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Differences in 15-Hydroxyprostaglandin Dehydrogenase Activity in Male and Female Rat Kidneys (41256)

LAUREN M. CAGEN, REBECCA L. MORGAN, AND PHILIP G. BAER

Department of Pharmacology, University of Tennessee, Center for the Health Sciences, Memphis, Tennessee 38163

Abstract. In adult Wistar rats, 15-hydroxyprostaglandin dehydrogenase (PGDH) activity of male kidney homogenates was 40 times greater than that of female kidney homogenates; in contrast, PGDH activity of male and female lung homogenates did not differ significantly. Perhaps causally related to the difference in renal PGDH activity, urinary excretion of prostaglandin (PG) E_2 by female rats was twice, and excretion of PGF_α 3 times, that by males. Female rat renal homogenate and female rat renal high-speed supernatant inhibited PGDH activity of male rat renal homogenate and the activity of partially purified bovine PGDH.

Prostaglandins (PG)E_2 and $F_{2\alpha}$ are major products of prostaglandin synthesis within the kidney (1–3); endogenous levels of PGE_2 and $PGF_{2\alpha}$ presumably reflect the balance between synthesis, physical removal, and metabolism, the last being initiated by the action of 15-hydroxyprostaglandin dehydrogenase (PGDH) (4, 5). Reduction in PGDH activity might be expected to lead to increased levels of prostaglandins in the kidney, reflected in the rate at which they are released into the urine or blood stream. We have noted that PGDH activity is greater in renal homogenates of adult male Wistar rats than in homogenates from age- and strain-matched females. Perhaps associated with this difference, urinary excretion of PGE_2 and PGF_α was higher in the female rats than in the males. In addition, we observed that when female rat kidney homogenate was added to male rat kidney homogenate, PGDH activity of the latter, clearly evidenced during separate incubation, was largely suppressed.

Materials and Methods. Five-month-old Wistar rats (Harlan), 12 female and 6 male, were placed in individual metabolic cages. As indicated by vaginal smears, estrous cycling in the female rats was normal. After a 4-day period of adaptation, three consecutive 24-hr urine collections were taken into containers surrounded by dry ice and kept frozen until thawed for determination of volume and concentrations of PGE_2 and PGF_α. After completion of the final 24-hr urine collection, the rats were killed by cervical dislocation. Both kidneys and the lungs were removed within 1 min, frozen on dry ice and acetone, and stored at −70°.

For determination of kidney and lung PGDH activity, organs were thawed into 4 vol of 0.01 *M* Tris–HCl buffer, pH 7.8, containing 0.1 m*M* dithiothreitol. Tissue was sliced into small fragments, disrupted with a Polytron homogenizer (Brinkmann, 30 sec at top speed), and centrifuged at 4250*g* for 15 min (apparent enzyme activity was higher in the 4250*g* supernatant than in supernatant obtained from centrifuging at 105,000*g* for 1 hr). Homogenates averaged 18.6 mg protein/ml. NAD^+ (2 μmole), [1-^{14}C]PGE_2 (New England Nuclear; 0.01 μCi, 200 pmole), and Tris–HCl–dithiothreitol buffer were incubated with lung or kidney supernatant in a total volume of 1 ml for 10 min at 37°. The reaction was terminated by addition of 10 μl of formic acid, and the acidified solutions were extracted three times with 1 ml of ethyl acetate. No nonenzymatic decomposition was observed during incubation at 37° with boiled enzyme. To control for possible dehydration of PGE_2 during acid extraction, 10 μl of formic acid was added to the reaction mixture in some tubes prior to addition of [1-^{14}C]PGE_2 and the solutions were extracted without incubation. After removal of solvent under a stream of nitrogen, residue in the organic

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phase was redissolved in 50 μl of methanol and applied to a silica gel G/Hy thin-layer plate (0.25 mm; Brinkmann) which was developed in ethyl acetate/isooctane/acetic acid/water (110/55/20/100; upper phase). Radioactive zones were located by radiochromatogram scanning (Packard Model 7201) and prostaglandin standards visualized with iodine vapor. Zones corresponding in mobility to PGE_2 (R_f:0.30), or to 15-keto-PGE_2 (R_f:0.57), 13,14-dihydro-15-keto-PGE_2 (R_f:0.61), or PGA_2 (R_f:0.64) standards were scraped from the plate and suspended in 10 ml Scintiverse cocktail (Fisher), and radioactivity was determined by liquid scintillation counting (Searle Delta 300). The extent of metabolism of added PGE_2 was determined from the equation: picomoles PGE_2 metabolized = (% of radioactivity in metabolite zones − % of radioactivity in metabolite zone in control incubations) × picomoles PGE_2 added to the reaction mixture. All values reported are the mean of duplicate determinations.

Urinary content of immunoreactive PGE_2 and PGF_α was determined by radioimmunoassay according to the method of Dray *et al.* (6) after extraction and partial purification by silicic acid chromatography. Two-milliliter aliquots of the urine sample were diluted to a final volume of 10 ml with water, acidified to pH 3.0 with formic acid after addition of [^{3}H]PGE_2 tracer, and extracted twice with cyclohexane–ethyl acetate (1/1). The organic phase was removed and evaporated, and the residue was redissolved for chromatography on 0.5-g silicic acid columns equilibrated with benzene–ethyl acetate (3/2). After development of the columns with 5.5 ml of benzene–ethyl acetate (3/2) to elute PGA and PGB, with 12 ml of benzene–ethyl acetate–methanol (30/20/1) to elute PGE, and with 5 ml of benzene–ethyl acetate–methanol (3/2/1) to elute PGF, the fractions of interest were dried under nitrogen and redissolved in assay buffer the day of assay. For the assay procedure, standards of PGE_2 and $PGF_{2\alpha}$ (0.95–500 pg), or sample, were incubated with the appropriate antibody (Pasteur Institute, Paris) for 2 hr at 4°. Bound and free antigen were separated with charcoal–dextran, and the concentration of antibody-bound antigen was determined by scintillation counting of the supernatant. Recovery through the extraction and purification procedures was determined for each sample from tracer PGE_2 recovery, and assay estimates of both PGE_2 and $PGF_{2\alpha}$ were individually corrected for losses. Average urinary prostaglandin excretion values were determined for each rat from the three consecutive collection values. The data in Table I are group mean values calculated from these individual average values. Since the PGF_α antiserum does not discriminate between $PGF_{1\alpha}$ and $PGF_{2\alpha}$, the results have been reported as PGF_α.

Values in the text, figures, and tables are expressed as mean ± SEM. Differences between group mean values were analyzed by Student's t test for unpaired observations; a P value of less than 0.05 was taken to indicate statistical significance.

Results. After incubation of [^{14}C]PGE_2 with 2.5 μl (~50 μg of protein) of male rat kidney homogenate, an average of 33.4% of the PGE_2 substrate was converted to the 15-keto metabolite, while with 2.5 μl of female kidney homogenate, only 0.8% was converted. When [^{14}C]PGE_2 was incubated with 900 μl (~17 mg of protein) of the male kidney homogenate, all of the substrate was consumed; after incubation with 900 μl of the female kidney homogenate only 39% was oxidized in 10 min. Mean PGDH activity of the male and female rat kidneys and lungs is shown in Table I. In contrast to the sex-related difference observed between the kidneys, no significant difference in PGDH activity was found between male and female rat lungs. When incubated with 50 μl (~0.95 mg of protein) of lung homog-

TABLE I. 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE ACTIVITY IN HOMOGENATES OF MALE AND FEMALE RAT LUNG AND KIDNEY

Lung		Kidney	
Males (5)	Females (9)	Males (5)	Females (9)
2.03 ±0.81[a]	2.64 ±0.56	107.0 ±43.7	2.52 ±0.92
N.S.		$P < 0.01$	

[a] Results expressed as nanomoles PGE_2 metabolized in 10 min/g tissue weight.

enate, average conversion of the [^{14}C]PGE_2 substrate to the 15-keto metabolite was 12.7% for the male tissue, compared with 16.5% conversion by the female tissue.

The lower PGDH activity of the female rat kidney, relative to that of the male, may be due to the presence of an inhibitor of PGDH. Figure 1 shows radiochromatogram scans of the products obtained after incubation of [^{14}C]PGE_2 with homogenates of male and female rat kidneys separately and in combination. The homogenates had been stored for several days at −15° prior to the experiments, with resultant loss of most of the initial PGDH activity of the female kidney homogenates. PGDH activity of the male kidney homogenate, clearly evidenced during separate incubation, was greatly suppressed when the male kidney homogenate (0.47 mg of protein) was incubated in combination with the female kidney homogenate (16.8 mg of protein). Bovine lung PGDH (Gallard–Schlesinger) was also inhibited by aliquots of female rat renal homogenate (Fig. 2A) or the 105,000*g* supernatant of the renal homogenate (Fig. 2B). The inhibitory capacity of the renal high-speed supernatant was eliminated by boiling for 2 min. Resuspended 105,000*g* pellet also inhibits bovine lung PGDH: the microsomal inhibitor may be distinct from that in the supernatant since it was not affected by boiling.

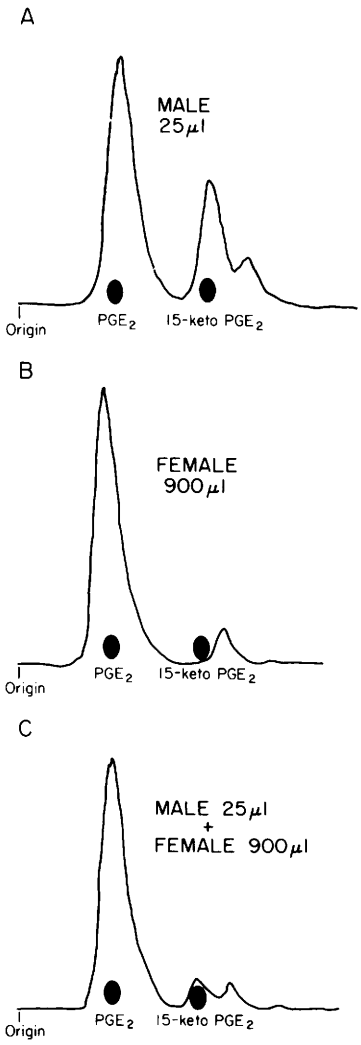

FIG. 1. Radiochromatogram scans of products obtained after incubation of [^{14}C]PGE_2 with male (A) or female (B) rat kidney homogenates separately or male and female rat kidney homogenates in combination (C). Solid ovals indicate chromatographic mobility of standards.

Shown in Table II, for the 12 female and 6 male rats studied over a period of 3 consecutive days, after correction for body weight differences, 24-hr urine volumes of the males and females were not different. The urinary concentrations of both PGE_2 and PGF_α were significantly greater for the female rats than for the males, and the 24-hr urinary excretion of PGE_2 and PGF_α by female rats exceeded that of the males by two- and threefold, respectively. In addition to the difference in absolute urinary prostaglandin excretion rates of male and female rats, these data indicate the possibility of a sex-related difference in the relative concentrations of PGE_2 and PGF_α in the urine. The average urinary PGF_α/PGE_2 concentration ratio for 36 separate observations in female rats was 3.5 ± 0.5, compared with 1.7 ± 0.2 for 18 observations in male rats; intragroup variability of this ratio was high, however, and the difference was significant only at the 0.05 level, despite the large number of observations.

Discussion. The present study was intended to confirm a previously reported sex-related difference in renal PGDH activity in the rat, and to determine whether the difference in PGDH activity is correlated with a difference in urinary excretion

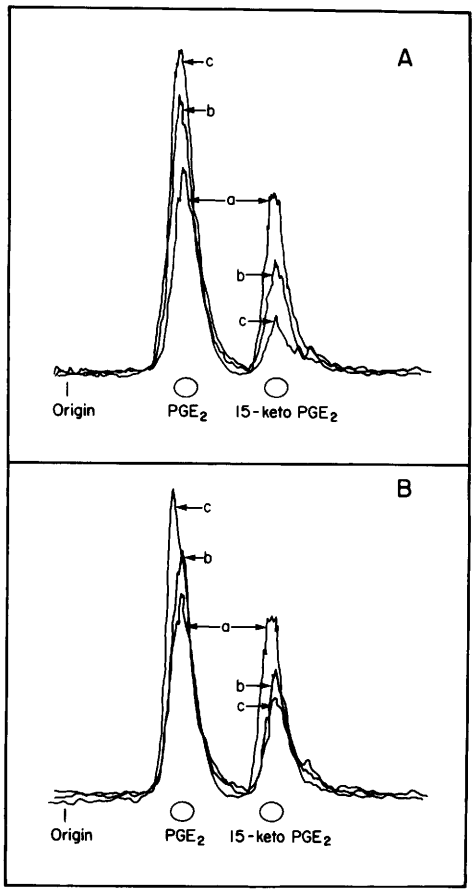

FIG. 2. Radiochromatogram scans of products obtained after incubation of bovine pulmonary PGDH alone (a) or in the presence of 10 μl (b) or 100 μl (c) of female rat renal homogenate (A) or the 105,000g supernatant (B). Reaction conditions were the same as those described under Materials and Methods except that the final PGE_2 concentration was 5.7 μM.

of unmetabolized prostaglandins. In agreement with an earlier report (7), we found that PGDH activity was much greater in homogenates of kidneys from male rats than in homogenates of female rat kidneys. Possibly as a result of the lower renal PGDH activity, urinary excretion of PGE_2 and PGF_α by the female rats was greater than that by the male rats. In addition, the kidneys of the female rats contain an inhibitor of PGDH activity, which may contribute to the sex-related difference in PGDH activity.

Interpretation of the difference in urinary prostaglandin excretion as an expression of the sex-related difference in renal PGDH activity is based on the following considerations. Urinary PGE_2 and $PGF_{2\alpha}$ are of renal origin (8), entering the tubular fluid in the loop of Henle (9). Histochemical techniques have demonstrated PGDH activity in the thick ascending limb of the loop of Henle and in distal portions of the collecting ducts (10), suggesting that metabolism of PGE_2 and $PGF_{2\alpha}$ might occur during passage through these portions of the nephron. The extent to which these prostaglandins are metabolized prior to excretion and, therefore, the extent to which variations in PGDH activity might be expected to alter urinary excretion of PGE_2 and $PGF_{2\alpha}$, is not known. If, in the males, half of the PGE_2 destined for excretion in the urine were metabolized prior to entering the tubular fluid or during passage through the nephron, complete suppression of PGDH activity would increase urinary PGE_2 levels a maximum of twofold. Although the data observed are consistent with this interpretation, the possible contribution of other factors should be considered, since kidneys of male and female rats may differ in the rate of prostaglandin synthesis, as well as the rate of its destruction. Gecse *et al.* re-

TABLE II. COMPARISON OF 24-hr URINARY PROSTAGLANDIN EXCRETION IN MALE AND FEMALE RATS

Sex (N)	Volume (ml/100 g body wt)	Concentration (ng/ml)		Excretion (ng/100 g body wt/day)	
		PGE_2	PGF_α	PGE_2	PGF_α
Males (6)	4.5	15	27	66	115
± SEM	0.4	2	6	8	27
Females (12)	4.2	35	104	149	435
± SEM	0.2	5	12	18	49
P	NS	<0.01	<0.001	<0.005	<0.001

ported that cyclooxygenase activity of renal medullary microsomes is also higher in male than in female rats (7), which would suggest higher rates of prostaglandin synthesis in kidneys of male rats, but others have found that the extent and direction of sex-related differences in estimates of cyclooxygenase activity depend on the conditions chosen for assay (11). It should also be noted that the rate of arachidonic acid release from phospholipid stores may be more important than the level of activity of cyclooxygenase in regulating the overall rate of prostaglandin synthesis.

Factors regulating renal PGDH activity are only partially understood. Renal PGDH is a labile enzyme in the rat, with a half-life of about 1 hr (12), and PGDH levels should be responsive to changes in the rate of transcription or translation of the appropriate mRNA. Steroid hormones, which exert their effects primarily at the transcriptional level, have been shown capable of altering PGDH activity in lungs and kidneys (13, 14). Differences between male and female renal PGDH activity may, therefore, reflect differences in the steroid hormonal backgrounds of the animals; however, the lack of a difference in pulmonary PGDH activity would still have to be accounted for. Perhaps of significance in this context is the report, by Moore and Hoult, that pretreatment of male Sprague–Dawley rats with prednisolone significantly increased levels of renal PGDH activity without affecting levels of PGDH activity in the lung (15).

Our findings indicate that an endogenous PGDH inhibitor is present in the kidneys of female rats; while it is possible that the activity of this inhibitor contributes to suppression of PGDH activity in the female rat kidneys, a definite conclusion cannot be drawn at this time. PGDH activity has also been found to be lower in kidneys of male New Zealand genetically hypertensive rats than in kidneys of male New Zealand normotensive rats (16), and it has been suggested that an endogenous PGDH inhibitor may account for that difference (17).

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