Prostaglandin-Related Renin Release from Rabbit Renal Cortical Slices

ERIC G. SPOKAS, PH.D., PATRICK Y-K WONG, PH.D., AND JOHN C. McGIFF, M.D.

SUMMARY The possibility that 6-keto-prostaglandin E, (6-keto-PGE,) affects renin release was studied using rabbit renal cortical slices, a preparation that eliminates hemodynamic, neural, and blood-borne factors that might influence renin release. The medium used for incubating the slices was collected for renin assay at the end of each of four successive 20-minute periods. Test agents were added only once, at the beginning of Period 3 (experimental period). Between Periods 3 and 4 (recovery period), the medium was aspirated and the slices rinsed with Krebs solution before replacing the medium. Renin release did not change in vehicle-treated slices. Unlike the PGI,-induced changes, the effects of 6-keto-PGE, on renin release were sustained in Period 4. Indomethacin potentiated renin stimulation induced by 10 μM concentrations of PGI, and 6-keto-PGE, in Period 3 and by 6-keto-PGE, in Period 4. Using platelet antiaggregatory activity as an index of stability, we found that PGI, was largely inactivated within 10 minutes under the conditions used for incubating the slices (pH 7.4, 37°C), while 6-keto-PGE, was stable. The results lend further support to the concept that 6-keto-PGE, is capable of releasing renin through a direct action.

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KEY WORDS • PGI, • prostacyclin metabolites • 6-keto-PGE, • inhibition of platelet aggregation

Evidence has been accumulating that in certain tissues a mechanism exists to sustain the biological actions of prostacyclin (PGI,). The evidence includes reports of prostacyclin-induced changes in platelet aggregation long outlasting prostacyclin's chemical half-life (about 3 minutes in aqueous solution, pH 7.6, 37°C), continued elevation of platelet cAMP despite immunological neutralization using anti-PGI antibodies, and prolonged platelet antiaggregatory activity and enhanced in vitro spasmogenic activity after incubation of prostacyclin in human plasma. Of special interest with respect to the present investigation is a report that the stimulatory effect of prostacyclin on renin release from rabbit renal cortical slices was time-dependent; i.e., the response was essentially linear for at least 30 minutes of incubation. Proposals that PGI, may be the physiologic or even obligatory mediator of renin secretion were based in part on these in vitro studies.

Studies by Wong et al. indicate, however, that PGI, can be transformed via 9-hydroxyprostaglandin dehydrogenase (9-OH PGDH) to an active and stable metabolite, 6-keto-prostaglandin E, (6-keto-PGE,). Like prostacyclin, 6-keto-PGE, exhibits a remarkable range of potent circulatory effects, inhibiting platelet aggregation, and reducing blood pressure and vascular resistance of organs and organ systems. According to a recent report by Jackson and coworkers, 6-keto-PGE, stimulates renin secretion from the in situ dog kidney at intraarterial infusion rates less than those required to demonstrate a prostacyclin effect. In the present study, we have examined the possibility that 6-keto-PGE, induces renin secretion from rabbit renal cortical slices. Effects on renin release were compared to those caused by prostacyclin. As the background levels of endogenous prostaglandins may influence the renin secretory response to exogenous prostaglandins, we also studied renin responses to 6-keto-PGE, and PGI, in slices treated with the cyclooxygenase inhibitor indomethacin. The in vitro preparation allowed the study of renin release in the absence of hemodynamic, neural, and blood-borne factors. The experimental design, modified from Weinberger et al., fortuitously afforded a ready comparison of response duration. The decay of human platelet antiaggregatory activity was used as an index of stability of PGI, and 6-keto-PGE, in the medium used for incubating the slices.
Methods

Renal Cortical Slices

Following anesthesia with sodium pentobarbital (25 mg/kg, i.v.), New Zealand white male rabbits (2.6–3.9 kg) were subjected to midline laparotomy. The left renal artery was cannulated with a 20-gauge Angiocath cannula, the ipsilateral renal vein ligated and transected distally, and the kidney flushed with 50 ml Krebs-Ringer solution. After excising and decapsulating the kidney, two slices (approximately 0.5 mm thick) were obtained from the superficial cortex using a Stadie-Riggs microtome. Each slice was subdivided and the sections added to flasks (1–2 tissues per flask, 10–30 mg dry weight) containing 5 ml Krebs-Ringer solution with 1 g/liter dextrose and 100 µg/ml BSA (KR-BSA). All flasks were placed in a 37°C water bath and the tissues gassed with 95% O₂/5% CO₂. After 20 minutes of preincubation, the medium was aspirated and all tissues were rinsed with 5.0 ml fresh KR-BSA solution. The rinse solution was discarded, and the tissues were then incubated with 5.0 ml fresh KR-BSA solution for four successive 20-minute periods (Periods 1, 2, 3, and 4). The medium was collected for renin assay at the end of each period, the medium being replaced with fresh (warmed and gassed) KR-BSA to start a new period.

In this experimental design, the first two periods were employed to indicate baseline release, and test agents and vehicle solutions were added (maximum volume 10 µl) at the start of Period 3 (the experimental period). At the conclusion of Period 3, all tissues were rinsed with an additional 5 ml KR-BSA solution before starting Period 4, which served as a “recovery” period. Each concentration of a test agent was studied using tissues from at least two rabbits. Aliquots of incubation medium withdrawn after each period were centrifuged briefly and used promptly to generate angiotensin I (Al) from homologous substrate.

Effects of Indomethacin on Prostaglandin-Evoked Renin Release

Four rabbits were pretreated with an i.v. injection of indomethacin, 5 mg/kg, 33 to 41 minutes before excising the kidney. From each kidney, cortical slices (dry weight/flask, 13.4 ± 0.5 mg, n = 20) were prepared as described above. Responses to both 6-keto-PGE₂ and PGJ₂, as well as to vehicles for these prostaglandins, were tested in each of the four pretreatment experiments, using cortical slices from a single rabbit (1 to 2 incubations/test agent). Medium from incubations of cortical slices was subjected to microfiltration through 0.2 µ pore diameter RCS58 filters (BioAnalytical Systems, West Lafayette, Indiana). For the indomethacin pretreatment experiments, indomethacin, 1 µg/ml, was also included in the KR-BSA buffer for preincubation and incubation periods. Indomethacin effectively inhibited prostaglandin synthesis in renal cortical slices as determined by radioimmunoassy of PGE₂ in the incubating medium. Control and indomethacin-treated levels of immuno-reactive PGE₂ in the cortical slice medium were 152 ± 22 pg/ml and 17 ± 11 pg/ml, respectively (n = 5, p < 0.001) using a method¹⁸ based on that of Dray et al.¹⁸

Renin Assay

Samples of medium (100 µl) were incubated at 37°C for 1 hour with 500 µl of nephrectomized rabbit plasma (pooled from four rabbits bilaterally nephrectomized 24 hours before bleeding) in the presence of disodium EDTA (3.5 mM), PMSF (3.0 mM), Tris-maleate pH 7.4 buffer (200 mM), and BSA (3 mg/ml). Reaction mixture volume was adjusted to 1.0 ml with 100 mM NaH₂PO₄-Na₂HPO₄, pH 7.4, buffer. The reaction was stopped by freezing (−20°C), and samples were stored at −20°C until assayed (less than 2 weeks).

Linearity of Al yield vs enzyme concentration was established using both a purified commercial preparation of lyophilized hog renal renin and KR-BSA medium (5–290 µl) from experiments with isoproterenol-treated slices. The substrate blank (no enzyme) and samples of slice medium incubated in the absence of nephrectomized rabbit plasma (enzyme blank) did not contain measurable amounts of Al. Recovery of Al, added to the reaction mixture as internal standard, was 95%.

Renin substrate concentration estimated by kinetic assay in the presence of a high concentration of partially purified hog renin was 2200–2640 ng Al equivalents ml⁻¹ (n = 5 pools), a value that compares favorably with the value of 2900 ng ml⁻¹ reported by Weber et al.²⁰

Angiotensin I generation was determined using the Squibb Al Immutope Kit. The interassay coefficient of variation for six consecutive radioimmunoassays performed with an 88 pg/tube quality control was 7.5%. Displacement of radioactivity by a 10 pg Al internal standard, was 95%.

Values termed “renin release” were corrected for dilution and expressed as ng Al-generated ml⁻¹ slice medium hr⁻¹ mg⁻¹ dry weight.

Platelet Aggregation Experiments

The stability of PGJ₂ and 6-keto-PGE₂ in the incubation medium used for our slice experiments (in the absence of tissue) was evaluated by following inhibition of human platelet aggregation. Aggregation tests were performed in triplicate for each prostaglandin at a final concentration in KR-BSA of 10 µM. A single stability trial was performed for 100 µM 6-keto-PGE₂. Vehicle (10 µl) containing either 6-keto-PGE₂ or PGJ₂ was added to 5 ml KR-BSA solution, which had been incubated and gassed for 20 minutes to fix the pH and temperature. Samples (20 µl) withdrawn after various time intervals were immediately added to ice-cold 50 mM Tris buffer (pH 9.3 at 5°C, total volume 1 ml). The Tris dilutions as well as blanks were then tested for inhibition of aggregation of human platelets.
Drugs

Prostaglandins (Upjohn, Kalamazoo, Michigan) were stored dessicated as the solid form at $-20^\circ$C. Solutions were freshly prepared on the day of the experiment. Prostacyclin sodium salt was dissolved in 20 ml 0.9% saline (reagent grade), was obtained from Miles Laboratories (Elkhart, Indiana), and purified hog kidney renin (lyophilized) from Schwartz-Mann (Spring Valley, New York). Indomethacin (71.4 mg, Sigma, St. Louis, Missouri) was dissolved in dry acetone (17.0 or 56.1 mg/ml) and diluted 10- or 100-fold with Krebs-Ringer solution. Bovine serum albumin, Fraction V (reagent grade), was obtained from Miles Laboratories (Elkhart, Indiana), and purified hog kidney renin (lyophilized) from Schwartz-Mann (Spring Valley, New York). Indomethacin (71.4 mg, Sigma, St. Louis, Missouri) was dissolved in 20 ml 0.9% saline containing 1.34 mg/ml sodium carbonate monohydrate (final concentration = 3.57 mg/ml).

Statistics

Results are expressed as means ± standard error of the mean (SE); p values less than 0.05 were considered significant. The statistical significance of the change in Period 3 from Period 2 was evaluated for control (no indomethacin) groups exposed to vehicle and prostaglandins using a one-way analysis of variance. Additional analyses based on one-way analysis of variance were carried out for the change in Period 3 or Period 4 from Period 2 in the indomethacin-treated group. Since the analyses indicated a significant overall difference among groups for the indomethacin data, simultaneous multiple comparisons were performed with modified \( t \) statistics using the Bonferroni method. The critical Bonferroni value was \( f_{0.017} \) as three comparisons were of interest (table 2).

Student's unpaired \( t \) test was used to compare values obtained by radioimmunoassay of PGE\(_4\) in experiments with or without indomethacin treatment.

Results

Linear release of renin is implicit in this experimental design as indicated by the stability of renin release from cortical slices of rabbits pretreated with indomethacin during Periods 1-4 of table 1. The corresponding renin values for renal cortical slices obtained from rabbits not treated with indomethacin, but exposed to vehicle, were 2.14 ± 0.36 (mean ± SE), 2.53 ± 0.44, 2.80 ± 0.58 and 2.73 ± 0.56 ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\) dry weight for Periods 1-4, respectively (n = 12).

Without indomethacin treatment, prostacyclin and 6-keto-PGE\(_4\) at 10 \( \mu \)M caused a consistent renin secretory effect during Period 3 (table 2). In response to PGI\(_2\), renin release increased by 37% to 90% over Period 2 values in five of the six preparations; the mean change = 0.84 ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\). During Period 4, a return of renin release toward control values was evident. In response to 6-keto-PGE\(_1\), renin secretion increased in Period 3 by 32% to 100% over Period 2; the mean change = 0.93 ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\). This response to 6-keto-PGE\(_1\) was observed in each of the five preparations. In response to 6-keto-PGE\(_1\), renin secretion in response to 6-keto-PGE\(_1\), renin secretion increased by 37% to 90% over Period 2; the mean change = 0.84 ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\). During Period 4, a return of renin release toward control values was evident. In response to 6-keto-PGE\(_1\), renin secretion increased in Period 3 by 32% to 100% over Period 2; the mean change = 0.93 ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\). This response to 6-keto-PGE\(_1\) was observed in each of the five preparations.

### Table 1. Renin Release (ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\) Dry Weight) from Indomethacin-Treated Rabbit Renal Cortical Slices Exposed to Vehicle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( \mu )M</th>
<th>No.</th>
<th>Period 3*</th>
<th>Period 4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle†</td>
<td>12 *</td>
<td>26</td>
<td>0.19 ± 0.24</td>
<td>0.19 ± 0.24</td>
</tr>
<tr>
<td>PGI(_2)</td>
<td>5</td>
<td>4</td>
<td>0.11 ± 0.43</td>
<td>0.27 ± 0.17</td>
</tr>
<tr>
<td>6-keto-PGE(_1)</td>
<td>1</td>
<td>6</td>
<td>0.34 ± 0.08</td>
<td>0.14 ± 0.12</td>
</tr>
<tr>
<td>Indomethacin treatment:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle†</td>
<td>7</td>
<td>18</td>
<td>0.13 ± 0.09</td>
<td>0.08 ± 0.11</td>
</tr>
<tr>
<td>PGI(_2)</td>
<td>10</td>
<td>6</td>
<td>1.40 ± 0.42</td>
<td>2.30 ± 0.43</td>
</tr>
<tr>
<td>6-keto-PGE(_1)</td>
<td>10</td>
<td>7</td>
<td>2.19 ± 0.58</td>
<td>2.30 ± 0.43</td>
</tr>
</tbody>
</table>

*Values shown for Periods 3 and 4 represent changes from Period 2 values.
†Either Krebs-acetone or Tris buffer added at the end of Period 2.

### Table 2. Prostaglandin and 6-Keto-PGE\(_1\)-Induced Changes (Mean ± SE) in Renin Release (ng ml\(^{-1}\) hr\(^{-1}\) mg\(^{-1}\) Dry Weight) from Rabbit Cortical Slices

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Treatment</th>
<th>( \mu )M</th>
<th>No.</th>
<th>Basal period</th>
<th>Experimental period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2*</td>
<td>3</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>0.8</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>4.1</td>
<td>5.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>0.9</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Prostaglandin</td>
<td></td>
<td></td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>1.35</td>
<td>1.49</td>
<td>1.67</td>
</tr>
</tbody>
</table>

*Either Krebs-acetone or Tris buffer added at the end of Period 2.

### Table 3. Decay of Human Platelet Antiaggregatory Activity in Krebs-Ringer Solution, pH 7.4, 37°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after addition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 Sec</td>
</tr>
<tr>
<td>PGI(_2)</td>
<td>10 (55.0-7.9 ng)</td>
</tr>
<tr>
<td>6-keto-PGE(_1)</td>
<td>10 (1.4-2.0 ng)</td>
</tr>
<tr>
<td>6-keto-PGE(_1)</td>
<td>10 (6.8 ng)</td>
</tr>
</tbody>
</table>

Values represent % inhibition of ADP (5 \( \mu \)M)-induced aggregation.
tions and persisted during Period 4. However, the Period 3 differences in renin release among the groups, vehicle, PG1, and 6-keto-PGE, were not significant when tested by a one-way analysis of variance.

Indomethacin potentiated stimulation of renin release by both PG1, and 6-keto-PGE, during Period 3 and the response to 6-keto-PGE, during Period 4 (table 2). In the face of cyclooxygenase inhibition, prostacyclin, at 10 μM, produced an 88% increase in renin release over the control level (Period 3 value, 2.99 ± 0.99 vs Period 2 value, 1.59 ± 0.65 ng ml⁻¹hr⁻¹mg⁻¹), an effect that was unsustained in Period 4 (1.67 ± 0.59 ng ml⁻¹hr⁻¹mg⁻¹). In contrast, renin release during Period 3 increased by 150% over Period 2 in response to 6-keto-PGE, and was maintained at this high level during Period 4 (table 2); 1.46 ± 0.63, 3.65 ± 1.17, 3.72 ± 0.95 ng ml⁻¹hr⁻¹mg⁻¹, values for Periods 2, 3, and 4, respectively. The Period 4 renin response to 6-keto-PGE, was significantly different from that induced by PG1,.

To assess the stability of PG1, and 6-keto-PGE, in the Krebs medium used for incubating the cortical slices, we studied the platelet antiaggregatory effects of samples obtained at various time intervals after addition of either prostaglandin to the medium (table 3). The platelet aggregation tests were carried out by adding aliquots (7-29 μl) of Tris buffer dilutions (containing 0.5-6.8 ng of either PG1, or 6-keto-PGE, estimated at zero time) to aggregometer cuvettes 1 minute before induction of platelet aggregation by adenosine 5'-diphosphate (ADP). Most of the antiaggregatory activity of PG1, was lost within 10 minutes, whereas 6-keto-PGE, showed no loss of activity at 20 minutes, suggesting that 6-keto-PGE, is considerably more stable than PG1, under the conditions used for incubating the renal cortical slices.

Discussion

We have demonstrated that 6-keto-PGE, is capable of exerting a prominent and sustained in vitro renin-releasing effect. The present study also confirms the report of Whorton et al. who observed prostacyclin-induced stimulation of renin release from rabbit renal cortical slices. The threshold concentration of 6-keto-PGE, which caused renin release was similar to the minimal effective concentration of 6-keto-PGE, stimulating adenylate cyclase activity in membrane fractions prepared from rat renal cortex. As cAMP may be an important component in the mechanism for stimulus-secretion coupling of renin at the level of the juxtaglomerular cell, the renin stimulatory effect of 6-keto-PGE, may result from elevation of intracellular cAMP levels. In the present study, comparison of the effects on renin release of 6-keto-PGE, with those of PG1, must be made cautiously because of inherent limitations in the experimental design; viz., progressive reduction in the effective concentration of prostacyclin throughout the 20-minute incubation period due to hydrolysis.

A primary determinant of the sensitivity of the preparation to exogenous prostaglandins may be the background level of endogenous prostaglandins. Thus, 6-keto-PGE, stimulated renin release to a greater extent in the face of cyclooxygenase inhibition. High concentrations of indomethacin have been reported to inhibit a number of renal cortical enzymes besides cyclooxygenase. While it is conceivable that the influence of indomethacin on prostaglandin-evoked renin release could be related to inhibition of cyclic nucleotide phosphodiesterase, the observation that basal release was generally reduced by indomethacin would seem inconsistent with elevation of cAMP levels. Weber et al. first reported lowering of basal renin release in vitro after inhibition of prostaglandin synthesis. Much higher concentrations of indomethacin seem to be required for inhibition of 15-hydroxyprostaglandin dehydrogenase or phosphodiesterase activity as compared to cyclooxygenase, at least 100-fold in some in vitro systems. The present study reveals a difference in the duration of renin responses evoked by PG1, and 6-keto-PGE, that is most evident in the experiments carried out using indomethacin-treated slices. Thus, renin release more than doubled in Period 3 in response to 6-keto-PGE, and continued at this level in Period 4, while the PG1, induced change recovered completely in Period 4. However, apparent differences that we observed between 6-keto-PGE, and PG1, in terms of threshold concentrations, potency, and duration of effect may arise from differences in chemical stability, penetrability of cellular barriers and metabolism of the agonists. Further, these differences may determine the renin response to exogenous but not to endogenous prostaglandins generated within or near the juxtaglomerular apparatus. For example, the failure of exogenous PG1, to have a prolonged effect on renin release, if this effect is dependent upon conversion to 6-keto-PGE, can also be accounted for by the operation of one or more of these factors. Thus, enzymic degradation of exogenous PG1, by 15-hydroxyprostaglandin dehydrogenase, which is very active in the renal cortex, would prevent its transformation to 6-keto-PGE,.

We speculate that in certain tissues prostacyclin may be subject to biotransformation to an active product. Recent work by Hoult et al. indicates that this conversion may take place in blood platelets. These investigators found prolongation of platelet antiaggregatory activity and enhanced spasmogenic effects after incubation of prostacyclin in human plasma, and tentatively identified the product of PG1, responsible for the alterations as 6-keto-PGE, A 9-OH PGDH capable of converting PG1, to 6-keto-PGE, in platelets has been purified and characterized by Wong et al. Further, Stoff et al. have described in patients with Bartter's syndrome a plasma factor responsible for a defect in platelet aggregation associated with abnormally high cAMP levels in platelet-rich plasma. The authors stress that the stability of the plasma factor argues against prostacyclin. In our platelet aggregation experiments,
we found no evidence for degradation of 6-keto-PGE, after 20 minutes of incubation under the conditions used for our experiments with cortical slices. In contrast, PGI₂ was largely inactivated in 10 minutes, as judged by decay of its antiaggregatory activity.

We have recently studied the distribution of 9-OH PGDH activity in cortical, medullary, and papillary zones of rabbit kidney and found that the intrarenal distribution of enzyme activity capable of generating 6-keto-PGE₃, parallels that of renin. Taken together, our results and those reviewed above suggest that 6-keto-PGE₃ may mediate renin release, possibly contributing to or accounting for PGI₂-induced renin release.

Acknowledgments

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References

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