Prostaglandin-Related Renin Release from Rabbit Renal Cortical Slices

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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_2-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_2 and 6-keto-PGE_1 in Period 3 and by 6-keto-PGE_1 in Period 4. Using platelet antiaggregatory activity as an index of stability, we found that PGI_2 was largely inactivated within 10 minutes under the conditions used for incubating the slices (pH 7.4, 37°C), while 6-keto-PGE_1 was stable. The results lend further support to the concept that 6-keto-PGE_1 is capable of releasing renin through a direct action.

Evidence has been accumulating that in certain tissues a mechanism exists to sustain the biological actions of prostacyclin (PGI_2). 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_2 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_2 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_2 can be transformed via 9-hydroxyprostaglandin dehydrogenase (9-OH PGDH) to an active and stable metabolite, 6-keto-prostaglandin E (6-keto-PGE_1). Like prostacyclin, 6-keto-PGE_1 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_1 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_1 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_1 and PGI_2 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_2 and 6-keto-PGE_1 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 Angio cath 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 slices 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 basal 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 as a single determination and expressed as ng Al-generated ml⁻¹ dry weight.

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 PGI₂, 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 RC58 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 radioimmunoassay 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 Immutlope 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 standard ranged from 4%–7% of maximum binding, and the intraassay coefficient of variation was 6%. Values termed "renin release" were corrected for dilution and expressed as ng Al-generated ml⁻¹ slice medium hr⁻¹mg⁻¹ dry weight.

Platelet Aggregation Experiments

The stability of PGI₂ 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 PGI₂ 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°C. Solutions were freshly prepared on the day of the experiment. Prostaglincylin sodium salt was diluted to 1.76 mg/ml with ice-cold Tris 50 mM, pH 9.3, buffer. The 6-keto-PGE$_1$ 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 dry weight for Periods 1-4, respectively (n = 12).

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 $t_{0.95,12}$ as three comparisons were of interest (table 2).

Student's unpaired t test was used to compare values obtained by radioimmunoassay of PGE$_2$ 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$_1$ at 10 μM caused a consistent renin secretory effect during Period 3 (table 2). In response to PG$	ext{I}_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 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 prepara-

| TABLE 1. Renin Release (ng ml$^{-1}$ hr$^{-1}$ mg$^{-1}$ Dry Weight) from Indomethacin-Treated Rabbit Renal Cortical Slices Exposed to Vehicle |
|-----------------|---|---|---|---|
| Basal period          | 1   | 2* | 3   | 4  |
| 0.8            | 0.5 | 0.8 | 0.7 |
| 1.2           | 1.2 | 1.2 | 0.9 |
| 0.5           | 0.7 | 1.2 | 1.0 |
| 1.0           | 0.8 | 0.8 | 0.7 |
| 4.1           | 5.2 | 5.5 | 5.5 |
| 0.9           | 1.3 | 1.5 | 1.7 |
| 0.9           | 0.8 | 0.8 | 0.8 |
| Mean         | 1.35 | 1.49 | 1.67 | 1.62 |
| ± SE         | 0.46 | 0.62 | 0.64 | 0.66 |

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

| TABLE 2. PG$	ext{I}_2$ 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 |
|-----------------|---|---|---|
| Treatment                  | μM | No | Period 3* | Period 4* |
| Vehicle†               | 12 | 0.26 ± 0.19 | 0.19 ± 0.24 |
| PG$	ext{I}_2$          | 5  | 0.08 ± 0.24 | 0.11 ± 0.43 |
| 6-keto-PGE$_1$        | 1  | 0.27 ± 0.12 | 0.34 ± 0.08 |

| PG$	ext{I}_2$          | 6  | 0.29 ± 0.10 | 0.14 ± 0.12 |
| 6-keto-PGE$_1$      | 10 | 0.93 ± 0.26 | 1.14 ± 0.41 |

| Indomethacin treatment: |
|-----------------|---|---|---|
| Vehicle†           | 7  | 0.18 ± 0.06 | 0.13 ± 0.09 |
| PG$	ext{I}_2$      | 10 | 1.40 ± 0.42 | 0.08 ± 0.11 |
| 6-keto-PGE$_1$     | 10 | 2.19 ± 0.58 | 2.30 ± 0.43 |

*Values shown for Periods 3 and 4 represent changes from Period 2 values.
†Either Krebs-acetone or Tris buffer.
‡Significantly different when compared to the corresponding period in vehicle-treated slices.

| TABLE 3. Decay of Human Platelet Antiaggregatory Activity in Krebs-Ringer Solution, pH 7.4, 37°C |
|-----------------|---|---|---|
| Time after addition |
| Treatment                  | 15 Sec | 10 Min | 20 Min |
| PG$	ext{I}_2$          | 91 ± 2  | 19 ± 6  | 4 ± 7   |
| (0.5-0.7 ng)           |        |        |        |
| 6-keto-PGE$_1$        | 53 ± 8  | 59 ± 7  | 51 ± 11 |
| (1.4-2.0 ng)         |        |        |        |
| 6-keto-PGE$_1$      | 89      | 89      | 88      |
| (6.8 ng)             |        |        |        |

Values represent % inhibition of ADP (5 μM)-induced aggregation.
tions and persisted during Period 4. However, the Period 3 differences in renin release among the groups, vehicle, PGI₁, and 6-keto-PGE₁, were not significant when tested by a one-way analysis of variance.

Indomethacin potentiated stimulation of renin release by both PGI₁ and 6-keto-PGE₁ during Period 3 and the response to 6-keto-PGE₁ 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⁻¹ hr⁻¹ mg⁻¹), an effect that was unsustained in Period 4 (1.67 ± 0.59 ng ml⁻¹ hr⁻¹ mg⁻¹). In contrast, renin release during Period 3 increased by 150% over Period 2 in response to 10 µM 6-keto-PGE₁ 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⁻¹ hr⁻¹ mg⁻¹, values for Periods 2, 3, and 4, respectively. The Period 4 renin response to 6-keto-PGE₁ was significantly different from that induced by PGI₁.

To assess the stability of PGI₁ and 6-keto-PGE₁ 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 PGI₁ or 6-keto-PGE₁ 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 PGI₁ was lost within 10 minutes, whereas 6-keto-PGE₁ showed no loss of activity at 20 minutes, suggesting that 6-keto-PGE₁ is considerably more stable than PGI₁ under the conditions used for incubating the renal cortical slices.

**Discussion**

We have demonstrated that 6-keto-PGE₁ 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-PGE₁, which caused renin release, was similar to the minimal effective concentration of 6-keto-PGE₁ 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-PGE₁ may result from elevation of intracellular cAMP levels. In the present study, comparison of the effects on renin release of 6-keto-PGE₁ with those of PGI₁ 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-PGE₁ 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 PGI₁ and 6-keto-PGE₁ 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-PGE₁, and continued at this level in Period 4, while the PGI₁-induced change recovered completely in Period 4. However, apparent differences that we observed between 6-keto-PGE₁ and PGI₁ 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 PGI₁ to have a prolonged effect on renin release, if this effect is dependent upon conversion to 6-keto-PGE₁, can also be accounted for by the operation of one or more of these factors. Thus, enzymic degradation of exogenous PGI₁ by 15-hydroxyprostaglandin dehydrogenase, which is very active in the renal cortex, would prevent its transformation to 6-keto-PGE₁.

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 platelet membranes. 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 PGI₁ responsible for the alterations as 6-keto-PGE₁. A 9-OH PGDH capable of converting PGI₁ to 6-keto-PGE₁ 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-PGE₁ after 20 minutes of incubation under the conditions used for our experiments with cortical slices. In contrast, PG₁₂ 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-PGE₁, parallels that of renin. Taken together, our results and those reviewed above suggest that 6-keto-PGE₁ may mediate renin release, possibly contributing to or accounting for PG₁₂-induced renin release.

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