Evidence for a Direct Stimulatory Effect of Prostacyclin on Renin Release in Man

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ABSTRACT The objectives of this investigation were: (*a*) to characterize the time and dose dependence of the effects of prostacyclin (PGI_2) on renin release in healthy men; (*b*) to define whether PGI_2-induced renin release is secondary to hemodynamic changes; (*c*) to determine the plasma and urine concentrations of 6-keto-$PGF_{1\alpha}$ (the stable breakdown product of PGI_2) associated with renin release induced by exogenous or pharmacologically enhanced endogenous PGI_2. Intravenous PGI_2 or 6-keto-$PGF_{1\alpha}$ infusions at nominal rates of 2.5, 5.0, 10.0, and 20.0 ng/kg per min were performed in each of six normal human subjects; in three of them, PGI_2 infusion was repeated after β-adrenergic blockade and cyclooxygenase inhibition. PGI_2, but not 6-keto-$PGF_{1\alpha}$, caused a time- and dose-dependent increase of plasma renin activity, which reached statistical significance at 5.0 ng/kg per min and was still significantly elevated 30 min after discontinuing the infusion. Although combined propranolol and indomethacin treatment significantly enhanced the hypotensive effects of infused PGI_2, it did not modify the dose-related pattern of PGI_2-induced renin release.

Plasma 6-keto-$PGF_{1\alpha}$ levels rose from undetectable levels (<7.5 pg/ml) in a stepwise fashion during increasingly higher infusion rates of PGI_2 or 6-keto-$PGF_{1\alpha}$. The threshold concentration of plasma 6-keto-$PGF_{1\alpha}$ associated with a statistically significant stimulation of renin release was ~200 pg/ml. Upon discontinuing PGI_2 or 6-keto-$PGF_{1\alpha}$ infusion, the disappearance of 6-keto-$PGF_{1\alpha}$ from blood showed an identical biphasic behavior, the initial phase having an apparent $t_{1/2}$ of 3.2 min. The intravenous infusion of furosemide, which is known to stimulate renin release via a cyclooxygenase-dependent mechanism, caused a three-to fourfold increase of urinary 6-keto-$PGF_{1\alpha}$ excretion rate, concomitant with the elevation of plasma renin activity levels, in six healthy women. 6-Keto-$PGF_{1\alpha}$ remained undetectable in peripheral venous plasma throughout the study.

We conclude that in human subjects: (*a*) PGI_2-induced renin release occurs with a dose and time dependence similar to its reported platelet effects; (*b*) PGI_2-induced renin release is not mediated by adrenergic stimuli or cyclooxygenase-dependent mechanisms secondary to hemodynamic changes; (*c*) furosemide-induced renin release is associated with increased renal PGI_2 formation; and (*d*) PGI_2 appears to act as a local modulator rather than a circulating hormone in controlling juxtaglomerular function.

INTRODUCTION

A large body of experimental as well as clinical evidence indicates that intrarenal metabolism of arachidonic acid via the cyclooxygenase pathway may participate in the control of renin release (1–3). However, the precise nature of the arachidonate metabolite(s) involved remains questionable. Thus, although a va-

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riety of vasoactive prostaglandins (PG)[1] stimulate renin release, when infused systemically or intrarenally in several animal species and man, conflicting results have been reported on their effects on renin release from renal cortical slices or cortical cell suspensions (1–3). The demonstration that rabbit cortical microsomes synthesize prostacyclin (PGI_2) from arachidonic acid and PGG_2 (4), both of which were previously found to stimulate renin release from cortical slices (5), has prompted the group of J. A. Oates to investigate the effects of PGI_2 on renin release in this isolated system. They found PGI_2 but not PGE_2 to cause a time-dependent stimulation of renin release over the range of 0.1–10 μM (6), and suggested that PGI_2 is the metabolite of arachidonic acid, which mediates its effect on the secretion of renin in rabbit. We have recently reported preliminary evidence suggesting a similar role for PGI_2 in man (7). The aims of the present investigation were: (*a*) to characterize the time and dose dependence of the effects of exogenous PGI_2 on plasma renin activity (PRA) in healthy men; (*b*) to define whether a secondary activation of the β-adrenergic mechanism of renin release or endogenously released cyclooxygenase products possibly mediating other mechanisms of renin release were responsible for PGI_2-induced PRA elevation; and (*c*) to determine the plasma and urine concentrations of 6-keto-$PGF_{1\alpha}$ (the stable breakdown product of PGI_2) associated with renin release induced by exogenous or pharmacologically enhanced endogenous PGI_2 (8). For this purpose we have infused PGI_2 or 6-keto-$PGF_{1\alpha}$, at four nominal rates into each of six normal human subjects and repeated PGI_2 infusion in three of them after β-adrenergic blockade and cyclooxygenase inhibition. In addition, furosemide, which is known to stimulate renin release by causing an acute overall activation of the renal PG-system (9), was infused i.v. into six healthy women.

These studies demonstrate that PGI_2-induced renin release is not mediated by adrenergic stimuli secondary to hemodynamic changes, and may represent a physiologic mechanism locally regulating juxtaglomerular function in man.

METHODS

Prostacyclin and 6-keto-prostaglandin $F_{1\alpha}$ infusions. Six healthy male physicians (age: 27, 31, 34, 35, 43, and 50 yr; weight 63, 72, 65, 80, 80, and 72 kg) each consented to four consecutive 30-min PGI_2 or 6-keto-$PGF_{1\alpha}$ infusions at nominal rates of 2.5, 5.0, 10.0, and 20.0 ng/kg per min. In a given subject, PGI_2 and 6-keto-$PGF_{1\alpha}$ infusions were separated by intervals of at least 1 mo. After an overnight fast, the subjects remained fasting and in the recumbent position from ~9 a.m. to 2 p.m. Intravenous catheters, one for infusion and one for sampling, were inserted into antecubital veins 60 min before the infusion. A sphygmomanometer cuff was placed around the left arm for arterial pressure recording. 0.5–1 mg of PGI_2 sodium salt was dissolved in appropriate volumes of sterile glycine buffer pH 10.5 (both obtained from the Wellcome Research Laboratories, Beckenham, England, through the courtesy of Dr. S. Moncada) immediately before each study and cooled at 4°C. This solution was infused with a Harvard infusion apparatus (Harvard Apparatus Co., Inc., S. Natick, Mass.) at rates of 0.1–0.8 ml/min. A thermostatic jacket was built around the syringe in order to keep its temperature as close as possible to 4°C. The stability of PGI_2 under these conditions was verified periodically by comparing the antiaggregatory activity of its solution taken at the end of each infusion period, with the activity of a freshly prepared standard. To obtain a sterile solution of 6-keto-$PGF_{1\alpha}$, the corresponding amount of PGI_2 was dissolved in sterile saline and boiled for 30 min; this concentrated solution was further diluted into an appropriate volume of glycine buffer, pH 10.5, and infused in an identical fashion to PGI_2. Conversion of PGI_2 into 6-keto-$PGF_{1\alpha}$ was verified by assessment of loss of platelet antiaggregatory activity and radioimmunological determination of 6-keto-$PGF_{1\alpha}$ content of this solution. No attempt was made at having the subjects blind as to the nature of the substance infused, in view of obvious and consistent subjective effects of PGI_2, i.e. skin flushing, especially marked on face, neck, and palms, accompanied by a sensation of warmness in the same areas. No serious untoward effects occurred, although the highest infusion rate commonly caused restlessness and/or headache.

Blood samples (5–10 ml) were drawn (and blood pressure recorded) at 15-min intervals before and during each infusion and at more frequent intervals for 30 min after each infusion. Blood was promptly distributed into iced tubes containing EDTA (0.02 ml of a 5% solution per milliliter of blood). All tubes were promptly centrifuged in a refrigerated centrifuge and the supernates frozen for subsequent analysis.

Urine voided during 2 h preceding the infusion, at the end of it, and up to 24 h after the infusion was collected and immediately frozen. Both urine and plasma samples were kept at −20°C until the time of the assays.

Three of the same subjects gave their informed consent to repeating the PGI_2 infusions after indomethacin and propranolol treatment in order to minimize the contribution of endogenous PG production and β-adrenergic receptors to renin release. The expected risk of enhanced vascular activity of infused PGI_2 was fully discussed among the participating investigators, and appropriate measures were taken in order to secure immediate termination of any severe hypotensive effect. The highest infusion rate was reduced to 15 min for safety considerations. Propranolol (inderal, icpharma) was given according to a conventional schedule: 40 mg on day 1, 120 mg on day 2, 240 mg on days 3 and 4, and 80 mg on day 5, 2 h before the infusion. Indomethacin, (Indocid, Merck Sharp & Dohme Canada Ltd., Montreal, Quebec, Canada) was given on day 3 (100 mg), day 4 (150 mg), and day 5 (50 mg, 2 h before the infusion). The reason for a shorter indomethacin treatment lies in the possible attenuation of its inhibitory effect on renal PG synthesis despite sustained administration (10). Blood and urine samples were obtained as described in the control study.

Furosemide studies. Informed consent was obtained

[1] *Abbreviations used in this paper*: MBP, mean blood pressure; PG, prostaglandin (used variously according to the identification of a given prostaglandin, i.e. PGE_2 or PGI_2); PRA, plasma renin activity; RIA, radioimmunoassay; TLC, thin-layer chromatography; GC/MS, gas chromatography/mass spectrometry; 6-keto-$PGF_{1\alpha}$-LI, 6-keto-$PGF_{1\alpha}$-like immunoreactivity; TX, thromboxane.

from six healthy women, i.e., nurses of the Nephrology Division of the Department of Medicine (University of Rome, Italy), aged 20–40 yr. They were placed on a controlled sodium and potassium intake (100 and 80 meq/d, respectively) for 5 d before the study. They were catheterized after an overnight fast and remained recumbent for the whole duration of the experiment. A 4-h control sample before and 12 consecutive 15-min urine samples after an i.v. injection of furosemide (Lasix, Hoechst Pharmaceutical Co., Kansas City, Mo.: 50 mg) were collected and immediately frozen. Urine samples obtained after 60 min were pooled into 30-min fractions before extraction. Samples of peripheral venous blood (10 ml) were drawn into iced tubes containing EDTA, before and 15, 30, 60, 120, and 180 min after furosemide. The separated plasma was frozen immediately.

Analyses. Plasma and urine concentrations of 6-keto-$PGF_{1\alpha}$ were determined by a recently developed radioimmunoassay (RIA) technique. This uses 5,000 dpm of 6-keto-[^{3}H]$PGF_{1\alpha}$ (New England Nuclear, Boston, Mass. 150 Ci/mM) and a rabbit anti-6-keto-$PGF_{1\alpha}$ serum diluted 1:400,000 in a final volume of 1.5 ml. Approximately 40% binding of the tracer is obtained after 16–24-h incubation at 4°C. Separation of free from antibody-bound 6-keto-[^{3}H]$PGF_{1\alpha}$ is obtained by rapid addition of 10 mg of uncoated charcoal (Norit A) and subsequent centrifugation at the same temperature, as previously described for other PG (10). The binding of 6-keto-[^{3}H]$PGF_{1\alpha}$ is inhibited by unlabeled 6-keto-$PGF_{1\alpha}$ in a linear fashion, over the range 0.5 to 50 pg/ml, with an IC_{50} of 8 pg/ml. This antiserum (AS 1) has an association constant of 1.9×10^{11} liters/M, as determined graphically by a Scatchard plot (Table I). The least detectable concentration that can be measured with 95% confidence (i.e., 2 SD at zero) is 0.5 pg/ml. The immunological specificity of AS 1 is reported in Table II. 25-ml urine samples were extracted and subjected to silicic acid column chromatography before the assay as described (10). The overall recovery was assessed by labeled as well as unlabeled 6-keto-$PGF_{1\alpha}$, and found to be identical to that of other urinary PG, i.e., ~70% (10). Recovery yields were determined for each extraction-purification run by adding a known amount of [^{3}H]$PGF_{1\alpha}$, and urinary PG concentrations were corrected accordingly. The silicic acid column eluates were assayed at a final dilution ranging from 1:60 to 1:300, depending upon the nature of the sample. Validation of RIA measurements was obtained by three independent criteria, i.e., comparison with other anti-6-keto-$PGF_{1\alpha}$ sera; characterization of the thin-layer chromatographic (TLC) pattern of distribution of the extracted 6-keto-$PGF_{1\alpha}$-like immunoreactivity (LI); comparison with gas-chromatography/mass spectrometry (GC/MS) determinations. Three additional anti-6-keto-$PGF_{1\alpha}$ sera (which will be referred to as AS 2, AS 3, and AS 4) were obtained from one European and two American laboratories. The binding characteristics and immunological specificities of these antisera are described in Table I and II in comparison with AS 1. The TLC pattern of distribution of urinary 6-keto-$PGF_{1\alpha}$-LI was assessed with four different antisera, showing varying degrees of cross-reactivity with other structurally related compounds, as previously described for urinary PGE_2 (10, 11). For this purpose, purified extracts obtained from 24-h urine collections as well as from pooled 15-min samples obtained after furosemide injections were subjected to TLC using the ascending technique, as described in detail elsewhere (10). The whole lanes corresponding to the chromatographed samples were then divided into 0.5–1-cm segments, the silica gel scraped off and eluted with methanol. Each eluate, from the origin to the solvent front, was then assayed for 6-keto-$PGF_{1\alpha}$-LI with four different antisera. For comparison with GC/MS determination, a 500-ml sample pooled from urine collections obtained from three subjects before PGI_2 infusion was subjected to extraction and silicic acid column chromatography. The column eluate corresponding to 6-keto-$PGF_{1\alpha}$ was then subjected to TLC. Silica gel corresponding to the 6-keto-$PGF_{1\alpha}$ area, as determined by comparison with cochromatographed authentic 6-keto-$PGF_{1\alpha}$, was scraped off and eluted with methanol. This was divided into two identical aliquots and evaporated to dryness. 6-keto-$PGF_{1\alpha}$ concentrations were determined by RIA in our laboratory, using AS 1, and by GC/MS in the Wellcome Research Laboratories through the courtesy of Dr. John Salmon. The eluates obtained from a side lane of the same plate were subjected to the same procedures and used as blank. For GC/MS analysis, the urine extract and the blank were derivatized by reaction with freshly prepared diazomethane methoxylamine hydrochloride and finally bis-(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS). Methoxylamine HCl, BSTFA, and TMCS were purchased from Pierce Chemical Co., Rockford, Ill. The derivatives were injected into a Hewlett-Packard model 5730 gas chromatograph combined with a VG Micromass 16F mass spectrometer (Hewlett-Packard Co., Palo Alto, Calif.). The gas chromatograph was fitted with a 1% OV17 column operated at 240°C. Selected ion monitoring of m/e 598 and 508 was performed at 40 eV; the source current and temperature were 500 μA and 210°C, respectively. The urine extract produced ions at both m/e 598 and 508 at a comparable retention time as authentic derivatized 6-keto-$PGF_{1\alpha}$. No such ions were detected in the blank. Moreover, the ratio

TABLE I
Binding Parameters of RIA Systems for 6-Keto-$PGF_{1\alpha}$

	Final dilution of antiserum	IC_{50}*	Ka‡
		pg/ml	*liters/M*
RIA 1	1:400,000	8	1.9×10^{11}
RIA 2	1:25,000	28	0.9×10^{11}
RIA 3	1:150,000	24	1.2×10^{11}
RIA 4	1:500,000	12	1.4×10^{11}

* Concentration required to displace 50% of bound 6-keto-[^{3}H]$PGF_{1\alpha}$.
‡ Association constant.

TABLE II
Immunological Specificity of Antisera Directed against 6-keto-$PGF_{1\alpha}$

Substance measured	AS 1	AS 2	AS 3	AS 4
	relative cross reaction			
	%			
6-keto-$PGF_{1\alpha}$	100	100	100	100
6-keto-PGE_1	1.9	16.6	0.8	18.1
PGE_2	0.3	1.0	0.2	2.1
PGD_2	0.2	0.3	0.2	1.0
13,14-DH-6,15-DK-$PGF_{1\alpha}$	0.1	0.7	8.7	3.3
6,15-DK-$PGF_{1\alpha}$	0.06	0.8	3.5	1.4
$PGF_{2\alpha}$	0.05	2.0	0.3	8.0
2,3-Dinor-6-keto-$PGF_{1\alpha}$	0.006	0.004	18.5	14.6
TXB_2	0.003	0.006	0.004	0.03

of the areas of peaks 508–598 were comparable in standard and unknown. Urinary excretion of PGE_2 was used as a control for monitoring renal PG synthesis under the influence of exogenous PGI_2. Urinary PGE_2 was measured by RIA as described (10). Unextracted samples of peripheral venous blood were assayed for 6-keto-$PGF_{1\alpha}$ by RIA, using AS 1, at a final dilution ranging from 1:15 (under basal conditions) to 1:150 (at the highest infusion rate). Standard curves were prepared for each assay, with the same amount of unextracted "PG-free" plasma in order to correct for nonspecific protein binding. All the samples drawn in each infusion study were assayed simultaneously in triplicate. The nature of plasma 6-keto-$PGF_{1\alpha}$-LI detected during PGI_2 or 6-keto-$PGF_{1\alpha}$ infusion was characterized by TLC, as recently described for serum thromboxane (TX) B_2 (12).

Intraassay and interassay variability of RIA measurements was evaluated by assay of several urine and plasma samples. Intraassay variability for AS 1 averaged 4%, whereas interassay variability averaged 8% over a range of 6-keto-$PGF_{1\alpha}$ concentrations from 15 to 200 pg/ml.

Plasma renin activity (PRA) was measured by RIA of angiotensin I, as described by Haber et al. (13), using a commercially available kit (Sorin Biomedica, Saluggia, Italy). Urinary sodium was determined by flame photometry. Arterial blood pressure was recorded manually by the same investigator throughout each study.

Results were analyzed using parametric analysis of variance. A paired Student's t test was used to compare changes in arterial blood pressure and PRA secondary to PGI_2 infusion to 6-keto-$PGF_{1\alpha}$ infusion performed in the same subjects at identical rates. The same test was used to compare changes secondary to PGI_2 infusion under control conditions with infusion of PGI_2 after indomethacin and propranolol treatment in the same subjects.

RESULTS

Mean ($\pm$SD)%changes of mean blood pressure (MBP) and PRA induced by PGI_2 or 6-keto-$PGF_{1\alpha}$ infusion at each of the four nominal infusion rates are illustrated in Fig. 1. PGI_2 caused a dose-related decrease of MBP, fully reversible within 15 min after discontinuing the infusion. In addition, it caused a dose-dependent increase of PRA, which reached statistical significance only at 5 ng/kg per min, and was still significantly elevated 30 min after discontinuing the infusion. Changes in PRA and MBP correlated in a statistically significant fashion ($r = 0.50$, $P < 0.01$, $n = 65$). Heart rate increased progressively during the infusion, reaching a maximum during the 20-ng/kg per min stage ($21 \pm 5\%, P < 0.01$). In contrast, 6-keto-$PGF_{1\alpha}$ infused into the same subjects at identical rates did not cause any statistically significant changes of the measured parameters. In addition to indicate that the stable breakdown product of PGI_2 does not contribute to its biological effects to any significant extent, these results also demonstrate that the glycine buffer pH 10.5 used to dissolve PGI_2 was virtually devoid of any effect on MBP or PRA.

Mean ($\pm$SD) plasma 6-keto-$PGF_{1\alpha}$ concentrations measured before, during, and after PGI_2 or 6-keto-$PGF_{1\alpha}$ infusion, are illustrated in Fig. 2. Plasma 6-keto-

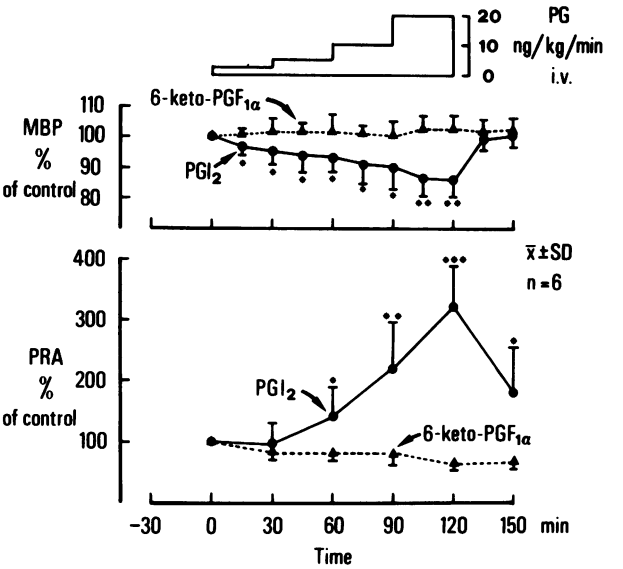

FIGURE 1 Prostacyclin (PGI_2) or 6-keto-$PGF_{1\alpha}$ infusions. Changes (%) in mean blood pressure (MBP) and plasma renin activity (PRA), measured during and after 30-min infusions at four nominal infusion rates as compared with control values. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$: PGI_2 vs. 6-keto-$PGF_{1\alpha}$.

$PGF_{1\alpha}$ levels rose from undetectable values (<7.5 pg/ml) in a stepwise fashion during increasingly higher infusion rates of both substances. When PGI_2 was infused into three subjects at a constant rate of 10 ng/kg per min for 80 min, it gradually raised plasma 6-keto-$PGF_{1\alpha}$ concentrations up to 20 min; thereafter, relatively constant levels were maintained throughout the infusion (14). Using the steady-state plasma concentrations of 6-keto-$PGF_{1\alpha}$ and the PGI_2 infusion rate we calculated a clearance rate of 24.4 ± 9.8 (SD) ml/kg per min. Upon discontinuing PGI_2 or 6-keto-$PGF_{1\alpha}$ infusion, the disappearance of 6-keto-$PGF_{1\alpha}$ from blood showed an identical biphasic behavior. The initial phase (10 min) had an apparent $t_{1/2}$ of 3.2 min after PGI_2 ($r = 0.80$, $P < 0.005$) or 6-keto-$PGF_{1\alpha}$ ($r = 0.91$, $P < 0.005$) infusion; the slower phase had an apparent $t_{1/2}$ of 15 min following PGI_2 ($r = 0.83$, $P < 0.005$) or 6-keto-$PGF_{1\alpha}$ ($r = 0.88$, $P < 0.005$) infusion. Plasma 6-keto-$PGF_{1\alpha}$ concentrations returned to undetectable levels 60 min after discontinuing the infusion. Plasma immunoreactive 6-keto-$PGF_{1\alpha}$, as detected by AS 1, met classical criteria of specificity for RIA measurements: i.e. it was recovered quantitatively when added to plasma, and its concentrations decreased in a linear fashion upon dilution. Furthermore, when subjected to extraction and TLC the vast majority (>90%) of the recovered immunoreactivity (60%) cochromatographed with authentic 6-keto-$PGF_{1\alpha}$.

The means ($\pm$SD)of diuresis and urinary excretion

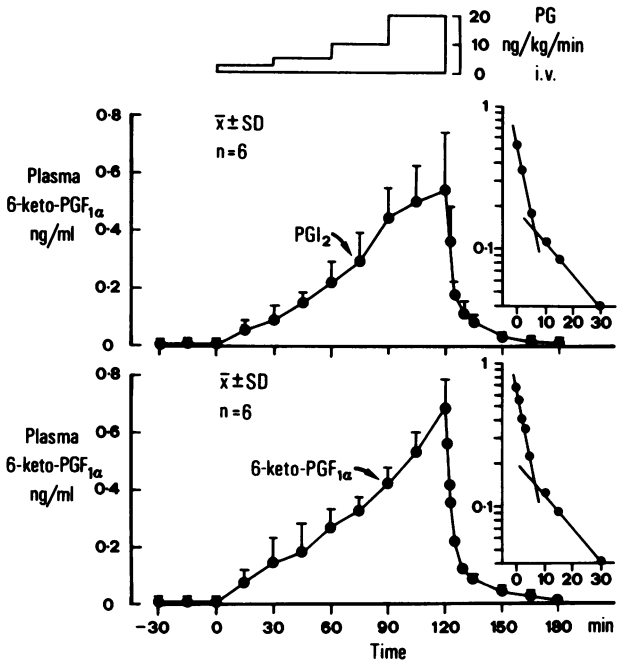

FIGURE 2 Prostacyclin (PGI_2) or 6-keto-$PGF_{1\alpha}$ infusions. Mean (±SD)plasma 6-keto-$PGF_{1\alpha}$ concentrations measured before, during, and after 30-min infusions at four nominal infusion rates. A semilogarithmic plot of the disappearance rate of plasma 6-keto-$PGF_{1\alpha}$, upon discontinuing the infusion, is also represented.

rates of Na, PGE_2, and 6-keto-$PGF_{1\alpha}$ before and during PGI_2 or 6-keto-$PGF_{1\alpha}$ infusion are shown in Table III, PGI_2 but not 6-keto-$PGF_{1\alpha}$, caused a statistically significant increase of urine flow and Na excretion rate. This was markedly attenuated in the three subjects receiving PGI_2 infusion, after β-adrenergic blockade and cyclooxygenase inhibition (not shown). Both PGI_2 and 6-keto-$PGF_{1\alpha}$ caused a marked elevation of the urinary 6-keto-$PGF_{1\alpha}$/PGE_2 ratio from 0.52 to 9.5 and from 0.58 to 31.6, respectively. When calculated over the total amount of PGI_2 or 6-keto-$PGF_{1\alpha}$ infused, percent recovery of unchanged urinary 6-keto-$PGF_{1\alpha}$ averaged 0.93 ± 0.34and 3.55 ± 1.12(Mean ± SD,$P < 0.01$), respectively. Approximately 95% of the recovered immunoreactive 6-keto-$PGF_{1\alpha}$ was excreted within 2 h after the end of the infusion for both PGI_2 and 6-keto-$PGF_{1\alpha}$.

The possible contribution of β-adrenergic stimuli and of secondary release of cyclooxygenase products of arachidonic acid metabolism to the observed PGI_2-induced renin release was investigated by repeating PGI_2 infusion in three subjects after pretreatment with a β-adrenergic blocking agent, i.e. propranolol and a cyclooxygenase inhibitor, i.e. indomethacin. After this combined treatment, basal PRA averaged 0.2±0.09 ng/ml per h (mean±SD), as compared with 0.83±0.25 of the previous experiment ($P < 0.025$). Diastolic blood pressure averaged 80±15 mm Hg as compared with 85±10 of the previous experiment (P = NS); heart rate averaged 52±5 beat/min as compared with 71±15 ($P < 0.01$) and indicated effective β-adrenergic blockade. The urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ averaged 14.2±6.3 and 6.1±3.5 ng/h before treatment and was reduced to 5.4±2.3 and 2.2±1.3, respectively, on day 3 of indomethacin treatment (mean±SD, $P <$ 0.01 for both compounds), thus indicating effective cyclooxygenase inhibition. Mean (±SD) percent changes of MBP and PRA induced by PGI_2 infusion, under control conditions and after propranolol and indomethacin treatment, are illustrated in Fig. 3. Although this combined pharmacologic treatment significantly enhanced the hypotensive effect of infused PGI_2 at higher infusion rates, it did not modify the dose-related pattern of PGI_2-induced renin release to any significant extent. Persistence of β-adrenergic blockade throughout the experiment was further demonstrated by failure of heart rate to reach basal con-

TABLE III

Mean (±SD) Values of Urine Flow, and Urinary Excretion Rates of Sodium, PGE_2 and 6-keto-$PGF_{1\alpha}$ before and during Multidose Infusions (2.5–20 ng/kg/min) of Prostacyclin (PGI_2) or 6-keto-$PGF_{1\alpha}$ in Six Healthy Subjects

Infusion	Urine flow	Urinary excretion rates of		
		Na^+	PGE_2	6-keto-$PGF_{1\alpha}$
	ml/min	*meq/min*	*ng/h*	*ng/h*
Control	0.62±0.27	0.09±0.04	13.7±6.3	6.6±4.7
PGI_2	1.47±1.01	0.15±0.04	23.3±22.6	222.2±112.8
P*	<0.05	<0.01	NS	<0.005
Control	0.69±0.19	0.11±0.03	21.2±13.9	12.4±5.2
6-keto-$PGF_{1\alpha}$	1.10±0.49	0.10±0.02	23.4±11.7	740.3±235
P*	NS	NS	NS	<0.005

* As determined by the paired t test.

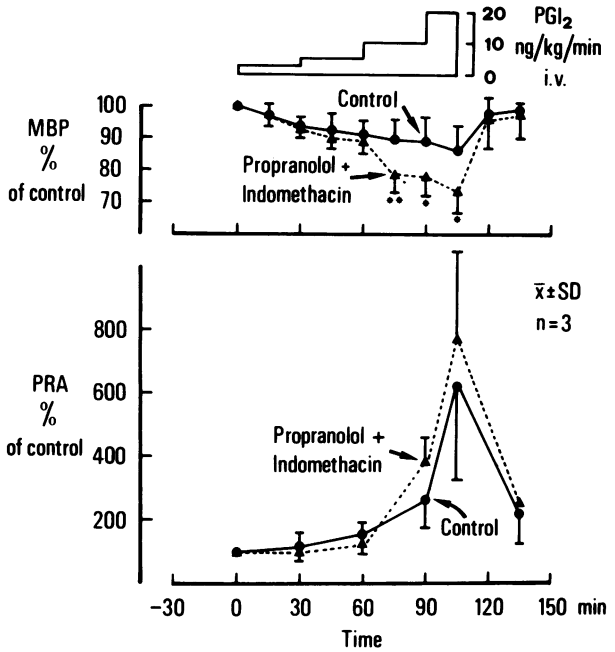

FIGURE 3 Prostacyclin (PGI_2) infusions. Changes (%) in mean blood pressure (MBP) and plasma renin activity (PRA), measured during and after PGI_2 infusions at four nominal infusion rates, under control conditions (solid line) and following propranolol and indomethacin treatment (broken line). $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$: Control vs. propranolol + indomethacin.

trol values, despite an appreciable drop of diastolic blood pressure (65 ± 7 beats/min at 55 ± 5 mm Hg at 10 ng/kg per min).

The fate of plasma and urinary 6-keto-$PGF_{1\alpha}$ after the administration of a pharmacologic stimulus known to stimulate renin release via a cyclooxygenase-dependent mechanism (1–3) was investigated by infusing furosemide into six healthy women. Mean (±SE) urinary 6-keto-$PGF_{1\alpha}$ and sodium excretion, and PRA levels are illustrated in Fig. 4. Furosemide caused a statistically significant increase of urinary 6-keto-$PGF_{1\alpha}$ and sodium excretion rates and PRA levels within the first 15 min. The excretion rate of 6-keto-$PGF_{1\alpha}$ increased three- to fourfold over basal levels, remained significantly elevated over a period of 60 min, and declined thereafter. PRA rose significantly after 15 min, and remained significantly elevated for the whole period of observation (3 h). 6-keto-$PGF_{1\alpha}$ remained undetectable (<7.5 pg/ml) in peripheral venous plasma throughout the study (not shown). Both the relative increase and time-course of urinary 6-keto-$PGF_{1\alpha}$ excretion after furosemide injection were quite similar to the pattern of urinary PGE_2, $PGF_{2\alpha}$, and TXB_2, previously measured in the same subjects and described elsewhere (9). When basal excretory values of 6-keto-$PGF_{1\alpha}$ were measured with other anti-6-keto-$PGF_{1\alpha}$ sera, i.e., AS 2, AS 3, AS 4, these gave consistently higher values by a factor of 2, 3, and 6, respectively, over AS 1. However, when highly diluted urine obtained after furosemide injection was assayed for 6-keto-$PGF_{1\alpha}$ by different antisera, they all gave quite similar results. As shown in Fig. 5, under basal conditions, only AS 1 recognized a single peak of immunoreactive material migrating in an identical fashion to authentic 6-keto-$PGF_{1\alpha}$, whereas AS 2, AS 3, and AS 4 also revealed the presence of increasing amounts of immunoreactivity in both more and less polar zones of the plate, thus indicating the presence of specifically interfering substances coeluting with 6-keto-$PGF_{1\alpha}$ from silicic acid columns.

Contrastingly, after furosemide injection the vast majority of 6-keto-$PGF_{1\alpha}$-LI detected by the less spe-

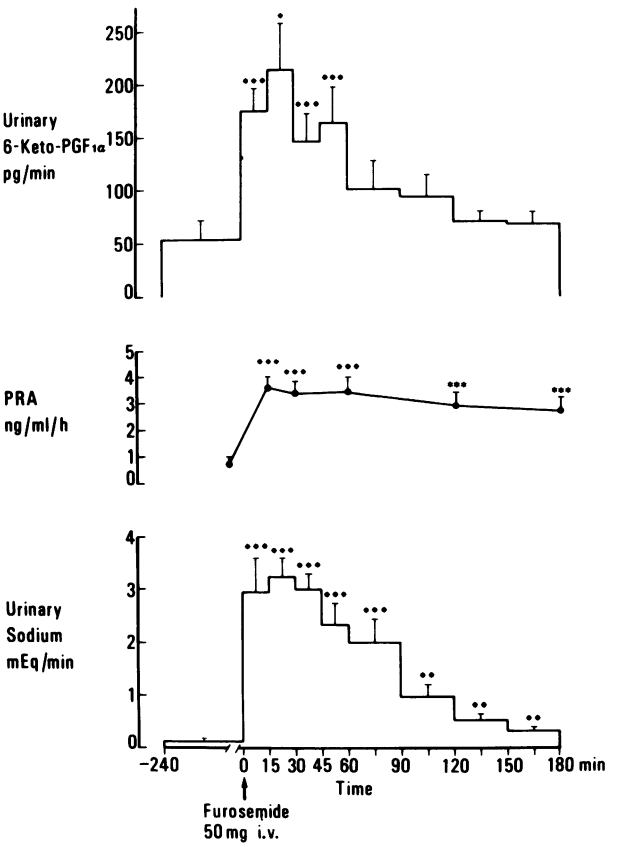

FIGURE 4 Furosemide infusion. Time-course of plasma renin activity (PRA), and urinary excretion rates of 6-keto-$PGF_{1\alpha}$ and sodium following the intravenous infusion of 50 mg furosemide at time zero in six healthy women. The level of significance of the difference between values obtained before and after furosemide was determined by the paired t test. Mean ± SEM. $n = 6$. $^{\circ}P < 0.025$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.005$.

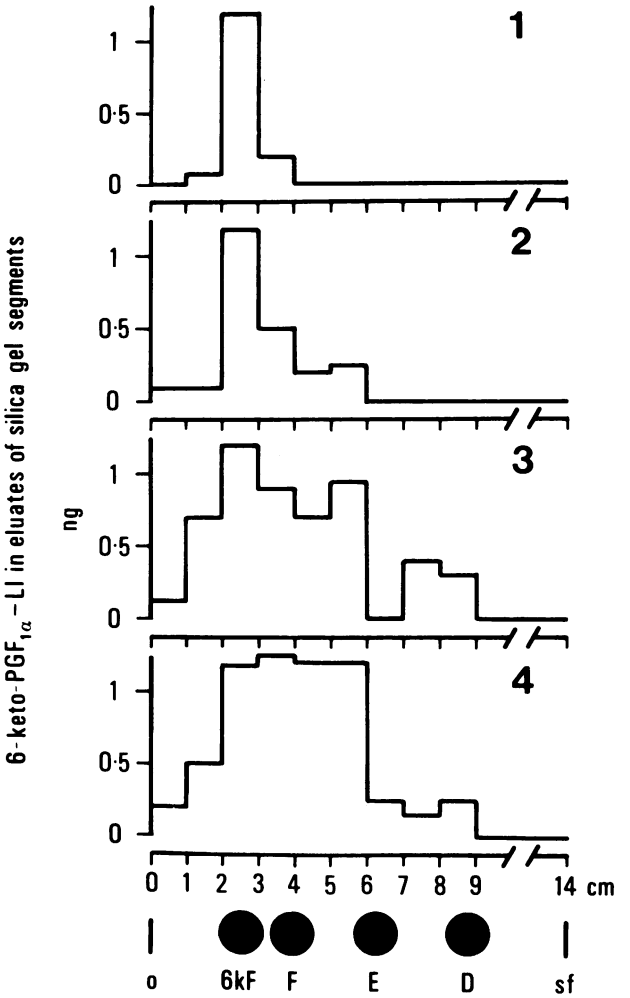

FIGURE 5 Heterogeneity of urinary 6-keto-$PGF_{1\alpha}$-like immunoreactivity (LI). Purified extracts, prepared from urine obtained under basal conditions, were subjected to thin-layer chromatography. The whole lane corresponding to one particular extract was divided into 1-cm segments, the silica gel scraped off and eluted with methanol. All the eluates were assayed for 6-keto-$PGF_{1\alpha}$-LI with four anti-6-keto-$PGF_{1\alpha}$ sera of different specificities. The vertical marks on the abscissa indicate the origin (o) and the solvent front (sf) of the plate. The dots indicate the location of cochromatographed authentic PGs: 6kF, 6-keto-$PGF_{1\alpha}$; F, $PGF_{2\alpha}$; E, PGE_2; D, PGD_2.

cific antisera comigrated with authentic 6-keto-$PGF_{1\alpha}$ and with the single peak of immunoreactivity detected by AS 1 (not shown). Failure of this anti-6-keto-$PGF_{1\alpha}$ antiserum to detect any appreciable amount of immunoreactivity in areas other than that corresponding to the homologous compound strongly supports the identification of the urinary immunoreactive material as 6-keto-$PGF_{1\alpha}$. Further evidence for this conclusion was obtained from GC/MS analysis. Almost identical amounts of 6-keto-$PGF_{1\alpha}$, i.e. 43 vs. 45 ng were measured by AS 1 and GC/MS in the same region of the TLC plate.

DISCUSSION

PGI_2 is a powerful vasodilator and inhibitor of platelet aggregation, in experimental animals as well as in man. In addition, a number of clinical studies have recently reported an increase of PRA during PGI_2 infusion, both in healthy (7, 15) and uremic subjects (16). This study has demonstrated that PGI_2, but not its stable breakdown product, 6-keto-$PGF_{1\alpha}$, induced a reproducible increase of PRA levels in healthy subjects, with a time- and dose-dependence quite similar to those reported by O'Grady et al. (17) for the inhibition of platelet aggregation. Thus, a dose-related inhibition of platelet aggregation was observed with infusion rates of PGI_2 at 2—16 ng/kg per min (17), as compared with stimulation of renin release at 2.5–20 ng/kg per min in our study. Moreover, in contrast to the rapid return of blood pressure and heart rate to control levels upon discontinuing PGI_2 infusion observed in both studies, inhibition of platelet aggregation, and significant elevation of PRA were still measurable 30 min after the end of PGI_2 infusion. The latter change may simply reflect the relatively long $t_{1/2}$ of renin compared with the short $t_{1/2}$ of PGI_2. The threshold concentration of plasma 6-keto-$PGF_{1\alpha}$ associated with a statistically significant stimulation of renin release was ~200 pg/ml. This would argue against a systemic origin of PGI_2 controlling renin release under normal circumstances in view of undetectable concentrations of 6-keto-$PGF_{1\alpha}$ in the basal state.

Although ample evidence exists to suggest that PGI_2 has a direct stimulant effect on the renin release mechanism (1–3), the possible contribution of hemodynamic effects and endogenously released PGI_2 to the observed changes of PRA levels had to be verified in man. The quite similar dose dependence for the reduction of MBP and increase of PRA measured during PGI_2 infusion makes it difficult to dissect out hemodynamically mediated from direct effects on renin release. However, the substantially unaltered increase of PRA levels observed during pharmacologic inhibition of β-adrenergic activity and PG synthesis is strongly suggestive of a direct effect of PGI_2 on the renin release mechanism. The possible contribution of α-adrenergic stimuli and of the macula densa mechanism to renin release should be minimized under these conditions, in view of their dependence on intact PG-synthesis (3). However, because the urinary excretion of 6-keto-$PGF_{1\alpha}$ and PGE_2 (and presumably, the renal synthesis of PGI_2 and PGE_2) were incompletely suppressed by indomethacin treatment, a partial contri-

bution of the residual cyclooxygenase activity of the kidney can not be ruled out. The results of the present study are consistent with the conclusion of Frölich et al. (18) that a cyclooxygenase-derived metabolite of arachidonic acid is a mediator of the nonadrenergic component of renin release in man. Gerber et al. (19) had previously provided evidence for such a direct effect in dogs, by showing an increase in renin production induced by intrarenal infusion of PGI_2 at 10 ng/kg per min in renally denervated, β-adrenergic blocked, indomethacin-treated dogs with unilateral nephrectomy.

To gain further insight into the participation of endogenous PGI_2 formation in the mechanism of renin release, we investigated the effects of furosemide on the urinary excretion of 6-keto-$PGF_{1\alpha}$ in healthy women. Both the relative increase and time-course of urinary 6-keto-$PGF_{1\alpha}$ excretion following furosemide injection were quite similar to the excretory pattern of other cyclooxygenase-derived products of renal arachidonic acid metabolism (9), thus suggesting a common origin. PGI_2 synthesis by human renal cortical and medullary microsomes has been reported recently by Hassid and Dunn (20). When comparing the urinary excretion rates of 6-keto-$PGF_{1\alpha}$ associated with furosemide- and PGI_2-induced renin release, it becomes obvious that similar increases of PRA levels are associated with quite different elevations of urinary 6-keto-$PGF_{1\alpha}$. It seems likely that only a fraction of the infused PGI_2 is necessary to cause renin release when enhanced precursor availability and/or diminished metabolism is induced within the kidney, by drugs or physiologic stimuli. The local nature of such activation is further supported by failure of furosemide to raise peripheral plasma levels of 6-keto-$PGF_{1\alpha}$. The possible contribution of enhanced PGE_2 formation to renin release measured under these circumstances should also be considered, although it is questionable whether this compound has a direct effect on renin release (1–3).

The identification of the immunoreactive material present in human urine as 6-keto-$PGF_{1\alpha}$ was obtained by several independent criteria: (*a*) its identical immunochemical behaviour with authentic 6-keto-$PGF_{1\alpha}$; (*b*) its identical chromatographic behaviour on TLC with authentic 6-keto-$PGF_{1\alpha}$; (*c*) its detection by four different anti-6-keto-$PGF_{1\alpha}$ sera; and (*d*) its detection by GC/MS. The presence of 6-keto-$PGF_{1\alpha}$ in human urine raises the question of its origin and significance. We had previously suggested that urinary 6-keto-$PGF_{1\alpha}$ might reflect a fraction of renal PGI_2 escaping intrarenal metabolism (7) similarly to urinary PGE_2 and $PGF_{2\alpha}$ (21) and TXB_2 (9). Evidence in favor of a renal origin of urinary 6-keto-$PGF_{1\alpha}$ is provided by its similar behaviour to other cyclooxygenase-derived products under a variety of pharmacologic and pathophysiologic conditions affecting renal arachidonic acid metabolism. These include: (*a*) furosemide injection (this study); (*b*) Bartter's syndrome (22); and (*c*) indomethacin (22) or sulindac (23) administration in healthy women as well as in patients with Bartter's syndrome. Evidence against a renal origin of urinary 6-keto-$PGF_{1\alpha}$ is provided by the recovery of significant amounts of this compound following PGI_2 infusion, as demonstrated by Rosenkranz et al. (24) and confirmed in our study. It should, however, be pointed out that both studies were carried out by infusing pharmacologic doses of PGI_2, 25–200 times higher than the maximal estimates of endogenous secretion rates.[2]

In this study, no 6-keto-$PGF_{1\alpha}$ could be detected in peripheral venous plasma, under basal conditions, in contrast to previous reports of circulating 6-keto-$PGF_{1\alpha}$ levels as detected by RIA (25) or GC/MS (26). In view of rapid disappearance of 6-keto-$PGF_{1\alpha}$ from the circulation, demonstrated in this study, with a behavior quite similar to that of PGE_1 (27), and relatively low rate of entry of endogenous PGI_2 into the circulation[2], it is not surprising to find undetectable levels of this compound in peripheral venous blood. Furosemide-induced renal PGI_2 release, as detected by increased urinary 6-keto-$PGF_{1\alpha}$ excretion, was not associated with circulating levels of this compound. A plasma metabolite with a longer $t_{1/2}$, such as 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$, would probably give some long term indication as to the amounts of PGI_2 released in man (28).

Although our findings clearly demonstrate that the stable hydrolysis product of PGI_2, 6-keto-$PGF_{1\alpha}$ does not contribute to the observed changes of renin release to any measurable extent, they do not allow us to exclude the possibility that enzymatic conversion of PGI_2 to a chemically stable and biologically active compound, such as 6-keto-PGE_1 (29), might contribute to some of its effects.

We conclude that in human subjects: (*a*) PGI_2-induced renin release occurs with a dose and time dependence similar to its reported platelet effects; (*b*) PGI_2-induced renin release is not mediated by adrenergic stimuli or cyclooxygenase-dependent mechanisms secondary to hemodynamic changes; (*c*) furosemide-induced renin release is associated with increased renal PGI_2 formation; and (*d*) PGI_2 appears to act as a local modulator rather than a circulating hormone in controlling juxtaglomerular function.

[2] Fitzgerald, G. A. et al. Poster presentation at the Winter Prostaglandin Conference, Clearwater, Fla., 1–5 March 1981.

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