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INCREASED $PGF_{2\alpha}$ SYNTHESIS IN RENAL PAPILLA OF SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—The increased urinary excretion of $PGF_{2\alpha}$ in Wistar–Okamoto hypertensive rats (SHR) has been confirmed using as normotensive control animals the Wistar–Kyoto rats (NTR-Kyoto). The renal cortical PGE_2-9-reductase activity (9-PGR) is lower in SHR from the fourth week of age as compared to age matched NTR. Renal papillary slices from 4–8-week-old SHR incubated in absence of exogenous arachidonic acid released more $PGF_{2\alpha}$ than did papillary tissues from age-matched NTR. Higher synthetic rate has also been found in renal papillary microsomes from SHR. The assessment of the kinetics of $PGF_{2\alpha}$ synthetase indicated higher steady state values in SHR.

In a previous paper [1] we reported that the urinary excretion of $PGF_{2\alpha}$ is significantly higher in hypertensive Wistar–Kyoto–Okamoto rats (SHR) than in normotensive COBS Wistar rats (NTR) at all the ages and even before the hypertension develops. Major defects in $PGF_{2\alpha}$ catabolism were not observed in SHR at variance with the findings of Armstrong *et al.* [2] in New Zealand hypertensive rats, thus suggesting the possible existence in SHR of an inherited abnormality in PG synthesis.

Other investigators have previously suggested that there is an increased prostaglandin synthesis in response to diverse types of experimental hypertension [3–5] including the genetic hypertension [6, 7]. Contrasting results have, however, been reported [8–10].

The purpose of the present investigation was 3-fold: (1) to investigate the intrastrain difference in urinary $PGF_{2\alpha}$ excretion using normotensive Wistar–Kyoto rats (NTR-Kyoto); (2) to evaluate the interconversion of PGE_2 to $PGF_{2\alpha}$ measuring the renal cortical 9-PGR activity, and (3) to measure $PGF_{2\alpha}$ synthesis in renal papillary slices *in vitro*.

MATERIALS AND METHODS

Animals. Spontaneously hypertensive SHR, and age-matched NTR-Kyoto or NTR of either sex were from Charles River, MA, U.S.A. and Margate, U.K. (SHR and NTR) and Møllegaards Avlslaboratorier, DK-4623 Ll. Skensved, Denmark (SHR and NTR-Kyoto).

Chemicals. PGE_2 (Unilever Research, Vlaardingen, Holland), NADPH, grade I, and glutathione, reduced form (Boehringer Mannheim GmbH, West Germany), $PGF_{2\alpha}$-radioimmunoassay kit (Clinical Assays, Inc., MA, U.S.A.), [9-^{3}H]$PGF_{2\alpha}$ (Radiochemical Centre, Amersham, U.K.) and angiotensin II (Hypertensin, Ciba–Geigy, Switzerland), arachidonic acid, grade I, *l*-epinephrine bitartrate, and bradykinin acetate (Sigma Chemical Co., MO., U.S.A.).

Arachidonic acid was stored at a concentration of 1 mg/ml in 99.9% ethanol sealed under nitrogen in a freezer (−25°). Immediately before use 30 μl of this solution was transferred to a glass tube, and the solvent was evaporated in a stream of nitrogen. Ten ml Krebs buffer was then added, and the tube was vigorously mixed to dissolve the acid.

Urinary $PGF_{2\alpha}$ excretion. The collection of urine and the urinary PGF-assay were performed as previously described [1], with the following modification: 2 vol. of 0.1 M citrate buffer, pH 3.0 was used for acidification of the samples prior to extraction.

9-PGR-assay. The activity of 9-PGR in the 105,000 *g* supernatant of renal cortex was measured by the method of Lee *et al.* [11] with the following modifications: The concentration of PGE_2 was 3.5 μg/ml, and NADPH 2.5 mM. The reaction took place in 0.2 ml 0.05 M KH_2PO_4/NaOH buffer, pH 7.4, containing 0.6 mg of supernatant protein. After appropriate dilution with gelatine-Tris buffer the $PGF_{2\alpha}$-formation was determined by radioimmunoassay.

PGS-activity in renal papillary slices. The rats were killed by decapitation and the kidneys excised and frozen in solid CO_2 within 30 sec. The organs were then slowly heated on ice so that cubes (5 · 5 · 5 mm) could be cut from the papillary–medullary sections with a razor. While still on ice the cubes were finally cut in slices 1 mm thick, washed rapidly in ice-cold 0.9% saline, and weighed.

The incubations were started by transferring the ice-cold slices to tubes containing 0.6 ml Krebs–Henseleit buffer with 1 mg/ml glucose, and maintained at 37° in an atmosphere of CO_2–O_2 (5 : 95). As indicated in the legends to the experimental data, arachidonic acid, angiotensin II, or bradykinin acetate was added to the buffer in some experiments,

Abbreviations—SHR—Spontaneously hypertensive Wistar–Okamoto rats; NTR—Charles River 'COBS' Wistar normotensive rats; NTR-Kyoto—Wistar-Kyoto normotensive rats; PG—Prostaglandin; 9-PGR—9-Keto-prostaglandin reductase; 15-PGDH—15-Hydroxy-prostaglandin dehydrogenase; PGS—Prostaglandin synthetase; RIA—Radioimmunoassay.

just as times of incubation, and changes of buffer during incubation, were varied.

Incubations were stopped by cooling the tubes to 0°, sedimenting the tissue slices by a brief centrifugation, and diluting the buffer appropriately with gelatine-Tris RIA-buffer. $PGF_{2\alpha}$ was subsequently measured by radioimmunoassay.

$PGF_{2\alpha}$ released from papillary microsomes. Renal papillae from 20 rats in each group were homogenized in 0.1 M KH_2PO_4/NaOH pH 8.0. The homogenate was centrifuged at 10,000 *g* for 20 min at 4°. The supernatant was centrifuged at 105,000 *g* for 90 min, and the pellet, containing the microsomal fraction, was lyophilized. The lyophilized powder was suspended in 150 μl of the above mentioned buffer at 1.8 mg microsomal protein/ml and incubated in the presence of *l*-epinephrine and reduced glutathione (0.5 mg/ml each) for 4 min at 37°. The suspension was then extracted three times with 3 vol. of hexane–ethyl acetate (2 : 1) to remove neutral and non-polar lipids, acidified with 6.7 vol. of 0.1 M citrate buffer, pH 3.0, and extracted three times with 1.5 vol. of ethyl acetate. The solvent was evaporated at 30° under reduced pressure, and the residue was dissolved in 10 ml gelatine-Tris buffer. This procedure yielded recoveries of $PGF_{2\alpha}$ of >90 per cent as judged from experiments with tritium-labelled tracer (not reported). The $PGF_{2\alpha}$-concentration was finally radioimmunoassayed.

$PGF_{2\alpha}$-radioimmunoassay. A commercially available kit was used for the radioimmunoassay. The kit protocol, which is a modification of the procedures of Jaffe [12], Levine [13], Caldwell [14] and Hickler [15], was followed. The recommended 1 : 2 standard serial dilutions from 2400 pg to 9.2 pg was altered to 1 : 1 serial dilutions from 2400 pg to 37.5 pg, thus providing two additional concentrations on the standard curve, and omitting the lowest recommended concentration (9.2 pg).

The standard curve fitting was provided by computer, using a log-logit transformation. As a test of goodness of fit, χ^2 was calculated for each assay. For nine consecutive assays χ^2 was 11.9 ± 1.5 (means ± S.E.M.) with twelve degrees of freedom, which indicates a satisfactory fit.

Cross-reaction with PGE, PGA, and PGB was less than 0.02 per cent, according to the antiserum manufacturer. In the case of PGE_2, this was confirmed by us at the 50 per cent binding level. Cross-reaction with $PGF_{1\alpha}$ is 42 per cent at this level, and it is therefore likely that some of the material reported as $PGF_{2\alpha}$ is in fact $PGF_{1\alpha}$, especially since the two compounds do not separate in the chromatographic system used [1]. Cross-reaction with compounds other than prostaglandins was unlikely, considering the high specificity of the antiserum, the chromatographic purification, and/or the sample dilution prior to the radioimmunoassay.

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

RESULTS

Urinary excretion of $PGF_{2\alpha}$ in SHR, NTR-Kyoto and NTR. The urinary excretion of $PGF_{2\alpha}$ in SHR has been compared in two separate experiments with that of NTR-Kyoto and NTR, respectively. The animals were 7 weeks old. As shown by the results reported in Table 1 the urinary excretion of $PGF_{2\alpha}$ is higher in SHR than in the two strains of normotensive animals. No significant difference has been observed in the $PGF_{2\alpha}$ excretion in the two separate experiments with SHR (P > 0.5). The results suggest that the excretion of $PGF_{2\alpha}$ is lower in NTR-Kyoto than in NTR, although not significantly.

Table 1. Urinary $PGF_{2\alpha}$-excretion in 6–7-week-old rats of various strains

Strain	μg $PGF_{2\alpha}$.kg^{-1}.3 hr^{-1}		P (.)
NTR-Kyoto	0.008 ± 0.004	(3)	
SHR	0.14 ± 0.03	(5)	< 0.025
NTR	0.024 ± 0.006	(3)	
SHR	0.17 ± 0.03	(9)	< 0.05

Values are means ± S.E.M. with the number of groups of rats in brackets: two rats/group (for details see Methods).
(.) Versus normotensive controls.

Table 2. Age-dependent activity profile of renal cortical 9-PGR

Age (weeks)	9-PGR activity (pg $PGF_{2\alpha}$.mg protein^{-1}.min^{-1}) NTR	SHR	P
3	72.2 ± 7.7 (6)	113.7 ± 6.1 (6)	< 0.005
4	60.1 ± 10.4 (5)	41.2 ± 4.2 (6)	N.S.
5	99.5 ± 18.9 (6)	19.7 ± 4.1 (6)	< 0.005
6	139.0 ± 19.9 (5)	54.9 ± 21.3 (5)	< 0.02
7	138.9 ± 14.5 (6)	70.9 ± 7.8 (6)	< 0.005

Values are means ± S.E.M. with number of rats in brackets.

Renal cortical 9-PGR activity in SHR and NTR. This enzyme shows in NTR an age-dependent profile different from that observed in SHR (Table 2). In NTR it increases from the fourth to sixth week, whereas in SHR it decreases markedly from the third to fifth week. From the fifth week the 9-PGR activity is significantly lower in SHR than in NTR. In earlier phases of the life it has been found to be not significantly different (4 weeks) or higher (3 weeks) in SHR than in NTR.

$PGF_{2\alpha}$ synthesis in renal papillary slices of NTR

Table 3. Renal papillary $PGF_{2\alpha}$-synthesis in the presence of 10 μM exogenous arachidonic acid (.)

Age (weeks)	pg $PGF_{2\alpha}$ formed.mg tissue^{-1}.min^{-1} NTR	SHR	P
3	45.0 ± 5.4	38.3 ± 3.4	N.S.
4	51.7 ± 4.1	43.3 ± 6.8	N.S.
5	61.7 ± 8.2	63.3 ± 4.8	N.S.
7	65.0 ± 5.4	73.3 ± 3.4	N.S.

Tubes were incubated for 1 hr with constant shaking at 37° in CO_2–O_2 (5 : 95). Values are mean ± S.E.M. of six kidney preparations in each group.
(.) Values not corrected for spontaneous production.

Table 4. Spontaneous release of $PGF_{2\alpha}$ from renal papillary slices

Age (weeks)	$PGF_{2\alpha}$-release (pg.mg tissue^{-1}.min^{-1}) at 2–5 min NTR	SHR	P	5–15 min NTR	SHR	P
4	5.8±0.9 (6)	23.2±4.4 (5)	<0.005	4.6±0.5 (6)	13.7±1.9 (5)	<0.001
5	15.2±3.0 (6)	27.6±2.5 (6)	<0.01	8.6±1.6 (6)	14.4±1.0 (6)	<0.01
6	5.0±0.7 (6)	35.9±4.4 (6)	<0.001	5.9±1.4 (6)	27.0±4.7 (6)	<0.005
8	7.6±0.8 (6)	17.3±2.0 (6)	<0.005	5.4±0.7 (6)	11.1±1.6 (6)	<0.01

At 2 min of incubation the buffer was discarded, fresh buffer being added immediately. This buffer was withdrawn at 5 min and kept for $PGF_{2\alpha}$-assay. Fresh buffer was again added, and withdrawn at 15 min for $PGF_{2\alpha}$-assay. No exogenous substrate was added in this experiment. Values are mean±S.E.M. Number of kidney preparations in brackets.

and SHR. Table 3 presents the results of experiments comparing the $PGF_{2\alpha}$ synthesis in renal papillary slices from SHR and NTR of different ages in presence of 10 μM of arachidonic acid. No difference has been observed between the two strains of rats.

In Table 4 we have reported the amounts of $PGF_{2\alpha}$ synthesized by renal papillary slices from SHR and NTR at four different ages when the addition of the exogenous substrate to the incubation medium was omitted. These values, assumed to reflect the spontaneous intrarenal synthesis of $PGF_{2\alpha}$, show a significantly higher activity in SHR at all ages, with the greatest disparity between the two strains at 6-weeks-old.

The kinetics of the $PGF_{2\alpha}$ synthetase over time in absence of exogenous substrate have been assessed in renal papillary slices obtained from 7-week-old SHR and NTR-Kyoto. A substantially greater activity has been observed in renal papillae from SHR at all points in time (Fig. 1). This finding

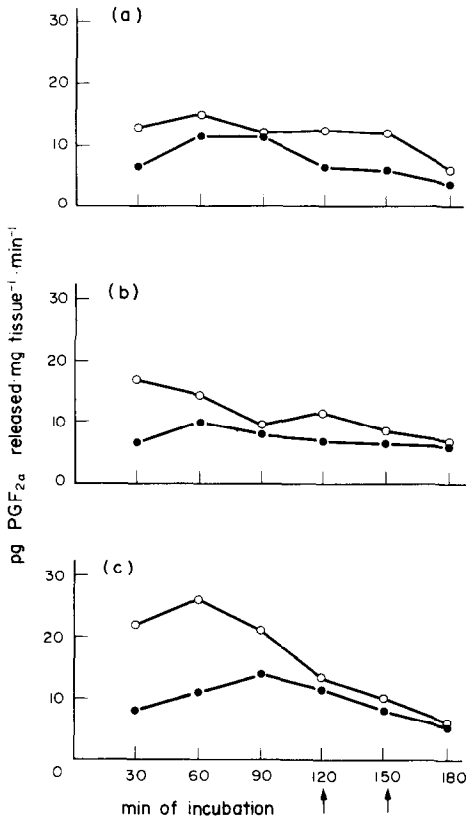

Fig. 1. Release of $PGF_{2\alpha}$ from renal papillary slices from 6–7-week-old rats after prolonged incubation at 37°, and upon addition of angiotensin II bradykinin acetate. The buffer was changed every 30 min, and angiotensin II (0.1 μg/ml) or bradykinin (1.0 μg/ml) were sometimes included in the fresh buffer added at 120 and 150 min (arrows).

A: Controls. B: angiotensin II. C: Bradykinin. Each point is the mean of two kidney preparations. Open symbols: SHR Closed symbols: NTR.

Table 5. Spontaneous release of $PGF_{2\alpha}$ from renal papillary slices after prolonged incubation

Time of incubation (min)	$PGF_{2\alpha}$-release (pg.mg tissue^{-1}.min) NTR	SHR	P
0–30	6.2±1.4 (6)	17.4±2.6 (6)	<0.005
30–60	10.8±0.7 (6)	18.4±3.2 (6)	<0.05
60–90	11.3±1.5 (6)	14.2±2.5 (6)	N.S.
90–120	8.3±1.3 (6)	12.4±0.9 (6)	<0.05
120–150	6.0–6.4 (2)	11.1–12.6 (2)	—
150–180	3.1–3.8 (2)	6.0–6.1 (2)	—

Kidney papillae from 7-week-old rats were used. Every 30 min the buffer was withdrawn for radioimmunoassay of $PGF_{2\alpha}$, and fresh buffer was added to the tissue slices. Values are mean±S.E.M. with the number of kidney preparations in brackets. At 120–150 and 150–180 min, instead of mean ±S.E.M. the range is given.

Table 6. $PGF_{2\alpha}$ in renal papillary microsomes from 7-week-old rats

Strain	ng$PGF_{2\alpha}$.mg protein^{-1}	P
NTR-Kyoto	51.2±1.6	
SHR	71.5±3.5	<0.001

Renal papillary microsomes were harvested from pooled kidneys of twenty 6–7-week-old rats in each group. The concentration of $PGF_{2\alpha}$-like material was measured in five separate experiments (each in duplicate) as described under Methods. Values are mean±S.D.

clearly indicates that the steady state values for the enzyme activity are significantly higher in SHR.

The effects of angiotensin II (0.1 μg/ml) and bradykinin (1 μg/ml) on $PGF_{2\alpha}$ release from the papilla of 7-week-old SHR and NTR-Kyoto, were assessed after four consecutive 30-min control periods. As shown in Fig. 1 (a–c), neither polypeptide was able to stimulate the prostaglandin output from SHR or NTR renal papillae. Instead the $PGF_{2\alpha}$-release decreased steadily from 120–180 min in a manner similar to the control preparations. At all times the $PGF_{2\alpha}$-release from SHR control papillae was higher than NTR (Table 5).

$PGF_{2\alpha}$ in renal papillary microsomes. We next sought to determine whether the increased output of $PGF_{2\alpha}$ from renal papillary slices of SHR was accompanied by a higher synthetic rate. We incubated renal papillary microsome suspensions from 7-week-old rats either SHR or NTR-Kyoto in absence of exogenous substrate and in presence of cofactors. As shown in Table 6 we found higher amount of $PGF_{2\alpha}$ in microsome preparations from hypertensive animals.

DISCUSSION

The higher urinary $PGF_{2\alpha}$ excretion in SHR as compared with NTR could merely reflect an inter-strain difference. The confounding effect of strain difference on biochemical measurement in studies involving spontaneously hypertensive rats is illustrated, for instance, by the investigations on urinary kallikrein excretion in SHR and various strain of normotensive rats [16, 17]. Furthermore, it has been shown that a deficiency of kidney 15-PGDH activity exists in New Zealand Wistar hypertensive rats [2], whereas the hypertension in SHR has been found not associated with decreased renal 15-PGDH activity [1, 2]. However, some other findings are at odds [18]. The use, as appropriate controls, of normotensive Wistar–Kyoto rats confirmed that the elevated excretion of $PGF_{2\alpha}$ is an inherent abnormality linked to the hypertensive condition.

The observed increase in urinary $PGF_{2\alpha}$ excretion may result from either an alteration in PGE_2 conversion to $PGF_{2\alpha}$, an increased intrarenal synthesis of $PGF_{2\alpha}$, or both. One factor influencing the prostaglandins formation is the PGE_2-9-Keto-reductase (9-PGR) which catalyses the interconversion of PGE_2 to $PGF_{2\alpha}$ [19]. An increase of 9-PGR activity may in fact shift the intrarenal prostaglandin formation from PGE_2 to $PGF_{2\alpha}$. The 9-PGR activity play a pivotal role in the regulation of NaCl and water balance [20], which is altered in SHR [17]. Disease states characterized by variations in sodium and volume homeostasis are accompanied by changes in PGE_2–$PGF_{2\alpha}$ ratio [21]. Our findings exclude the possibility that an increased conversion of PGE_2 to $PGF_{2\alpha}$ may occur in the renal cortex of SHR. The 9-PGR activity is in fact remarkably lower in SHR than in NTR from the fourth week of age. In the same period of the life we have previously observed [1] a continuous increase of $PGF_{2\alpha}$ excretion. This may perhaps suggest the existence of feedback regulatory mechanism of enzyme activity.

When the $PGF_{2\alpha}$ synthetase activity in renal papillary tissues was assessed in the presence of high substrate concentration no difference was found between SHR and NTR 3–7-weeks-old.

The results of these experiments, which measure the true synthetic capacity of renal tissue confirm previous observations of Dunn [6] in animals 1–2 months old. The same author reported that in older animals (3–7 months old) the prostaglandin synthetase activity is higher in SHR.

We found, however, that in the absence of exogenous substrate the papilla from 4–8-week-old SHR elaborate much more $PGF_{2\alpha}$ than the papillae from age matched NTR during short term incubation and that the steady state values for $PGF_{2\alpha}$ output in 7-week-old animals were higher in SHR than in NTR. We have also found a 40 per cent greater $PGF_{2\alpha}$ content in SHR papillary microsomes obtained from 7-week-old animals than in controls. It appears therefore that an increased synthesis of $PGF_{2\alpha}$ occurs in the kidney of SHR. Mandal *et al.* [22, 23] reported a decreased osmiophylic granularity in the renal medullary interstitial cells of SHR and suggested that it may reflect increased PG synthesis. Direct measurement of intrarenal prostaglandin concentration in 17-week-old SHR and NTR Kyoto showed a three times higher intrarenal $PGF_{2\alpha}$ concentration in hypertensive animals whereas PGE_2 concentrations were about the same [7].

We could not accurately measure blood pressure by the tail cuff method in 3–5-week-old animals because of their size. Direct artheial measurements by Simpson [24] show no significant difference of blood pressure between SHR and NTR at this age. This clearly indicated that alterations in PG synthesis occur before hypertension develops and that they are not a response to the progressive development of the hypertension as previously suggested [6, 25].

Prostaglandin biosynthesis depends on the availability of the substrate. It has been suggested that polypeptides, like angiotensin II and bradykinin, increase PG synthesis in isolated tissues by making more substrate available to the synthetase through an activation of intracellular phospholipase and/or acylhydrolase [26–28]. In our experimental conditions we did not find any increase of $PGF_{2\alpha}$ output from renal papillary slices of either SHR and NTR upon addition of angiotensin II or bradykinin. We were, however, measuring $PGF_{2\alpha}$ by a specific radioimmunoassay method and it has been reported that angiotensin II and bradykinin stimulation produces predominantly E-type prostaglandins [27, 28] whereas exogenous arachidonic acid is metabolized at a similar extent to both PGF- and PGE-like materials [28, 29].

It may be suggested that an increased substrate availability is the primary cause of the increased $PGF_{2\alpha}$ synthesis in SHR papillae.

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