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MASS FRAGMENTOGRAPHIC DETERMINATION OF PROSTAGLANDIN $F_{2\alpha}$ IN HUMAN AND RABBIT URINE

B. SJÖQUIST, E. OLIW*, I. LUNDÉN and E. ÄNGGÅRD

Department of Alcohol and Drug Addiction Research and Department of Pharmacology, Karolinska institutet, 104 01 Stockholm (Sweden)

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SUMMARY

Analysis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in urine is a useful indicator of renal prostaglandin synthesis. A mass fragmentographic method for $PGF_{2\alpha}$ analysis in human urine was developed using [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$ as an internal standard and carrier. $PGF_{2\alpha}$ was extracted from urine (20 ml) with chloroform, purified by preparative thin-layer chromatography and converted to the methyl ester trimethylsilyl ether before analysis by gas chromatography—mass spectrometry. The specificity of the urine analysis was demonstrated by retention time and the use of two pairs of fragments *m/e* 494/498 and 513/517 with the same results. The coefficient of variation for duplicate analysis averaged 12.6%, n = 17. Urine from recumbent women contained 4.9 ± 2.6 (S.D.) ng/ml or 4.1 ± 1.0 ng $PGF_{2\alpha}$ per mg creatinine (n = 10) with little diurnal variation. Male urine contained 5.0 ± 2.7 (S.D.) ng/ml or 3.7 ± 2.1 ng/mg creatinine (n = 10). Similar concentrations were found in boys and in girls. These observations indicate that urinary $PGF_{2\alpha}$ originates from the kidneys with little contribution from the male accessory sexual glands. This method can also be applied to analysis of $PGF_{2\alpha}$ in rabbit urine.

INTRODUCTION

The kidneys have a large capacity to synthesize prostaglandins (PGs) [1, 2]. The renal PGs may be of physiological importance in the regulation of renal blood flow, renin release and sodium excretion [3,4]. Recently PGE_2 and $PGF_{2\alpha}$ were conclusively identified in human urine [5]. Stop-flow experiments indicate that PGs formed in the kidney enter the urine via the loop of Henle [6], while PGs infused into the renal artery are mainly recovered in urine as metabolites [7, 8]. Urinary excretion of primary PGs might therefore be used as a valuable indicator of renal PG synthesis [4, 5]. In this paper we describe a convenient and rapid mass fragmentographic method for analysis of $PGF_{2\alpha}$ in human and rabbit urine.

*To whom correspondence should be addressed.

METHODS

Materials

All reagents were of analytical grade. $PGF_{1\alpha}$, $PGF_{2\alpha}$, and [3,3,4,4-$^2H_4$]-$PGF_{2\alpha}$ were obtained from Upjohn (Kalamazoo, Mich., U.S.A.) and [^{3}H]-$PGF_{2\alpha}$ (150 Ci/mmol) from New England Nuclear (Darmstadt, G.F.R.). The purity of the compounds was checked by thin-layer chromatography (TLC) or by gas chromatography—mass spectrometry (GC—MS) (cf. Fig. 1). Thin-layer silicic acid plates (0.25 mm DC-Fertigplatten Kieselgel 60, Art. 5721) were obtained from Merck (Darmstadt, G.F.R.). The silylation reagent, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), was obtained from Pierce (Rockford, Ill., U.S.A.).

Sample purification

Human urine. Fresh urine samples were obtained from healthy men, women and children before lunch. Urine was not collected from women during menstruation. The first few millilitres of urine were discarded. For diurnal analysis, urine was collected every 4 h during the day and overnight after 8 h and stored frozen.

All analyses were run in duplicate. To 20-ml portions of urine was added 0.50 μg of [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$. The urine was acidified to pH 3 with 5—10 drops of 50% formic acid and extracted with chloroform (2 × 20 ml). To obtain phase separation, centrifugation at 1500 *g* for 15 min was often necessary. The combined chloroform extracts were dried by filtering through 4 g sodium sulphate on a filter paper and then evaporated. The sample was dissolved in 0.5 ml methanol and methylated with a fresh ethereal solution of diazomethane and evaporated to dryness under a stream of nitrogen. The samples were purified by TLC using ethyl acetate—methanol—water (80:13:50). In this system $PGF_{1\alpha}$ and $PGF_{2\alpha}$ methyl esters gave spots of the same R_F (R_F 0.67). Reference $PGF_{1\alpha}$ methyl ester was therefore put onto both sides of the TLC plate. The part of the plate containing the urine sample was covered and the references developed by spraying with phosphomolybdic acid and gentle heating of the sides of the plate. The zone corresponding to the $PGF_{1\alpha}$ and $PGF_{2\alpha}$ methyl ester was then scraped off and eluted twice with 2 ml methanol.

The sample was evaporated to dryness and the trimethylsilyl ethers were prepared by treatment with 50 μl BSTFA for 1 h at room temperature. After evaporation the residue was dissolved in 30 μl *n*-hexane and 5 μl was injected into an LKB 2091 combined gas chromatograph—mass spectrometer.

In some experiments [^{3}H] $PGF_{2\alpha}$ (2 × 10^5 dpm) was also added to the urine prior to extraction to obtain data on recovery. The recovery of [^{3}H]$PGF_{2\alpha}$ throughout the procedure averaged 35% with about 30% of the total radioactivity lost in the chloroform extractions and about 40% of the remaining activity lost during TLC.

Rabbit urine. Female non-pregnant white New Zealand rabbits (2—3 kg), were housed in metabolic cages. The cages were cleaned mechanically daily and rinsed with water. Urine was collected daily in traps on dry ice and kept frozen until analysis [9]. All samples were run in duplicate. To 5 ml of rabbit urine

were added 15 ml of water and 1.0 μg [2,3,4,4-$^2H_4$]$PGF_{2\alpha}$ but the analysis was otherwise performed exactly as described above for human urine.

GC—MS analysis

The samples were run on an LKB 2091 gas chromatograph—mass spectrometer equipped with a multiple ion detector. A 1% OV-17 column, 80 cm × 3 mm, operated at 240°, was used. The energy of the electrons was 22.5 eV and the trap current was 100 μA. The instrument was initially focused both on *m/e* 494/498 (M^+ —90) and 513/517 (M^+ —71), but for routine analysis only on *m/e* 494/498. A mass spectrum of the [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$ methyl ester trimethylsilyl ether, demonstrating the main ion intensities, is shown in Fig. 1. The deuterated $PGF_{2\alpha}$ standard contained 1% of the protium form.

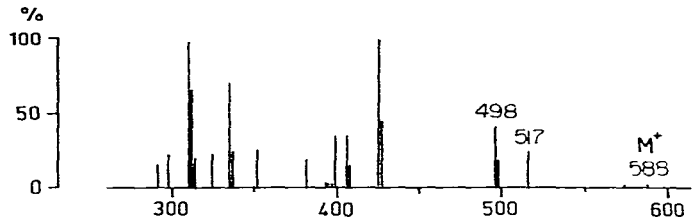

Fig. 1. Mass spectrum of [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$ methyl ester trimethylsilyl ether. The deuterated $PGF_{2\alpha}$ contained 1% of the protium form. For mass fragmentographic analysis *m/e* 494/498 (M^+ —90) were used.

Standard curves were prepared by plotting the ratio between the maximum ion intensities of the ions in focus on the ordinate and the known ratio between unlabelled and deuterium-labelled $PGF_{2\alpha}$ in the standard solutions on the abscissa. Linearization by the least squares method gave lines with slope 1.05 ± 0.09 (S.D.) and intercept of ordinate 0.023 ± 0.012. The regression coefficient was 0.996 ± 0.006.

Other analyses

Radioimmunoassay of $PGF_{2\alpha}$ in rabbit urine was performed as previously described [10]. The antibodies to $PGF_{2\alpha}$ were raised and characterized in our laboratory [10]. To reduce the error due to interradioimmunoassay variation, the urine samples were analyzed by four assays and the mean values compared with mass fragmentography (Table I).

Creatinine in urine was measured spectrophotometrically by the Jaffé colour reaction according to Poulsen [11]. Data are expressed as mean ± S.D.

RESULTS

Evaluation of the method

The typical mass fragmentogram in human urine gave $PGF_{2\alpha}$ as the main peak with little background interference. This is illustrated in Fig. 2a using *m/e* 494/498. The specificity of the urine analysis was demonstrated by using two different ion pairs (*m/e* 513/517, M^+ —71 and 494/498, M^+ —90) as shown in Fig. 2b.

TABLE I

URINE CONCENTRATION OF $PGF_{2\alpha}$ IN URINE SAMPLES OF SEVEN RABBITS MEASURED BY GC—MS AND RADIOIMMUNOASSAY

Sample	GC—MS (ng/ml)	RIA (ng/ml)	Difference* (%)
1	36	36	0
2	34	21	—38
3	37	37	0
4	23	20	—13
5	28	27	— 4
6	39	32	—18
7	37	26	—30
7 + 588 ng/ml	631	690	9
Recovery	101%	113%	

*$\overline{x}$ = —12%.

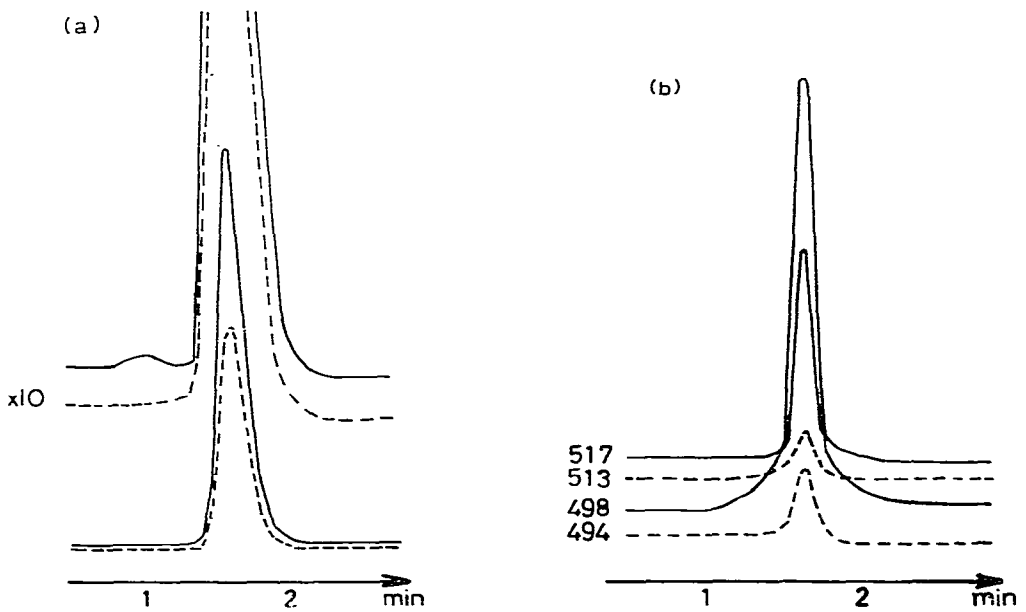

Fig. 2. (a) Mass fragmentogram from human urine using *m/e* 494/498. The main peak corresponds to $PGF_{2\alpha}$ derivatives. (b) Mass fragmentogram from human urine demonstrating the ratio between two different ion pairs *m/e* 513/517 (M^+ —71) and *m/e* 494/498 (M^+ —90).

The precision of the analysis was evaluated by replicate (n = 8) analyses of the same urine sample which gave 1.57 ± 0.31 (S.D.) ng/ml. In seventeen duplicate urine analyses the coefficient of variation was found to be 12.6%.

Unlabelled $PGF_{2\alpha}$ added to the urine was quantitatively recovered. The sensitivity of the analysis was usually adequate to measure 0.5 ng $PGF_{2\alpha}$ per ml corresponding to double background. Most of the background signal originated from the protium in the deuterium-labelled internal standard.

The stability of $PGF_{2\alpha}$ derivatives for GC—MS analysis was not particularly studied. However, the derivatives could be kept in a desiccator at 4° for at least one week, without decaying.

Analysis in human urine

The method was used to analyse the excretion of $PGF_{2\alpha}$ in urine from men, women and children. The results are shown in Table II and III. In the group of women aged 20—50 years the urinary concentration was 4.1 ± 1.0 mg $PGF_{2\alpha}$ per mg of creatinine. Young girls between the ages of 3 and 11.5 years had levels of the same order of magnitude, 4.6 ± 1.3 ng/mg of creatinine. The men (Table III) had slightly lower values than the women 3.7 ± 2.1 ng/mg of creatinine. It should be noted that there was greater tendency towards inter-individual variation among the men. Three boys between 3.5 and 7 years had $PGF_{2\alpha}$ levels between 4.7 and 5.6 ng/mg creatinine.

The possibility of a diurnal variation was explored in four healthy young women. The hourly $PGF_{2\alpha}$ output is shown in Fig. 3. No marked variation was seen. Two of the women had higher levels in the evening but the other two had not. One woman had an excretion rate of $PGF_{2\alpha}$ which was about 5 times higher than that of the other subjects. The reason for this was not apparent but it could be noted that she had had recent history of acute pyelonephritis.

Analysis in rabbit urine. Rabbit urine was generally found to contain higher concentrations of $PGF_{2\alpha}$ than human urine. A comparison between GC—MS analysis and radioimmunoassay of $PGF_{2\alpha}$ in rabbit urine (Table I) demon-

TABLE II

URINARY CONCENTRATION OF $PGF_{2\alpha}$ MEASURED BY MASS FRAGMENTOGRAPHY IN FEMALES

Females	Age	ng/ml	ng/mg creatinine
Women	20	7.9	3.2
	23	4.0	5.7
	26	8.5	3.1
	28	3.5	4.5
	31	4.9	3.2
	32	4.1	3.3
	33	8.3	4.1
	34	4.8	5.6
	38	0.9	3.8
	50	2.2	4.3
Mean ± S.D.		4.9 ± 2.6	4.1 ± 1.0
Girls	3	6.8	5.2
	5.5	1.6	2.3
	6	6.7	5.0
	10	6.8	5.5
	11.5	6.7	5.0
Mean ± S.D.		5.7 ± 2.3	4.6 ± 1.3

TABLE III

URINARY CONCENTRATIONS OF $PGF_{2\alpha}$ MEASURED BY MASS FRAGMENTOGRAPHY IN MALES

Males	Age	ng/ml	ng/mg creatinine
Men	23	8.8	3.9
	27	4.1	2.6
	29	1.5	3.2
	29	7.4	5.7
	30	5.1	1.7
	31	1.8	1.3
	31	2.9	4.1
	33	3.2	1.9
	38	8.4	8.2
	42	6.4	4.2
Mean ± S.D.		5.0 ± 2.7	3.7 ± 2.1
Boys	3.5	4.9	5.6
	5	3.0	4.7
	7	4.8	4.8
Mean ± S.D.		4.2 ± 1.1	5.0 ± 0.5

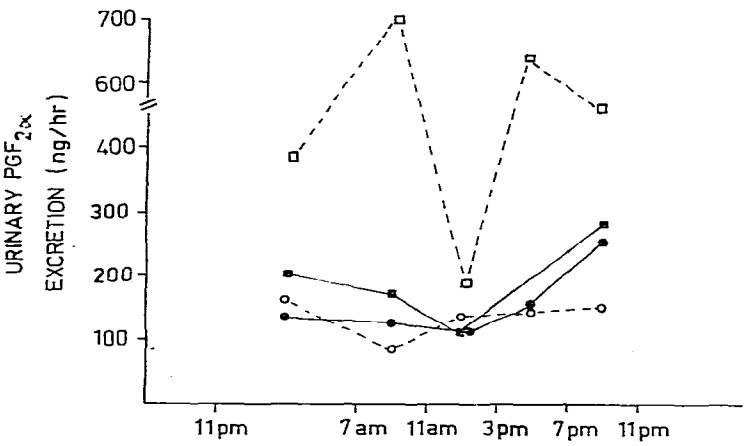

Fig. 3. Diurnal variation of urinary $PGF_{2\alpha}$ excretion in four women, measured by mass fragmentography.

strates little systematic difference between the two methods. Recovery of large amounts of $PGF_{2\alpha}$ (588 ng/ml) added to urine was quantitative with GC—MS but slightly overestimated by radioimmunoassay (Table I).

DISCUSSION

The present paper describes a mass fragmentographic method for analysis of $PGF_{2\alpha}$ in human and rabbit urine. $PGF_{2\alpha}$ has previously been identified in human urine [5] and analysed with GC—MS methods after different purifica-

tion procedures [12, 13]. The present method demonstrates that mass fragmentographic analysis of $PGF_{2\alpha}$ in urine is possible after solvent extraction, TLC and derivatization.

The concentration of $PGF_{2\alpha}$ in urine using this method is slightly higher than has previously been reported by GC—MS analysis [5]. The reason for this discrepancy is unknown. Dietary factors may be of importance since urinary $PGF_{2\alpha}$ excretion seems be inversely related to sodium excretion [9]. However, the difference is not likely to depend on diurnal changes in $PGF_{2\alpha}$ excretion.

In a recent publication [14] urinary PGE_2 excretion was found to change parallel to the diurnal changes in water and sodium excretion. Urine flow and sodium excretion were low during the night and increased after noon [15]. In this work $PGF_{2\alpha}$ excretion did not exhibit diurnal rhythm but remainded rather stable throughout the day.

The human semen and menstrual fluid contain large quantities of $PGF_{2\alpha}$. Urine was therefore not collected during menstruation and the first millilitres of voided urine were also discarded to avoid contamination with genital secretion. This is also important in males, where $PGF_{2\alpha}$ from the seminal fluid and the accessory sexual glands could give spuriously high values in urine. For urine collected in this way between 8 and 12 a.m. similar concentrations of $PGF_{2\alpha}$ were found in men, women and children. This indicates that the contribution of $PGF_{2\alpha}$ from the seminal fluid to the urine could be of little importance under these conditions.

For duplicate analysis the method had a coefficient of variation of about 12%. This variation is comparatively high for a mass fragmentographic analysis. There are several reasons for the low precision: the levels of $PGF_{2\alpha}$ in urine are relatively low, only a few ng/ml and the recovery after extraction and purification on TLC was only 35%. Although the mass fragmentograms showed stable baselines with little background interference, the high coefficient of variation indicates the appearance of background noise from other compounds in the $PGF_{2\alpha}$ peaks. The background contribution from the protium form was of minor importance (1%), i.e. 0.5 μg $[^2H_4]PGF_{2\alpha}$ added to 20 ml urine contributed with 0.25 ng/ml. For a rapid and convenient method, a variation of 12% can often be accepted.

Many radioimmunoassays for primary PGs or PG metabolites in urine have been reported [16, 17]. Data on the concentration of PGs in urine or in other body fluids obtained by radioimmunoassays differ [16]; it is therefore important to validate radioimmunoassays by other methods. This is emphasised by Gill et al. [18], who found PGE_2 in human urine to be considerably higher by radioimmunoassay than by GC—MS. One application of the present method could therefore be to validate radioimmunoassays of $PGF_{2\alpha}$ in urine.

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