Increased Renal Vascular Reactivity to Angiotensin II but not to Nerve Stimulation or Exogenous Norepinephrine in Renal Hypertensive Rats

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SUMMARY We isolated and perfused both the "clipped" and "contralateral" kidneys from Goldblatt renal hypertensive and sham-operated control rats, 1-104 days postoperatively. Responses to renal nerve stimulation were depressed in clipped kidneys from hypertensive rats (1 day postoperative), and these kidneys were supersensitive to exogenous norepinephrine (1-31 days) when compared with the contralateral organ of the same animal. Similar alterations were found between clipped and contralateral kidneys from sham-operated control rats. There was no difference in responses to renal nerve stimulation of norepinephrine between clipped kidneys from hypertensive and control rats, but clipped kidneys from hypertensive rats were supersensitive to angiotensin II (17 and 31 days). Comparison of contralateral kidneys from hypertensive and control rats revealed no change in norepinephrine sensitivity or in responses to renal nerve stimulation, but there was a reduction in the slope of the dose-response curve to norepinephrine and of the maximal effect of the catecholamine (104 days) and a pronounced supersensitivity to angiotensin II (17-104 days) in the hypertensive rats. These results indicate that (1) renal nerve function and norepinephrine sensitivity of the isolated renal vasculature are unchanged in renal hypertension, but clipping partially denervates the kidney causing depressed nerve function and unilateral norepinephrine supersensitivity, unrelated to hypertension; (2) the prolonged high pressure load on the contralateral kidney may impair the function of the vascular smooth muscle; and (3) bilateral supersensitivity to angiotensin II is associated with hypertension but is not solely a consequence of the high pressure.

AN exaggerated response to vasoactive stimulation (increased vascular reactivity) has been demonstrated in a variety of vascular preparations from hypertensive rats. One explanation of this increase in reactivity is that the vascular smooth muscle is supersensitive, shortening to a greater than normal extent, in response to a submaximal stimulus. A functional change in the vascular smooth muscle can cause nonspecific increases in responsiveness to a variety of agonists, whereas changes in receptor affinity or agonist disposition will cause specific supersensitivity. The supersensitivity is unlikely to be a consequence of hypertension since it peaks as the blood pressure is rising. Alternatively, the blood vessel wall may have thickened, by medial hypertrophy, amplifying the essentially normal smooth muscle response to all vasoconstrictor agonists. A structural vascular change of this type probably is a consequence of high pressure because it occurs after the blood pressure has stabilized and is prevented by protection of the vascular bed from the high pressure.

When renal hypertension is induced by clipping one renal artery and leaving the contralateral kidney untouched, the clipped kidney is protected from the high pressure, whereas the contralateral organ is exposed to it. We have examined responses to renal nerve stimulation, norepinephrine, and angiotensin II in both kidneys from rats, 1-104 days after the induction of renal hypertension. By comparing changes in clipped (protected) and contralateral (exposed) kidneys, we hoped to separate pressure-dependent from pressure-independent changes in renal vascular reactivity.

Methods

The Induction of Renal Hypertension

Wistar rats of both sexes (160-240 g) were anesthetized (halothane-oxygen) and a silver clip was applied to one renal artery. Constricting clips, designed to induce hypertension, had apertures of 0.2 mm; nonconstricting clips (1- to 2-mm aperture) were used for sham-operated control rats. Systolic blood pressure was measured in the unanesthetized rat by a tail cuff method.

The Perfused Kidney Preparation

Renal hypertensive and sham-operated control rats were anesthetized (pentobarbital sodium, 50 mg/kg, ip) at various time periods after the clipping procedure (see Table 1), and the blood pressure was...
measured by direct carotid manometry. The renal artery clip was removed, and both renal arteries were cannulated. Both kidneys from each rat were perfused simultaneously with Tyrode's solution at 37°C using separate, identical constant flow perfusion systems. The Tyrode's solution had the following composition (mm): NaCl, 137; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.1; NaHCO₃, 12.0; NaH₂PO₄, 0.42; D(+)-glucose, 5.6; and was aerated with 5% CO₂ in O₂. The kidneys were isolated and placed in separate chambers, containing Tyrode's solution at 37°C. Electrodes placed around the renal artery, distal to the cannula, were used to stimulate the renal nerves. Renal vascular constrictor responses were recorded as increases in perfusion pressure, downstream from the perfusion pump.

Optimal perfusion conditions were determined for each kidney by step-wise increases in perfusion flow rate. A standard electrical stimulus (16 Hz, 2 msec, 10 V, 10-second duration) was applied at each flow rate, and the minimal flow that permitted a maximal constrictor response was taken as the optimum and, subsequently, used. An equilibration period of 30–45 minutes was allowed before commencing the experiment.

Frequency-response curves to electrical stimulation were obtained by increasing frequencies (2–16 Hz) of periarterial nerve stimulation for 60-second periods every 4 minutes. Agonists were administered by rapid injection of 0.02 ml into the perfusion system, close to the kidney. Injection of this volume caused a small transient increase in perfusion pressure which preceded the agonist-evoked response. This injection artifact was the only change in perfusion pressure observed when Tyrode's solution was injected into the system. Dose-response curves were made to (-)-norepinephrine bitartrate (Fluka) and then to angiotensin II amide (Ciiba). Both drugs were dissolved in Tyrode's solution; doses of norepinephrine are expressed as base, and of angiotensin II as salt. Minimum dosing cycles of 2 minutes for norepinephrine and 8 minutes for angiotensin II amide were used. When necessary, these time periods were extended until the previous response had disappeared.

Kidneys from normal Wistar rats have been used previously to evaluate the viability of this preparation. Optimum perfusion flow rate was reached at 4–5 ml/g of kidney weight per minute and generated perfusion pressures of 80–120 mm Hg (n = 9). At the optimal flow rate, basal perfusion pressure remained stable for 5–6 hours, and there was minimal evidence of edema formation, since the weight of the perfused kidneys did not significantly increase over the period of the experiment.

The Effects of Circulating Angiotensin II on Isolated Kidney Responses

To investigate any possible influence that circulating angiotensin II levels might have on responses in the isolated kidney preparation, normotensive anesthetized rats (pentobarbital, 50 mg/kg, ip) were injected with angiotensin II amide (10 μg/kg, i.v.). Immediately after the peak blood pressure response (measured by carotid manometry), the left renal artery was cannulated and the kidney isolated and perfused. The kidney was perfused for a period equivalent to the equilibration time and the time necessary for a frequency response curve and a norepinephrine dose-response curve to be made (1.5–2 hours). A dose-response curve to angiotensin II was then made and compared with angiotensin II dose-response curves in perfused kidneys from untreated rats.

Analysis of Results

An increase in vascular sensitivity to an agonist will cause a parallel leftward shift of the dose-response curve, without a change in maximal response amplitude. The magnitude of the dose-response curve shift and, consequently, of the supersensitivity of the perfused kidney preparation, was evaluated by calculating the dose of agonist which evoked 50% maximal response (ED₅₀). The ED₅₀ values are normally distributed only when converted to logarithms. To convert the log ED₅₀ to a positive integer, it is convenient to quote the negative logarithm (also known as pD₅₀). An increase in the numerical value of the −log ED₅₀ (pD₅₀) indicates a leftward shift of the agonist dose-response curve, and thus supersensitivity.

A structural vascular adaptation, involving a thickening of the vessel wall, will cause a greater maximal response amplitude and an asymmetric shift of the dose-response curve to the left, due to an increased gradient. The dose-response curve slope was evaluated by fitting a regression line to its linear portion.

Differences between means were analyzed by Student's t-test, paired when comparing kidneys from the same rat, and unpaired when comparing kidneys from hypertensive and control rats. P < 0.05 was considered to be significant. Values are quoted as mean ± SEM throughout this paper.

Results

There was no significant difference in body weight between hypertensive (direct mean blood pressure significantly greater than sham control) and sham control rats at any time after the clipping procedure (Table 1). Kidneys from a hypertensive and a sham control rat were perfused on the same day using the same solutions, in 68% of the experiments.

Rats with a constricting renal artery clip were hypertensive 5.5 days after the clipping operation (Table 1). The major increase in the blood pressure occurred in the first 31 days after clipping, after which pressure stabilized. A small number of rats with constricting clips on the renal artery exhibited an elevated systolic blood pressure (unanesthetized), but were normotensive after pentobarbital
Table 1  Body Weight, Indirect Systolic Blood Pressure, and Direct Mean Blood Pressure of Renal Hypertensive and Sham-Operated Rats

<table>
<thead>
<tr>
<th>Days post-clipping</th>
<th>Group</th>
<th>Body wt (g)</th>
<th>Indirect systolic BP (mmHg)</th>
<th>Direct mean BP (mmHg)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 ± 0</td>
<td>H</td>
<td>216.0 ± 27.7</td>
<td>140.0 ± 7.0</td>
<td>77.6 ± 8.0</td>
<td>5</td>
</tr>
<tr>
<td>1.0 ± 0</td>
<td>S</td>
<td>227.0 ± 26.3</td>
<td>140.2 ± 2.5</td>
<td>83.6 ± 11.5</td>
<td>5</td>
</tr>
<tr>
<td>5.5 ± 0.9</td>
<td>H</td>
<td>230.5 ± 21.3</td>
<td>151.7 ± 8.2</td>
<td>119.7 ± 3.6*</td>
<td>6</td>
</tr>
<tr>
<td>5.5 ± 1.0</td>
<td>S</td>
<td>224.6 ± 25.4</td>
<td>134.5 ± 5.0</td>
<td>101.0 ± 4.6</td>
<td>6</td>
</tr>
<tr>
<td>16.7 ± 1.5</td>
<td>H</td>
<td>257.9 ± 14.2</td>
<td>173.7 ± 4.9*</td>
<td>136.2 ± 4.4*</td>
<td>6</td>
</tr>
<tr>
<td>17.0 ± 1.8</td>
<td>S</td>
<td>266.0 ± 9.2</td>
<td>131.3 ± 5.9</td>
<td>107.0 ± 3.3</td>
<td>6</td>
</tr>
<tr>
<td>31.3 ± 1.4</td>
<td>H</td>
<td>246.4 ± 5.1</td>
<td>183.3 ± 10.7*</td>
<td>164.0 ± 8.2*</td>
<td>6</td>
</tr>
<tr>
<td>31.7 ± 1.0</td>
<td>S</td>
<td>234.8 ± 5.3</td>
<td>134.7 ± 3.2</td>
<td>110.2 ± 8.3</td>
<td>6</td>
</tr>
<tr>
<td>61.8 ± 2.1</td>
<td>H</td>
<td>291.2 ± 14.2</td>
<td>170.2 ± 8.0*</td>
<td>152.6 ± 10.1*</td>
<td>5</td>
</tr>
<tr>
<td>59.2 ± 1.5</td>
<td>S</td>
<td>335.0 ± 55.8</td>
<td>134.4 ± 2.2</td>
<td>109.4 ± 7.3</td>
<td>5</td>
</tr>
<tr>
<td>104.5 ± 1.6</td>
<td>H</td>
<td>345.5 ± 23.2</td>
<td>199.0 ± 4.1*</td>
<td>168.2 ± 7.1*</td>
<td>6</td>
</tr>
<tr>
<td>104.0 ± 1.8</td>
<td>S</td>
<td>353.3 ± 32.0</td>
<td>136.3 ± 5.8</td>
<td>106.7 ± 5.5</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. BP = blood pressure; H = hypertensive; S = sham.
* Significantly different from sham-operated control rat (P < 0.05).

Table 2  The Weight of Clipped and Contralateral Kidneys from Renal Hypertensive and Sham-Operated Rats

<table>
<thead>
<tr>
<th>Days post-clipping</th>
<th>Group</th>
<th>Weight (% body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 ± 0</td>
<td>H</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>1.0 ± 0</td>
<td>S</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>5.5 ± 0.9</td>
<td>H</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>5.5 ± 1.0</td>
<td>S</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>16.7 ± 1.5</td>
<td>H</td>
<td>0.41 ± 0.02†</td>
</tr>
<tr>
<td>17.0 ± 1.8</td>
<td>S</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>31.3 ± 1.4</td>
<td>H</td>
<td>0.45 ± 0.02†</td>
</tr>
<tr>
<td>31.7 ± 1.0</td>
<td>S</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>61.8 ± 2.1</td>
<td>H</td>
<td>0.39 ± 0.01†*</td>
</tr>
<tr>
<td>59.2 ± 1.5</td>
<td>S</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>104.5 ± 1.6</td>
<td>H</td>
<td>0.34 ± 0.01*</td>
</tr>
<tr>
<td>104.0 ± 1.8</td>
<td>S</td>
<td>0.40 ± 0.01</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM; for n see Table 1. H = hypertensive; S = sham.
* Significantly different from sham-operated control.
† Significantly different from contralateral kidney (P < 0.05).

anesthesia. The results from these rats were not included.

Hypertensive Rats, Clipped and Contralateral Kidneys

The clipped kidneys of the renal hypertensive rats were significantly lighter than the contralateral organs from the same animals from 16.7 to 104 days after the clipping procedure (Table 2). There was no significant difference in optimal flow rate or basal resistance to flow between clipped and contralateral kidneys at any time (Table 3). One day after the clipping operation, the amplitude of the response to renal nerve stimulation in clipped kidneys was significantly smaller than that in contralateral kidneys from hypertensive rats (Fig. 1). This reduction was still significant when the response amplitude evoked by an 8-Hz stimulation was expressed as a percentage of the maximal norepinephrine-evoked response amplitude (clipped kidney = 22.0 ± 11.5%; contralateral kidney = 49.0 ± 6.8%). There was no significant difference between the amplitudes of responses to renal nerve stimulation between clipped and contralateral kidneys at all subsequent times (Fig. 1).

Responses evoked by submaximal doses of norepinephrine were of significantly greater amplitude in clipped than in contralateral kidneys from hypertensive rats from 1 to 104 days after the clipping procedure (Table 4). This resulted in a higher —log ED50 in the clipped kidney, which was significantly different from the value for the contralateral kidney at 1, 17, and 31 days (Fig. 2). From 1 to 61 days postoperatively, there was no significant difference between the norepinephrine dose-response curve slopes or maximal responses in clipped and contralateral kidneys from hypertensive rats. At the 104-day stage, the clipped kidneys had a significantly
### Table 3  The Optimal Flow Rate and Basal Resistance to Flow of Clipped and Contralateral Kidneys from Renal Hypertensive and Sham-Operated Control Rats

<table>
<thead>
<tr>
<th>Days post-clipping</th>
<th>Group</th>
<th>Clip</th>
<th>Contralateral</th>
<th>Clip</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 ± 0.0</td>
<td>H</td>
<td>4.3 ± 0.4</td>
<td>4.6 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>1.0 ± 0.0</td>
<td>S</td>
<td>4.6 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>5.5 ± 0.9</td>
<td>H</td>
<td>4.7 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>5.5 ± 1.0</td>
<td>S</td>
<td>4.6 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>16.7 ± 1.5</td>
<td>H</td>
<td>5.3 ± 0.4</td>
<td>4.8 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>17.0 ± 1.8</td>
<td>S</td>
<td>4.7 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>31.3 ± 1.4</td>
<td>H</td>
<td>4.0 ± 0.3</td>
<td>4.3 ± 0.4</td>
<td>2.4 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>31.7 ± 1.0</td>
<td>S</td>
<td>4.2 ± 0.4</td>
<td>4.7 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>61.8 ± 2.1</td>
<td>H</td>
<td>4.4 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>58.2 ± 1.5</td>
<td>S</td>
<td>4.0 ± 0.2</td>
<td>4.4 ± 0.4</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>104.5 ± 1.6</td>
<td>H</td>
<td>4.5 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>104.0 ± 1.8</td>
<td>S</td>
<td>4.5 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM (for n see Table 1). A detailed description of optimal flow rates of basal resistance to flow (Table 3) and of norepinephrine and angiotensin II dose-response curve slopes and maximum (Table 4) in clipped and contralateral kidneys of hypertensive (H) and sham control (S) rats from 1 to 104 days postoperatively is available from the Library of Congress.

There was no significant difference in the weight, optimal perfusion flow rate, or basal perfusion pressure between clipped and contralateral kidneys from sham-operated control rats from 1 to 104 days postoperatively. One and 17 days after the clipping operation, renal nerve stimulation evoked responses in clipped kidneys whose amplitudes were significantly smaller than those in contralateral kidneys from control rats (Fig. 1). This reduction was still significant when the response amplitude evoked by an 8-Hz stimulation was expressed as a percentage of the maximal norepinephrine-evoked response amplitude (clipped kidneys = 20.6 ± 7.4%; contralateral kidney = 57.0 ± 10.4%, 1 day after clipping; clipped kidney = 30.8 ± 9.8%, contralateral kidney = 72.8 ± 9.6%, 17 days). There was no significant difference between responses of clipped and contralateral kidneys to renal nerve stimulation at all subsequent times (Fig. 1).

Responses evoked by submaximal doses of norepinephrine were of significantly greater amplitude in clipped than in contralateral kidneys from 17 to 31 days postoperatively. At 31 days postoperatively, the —log EDso of clipped kidneys from hypertensive rats was significantly smaller than that of the contralateral kidneys from the same animals (Figs. 3 and 4). There was no significant difference in the angiotensin II dose-response curve slope between clipped and contralateral kidneys from hypertensive rats at any time point. At the 104-day stage, the maximal angiotensin II-evoked response amplitude of the contralateral kidneys (108.3 ± 12.7 mm Hg) was significantly smaller than that of the clipped kidneys from the same group of rats (136.7 ± 7.7 mm Hg).

**Sham-Operated Control Rats, Clipped and Contralateral Kidneys**

There was no significant difference in the weight, optimal perfusion flow rate, or basal perfusion pressure between clipped and contralateral kidneys from sham-operated control rats from 1 to 104 days postoperatively. One and 17 days after the clipping operation, renal nerve stimulation evoked responses in clipped kidneys whose amplitudes were significantly smaller than those in contralateral kidneys from control rats (Fig. 1). This reduction was still significant when the response amplitude evoked by an 8-Hz stimulation was expressed as a percentage of the maximal norepinephrine-evoked response amplitude (clipped kidneys = 20.6 ± 7.4%; contralateral kidney = 57.0 ± 10.4%, 1 day after clipping; clipped kidney = 30.8 ± 9.8%, contralateral kidney = 72.8 ± 9.6%, 17 days). There was no significant difference between responses of clipped and contralateral kidneys to renal nerve stimulation at all subsequent times (Fig. 1).
104 days after the clipping procedure. One day postoperatively, the clipped kidney of the sham control rats had a significantly smaller maximal norepinephrine-evoked response amplitude (143.2 ± 38.3 mm Hg) than the contralateral kidney (235.6 ± 6.0 mm Hg). The −log ED₅₀ was always larger in clipped than in contralateral kidneys (Fig. 2) and this difference was significant at 1, 17, and 31 days. There was no significant difference in the norepinephrine dose-response curve slopes of clipped and contralateral kidneys from sham control rats at any time. There was no significant difference in angiotensin II dose-response curve slope, maximum nor in the −log ED₅₀ between clipped and contralateral kidneys from sham control rats at any time point investigated (Figs. 3 and 4).

Differences between Matched Kidneys from Hypertensive and Sham Control Rats

From 61 to 104 days after the clipping operation, clipped kidneys from hypertensive rats weighed significantly less than those from control rats (Table 2). At 104 days, the contralateral kidneys of hypertensive rats were significantly heavier than those of the control rats (Table 2). There was no significant difference in optimal flow rate or in basal resistance to flow between matched kidneys from hypertensive and sham control rats, at any time point. The amplitudes of responses evoked by renal nerve stimulation in matched kidneys from hypertensive and control rats were not significantly different at any time (Fig. 1). There was no significant difference in norepinephrine dose-response curve slope, maximum, or −log ED₅₀ between clipped kidneys from hypertensive and control rats from 1
VASCULAR REACTIVITY IN RENAL HYPERTENSIVE RATS/Collis and Vanhoutte

FIGURE 3  Log dose-response curves to angiotensin II in clipped and contralateral kidneys from renal hypertensive and sham control rats, 17, 31, and 61 days postoperatively. Mean increases in perfusion pressure of clipped kidneys from hypertensive rats ○—○, clipped kidneys from control rats ○—○, contralateral kidneys from hypertensive rats □—□, and contralateral kidneys from control rats □—□. Significant differences (P < 0.05) between matched kidneys from hypertensive and control rats are indicated by *. Significant differences (P < 0.05) between clipped and contralateral kidneys from the same group of rats are indicated by • instead of ○ for the clip data. (For n see Table 1.)

to 104 days and between contralateral kidneys from hypertensive and control rats from 1 to 61 days postoperatively (Fig. 2). At 104 days after the clipping procedure, the maximal response (187.3 ± 10.1 mm Hg) and the dose-response curve slope (113.4 ± 17.6 mm Hg/log dose) of contralateral kidneys from hypertensive rats were significantly less than the maximum (218.7 ± 8.4 mm Hg) and slope (187.3 ± 10.6 mm Hg/log dose) of the norepinephrine

dose-response curve in contralateral kidneys from control rats.

One and 5 days postoperatively, there was no significant difference between angiotensin II dose-response curves in matched kidneys from hypertensive and sham control rats. Seventeen, 31, and 61 days after clipping, both kidneys from the hypertensive rats responded to submaximal doses of angiotensin II with significantly greater response amplitudes than matched kidneys from sham control rats (Fig. 3). This caused a significant increase in the –log ED$_{50}$ of contralateral kidneys at 17, 31, and 61 days, and in clipped kidneys at 17, 31, but not at 61 days (0.1 > P > 0.05; Fig. 4). The –log ED$_{50}$ for angiotensin II was also significantly increased in contralateral but not in clipped kidneys from hypertensive rats, compared with matched kidneys from control rats, at the 104-day stage (Fig. 4). The angiotensin II dose-response curve slope and maximum were not significantly different between matched kidneys from hypertensive and control rats from 1 to 104 days after the clipping procedure.

The Effects of Circulating Angiotensin II on Isolated Kidney Responses

An injection of angiotensin II amide, 10 μg/kg, iv, caused the mean carotid blood pressure of normotensive anesthetized rats to rise from 96.8 ± 10.4 to 168.2 ± 7.4 mm Hg (n = 6). Kidneys from these rats were perfused and compared with simultaneously perfused kidneys from untreated rats (n = 6, mean blood pressure = 108.2 ± 7.5 mm Hg). There was no significant difference in the angiotensin II dose-response curve or in the sensitivity of kidneys from the angiotensin II-treated rats (slope = 41.3 ± 8.7 mm Hg/log dose; maximal response = 113.0 ± 14.1 mm Hg; –log ED$_{50}$ = 9.33 ± 0.17 g) and those of untreated control rats (slope = 40.4 ± 5.4 mm Hg/log dose; maximal response = 112.3 ± 10.4 mm Hg; –log ED$_{50}$ = 9.52 ± 0.16 g).

Discussion

The aim of this study was to separate pressure-dependent from pressure-independent changes in responsiveness of the renal vasculature during the development of two-kidney renal hypertension in the rat, and to assess whether the observed changes were due to structural variations or to alterations in the function of the renal vascular smooth muscle and its adrenergic innervation.

A high pressure load can cause a structural vascular adaptation, characterized by increases in basal resistance to flow and in the dose-response curve slope and maximum response to vasoactive agonists. The contralateral kidney of the two-kidney Goldblatt renal hypertensive rat was exposed to a high pressure load but exhibited no evidence of structural adaptation, in contrast to previous findings suggesting an increased wall-to-lumen ratio in
other perfused vascular preparations from 4- to 6-week-old renal hypertensive rats. Therefore, the renal vascular bed may not adapt to a high pressure load, or may adapt more slowly than other vascular circuits. Likewise, the normality of optimal perfusion flow rates in both kidneys from the renal hypertensive rats indicate that no change has occurred in the length-tension relationship of the renal vascular wall.

The present study demonstrates that, whereas the responsiveness of the renal vasculature to adrenergic stimulation is basically unaltered, a significant increase in its sensitivity to angiotensin II develops during hypertension. When clipped kidneys from hypertensive rats were compared with the contralateral kidneys from the same animals, they were found to be supersensitive to norepinephrine, with an increased $-\log ED_{50}$ from 1 to 31 days postoperatively. This unilateral supersensitivity is not related to hypertension as it also occurred in the sham-operated control rats. The origin of this supersensitivity is probably a partial renal denervation, which occurred when the clip was applied to the renal artery, as also suggested by the early depression of the responses to nerve stimulation in clipped kidneys from both hypertensive and control rats. There is reported histological evidence of a renal denervation after the application of a constricting renal artery clip, and of a later re-innervation; the latter would explain the recovery of the response to nerve stimulation and the later normalization of norepinephrine sensitivity. The inconsistent depression of the norepinephrine maximum response, 1 day postoperatively, probably also was a consequence of the manipulation involved in applying the clip to the renal artery. This change was not the major cause of the depressed responses to nerve stimulation since the latter was still significant when the data were expressed as a percentage of the norepinephrine maximum.

Except for the changes that can be explained by partial denervation on clipping the renal artery, the present study indicates that the sensitivity to norepinephrine is not augmented in both kidneys from renal hypertensive rats. An unchanged sensitivity to norepinephrine has been reported in the perfused hindquarters preparation from the two-kidney renal hypertensive rat, which would confirm the results of the present study. In contrast, norepinephrine sensitivity is increased in perfused hindquarters and mesenteric vasculature preparations from rats in which the function of one kidney has been compromised and the other kidney removed. This difference in norepinephrine sensitivity of tissues from rats with one- and two-kidney renal hypertension may be a consequence of the sodium retention which occurs in the former but not in the latter, as sodium loading is known to increase norepinephrine sensitivity in isolated vascular preparations.

Periartrial electrical stimulation in the perfused kidney preparation has been shown to activate sympatheic nerves which release norepinephrine, since the evoked responses are blocked by guanethidine and phentolamine, and by pretreatment of the rats with 6-hydroxydopamine or reserpine. There was no significant difference between the response amplitudes evoked by renal nerve stimulation in matched kidneys from renal hypertensive and sham control rats. When combined with the normal sensitivity of the renal vasculature to exogenous norepinephrine, these observations imply that, in this experimental model, the intrinsic function of the adrenergic nerves subserving the isolated kidney preparation is not altered by the hypertensive process. These in vitro experiments cannot rule out an in vivo alteration in adrenergic nerve function by humoral modulators in renal hypertension.

The only significant changes in responsiveness to norepinephrine were the reductions of the maximal response and dose-response curve slope for the catecholamine in contralateral kidneys from 104-day renal hypertensive rats. These alterations could be interpreted as evidence for a decrease in the vascular wall-to-lumen ratio. A structural change of this type would be expected to reduce the slope and maximal response to all vasoconstrictor agonists; however, for angiotensin II, these parameters were not significantly different between contralateral kidneys from hypertensive and control rats. Another explanation of the results is that the high pressure load on the contralateral kidney causes damage to the vascular smooth muscle. Vascular strip preparations from chronic renal hypertensive rats have been reported to respond less powerfully than the controls to maximal stimulation with norepinephrine. In the intact perfused vessel, the consequences of this impairment of the vascular smooth muscle could easily be masked by the increase in wall-to-lumen ratio which often occurs in hypertension. No evidence of an increased wall-to-lumen ratio was found in the contralateral kidney of the 104-day renal hypertensive rats (see above) and, subsequently, the underlying damage to the vascular smooth muscle was revealed. The observation that the dose-response curve slope and maximum are reduced for norepinephrine, but not for angiotensin II, which produces a lesser maximal effect, indicates that this damage mainly impairs the maximal contractile ability of the smooth muscle cells.

To judge from the increase in the $-\log ED_{50}$ value and the shift to the left of the dose-response curve to angiotensin II, supersensitivity to the octapeptide occurs early in the kidneys from the hypertensive animals; thus it is likely to be involved in the development of high blood pressure. Increased sensitivity to angiotensin II is probably not confined to the renal vascular bed, as in vivo experiments using renal hypertensive rats have suggested a systemic increase in sensitivity to angiotensin II.

Supersensitivity to angiotensin II was not found
in kidneys from control rats; thus it is not related to the clipping procedure. It occurred both in the clipped and the contralateral kidneys from the hypertensive rats, which implies that it is not totally caused by the increased pressure load. In view of the normality of the responses to norepinephrine, it is not due to a nonspecific increase in the responsiveness of the renal vascular smooth muscle. Since prostaglandins of the E series augment the renal vasoconstrictor response to both angiotensin II and norepinephrine, it seems unlikely that an increased production of the former compounds resulting from kidney ischemia upon clipping could explain the specific increase in sensitivity to angiotensin II. Endogenous angiotensin II may reside on the receptors of the renal vascular smooth muscle cells and modulate their sensitivity to the exogenous peptide. When doses of the peptide that give abnormally high in vivo plasma levels were injected into normal animals prior to the removal of the kidneys, a marked increase in blood pressure was obtained, indicating that the peripheral receptors for angiotensin II were activated. However, no significant alteration in the angiotensin dose-response curve was observed in the isolated perfused kidneys taken from these rats. This then suggests that the experimental conditions used, and in particular the initial wash-out period imposed, rule out possible acute effects of altered levels of circulating angiotensin II at the time the kidneys are removed from the donor animal.

Supersensitivity to angiotensin II persisted longer and was more pronounced in contralateral than in clipped kidneys from the renal hypertensive rats. It is unlikely that this difference is a consequence of the high pressure load on the contralateral kidney, since its supersensitivity decreased in the later stages of hypertension, even though the pressure load on the kidney was maintained. The clipped kidney of the renal hypertensive rat is reported to have a high endogenous renin level, whereas the contralateral organ usually is depleted. A low level of circulating renin can cause supersensitivity to angiotensin II, and the low renin content of the contralateral kidney may have had a similar local effect. The renin-depleted kidney would still, however, be exposed to circulating renin, which may be high or normal in this form of hypertension. Angiotensin II is mainly inactivated in vascular beds, rather than by circulating angiotensinasases, in the renal bed, in particular, this inactivation is very efficient. A reduction in the efficiency of angiotensin inactivation could provide a partial explanation of the supersensitivity to the peptide. There is evidence for a decreased inactivation of angiotensin II by the contralateral but not the clipped kidney of the renal hypertensive rat. This could explain why the supersensitivity was more pronounced and prolonged in the contralateral than in the clipped kidney. For the latter, a change in the number of receptors or in their affinity could provide an explanation of the results.

The renal hemodynamic importance of an increase in sensitivity of the isolated kidney to angiotensin II will depend on its interaction with the endogenous levels of angiotensin II and on the other humoral modulators of vascular smooth muscle sensitivity. Neither plasma renin nor angiotensin II was measured in this study, but both are reported to show an acute rise which is often transient in this form of hypertension. Specific angiotensin II antagonists lower blood pressure during the early but not the late (120 days or more) stage of two-kidney renal hypertension in the rat.

Acknowledgments

We thank L. Van den Eynde for typing this paper and F. Jordaens and L. Zonnekeyn for technical assistance. Angiotensin II was kindly provided by Ciba-Geigy.

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doi: 10.1161/01.RES.43.4.544

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1978 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

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