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Urinary prostaglandins. Identification and origin.

J C Frölich, … , J T Watson, J A Oates

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Research Article

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Urinary Prostaglandins

IDENTIFICATION AND ORIGIN

JÜRGEN C. FRÖLICH, THOMAS W. WILSON, BRIAN J. SWEETMAN, MURRAY SMIGEL, ALAN S. NIES, KEITH CARR, J. THROCK WATSON, and JOHN A. OATES

From the Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee 37232

ABSTRACT Human urine was analyzed by mass spectrometry for the presence of prostaglandins. Prostaglandin E_2 and $F_{2\alpha}$ were detected in urine from females by selected ion monitoring of the prostaglandin E_2-methylester-methoxime bis-acetate and the prostaglandin $F_{2\alpha}$-methyl ester-Tris-trimethylsilylether derivative. Additional evidence for the presence of prostaglandin $F_{2\alpha}$ was obtained by isolating from female urine an amount of this prostaglandin sufficient to yield a complete mass spectrum. The methods utilized permitted quantitative analysis.

The origin of urinary prostaglandin was determined by stimulating renal prostaglandin synthesis by arachidonic acid or angiotensin infusion. Arachidonic acid, the precursor of prostaglandin E_2, when infused into one renal artery of a dog led to a significant increase in the excretion rate of this prostaglandin. Similarly, infusion of angiotensin II amide led to a significantly increased ipsilateral excretion rate of prostaglandin E_2 and $F_{2\alpha}$ in spite of a simultaneous decrease in the creatinine clearance. In man, i.v. infusion of angiotensin also led to an increased urinary elimination of prostaglandin E.

These results show that urinary prostaglandins may originate from the kidney, indicating that renally synthesized prostaglandins diffuse or are excreted into the tubule. Thus, urinary prostaglandins are a reflection of renal prostaglandin synthesis and have potential as a tool to delineate renal prostaglandin physiology and pathology.

INTRODUCTION

When prostaglandins (PG) [1] are infused i.v., they cannot be recovered unchanged in the urine (1, 2), indicating little direct excretory clearance of the primary prostaglandins from the circulation. The kidney itself, however, is a prime site of prostaglandin synthesis (3–5), and it was conceived that PG synthesized in the kidney might diffuse directly into the urine. Certainly some substances such as tryptamine (6) and cyclic AMP (7) appear in the urine totally or in part as a reflection of their intrarenal synthesis.

Accordingly, an attempt was made to ascertain whether renal production of PG might result in their release directly into the tubular fluid. We have previously obtained preliminary evidence for the presence of primary prostaglandins in human urine (8, 9), and others have obtained bioassay (10) and radioimmunoassay (11) data suggesting the presence of PG in the urine of other species. This report provides definitive evidence regarding the existence and quantity of PG in human urine, and demonstrates that PG in the urine can originate from intrarenal synthesis.

METHODS

Analytical methods

The identification of PGE_2 and $PGF_{2\alpha}$ was based on mass spectrometry. PGE_2 and $PGF_{2\alpha}$ were purified from 200-ml samples of urine, employing 2.5 μg of 3,3,4,4-tetradeutero PGE_2 and 3,3,4,4-tetradeutero $PGF_{2\alpha}$ as internal standards and carriers, together with tritiated PGE_1 and tritiated $PGF_{2\alpha}$ (Amersham/Searle Corp., Arlington Heights, Ill.; specific activity 60 and 15 Ci/mmol, respectively) as tracers during purification. Purification consisted of washing with an equal volume of benzene: butylchloride 1:1 (vol/vol) at pH 6.5. The pH of the urine was then adjusted to 3.2 with formic acid and extraction was performed with an equal volume of chloroform. The chloroform was removed by evaporation and the residue dissolved in $CHCl_3$: heptane: ethanol: acetic acid (100:100:30:2) (vol/vol) and applied to an

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[1] *Abbreviations used in this paper:* CPBA, competitive protein-binding assay; PG, prostaglandins; PGE_2-ME-MO-bis-Ac, PGE_2 methyl ester converted to methoxime bis-acetate; $PGF_{2\alpha}$-ME-Tris-TMS, $PGF_{2\alpha}$ methyl ester converted to its Tris-trimethylsilylether; SIM, selected ion monitoring.

LH-20 column (13 × 1 cm) packed in the same solvent mixture (12). All the prostaglandins were eluted in one peak. Material from this peak was separated into PGE and PGF by reversed phase partition chromatography on 4.5 g of support using solvent system C50 (13, 14). Because [^{3}H]PGE_1 was used for location of PGE_2 during chromatography, and because PGE_1 and PGE_2 separate on this column, the relative retention volume of PGE_2 was established by applying a mixture of [^{3}H]PGE_1 and PGE_2 on a separate reversed phase column. An aliquot of each fraction was analyzed for radioactivity and another aliquot (after conversion to PGB by treatment with 0.5 M methanolic KOH) for absorbance at 278 nm. PGE_2 had a retention volume of 65–100 ml and PGE_1 of 95–125 ml. The fractions containing PGE_2 and $PGF_{2\alpha}$ as well as their deuterated internal standards were derivatized to the methyl esters with redistilled diazomethane prepared from *N*-nitroso-*N*-methylurea (15). The $PGF_{2\alpha}$ methyl ester was further converted to its Tris-trimethylsilylether ($PGF_{2\alpha}$-ME-Tris-TMS) by treatment with *N,O*-bis-trimethylsilylacetamide in pyridine (1:1, vol/vol) (9). The PGE_2 methyl ester was converted to the methoxime bis-acetate (PGE_2-ME-MO-bis-Ac) by treatment with methoxyamine hydrochloride and acetic acid anhydride (16). Final analysis was accomplished using a LKB 9,000 mass spectrometer-PDP 12-computer system (LKB Instruments, Inc., Rockville, Md.) adjusted for selected ion monitoring (SIM) (17). For analysis of PGE_2-ME-MO-bis-Ac by SIM at *m/e* 419/423, a 0.5-m, 2-mm (ID) column of 1.2% Dexsil on Supelcoport 100/200 mesh (Supelco Inc., Bellefonte, Pa.) was used at 220°C with the helium flow rate at 20 ml/min. Source temperature was 250°C, trap current 60 μA, and ionizing voltage 32.5 eV. The multiplier slit was adjusted to a setting slightly wider than for normal scan use. Source focus was adjusted to optimize both sensitivity and resolution at *m/e* 419. The magnet was equilibrated at this setting for about 12 h before the run. During the run stability and focus were checked frequently by the use of appropriate standard mixtures.

For the analysis of $PGF_{2\alpha}$ as $PGF_{2\alpha}$-ME-Tris-TMS by SIM at *m/e* 423/427 and *m/e* 513/517, a 2-m, 2-mm (ID) column of 2% OV-7 on chromosorb G was used at 245°C with a helium flow rate of 20 ml/min. Otherwise, instrumental conditions were similar to those used for PGE_2-ME-MO-bis-Ac.

In addition to data obtained by selected ion monitoring, a sufficient amount of $PGF_{2\alpha}$ was isolated and purified to obtain a complete mass spectrum. For this, 2 μg tetradeutero $PGF_{2\alpha}$ were added to 1,500 ml of human urine from females. The urine was acidified to pH 3.4 and percolated through an Amberlite XAD-2 column (Mallinkrodt Chemical Works, St. Louis, Mo.) (18). Subsequent purification consisted of LH-20 chromatography on a column 5 cm in diameter and 20 cm in height, reversed phase partition chromatography on system C40 (14), methylation with diazomethane, chromatography on reversed phase system F55 (14), and final purification on a 500-mg silicic acid column which was eluted with redistilled ethylacetate: benzene, 7:3 (vol/vol).

To analyze aliquots of urine smaller than 50 ml, mass spectrometry was impractical and therefore alternate methods were found. For analysis of PGE a newly developed competitive protein-binding assay (CPBA) was employed (19). The PGE assay utilized a high-affinity binding site on rat-liver cell membranes that has selective affinity for PGE_1 and PGE_2. The affinities of this binding site for $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGB_2, 15-keto-PGE_2, and arachidonic acid were 100–1,000 times less than those for PGE_2. The sensitivity extends into the low picogram range.

The radioimmunoassay for $PGF_{2\alpha}$ utilizes a commercial antibody (Calbiochem, San Diego, Calif.) specific for $PGF_{2\alpha}$. $PGF_{2\alpha}$ standards in ethanol (25–400 pg) in triplicate and aliquots of the unknown were evaporated under reduced pressure in 12 × 75-mm polystyrene tubes. Amounts of antibody sufficient to bind 50% of tritiated $PGF_{1\alpha}$ (60 Ci/mmol, Amersham/Searle Corp., Arlington Heights, Ill.) were added dissolved in 0.6 ml of buffer (0.01 M sodium phosphate, 0.1% gelatin, 1 mM EDTA, pH 7.5). After incubation for 4 h at 4°C, 0.5 ml of charcoal/dextran was added to all but the tubes used for the counting of total radioactivity. The tubes were centrifuged for 20 min at 2,500 *g*, and the supernate was poured into liquid scintillation vials and analyzed by liquid scintillation counting.

The counting results of both assays were evaluated by computerized Scatchard regression that incorporates a correction for the [^{3}H]PGE_1 added to urine for estimation of recovery (20). The purity of the [^{3}H]PGE_1 used was checked periodically using thin-layer radio chromatography (21). The material was purified by thin-layer chromatography if a contamination of >5% was detected. Both assays show linear Scatchard regressions between 25 and 250 pg. The SE of 10 replicate determinations of the same urine is ±5%.

Biological studies

Animal studies

Arachidonic acid infusion. Mongrel dogs ($n = 6$) were anesthetized with sodium pentothal by i.v. drip. An endotracheal tube was inserted and artificial respiration instituted. A renal artery was exposed by abdominal approach and the ipsilateral ureter catheterized. In a 90-min equilibration period 0.225% NaCl was infused at a rate sufficient to produce a urine flow of 0.3–1 ml/min from the investigated kidney. Creatinine was infused at a rate of 7.04 mg/min. After a 30-min control period sodium arachidonate was infused at the rate of 10 μg/min in 0.2 ml of saline into the renal artery via a 25-gauge needle for 30 min. Sodium arachidonate was prepared freshly for each experiment under conditions that avoided in vitro formation of PGE_2 from arachidonic acid (5,8,11,14-tetraenoic acid, Nu Check Prep Inc., Elysian, Minn.) by opening the vials under nitrogen atmosphere, dissolving the material in 80% ethanol with phenolphthalein indicator, and titration to pH 7 with 0.1 N NaOH. The solution was evaporated under a gentle stream of nitrogen and dissolved in saline. Analysis of this material by gas chromatography-mass spectrometry revealed that it contained less than 0.025% PGE_2 (22). Blood was drawn in the middle of the control and infusion period for estimation of glomerular filtration rate.

Angiotensin infusion. Similarly prepared dogs ($n = 6$) were infused with angiotensin (angiotensin II amide, Hypertensin, Ciba Pharmaceutical Co., Summit, N. J.) at a rate of 0.5, 5, and 50 ng/kg per min into a renal artery, and ipsilateral urine was collected at each dose level for 30 min after a 30-min control period.

Another group of dogs ($n = 5$) received angiotensin (20 ng/kg per min) into one renal artery during a 30-min period. In these animals urine was collected from both ureters.

Human studies

Studies in man were approved by the Vanderbilt Human Investigation Committee, Nashville, Tenn. Informed consent was obtained from the volunteers.

Human urines were collected under refrigeration from females only. Participants in the studies were instructed to abstain from sexual intercourse 2 days before and during the study. During acute studies a vaginal tampon was inserted. Urines were collected only outside the menstrual period. Urine specimens were obtained by voiding and kept at −20°C until analyzed. 24-h urine specimens were collected from female volunteers who had not been taking any drugs for at least 14 days.

Angiotensin was given i.v. to six subjects in a total of nine studies. The subjects fasted overnight. After voiding in the morning they received 500 ml of 0.45% saline in 5% dextrose orally, and subsequently 50 ml 5% dextrose every 30 min for 2 h. 3 h later urine was collected, 500 ml of 0.45% saline in 5% dextrose were given again, and an angiotensin infusion (Hypertensin, Ciba, Summit, N. J.) was started at the rate of 2.3 ng/kg per min. Blood pressure was taken every 2 min, and after 30 min the infusion rate was increased to 4.4 ng/kg per min in 1.1 ml of 5% dextrose. Urine was collected for 3 h after the beginning of the angiotensin infusion.

RESULTS

Identification and measurement of PGE_2 and $PGF_{2\alpha}$ in human urine. PGE_2 and $PGF_{2\alpha}$ were identified in urine of human females by mass spectrometry as the PGE_2-ME-MO-bis-Ac and $PGF_{2\alpha}$-Me-Tris-TMS. Representative data from selective ion monitoring are shown in Fig. 1 and Fig. 2. These are photographs taken from the computer oscilloscope (17) showing ion profiles obtained in the analysis of PGE_2 (ions *m/e* 419 and 423, Fig. 1) and $PGF_{2\alpha}$ (ions *m/e* 423 and 427, Fig. 2). It was also possible to demonstrate the presence of PGE_2 and $PGF_{2\alpha}$ by monitoring ions *m/e* 328 and *m/e* 513, respectively. These findings confirm earlier SIM data obtained by converting urinary PGE_2 to PGB_2 (analysis as PGB_2-ME-TMS) and $PGF_{2\alpha}$ to $PGF_{2\alpha}$-Me-triacetate (8). Repeated analysis ($n = 10$) of the same urine sample revealed that a level of 590 pg/ml of urine can be measured with a SD of 6.4%. Based on the amount of deuterated internal standard added to each aliquot of urine, 24-h urine samples from ambulatory females were found to have levels of PGE_2 of 374±77 pg/ml ($n = 8$, mean±SE) and were thus in the same range as the levels obtained by CBPA (319±53 pg/ml, $n = 8$). Levels of $PGF_{2\alpha}$ measured by SIM were 386±78 pg/ml ($n = 10$).

In order to obtain a complete mass spectrum of $PGF_{2\alpha}$ from human urine, this prostaglandin was purified from a 1,500-ml sample of pooled female urine after addition of tetradeutero $PGF_{2\alpha}$. Fig. 3 shows that the mass spectra of $PGF_{2\alpha}$-Me-Tris-TMS standard and the material extracted from urine are virtually identical. The spectrum of the tetradeutero $PGF_{2\alpha}$ added to the urine and isolated together with the urinary $PGF_{2\alpha}$ provided additional support for the identification.

Investigation of the renal origin of urinary prostaglandins. Infusion of sodium arachidonate into the

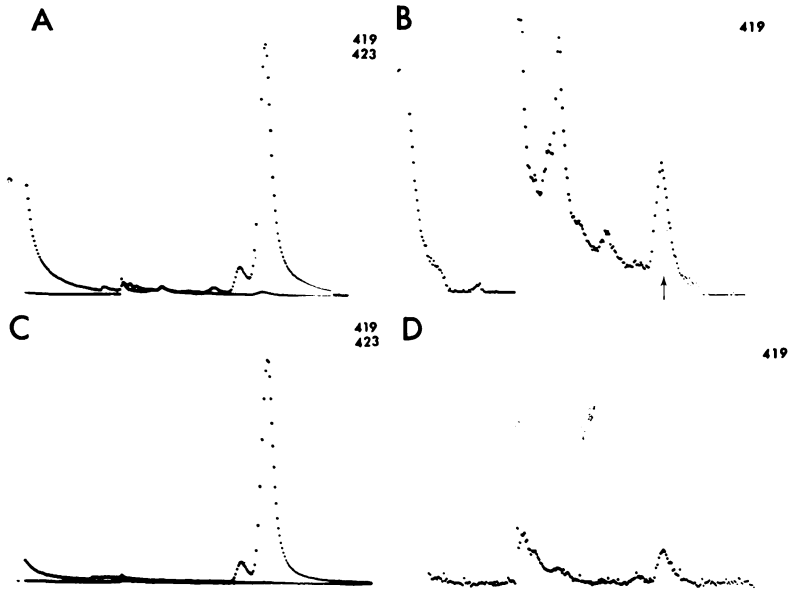

FIGURE 1 PGE_2 from human urine. The vertical axis represents abundance, the horizontal axis time. Ions monitored are *m/e* 423 for the deuterated internal standard and *m/e* 419 for the protonated (biological) prostaglandin derivative. (A) Biological sample with internal standard (*m/e* 419 and 423). (B) Same as A, but magnified 32 times, *m/e* 419 only. Arrow indicates the expected retention time of PGE-ME-MO-bis-Ac. Ion *m/e* 423 is deleted by the computer. (C) Internal standard alone (*m/e* 423). (D) Protium blank created by internal standard, magnified 32 times (*m/e* 419).

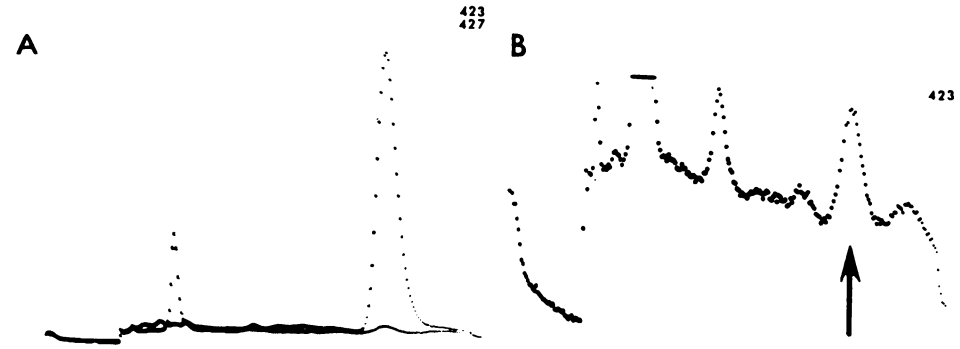

FIGURE 2 $PGF_{2\alpha}$ from human urine. The vertical axis represents abundance, the horizontal axis time. Ions monitored are *m/e* 423 for the internal standard and *m/e* 427 for the protonated (biological) prostaglandin derivative. (A) Biological sample with internal standard (*m/e* 423 and 427). (B) Same as A, but magnified 32 times (*m/e* 423 only). Arrow indicates expected retention time of $PGF_{2\alpha}$-Me-Tris-TMS. Blank correction as in Fig. 1.

renal artery lead to a significantly increase in ipsilateral concentration and excretion rate of PGE (measured by CPBA) (Fig. 4). It is known that arachidonic acid may be converted to prostaglandin-like material in vitro (23). In order to exclude PG formation from arachidonate in the urine or during the analytical procedure as a possible explanation for our findings, tritiated arachidonate (100 Ci/mmol, New England Nuclear, Boston, Mass.) was added to unlabeled arachidonate and infused into the renal artery of a dog as

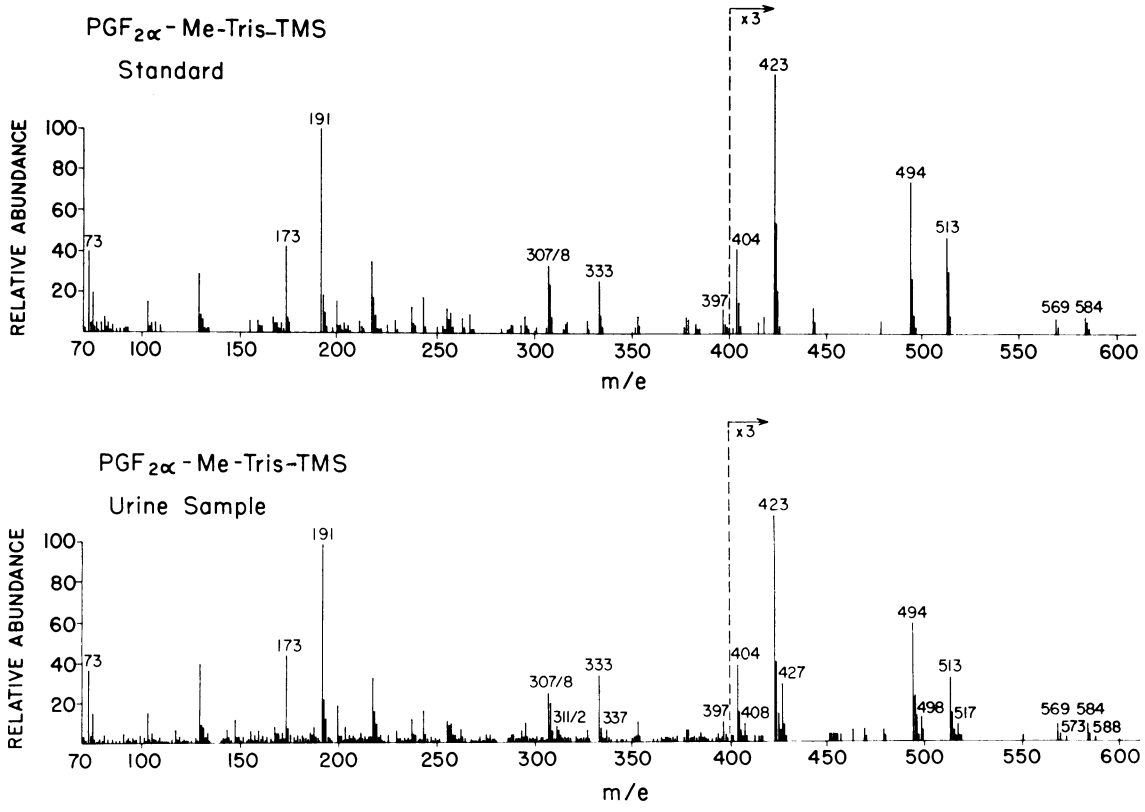

FIGURE 3 Mass spectrum of $PGF_{2\alpha}$ from human urine. Top is mass spectrum of $PGF_{2\alpha}$-Me-Tris-TMS standard with prominent ions *m/e* 584 (M^+), 569, 513, 494, 423, and 404. Bottom is mass spectrum of deuterated internal standard with prominent ions *m/e* 588 (M^+), 573, 517, 498, 427, and 408 together with mass spectrum of protonated (biological) prostaglandin with prominent ions *m/e* 584 (M^+), 569, 513, 494, 423, and 404.

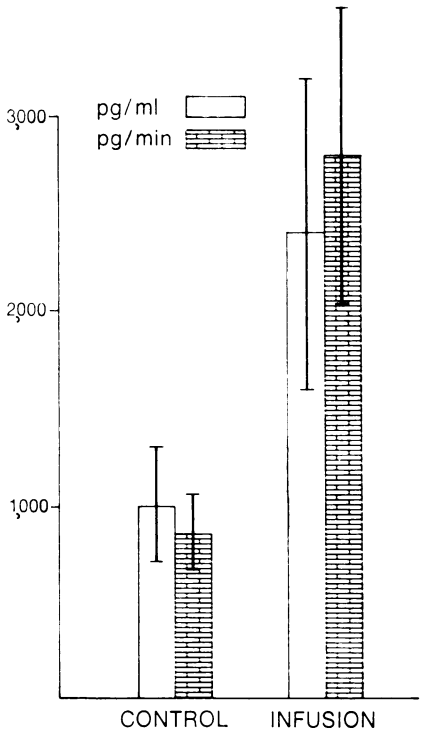

FIGURE 4 Effect of infusion of sodium arachidonate on urinary concentration and excretion rate of PGE in the dog ($n = 6$, mean±SE). Blank bars: concentration in pg/ml, and cross-labeled bars: excretion rate in picogram per minute.

in the other experiments. Coincident with the beginning of the infusion a portion of the labeled and unlabeled arachidonic acid was added to the dogs urine collected during the control period. Immediately at the end of the infusion both urine specimens were extracted by the methods described above, the material applied to 1-g silicic acid columns (6 × 100 mm) and eluted with a discontinuous gradient of ethylacetate and toluene. This column separates arachidonic acid, PGE, and PGF (24). The results of this chromatography are shown in Fig. 5 and indicate that only the urine obtained after intra-arterial infusion of arachidonate contained significant amounts of PGE and PGF. Therefore, generation of PGE_2 from arachidonate in the urine or during the isolation procedure cannot account for the increased amounts of PGE_2 appearing in urine during arachidonate administration.

The results of infusing increasing amounts of angiotensin into the canine renal artery are shown in Table I. Both PGE (measured by CPBA) and PGF (measured by radioimmunoassay) excretion rates increased in a dose-dependent manner in response to angiotensin. This increase became significant ($P < 0.05$) for PGF at the 5 ng/kg per min and for PGE at the 50 ng/

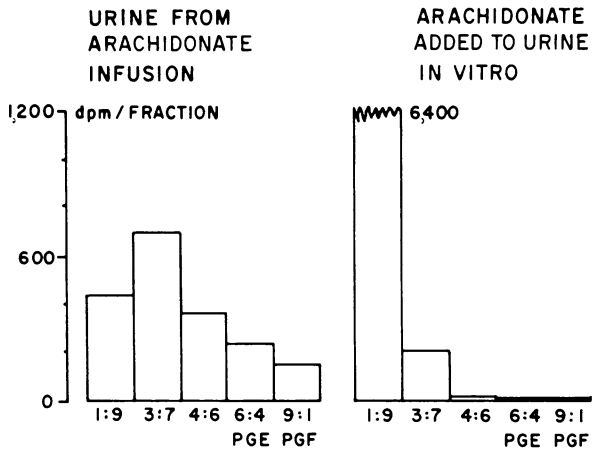

FIGURE 5 Silicic acid column chromatography of extract from ureteral urine after administration of tritiated sodium arachidonate into the ipsilateral renal artery or after its addition directly to the urine. Numbers on the abscissa indicate ratios of ethylacetate to toluene in the eluating fractions. Fraction volume is 35 ml.

kg per min infusion rate of angiotensin. These changes also were seen when the excretion rate was expressed in terms of nanograms of prostaglandin per minute per milligram creatinine.

When angiotensin was infused at a rate of 20 ng/kg per min into one renal artery and urine collected from both ureters, only the ipsilateral excretion rate of PGE (measured by CPBA) increased (Fig. 6). PGF excretion rate was measured in three experiments by radioimmunoassay and increased in each instance. These increases in PG excretion occurred even though creatinine clearance fell (by 38%) during the infusion of angiotensin.

The effect of angiotensin on urinary prostaglandin excretion in man. Angiotensin infusion (4.4 ng/kg per min i.v.) in man also led to an increased rate of PGE excretion (measured by CPBA) from a control rate of 12.5±2.6 ng/h to 27±3.8 ng/h ($n = 9$, $P < 0.01$) (Fig. 7). This dose of angiotensin elicited only a minimal increase in blood pressure which was not

TABLE I

Effect of Infusion of Angiotensin into the Renal Artery on Ipsilateral Excretion Rates of Prostaglandins E and F

Angiotensin	PGE	PGF
ng/kg/min	*ng/min*	
0	1.7±0.3	0.12±0.03
0.5	2.3±0.7	0.17±0.04
5	5.0±1.6	0.25±0.03*
50	7.5±2.3*	0.53±0.06*

* $P < 0.05$.

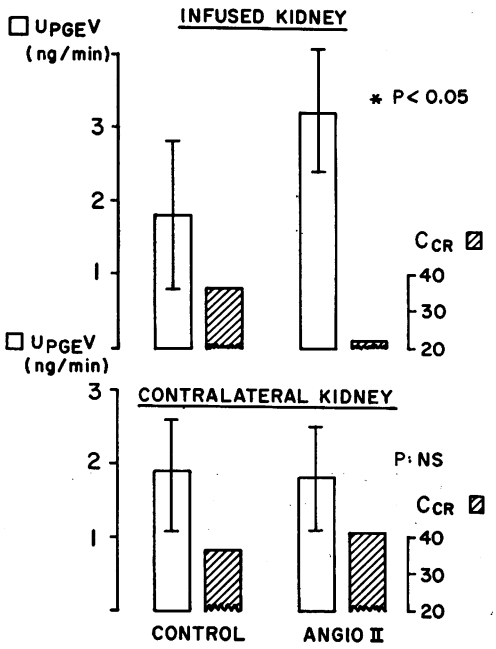

FIGURE 6 Effect of infusion of angiotensin into dog renal artery on ipsilateral and contralateral PGE excretion rates and creatinine clearances.

statistically significant. Urine flow rate was not significantly altered by angiotensin.

DISCUSSION

Our preliminary report of PGE_2 and $PGF_{2\alpha}$ in human urine was based on gas chromatographic analysis employing selected ion monitoring with the mass spectrometer of the methyl ester trimethylsilylether of PGB_2, which was obtained by converting PGE_2 to PGB_2 and the methyl ester Tris-acetate derivative of $PGF_{2\alpha}$ (8). Although these data provided evidence for the presence of PGE_2 and $PGF_{2\alpha}$ in urine, these approaches were not useful for quantification. The present communication provides a more complete identification of urinary prostaglandins by measuring two additional derivatives, namely the methyl ester-methoxime-bis acetate of PGE_2 and the methyl ester-Tris-TMS of $PGF_{2\alpha}$. Selected ion monitoring of both derivatives confirmed the presence of PGE_2 and $PGF_{2\alpha}$ in urine and offered a precise method for quantification. In the case of $PGF_{2\alpha}$ a complete mass spectrum was obtained.

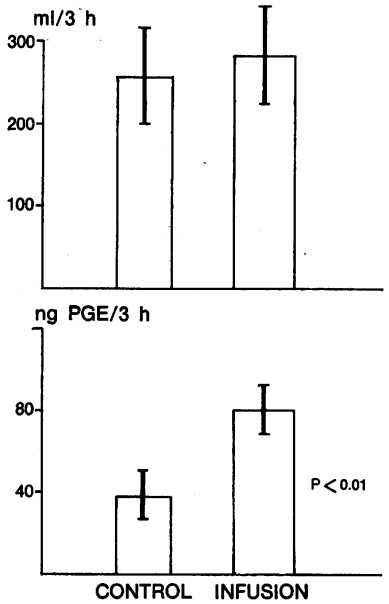

FIGURE 7 Effect of angiotensin on urinary excretion rate of PGE in human females ($n = 9$, mean±SD). Top is urine flow (ml/3 h) and bottom is PGE excretion rate (ng/3 h).

A renal origin for urinary PG was suggested by the finding that the primary PG are not recovered unchanged in the urine after their i.v. administration (1, 2). This implied that the high capacity of the lung (14) and kidney (25) to metabolize PG as well as the binding of PG to plasma protein (26) left little opportunity for clearance of unmetabolized PG directly into the urine. However, because we have found that infusion of large amounts of PGE_2 directly into the renal artery can lead to an increase in urinary PGE_2 (unpublished observation), additional evidence was sought in support of the release of PG synthesized in the kidney directly into the urine. One approach would be to determine the clearance of PG from the blood into the urine and to compute from that clearance the separate contribution of the kidney and the blood to urinary PG, analogous to the method for assessing renal 3',5'-cyclic AMP production (7). This would require, however, precise quantification of prostaglandins present in arterial blood, and because of ready release of PG from blood platelets (27) and that the most specific method for measurement (by mass spectrometry) (28) yields values only a fraction higher than the blanks, attempts to assess prerenal contribution to urinary PG from clearance information was thought to be impractical. Accordingly, the release of renal PG directly into the urine was evaluated by determining whether urinary PG excretion could be increased by two maneuvers that increase renal prostaglandin synthesis by quite different mechanisms.

An increase in renal PG synthesis by infusion of the PG precursor, arachidonic acid, was found to produce an increase in urinary PG excretion. In separate experiments, it was demonstrated that glomerular filtration is little affected by arachidonic acid infusion, but urine flow rate and sodium excretion do increase only on the ipsilateral side, effects blocked by inhibiting PG synthesis (29).

Confirmation of the release of renally synthesized PG directly into the urine was obtained by stimuling renal PG formation with angiotensin II (30). Infusion of angiotensin II into the renal artery raised urinary PG excretion rate even though creatinine clearance on that side decreased. The increase in PG excretion only on the ipsilateral side precludes a prerenal source for this increase in urinary PG.

These experiments show that an increase in renal PG synthesis is reflected by an increase in urinary PG excretion rates. That a similar response to angiotensin occurs in man is suggested by the finding of an increased urinary excretion rate of PGE during angiotensin infusion.

The finding of PG in urine adds a new dimension to assessment of their overall synthesis. Production rates based on measurements of the major urinary metabolites does not adequately describe the turnover of PG since a fraction of the primary PG is excreted directly with the urine. The amount excreted with urine in man is not large and represents only a small fraction of the secretion rate, as measured by the major metabolites (31). The direct excretion into the urine of renally synthesized PG, however, raises the possibility that some early metabolites also may exit directly into the urine without entering into the pool from which the major metabolite if formed.

While the quantitative aspects of urinary PG in terms of total body synthesis appear to be of minor importance, the close association with renal PG production suggests that urinary PG might serve as indicators of renal PG synthesis. Clearly, measurement of PG in urine can be accomplished more readily than in plasma where platelets generate PG rapidly (27, 32) and may be an important factor in causing the large discrepancies in reported plasma levels (28, 33). Also, renal venous blood sampling is limited to the animal model or to the acute study in man. In contrast, urinary levels of PG are found to be much higher than plasma levels, and repeated or prolonged sampling of urine can easily be accomplished facilitating studies in man.

To ascertain more precisely the extent to which urinary PG may be employed as indicators of renal PG synthesis, other parameters that influence their excretion need to be investigated. In particular the weak acidic properties of PG suggests that their excretion could be influenced by urinary pH and flow rate.

Studies from this and other laboratories have demonstrated that PG synthesized intrarenally will affect blood flow to the cortex, chiefly that to the juxtamedullary nephrons (29, 34). As the synthesis of PG in the kidney occurs almost entirely in the medulla (35), the question arises as to how the PG are transported from medulla to the point of blood flow regulation in the cortex. The finding of appreciable quantities of PG in urine provides the basis for a hypothesis that the medullo-cortical transport occurs via the tubular fluids to the site where the tubule is in direct apposition to glomerular arterioles.

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REFERENCES

1. Granström, E. 1967. On the metabolism of prostaglandin E_1 in man. Prostaglandins and related factors. *Prog. Biochem. Pharmacol.* **3**: 89–93.
2. Granström, E., and B. Samuelsson. 1969. The structure of a urinary metabolite of prostaglandin $F_{2\alpha}$ in man. *J. Am. Chem. Soc.* **91**: 3398–3400.
3. Daniels, E. G., J. W. Hinman, B. E. Leach, and E. E. Muirhead. 1967. Identification of prostaglandin E_2 as the principal vasodepressor lipid of rabbit renal medulla. *Nature (Lond.)*. **215**: 1298–1299.
4. Hamberg, M. 1969. Biosynthesis of prostaglandins in the renal medulla of rabbit. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **5**: 127–130.
5. Änggård, E., S. O. Bohman, J. E. Griffin, III, C. Larsson, and A. B. Maunsbach. 1972. Subcellular localization of the prostaglandin system in the rabbit renal papilla. *Acta Physiol. Scand.* **84**: 231–246.
6. Oates, J. A. 1961. Measurement of urinary tryptamine, tyramine, and serotonin. *Methods Med. Res.* **9**: 169–174.
7. Broadus, A. E., N. I. Kaminsky, J. G. Hardman, E. W. Sutherland, and G. W. Liddle. 1970. Kinetic parameters and renal clearances of plasma adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate in man *J. Clin. Invest.* **49**: 2222–2236.
8. Frölich, J. C., B. J. Sweetman, K. Carr, J. Splawinski, J. T. Watson, E. Änggård, and J. A. Oates. 1973. Occurrence of prostaglandins in human urine. *In* Adv. Biosc. **9**: 321–330.
9. Sweetman, B. J., J. T. Watson, K. Carr, J. A. Oates, and J. C. Frölich. 1973. Quantitative vapor-phase analysis of prostaglandin $F_{2\alpha}$ in female human urine. *Prostaglandins.* **3**: 385–387.
10. Magotteaux, G., C. Deby, and G. Barac. 1971. Urinary elimination of smooth muscle-acting prostaglandins in the dog. *Arch. Int. Physiol. Biochim.* **79**: 799–801.
11. Skarnes, R. C., and M. J. K. Harper. 1972. Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. *Prostaglandins.* **1**: 191–203.
12. Jouvenaz, G. H., D. H. Nugteren, and D. A. van Dorp. 1973. Gas chromatographic determination of nanogram amounts of prostaglandin E and F. *Prostaglandins.* **3**: 175–187.

13. Norman, A. 1953. Separation of conjugated bile acids by partition chromatography. *Acta Chem. Scand.* **7**: 1413–1419.
14. Änggård, E., and B. Samuelsson. 1964. Prostaglandins and related factors. Metabolism of prostaglandin E_1 in guinea pig lung. The structure of two metabolites. *J. Biol. Chem.* **239**: 4097–4102.
15. Sweetman, B. J., J. C. Frölich, and J. T. Watson. 1973. Quantitative determination of prostaglandins A, B, and E in the sub-nanogram range. *Prostaglandins.* **3**: 75–87.
16. Axen, U., K. Greén, D. Hörlin, and B. Samuelsson. 1971. Mass spectrometric determination of picomole amounts of prostaglandin E_2 and $F_{2\alpha}$ using synthetic deuterium labeled carriers. *Biochem. Biophys. Res. Commun.* **45**: 519–525.
17. Watson, J. T., D. R. Pelster, B. J. Sweetman, J. C. Frölich, and J. A. Oates. 1973. A display-oriented data system for multiple ion detection with gas chromatography-mass spectrometry in quantifying biomedically-important compounds. *Anal. Chem.* **45**: 2071–2078.
18. Greén, K. 1971. The metabolism of prostaglandin $F_{2\alpha}$ in the rat. 1971. *Biochim. Biophys. Acta.* **231**: 419–444.
19. Frölich, J. C., T. W. Wilson, M. Smigel, and J. A. Oates. 1974. A competitive protein binding assay specific for prostaglandin E. *Biochim. Biophys. Acta.* **348**: 241–248.
20. Smigel, M., J. C. Frölich, and S. Fleischer. 1974. Characterization of prostaglandin E receptors in membranes and their use in the assay of prostaglandin E. Methods Enzymol. **32**: 109–123.
21. Greén, K., and B. Samuelsson. 1964. Prostaglandins and related factors. XIX. Thin layer chromatography of prostaglandins. *J. Lipid Res.* **5**: 117–120.
22. Splawinski, J. A., A. S. Nies, B. J. Sweetman, and J. A. Oates. 1973. The effects of arachidonic acid, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ on the longitudinal stomach strip of the rat. *J. Pharmacol. Exp. Ther.* **187**: 501–510.
23. Nugteren, D. H., H. Vonkeman, and D. A. van Dorp. 1967. Nonenzymic conversion of al-cis 8, 11, 14 eicosatrienoic acid into prostaglandin E_1. *Rec. Trav. Chim.* **86**: 1237–1245.
24. Bygdeman, M., and B. Samuelsson. 1966. Analyses of prostaglandin in human semen. *Clin. Chim. Acta.* **13**: 465–474.
25. Änggård, E., C. Larsson, and B. Samuelsson. 1971. The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin-Δ^{13}-reductase in tissue of the swine. *Acta. Physiol. Scand.* **81**: 396–404.
26. Raz, A. 1972. Interactions of prostaglandins with blood plasma proteins *Biochem. J.* **130**: 631–636.
27. Smith, J. B., and A. L. Willis. 1970. Formation and release of prostaglandins by platelets in response to thrombin. *Br. J. Pharmacol.* **40**: 545P (Abstr.)
28. Greén, K., E. Granström, and B. Samuelsson. 1973. Methods for quantitative analysis of $PGF_{2\alpha}$, PGE_2, 9_α, 11_α-dihydroxy-15-ketoprost-5-enoic acid, and 9_α,11_α,15-trihydroxy-prost-5-enoic acid from body fluids using deuterated carriers and gas-chromatography-mass spectrometry. *Anal. Biochem.* **54**: 434–453.
29. Nies, A. S., J. Tannenbaum, J. Splawinski, and J. A. Oates. 1973. Prostaglandin formed endogenously can affect renal function. *Clin. Res.* **21**: 701. (Abstr.)
30. McGiff, J. C., K. Crowshaw, N. A. Terragno, and A. J. Lonigro. 1971. Renal prostaglandins: their biosynthesis, release, effects and fate. *In* Renal Pharmacology. J. W. Fisher and E. J. Cafruny, editors. New York. Appleton-Century-Crofts, Inc. 211–240.
31. Hamberg, M., and B. Samuelsson. 1971. On the metabolism of prostaglandin E_1 and E_2 in man. *J. Biol. Chem.* **246**: 6713–6721.
32. Silver, M. J., J. B. Smith, C. Ingerman, and J. J. Kocsis. 1972. Human blood prostaglandins: formation during clotting. *Prostaglandins.* **1**: 429–436.
33. Jaffe, B. M., H. R. Behrman, and C. W. Parker. 1973. Radioimmunoassay measurement of prostaglandins E, A, and F in human plasma. *J. Clin. Invest.* **52**: 398–405.
34. Lonigro, A. J., H. D. Itskovitz, K. Crowshaw, and J. McGiff. 1973. Dependency of renal blood flow on prostaglandin synthesis in the dog. *Circ. Res.* **32**: 712–717.
35. Crowshaw, K., and J. Z. Szlyk. 1970. Distribution of prostaglandins in rabbit kidney. *Biochem. J.* **116**: 421–424.