The Prostaglandin System

A Role in Canine Baroreceptor Control of Renin Release

JOANN L. DATA, JOHN G. GERBER, WILLIAM J. CRUMP, JÜRGEN C. FROLICH, JOHN W. HOLLIFIELD, AND ALAN S. NIES

SUMMARY We used the nonfiltering kidney model with contralateral nephrectomy to investigate the site where prostaglandins influence renin release. Adrenergic influences on renin release were excluded by renal denervation, bilateral adrenalectomy, and a continuous propranolol infusion. In this model, reduction of renal perfusion pressure by 50% increased renal venous renin activity from 3.10 ± 0.66 to 13.14 ± 3.8 ng of angiotensin I/ml per hour within 10 minutes (P < 0.05). This increase in renin activity was abolished by pretreatment with indomethacin, 8 mg/kg, but not by the sodium carbonate buffer in which indomethacin was dissolved. Infusion of arachidonic acid into the artery of the nonfiltering kidney at a rate of 10⁻³ g/kg per minute for 20 minutes also increased the renal venous renin activity from a baseline of 2.35 ± 0.37 to 5.45 ± 2.45 ng of angiotensin I/ml per hour by the end of the infusion. This effect of arachidonic acid was blocked by indomethacin. A fatty acid, 11,14,17-eicosatrienoic acid, which is not a substrate for cyclooxygenase, had no effect on renin release in this model. These data indicate that the prostaglandin system can affect the renal baroreceptor mechanism for renin release. Stimulation of prostaglandin synthesis by providing arachidonic acid increased renin secretion, and inhibition of cyclooxygenase abolished the ability of the renal baroreceptor to respond to a reduced perfusion pressure with renin release. Furthermore, this interaction is probably due to products of the renal cortical cyclooxygenase since transport of prostaglandins from the medulla to the cortex in tubular fluid cannot occur in the nonfiltering kidney.

RENNIN IS A proteolytic enzyme released from specialized cells in the juxtaglomerular apparatus of the kidney in response to a variety of stimuli. At least three mechanisms for renin release have been postulated: (1) a vascular "baroreceptor" in the afferent arterioles senses changes in arterial pressure or wall tension and releases renin in response to decreased pressure; (2) a β-adrenergic receptor responds to catecholamines by releasing renin, and (3) chemoreceptors at the macula densa of the distal convoluted tubules sense changes in tubular sodium and/or osmolality and respond with renin release.1,2 Many of the same stimuli that result in the release of renin by the kidney also increase the secretion of renal prostaglandins into urine and renal venous blood, and this suggests a link between the renin-angiotensin and renal prostaglandin systems.2-6

Several recent observations indicate that prostaglandins may play a role in the release of renin. Arachidonic acid infused into the renal artery of rabbit,7 rats,7 and dogs8 increases renin release. Indomethacin, an inhibitor of cyclooxygenase, inhibits the renin-releasing effects of arachidonic acid, hemorrhage and partial renal artery occlusion.9-10 We found that indomethacin significantly reduces renal cyclooxygenase activity in man and simultaneously reduces plasma renin activity (PRA). However, indomethacin treatment is associated with considerable sodium retention, suggesting that at least part of the reduction in PRA is secondary to volume expansion.11 More recently we found that in normal volunteers in 10 mEq sodium balance, indomethacin has no effect on supine or upright PRA. Because sodium depletion is associated with increased sympathetic tone,12 this lack of an effect of indomethacin could be the consequence of catecholamine-induced renin release. This possibility was studied in a group of hypertensive patients in 10 mEq sodium balance on high doses of propranolol, and it was found that indomethacin causes a 4-fold reduction in PRA and 65% reduction of cyclooxygenase activity without affecting sodium excretion.13 Thus, when body volume and β-sympathetic tone are kept constant, indomethacin treatment reduces PRA. This finding suggests that indomethacin exerts its effect on renin release by a mechanism independent of the sympathetic nervous system.

To investigate the possibility that indomethacin affects the baroreceptor mechanism for renin release, we used the nonfiltering kidney model of Blaine et al.14,15 In this model, the macula densa is not functional because of lack of tubular fluid flow, but the baroreceptor mechanism is intact. When combined with renal sympathectomy, adrenalectomy, and β-adrenergic blockade, the nonfiltering kidney is an effective model for isolation of the renal baroreceptor from the other major mechanisms of renin release; we used aortic clamping as the baroreceptor stimulus in this model and studied the effect of inhibiting cyclooxygenase on renin release. We also investigated the effects of arachidonic acid infusion on renin release in the nonfiltering kidney.
Methods

Twenty-six mongrel dogs weighing 16.5–30 kg were divided into four groups. On day 1 the dogs were anesthetized with pentobarbital (25 mg/kg, iv), and with aseptic technique the left renal artery, vein, and ureter were exposed through a flank incision. The ureter was ligated and severed, and the renal artery was occluded completely with a clamp for 2 hours and then released. We denervated the kidney by severing all tissue connections except the vasculature and then applied a 5% phenol solution to the vessels. The incision was closed, and the dogs were allowed to recover. On days 1–3, cortisone acetate (250 mg/day) was administered, im. On day 4, cortisone acetate (500 mg) was given 1 hour before pentobarbital (25 mg/kg, iv) anesthesia. Dogs were intubated and ventilated at 10–12 insufflations per minute. The normal kidney and the adrenals were removed through a midline incision. The brachial and femoral arteries were catheterized to measure blood pressure, and the femoral veins were catheterized for drug administration. The left renal vein was catheterized for blood sampling. Propranolol (gift from Ayerst Laboratories, New York, N.Y.) was given as a bolus of 0.3 mg/kg, followed by an infusion of 3 μg/kg per minute for the remainder of the experiment. Plasma propranolol concentrations were measured fluorimetrically10 to document that the levels were >100 ng/ml during the protocol, a concentration associated with β-adrenergic blockade.17

After a 1-hour stabilization period, the experiments were begun. In groups I and II, a supraprenal aortic clamp was applied to reduce the renal artery perfusion pressure by 50%. Renal venous blood samples were obtained for PRA determination at −10, −5, 0, +10, +20, +30, +45, and +60 minutes after clamp application. The clamp was released for 1 hour, after which indomethacin (8 mg/kg in sodium carbonate for group I or its vehicle, sodium carbonate, dose for group II) was administered intravenously. One hour after indomethacin or sodium carbonate was given, the aortic clamp was reapplied to produce a 50% reduction in perfusion pressure, and renal venous blood samples were obtained at the same time periods as before. Blood loss was replaced with equal volumes of Dextran 40.

In groups III and IV, a 25-gauge needle was placed into the renal artery of the nonfiltering kidney for infusion of physiological saline or fatty acid solutions at 0.116 ml/min. After a period of 1 hour for stabilization, sodium arachidonate was infused into the renal artery at 10−5 g/kg per minute for 20 minutes. Renal venous blood was obtained for PRA determination at −10, −5, 0, 5, 10, 15, 20, 30, 45, and 60 minutes after the start of the infusion. Indomethacin then was given intravenously as in group I, and after 1 hour the sodium arachidonate infusion and blood sampling were repeated. Sodium arachidonate was prepared from arachidonic acid (Nu Chek Prep, Inc.) as previously described.18 Dogs in group IV received a 20-minute infusion of the sodium salt of 11,14,17-eicosatrienoic acid (10−3 g/kg per minute) into the renal artery during the first half of the experiment and sodium arachidonate (10−5 g/kg per minute) in the second half without receiving indomethacin. Renal venous blood was sampled at equivalent times from dogs in groups III and group IV. Since 11,14,17-eicosatrienoic acid is not a substrate for cyclooxygenase, group IV served as a control for both the nonspecific effects of a fatty acid infusion and the effects of time of our experimental model.

Prior to the termination of the experiment, 5% indigo carmine (2 ml) was injected intravenously. Fifteen to 20 minutes later, the dogs were killed, and the kidney was examined grossly for blue discoloration of the tubules and the remainder of the filtrate. The cortex also was sent to pathology for examination. None of the kidneys showed any blue discolorations, indicating no significant filtration, and histological sections of all the nonfiltering kidneys showed intact glomeruli but most of the tubules were entirely filled with proteinaceous material. Renal venous PRA samples were collected in chilled tubes containing EDTA. The 1-hour incubation step of the renin activity assay was performed at pH 5.5 with disopropylfluorophosphate, dimercaprol, and 8-hydroxyquinoline as inhibitors of angiotensinases. Quantification of angiotensin I generated was accomplished by radioimmunoassay, using an antibody generously supplied by Dr. A. M. Michelakis. This assay is not affected by indomethacin.19 The effects of aortic clamping, indomethacin, and fatty acid infusion were evaluated statistically by the Wilcoxon Sign test because of the large interanimal variability in renin response. Significance was defined as P < 0.05.

Results

The results from groups I and II are presented in Table 1. The values for renin activity at times −10, −5, and 0 were averaged to give the preclamp baseline value for each dog. The renal venous PRA increased markedly in all dogs within 10 minutes after clamping the aorta and remained significantly elevated for 60 minutes. After indomethacin (group I), baseline renal venous PRA was not significantly different from the control baseline value, but the response of the renal venous PRA to a reduced renal perfusion pressure was inhibited. To ensure that this change was not the result of deterioration of the experimental preparation, we evaluated a second group of dogs (group II) in which sodium carbonate was substituted for indomethacin. In these animals, as in group I, there was a significant increase in renal venous PRA within 10 minutes after renal perfusion pressure was reduced. Renin activity declined over the next 50 minutes but always remained significantly elevated over the baseline value. Following sodium carbonate, aortic constriction produced a similar increase in renal venous PRA with the result that there were no significant differences in renal venous PRA at corresponding time periods in the two parts of the experiment. Thus, the blunted renin response in the presence of indomethacin could not be attributed to deterioration of the animal model or to the carbonate buffer. Arterial pressure was not significantly altered by indomethacin or the buffer. The aortic clamp was adjusted so that the renal perfusion pressure was always reduced to the same extent (Table 2A).

In group III the effects of arachidonic acid were inves-
Table 1  Renin Release from Nonfiltering Kidney with Reduction of Perfusion Pressure

<table>
<thead>
<tr>
<th>Clamp</th>
<th>Baseline</th>
<th>+10 min</th>
<th>+20 min</th>
<th>+30 min</th>
<th>+40 min</th>
<th>+60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.10 ± 0.66</td>
<td></td>
<td>13.14 ± 3.8*</td>
<td>10.59 ± 2.56*</td>
<td>10.98 ± 2.32*</td>
<td>9.21 ± 1.93*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2.44 ± 0.47</td>
<td>4.14 ± 0.7†</td>
<td>4.28 ± 0.87†</td>
<td>4.90 ± 0.94†</td>
<td>4.04 ± 0.85†</td>
<td>4.49 ± 1.35</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.35 ± 1.52</td>
<td>17.83 ± 9.45*</td>
<td>16.78 ± 9.85*</td>
<td>14.75 ± 7.18*</td>
<td>11.22 ± 5.20*</td>
<td>9.96 ± 4.08*</td>
</tr>
<tr>
<td>Carbonate buffer</td>
<td>3.99 ± 0.87</td>
<td>22.47 ± 17.30</td>
<td>20.37 ± 15.25</td>
<td>14.49 ± 9.20</td>
<td>10.52 ± 5.55</td>
<td>5.11 ± 2.16</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
*P < 0.05 as compared to preclamp baseline.
†P < 0.05 as compared to the corresponding value prior to indomethacin or carbonate.

tigated (Fig. 1). Infusion of arachidonic acid into the renal artery of the nonfiltering kidney produced a rapid rise in renal venous PRA within 5 minutes. Renin activity remained significantly elevated throughout the infusion and gradually fell to control levels after the infusion. Indomethacin significantly decreased the baseline renal venous PRA and prevented the rise in PRA induced by the arachidonate infusion. To ascertain that the renin response was specific to arachidonic acid and not an effect of any unsaturated fatty acid and to verify the stability of the preparation with time, group IV received 11,14,17-eicosatrienoic acid during the first half of the experiment and arachidonic acid in the second half. None of the dogs showed an increase in renal venous PRA during administration of 11,14,17-eicosatrienoic acid. However, administration of sodium arachidonate in the second half of the experiment again produced a rapid rise in renal venous PRA (Fig. 2). Neither fatty acid altered the systemic arterial pressure (Table 2B).

Discussion

We have shown that renin release in response to stimulation of the intrarenal baroreceptor is linked closely to the prostaglandin system. In the nonfiltering kidney model, the macula densa mechanism for renin release was nonfunctional because of lack of tubular fluid flow. The sympathetic influences on renin release were inactivated by a combination of adrenalectomy, renal sympathetic denervation, and propranolol infusion. In spite of these modifications, the nonfiltering kidney still released renin when renal perfusion pressure was reduced, indicating that the vascular baroreceptor mechanism for renin release remained intact. Indomethacin virtually abolished the release of renin in this model. Although indomethacin has been reported to inhibit renin release in vivo, the site at which indomethacin alters renin secretion has not been investigated previously. In many reported studies, indomethacin resulted in sodium retention that might

Table 2  Arterial Pressures (mm Hg) during Experimental Maneuvers

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After aortic clamp</th>
<th>During infusion of fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td>Brachial</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>121.1 ± 4.0</td>
<td>60.6 ± 1.2</td>
<td>140.3 ± 7.0</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>125.9 ± 6.2</td>
<td>57.9 ± 2.4</td>
<td>138.3 ± 8.0</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>124.9 ± 6.8</td>
<td>61.3 ± 2.6</td>
<td>142.3 ± 8.7</td>
</tr>
<tr>
<td>Carbonate</td>
<td>112.3 ± 7.9</td>
<td>61.3 ± 2.8</td>
<td>128.8 ± 12.8</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>127.8 ± 2.5</td>
<td>130.0 ± 2.7</td>
<td>134.2 ± 4.8</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>133.2 ± 4.5</td>
<td>134.2 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td>114.8 ± 7.6</td>
<td>116.2 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>112.7 ± 3.4</td>
<td>107.5 ± 5.3</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. EA = 11,14,17-eicosatrienoic acid; AA = arachidonic acid.
The demonstration that arachidonic acid, the substrate for cyclooxygenase, stimulated renin release adds further credence to the hypothesis that the prostaglandin system is intimately involved in the baroreceptor-mediated release of renin.

The effect of arachidonic acid was blocked by indomethacin and was not mimicked by another 20 carbon unsaturated fatty acid that is not a substrate for cyclooxygenase. This inhibitory effect of indomethacin on renin is probably specific for cyclooxygenase inactivation, because it has been shown by Yun et al. that indomethacin-treated dogs still release renin in response to intra-arterial prostaglandin E2. Therefore, a product of the arachidonic acid-cyclooxygenase interaction is the mediator of the arachidonic acid effects on renin.

The product of arachidonic acid primarily involved in release of renin is unknown. Published reports have shown that neither prostaglandin E2 nor prostaglandin F2α stimulates release of renin by rabbit renal cortical slices, although arachidonic acid and prostaglandin endoperoxide do result in renin release. In vivo, the findings are inconclusive; some reports indicate that large amounts of prostaglandin E1 or E2 will cause renin release and others show that small amounts of prostaglandin E1 or E2 do not release renin. Our data indicate that a product of arachidonic acid and cyclooxygenase results in renin release, and blockade of cyclooxygenase inactivates the intrarenal baroreceptor for renin release. In view of the recent interest in products of arachidonic acid other than the primary prostaglandins, it certainly is possible, that the effects we and others have observed are due to prostacyclin, thromboxane, or other cyclooxygenase-arachidonate products.

Our results also indicate that the link between the cyclooxygenase system and renin release is located in the cortex and not in the medulla. Although the renal medulla is a rich source of cyclooxygenase and accounts for the prostaglandins present in urine, it seems improbable that prostaglandins produced in the medulla could affect the renal baroreceptor in the juxtaglomerular apparatus of the nonfiltering kidney. Whereas, in the normal kidney, prostaglandins might influence renin by being transported in tubular fluid to the macula densa, such a mechanism of transport did not exist in our experiments. Other potential pathways from medulla to cortex include the vasa rectae and lymphatics, but neither of these seems likely to allow access of medullary prostaglandins to the juxtaglomerular apparatus. Therefore, the effects of arachidonic acid and indomethacin we have reported are most probably due to the cyclooxygenase system in the renal cortex.

Our data do not rule out an effect of the cyclooxygenase system on renin release that results from stimulation of the sympathetic nerves or the macula densa chemoreceptor. However, we can at least localize one site of renin-prostaglandin interaction to the renal baroreceptor.

The physiological and pathophysiological consequences of therapy with nonsteroidal anti-inflammatory drugs currently are being investigated intensively in a number of laboratories. The inhibition of the baroreceptor mechanism for renin release must now be incorporated into any schema explaining the overall effects of cyclooxygenase inhibition.

Acknowledgments

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