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AGE-DEPENDENT CHANGES IN THE SYNTHESIS AND CATABOLISM OF 6 OXO PGE_1 AND OTHER PROSTANOIDS BY THE RAT KIDNEY *IN VITRO*

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Abstract—Synthesis and catabolism of 6 oxo PGE_1 was assessed in 100,000 *g* cell-free supernatant fractions of kidneys obtained from rats aged 20, 34 and 70 days. In addition the release of PGI_2, TxA_2 (measured as 6 oxo $PGF_{1\alpha}$ and TxB_2, respectively), PGE_2 and $PGF_{2\alpha}$ from kidney slices prepared from these three groups of rats was determined using specific radioimmunoassays. The conversion of PGI_2 to 6 oxo PGE_1 (but not 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to 13,14 dihydro 15 oxo PGE_2) was detected in supernatant fractions of kidneys from 20 day rats. Slices prepared from the kidneys of these animals spontaneously released significant amounts of three prostanoids (6 oxo $PGF_{1\alpha} > PGE_2 > PGF_{2\alpha} > TxB_2 = 0$). No formation of 6 oxo PGE_1 from exogenous PGI_2 was demonstrated in renal 100,000 *g* supernates from 34 and 70 day rats even though these supernates avidly oxidised 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to 13,14 dihydro 15 oxo PGE_2. In these animals the rank order of prostanoid release from kidney slices was $PGE_2 >$ 6 oxo $PGF_{1\alpha} > PGF_{2\alpha} > TxB_2 = 0$. The catabolism of 6 oxo PGE_1 is also age-dependent. In 20 and 34 day old rats 6 oxo PGE_1 and PGE_1 incubated with renal 100,000 *g* supernates undergo loss of biological activity as determined by the ability to inhibit ADP induced human platelet aggregation. In contrast, kidney 100,000 *g* supernates prepared from 70 day rats convert 6 oxo PGE_1 to an unidentified metabolite with more potent anti-aggregatory activity. The possibility that 6 oxo PGE_1 has a biological role in the developing rat kidney is discussed.

Prostacyclin (PGI_2) is converted by the enzyme 9-hydroxy prostaglandin dehydrogenase (9-PGDH) to 6 oxo prostaglandin E_1 (6 oxo PGE_1) by cell-free, 100,000 *g* supernatant fractions of homogenised human and rabbit kidney [1, 2]. Since 6 oxo PGE_1 retains considerable potency as an inhibitor of platelet aggregation [3], renal vasodilator [4] and renin secretagogue [5] it has been suggested that this prostaglandin may have a physiological role to play in renal homeostasis. Although we were recently unable to demonstrate conversion of PGI_2 to 6 oxo PGE_1 by rat kidney 100,000 *g* supernates [2] this reaction has previously been reported to occur in rat kidney *in vitro* [6]. We have, therefore, investigated the reasons for this discrepancy by studying the *in vitro* formation and catabolism of 6 oxo PGE_1 in kidneys taken from rats of different ages.

MATERIALS AND METHODS

Materials. Prostaglandin (PG) E_2, $PGF_{2\alpha}$ and thromboxane B_2 (TxB_2) were purchased from Sigma Chemical Co. (Poole, U.K.). PGI_2, 6 oxo $PGF_{1\alpha}$ and 6 oxo PGE_1 were the generous gifts of Dr. J. O'Grady and Dr. B. J. R. Whittle (both Wellcome Ltd., Beckenham, U.K.) and Dr. J. E. Pike (Upjohn Co., Kalamazoo, MI), respectively. PGI_2 was stored in 0.1 M NaOH (pH 12.0) at −20°. Ph CH 44A (homosulphasalazine) [7] was obtained from Pharmacia AB (Uppsala, Sweden). ADP, NAD^+ and $NADP^+$ were purchased from Sigma Chemical Co. (5,6,8,11,12,14,15 n-^{3}H) PGE_2 sp.act. 170 Ci/mmole, (5,6,8,9,11,12,14,15 n-^{3}H) $PGF_{2\alpha}$ sp.act. 180 Ci/mmole, (5,8,9,11,12,14,15 n-^{3}H) 6 oxo $PGF_{1\alpha}$ sp.act. 180 Ci/mmole and (5,6,8,9,11,12,14 n-^{3}H) 13,14 dihydro 15 oxo $PGF_{2\alpha}$ sp.act. 80 Ci/mmole were purchased from Amersham International U.K. (5,6,8,9,11,12,14,15 n-^{3}H) TxB_2 sp.act. 139 Ci/mmole and (9β-^{3}H) PGI_2 methyl ester, sp.act. 12 Ci/mmole were obtained from New England Nuclear Co. (Boston, MA). All organic reagents were Analar grade.

Preparation of organ slices, homogenates and supernates. Male, Sprague–Dawley rats were used in this study. Three groups of animals aged 20, 34 and 70 days were sacrificed by a blow to the head and exsanguinated. The mean body wt of animals per group (N = 15–26) was 55.4 ± 1.9 g, 142.5 ± 2.8 g and 248.0 ± 5.6 g, respectively. Kidneys were removed on ice, freed from fat and connective tissue, weighed and homogenised in 4 vol. phosphate buffer (composition mM: K_2HPO_4 40, KH_2PO_4 10, pH 7.4) using 3–4 strokes of an Ultra-Turrax homogeniser (type 18/2N). Crude homogenate was centrifuged twice at 4° (first at 3000 *g* for 10 min then at 100,000 *g* for 45 min) to prepare cytosolic, high speed supernates (HSSN's) which were stored in 1 ml aliquots at −20°. The concentration of protein in HSSN was determined by the method of Lowry *et al.* [8] using bovine serum albumin as standard. In some experiments kidneys were removed and cut longitudinally into two halves. Each kidney half was then sectioned parallel to the first cut and the outer slices (comprising mainly renal cortex) were discarded. Nor-

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mally 2 slices approx. 3 mm dia. were obtained from each kidney.

Incubation of 100,000 g supernates with prostaglandins: extraction and bioassay. The conversion of PGI_2 to 6 oxo PGE_1 was routinely assayed by a loss of radioactivity method as described elsewhere [2]. Briefly, thawed HSSN (0.2 ml) was incubated with PGI_2 (1 μg/ml) containing 0.03–0.045 μCi (9β-^{3}H) PGI_2 methyl ester at 37° for up to 120 min. Incubations also contained NAD^+ or $NADP^+$ (5 mM) or an appropriate volume of 0.9% (w/v) saline. At the end of 60 min, incubations were acidified to pH 3.4 by addition of 0.2 ml 1 M formic acid and extracted twice into 0.8 ml ethyl acetate. The combined organic phase was evaporated to dryness under a stream of air at 30° and the dried residue resuspended in 200 μl methanol, 50 μl of which was transferred to vials containing scintillant (toluene containing 0.5% 2,5 diphenyloxazole w/v 0.01% 1,4-di-2-(5 phenyloxazoyl)-benzene w/v and 30% v/v ethoxyethanol) and radioactivity counted in a Beckmann LS230 liquid scintillation counter. The formation of 6 oxo PGE_1 was calculated as the difference in extracted radioactivity of samples incubated in this way and zero time samples extracted on ice.

In some experiments dried residues obtained after extraction of incubations were resuspended in 50 μl 0.9% (w/v) saline and aliquots bioassayed for ability to inhibit ADP induced human platelet aggregation [2, 9].

The conversion of 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to the corresponding PGE_2 metabolite was also assayed as described previously [10]. Thawed HSSN (0.2 ml) was incubated with 13,14 dihydro 15 oxo $PGF_{2\alpha}$ (10 μg/ml) containing 0.07 μCi ^{3}H 13,14 dihydro 15 oxo $PGF_{2\alpha}$ and NAD^+ (5 mM) for up to 120 min at 37°. At timed intervals incubations were acidified and extracted and the dried residues resuspended in 15 μl methanol of which 5 μl was applied to silica coated, plastic-backed chromatography sheets (Kodak Ltd., type 13181) and chromatographed in solvent F6 (ethyl acetate:acetone:acetic acid, 90:10:1 v/v) in glass tanks for a distance of 10 cm. Unlabelled 13,14 dihydro 15 oxo $PGF_{2\alpha}$ ($R_f = 0.41$) and 13,14 dihydro 15 oxo PGE_2 ($R_f = 0.51$) applied to separate channels were visualised by staining in iodine. Zones corresponding to both prostaglandins were cut out, transferred to scintillation vials and radioactivity measured as described above.

The biological inactivation of PGE_1 and 6 oxo PGE_1 was determined in incubations (37°, up to 60 min) containing thawed HSSN (0.2 ml), prostaglandin (10 μg/ml) and NAD^+ (5 mM). Some incubations contained in addition to sulphasalazine analogue pH CH 44A (1–200 μM) or an appropriate volume of 0.5 (w/v) Na_2CO_3. After acidification and extraction as described above, dried residues were resuspended in 200 μl 0.9% (w/v) saline and assayed for ability to inhibit ADP induced human platelet aggregation. These standardised assays have been described elsewhere [2].

Incubation of kidney slices: radioimmunoassay. Rat kidney slices weighing 400–600 mg were incubated in 1 ml Krebs' solution (composition mM: NaCl 121, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.1, $CaCl_2$ 2.7, glucose 11.1) at 37° for 30 min. At the end of this period slices were removed, blotted and weighed and the incubation medium acidified to pH 3.4 with 1 drop of concentrated formic acid and extracted twice into 2 ml ethyl acetate. After evaporation under air at 30° dried residues were resuspended in 1 ml water and 5 μl and 10 μl aliquots radioimmunoassayed for PGE_2, $PGF_{2\alpha}$, 6 oxo $PGF_{1\alpha}$ and TxB_2 by a double antibody method [11] using antibodies kindly supplied by Dr. F. D. C. Lytton. The extraction efficiency of radiolabelled prostanoids was also determined. For these experiments 0.01 μCi of the appropriate prostanoid was added to 1 ml Krebs' solution and extracted as described above. No statistically significant differences in the percentage recovery of PGE_2 (79.2 ± 6.1%, N = 6), $PGF_{2\alpha}$ (80.2 ± 2.2%, N = 6). 6 oxo $PGF_{1\alpha}$ (81.4 ± 4.6%, N = 6) and TxB_2 (78.4 ± 4.2%, N = 6) was observed. Results are not corrected for extraction efficiency. The inter and intra (in parentheses) assay variations within these experiments were as follows, PGE_2, 8.7% (13.8%), $PGF_{2\alpha}$ 13.5% (9.6%), 6 oxo $PGF_{1\alpha}$ 21% (13.1%) and TxB_2 27% (14.2%). The cross reactivities of antibodies used in this study are shown in Table 1.

Table 1. Percent cross reactivities of prostaglandin antibodies

	Antibody			
Prostaglandin	6 oxo $PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	TxB_2
6 oxo $PGF_{1\alpha}$	100	<0.2	1.2	<2
PGE_2	6.6	100	0.4	<2
$PGF_{2\alpha}$	5.8	7.0	100	<2
TxB_2	<0.1	<0.2	<0.3	100
6 oxo PGE_1	<0.2	8.9	0.4	<2
PGD_2	<0.1	0.1	<0.2	<2

Statistics. Results show mean ± S.E. and the number of observations are given in parentheses. Statistical analysis of differences between groups was assessed using unpaired Student's *t* test. A probability (P) value of 0.05 or less was taken to indicate statistical significance.

RESULTS

Synthesis of prostanoids

The conversion of PGI_2 to 6 oxo PGE_1 following incubation with rat kidney HSSN assessed both by the loss of radioactivity method and by the appearance of an acid-stable, ethyl acetate-extracted anti-aggregatory substance is shown in Fig. 1. Both assay methods revealed that significant, time related biosynthesis of 6 oxo PGE_1 occurred in kidney HSSN prepared from 20 day old rats. The percentage conversion of PGI_2 to 6 oxo PGE_1 in the absence of added cofactors in these animals peaked after 60 min incubation (61.2 ± 1.3%, N = 8) as determined by the loss of radioactivity method. Inclusion of either NAD^+ (73.4 ± 2.1%, N = 8, $P < 0.05$) or $NADP^+$ (71.1 ± 1.1%, N = 8, $P < 0.05$) in the incubation mixture stimulated 6 oxo PGE_1 formation. No loss of extractable radioactivity or generation of anti-aggregatory activity occurred following incubation of (9β-^{3}H) PGI_2 with 34 days or 70 day rat kidney

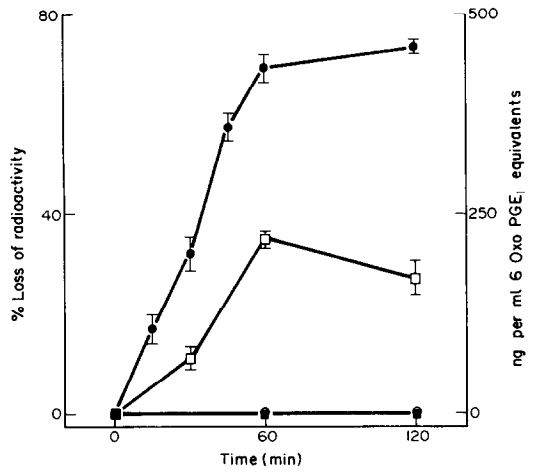

Fig. 1. Conversion of PGI_2 to 6 oxo PGE_1 by kidney 100,000 *g* supernates prepared from 20 day old rats determined by the loss of radioactivity method (●) and by the appearance of an acid-extracted, anti-aggregatory substance (□). Biological activity was measured as the ability to inhibit ADP induced human platelet aggregation and is expressed as ng/ml 6 oxo PGE_1 equivalents. Conversion of PGI_2 to 6 oxo PGE_1 assessed by the loss of radioactivity method is also shown for renal 100,000 *g* supernates prepared from 34 day (○) and 70 day (■) animals. Results show mean ± S.E.M., N = 8.

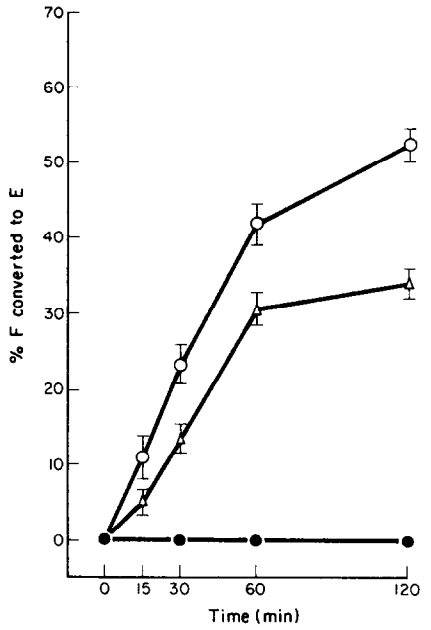

Fig. 2. Conversion of 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to 13,14 dihydro 15 oxo PGE_2 by kidney 100,000 *g* supernates prepared from 20 day (●), 34 day (△) and 70 day (○) rats. Results show mean ± S.E.M., N = 6.

HSSN in the presence or absence of added cofactors (Fig. 1), or in 20 day rat kidney HSSN (boiled for 2 min) or in 50 mM phosphate buffer (pH 7.4) (data not shown).

Markedly different results were obtained when renal 9PGDH activity was determined using 13,14 dihydro 15 oxo $PGF_{2\alpha}$ as substrate. In this case no formation of 13,14 dihydro 15 oxo PGE_2 was apparent in renal HSSN from 20 day old rats. In contrast, time-related biosynthesis of 13,14 dihydro 15 oxo PGE_2 did occur in renal HSSN from older (both 34 and 70 day) animals (Fig. 2). The percentage conversion of 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to 13,14 dihydro 15 oxo PGE_2 measured after 60 min incubation was 31.0 ± 2.6%, N = 6 and 42.9 ± 2.4%, N = 6 in kidneys from 34 and 70 day old rats, respectively.

The spontaneous release of PGE_2, $PGF_{2\alpha}$ and 6

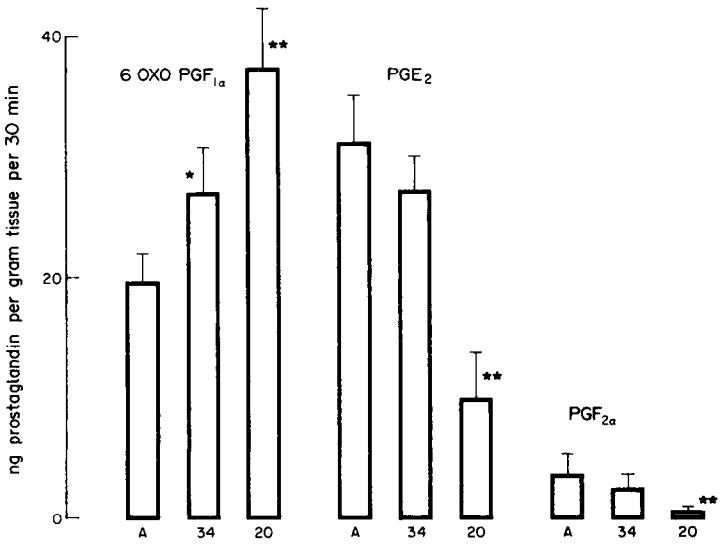

Fig. 3. Release of 6 oxo $PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ from kidney slices prepared from adult 70 day (A), 34 and 20 day old rats. Results show ng prostanoid released per g of kidney after incubation at 37° for 30 min. Results show mean ± S.E.M., N = 16. *P < 0.05, **P < 0.01 (cf. adult).

oxo $PGF_{1\alpha}$ by rat kidney slices is shown in Fig. 3. Animal age did not affect the sum total of cyclo-oxygenase products (COP) formed from kidney slices. Release of COP over the 30 min incubation period was 1.65 ± 0.2 pg/mg tissue/min, 1.89 ± 0.15 pg/mg tissue/min and 1.82 ± 0.11 pg/mg tissue/min (all N = 16) from slices prepared from 20, 34 and 70 day rats, respectively. However, the synthesis of individual prostanoids did vary from one age group to the next. In adult animals the rank order of renal prostanoid biosynthesis was PGE_2 > 6 oxo $PGF_{1\alpha}$ > $PGF_{2\alpha}$. In contrast, renal slices prepared from 20 day animals released a significantly larger amount of 6 oxo $PGF_{1\alpha}$ when compared with kidneys of adult animals. The increased formation of PGI_2 by kidney slices of immature rats probably occurs at the expense of both PGE_2 and $PGF_{2\alpha}$ significantly smaller amounts of which were released from 20 day rat kidney slices compared with adult animals. The release of TxB_2 from kidney slices of all animals was below the detection limits of the assay (<5 pg).

Catabolism of prostanoids

Incubation of 6 oxo PGE_1 with rat kidney HSSN obtained from adult (70 day) animals resulted in biological activation as evidenced by an increase in anti-aggregatory activity of incubations extracted at timed intervals for up to 120 min. In marked contrast, kidney HSSN prepared from 24 and 34 day old rats did not convert 6 oxo PGE_1 to a more biologically active metabolite. Indeed, 6 oxo PGE_1 underwent loss of anti-aggregatory activity when incubated in this way (Fig. 4). The initial rate of 6 oxo PGE_1 breakdown measured over the first 15 min incubation was 35.5 ± 0.7 ng/mg protein/min, (N = 6) and 8.9 ± 0.4 ng/mg protein/min, N = 6, in 20 and 34 day rats, respectively. The biological inactivation of 6 oxo PGE_1 incubated with rat kidney HSSN was dose dependently inhibited by Ph CH 44A, a selective inhibitor of the enzyme 15 hydroxy prostaglandin dehydrogenase. The concentration of Ph CH 44A required to produce 50% inhibition of 6 oxo PGE_1 inactivation was 10.1 ± 2.4 μM, N = 6. Additionally the catabolism of PGE_1 by rat kidney HSSN was also age-dependent (Fig. 5). Greatest biological inactivation measured by loss of anti-aggregatory activity in extracted incubate occurred in 20 days (57.8 ± 4.6 ng/mg protein/min, N = 8), followed by 34 days (21.3 ± 1.5 ng/mg protein/min, N = 8) and adult animals (12.9 ± 0.8 mg protein/min, N = 8).

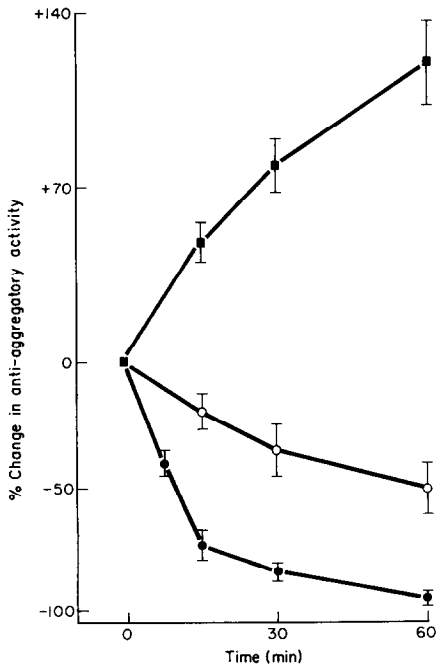

Fig. 4. Catabolism of 6 oxo PGE_1 incubated with renal 100,000 *g* supernates prepared from kidneys of 70 day (■), 34 day (○) and 20 day (●) rats. Results show the percentage change in anti-aggregatory activity in the incubation medium compared with activity in zero time samples. Mean ± S.E.M., N = 6.

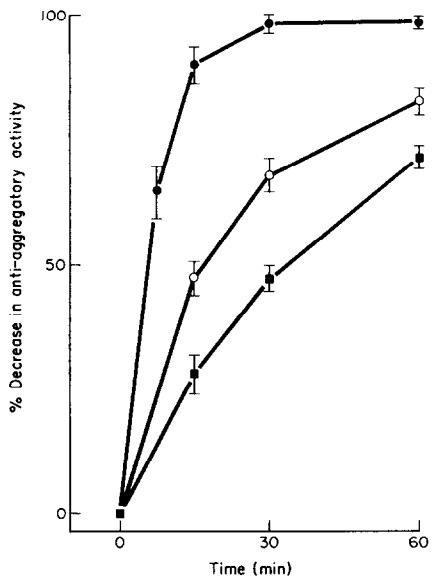

Fig. 5. Catabolism of PGE_1 incubated with renal 100,000 *g* supernates prepared from kidneys of 70 day (■), 34 day (○) and 20 day (●) rats. Results show the percentage decrease in ability to inhibit ADP induced human platelet aggregation compared with anti-aggregatory activity in zero time samples. Mean ± S.E.M., N = 8.

DISCUSSION

Age-dependent changes in the activity of prostaglandin catabolising enzymes in the rat kidney have been reported previously. The enzyme, 15 hydroxy-prostaglandin dehydrogenase (15-PGDH) which catalyses the rate-limiting step in prostaglandin inactivation shows peak activity in the kidneys of 19 day old rats and thereafter declines rapidly to reach the minimum adult concentration by day 48 [12]. In this study, 9PGDH activity (assessed using 13,14 dihydro 15 oxo $PGF_{2\alpha}$ as substrate) was not detectable in the rat kidney at 20 days of age and then progressively increased in concentration in 34 and 70 day old animals. These results confirm those of Pace-Asciak [13] who was unable to detect 9PGDH activity using

this prostaglandin as substrate in rat kidney homogenate until the animals were 19 days of age. Thereafter a gradual increase in renal enzyme activity occurred to reach adult levels by 45 days. In contrast, 9PGDH (assessed using PGI_2 as substrate) was present in kidneys from 20 day old rats and declined (not increased) as the animals matured. Indeed, we were unable to detect any conversion of PGI_2 to 6 oxo PGE_1 in kidney HSSN's prepared from 34 and 70 day old animals. Since 9PGDH isolated from several sources differs with respect to substrate and cofactor requirements and susceptibility to drugs [2, 14] it is likely that this enzyme exists in two or more isoenzyme forms with different age-dependent profiles in the developing rat kidney.

The catabolic fate of 6 oxo PGE_1 incubated with rat kidney 100,000 *g* HSSN's also depends to a large extent upon the age of the animal. We have previously reported that 6 oxo PGE_1 is converted to an unidentified metabolite which potently inhibits ADP induced human platelet aggregation following incubation at 37° with rat kidney HSSN in the presence of NAD^+ as cofactor [10]. For these experiments kidneys from adult (> 65 days) rats were used. However, in the present experiments we were unable to detect similar biological activation of 6 oxo PGE_1 following incubation with kidney HSSN prepared from immature (20 or 34 day) animals. In contrast, 6 oxo PGE_1 incubated in this way lost platelet anti-aggregatory activity. This biological inactivation was inhibited by Ph CH 44A which suggests that the enzyme 15-PGDH was responsible. The catabolism of PGE_1 was similarly age dependent in the rat kidney *in vitro*.

Since both the synthesis and catabolism of 6 oxo PGE_1 occurs in kidneys of 20 day old rats it was of interest to determine whether the kidney is capable of synthesising precursor PGI_2 in animals of this age. Interestingly, the formation of PGI_2 by kidney slices (as determined by radioimmunoassay of 6 oxo $PGF_{1\alpha}$) was at its greatest in 20 day rats and progressively declined in 34 day and 70 day animals. Thus, prostaglandin endoperoxides formed by the action of cyclooxygenase in kidneys of immature rats may be preferentially converted to PGI_2 whereas in older rats prostaglandin endoperoxide catabolism is redirected towards classical prostaglandins especially PGE_2. However, we cannot exclude the possibility that PGE_2 and $PGF_{2\alpha}$ levels in these experiments are underestimated because of the very high 15-PGDH activity in renal HSSN from 20 and 34 day rats. Similar age-dependent changes in renal PGI_2 synthesis have been reported in other species. For example, renal cortex slices prepared from foetal pigs avidly convert arachidonic acid to PGI_2 whereas formation of this prostanoid was almost undetectable in the renal cortex of adult animals. In man also, urinary excretion of 6 oxo $PGF_{1\alpha}$ (frequently used as an index of kidney PGI_2 formation) was significantly greater in the first week of life than in either 1 yr old children or in adults [16]. In contrast, our present results are diametrically opposed to the reported age dependent changes in vascular PGI_2 formation in the rat. For example, aortic PGI_2 biosynthesis in this species was low in foetal animals and began to increase only after 4 weeks to reach a maximum when the animals were 10 weeks old [17]. Similar results have been reported in spontaneously hypertensive rats [18].

We believe that these results suggest a physiological role for 6 oxo PGE_1 in the developing rat kidney. If this is the case then the inactivation of 6 oxo PGE_1 in kidney HSSN from 20 day rats (as opposed to the biological activation which occurs in adult animals) may represent the means of rapidly terminating the effect of 6 oxo PGE_1 at its site of action. The precise role of 6 oxo PGE_1 in the developing rat kidney is not clear. Two possible physiological functions may be proposed. Firstly, 6 oxo PGE_1 is a potent renal vasodilator and thus may protect the developing rat kidney from vasoconstrictor substances with otherwise could cause ischaemia and hinder proper structural development of the kidney. An alternative physiological role is suggested by a recent report [19] that 6 oxo PGE_1 induces erythropoietin formation in mice. Since the renal glomerulus is an important site for the synthesis of both erythropoietin [20] and PGI_2 [21] and the kidney is a prime site for erythropoetin production in the first few weeks of life [22] it is possible that 6 oxo PGE_1 plays a part in the elaboration of this hormone in the kidneys of immature rats.

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REFERENCES

1. A. Hassid and M. J. Dunn, in *Prostaglandins and the Kidney* (Eds. M. J. Dunn, C. Patrono and G. A. Cinotti), p. 3. Plenum Press, New York (1983).
2. R. J. Griffiths and P. K. Moore, *Br. J. Pharmac.* **79**, 149 (1983).
3. O. V. Miller, J. W. Aiken, R. J. Shebuski and R. R. Gorman. *Prostaglandins* **20**, 391 (1980).
4. C. P. Quilley, P. Y-K. Wong and J. C. McGiff, *Eur. J. Pharm.* **57**, 273 (1979).
5. E. K. Jackson, W. A. Herzer, J. B. Zimmerman, R. A. Branch, J. A. Oates and J. F. Gerkens, *J. Pharmac. exp. Ther.* **216**, 24 (1981).
6. K. R. Gans and P. Y-K. Wong, *Fed. Proc.* **40**, 681 (1981).
7. P. K. Moore, E. Ramcharan and J. R. S. Hoult, *Eur. J. Pharmac.* **61**, 287 (1980).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
9. P. K. Moore, *Thromb. Haemostas.* **47**, 76 (1982).
10. P. K. Moore and J. R. S. Hoult, *Biochem. Pharmac.* **27**, 1839 (1978).
11. K. K. Dighe, H. A. Emslie, L. K. Henderson, F. Rutherford and L. Simon, *Br. J. Pharmac.* **55**, 503 (1975).
12. C. Pace-Asciak, *Prostaglandins* **13**, 649 (1979).
13. C. Pace-Asciak, *J. biol. Chem.* **250**, 2795 (1975).
14. B. Yuan, C. L. Tai and H. H. Tai, *J. biol. Chem.* **255**, 7439 (1980).
15. N. A. Terragno, J. C. McGiff and A. Terragno, *Clin. Res.* **26**, 545 (1978).
16. B. Scherer, S. Fischer, W. Seiss and P. C. Weber, *Prostaglandins* **23**, 41 (1982).
17. H. Deckmyn, L. Font, C. van Hemelen, L. O. Carreras, G. Defreyn and J. Vermylen, *Life Sci.* **33**, 1491 (1983).
18. C. R. Pace-Asciak and M. C. Carrara, in *Advances in*

Prostaglandin and Thromboxane Research (Eds. B. Samuelson, P. W. Ramwell and R. Paoletti), Vol. 7, p. 797. Raven Press, New York (1980).
19. P. K. Nelson, J. Brookins and J. W. Fisher, *J. Pharmac. exp. Ther.* **226**, 493 (1983).
20. R. W. Busuttil, B. L. Roh and J. W. Fisher, *Proc. Soc. exp. biol. Med.* **137**, 327 (1971).
21. J. C. McGiff, *Ann. Rev. Pharm.* **21**, 479 (1981).
22. D. F. Gruber, J. R. Zucali, J. Wleklinski, V. La Russa and E. A. Mirand, *Exp. Haematol.* **5**, 399 (1977).