Role of Prostaglandins and Kinins in the Renal Pressor Reflex

JAMES E. FABER

SUMMARY In previous studies we identified an afferent renal nerve–dependent pressor reflex elicited by acute unilateral renal artery stenosis (50% decrease in renal blood flow) in conscious, instrumented rats with reduced responsiveness of arterial baroreceptor reflexes and the renin-angiotensin system. The pressor reflex involves a neurogenic increase in peripheral resistance. The present study examined the nature of the intrarenal stimulus underlying this renal pressor reflex. Rats were subjected to sinoaortic denervation and, 7 to 10 days later, were chronically instrumented with Doppler flow probes on the right renal artery, superior mesenteric artery, and abdominal aorta and with an occluder on the right renal artery. Following surgical recovery and inhibition of the renin-angiotensin system (captopril), animals received intravenous isotonic saline, 6% of body weight over 60 minutes. Saline infusion did not alter baseline hemodynamics, vascular neurogenic tone, or responsiveness to tyramine, but it attenuated the reflex by 70%. A second series of experiments examined a possible role for intrarenal prostaglandins, kinins, or adenosine in the activation of renal sensory receptors during renal stenosis. Prostaglandin inhibition with intravenous administration of indo-methacin and meclofenamate virtually abolished the reflex in the face of enhanced tyramine responsiveness, whereas kallikrein inhibition (aprotinin) attenuated the reflex pressor response by 33%. Adenosine inhibition with aminophylline or adenosine deaminase had no effect on the reflex; these agents and aprotinin did not affect vascular neuroeffector responsiveness (tyramine). The data suggest that the renal pressor reflex may be mediated by renal sensory nerves, possibly chemoreceptors, whose activation could depend on renal excretory function and synthesis of prostaglandins and kinins. (Hypertension 10: 522–532, 1987)

KEY WORDS • afferent renal nerves • sympathetic nervous system • aprotinin • adenosine • volume expansion • saline infusion

Two types of renal chemoreceptors, designated R₁ and R₂, have been identified in the rat.¹⁻² R₁ chemoreceptors have no basal discharge and are unresponsive to changes in renal arterial, venous, or intrapelvic pressure, but they become active after renal blood flow (RBF) is stopped or reduced to very low levels for 20 to 30 seconds. In contrast, the more numerous R₂ chemoreceptors exhibit a basal discharge and are activated by introduction into the renal pelvis of hypertonic urine or sodium solutions (≥600 mEq/L) or potassium solutions (≥50 mEq/L). Renal mechanoreceptors are sensitive to changes in pressure in the renal artery, renal vein, and ureter and have been described in several species.³⁻⁶ Compared with mechanoreceptors, which exhibit rapid adaptation to a maintained stimulus,² R₂ chemoreceptors continue to discharge in the face of a maintained stimulus.¹² The physiological significance of afferent renal nerves (ARNs) to renal-cardiovascular integration remains unclear. Activation of chemoreceptor ARNs, by back-perfusion of the renal pelvis with either hypertonic saline or concentrated urine, and activation of mechanoreceptor ARNs, by increasing ureteral pressure, both elicit changes in effluent renal nerve activity to the opposite kidney (i.e., renorenal reflexes) and alterations in salt and water excretion.¹⁻⁴ However, no consistent effects on arterial pressure, vascular re-
sistance, or RBF have been observed in response to these stimuli. Recent studies have suggested that alteration of ARN activity during reduced RBF and perfusion pressure may initiate a sympathoexcitatory reflex. Acute, unilateral reduction of RBF by 50% in conscious, instrumented rats with reduced arterial baroreceptor reflex sensitivity and interruption of the renin-angiotensin system produces a sustained increase in arterial pressure and peripheral vascular resistance by a mechanism dependent on ARNs from the stenotic kidney. This response consists of a sympathoexcitatory vasoconstrictor reflex that is integrated at both spinal and supraspinal levels and that is inhibited by anesthesia.

The purpose of the present study was to examine the nature of the intrarenal stimulus for this renal pressor reflex. The reflex may involve sustained activation of R_{1} renal chemoreceptors, since in recent electrophysiological studies only these receptors exhibited persistent activation during modest reductions in renal artery pressure. Acute stenosis could, by decreasing glomerular filtration and tubular fluid flow, increase the ionic strength of tubular and interstitial fluid and activate R_{2} chemoreceptors. Acute intravenous saline volume expansion, which has been shown to inhibit activation of R_{1} chemoreceptors, was examined in the present study for an effect on the renal pressor reflex. A second series of experiments examined whether prostaglandins, kinins, or adenosine, possibly released intrarenally during renal artery stenosis (RST), could participate in reflex activation. Prostaglandins and adenosine are released by the kidney during reductions in RBF, and all three substances can directly activate or increase the sensitivity of thinly myelinated or unmyelinated sensory nerve endings, including those in the kidney. Thus, pharmacological modification of the synthesis or degradation of these agents was employed to examine their potential role in the reflex.

**Materials and Methods**

**Chronic Surgical Preparation**

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used for these studies and weighed 325 to 375 g at the time of the experimental protocols. Arterial baroreceptor reflex gain was reduced by sinoaortic denervation (SAD), as described in detail elsewhere. Animals were allowed 7 to 10 days to recover during which body weight was recorded daily. This was sufficient time for animals to at least regain their pre-SAD body weight; animals not exhibiting normal weight gain were excluded from the study.

After recovery from SAD, the abdomen was opened ventrally and the right renal artery, superior mesenteric artery, and aorta below the left renal artery were isolated along 2 to 4 mm of their length. Miniaturized pulsed-Doppler flow probes were implanted around each vessel, and a perivascular pneumatic microballoon occluder was implanted around the right renal artery distal to the renal flow probe. Details of the construction and implantation of the flow probes and microoccluders are described elsewhere. Fine glass tools were used to isolate any parallel-running nerves from the vessels and position them beyond the flow probe cuffs and occluder. Previous studies have shown that direct mechanical activation of any remaining intramural renal nerves by occluder inflation does not contribute to the renal pressor reflex. The left femoral vein was cannulated with two pulled-out PE-50 catheters. The flow probe wire leads, occluder tubing, and venous catheters were brought through the dorsolateral abdominal wall and led subcutaneously to the back of the neck, where the occluder tubing and catheters were anchored and exteriorized. Flow probe leads were soldered to a miniature, skull-mounted connector pin. Animals were allowed 5 days to recover from the operation; only those that recovered satisfactorily, as judged by resumption of normal weight gain within 2 to 3 days after the operation, were used in the protocol. One to 2 days before the experimental protocol, a PE-10 catheter was positioned in the descending thoracic aorta through the left carotid artery, with the rats under ether anesthesia, and exteriorized behind the left ear. All implanted materials were gas-sterilized, surgical procedures were conducted aseptically, and animals received penicillin after each surgical procedure.

**Experimental Protocol**

The following procedures were common to all of the individual protocols. 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. Changes in electronically averaged blood flow velocity were recorded on an oscillograph. Mean arterial pressure (MAP) was derived from a pressure transducer, and average heart rate (HR) was determined by a cardiograph. A CFS-06 Intraflow device (Sorenson, Salt Lake City, UT, USA) 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. An interval of at least 120 minutes was observed before beginning a protocol to ensure stabilization of hemodynamics. Animals were studied between 0800 and 1800 in a quiet room and generally were asleep or resting quietly during the experiments. During the stabilization period, right 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, was the same as actual zero flow.

As described in previous studies, full expression of the renal pressor reflex requires interruption of the renin-angiotensin system, which is otherwise coactivated during acute RST. The angiotensin I converting
envelope inhibitor captopril (kindly donated by Squibb, Princeton, NJ, USA) was intravenously administered during the stabilization period in all experiments as a 10 mg/kg bolus (in 95–120 μl saline), followed by a continuous infusion of 9 mg/kg/hr (approximately 2.2 μl/min). This dose is sufficient to produce inhibition of pressor responses to angiotensin I (25–300 ng/kg i.v.) in conscious animals that have undergone SAD.7,8 Converting enzyme inhibition was confirmed in the present studies by periodic administration of angiotensin I (Bachem, Torrance, CA, USA), 300 ng/kg i.v. in 100 to 120 μl of saline, which was without effect.

For all animals the effectiveness of the SAD procedure to depress baroreceptor reflexes was assessed before any interventions, by obtaining the maximal changes in MAP and HR to pressor and depressor challenges produced by i.v. administration of phenylephrine (Sigma Chemical, St. Louis, MO, USA), 3 μg/kg in 28 to 35 μl of saline, and nitroglycerin (Nitrostat; Parke, Davis, Detroit, MI, USA), 120 μg/kg in 30 to 35 μl of saline, respectively. The order of administration was randomized. All drugs used in this study were flushed into the animal with 175 μl of saline. After reestablishment of control conditions, the pressor and regional vasoconstrictor responses to bolus administration of phenylephrine (Sigma Chemical, St. Louis, MO, USA) were observed, with at least 5 minutes separating each dose. Phenylephrine causes displacement of extravascular norepinephrine from sympathetic nerve endings and was used to evaluate a possible confounding effect of the interventions discussed below to alter vascular smooth muscle responsiveness to norepinephrine released at the vascular neuroeffector junction.

Experiment 1: Saline Infusion

The control renal pressor reflex response during eu­volemic conditions was obtained by reducing right RBF to 50% of control within 30 to 60 seconds (n = 8). This was achieved by raising the pressure in the vascular microoccluder with a pressure-regulating device19 that allowed occluder pressure to be monitored continuously and held constant throughout the period of RST. The stenosis was reversed after 90 minutes by depressurizing the occluder line. After reflex reversal and an additional 30 to 60 minutes, animals were subjected to acute extracellular fluid volume expansion with sterile isotonic saline. Saline was administered intravenously at 6% of body weight over 60 minutes, followed by a maintenance infusion of 40 μl/100 g/min. The RST procedure was then repeated, followed by destenosis, recovery, and evaluation of tyramine responses during continuous saline infusion.

Saline infusion may decrease baseline neurogenic vasoconstrictor tone, secondary to a rise in venous pressure and activation of low pressure venous and cardiopulmonary baroreceptors, and attenuate the renal pressor reflex. To test this possibility, a second group of five conscious, instrumented rats that had undergone SAD were prepared and submitted to the protocol as previously described, with the exception of RST. Instead, baseline MAP, HR, regional resistances, venous pressure, and hematocrit were obtained during euvoelema. Femoral venous pressure was measured with a pressure transducer (Model P23Gb ultralow volume displacement; Gould, Oxnard, CA, USA) and recorded on an oscillograph, and hematocrit was measured from duplicate microcapillary tube blood samples obtained from the arterial pressure catheter.

Neurogenic vascular tone was then assessed by measuring decreases in MAP and regional resistances during ganglionic blockade with intravenous administration of the fast-acting ganglionic blocking agent trimethaphan camsylate (Arfonad; Roche, Nutley, NJ, USA), 1.7 mg/kg bolus (100–125 μl saline) plus 1.4 mg/min (95 μl/min). Pilot experiments in both baroreceptor reflex-intact conscious rats and rats that had undergone SAD demonstrated that this dose produced a maximal and sustained drop in MAP and regional resistances within 30 to 60 seconds after administration and blocked reflex changes in HR to bolus i.v. infusions of phenylephrine and nitroglycerin. In the present experiments the infusion was stopped after 5 minutes of ganglionic blockade, and all parameters returned to control within 15 minutes. The blocking efficacy and reversibility of ganglionic blockade established in these experiments are in agreement with the effect of trimethaphan on effenter renal nerve activity in rats.21 After an additional 15 minutes, animals underwent saline volume expansion, as already described. Thirty minutes into the saline maintenance infusion, hematocrit was obtained and ganglionic blockade was repeated.

Experiment 2: Pharmacological Blockade of Potential Intrarenal Chemoreceptor Stimulants

Three groups of animals that had undergone SAD were chronically instrumented as outlined previously, and on the day of the experiment each rat was allowed to stabilize during 2 hours of continuous captopril infusion. During this period, animals were subjected to converting enzyme inhibition and tested for baroreceptor reflex gain and tyramine responses as described for previous groups.

Group 1: Prostaglandin Inhibition

To determine whether production of prostaglandins is involved in the renal pressor reflex, blockade of cyclooxygenase was produced by systemic administration of indomethacin or meclofenamate (n = 12). The vehicle for indomethacin (sodium carbonate, 10 mg/ml i.v. in saline, 169–194 μl; n = 8) or meclofenamate (in saline, 94–109 μl i.v.; n = 4) was administered, and 15 minutes later, RST was produced for 1 hour as described. Following destenosis and a 60-minute recovery period, either indomethacin (Sigma; 5 mg/kg i.v., plus 2.5 mg/kg supplement 30 minutes later) or meclofenamate (Sigma; 9 mg/kg i.v., plus 4.5 mg/kg supplement 30 minutes later) was administered, and 15 minutes later, RST was repeated for 1 hour, followed by destenosis. Thirty to 60 minutes after re-
versal of stenosis, tyramine responses were retested in the presence of prostaglandin inhibition. Both indomethacin and meclofenamate at doses (2–4 mg/kg) lower than those used in the present study have been shown to decrease renal prostaglandin synthesis in the rat by approximately 80% (R. E. Colindres, personal communication, 1985).

Group 2: Kallikrein Inhibition

As a test for involvement of kinins in the activation of renal chemoreceptors during RST, the serine protease inhibitor aprotinin was administered to inhibit renal kallikrein (n = 9). A protocol identical to that for prostaglandin inhibition was followed, consisting of i.v. aprotinin vehicle administration (saline, 100–120 µl bolus plus 10–12 µl/min) and, after 15 minutes, application of RST for 60 minutes. After destenosis and a 60-minute recovery period, aprotinin (Sigma A1153; 10,000 kallikrein inhibitory units [KIU]/bolus plus 1000 KIU/kg/min) was administered and, 15 minutes later, RST was repeated for 1 hour. This dose is slightly in excess of a dose (5000 KIU/kg bolus plus 1000 KIU/kg/min) that produces 80% inhibition of urinary kallikrein activity in the rat.22 After stenosis reversal and recovery, tyramine responses were retested in the presence of kallikrein inhibition.

Group 3: Adenosine Inhibition

To determine whether release of adenosine during RST plays a role in the reflex, aminophylline (Sigma; 10 mg/kg i.v.) was administered to block adenosine receptors (n = 3) or adenosine deaminase (Sigma Type III or IV; 20 µg/kg i.v. bolus plus 2 µg/kg/min) was administered to prevent intrarenal accumulation of adenosine (n = 8). This dose of aminophylline produces complete inhibition of systemic hemodynamic responses to adenosine (1 mg) and inhibits the coronary vasodilator response by 80% in conscious dogs.23 This dose of adenosine deaminase, when given intrarenally, significantly lowers urinary adenosine concentration and excretion by more than 99% (R. E. Katholi, personal communication, 1985). A protocol identical to the cyclooxygenase and kallikrein inhibition protocol was followed. The aminophylline vehicle (saline) or adenosine deaminase vehicle (heat-denatured adenosine deaminase in 50% glycerol and 0.01 M potassium phosphate, pH 6.0) was given, and after 15 minutes, RST was produced for 60 minutes. Following destenosis and a 60-minute recovery period, either aminophylline or adenosine deaminase was administered and, 15 minutes later, RST was repeated for 1 hour. After destenosis and recovery, tyramine was retested.

Group 4: Dual Renal Stenosis

A final group of animals (n = 8) selected from the preceding groups was studied 48 hours after the preceding protocol as time controls during two successive periods of RST and recovery 75 minutes apart. That is, these animals were subjected to a protocol identical to that used in the pharmacological protocol of Experiment 2; however, with the exception of captopril, no vehicles or drugs were administered.

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 of change in resistance at time X was normalized to baseline resistance. Resistance at time X represents the resistance at specified intervals after RST, sham stenosis, or drug administration for analysis of changes in resistance over time, or it represents the maximal change in resistance, irrespective of time, for analysis of the effects of tyramine and trimethaphan. This calculation provides a valid measurement of percent changes in regional vascular resistance, since percent changes in Doppler shift (blood velocity) obtained with the Doppler flow probe have been shown to be equivalent to true percent changes in volume flow.20 All baseline (i.e., control) hemodynamic values before captopril administration and before RST or pharmacological inhibition (see Tables 1 and 2) represent averages of five determinations taken at 5-minute intervals in the 20 minutes immediately preceding the intervention. Values for the effect of captopril (see Table 1) represent averages of the final 20-minute interval of the initial 120-minute stabilization period just prior to RST. Hemodynamic values after pharmacological inhibition but before the second period of RST (see Table 2) represent averages of 10 determinations taken at 1-minute intervals 5 to 15 minutes after drug administration.

Data were analyzed with paired and grouped t tests and analysis of variance where appropriate. Preplanned paired comparisons between multiple groups were tested for significance using the Dunn-Bonferroni procedure.24 Nonlinear polynomial regression analysis and analysis of variance were employed to determine the effect of RST on hemodynamics over time for various groups and to aid in construction of curves. Significance was defined as a p level below 0.05. All values are expressed as means ± SE.

Results

Baroreceptor reflex gain was assessed after animals had become stable and before any experimental interventions to determine the effectiveness of the SAD procedure. For animals used in the saline infusion experiments (n = 13), phenylephrine administration increased MAP by 45 ± 2 mm Hg and decreased HR by 10 ± 4 beats/min, for a pressor reflex ratio of -0.23 ± 0.10 beats/min/mm Hg. This value was significantly lower (p<0.001) than the similarly obtained pressor ratio in baroreceptor reflex—intact sham-denervated animals (-1.31 ± 0.17) obtained in another study.19 Nitroglycerin decreased MAP by 42 ± 3 mm Hg and increased HR by 29 ± 5 beats/min for a depressor reflex ratio of -0.77 ± 0.12, which was also significantly (p<0.001) lower than the depressor ratio (-2.13 ± 0.27) in baroreceptor
reflex–intact conscious animals.19 Consistent with our previous studies,7 8 18 the pressor and depressor ratios were thus reduced by 82 and 64%, respectively, for an overall 73% impairment of arterial baroreceptor reflexes.

Experiment 1: Saline Infusion

Effect of Saline Infusion on Renal Pressor Reflex

Following the baroreceptor reflex test, captopril was administered and hemodynamic values averaged 40 to 60 minutes later over a 20-minute interval just prior to RST (Table 1). Before intravenous saline infusion, captopril had no significant effect on any hemodynamic parameter, although there was a tendency for MAP and resistances to be reduced. Saline expansion had no effect on baseline hemodynamics, and captopril also had no effect with the exception of slightly increased renal resistance and decreased hindquarter resistance (see Table 1). Hemodynamic values prior to RST (captopril values) were similar for euvolemic and saline-infused animals, except for a higher right renal resistance after saline infusion (see Table 1). In the euvolemic state, the renal pressor reflex was evident as an increase in arterial pressure that persisted for the duration of the stenosis and reversed upon removal of the stenosis (Figure 1). The pressor reflex was associated with moderate bradycardia and significant increases in mesenteric and hindquarter resistances (Figure 2) that rose in parallel with the pressure increases. This reflex pattern was similar to previously reported responses.7 9 Despite similar hemodynamic starting values, however, saline infusion significantly attenuated the pressor reflex (see Figure 1). The reflex elevation in mesenteric resistance was similarly attenuated, whereas the attenuations in hindquarter vasoconstriction and bradycardia did not attain statistical significance (see Figure 2). This attenuation of the reflex was not due to depressed responsiveness of the vascular neuroeffector junction. Instead, there was a clear tendency, though not significant, toward accentuated responses to I.V. tyramine administration during saline expansion (Figure 3).

Effect of Saline Infusion on Venous Pressure and Sympathetic Tone

The attenuation of the reflex by saline infusion could have been a consequence of decreased baseline neurogenic tone secondary to a rise in venous pressure and activation of cardiopulmonary baroreceptors. Thus, in a second group of animals we measured venous pressure and estimated peripheral sympathetic vascular tone by the reduction in arterial pressure and regional resistances during ganglionic blockade, before and after saline infusion (Figure 4). As in the previous group (see Table 1), euvolemic and saline-infused animals had similar values (control) for MAP, regional

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Right renal resistance (mm Hg/kHz)</th>
<th>Mesenteric resistance (mm Hg/kHz)</th>
<th>Hindquarter resistance (mm Hg/kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before saline expansion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>126 ± 8</td>
<td>335 ± 12</td>
<td>16 ± 2</td>
<td>16 ± 1</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Captopril</td>
<td>114 ± 10</td>
<td>325 ± 12</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-10</td>
<td>-3</td>
<td>-6</td>
<td>-12</td>
<td>7</td>
</tr>
<tr>
<td>No. of rats</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>After saline expansion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>110 ± 10</td>
<td>317 ± 12</td>
<td>18 ± 1</td>
<td>18 ± 2</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>Captopril</td>
<td>115 ± 11</td>
<td>325 ± 10</td>
<td>21 ± 1*†</td>
<td>16 ± 2</td>
<td>27 ± 3*</td>
</tr>
<tr>
<td>Change (%)</td>
<td>5</td>
<td>3</td>
<td>17</td>
<td>-11</td>
<td>-13</td>
</tr>
<tr>
<td>No. of rats</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Change indicates percentage of change from control.

* p < 0.05, compared with control values.

† p < 0.05, compared with pre-saline expansion captopril values.
resistances, and HR (see Figure 4). Saline infusion elevated femoral venous pressure only slightly (9 vs 11 mm Hg), and hematocrit was reduced from 37 to 36%. Ganglionic blockade with trimethaphan produced similar absolute and percentage-of-control changes in hemodynamics in euvolemic and saline-infused states. These data indicate that the level of saline expansion did not alter baseline hemodynamics or vascular neurogenic tone.

Experiment 2: Pharmacological Blockade of Potential Intrarenal Chemoreceptor Stimulants

Three groups of animals (n = 31) were studied to determine whether the neural elements within the kidney that are sensitive to reduced RBF might depend on intrarenal production of prostaglandins, kinins, or adenosine to initiate the reflex. The SAD procedure was effective in reducing overall baroreceptor reflex responsiveness in these animals by 79% relative to baroreceptor reflex-intact conscious rats.19 Phenylephrine elevated MAP by 44 ± 3 mm Hg and decreased HR by 12 ± 3 beats/min for a pressor ratio of −0.30 ± 0.06 beats/min/mm Hg. Blood pressure fell 49 ± 3 mm Hg, and HR increased 18 ± 4 beats/min with nitroglycerin, for a depressor ratio of −0.42 ± 0.08 beats/min/mm Hg. In the experimental groups in this series, captopril administration was begun immediately on connecting the animals to the recording devices, and thus its minor effects, as evidenced in Table 1, were not quantified.

Group 1: Prostaglandin Inhibition

Since either indomethacin (n = 8) or meclofenamate (n = 4) had effects on the reflex that did not differ significantly, the groups were combined. After vehicle administration, RST produced the expected increase in MAP and regional resistance (control values in Figures 5 and 6). However, during prostaglandin inhibition, the pressor response was attenuated by 78% and mesenteric and hindquarter vasoconstriction were abolished (see Figures 5 and 6). Prostaglandin inhibition alone produced small but significant increases in MAP and hindquarter resistance and decreased HR (Table

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Hemodynamic values averaged (± SE) for final 30 minutes of renal stenosis before (euvolemia) and after saline expansion. Change expressed as a percentage of control values (control values given in Table 1). Statistical analysis by two-tailed, unpaired t tests.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Vascular neuroeffector junction responsiveness to two i.v. doses of tyramine in euvolemic and saline-expanded animals. Values are means ± SE. Number of animals is given at the base of each column. Statistical analysis by two-tailed, unpaired t tests.

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** Effect of saline expansion on neurogenic tone as estimated by hemodynamic response to ganglionic blockade with i.v. infusion of trimethaphan. RRR = right renal resistance; MR = mesenteric resistance; HQR = hindquarter resistance; VP = femoral venous pressure; HCT = arterial hematocrit. Control values (means ± SE) indicate pretreatment values. Changes in parameters during trimethaphan treatment are shown as absolute values and are given at right of graphs as percent changes from control. Statistical analysis by two-tailed, paired t tests for control versus trimethaphan (single and double asterisks) and euvolemia versus saline expansion (dagger).
FIGURE 5. Reflex response to right renal artery stenosis (RST; upward-pointing arrow) and stenosis reversal (downward-pointing arrow) before (control) and during prostaglandin synthesis inhibition (PGX) with indomethacin or meclofenamate. Values are means for 12 animals. Data for both groups during stenosis fitted by least-squares polynomial regression analysis. ANOVA indicated significant increases in all hemodynamics during RST (p<0.001) only during control period; no significant (p>0.05) changes occurred during RST after PGX. Prestenosis control values are given in Table 2. Lines after stenosis reversal were drawn by hand.

Hence, baseline values for arterial pressure and regional resistances before RST were slightly increased during prostaglandin inhibition (see Table 2). The inhibitory effects of prostaglandin synthesis inhibition on the pressor reflex cannot be attributed to decreased vascular norepinephrine responsiveness. Cyclooxygenase inhibition instead increased pressor responses to intermediate doses of tyramine (Figure 7).

Group 2: Kallikrein Inhibition

Administration of the kallikrein inhibitor aprotinin had no effect on baseline hemodynamics prior to RST (see Table 2). However the pressor portion of the reflex was attenuated by 33%, although attenuations in regional vasoconstrictor responses did not attain significance (see Figure 6). In four experiments, the rate of aprotinin infusion was doubled after 60 minutes of RST and stenosis was maintained for an additional 30 minutes. The average increase in MAP over pre-RST values for these animals after 60 minutes of RST was 20 ± 3 mm Hg. After an additional 30 minutes of RST with the infusion rate for aprotinin doubled, the pressure increase (17 ± 3 mm Hg) was not significantly different. Thus, the partial inhibition of the reflex after 60 minutes of RST was not due to incomplete blockade of kinin production. Also, the inhibition was not due to an effect of aprotinin on responses of the vascular reactivity to endogenous norepinephrine, since tyramine pressor effects were not altered by aprotinin (see Figure 7).

Group 3: Adenosine Inhibition

The vehicle for aminophylline (saline) or adenosine deaminase (heat-denatured enzyme) had no effect. Arterial pressure before vehicle administration (115 ± 6 mm Hg) was not altered after a 15-minute infusion (111 ± 6 mm Hg). Neither aminophylline (n = 3) nor adenosine deaminase (n = 8) administration had an effect on the reflex response to RST; thus, the data for the two agents were combined (see Figure 6). Adenosine inhibition had no significant effect on baseline hemodynamic parameters, except for a slight decrease in MAP that became significant relative to arterial pressure prior to RST during vehicle administration (see Table 2). The lack of an effect of adenosine deaminase on the reflex was not from insufficient enzyme levels to prevent actions of endogenous or exogenous adenosine. In three experiments the rate of adenosine deaminase infusion was doubled after 60 minutes of RST for an additional 10-minute period of RST. The increase rate of infusion had no effect on the reflex. In three other experiments exogenous adenosine (150 μg/kg i.v.) was evaluated. Control responses to adenosine consisted of peak decreases in MAP and mesenteric resistance of −45 ± 11 and −56 ± 3 mm Hg/kHz, respectively. Renal and hindquarter responses were biphasic (constriction followed by dilation) and variable. During adenosine deaminase administration (20 μg/kg bolus plus 2 μg/kg/min), the depressor and mesenteric vasodilator responses were reversed (14 ± 8 and 69 ± 27 mm Hg/kHz, respective-
TABLE 2. Baseline Hemodynamics Before Right Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Right renal resistance (mm Hg/kHz)</th>
<th>Mesenteric resistance (mm Hg/kHz)</th>
<th>Hindquarter resistance (mm Hg/kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RST1 (n=12)</td>
<td>98±4</td>
<td>307±10</td>
<td>18±2</td>
<td>15±1</td>
<td>42±6</td>
</tr>
<tr>
<td>Before PGX (n=12)</td>
<td>99±5</td>
<td>310±10</td>
<td>21±2</td>
<td>17±2</td>
<td>45±7</td>
</tr>
<tr>
<td>After PGX, before RST2 (n=12)</td>
<td>107±3●</td>
<td>289±81●</td>
<td>22±2●</td>
<td>20±2●</td>
<td>57±9●</td>
</tr>
<tr>
<td>KNX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RST1 (n=8)</td>
<td>100±5</td>
<td>326±12</td>
<td>17±2</td>
<td>16±1</td>
<td>26±2</td>
</tr>
<tr>
<td>Before KNX (n=8)</td>
<td>105±4</td>
<td>310±14</td>
<td>22±2</td>
<td>18±2</td>
<td>28±2</td>
</tr>
<tr>
<td>After KNX, before RST2 (n=8)</td>
<td>108±4</td>
<td>316±12</td>
<td>21±2●</td>
<td>17±2●</td>
<td>29±3</td>
</tr>
<tr>
<td>ADX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RST1 (n=11)</td>
<td>108±5</td>
<td>338±9</td>
<td>15±2</td>
<td>14±1</td>
<td>30±5</td>
</tr>
<tr>
<td>Before ADX (n=11)</td>
<td>105±5</td>
<td>328±11</td>
<td>15±2</td>
<td>14±1</td>
<td>31±4</td>
</tr>
<tr>
<td>After ADX, before RST2 (n=11)</td>
<td>98±4∥</td>
<td>340±12</td>
<td>14±2</td>
<td>14±1</td>
<td>30±4</td>
</tr>
<tr>
<td>Dual RST (time control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RST1 (n=8)</td>
<td>105±6</td>
<td>313±15</td>
<td>15±1</td>
<td>15±1</td>
<td>42±6</td>
</tr>
<tr>
<td>RST2 (n=8)</td>
<td>105±7</td>
<td>315±12</td>
<td>15±1</td>
<td>16±1</td>
<td>49±7</td>
</tr>
</tbody>
</table>

Values are means ± SE. PGX = prostaglandin inhibition with indomethacin or meclofenamate; KNX = kinin inhibition with aprotinin; RST = renal artery stenosis; RST1 = first 60 minutes of RST; RST2 = second 60 minutes of RST, separated from RST1 by a 60-minute recovery period; ADX = adenosine inhibition with adenosine deaminase or aminophylline.

* p<0.05, compared with pre-PGX values; † p<0.01, § p<0.001, || p<0.05, compared with pre-RST1 values; □ p<0.01, compared with pre-ADX values.

Discussion

The purpose of this study was to examine the nature of the intrarenal stimulus produced by acute RST that initiates the renal pressor reflex. Based on electrophysiological studies, renal R2 chemoreceptors appear to be specifically activated by acute RST. However, no effects on arterial pressure were observed in these studies, perhaps owing to the short duration (several minutes) of stenosis and presence of intact baroreceptor reflexes and anesthesia, which inhibit the renal pressor reflex. Recordati et al.1-2 showed that activation of R2 chemoreceptors by back-perfusion of the pelvic region with concentrated urine or high sodium and potassium solutions was inhibited by intravenous saline infusion (5% of body weight). Saline infusion also reduced the basal discharge of R2 receptors by 55% in association with a sevenfold increase in urine flow and 45 to 70% decreases in urinary osmolality and potassium and urea concentration. These authors suggested that during pelvic perfusion with concentrated urine or potassium solutions, solutes such as potassium may cross the pelvic epithelium to excite or increase the sensitivity of R2 chemoreceptors. They also speculated that the inhibitory effect of intravenous saline infusion on R2 activation resulted from a reduction in the ionic strength of the pelvic urine or medullary interstitial fluid. In the present study saline infusion markedly attenuated the renal pressor reflex. We interpret the similar inhibitory effect of saline infusion on renal chemoreceptor activation that was observed previously2 together with electrophysiological evidence for ac-
tivation of R, chemoreceptors during RST\textsuperscript{10} to suggest that renal chemoreceptor activation may underlie the renal pressor reflex. The inhibitory effect of saline infusion in the present study was not simply due to a volume expansion—mediated interruption of the reflex at the effector level (i.e., by a reduction in sensitivity of resistance vessels to the neurotransmitter). Rather, vascular neuroeffector responsiveness, as indicated by tyramine responses, was unaffected by volume expansion.

It could be argued that the 2 mm Hg increase in venous pressure produced by saline infusion elicited cardiopulmonary baroreceptor reflex sympathoinhibition, and that this accounted for the inhibition of the renal pressor reflex during saline infusion. Several observations make this appear unlikely. Although atrial pressure was not measured, one would expect a proportionately smaller increase in atrial pressure than in femoral venous pressure. There is disagreement concerning the degree to which atrial pressures must be increased to evoke a cardiopulmonary baroreceptor reflex reduction in neurogenic tone. In rats with intact arterial baroreceptor reflexes that were subjected to a larger and more rapid saline infusion (10% body weight over 30 minutes) than we used in our study, increases in right atrial pressure (\( \approx 1.5 \) mm Hg) were sufficient to reduce efferent renal nerve activity.\textsuperscript{25} In contrast, Ricksten et al.\textsuperscript{26} observed that when rats that had undergone SAD were given a large, rapid i.v. infusion of plasma (2–4 ml/min over several minutes), which immediately increased MAP by approximately 25 mm Hg, increases in left atrial pressure in excess of 3 mm Hg were required to produce a detectable decrease in efferent renal nerve activity. Furthermore, neurogenic tone to the kidney is under greater cardiopulmonary baroreceptor reflex regulation than other beds.\textsuperscript{27} Hence, larger increases in venous pressure than those observed in the present study would likely be required to reduce neurogenic tone to other vascular beds sufficiently to account for the marked inhibition of the renal pressor reflex. Most importantly, in the present study the small increase in venous pressure was not sufficient in magnitude of rate of increase to produce a decrease in vascular resistance and neurogenic tone (see Table 1 and Figure 4), at least as indirectly estimated by ganglionic blockade. The slow rate of infusion may have allowed resetting of cardiopulmonary baroreceptors\textsuperscript{28} and prevented any sustained sympathoinhibition.

There is evidence in nonrodent species that intrarenal arteries may possess baroreceptorlike mechanoreceptors that discharge in direct proportion to renal artery pressure and activate a depressor reflex when stimulated.\textsuperscript{3,4} Thus, in the present study it could be argued that RST, by reducing intrarenal arterial pressure, inhibited renal arterial "baroreceptors" and activated the renal pressor reflex. Although the present study does not rule out an involvement of renal mechanor eceptors, the inhibitory effect of volume expansion on the reflex does not support this hypothesis. Extracellular fluid volume expansion with intravenous saline administration is known to increase renal interstitial fluid pressure and would be expected to decrease intrarenal artery transmural pressure. Hence, saline infusion should have accentuated inhibition of intrarenal baroreceptors during RST and augmented rather than inhibited the renal pressor reflex. Instead, it is possible that saline infusion, by increasing the rate of renal tubular fluid, lymph, and urine flow and by decreasing the ionic and osmotic strength of the medullary interstitium and urine,\textsuperscript{2,29,30} minimizes activation of renal chemoreceptors during renal stenosis by opposing intrarenal accumulation of a chemoreceptor stimulant. This hypothesis remains speculative, however, since the effects of RST and saline infusion on urinary solute concentration were not determined, and since direct recording of ARN activity was not possible.

Prostaglandins, kinins, and adenosine were evaluated as possible candidates for activation of ARN during RST. Renal stenosis increases the intrarenal release of prostaglandins\textsuperscript{11} and adenosine,\textsuperscript{12,13} and all three types of substances can excite or increase the sensitivity of visceral or renal afferent nerves, or both.\textsuperscript{12,14-18} Although evidence suggests that intrarenal kinin release may decrease with RST\textsuperscript{11} renal kinin activity could have been potentiated in the present study by the use of captopril, which inhibits the enzyme responsible for both angiotensin I conversion and kinin degradation.

Neither blockade of adenosine receptors with amrinone nor potentiation of adenosine degradation with adenosine deaminase affected the reflex response to RST. This failure was not due to insufficient drug administration, as efficacy against exogenous adenosine was demonstrated for adenosine deaminase, and doubling the infusion rate had no effect on the reflex. Thus, these data do not support an involvement of adenosine in the activation of ARN during acute RST. However, Katholi\textsuperscript{12} has obtained evidence that suggests that the maintenance of chronic one-kidney, one clip renal hypertension depends, in part, on the activation of ARN by accentuated intrarenal release of adenosine. The reason for this disparity is not obvious, but it may reside in the type of ARN involved or in the mechanism of activation under acute versus chronic RST. Also, activation of ARN by intrarenal adenosine infusion in dogs causes hypertension associated with a rise in HR and cardiac output but no change in peripheral resistance.\textsuperscript{14} Thus, this adenosine-dependent reflex differs substantially from the renal pressor reflex. Inhibition of prostaglandin synthesis with two structurally dissimilar cyclooxygenase inhibitors markedly reduced the pressor response and abolished the mesenteric and hindquarter vasoconstrictor responses to RST. The site of this inhibitory action does not appear to be the resistance vessels. Blockade of prostaglandin synthesis alone caused modest hindquarter vasoconstriction and pressure elevation that were insufficient to account for the attenuated reflex. Also, tyramine responses were enhanced during inhibition of prostaglandin synthesis, which would favor accentuation of the renal pressor reflex.
The potent antagonistic effect of inhibition of prostaglandin synthesis on the renal pressor reflex suggests that activation of intrarenal chemoreceptors during acute RST involves prostaglandins, possibly prostaglandin E₂ or prostanolcyn. This conclusion is supported by recent electrophysiological studies in which blockade of prostaglandin synthesis inhibited renal chemoreceptor stimulation by acute RST but not by other stimuli. At this time it is only possible to speculate on how intrarenal prostaglandins might activate ARN during RST. Prostaglandins (and bradykinin) have been shown to produce direct activation and increase the sensitivity of splanchnic chemoreceptorlike afferents to various stimuli. Comparable evidence for kidney afferents is not available. However, renal prostaglandins could enhance ARN discharge by one of several mechanisms, including a direct excitation of ARN endings, an action to increase ARN sensitivity to some other substance released by or accumulating during RST, or by altering tubular reabsorption of solute and water.

Substantial decreases in renal perfusion pressure in both the presence and absence of converting enzyme activity are associated with decreases in glomerular filtration rate, tubular fluid, and vasa rectae flow and urinary sodium excretion. These conditions could increase the ionic or osmotic composition of fluid in the environment near R₂ chemoreceptors, possibly located in distal nephron and pelvic regions. Further studies will be required to substantiate these initial findings suggestive of an involvement of prostaglandins in renal chemoreceptor stimulation and reflex activation during acute RST.

Some support for a partial contribution of kinins to the renal pressor reflex was indicated by the ability of aprotinin to attenuate the reflex by approximately 33%. This effect could not be ascribed to a nonspecific effect of aprotinin on baseline hemodynamics or vascular neuroeffector responsiveness. It is also unlikely that the dose employed in these studies was sufficient to attenuate prostaglandin synthesis and thereby inhibit the reflex. Indirect evidence suggests that alprostin, when administered in significantly higher concentrations, may inhibit renal cyclooxygenase activity. However, we observed no further attenuation of the reflex when the alprostin infusion rate was doubled. This and other evidence argue that the partial attenuation of the reflex was not due to incomplete kallikrein inhibition and that nonspecific cyclooxygenase inhibition was not involved, since the reflex was not attenuated further when alprostin administration was doubled. A potential involvement of intrarenal kinins in the pressor reflex is supported by a study demonstrating a renal nerve-dependent pressor effect of administration of bradykinin through the supraparenal artery into the kidney of conscious, instrumented rats. However, insofar as alprostin may inhibit other enzymes besides kallikrein, further studies are needed to substantiate any role for kinins in the reflex response to RST, and this finding should be taken cautiously. Moreover, since the pharmacological agents used in the present study to examine potential intrarenal chemoreceptor excitants were administered systemically, extrarenal effects beyond those at the vascular neuroeffector junction remain a possibility.

Acknowledgments

The author thanks David Gettes for technical assistance and Janet Leach for typing the manuscript.

References

23. Eikens E, Wikken DEL. Myocardial reactive hyperemia in conscious dogs: effect of diprydiamole and aminophylline on
Role of prostaglandins and kinins in the renal pressor reflex.

J E Faber

Hypertension. 1987;10:522-532
doi: 10.1161/01.HYP.10.5.522

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/10/5/522

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/