Renal Prostaglandins and the Control of Renin Release

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SUMMARY This study examines the hypothesis that the renal prostaglandins function as essential mediators in stimulus-secretion coupling for one or more of the basic receptor mechanisms in the control of renin release. Changes in plasma renin activity (PRA) were evaluated in response to suprarenal aortic constriction before and after indomethacin administration in conscious dogs with either a single denervated nonfiltering kidney or with intact filtering kidneys. Suprarenal aortic constriction was adjusted to reduce renal perfusion pressure below the autoregulatory range in both groups of dogs. Inhibition of cyclooxygenase with indomethacin significantly decreased urinary prostaglandin E$_2$ (PGE$_2$) excretion, but indomethacin failed to block or attenuate the increase in PRA in response to a decrease in renal perfusion pressure in either group of dogs. These results fail to support the hypothesis that the renal prostaglandins function as essential mediators of the intrarenal receptor mechanisms for renin release which are activated by a decrease in renal perfusion pressure below the autoregulatory range. (Hypertension 4 (suppl II): II-106-II-112, 1982)

KEY WORDS • renal baroreceptor mechanism • macula densa • beta-adrenergic mechanism • conscious dogs • nonfiltering kidney • urinary PGE$_2$ excretion • aortic constriction

CONTROL of renin release is complex and multifactorial. Stimuli for renin release act through three groups of mechanisms which include: 1) two intrarenal receptors, the renal vascular receptor in the afferent arteriole and the macula densa; 2) the renal nerves and a renal $\beta$-adrenergic receptor; and 3) a group of humoral agents. In the past decade, attention has been focused on the potential role of the renal prostaglandins in renin release, both as primary stimuli and as essential mediators in stimulus-secretion coupling for the renal vascular receptor, the macula densa, and the renal $\beta$-adrenergic receptor. It seems likely that the renal prostaglandins function as primary stimuli for renin release, both by a direct action on the juxtaglomerular (JG) cells and possibly also by an indirect action to amplify or attenuate the signal for other receptor mechanisms, i.e., prostaglandins alter both urinary electrolyte excretion and renal arteriolar tone and thus might indirectly influence the macula densa and the renal vascular receptor, respectively.

More controversial is the concept of the renal prostaglandins as essential mediators in stimulus-secretion coupling for one or more of the basic receptor mechanisms in the control of renin release. The fundamental importance of the autonomous renal vascular receptor mechanism for renin release led to early studies of its relationship to the renal prostaglandins, and one of the first suggestions that prostaglandins function as mediators for stimulus-secretion coupling was for the renal vascular receptor. Pretreatment with indomethacin was reported to block the renin responses to both stimuli, and it was suggested that renal vascular receptor stimulation of renin release is mediated by a cyclooxygenase product, presumably a prostaglandin. These acute experiments were performed in anesthetized dogs following surgery, conditions that stimulate basal levels of renin and prostaglandin release. Comparable studies have not been performed under more basal conditions in the conscious dog.
In the present study, experiments were designed to evaluate further the potential contribution of the renal prostaglandins in the renin response to reduced renal perfusion pressure in trained conscious dogs. Renin response to an acute reduction in renal perfusion pressure was determined before and after indomethacin administration in conscious dogs with intact filtering kidneys and in conscious dogs with a single denervated nonfiltering kidney.

**Methods**

All experiments were performed in adult female mongrel dogs. Each dog was housed in a stainless steel metabolic cage and maintained on a diet providing 60 mEq sodium per day; water was available ad libitum. After sodium balance was attained and the animals anesthetized with sodium pentobarbital (30 mg/kg i.v.), and catheters were advanced via the femoral vessels into the abdominal aorta and inferior vena cava to a level below the kidneys; the external portions of the catheters were extended subcutaneously to the back of the neck and exteriorized through a small incision, for chronic use. A left retroperitoneal flank incision was made, and a polyethylene snare (PE 90) was placed around the exposed suprarenal abdominal aorta. The snare was exteriorized to the surface through a larger diameter polyethylene tubing (PE 240). The dogs were allowed to recover from surgery for at least four days before the acute experiment was performed (Group 1).

Group 2 animals were fitted with chronic vascular catheters and a suprarenal snare in a similar manner. In addition, the left kidney was denervated and made nonfiltering by the method of Blaine et al. The denervated nonfiltering kidney preparation was developed in this laboratory and has been widely used to isolate the intrarenal vascular receptor mechanism from the influences of the renal nerves and the macula densa. Briefly, the left renal artery was occluded completely for 2 hours. Following restoration of blood flow, the ureter was ligated and sectioned. The kidney was denervated by stripping all visible nerves from the renal vessels, then painting the vessels with 5% phenol. The generated angiotensin 1 was separated by centrifugation and stored frozen until assay for PRA as described previously. Briefly, plasma samples were dialyzed for 18 hours against a phosphate buffer of pH 5.3 and incubated for 60 minutes at 37°C in the presence of diisopropylfluorophosphate (DFP). The plasma was separated by centrifugation and stored frozen until assay for PRA as described previously. Briefly, plasma samples were dialyzed for 18 hours against a phosphate buffer of pH 5.3 and incubated for 60 minutes at 37°C in the presence of diisopropylfluorophosphate (DFP). The generated angiotensin I was quantitated by radioimmunoassay, and PRA is expressed as nanograms of angiotensin I (AI) per milli-
Urinary PGE₂ content was determined on frozen urine samples by the radio-immunoassay method of Dray et al.\textsuperscript{14} following extraction with organic solvents and purification by silicic acid column chromatography. Individual recovery values for each sample were determined by adding \textsuperscript{[3H]}PGE₂ (New England Nuclear) before extraction, and the reported values for PGE₂ were corrected for losses incurred during purification. Urinary PGE₂ excretion rates are expressed as picograms of PGE₂ excreted per minute (pg/min). Plasma and urine electrolytes, creatinine, and PAH were determined by standard analytical procedures.

Results are expressed as means ± SEM. Data were subjected to analysis of variance and least significant differences (LDS) tests to determine statistical significance. Student’s \textit{t} test for paired observations was used to determine statistical significance of changes in urinary PGE₂ excretion. The null hypothesis was rejected when the \( p \) value was less than 0.05.

Results

Figure 1 depicts the changes in renin release and renal perfusion pressure during suprarenal aortic constriction in conscious dogs with intact filtering kidneys (Group 1). Prior to indomethacin administration, renal perfusion pressure was decreased to 50 ± 2 mm Hg for 30 minutes and PRA increased from control levels of 0.50 and 0.63 ng/ml hr\(^{-1}\) to 8.94 ng/ml hr\(^{-1}\) (\( p < 0.01 \)). Following release of the constriction, recovery PRA decreased toward control levels but were still statistically elevated after 30 and 60 minutes. This is not surprising since the half-life of renin in the dog has been reported to be as long as 80 minutes.\textsuperscript{17} Indomethacin was administered (8 mg/kg, i.v.) at this time. At 30 and 60 minutes after indomethacin administration, PRA appeared to remain above control value at 3.72 and 3.34 ng/ml hr\(^{-1}\), respectively, but these values were not statistically different (\( p > 0.05 \)) from control PRA values. Additionally, renal perfusion pressure returned to baseline levels. At this time, renal perfusion pressure again was decreased to 48 ± 2

\[ \text{Figure 1. Changes in plasma renin activity and renal perfusion pressure in response to suprarenal aortic constriction (stippled bars) in conscious dogs with intact filtering kidneys before and after indomethacin administration (n = 5).} \]
mm Hg for 30 minutes, and PRA increased strikingly to 8.39 ng/ml hr⁻¹ (p < 0.01 compared to both pre- and post-indomethacin controls). This level of response (ΔPRA = 5.05 ng/ml hr⁻¹) was not different statistically (p > 0.05) from the response (ΔPRA = 8.31) that was observed before the administration of indomethacin.

The other renal function data for these Group 1 dogs are presented in table 1. Indomethacin reduced urinary PGE₂ excretion from control levels of 1336 to 601 pg/min (p < 0.05) in three dogs. This 55% decrease in urinary PGE₂ excretion is similar to a 58% decrease previously reported following the administration of 5 mg/kg body weight of indomethacin to conscious dogs. Also, unpublished data from this laboratory in another series of conscious dogs (n = 5) indicated a 65% decrease in urinary PGE₂ excretion from 844 ± 140 to 282 ± 19 pg/min (p < 0.01) between 45 and 90 minutes after 5 mg/kg body weight of indomethacin. Control levels of perfusion pressure, creatinine, and paraminohippurate (PAH) clearances, and the filtered load of sodium, were not altered significantly (p < 0.05) by indomethacin. Clearances of creatinine and PAH and the filtered load of sodium fell significantly (p < 0.01) during suprarenal aortic constriction to similar levels before and after indomethacin administration, and these responses were not significantly different (p < 0.05).

Figure 2 depicts the changes in renin release and renal perfusion pressure during suprarenal aortic constriction in conscious dogs with a single denervated nonfiltering kidney (Group 2). Prior to indomethacin administration, renal perfusion pressure was reduced to 50-57 mm Hg for 40 minutes and PRA increased from control levels of 0.32 and 0.32 ng/ml hr⁻¹ to 1.50 and 1.81 ng/ml hr⁻¹ after 30 and 40 minutes, respectively (p < 0.01 for both values). Following release of the constriction, PRA returned to a level not significantly different (p > 0.05) from the baseline control. After this 90-minute recovery period, indomethacin was administered (8 mg/kg, i.v.) and post-indomethacin baseline measurements of PRA.
and perfusion pressure were not altered significantly (p > 0.05) after 30 and 60 minutes from the original baseline controls. At this time, renal perfusion pressure again was decreased to 52-58 mm Hg for 40 minutes and PRA increased strikingly to 0.97 and 1.38 ng/ml hr⁻¹ after 30 and 40 minutes, respectively (p < 0.05-0.01 compared to both pre- and post-indomethacin controls. Again, the magnitude of the responses (ΔPRA) to suprarenal aortic constriction was not different (p > 0.05) before and after indomethacin administration. Following release of the constriction, PRA returned to levels not different (p > 0.05) from baseline controls.

**Discussion**

The role of the renal prostaglandins in the control of renin release has been studied extensively. The prostaglandins appear to be important stimuli for renin release in several experimental animal models and in patients with Bartter’s syndrome. In both the conscious sodium deplete dog and the conscious dog with constriction of the thoracic inferior vena cava, inhibition of the cyclooxygenase system produced a striking decrease in PRA and also a striking decrease in renal hemodynamic function. However, in both animal models basal PRA levels never returned to normal control levels even with deletion of both adrenergic and prostaglandin pathways. It seems possible that indomethacin attenuated PRA in the sodium deplete dog and the dog with chronic constriction of the thoracic inferior vena cava by blocking synthesis of prostaglandins that stimulated renin release by a direct action on the juxtaglomerular (JG) cell.

In the present study, indomethacin administration did not alter significantly the baseline levels of PRA or renal perfusion pressure in conscious dogs with either intact filtering kidneys or with a single denervated nonfiltering kidney; baseline levels of PAH and creatinine clearances and the filtered load of sodium were unchanged by indomethacin in the dogs with filtering kidneys. Previous studies also have demonstrated no significant effect of indomethacin or meclofenamate administration on basal levels of renal function and PRA in conscious sodium replete dogs. Thus, the present results and earlier observations demonstrate a minimal influence of basal prostaglandin synthesis on PRA and renal function in conscious sodium replete dogs. In our present study, indomethacin administration or in their time control group after administration of vehicle alone. In addition, Data et al. measured renal vein renin but did not measure renal blood flow in their experiments.

In our present study, indomethacin administration failed to block or attenuate the renin response to a reduced renal perfusion pressure below the auto-

**Table 1. Renal Function Changes Produced by Alterations in Renal Perfusion Pressure in Conscious Dogs With Intact Filtering Kidneys**

<table>
<thead>
<tr>
<th>Effect of indomethacin treatment</th>
<th>RPP (mm Hg) (n = 5)</th>
<th>C\text{Cr} (ml/min) (n = 5)</th>
<th>C\text{PAH} (ml/min) (n = 5)</th>
<th>Filtered sodium (mEq/min) (n = 5)</th>
<th>UV\text{PGE}_2 (pg/min) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>109 ± 5</td>
<td>76 ± 8</td>
<td>226 ± 17</td>
<td>10.96 ± 1.09</td>
<td>1336 ± 203</td>
</tr>
<tr>
<td>Aortic constriction</td>
<td>50 ± 2†</td>
<td>25 ± 13†</td>
<td>69 ± 36†</td>
<td>3.65 ± 1.97†</td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>125 ± 5</td>
<td>60 ± 5</td>
<td>192 ± 10</td>
<td>8.83 ± 0.62†</td>
<td></td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 2</td>
<td>66 ± 5</td>
<td>190 ± 27</td>
<td>9.72 ± 0.83</td>
<td>601 ± 169*</td>
</tr>
<tr>
<td>Aortic constriction</td>
<td>48 ± 2†</td>
<td>25 ± 12†</td>
<td>84 ± 32†</td>
<td>3.80 ± 1.82†</td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>126 ± 4</td>
<td>56 ± 11</td>
<td>194 ± 34</td>
<td>8.22 ± 1.65</td>
<td></td>
</tr>
</tbody>
</table>

RPP = renal perfusion pressure; C\text{Cr} = creatinine clearance; C\text{PAH} = paraaminohippurate clearance; UV\text{PGE}_2 = urinary excretion rate of prostaglandin E₂.

* p < 0.05; † p < 0.01; ‡ p < 0.001.
regulatory range in the conscious dog with a denervated nonfiltering kidney. Measurements of urinary PGE_{2} excretion were not possible in these studies on dogs with nonfiltering kidneys, but there is no reason to believe that indomethacin did not inhibit prostaglandin synthesis in these animals to levels comparable to those observed in the filtering kidney dog series (see below). Because the present study was performed in dogs with intact adrenal glands, it is possible that circulating levels of epinephrine contributed to the renin response. This possibility seems remote however since it is unclear how suprarenal aortic constriction might activate the sympathoadrenal system. Also, suprarenal aortic constriction stimulates renin release in both intact and bilaterally adrenalectomized dogs with a single denervated nonfiltering kidney, and this finding does not support a major role for circulating epinephrine in the renin response to reduced renal perfusion pressure. For these reasons, it seems reasonable to conclude that, in the conscious dog with a single denervated nonfiltering kidney, an acute reduction in renal perfusion pressure stimulates renin release primarily through the renal vascular receptor mechanism. Thus, the present results fail to support an essential role for the prostaglandin system in stimulus-secretion coupling during activation of the renal vascular receptor mechanism for renin release.

Our present study also provides the first evidence that renin release stimulated by an acute reduction in renal perfusion pressure in conscious dogs with intact filtering kidneys is not blocked or attenuated by the cyclooxygenase inhibitor indomethacin. An acute reduction in renal perfusion pressure stimulates renin release primarily through the renal vascular receptor mechanism. Thus, the present results fail to support an essential role for the prostaglandin system in stimulus-secretion coupling during activation of the renal vascular receptor mechanism for renin release.}

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constriction when perfusion pressure was reduced below the autoregulatory range in anesthetized dogs with filtering kidneys; it seems likely that both the renal vascular receptor and the macula densa were activated in these experiments, also. Within the autoregulatory range of perfusion pressure, however, cyclooxygenase inhibition did attenuate the renin response to aortic constriction in anesthetized dogs. Given the possible multifactorial mechanisms involved in the renin response to suprarenal aortic constriction in these experiments, inhibition of renin release within the autoregulatory range may simply reflect removal of the direct action of the prostaglandins on the JG cell. With greater reductions in perfusion pressure, the renal vascular receptor and the macula densa mechanisms probably become fully activated.

Thus, the present results obtained in conscious dogs with filtering kidneys are consistent with earlier studies in anesthetized dogs with intact kidneys and suggest that reduced renal perfusion pressure below the autoregulatory range stimulates renin release by mechanisms not mediated by increased prostaglandin synthesis, possibly the renal vascular receptor and the macula densa. Although both the renal nerves and the adrenal glands were intact, the possibility that norepinephrine and epinephrine were involved in the renin response to suprarenal aortic constriction seems remote for reasons considered previously.

The precise relationship between the renal prostaglandins and the macula densa pathway to renin release is unclear. Recently, Olson et al. reduced renal perfusion pressure and decreased urinary sodium excretion in dogs undergoing intrarenal infusion of papaverine and pretreated with either propranolol or with propranolol plus a cyclooxygenase inhibitor (indomethacin or meclofenamate). In these studies, renin release did not increase in the presence of the cyclooxygenase inhibitors, and it was concluded that macula densa stimulation of renin release is mediated by the prostaglandin system. However, it should be noted that there is no assurance that the macula densa mechanism was isolated in these experiments by Olson et al. Also, at least one well-defined signal to the macula densa appears to modulate renin release by some mechanism other than altering the availability of prostaglandins. Intrarenal infusions of nonhypotensive doses of PG Il stimulated renin secretion markedly in dogs with either a filtering kidney or a single denervated nonfiltering kidney. Superimposition of intrarenal hypertonic sodium chloride infusion increased the tubular load of sodium chloride and completely inhibited renin release in the filtering kidney, but did not inhibit or attenuate PG Il-stimulated renin secretion in the nonfiltering kidney without a functional macula densa mechanism. Thus, a specific signal to the macula densa inhibits renin secretion when renin stimulating levels of PG Il are maintained constant by exogenous infusion. It appears unlikely that the renal prostaglandin system functions as the sole mediator of the macula densa mechanism in control of renin release.
There is a remote possibility that the renal nerves or circulating epinephrine influenced renin release in this study. Recent studies have clarified several aspects of the relationship between the renal prostaglandins and the beta-adrenergic pathway to renin release. Several studies in anesthetized dogs have failed to demonstrate blockade of isoproterenol-stimulated renin release with indomethacin. In addition, indomethacin failed to attenuate the renin response to isoproterenol in normotensive humans maintained on a 10 mEq Na/day intake. More recently, Seymour et al demonstrated that indomethacin administration did not block or attenuate the renin response to stimulation with isoproterenol infusion in conscious dogs. In agreement with these pharmacological studies with isoproterenol is a preliminary report that cyclooxygenase inhibitors do not block the renin response to direct low level electrical stimulation of the renal nerves in the anesthetized dog; however, the renin response was blocked by the beta receptor antagonist propranolol. Thus, the evidence in the dog supports the concept that the beta-adrenergic receptor mechanism for renin release is prostaglandin independent. This relationship is less clear in the rat, however, and indomethacin has been reported to attenuate or to have no effect on isoproterenol-stimulated renin release in this species.

The results of the present study do not exclude the possibility that other prostaglandins not measured in this study could have influenced renin release in our experiments. It has been suggested that PGF₂α may play an important role in prostaglandin control of renin release. Since urinary PGF₂α measurements were not made in the present study, the role of PGF₂α in the renin response to supraprenal aortic constriction cannot be assessed. Presumably, however, cyclooxygenase inhibition with indomethacin would have attenuated PGF₂α synthesis also.

From the present considerations, it seems reasonable to suggest that the renal prostaglandins do function as important stimuli to increase renin release by both direct and indirect pathways. However, the total evidence and the present findings fail to support a role for the renal prostaglandins as essential mediators of the other basic receptor mechanisms for renin release.

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