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RENAL PROSTAGLANDIN METABOLISM IN SPONTANEOUSLY HYPERTENSIVE RATS

I. AHNFELT-RØNNE and E. ARRIGONI-MARTELLI
Department of Pharmacology, Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark

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Abstract—Urinary excretion of $PGF_{2\alpha}$ and kidney 15-PGDH and 9-PGDH have been compared in Wistar Okamoto hypertensive rats (SHR) of different ages and age-matched Wistar normotensive rats (NTR). The urinary $PGF_{2\alpha}$ excretion is increased at all the ages. The 15-PGDH is unaltered whereas the 9-PGDH is decreased significantly in adult SHR. It is suggested the existence in Wistar Okamoto hypertensive rats of an inherited abnormality in renal prostaglandin metabolism different from that reported in New Zealand hypertensive rats.

Modifications of kidney functions are probably involved in the initiation and maintenance of high blood pressure in spontaneously hypertensive rats (SHR). There are changes in plasma renin activity [1, 2], water and sodium balance [2] and hemodynamic parameters [3] during the development of hypertension, and excretion of vasoactive polypeptides is variably altered [4, 5].

Age related changes in PG catabolizing enzymes occur in kidneys of normotensive rats [6]. PG-15-hydroxydehydrogenase (15-PGDH) and Δ-13, 14-reductase (13-PGR) are present in the developing kidney, whereas PG-9-hydroxydehydrogenase (9-PGDH) is characteristic of the adult rat kidney. Capacity for PG biosynthesis does not vary appreciably with age [6]. PGs of both the F and E types cause vasoconstriction in the rat kidney [7] in contrast to other species [8]. Unless catabolized they might interfere with blood flow to the renal cortex and affect renal functions and nephrogenesis not yet complete at birth [9].

Kidney homogenates of New Zealand genetic hypertensive rats have low 15-PGDH activity [10], the most important enzyme in metabolism of renal prostaglandins [11], but the kidney enzyme level is normal in Wistar-Okamoto hypertensive rats [10]. The present results show that in the Wistar-Okamoto rats renal PG metabolism is altered in a different way.

MATERIALS

1. *Animals.* Spontaneously hypertensive Wistar-Okamoto rats of either sex and normotensive Wistar rats (Charles River) matched for age were used. The animals were housed in cages maintained at 22°, relative humidity 60%. Food and water were given *ad lib.*

2. *Reagents.* $PGF_{2\alpha}$ radioimmunoassay kit (Clinical Assays, Inc., MA, USA), [9-^{3}H]prostaglandin $F_{2\alpha}$, 15 Ci/m-mole (Radiochemical Centre, Amersham, England), unlabelled $PGF_{2\alpha}$ (Unilever Research, Vlaardingen, Holland), L-epinephrine bitartrate (Sigma Chemical Co., St. Louis, MO, USA), β-NAD^+, grade I, lactic dehydrogenase (LDH), 550 units/mg, prepared from rabbit muscle, and reduced glutathione (Boehringer Mannheim GmbH), sodium pyruvate, 99% purity, and precoated silica plates (0.25 and 2 mm thickness, E. Merck, Darmstadt, West Germany), 'InstaGel' water-miscible scintillation cocktail (Packard Instrument Co., USA).

METHODS

1. *Urinary $PGF_{2\alpha}$ determination.* Rats were placed in metabolic cages from 9.00 a.m. to 12.00 noon. The number of rats per cage depended on the weight of the animals, in such a way that the total weight of animals was 200–300 g/cage. The rats had free access to water in the cages. At the end of urine collection, 2000 cpm [^{3}H]$PGF_{2\alpha}$, of the same sp. act. as the radiolabelled antigen used later for the radioimmunoassay, was added to the urine specimens. These were acidified to pH 3 with dilute hydrochloric acid, and extracted once with 4 vol. of ethyl acetate by vigorous Vortex mixing. The phases were separated by centrifugation at 3000 *g*. The organic layers were transferred to small conical tubes on a waterbath at 55° where the solvent evaporated in a gentle stream of nitrogen. The residues were dissolved in 100 μl of chloroform/methanol (1:1) each, and applied to preparative silica plates (2 mm thickness, 20 × 20 cm). 10 μg $PGF_{2\alpha}$ was spotted as standard in a separate compartment on each plate. After development in the solvent mixture chloroform/methanol/acetic acid/water (90:9:1:0.65) all parts of the plates, except the $PGF_{2\alpha}$-standard compartments, were covered with glass, and the standard spots visualized by exposure to iodine vapor. Corresponding zones of the urine-extract compartments were scraped into plastic tubes and eluted 3 times with 1 ml methanol. The combined eluates, separated by centrifugation, were evaporated at 55° in N_2, and the residues were dissolved in the

List of abbreviations: SHR = Spontaneously hypertensive rats; NTR = Normotensive rats; PG = Prostaglandin; 15K-PG = 15-keto-prostaglandin; 15KD-PG = 15-keto-13,14-dihydro-prostaglandin; 15-PGDH = 15-hydroxy-prostaglandin dehydrogenase; 9-PGDH = 9-hydroxy-prostaglandin dehydrogenase; PGS = Prostaglandin synthetase; 13-PGR = Δ-13, 14-prostaglandin reductase; 9-PGR = 9-oxy-prostaglandin reductase.

assay buffer supplied with the $PGF_{2\alpha}$ RIA kit. One aliquot of the solution was used for quantitation of ^3H in a liquid scintillation spectrometer (Beckman LS-230) allowing of the exact calculation of $PGF_{2\alpha}$-recovery in each purified urine-extract. Another aliquot was used for radioimmunoassay according to the kit instructions. The amount of $[^3H]PGF_{2\alpha}$-antigen added to each RIA tube was calculated taking into consideration the $[^3H]$-tracer already present in each tube. The total dpm of $[^3H]PGF_{2\alpha}$ was thus constant in each standard and assay tube. RIA results were calculated by computer using a log-logit transformation, and each result was finally corrected to 100 percent recovery with the actual tracer-recovery value. As the radioactive tracer method allows rapid and reliable calculation of recovery it was not considered necessary to apply more extensive ethyl acetate-extraction than described above. This method furnished recoveries in the range of 40–60 per cent, with enough $PGF_{2\alpha}$ in each extract to ensure accurate measurement by radioimmunoassay.

2. *Preparation and fractionation of rat kidney homogenates.* The rats were killed by decapitation and the right kidneys rapidly excised, freed from fat and connective tissue, cut open, and placed in ice-cold 50 mM KH_2PO_4/NaOH buffer, pH 7.4 (KP-buffer). After several washings in buffer the kidneys were weighed and homogenized for 10 sec in 20 vol. KP-buffer with an Ultra Turrax tissue homogenizer. The cold homogenates were filtered through a thin layer of glass wool, and the filtrates were centrifuged at 10.000 *g* and 4° for 20 min. The precipitates were discarded and the supernatants recentrifuged at 105.000 *g* and 4° for 60 min. The high speed supernatants were lyophilized and stored at −25°. Immediately before use the freeze-dried material was dissolved in ice-cold water to the original vol.

3. *15-PGDH assay.* 15-PGDH activity was measured in the high speed supernatants of kidney homogenates. The protein concentration was measured by the method of Lowry [12] and adjusted to 2.5–6.0 mg/ml with KP-buffer. 100 µl of the supernatants were then incubated for 10 min at 37° with shaking together with $[^3H]PGF_{2\alpha}$ 10^5 cpm, 5.7 µmole, 15K-$PGF_{2\alpha}$ 2 nmole, and NAD^+ 3 µmole, in a total vol of 350 µl KP-buffer. The final protein concentration was thus 0.7–1.7 mg/ml which in preliminary studies was shown to ensure a constant reaction rate for at least 10 min. Ethanol (5 vol.) were added to stop the reaction and the denatured protein was precipitated by centrifuging 5000 rev/min for 30 min at ambient temperature. The ethanolic supernatants were evaporated to dryness at 55° in a stream of nitrogen, the residues dissolved in 100 µl chloroform/methanol (1:1) and quantitatively applied to silica-coated thin layer plates with previously spotted standards of $PGF_{2\alpha}$ and 15K-$PGF_{2\alpha}$ (10 µg each). The latter was biosynthesized as described by Pace-Asciak [6]. After development in the above-mentioned solvent areas corresponding to the standards were scraped into scintillation vials and counted in 10 ml InstaGel. 15-PGDH activity was calculated by the ratio $[^3H]$15K-$PGF_{2\alpha}$/($[^3H]$15K-$PGF_{2\alpha}$ + $[^3H]PGF_{2\alpha}$) multiplied by the actual concentration of $PGF_{2\alpha}$. Activity was expressed in units of nmoles 15K-$PGF_{2\alpha}$ formed/mg protein/min.

4. *9-PGDH assay.* [9-^3H]15KD-$PGF_{2\alpha}$ was biosynthesized from [9-^3H]$PGF_{2\alpha}$ (Pace-Asciak, 6). The rate of conversion was measured in high speed supernatants of renal homogenates by incubating with labelled substrate, NAD^+, lactic dehydrogenase, and pyruvate. The ^3H-atoms were thus transferred to pyruvate ions, and since more than 99% lactate remained in the aqeous phase when extracted at pH 3.5 with ethyl acetate, the excess [^3H]15KD-$PGF_{2\alpha}$ was removed selectively after incubation. The assay tubes contained [9-^3H]15KD-$PGF_{2\alpha}$ 10.000 c.p.m., 150 pmole; 0.4–0.8 mg protein; NAD^+ 1 µmole; pyruvate 1 µmole; LDH 1.3 Units, in a final vol. of 250 µl 50 mM KH_2PO_4/NaOH, pH 7.4. Homogenate, LDH, and radiolabelled substrate were pre-incubated at 30° for 5 min prior to addition of pre-heated co-factors. The tubes were then incubated for 6 min at 30°. In preliminary experiments the reaction rate proved constant during this incubation period. The reaction was terminated by acidification to pH 3.5 with 40% trichloroacetic acid. Ethyl acetate, 2.5 vol, was then added to each tube, and after Vortex mixing for 20 sec the tubes were centrifuged. The organic layer was discarded, and the extraction repeated twice. An aliquot of the aqueous phase was finally mixed with InstaGel, and the radioactivity determined.

5. *General.* All *in vitro* experiments were performed in duplicate. Radioactivity was measured in a Beckman liquid scintillation spectrometer (LS-230). Samples were counted to a standard deviation of 0.5% or less (>40.000 counts), and quench corrections were performed by the external standard ratio method.

Results were evaluated using Student's *t*-test.

RESULTS

1. *Urinary excretion of $PGF_{2\alpha}$ in spontaneously hypertensive rats (SHR) and in normotensive rats (NTR) at different ages.* The urinary excretion of $PGF_{2\alpha}$ was significantly higher at all the ages in SHR than in NTR. Rats of both strains excreted least $PGF_{2\alpha}$-like material at 3 weeks of age. From 4 weeks the excretion increased to a plateau at 6–12 weeks and then decreased (17–21 weeks), although these

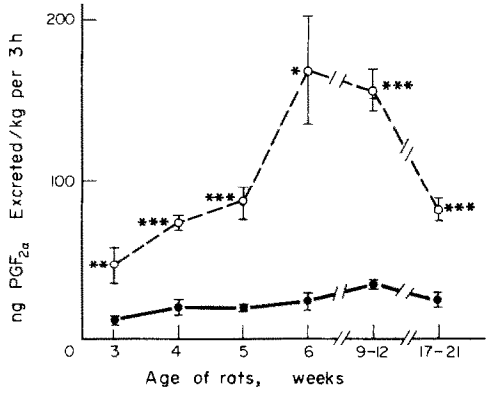

Fig. 1. Age-dependent excretion of $PGF_{2\alpha}$-like material in SHR (○---○) compared with NTR rats (●——●). Each point represents the arithmetic mean of 6–8 groups of rats with a total body weight of 200–300 g per group. Vertical bars are S.E.M. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

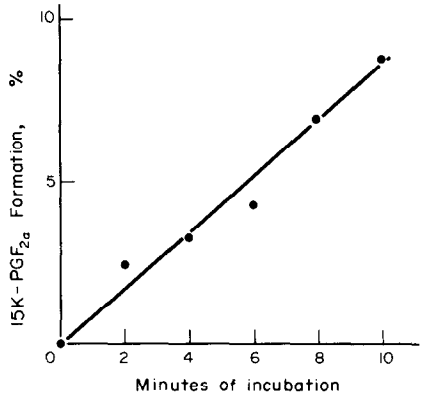

Fig. 2. Linearity of 15-PGDH assay in a typical high speed supernatant of 6 week old NTR kidney homogenate. At the times indicated samples were withdrawn for TLC analysis of [^{3}H]15K-$PGF_{2\alpha}$ formation. Each point represents the mean value of duplicate samples. For details of assay see Methods.

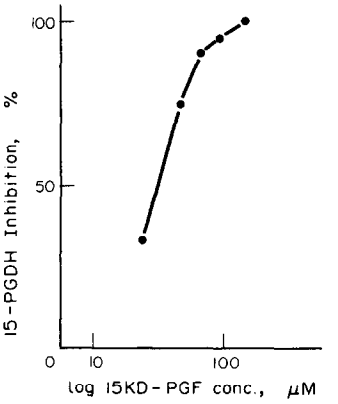

Fig. 4. Inhibition of 15-PGDH by 15KD-$PGF_{2\alpha}$. Increasing amounts of unlabelled 15KD-$PGF_{2\alpha}$ were added to the standard assay mixture devoid of unlabelled $PGF_{2\alpha}$ (see Methods), and the labelled $PGF_{2\alpha}$ and 15K-$PGF_{2\alpha}$ separated by TLC after 10 min incubation. The points are mean values of duplicate assay samples obtained with the pooled cytosol fraction of renal homogenates of 3 NTR 3 weeks old.

fluctuations were only statistically significant in SHR ($P < 0.05$ from 3–5 weeks; $P < 0.005$ from 5–12 weeks; $P < 0.001$ from 12–17 weeks) (Fig. 1).

2. *15-PGDH activity in renal homogenates of SHR and NTR.* Oxidation of $PGF_{2\alpha}$ by 15-PGDH in the high speed supernatant fraction occurred at constant rate for at least 10 min (Fig. 2). This enzyme activity decreased markedly in both SHR and NTR from 3 to 5 weeks of age. In SHR 4 weeks old the activity was significantly lower ($P < 0.05$) than in age-matched NTR. From 6 weeks the 15-PGDH activity remained almost constant. It was significantly higher ($P < 0.05$) in SHR at 6 and 17–21 weeks than in age-matched NTR (Fig. 3).

3. *Inhibition of 15-PGDH by 15KD-$PGF_{2\alpha}$.* The product of $PGF_{2\alpha}$ metabolism 15KD-$PGF_{2\alpha}$ inhibited renal 15-PGDH activity in a concentration-dependent manner (Fig. 4). The calculated IC_{50} was 32 μM.

4. *9-PGDH activity in renal homogenates of SHR and NTR.* The 9-PGDH activity has been assayed in high speed supernatants of 8 pooled kidney homogenates of SHR and NTR, 6 weeks old, and in individual kidney homogenates of SHR and NTR, 9–12 weeks old and 17–21 weeks old. As shown in Table 1 there seemed to be no great difference between the activities measured in the 6-week-old kidney pools. In adults, 9-PGDH activity is moderately but significantly less in SHR.

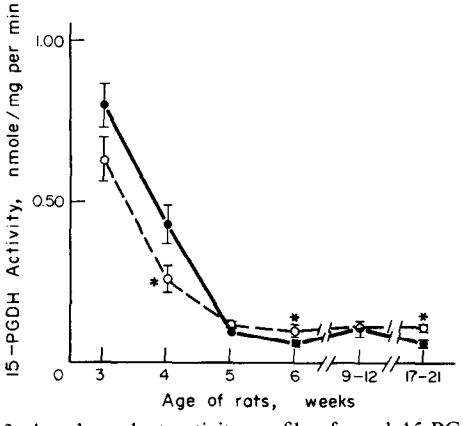

Fig. 3. Age-dependent activity profile of renal 15-PGDH activity of SHR (○---○) and NTR (●——●). Each point is the mean of 6–8 kidney homogenates, vertical bars, S.E.M. For details of assay see Methods.

DISCUSSION

It is generally accepted that urinary prostaglandins are of renal origin, since the half-life of circulating prostaglandins is less than 1 min [14] and systemically administered radiolabelled prostaglandins reach the urine already metabolized [15]. The site of entry into tubular urine of renomedullary prostaglandins is, at least in dogs, the loop of Henle [16]. We have found that the urinary $PGF_{2\alpha}$ excretion in SHR is significantly greater at all the ages examined compared to age-matched NTR. No attempt was made to quantitate urinary PGE_2 due to its relatively low stability in the urine, and it is not known whether the high levels of urinary $PGF_{2\alpha}$ in SHR reflect a higher urinary excretion of total prostaglandins.

The PGE_2 synthesis was found depressed by Sirois and Gagnon [17] in slices of papilla in Wistar Okamoto hypertensive rats and in Goldblatt hypertensive rats [17, 18]. PGE_2 and $PGF_{2\alpha}$ are formed from a common endoperoxide precursor [19]. The reduction of PGE_2 to $PGF_{2\alpha}$ by the enzyme 9-keto-prostaglandin-reductase (9-PGR) has been reported to occur in the tissue of several different mammals, including the rat [20–23]. A likely possibility is therefore the occurrence of an imbalance in the ratio PGE_2:$PGF_{2\alpha}$in the kidney of SHR.

Major defects in prostaglandin catabolism seem absent in SHR. In agreement with the findings of Armstrong *et al.* [10] we have observed normal 15-PGDH activity in the kidney homogenates of Wistar Okamoto hypertensive rats. Furthermore the age-dependent activity profile is similar to that demonstrated by Pace-Asciak [6] in Wistar normotensive rats.

Table 1. 9-PGDH activity in kidney homogenates

Age Weeks	pmoles 15KD-$PGF_{2\alpha}$ metabolized·mg protein^{-1}·min^{-1} SHR	NTR	P
6	9.48	9.26	–
9–12	6.40 ± 0.17	7.08 ± 0.29	< 0.05
17–21	6.09 ± 0.22	7.05 ± 0.24	< 0.01

High speed supernatants of kidney homogenates from 8 animals were pooled to assay the renal 9-PGDH activity in 6-week-old rats. In the adult groups the values represent the mean ± S.E.M. of 16 rats in each group.

9-PGDH, which further metabolizes 15KD-$PGF_{2\alpha}$ [24] was moderately but significantly less in adult SHR (9–12 and 17–21 weeks old) compared to age-matched NTR. 15KD-$PGF_{2\alpha}$ inhibits 15-PGDH, and might thus act as a feed-back regulatory mechanism of enzyme activity. It is doubtful whether the small decrease of 9-PGDH activity causes accumulation of 15KD-$PGF_{2\alpha}$ in adult SHR. It is more likely that an accumulation of 15KD-$PGF_{2\alpha}$ and the consequent inhibition of 15-PGDH occur due to increased $PGF_{2\alpha}$ synthesis in developing SHR.

The present investigations suggest the existence in Wistar Okamoto hypertensive rats of an inherited abnormality in renal prostaglandin metabolism different from that reported in New Zealand hypertensive rats [10]. We are now measuring the activity of the enzymes involved in renal $PGF_{2\alpha}$ formation and kidney 15KD-$PGF_{2\alpha}$ in Wistar Okamoto hypertensive rats in an attempt to clarify this difference.

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