INHIBITION OF PROSTAGLANDIN SYNTHESIS IN MAN

Mats Hamberg

Department of Chemistry, Karolinska Institutet,

Stockholm, Sweden

Received August 30,1972

SUMMARY: 7α-Hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid, the major urinary metabolite of prostaglandins E_1 and E_2 in man, was determined in human urine by a method based on the use of the bis(O-$^2H_3$-methyloxime) derivative of dimethyl 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioate as internal standard and determination of the ratio between unlabeled and deuterium-labeled molecules by multiple-ion analysis. Male subjects excreted larger amounts of the metabolite (6.5-46.7 μg/24 hours, n=10) than did female subjects (2.5-5.3 μg/24 hours, n=10). The excretion rate was strongly suppressed following oral administration of therapeutic doses of indomethacin, aspirin and sodium salicylate.

Tritium-labeled prostaglandin E_2 administered intravenously to human subjects is rapidly converted into 11α-hydroxy-9,15-diketo-prost-5-enoic acid (1). This compound is further degraded into several metabolites which are mainly excreted in the urine. The major urinary metabolite has been identified as 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid (1,2). This compound is also the major urinary metabolite formed from prostaglandin E_1 and the metabolites 11α-hydroxy-9,15-diketoprostanoic acid, 11α-hydroxy-9,15-diketoprost-5-enoic acid and 11α-hydroxy-9,15-diketoprosta-5,13-dienoic acid (1). The amounts of 7α-hydroxy--5,11-diketotetranor-prostane-1,16-dioic acid in 24 hour samples of urine from three human subjects was found to range between 7-27 μg (1,3).

Recently an improved method for quantitative determination of the metabolite in human urine was developed (4). This method was based on the use of a deuterium-labeled derivative of the metabolite

as internal standard and mass spectrometric determination of the ratio between unlabeled and deuterium-labeled molecules. In this communication we wish to report application of the new method to determine the basal excretion of the major urinary metabolite as well as inhibition of the excretion by indomethacin and other analgesics which inhibit prostaglandin biosynthesis (5-8).

EXPERIMENTAL AND RESULTS

One twentieth of 24 hour samples of urine was applied to a column of Amberlite XAD-2 (cf. ref. 1). Material in the ethanol eluate was treated with 30 mg of O-methyl hydroxylamine hydrochloride in 3 ml of pyridine for 18 hours. The pyridine was evaporated and the residue dissolved in methanol and treated with an excess of ethereal diazomethane. After evaporation methanol and water were added and the solution extracted with three portions of diethyl ether. The bis(O-$^{2}H_{3}$-methyloxime) derivative of tritium-labeled dimethyl 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioate (ref. 4, 5.9 nmoles, 2.4 μg) was added to the extracted material and the mixture subjected to reversed phase partition chromatography (cf. ref. 1)(column, 9 g of hydrophobic Hyflo Super-Cel; solvent system, methanol-water-chloroform-n-heptane (360:240:75:25, v/v/v/v)). Material in the peak of radioactivity (90-120 ml effluent) was isolated by extraction with diethyl ether and subjected to thin-layer chromatography (solvent system, organic layer of ethyl acetate-2,2,4-trimethylpentane-water (100:50:100, v/v/v); R_F of major isomer of the bis(O-methyloxime) derivative, 0.73). The purified bis(O-methyloxime) derivative of dimethyl 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioate was converted into the trimethylsilyl ether derivative and subjected to multiple-ion analysis on an LKB 9000 instrument with a 1% SE 30 column.

The mass spectrum of the trimethylsilyl ether - bis(O-$^2H_3$-methyloxime) derivative of dimethyl 7α-hydroxy-5,11-diketotetranorprostane-1,16-dioate (fig. 1) had a base peak at m/e 368 (elimination of $\cdot OC^2H_3$ from one of the O-$^2H_3$-methyloxime groups plus trimethylsilanol). By analysis of the ratio between the ions at m/e 365 and m/e 368 of known mixtures of unlabeled and deuterium-labeled derivatives a standard curve was constructed (fig. 2). Fig. 3 shows a recording of m/e 365/368 from an analysis of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in a sample of human urine. Methods for quantitative analysis of the major urinary metabolites of prostaglandins E_1 and E_2 and prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ will be described in detail shortly (4).

Table I shows the results of determinations of the metabolite in 20 different samples of urine. Male subjects consistently excreted larger amounts (range, 6.5-46.7 µg/24 hours, n=10) than did female subjects (range, 2.5-5.3 µg/24 hours, n=10; mean value, 4.1 $\pm$ 0.8 (S.D.) µg/24 hours).

Table II lists data obtained with subjects receiving indomethacin (Indomee, Merck, Sharp & Dohme; 200 mg/24 hours divided in four doses), aspirin (3 g/24 hours divided in four doses) and sodium salicylate (3 g/24 hours divided in four doses). Indomethacin gave 77-98% inhibition of the basal excretion. Corresponding figures for aspirin and sodium salicylate in one subject was 86% and 88-98%, respectively.

DISCUSSION

Preliminary studies with gas-liquid chromatographic determination of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in human urine showed values of 7-27 µg/24 hours (1,3). The relatively small amounts necessitated development of a more sensitive method. The present method in which a deuteri-

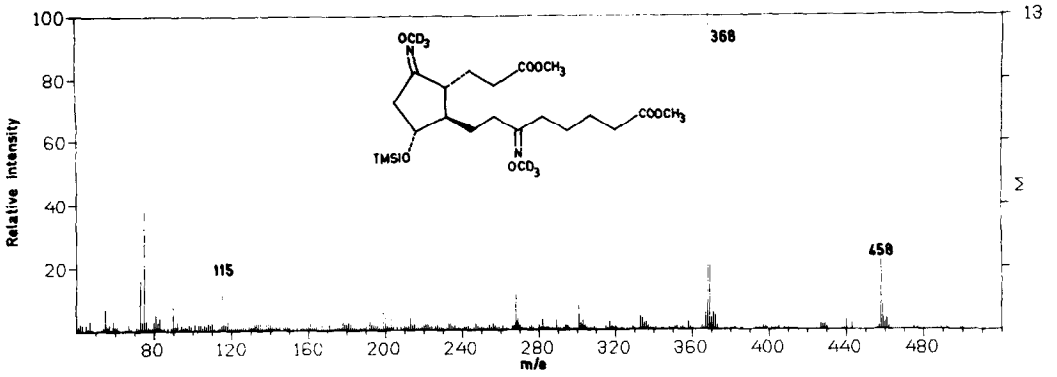

Fig. 1. Mass spectrum of major isomer of bis(O-$^{2}H_{3}$-methyl-oxime) - trimethylsilyl ether derivative of dimethyl 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioate.

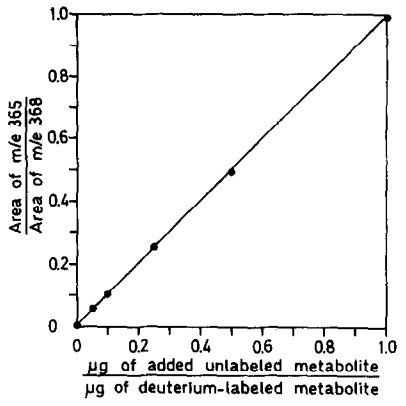

Fig. 2.

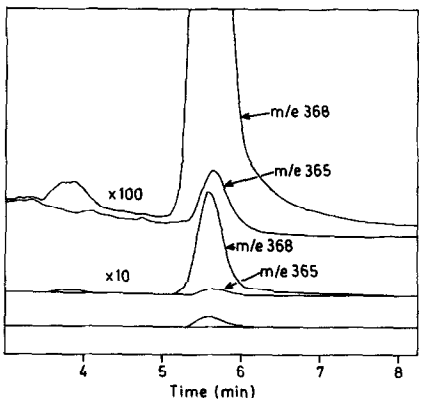

Fig. 3.

Fig. 2. Standard curve in multiple-ion analysis. Known mixtures of unlabeled and deuterium-labeled derivatives (ratios, 0, 0.05, 0.10, 0.25, 0.50 and 1.0) were injected and the intensities of m/e 365 and m/e 368 were followed vs. time.

Fig. 3. Multiple-ion analysis in quantitative determination of the major urinary metabolite in a sample of human urine. Electron energy, 30 eV. Trap current, 60 μA. Column, 1% SE 30. Column temperature, 232°.

um-labeled derivative of the metabolite is used as internal standard and where ratios between unlabeled and labeled mole-

TABLE I. Excretion of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in 20 healthy human subjects.

Sex	Amount (μg/24 hours)									
Males	6.5	7.3	7.4	8.1	9.6	10.9	11.9	12.3	34.0	46.7
Females	2.5	3.4	3.9	4.0	4.1	4.1	4.2	4.5	4.8	5.3

TABLE II. Excretion of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in subjects receiving analgesics. Indomethacin (a, 4x50 mg/24 hours), aspirin (b, 4x0.75 g/24 hours), and sodium salicylate (c, 4x0.75 g/24 hours) were given as indicated by asterisk.

Subject	Amount of metabolite (μg/24 hours)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
I[a] (♀)	4.8	4.8	1.8*	1.1*	1.5*	2.7	4.1
II[a] (♀)	3.9	4.4	0.7*	0.7*	0.7*	3.1	6.5
III[a] (♀)	3.8	3.0	0.5*	0.3*	0.3*	0.8	1.1
IV[a] (♂)	-	47.0	18.1*	3.5*	-	-	-
IV[a] (♂)	24.0	23.0	6.2*	0.5*	0.5*	4.1	20.0
IV[b] (♂)	-	47.0	19.5*	6.4*	6.4*	16.6	33.3
IV[c] (♂)	-	33.5	21.2*	3.7*	0.6*	-	-
IV[c] (♂)	34.8	32.0	26.1*	9.4*	4.3*	3.9	13.3

cules are determined by multiple-ion analysis appears to offer a significant improvement with respect to sensitivity, precision and accuracy. Analogous methods have earlier been described for determination of prostaglandin E_1 (9), prostaglandins E_2 and $F_{2\alpha}$ (10), plasma metabolites of prostaglandin $F_{2\alpha}$ (11) as well as the major urinary metabolite of prostaglandins E_1 and E_2 in the guinea pig (12).

Examination by the present method of the diurnal excretion in urine of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid showed values of 6.5-46.7 µg in male subjects and 2.5-5.3 µg (mean, 4.1 ± 0.8 (S.D.) µg) in female subjects. The reason for the sex difference in the excretion has to be further explored.

It was earlier found that 13-20% of intravenously injected tritium-labeled prostaglandin E_1, prostaglandin E_2, 11α-hydroxy-9,15-diketoprostanoic acid and 11α-hydroxy-9,15-diketoprost--5-enoic acid could be recovered as 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in urine (1). On basis of these figures the sum of the daily production rate of prostaglandins E_1 and E_2 may be estimated to 50-330 µg in males and 20-40 µg in females. These values are lower than those reported for the rat (minimum figures, 10-20 µg/kg)(13) and guinea pig (5-10 µg/kg)(12).

Administration of indomethacin in a dose used in therapeutic practice (4x50 mg/24 hours) to healthy human subjects resulted in a pronounced suppression of the levels of the major metabolite in urine (77-98% inhibition, Table II). Much higher doses of indomethacin (30-100 mg/kgx24 hours) were required to accomplish a similar inhibition in the guines pig (12). In man, therapeutic doses of aspirin and sodium salicylate (4x0.75 g/24 hours) also gave a strong inhibition of the excretion of the major urinary metabolite (Table II). Sodium salicylate was reported to be a very poor inhibitor of prostaglandin biosynthesis in guinea pig lung homogenates which posed a problem when attempts were made to relate the effectiveness of analgesics with their ability to inhibit prostaglandin biosynthesis (5). The postulated relationship is supported by the present results which show that sodium salicyl-

ate is at least as effective as aspirin in inhibiting prostaglandin formation *in vivo*.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council (project no. 03X-2828 and 13X-217), by Knut and Alice Wallenbergs Stiftelse and by Stiftelsen Riksbankens Jubileumsfond. The skilful technical assistance of Miss Kerstin Wahlberg is gratefully acknowledged.

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