery stenosis (fourth panel, Fig. 8). Compared to hypertensive rats with unprotected hindquarters, the second-order arterioles in rats with protected hindquarters tended to exhibit more dilation during the hypertensive period; however, a difference could not be demonstrated statistically (fourth panel, Fig. 8).

Third-order arterioles in rats with protected hindquarters exhibited an initial period (30 minutes) of vasoconstriction following stenosis of the renal artery which was smaller in magnitude than the constriction observed in rats with unprotected hindquarters (bottom panel, Fig. 8). However, the diameters during this period of constriction were not different from those observed in sham normotensive rats over a similar time interval (bottom panel, Fig. 8).
5). After the initial period of vasoconstriction, third-order arterioles in rats with protected hindquarters dilated compared to their prestenosis control diameter (bottom panel, Fig. 8). Fourth-order arterioles in the rats with protected hindquarters did not exhibit the initial period of vasoconstriction which was observed in rats with unprotected hindquarters (bottom panel, Fig. 6). Instead, fourth-order arterioles in the rats with protected hindquarters dilated compared to their prestenosis control value (bottom panel, Fig. 6). Compared to hypertensive rats with unprotected hindquarters, both third- and fourth-order arterioles demonstrated a strong tendency to dilate in rats with protected hindquarters, but this trend was not statistically significant over the entire period of renal artery stenosis (bottom panel, Fig. 6).

Venule diameters in hypertensive rats with protected hindquarters were not different from venule diameters in rats with unprotected hindquarters at any branch level (Fig. 9).

Discussion

Autoregulation and Hypertension: Macrocirculatory Evidence

By definition, autoregulation is the ability of a tissue to maintain perfusion at levels appropriate for its metabolic need in the face of changes in perfusion pressure or flow (Johnson, 1964). According to the
theory of whole body autoregulation, increased resistance during the development of hypertension results from an increased cardiac output that causes tissue perfusion to exceed metabolic requirements, which, in turn, produces vasoconstriction (Granger and Guyton, 1969; Coleman et al., 1971; Coleman et al., 1979; Cowley, 1980). As stated, the theory of whole body autoregulation is contingent upon a period of increased cardiac output to cause the tissue overperfusion. However, the presence of an increased cardiac output during the early stages of renal hypertension remains controversial and, consequently, has continued to make a role for autoregulation during the development of hypertension debatable (Haddy and Overbeck, 1976; Davis, 1977; Ferrario and Page, 1978; Freeman et al., 1982).

One alternative possibility that we feel would permit a contributory role for autoregulation during the onset of renal hypertension could be that there is an increase in mean arterial pressure resulting from nonuniform development of increased resistance within different regional circulations in the early stages of hypertension. In this situation, the potential for overperfusion exists in those regions in which local arterial pressure increases in disproportion to local changes in resistance. In our study, mean arterial pressure increased proportionally more than hindquarters resistance during the development of hypertension. Thus, the conditions for overperfusion and, hence, autoregulation exist in the hindquarters region during the early developmental phase of renal hypertension. Furthermore, these data suggest that other regional circulations are undergoing more intense increases in resistance than the hindquarters region. A nonhomogeneous development of increased resistance among differ-
ent organs has been reported for salt-loading hypertension in the dog (Liard, 1981) and for renal hypertension in the rat (Bralet et al., 1973; Faber and Brody, 1983).

We investigated the possibility that an autoregulation-mediated increase in vascular resistance was elicited by overperfusion or increased pressure within the hindquarters region by protecting the hindquarters from the elevated arterial pressure associated with developing hypertension. Compared to hypertensive rats with unprotected hindquarters, the development of increased vascular resistance in the hindquarters was clearly blunted in magnitude in rats with protected hindquarters (third panel, Fig. 2). Thus, these data strongly suggest that an autoregulatory component is one vasoconstrictor mechanism operating in the hindquarters region to elevate resistance.

An additional possibility is that the aortic stenosis per se may have altered neural and/or humoral vascular mechanisms by affecting the distribution of systemic pressure or flow. As such, the lower hindquarters resistance could have been the combined result of an autoregulatory process and an altered neurohumoral state. However, we did not note a significant effect of the stenosis on arterial pressure. In addition, further evidence for an autoregulatory mechanism was provided by sudden deflation of the aortic balloon occluder to produce a step increase in hindquarters arterial pressure from normotensive to hypertensive levels. Following sudden occluder deflation, hindquarters arterial pressure abruptly increased, producing a transient surge in aortic flow velocity and then a return to flow velocity toward the predilation value (Fig. 3). This return toward the control velocity represented a 69% compensation (calculated as 1 - [% A flow/% A pressure]) for the increase in pressure and was produced by a 22% increase (calculated as [R2 - R1]/R1 X 100 where R1 = pre-release resistance and R2 = post-release resistance) in hindquarters resistance. As a corollary to this, in several rats we observed that returning the hindquarters pressure to normotensive levels by reinflating the aortic occluder reduced hindquarters resistance (Fig. 4). Thus, the rapid time course of this phenomenon makes it unlikely that it is attributable to the appearance or disappearance of a circulating humoral factor which might be a consequence of the aortic stenosis.

## Autoregulation and Hypertension: Microcirculatory Evidence

### Evidence for Increased Vasoconstrictor Tone in Small Arterioles

Our data indicate that increased hindquarters resistance during the acute onset of renal hypertension is associated with vasoconstriction and increased arteriolar tone that is confined to the smaller third- and fourth-order arterioles in the cremaster muscle.
It is apparent that the vasoconstriction is not maintained throughout the early development of the hypertension. The progressive increase in the diameters of the third- and fourth-order arterioles over time could indicate increased dominance of vasodilatory processes in skeletal muscle during the development of the hypertension (e.g., baroreflex withdrawal or a buildup of locally generated vasodilatory substances).

The presence of a similar progressive increase in the diameters of comparable arterioles in the sham normotensive control rats also suggests that our cremaster preparation may slowly lose vascular tone. Two factors which could contribute to this phenomenon in our study include the long exposure time of the cremaster to the bath and/or the composition of the bathing solution. However, it is also possible that this loss of vascular tone is unrelated to the cremaster preparation and that it is a manifestation of more general systemic changes which could result from the presence of anesthesia or surgical stress. For example, the loss of arteriolar tone in the sham normotensive rats corresponds with a gradual reduction in hindquarters vascular resistance in these animals.

The large variance in the diameter data for the third- and fourth-order arterioles also complicates the comparisons between the groups. Two reasons for this large variance could be related to the presence of time-dependent vasomotor phenomena. In some of the sampled third- and fourth-order arterioles, cyclic vasomotion was observed, and it is possible that the response of the vasomoting vessels was different from that of the nonvasomoting vessels. Second, we observed over time that there were spontaneous changes in vascular diameter for the third- and fourth-order arterioles. These spontaneous changes appeared as periods of relative vasoconstriction and/or vasodilation, compared to the control period diameter. However, these changes were not rhythmic; neither did they demonstrate any regular pattern similar to what is typically described for vasomoting vessels. Using a mathematical model of the microcirculation, Popel and coworkers (Popel and Levin, 1982; Levin and Popel, 1982, 1983) have recently shown that increasing the diameter spread (i.e., population variance) for a given branch order of microvessels without changing the mean diameter will increase the resistance in a network of microvessels. In our study, variance increased from 10% to 30% in the third-order arterioles of the hypertensive rats. According to Popel's...
model, this change, alone, could account for a 30% increase in network resistance (A. S. Popel, personal communication).

Our data show that there are some differences between the overall time waveforms of the arteriolar data and the systemic hemodynamic data. For example, the diameter of the first-order arteriole increases as resistance increases in the hypertensive rats. We feel these differences serve to emphasize the value of combining microcirculatory and macrocirculatory techniques to identify those microvascular changes that are consistent with the regional hemodynamic responses. The arteriolar changes that are not consistent with the regional responses are no less real, but provide a good example of how the behavior of an individual microvessel can be aver-
Evidence for Autoregulation

In hypertensive rats with protected hindquarters, third- and fourth-order arteriolar vasoconstriction was attenuated or prevented following stenosis of the renal artery. Combined with our observation of reduced hindquarters vascular resistance in rats with protected hindquarters, we have interpreted this as evidence for less vasoconstrictor tone in these small arterioles. Furthermore, we suggest that the decrease in overall vascular tone is due to a net decrease in the vasoconstrictor tone contributed by a local pressure-dependent autoregulatory mechanism.

The property of autoregulation has been recently demonstrated in cremaster arterioles by Morff and Granger (1982). In their study, second- and third-order arterioles responded to decreased arterial pressure with vasodilation and to increased PO$_2$ and elevated venous pressure with vasoconstriction. The first-order arterioles were unresponsive to these changes. Moreover, their data indicated that the autoregulatory responses of the third-order arterioles were more intense than in the second-order arterioles, suggesting a greater capacity for local autoregulation in the smaller vessels. This may in part explain our observation of vasoconstriction in third- and fourth-order arterioles, but not in first- or second-order arterioles.

Possible Mechanisms and Implications

Whereas our data demonstrate that maintenance of normal pressure in the hindquarters during the onset of hypertension significantly attenuates the development of increased hindquarters vascular resistance, there is a progressive rise in the hindquarters resistance of rats with protected hindquarters during the period of renal artery stenosis. In this regard, the overall appearance of the time waveforms for hindquarters vascular resistance is similar for hypertensive rats with protected and unprotected hindquarters (third panel, Fig. 2). We have interpreted the attenuation of the development of increased hindquarters vascular resistance as evidence for the existence of a significant pressure-dependent
autoregulatory component. On the other hand, we view the similarity in the overall time waveforms as evidence for similar neural and humoral changes which should be identical in both groups. Our current hypothesis is that the local pressure-dependent phenomenon is triggered by the increase in mean arterial pressure which is initiated by the humoral and/or neural mechanisms that are set in motion after renal artery stenosis. The net effect of the autoregulatory phenomenon may be to minimize local disturbances in tissue flow, but the result is that it potentiates the overall disturbance in blood pressure control.

Since the early development of renal hypertension is generally believed to be mediated by increased activity of the renin-angiotensin system (Liard et al., 1974; Mohring et al., 1975; Carretero and Romero, 1977; Davis, 1977; Johnston et al., 1978), a vasoconstrictor component associated with high circulating levels of angiotensin II is one likely candidate for producing the similarity in the overall time waveform of the hindquarters resistance response in protected and unprotected hindquarters. There is also evidence to suggest that sympathetic activity is inappropriately elevated during renal hypertension (Ferrario and McCubbin, 1974; Brody et al., 1980). In this regard, there is increasing support for the idea that increased levels of angiotensin II may be modulating sympathetic activity both peripherally (McCubbin and Page, 1963; Zimmerman, 1981, 1983) and centrally (Brody and Johnson, 1980). A recent study has suggested that sympathetic activity in the hindquarters, mesenteric, and renal vascular regions may be inappropriately elevated within 5 hours after renal artery stenosis (Faber and Brody, 1983) and, thus, may also be a factor in contributing to the shape of the time waveform for the hindquarters resistance response in our study.

In previous work (Meininger et al., 1984), the distribution of microvascular pressure and diameter was measured across three successive branch orders of arterioles and venules in the cremaster skeletal muscle of rats with established (4 weeks) renal hypertension. These studies showed that there was a selective decrease in diameter for larger first- and second-order arterioles which acted to prevent the elevation of intravascular pressure in smaller third-order arterioles and in downstream venules. Our present study of acute renal hypertension suggests that vasoconstriction and increased arteriolar tone was confined to smaller arterioles and appeared to be the result of an autoregulatory process. One possible interpretation of the microcirculatory changes observed in skeletal muscle for acute and chronic renal hypertension is that the alterations (perhaps structural) in larger arterioles develop as hypertension becomes established, and they consequently alter the distribution of pressure and remove the local stimulus for autoregulation. As a result, the autoregulation-mediated component of vasoconstriction in smaller arterioles may disappear during the transition to more established phases of renal hypertension.

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