Afferent Renal Nerve-Dependent Hypertension following Acute Renal Artery Stenosis in the Conscious Rat

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SUMMARY. Anatomical and electrophysiological evidence indicates that the kidneys contain both mechano- and chemoreceptor nerve endings. We conducted the present study to determine whether conditions of reduced renal blood flow elicit cardiovascular alterations that are dependent on afferent renal nerves. Removal of the renin-angiotensin system with the angiotensin I-converting enzyme inhibitor, captopril, and/or reduction in baroreflex gain by sinoaortic denervation, were combined in conscious rats with acute renal artery stenosis to prevent these systems from potentially obscuring any afferent renal nerve-dependent effects. One week after sinoaortic denervation or sham sinoaortic denervation, each rat was chronically instrumented with Doppler flow probes on the lower abdominal aorta and superior mesenteric and right renal arteries, as well as with intravascular catheters, and a perivascular balloon occluder on the right renal artery. After surgical recovery, sham sinoaortic-denervated animals during captopril administration responded to stenosis with substantial increases in arterial pressure (25-30 mm Hg) and regional resistance (30-50%) that were unrelated to the renin-angiotensin system, but which were abolished after denervation of the stenotic kidney. The data suggest that acute reductions in renal blood flow activate an afferent renal nerve-dependent cardiovascular response that is strongly expressed under conditions of reduced gain of the renin-angiotensin and baroreflex systems. We speculate that this reflex may assume particular importance in chronic renal hypertension when baroreflexes become impaired and activation of the renin-angiotensin system is reduced. (Circ Res 57: 676-688, 1985)

IT is well recognized that efferent renal nerves strongly influence cardiovascular regulation through their effects on renal vascular resistance, renin release, and salt and water retention (DiBona, 1982). Renal nerves also contain a significant population of afferent fibers. However, unlike the efferent nerves, a physiological role for afferent renal nerves (ARN) in cardiovascular regulation remains an area of much recent controversy (for reviews see: DiBona, 1982; Moss, 1982; Katholi, 1983). Arterial pressure has been reported to decrease during ARN electrical stimulation in the rat (Mahoney et al., 1981; Webb et al., 1982), whereas others have observed the opposite response (DiBona and Francisco, 1979; Hermansson et al., 1984). Similar conflicting evidence exists for other species (Aars and Akre, 1970; Ueda et al., 1967b; Beacham and Kunze, 1969; Calaresu et al., 1976, 1978). Such variations may, in part, reflect stimulation of mixed afferent populations, since there is now clear evidence that the renal nerves contain a heterogeneous group of mechano- and chemoreceptor afferent fibers (Calaresu et al., 1978; Recordati et al., 1980).

There is also disagreement regarding the reflex response to more selective activation of renal mechano- and chemoreceptor ARN with "natural" stimuli. Thus, although renal compression and raising renal venous or ureteral pressure have generally been reported to increase ARN activity (Beacham and Kunze, 1969; Niijima, 1971; Recordati et al., 1980; Kostreva et al., 1981; Kopp et al., 1984), no consistent effects of these stimuli on blood pressure or vascular resistance have emerged from previous studies. Both Pines (1960) and Niijima (1971) demonstrated a direct relationship between ARN activity and renal perfusion pressure in vascularly isolated kidneys in the cat and rabbit, respectively; however, cardiovascular parameters were not quantified in those studies.

The functional significance of altered ARN activity during reduced renal blood flow (RBF) remains unknown. This possibility has received little or no
consideration, however, since it has become generally accepted that a single system, the renin-angiotensin system, underlies the cardiovascular changes (e.g., hypertension) elicited by acute renal artery stenosis (RSt) (Caravaggi et al., 1976; Barger, 1979). Two types of experiments performed in conscious dogs and primates support this conclusion; pharmacological interruption of the renin-angiotensin system abolished the pressor response to RSt (Freeman et al., 1977; Cody et al., 1982), and the transfer function relating plasma angiotensin II (All) and arterial pressure did not differ significantly regardless of whether plasma All was elevated by varying infusion rates of exogenous All or by varying the degree of RSt (Caravaggi et al., 1976), at least for levels of stenosis or All infusion that produce relatively small (10–20 mm Hg) increases in mean arterial pressure (MAP).

It is possible, however, that over the time period that RSt was employed in the studies of Freeman et al. (1977) and Cody et al. (1982), activation of arterial baroreflexes during acute RSt (Faber and Brody, 1984) could have effectively buffered any system attempting to alter arterial pressure in the absence of the renin-angiotensin system. In addition, the data of Caravaggi et al. (1976) suggest that RSt sufficient to raise MAP in excess of 20 mm Hg may be associated with lower plasma levels of endogenous All (as much as 2-fold) than required to produce the same pressure rise via infusion of exogenous peptide. This could be interpreted as evidence that another system, in addition to the renin-angiotensin system, participates in the hypertensive response to acute RSt. Moreover, in a previous study, we demonstrated that the elevated arterial pressure and vascular resistance accompanying acute RSt in conscious rats is dependent not only on the renin-angiotensin system, but to a large degree on elevated neurogenic vasoconstrictor tone (Faber and Brody, 1983). Although this sympathetic excitation clearly involves a central neural action of All (Faber and Brody, 1984; Hartle and Brody, 1984), and perhaps a peripheral interaction of All with sympathetic nerve endings, the participation of ARN from the stenotic kidney was not ruled out in these studies.

The above considerations, together with evidence that certain conditions likely to be present during RSt (e.g., reduced RBF, altered intrarenal pressures and chemical environment) could affect the discharge of renal chemo- and/or mechanoreceptors, suggest that an ARN mechanism might contribute to the hypertension elicited by RSt. Since no studies have evaluated this possibility, the present experiments were designed to determine whether an acute RSt can elicit cardiovascular alterations that are dependent on an ARN mechanism. A method previously developed by us for production of acute RSt in conscious rats (Faber and Brody, 1983, 1984) was used to avoid the complications of anesthesia. Animals were studied in the presence and absence of both the renin-angiotensin and arterial baroreflex systems which, when activated during RSt, would be expected to attenuate or mask any ARN-dependent cardiovascular response.

**Methods**

**Animals**

Experiments were performed on male, Sprague-Dawley rats (Charles River) weighing 300–350 g at the time of the experimental protocols. Animals were housed individually in 46 × 24 × 21 cm Plexiglas cages, maintained under a 12-hour light-dark cycle (lights on from 7 a.m. to 7 p.m.) and were given free access to standard rat chow (Purina) and tap water, except during the experimental protocols.

**Chronic Surgical Procedures**

**Sinoaortic Baroreceptor Deafferentation**

Sinoaortic baroreceptor deafferentation (SAD) was performed by a modification of the method of Krieger (1964). Animals were anesthetized with 0.11 ml/100 g, im, of a solution of ketamine (100 mg/ml) and acepromazine (1 mg/ml), and received atropine (1.3 mg/kg, ip). Using aseptic technique, a midventral neck incision was made, and the right and left carotid sinuses were exposed. All connective tissue and nerves were stripped from the thyroid, occipital, internal, external, and common carotid arteries along 1–4 mm of their lengths extending from the sinus region. The vessels were painted with 10% phenol in alcohol, avoiding contact with the nearby vagi and other nerves. The superior laryngeal nerves were cut bilaterally, as were the cervical sympathetic chains and any aortic depressor nerves that were occasionally found traveling with the vagus. Two-millimeter segments of the nerves were removed. After closure of the wound, animals received 80,000 U of penicillin (Flo-cillin, Bristol). Sham SAD was performed by exposing the carotid sinuses bilaterally with no dissection of the region. Baroreceptor deafferented and sham-treated animals were chronically instrumented (see below) 7–10 days after the surgery (Fig. 1).

**Flow Probe Implantation**

Animals were anesthetized with ketamine-acepromazine and atropine (as above) and supplemented as required. Under aseptic procedures, a midline ventral laparotomy was made and the right and left renal arteries, superior mesenteric artery, and aorta below the iliacal arteries were isolated along 2–4 mm of their length with the aid of a stereomicroscope. Miniature pulsed-Doppler flow probes were placed around each vessel, and flow probe cuffs were sutured closed with 6-0 ophthalmic silk. The paired fine-wire leads from each probe were sutured laterally, as were the cervical sympathetic chains and any aortic depressor nerves that were occasionally found traveling with the vagus. Two-millimeter segments of the nerves were removed. After closure of the wound, animals received 80,000 U of penicillin (Flo-cillin, Bristol). Sham SAD was performed by exposing the carotid sinuses bilaterally with no dissection of the region. Baroreceptor deafferented and sham-treated animals were chronically instrumented (see below) 7–10 days after the surgery (Fig. 1).

**Renal nerves running along the right renal artery were carefully freed from the vessel and positioned outside of the occluder cuff (and flow probe) to prevent potential mechanically induced changes in ARN activity due to**
nerve compression during inflation of the occluder. Mechanical effects on any remaining small ARN within the walls of the renal artery were not involved in the hemodynamic response to RSt, since prior painting of the renal artery itself with 10% phenol did not alter the ARN reflex effect of RSt \( (n = 4) \). Animals were allowed at least 4 days to recover from the surgery. We have repeatedly observed that the kidneys of such implanted animals demonstrate the presence of functional renal innervation as evidenced by strong renal vasoconstriction during the startle response or systemic administration of tyramine.

The flow probe wire leads and occluder tubing were brought through an incision in the dorsolateral lower abdominal wall and led subcutaneously to exit at the back of the neck. Wire leads were soldered to a miniature connector plug which was fixed to the skull with jewelers screws and cranioplastic cement. A catheter (pulled out PE-50) was advanced via the left femoral vein into the lower abdominal vena cava and exteriorized at the back of the neck. Each rat received 3 ml of sterile saline, ip, 80,000 U penicillin, and was allowed 4 days to recover from the surgery (Fig. 1). Recovery was judged satisfactory if animals were gaining weight and exhibiting normal water intake; otherwise, animals were excluded from the study.

One to 2 days before the initiation of the experimental protocol, a catheter (PE-10 welded to #1857 Microline, Thermoplastic Scientifics) was positioned in the thoracic aorta via the left common carotid artery under ether anesthesia and exited behind the left ear; penicillin was administered as above. Catheters were filled with sterile heparinized saline (50 U/ml). All implanted materials were gas sterilized before implantation.

Experimental Protocols (Fig. 1)

On the day of the experiment, each conscious rat was connected to the appropriate recording devices by way of a lightweight, flexible, spring-guarded umbilical line that contained the flow probe connector leads, catheter, and occluder lines. The umbilical line was suspended from the top of the animal's home cage to permit freedom of movement. Our procedures for study of conscious animals were in accordance with guidelines set by the National Institutes of Health and the University of North Carolina. The flow probe leads were connected to a flowmeter (University of Iowa Bioengineering Facility). Changes in blood flow velocity (Doppler shift in kHz) measured by the flow probes which are directly and linearly related to volume flow (Haywood et al., 1981) were recorded on an oscillograph (Gould 2600 or Beckman RM) as the electronically averaged mean flow velocity. MAP was electronically derived from a pressure transducer (Gould, P23Db, or Century, CP01), and average heart rate (HR) was determined by a cardiometer. A CFS-06 Infraflow device (Sorenson) was interposed between the arterial catheter and pressure transducer and provided a continuous infusion of saline (0.20 ml/hr) to maintain patency of the arterial catheter. At least 60 minutes were observed before beginning a protocol to ensure stabilization of hemodynamics. Animals were studied between 8 a.m. and 6 p.m. in a quiet room, and generally were asleep or resting quietly during the experiments. During the stabilization period, right renal blood flow (RBF) was reduced to zero for several seconds by pressurizing the occluder line (see below) to verify that electrical zero, achieved with interruption of excitation current to the renal flow probe piezoelectric crystal, was the same as actual zero flow.

Renal Artery Stenosis

We assessed the effect of SAD or the sham procedure on baroreflexes prior to any interventions by obtaining the maximal changes in MAP and HR to pressor and depressor challenges produced by iv administration of phenylephrine (Sigma) \((3 \, \mu g/kg, 28-35 \, \mu l)\) and nitroglycerin (Nitrostat, Parke-Davis, \(120 \, \mu g/kg, 30-35 \, \mu l\)) respectively. The order of administration was randomized. Each bolus was flushed into the animal with 175 \(\mu l\) of drug vehicle (saline).
Renal Denervation

To determine whether intact renal innervation of the right kidney possessing the renal artery vascular occluder influenced the response to RSt in SAD animals during CEI, we divided SAD animals into two groups (Fig. 1). Under ether anesthesia, one group underwent right renal denervation via a retroperitoneal approach. Denervation was aided by the use of a dissection microscope, and consisted of removal of the renal capsule and of stripping all nerves and connective tissue from between and around the renal artery and vein along their lengths extending to their branches at the hilum. The cleaned renal artery, vein, and their branches then were painted with 10% phenol in alcohol. We subjected a second group of SAD animals to sham renal denervation via a retroperitoneal approach. Denervation-intact sham SAD animals.

To allow analysis of the effect of RSt in the absence of the renin-angiotensin system, animals were treated with the AI-converting enzyme inhibitor, captopril (12.5 mg/kg, 130-175 µl, iv), with supplemental doses (3 mg/kg) administered every 60 minutes. This dose of captopril (12.5 mg/kg) is equivalent to 568 times the ID₉₀ of captopril (22 µg/kg) for the pressor response in rats to angiotensin I (310 ng/kg, iv) (Rubin et al., 1978). The adequacy of converting enzyme inhibition (CEI) was assessed in the present study by comparison of the pressor responses to bolus administration of AI (25, 75, 150, and 300 ng/kg, iv) before and at periodic intervals after the initial and supplemental doses of captopril. Forty-five minutes after the initial captopril injection, animals were subjected to acute RSt for 60 minutes, as described above.

Plasma Renin Determination

Plasma renin activity (PRA) was determined from blood samples obtained from conscious animals during the 55-to 60-minute interval during RSt or sham stenosis. A 0.5-ml blood sample was withdrawn within 20-30 seconds from the arterial catheter into a chilled heparinized syringe, transferred into a chilled centrifuge tube, centrifuged (5000 g, 5 minutes, 4°C), and the plasma was collected and stored at —20°C until assay. Blood cells were resuspended in isotonic saline and returned to the animal.

Data Analysis

Changes in regional vascular resistance are expressed as a percentage of baseline resistance, where resistance for a given vascular bed is calculated as MAP/Doppler shift (mm Hg/kHz). The percentage change in resistance (R) relative to baseline R was calculated as follows:

\[
\frac{\text{baseline } R - R \text{ at time } 'x' \times 100}{\text{baseline } R} = \% \Delta \text{ in resistance at time } 'x' 
\]

Resistance at time ‘x’ represents the resistance at specified time intervals after RSt or sham stenosis for analysis of changes in R over time, or represents the maximum change in resistance, irrespective of time, for analysis of the effects of pharmacological interventions, except for captopril. This calculation provides a valid measurement of percentage changes in regional vascular resistance, since percentage changes in Doppler shift (blood velocity) obtained with the Doppler flow probe have been shown to be equivalent to true percentage changes in volume flow (Haywood et al., 1981). All baseline (i.e., control) hemodynamic values prior to captopril administration and RSt or sham stenosis (Tables 1 and 2) represent averages of five determinations taken at 5-minute intervals over the 20-minute interval immediately preceding the intervention. Such averaging was necessary to obtain a good estimate of baseline values in the SAD animals which, due to reduced baroreflex buffering, exhibited a greater degree of blood pressure and flow variability than baroreflex-intact sham SAD animals.

Analysis of phenylephrine and nitroglycerin responses were made with group t-tests. Analysis of variance was used to test responses to AI before and after captopril and
### Table 1

**Effect of Captopril on Baseline Hemodynamics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Regional resistance (mm Hg/kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left renal</td>
</tr>
<tr>
<td>Sham SAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>107 ± 5</td>
<td>319 ± 7*</td>
<td>16 ± 2*</td>
</tr>
<tr>
<td>Captopril</td>
<td>105 ± 6</td>
<td>336 ± 10</td>
<td>15 ± 2†</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAD-renal den.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>106 ± 3†</td>
<td>350 ± 11†</td>
<td>17 ± 1†</td>
</tr>
<tr>
<td>Captopril</td>
<td>98 ± 4</td>
<td>366 ± 10</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAD-sham renal den.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>114 ± 7†</td>
<td>343 ± 16*</td>
<td>21 ± 4*</td>
</tr>
<tr>
<td>Captopril</td>
<td>104 ± 7</td>
<td>381 ± 23</td>
<td>16 ± 2†</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. Abbreviations: SAD, sinoaortic denervation; renal den., right renal denervation; n = number of animals. P values: *P < 0.05, †P < 0.01, ‡P < 0.001 for control vs. captopril (unless bracketed otherwise).

### Table 2

**Baseline Hemodynamics Prior to Renal Stenosis (RSt) or Sham Stenosis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Regional resistance (mm Hg/kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left renal</td>
</tr>
<tr>
<td>Sham SAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-RSt, no CAP</td>
<td>108 ± 6 (8)</td>
<td>338 ± 9 (7)</td>
<td>16 ± 1 (7)</td>
</tr>
<tr>
<td>Pre-RSt CAP</td>
<td>106 ± 6 (8)</td>
<td>329 ± 7 (7)</td>
<td>16 ± 2 (7)</td>
</tr>
<tr>
<td>Pre-Sham RSt, no CAP</td>
<td>109 ± 6 (8)</td>
<td>322 ± 8 (7)</td>
<td>15 ± 1 (7)</td>
</tr>
<tr>
<td>SAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-RSt, no CAP</td>
<td>108 ± 3 (15)</td>
<td>346 ± 11 (15)</td>
<td>18 ± 1 (15)</td>
</tr>
<tr>
<td>Pre-RSt, CAP</td>
<td>97 ± 4* (15)</td>
<td>353 ± 11 (15)</td>
<td>16 ± 1 (15)</td>
</tr>
<tr>
<td>Pre-Sham RSt, CAP</td>
<td>94 ± 3* (9)</td>
<td>325 ± 6 (9)</td>
<td>18 ± 1 (8)</td>
</tr>
<tr>
<td>SAD-renal den.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-RSt, CAP</td>
<td>103 ± 9 (8)</td>
<td>375 ± 13 (8)</td>
<td>23 ± 4 (6)</td>
</tr>
<tr>
<td>SAD-sham renal den.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-RSt, CAP</td>
<td>103 ± 7 (5)</td>
<td>345 ± 16 (5)</td>
<td>17 ± 2 (5)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Abbreviations: *no CAP,* without captopril pretreatment; CAP, with captopril pretreatment; for other abbreviations, see Table 1. Numbers in parentheses = number of animals. *P < 0.05, †P < 0.01 vs. Pre-RSt, no CAP (unless bracketed otherwise).
angiotensin system on the response to RSt. Control responses \((n = 7)\) to AI \((25, 75, \text{and } 150 \text{ ng/kg iv bolus})\) increased MAP by \(33 \pm 5, 58 \pm 5, \text{and } 62 \pm 4 \text{ mm Hg, respectively.}\) After the initial dose of captopril \((12.5 \text{ mg/kg, iv})\), pressor responses to AI were completely eliminated for at least 75 minutes after CEI. Subsequent supplemental doses of captopril \((3.0 \text{ mg/kg, iv, at 60-minute intervals})\) were sufficient to maintain complete CEI, as judged by total lack of responsiveness to supramaximal pressor doses of AI \((150 \text{ or } 300 \text{ ng/kg})\) which were administered before the last supplementation in all experiments. Values for the effect of captopril on baseline hemodynamics \((Table 1)\) represent the average of four values taken at 5-minute intervals between 15 and 30 minutes after administration. Captopril had no effect on blood pressure in sham SAD animals, but produced slight though significant decreases in renal resistances and an increase in heart rate \((HR)\) \((Table 1, \text{sham SAD group})\). These data suggest that the renin-angiotensin system assists in the regulation of arterial pressure under conscious unstressed conditions, an observation consistent with the work of others \((\text{Finke et al., 1983})\). However, other putative actions of captopril \((e.g., \text{kininase II inhibition})\) may be involved. The influence is small as evidenced by the lack of a decrease in MAP in association with regional vasodilation, probably as a result of baroreflex compensation. This thesis is supported by the observations that MAP does drop significantly in SAD animals \((Table 1, \text{and below})\).

Prior to CEI with captopril, an initial 50% reduction in right RBF was associated with a detectable increase in mesenteric resistance and arterial pressure within 1–3 minutes. Over the subsequent 60 minutes of RSt, MAP increased significantly by 13 ± 2 mm Hg \((\text{Fig. 2})\), and the reduction in right RBF was ameliorated as renal perfusion pressure \((\text{MAP})\) increased. Heart rate decreased; this response has been shown in previous studies to be baroreflex mediated \((\text{Faber and Brody, 1984})\). Vascular resistance increased in the contralateral kidney, superior mesenteric and hindquarters beds by approximately 40–60% \((\text{Fig. 3})\). None of the cardiovascular param-
Plasma renin activity (ng Al/ml plasma per hr) in the sham SAD animals prior to RSt (0.6 ± 0.1) increased 5-fold (3.2 ± 1.1) after 60 minutes of RSt (Table 3). After captopril treatment, baseline PRA increased 14-fold (8.6 ± 7.7). Compared to this elevated baseline, PRA increased an additional 3-fold (26.6 ± 4.6) after 60 minutes of RSt during CEI.

The lack of a change in arterial pressure during acute RSt in the presence of CEI does not rule out the possibility that a slowly developing pro-hypertensive mechanism could be activated during RSt plus CEI, but that it might not produce increases in arterial pressure or peripheral resistance large enough to be detectable, because of the strong buffering action of the sinoaortic baroreflex. The decrease in HR and increase in mesenteric resistance in the captopril-treated animals during RSt (Figs. 2 and 3) and the increase in mesenteric resistance in the captopril-treated animals during RSt (Figs. 2 and 3) are consistent with this possibility. To examine this hypothesis directly, we studied animals with reduced baroreflex gain secondary to SAD under conditions of acute RSt.

Response to Renal Stenosis in Conscious Rats with Impaired Baroreflexes

Animals with sinoaortic baroreceptor denervation were subjected to acute RSt in the presence and absence of CEI to determine whether reduced RBF could produce cardiovascular changes independent of the renin-angiotensin system.

Renin-Angiotensin System Intact

Eleven to 12 days after the SAD or sham SAD procedure, animals were tested for baroreflex responsiveness. Baseline pressures prior to CEI did not differ between the two groups (sham SAD vs. SAD), HR was significantly elevated in the SAD animals, and baseline regional resistances were similar, with the exception of reduced hindquarters resistance in the SAD group (Table 1). Phenylephrine administration in sham SAD rats (n = 8) produced increases in MAP (48 ± 5 mmHg) and reductions in HR (—64 ± 11 beats/min) representing a baroreflex gain (Δ heart rate/Δ MAP) for pressor challenges of —1.31 ± 0.17. Sinoarotic-denervated animals (n = 15) exhibited larger increases in MAP (56 ± 6 mm Hg) but much smaller reductions in HR (—8 ± 4 beats/min) for a baroreflex pressor gain of —0.18 ± .03, representing an 86% decrease (P < 0.001). Likewise, nitroglycerin administration in the baroreflex intact animals (n = 8) reduced MAP by 32 ± 3 mm Hg and increased HR by 65 ± 7 beats/min, for a baroreflex depressor gain of —2.13 ± 0.27. Sinoartic-denervated rats (n = 15) showed a larger decrease in MAP of —49 ± 5 mmHg but smaller increase in HR of 16 ± 3 beats/min, for a baroreflex depressor gain of —0.37 ± 0.08, representing an 83% decrease (P < 0.001).

Sinoaortic-denervated rats responded to RSt with accentuated increases in arterial pressure and regional resistances (Figs. 4 and 5) relative to the baroreflex-intact animals (Figs. 2 and 3), and the bradycardia in sham SAD animals was abolished after SAD, indicating the reflex nature of the bradycardia. The increased pressor response to acute RSt and elimination of bradycardia in rats with reduced baroreflex gain relative to reflex-intact animals is in agreement with previous findings in rats (Faber and Brody, 1984) and dogs (Liard et al., 1974). These accentuated hemodynamic responses were associated with a much smaller (1.6-fold) increase in PRA (Table 3) compared to sham SAD animals (5-fold...
Renin-Angiotensin System Impaired

Converting enzyme inhibition with captopril produced significant reductions in arterial pressure and regional resistances in the SAD group and significantly elevated HR (Table 1). As with the baroreflex-intact group (Figs. 2 and 3), sham RSt had no significant effect in the SAD group on any parameter (Figs. 4 and 5), substantiating the hemodynamic stability of the animals over time. However, in sharp contrast to absence of significant changes in arterial pressure or regional resistance during RSt in sham SAD animals in the presence of CEI, animals with depressed baroreflex function, plus CEI, exhibited significant elevations in arterial pressure and regional resistances that were presumably independent of AI production (Figs. 4 and 5). Values for PRA in the presence of captopril both before (control) and during RSt were similar to those obtained for sham SAD animals (Table 3). Baseline hemodynamics prior to RSt or sham RSt for the SAD groups presented in Figures 4 and 5 are given in Table 2. With the exception of slightly lower arterial pressures in the captopril-treated groups, baseline hemodynamics were similar.

Effect of Renal Denervation on the Non-AII-Dependent Hypertension Produced by Renal Stenosis

To determine whether the pressor response to RSt during CEI in SAD animals was dependent on renal nerves, responses to RSt were compared with those obtained after either denervation or sham denervation of the right (stenotic) kidney. The "offset" transients upon removal of the stenosis were also documented in these experiments. Control hemodynamics prior to captopril did not differ significantly among the three groups [SAD, SAD-renal denervation, SAD-sham renal denervation (Table 1)]. Cap-
topril produced similar significant reductions in MAP and regional resistances and increases in HR in all groups (Table 1). Figures 6 and 7 compare responses to acute RSt in the presence and absence of renal innervation of the stenotic kidney. Renal stenosis produced comparable increases in MAP and regional resistances in the control and sham renal-denervated groups that reversed upon removal of the stenosis. However, after renal denervation of the stenotic kidney, the pressor and vasoconstrictor responses were eliminated. This effect is shown in Figure 8 for average "steady state" values during the last 10 minutes of RSt. Before RSt (Pre-RSt), resting hemodynamics were similar among the three groups (Table 2). Renal denervation did not alter the increase in PRA during RSt in SAD animals pretreated with captopril (Table 3).

Effect of Angiotensin Receptor Blockade On RSt-induced Hypertension during Captopril Treatment

The possibility that the pressor response to RSt in SAD animals during CEI might be dependent on high levels of AI [or All, although the demonstration of complete CEI (see above) makes this unlikely] was evaluated with saralasin infusion during RSt-induced hypertension (n = 8). Mean arterial pressure prior to RSt (93 ± 9 mm Hg) increased to 115 ± 10 mm Hg (P < 0.05) after 60 minutes of RSt. Saralasin had no effect on the renal nerve-dependent hypertension (i.e., MAP was 116 ± 11 mm Hg after a 20-minute infusion of saralasin) nor on increases in regional resistances, indicating that the pressor response was not angiotensin dependent. Relative to control, there were no significant changes in heart rate during RSt or stenosis-plus-saralasin administration.

Discussion

The major finding of this study is that acute unilateral renal artery stenosis (RSt) during interruption of the renin-angiotensin system (captopril) produced a sustained increase in arterial pressure and vascular resistance in the hindquarters, mesenteric, and contralateral renal circulations. This response
was evident in conscious chronically instrumented animals with attenuated arterial baroreflexes produced by prior surgical sinoaortic denervation (SAD). The response was abolished after the nerves to the stenotic kidney were sectioned, presumably by interruption of ARN (see below). These findings represent the first demonstration that alterations of RBF can affect cardiovascular regulation through an influence of ARN activity.

In agreement with our previous results, in the present study, RSt in animals with an intact renin-angiotensin system and normal baroreflex gain exhibited hypertension and regional vasoconstriction, as well, but these changes were strongly dependent on increased activity of the renin-angiotensin system, as evidenced by the elevation in PRA and previous findings that blockade of central and peripheral ATII receptors antagonizes the hypertension (Faber and Brody, 1983, 1984). The increase in arterial pressure after 60 minutes of RSt in the present study (13 mm Hg) is smaller than observed in our previous studies (33-40 mm Hg), owing to the smaller degree of RSt in the present study (one initial 50% reduction in RBF, vs. two successive stenoses over 30 minutes after the initial reduction in our previous studies). Under conditions of converting enzyme inhibition (CEI) with captopril, RSt produced no significant changes in arterial pressure (Fig. 2). This latter finding is similar to those of other studies conducted in conscious dogs and monkeys wherein blockade of the renin-angiotensin system with saralasin prevented arterial pressure from rising (Freeman et al., 1977), and where CEI with teprotide reversed the pressure rise produced by RSt (Cody et al., 1982). These and other studies support the widely held view that the hemodynamic changes associated with acute RSt are entirely dependent on exclusive activation of the renin-angiotensin system (Caravaggi et al., 1967; Barger, 1979). However, a secondary pro-hypertensive mechanism activated by acute RSt could be effectively buffered by arterial baroreflexes, and thus escape detection without other measurements such as heart rate and vascular neurogenic tone. That such a secondary mechanism may be activated but obscured by baroreflexes is suggested by the progressive decline in heart rate, possibly indicating a decrease in cardiac output, during RSt in captopril-treated rats with intact baroreflexes (Fig. 2), and the tendency of mesenteric and hindquarters resistance to increase slightly, as well (Fig. 3). Accordingly, under conditions of reduced baroreflex gain, the effects of activation of a secondary mechanism would become unmasked. This is clearly evident in the renal stenosis-dependent increase in arterial pressure and regional resistance seen in captopril-treated SAD animals (Figs. 4-7).

It should be noted that it is unlikely that the increases in regional resistance that characterize the ARN-dependent hypertensive reflex are initiated by autoregulatory responses to an elevation in systemic pressure secondary to increased cardiac output. Either no change or a decrease in cardiac output might be expected, based on the bradycardia response. Moreover, initial elevations in flow were not observed in any vascular bed, and flow declined from baseline values during the stenosis period in all beds, as indicated by the greater percentage increases in vascular resistances (30-50%) than in arterial pressure (25%) (Fig. 8). Hence, although myogenic responses to the rise in arterial pressure may contribute to the regional vasoconstrictor responses (Meininger et al., 1984), the increase in peripheral resistance may be viewed to involve substantial humorally and/or neurally mediated vaso-constriction.

Interpretation of the results of this study rests strongly on demonstration of absence of involvement of the peripheral and brain renin-angiotensin
systems in the reflex response to RSt under conditions of CEI and depressed baroreflex gain. Several findings emphasize that this was the case. There is evidence that the dose of captopril used in the present study, which produced complete peripheral CEI, is also in excess of that required to achieve central CEI (Scholens et al., 1983). Also, in two conscious animals, intraventricular administration of captopril (50 μg/kg) during intravenous administration had no effect on the RSt-induced pressure rise, indicating that the reflex response was not due to central conversion of AI to AII and subsequent activation of the central AII pressure response.

Since plasma AI levels would be expected to be elevated during CEI and to increase further with RSt, it could be hypothesized that further increases in PRA during RSt would result in plasma levels of AI sufficient to produce vasoconstriction through an action on AII receptors in the periphery and/or CNS. Furthermore, several groups have reported that renin release in response to a variety of stimuli, including reduced renal perfusion pressure, is lower after renal denervation (Ueda et al., 1967a; Stella et al., 1976; Thames and DiBona, 1979). Hence, in the present study, the elimination of the reflex response to RSt by renal denervation could be viewed as consistent with the hypothesis that AI is a partial mediator of the ARN-hypertensive response, since less renin release from the denervated stenotic kidney would in turn lead to smaller increases in AI during RSt and less vasoconstriction. Several points indicate that this hypothesis is not valid. Intravenous infusion of the angiotensin receptor antagonist, saralasin, at a dose which occupies both peripheral and central receptors (Van Houten et al., 1980) had no effect on the reflex. Also, in contrast to data obtained in anesthetized animals (Ueda et al., 1967a; Stella et al., 1975; Thames and DiBona, 1979), there was no deficit in the release of renin during stenosis of denervated kidneys (Table 3). Collectively, these considerations rule out participation of the renin-angiotensin system in the reflex response.

The hypertensive response to RSt that is independent of the renin-angiotensin system appears to rely on afferent renal nerves, since denervation of the stenotic kidney abolished the response. Although we cannot, with certainty, rule out a role for efferent renal nerves, this possibility seems unlikely, since the known actions of efferent renal nerves (renin release, antinatriuresis, antidiuresis, and renal vasoconstriction) have either been eliminated or would probably not account for either the rapidity or magnitude of the rise in regional resistances and arterial pressure that characterize the response. Thus, the effect of renal denervation is most consistent with removal of an influence of afferent renal nerves. However, further studies are needed that employ dorsal rhizotomy to achieve selective removal of ARN (Lappe et al., in press).

The results suggest that RSt alters ARN activity in the affected kidney by a specific effect on intrarenal nerve endings to produce an ARN-dependent cardiovascular reflex. This effect is unlikely to be due to activation of a renal nociceptive response, as animals did not show any altering/arousal reactions during RSt. Recent work also indicates that the ARN reflex remains fully intact in animals with chronic T4 spinal cord transection, an intervention which would presumably eliminate perception of renal pain (Faber, 1984). It is well-known that marked reduction of RBF in conscious humans is not accompanied by renal sensation or pain. In conscious humans, no discomfort of any type was noted when renal perfusion to one kidney was reduced by 50% of control for 60 minutes (a RSt protocol similar to ours) with a balloon-tipped catheter placed in one renal artery (Guazzi et al., 1981).

There is abundant electrophysiological evidence to support the existence of both mechan- and chemoreceptor mechanisms for activation of ARN in all species studied, although there is much disagreement regarding the adequate stimuli for ARN or the physiological responses to activation (DiBona, 1982; Moss, 1982; Katholi, 1983). Raising renal venous pressure in the dog decreased efferent renal nerve activity, contralateral renal resistance, and MAP (Ueda et al., 1967b; Kostreva et al., 1981) while having no effect in another study (Kopp et al., 1984). In the cat, ipsilateral efferent renal nerve activity of single fibers was increased, decreased, or unchanged (Beacham and Kunze, 1969). Similar disagreement exists among studies examining reflex effects of increased ureteral pressure, which can activate both renal mechan- and chemoreceptors (Recordati et al., 1980; Moss, 1982). When renal chemoreceptors were activated selectively by introducing concentrated urine or high ionic strength solutions into the renal pelvis of the rat, Recordati et al. (1980) observed an excitatory "reno-renal reflex" involving increase in contralateral efferent renal nerve activity; however, for the same stimulus and species, an inhibitory reno-renal reflex was observed by another group (Kopp et al., 1984) involving exactly opposite contralateral responses. The results of the present study do not allow any conclusions to be drawn concerning the type of ARN affected by acute RSt, since this stimulus could conceivably alter the discharge of mechano- and/or chemoreceptor afferents.

A hypothetical model which presents the key features of the ARN-dependent hypertensive response is shown in Figure 9. The model proposes that reduced RBF activates both the renin-angiotensin system and ARN. The resulting increase in circulating AII causes arteriolar constriction and hypertension (e.g., Figs. 2 and 3) by peripheral actions and by a centrally induced increase in neurogenic vasoconstrictor tone (Faber and Brody, 1984; Hartle and Brody, 1984). Simultaneously, ARN activity is altered and, through projections to spinal and supraspinal centers, promotes an increase in preganglionic sympathetic outflow as well. However, with
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Figure 9. Hypothetical model of the effect of reduced renal blood flow on peripheral resistance through activation of the renin-angiotensin system (RAS) and the afferent renal nerve-dependent renal hypoperfusion reflex. The ANG II (All) lines depict peripheral vasoconstrictor mechanisms and indirect sympatho-excitatory central nervous system (CNS) actions of ANG II. Afferent renal nerves, baroreceptor afferents and descending hypothalamic neural projections are depicted as influencing sympathetic outflow in an excitatory (+) or inhibitory (−) manner. The graphs summarize the findings of the present study, with regard to arterial pressure, that support the model.

Time

Normally operating arterial baroreflexes, this ARN excitatory influence on neurogenic tone is opposed, at least acutely. Thus, the effect of reducing RBF in the presence of intact baroreflexes and the renin-angiotensin system—an increase in vascular resistance and arterial pressure—is prevented in the presence of CEI, perhaps due to offsetting effects of afferent renal and arterial baroreceptor nerves. The anatomical substrate for such an interaction between baroreceptor and ARN is supported by the electrophysiological studies of Calaresu and Ciriello (1981) who showed that more than 50% of medullary neurons receiving ARN inputs were also affected by direct baroreceptor afferent stimulation. With impaired baroreflexes, e.g., in SAD animals, RSt produces an accentuated rise in arterial pressure (Fig. 4) due to the absence of the buffering influence of these reflexes on both ARN and angiotensin vasoconstrictor mechanisms. However, when the gains of both the baroreflexes and renin-angiotensin system are reduced, vascular resistance and arterial pressure still increase, but now by a mechanism dependent on ARN (Figs. 4 and 5), since renal denervation eliminates the response (Figs. 6–8).

The model proposes that the hemodynamic responses produced by RSt in SAD, captopril-treated animals results from an ARN "reflex" involving spinal/supraspinal integration of ARN signals and excitation of efferent vasoconstrictor nerves. Recent studies (Faber, 1984) support this hypothesis. In conscious SAD rats with chronic T4 spinal cord transections and CEI, the ARN-dependent increase in arterial pressure during RSt was completely preserved, but the pattern of associated regional resistance changes was altered. Furthermore, the ARN response was totally abolished by ganglionic blockade or anesthesia.

In conclusion, we have shown that, although the renin-angiotensin system constitutes the dominant mechanism activated by acute RSt in normal animals, when this system and arterial baroreflexes are impaired, a secondary hypertensive mechanism is unmasked. This mechanism appears to depend on an ARN-dependent sympathoexcitatory reflex, although this and other possibilities await further analysis. The reflex, by increasing renal perfusion pressure, lessens the deficit in renal blood flow. We propose that the ARN-dependent hypertensive reflex may serve as an alternative or adjunct system to the renin-angiotensin system for the maintenance of renal blood flow. This reflex could predominate when baroreflexes and the renin-angiotensin system become impaired. In this regard, Katholi and coworkers (Katholi, 1983) have demonstrated that renal denervation markedly attenuates established renal hypertension produced by renal artery clipping, and have proposed that removal of an ARN signal from the affected kidney underlies the blood pressure-lowering effect of renal denervation. It is interesting to note that established renal hypertension is characterized by reduced baroreflex sensitivity (Angell-James and George, 1981), disappearance of renin in the contralateral kidney, loss of renin-angiotensin stimulation, and return to normal or near-normal PRA (Davis, 1977). Thus, the ARN hypertensive reflex may become dominant under these conditions and assume pathophysiological importance in the maintenance of renal hypertension.

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