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Formation of 6-keto prostaglandin E_1 in mammalian kidneys

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1 The metabolism of prostacyclin (PGI_2) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) was studied in cell-free homogenates of rat, rabbit and guinea-pig kidney.

2 Rabbit kidney converted both PGI_2 and 6-keto $PGF_{1\alpha}$ to a stable metabolite with chromatographic and biological activity identical to that of authentic 6-keto PGE_1. Activity was found in the kidney cortex but not medulla, was inhibited by NAD^+ or $NADP^+$ (5 mM) and showed an optimum temperature requirement of 37°C.

3 Guinea-pig kidney converted PGI_2 but not 6-keto $PGF_{1\alpha}$ to a labile, biologically active metabolite which was not 6-keto PGE_1.

4 No conversion of prostacyclin or 6-keto $PGF_{1\alpha}$ to biologically active metabolites occurred in cell-free homogenates of rat kidney, liver and colon or guinea-pig liver and colon.

5 6-keto PGE_1 rapidly lost spasmogenic activity on the rat stomach strip following incubation with rabbit or guinea-pig kidney supernatant in the absence of added cofactors. No loss of activity occurred on incubation with rat kidney.

6 Rutin (50 μM) potently inhibited synthesis of 6-keto PGE_1 from added PGI_2 by rabbit kidney cortex. This reaction was potentiated by a similar concentration of sulphasalazine, carbenoxolone, imidazole, papaverine or indomethacin.

7 The relevance of these findings for the possible physiological and pathological roles of 6-keto PGE_1 in the kidney is discussed.

Introduction

The biotransformation of prostacyclin (PGI_2) has been studied in experimental animals (Sun, Taylor, Sutter & Weeks, 1980; Machleidt, Forstermann, Anhut & Hertting, 1981) and man (Myatt, Jogee, Lewis & Elder, 1982) but this is still incompletely understood. The first step in the metabolism of prostacyclin may be spontaneous chemical hydrolysis to 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) or alternatively enzymatic conversion by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) to yield 15-keto PGI_2. Both metabolites may be further broken down before excretion in the urine.

Recently, a novel metabolite of prostacyclin, 6-keto prostaglandin E_1 (6-keto PGE_1) has been detected in the perfusate of rabbit liver (Wong, Malik, Desiderio, McGiff & Sun, 1980b) and in incubations of human platelets (Wong, Lee, Chao, Reiss & McGiff, 1980a). The enzyme responsible, prostaglandin 9-hydroxydehydrogenase (PG-9HDH), occurs throughout the body but is present in highest concentration in kidney and liver.

We have now studied the pathway of prostacyclin and 6-keto $PGF_{1\alpha}$ metabolism in cell-free supernatants of rat, rabbit and guinea-pig kidney using a combination of biological and radiochemical assays.

Methods

Preparation of organ homogenates and supernatants

Male rats (Wistar, 200–250 g), guinea-pigs (Dunkin-Hartley, 300–500 g) and rabbits (New Zealand White, 1.5–3.5 kg) were used in this study. Animals were killed by a blow to the head and exsanguinated. Kidneys and other organs were removed, weighed and homogenized in 4 volumes (w/v) of 50 mM phosphate buffer (pH 7.4) using 3–4 strokes of an Ultra-Turrax homogenizer (type 18/2N). Rabbit kidney medulla and cortex were obtained by scissor dissection discarding overlapping regions. Homogenates were centrifuged twice at 4°C,

first at 3000 g for 10 min and the supernatant at 100,000 g for 45 min in a Beckman SS50 ultracentrifuge to prepare cell-free, high speed supernatants (HSSN) which were stored at −20°C. No loss of enzyme activity occurred in supernatants kept in this way for up to 20 days.

Incubation of supernatants with prostaglandins: extraction and thin layer chromatography

Incubations (1.0–1.5 ml) contained thawed high speed supernatant, prostacyclin, 6-keto prostaglandin $F_{1\alpha}$ (both 1 μg/ml) or 6-keto prostaglandin E_1 (10 μg/ml) and were carried out at 37°C. In some experiments incubations contained in addition 0.03–0.045 μCi [9β−^{3}H]-prostacyclin tetramethylammonium salt. In order to determine the temperature and cofactor requirements of PG-9HDH some incubations were carried out over a range of temperatures (32°C–42°C) and in the presence of NAD^+, $NADP^+$ or NADH (all 5 mM).

In separate experiments, aliquots (0.2 ml) of incubation mixture were removed on ice at the start of the incubation and at timed intervals at 37°C and extracted twice into 0.8 ml ethyl acetate after addition of 0.2 ml ethanol and acidification to pH 3.4 with 1 N formic acid. The combined organic phase was evaporated under a stream of air at 30°C and the dried residue stored at −20°C before thin layer chromatography and bioassay or determination of radioactivity as described below. For thin layer chromatography, residues were resuspended in 15 μl methanol and 5–10 μl spotted onto plastic-backed silica gel-coated thin layer chromatography sheets (Kodak, type 13181) together with 5–10 μg of authentic prostaglandin tandards. Chromatography was carried out in a Shandon Southern tank containing 100 ml of solvent F6 (composition (ml): ethyl acetate 90, acetone 10, glacial acetic acid 1). After development for a distance of 10 cm, standard prostaglandins were visualized in iodine and the chromatogram cut into 0.5–1.0 cm sections from the origin to the solvent front. Each section was eluted twice into 1 ml methanol and the combined methanol phase evaporated to dryness at 30°C. The R_F values of authentic prostaglandins in this solvent were as follows: 6-keto $PGF_{1\alpha}$ 0.18, 6-keto PGE_1 0.24, PGE_2 0.38, $PGF_{2\alpha}$ 0.15. All figures are the means of 6 observations. Residues were stored at −20°C before bioassay.

Bioassay of incubations

Biological activity was determined either by direct assay of unextracted incubate or after acidification and extraction. Samples were assayed on the rat stomach strip preparation bathed in warmed, gassed (95% O_2/5% CO_2) Krebs solution containing a mixture of antagonists to prevent the action of other spasmogens as previously described (Hoult & Moore, 1977). Incubates were also assayed for anti-aggregatory activity against ADP-induced human platelet aggregation. Blood (20 ml) was obtained by clean venepuncture from human, drug-free volunteers and anti-coagulated with 3.8% (w/v) trisodium citrate. Platelet rich and platelet poor plasma (PRP and PPP) were prepared by differential centrifugation of anti-coagulated blood (Moore, 1982). Aggregation was performed on 0.1 ml aliquots of PRP warmed to 37°C and stirred at 1100 rev/min in a Payton dual channel aggregometer (model 300BD). To determine the anti-aggregatory activity of incubates or extracts, aliquots (0.5–5.0 μl) were preincubated with PRP for 1 min before the addition of 5–10 μM ADP and the degree of inhibition determined from the control ADP response. Inhibition of ADP-induced platelet aggregation by PGI_2 or 6-keto PGE_1 was also determined and the data plotted as percentage inhibition versus log dose of prostaglandin. Both anti-aggregatory and spasmogenic activity of incubates or extracts were assayed against authentic PGI_2 unless otherwise stated.

Conversion to prostaglandin E compounds: loss of radioactivity method

Metabolism of [9β−^{3}H]-PGI_2 to non-radioactive 6-keto PGE_1 was determined as previously described for the conversion of $PGF_{2\alpha}$ to PGE_2 (Hoult & Moore, 1977). Briefly, extracts were resuspended in 200 μl methanol and 20 μl transferred to vials containing 7 ml scintillant and counted in a Beckman LS230 liquid scintillation counter. The counts obtained were corrected for background and quenching as appropriate, compared with those of the zero time sample and the difference taken as the amount of PGI_2 converted to 6-keto PGE_1 (or metabolites).

Effect of drugs

Several drugs were tested for their effect on rabbit renal PG-9HDH. Formation of 6-keto PGE_1 was detected by the loss of radioactivity method, following incubation at 37°C for 150 min. Drugs used were sulphasalazine, carbenoxolone, rutin, indomethacin, papaverine and imidazole (all 50 μM).

Statistics

Results show mean ± s.e.mean. Number of observations are in parentheses. Statistically significant differences between groups was determined using Student's t test. A probability (P) value of 0.05 or less was taken to indicate statistical significance.

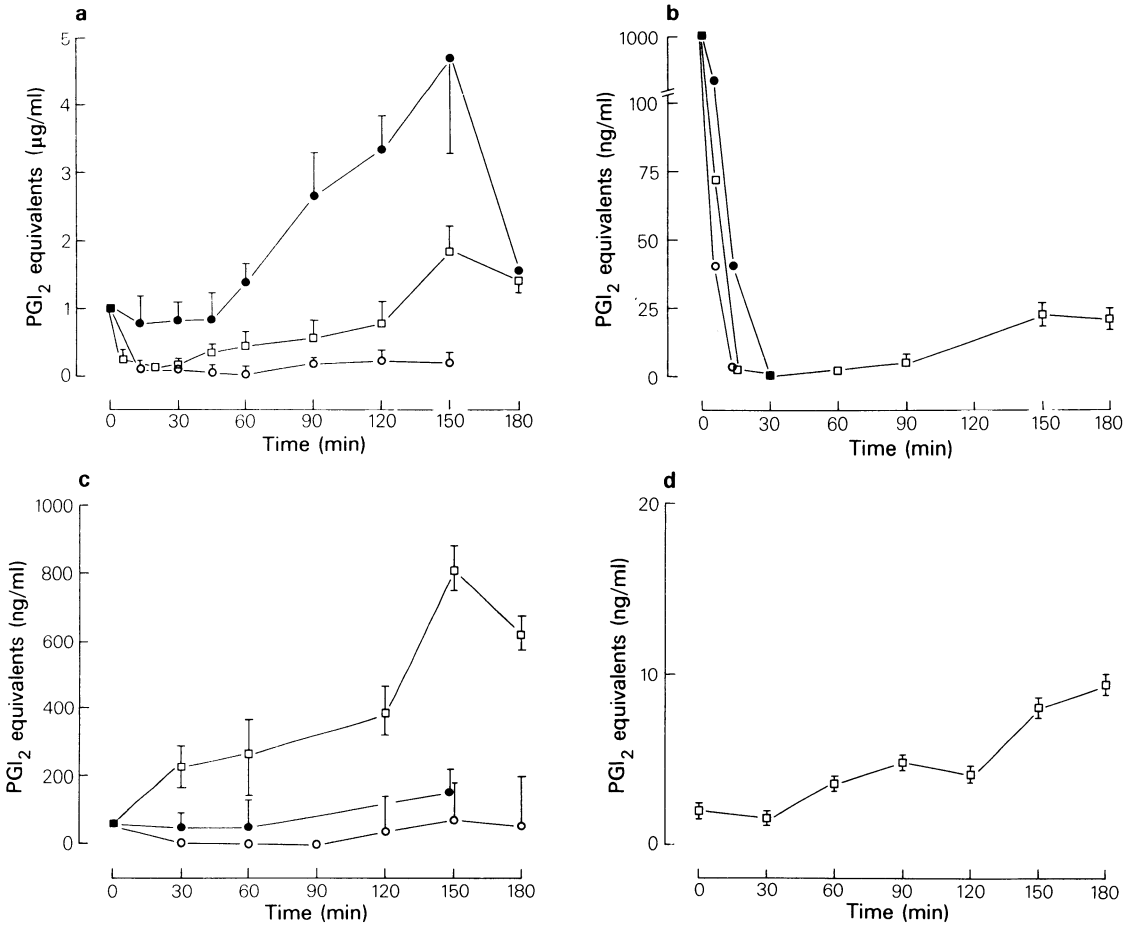

Figure 1 Incubation of 1 μg/ml PGI_2 (a,b) or 6-keto $PGF_{1\alpha}$ (c,d) with rat (○), rabbit (□) and guinea-pig (●) kidney 100,000 g supernatant. Spasmogenic activity on the rat stomach strip (a,c) and anti-aggregatory activity againt ADP-induced human platelet aggregation (b,d) were assayed against authentic PGI_2. $n = 7-14$ (spasmogenic assay) and 4–8 (anti-aggregatory assay). All values are corrected for endogenous spasmogenic activity in the renal supernatants.

Materials

Prostacyclin sodium salt was obtained from Wellcome Ltd and stored in 0.01 N NaOH (pH 12) at −20°C. 6-keto $PGF_{1\alpha}$ (Wellcome Ltd) and 6-keto PGE_1 (Upjohn Co.), PGE_2 and $PGF_{2\alpha}$ (Sigma) were dissolved in ethanol at −20°C. [9β−^{3}H]-prostacyclin tetramethylammonium salt (sp. act. 12 Ci/mmol) was obtained from New England Nuclear. Papaverine, indomethacin, rutin and imidazole (Sigma), sulphasalazine (Pharmacia Ltd) and carbenoxolone (May and Baker Ltd) were dissolved in 0.5% sodium carbonate solution. ADP, NAD^+, $NADP^+$ and NADH were purchased from Sigma. All organic reagents were analytical grade.

Results

Bioassay of renal supernatants

Aliquots (20–50 μl) of high speed supernatant of rat, rabbit and guinea-pig kidney contracted the rat stomach strip. Spasmogenic activity determined as μg PGI_2 equivalents/ml was 0.57 ± 0.09, $n = 10$; 0.34 ± 0.067, $n = 11$ and 1.97 ± 0.19, $n = 13$ respectively. No change in spasmogenic activity was detected in supernatant incubated for 150 min (0.66 ± 0.08, $n = 6$; 0.32 ± 0.07, $n = 6$; 1.79 ± 0.24, $n = 7$ respectively). Neither rat nor guinea-pig renal supernatant showed anti-aggregatory activity (<0.01 ng/ml PGI_2 equivalents). Rabbit kidney

supernatant inhibited ADP-induced platelet aggregation (2.1 ± 0.3 ng/ml PGI_2 equivalents, $n = 6$). No change in anti-aggregatory activity was observed following incubation at 37°C for 150 min (2.21 ± 0.46 ng/ml PGI_2 equivalents, $n = 6$) and no anti-aggregatory activity was detected in samples incubated for 150 min and then acidified and extracted.

Incubation with PGI_2 and 6-keto $PGF_{1\alpha}$

The time course of PGI_2 and 6-keto $PGF_{1\alpha}$ metabolism assayed by parallel bioassay on the rat stomach strip and against ADP-induced platelet aggregation is shown for rat, rabbit and guinea-pig in Figure 1.

(i) *Rat* There is a good correlation between the anti-aggregatory and spasmogenic bioassays in rat kidney. Biological activity delined rapidly ($T_{\frac{1}{2}} = 4.9 \pm 0.4$ min, $n = 7$ assayed on rat stomach strip and 5.1 ± 0.3 min, $n = 8$ assayed by inhibition of platelet aggregation). Similar results were obtained when prostacyclin was incubated in 50 mM phosphate buffer ($T_{\frac{1}{2}} = 3.6 \pm 0.72$ min, n =4 and 4.6 ± 0.28 min, $n = 6$ respectively). No change in spasmogenic or generation of anti-aggregatory activity occurred on incuation of rat kidney supernatant with 6-keto $PGF_{1\alpha}$ for up to 60 min. The spasmogenic activity of 6-keto PGE_1 was unaltered following incubation for 60 min with rat renal supernatant.

(ii) *Guinea-pig* A similar profile of biological activity was not observed in guinea-pig kidney supernatant incubated with PGI_2. Instead spasmogenic activity declined at a slower rate over the first 45 min (0.81 ± 0.35 μg/ml PGI_2 equivs, $n = 7$) and then increased rapidly to reach a peak after 150 min (4.7 ± 1.5 μg/ml, $n = 7$). In contrast, anti-aggregatory activity of added PGI_2 declined more rapidly ($T_{\frac{1}{2}} = 14.6 \pm 1.2$ min, $n = 6$) and was undetectable (<0.001 ng/ml PGI_2 equivs) after 10 min. Some return of anti-aggregatory activity was detected towards the end of the incubation period (30 ± 9.6 ng/ml PGI_2 equivs, $n = 5$, at 120 min). No increase in spasmogenic or return of anti-aggregatory activity occurred in boiled (100°C, 1 min) supernatant incubated with PGI_2 or in incubations containing 6-keto $PGF_{1\alpha}$. Spasmogenic activity of 6-keto PGE_1 was reduced by $53.5 \pm 4.9\%$, $n = 8$ following incubation in guinea-pig renal supernatant for 60 min.

(iii) *Rabbit* Spasmogenic and anti-aggregatory activity of PGI_2 incubated with rabbit kidney supernatant declined gradually with $T_{\frac{1}{2}}$ values of 3.9 ± 0.2 min, $n = 7$ and 3.6 ± 0.2 min, $n = 6$ respectively. Spasmogenic activity began to increase again after 60 min and reached a peak after 150 min (1.86 ± 0.4 μg/ml PGI_2 equivs, $n = 7$, cf. 0.91 ± 0.23 μg/ml PGI_2 equivs, $n = 16$ at $T = 0$, $P < 0.05$). Aliquots of incubate removed at 150 min also inhibited ADP-induced platelet aggregation (22 ± 8 ng/ml PGI_2 equivs, $n = 4$, cf. 1220 ± 20 ng/ml PGI_2 equivs, $n = 5$ at $T = 0$, figures corrected for basal anti-aggregatory activity). Unlike rat and guinea-pig, rabbit kidney supernatant converted 6-keto $PGF_{1\alpha}$ to a metabolite with spasmogenic and anti-aggregatory activity. Assayed on the rat stomach strip, aliquots removed after 150 min contained 815.5 ± 64.7 ng/ml PGI_2 equivs, $n = 6$ compared with 95.5 ± 38.7 ng/ml PGI_2 equivs, $n = 6$ at the start of the incubation. Similarly, incubates contained increased anti-aggregatory activity (9.8 ± 0.1 ng/ml PGI_2 equivs, $n = 6$ cf. 2.0 ± 0.15 ng/ml PGI_2 equivs at $T = 0$, $P < 0.01$). The spasmogenic activity of 6-keto PGE_1 incubated with rabbit kidney supernatant declined by $22 \pm 3.6\%$, $n = 8$ after 60 min.

Conversion of prostacyclin to E-type prostaglandins by loss of radioactivity method

The oxidation of [9β-^{3}H]-PGI_2 to PGE derivatives by renal supernatants prepared from rat, rabbit and guinea-pig is shown in Figure 2. No reduction in extracted radioactivity was detected for up to 150 min in rat or guinea-pig; in contrast, radioactivity in extracts of rabbit renal high speed supernatants progressively decreased on incubation.

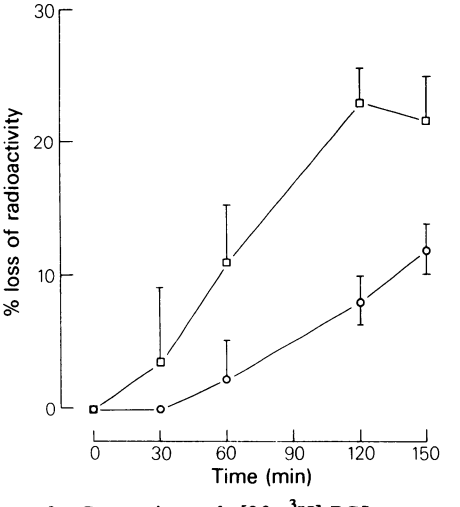

Figure 2 Conversion of [9β-^{3}H]-PGI_2 to non-radioactive 6-keto PGE_1 by the loss of radioactivity method. Aliquots removed from the incubation at timed intervals were acidified, extracted,resuspended in methanol and a small volume counted for radioactivity. Results show percentage reduction in radioactivity following incubation at 37°C compared with a sample extracted on ice at zero time. Rabbit whole kidney (○), rabbit kidney cortex (□), $n = 6$–12. No loss of radioactivity was observed in incubations containing rabbit kidney medulla or rat or guinea-pig kidney supernatant.

Identification of 6-keto PGE_1

Aliquots removed after 150 min incubation of rabbit and guinea-pig kidney supernatant in the presence of PGI_2 were extracted and subjected to thin layer chromatography. No anti-aggregatory and only small amounts of spasmogenic (< 1 ng/ml PGI_2 equivs) were detected on chromatograms of extracted guinea-pig kidney incubations. Activity on the rat stomach strip was confined to zones which co-chromatographed with PGE_2 and $PGF_{2\alpha}$. No such activity was found in the 6-keto PGE_1 zone. In contrast, both anti-aggregatory (12.4 ± 2.6 ng 6-keto PGE_1 equivs, $n = 6$) and spasmogenic (20.0 ± 3.8 ng 6-keto PGE_1 equivs, $n = 6$) activity was detected in the 6-keto PGE_1 zone of extracts of rabbit kidney supernatant incubated with PGI_2.

Effects of temperature and cofactors

All cofactors tested inhibited the conversion of PGI_2 to 6-keto PGE_1 by rabbit renal PG-9HDH estimated by the loss of radioactivity technique (Figure 3a). Maximum enzyme activity occurred at 37°C (Figure 3b).

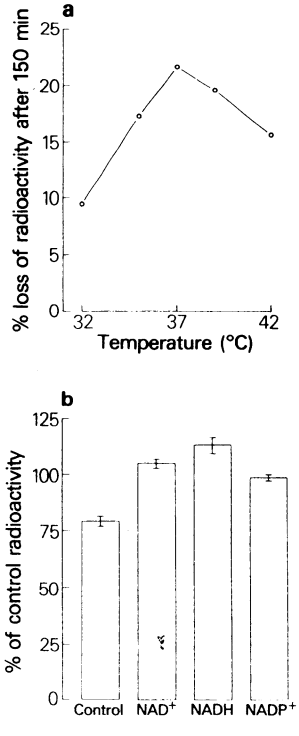

Figure 3 (a) Effect of temperature on conversion of PGI_2 to 6-keto PGE_1 by rabbit kidney cortex 100,000 g supernatant, measured by the loss of radioactivity method. $n = 8-12$. (b) Effect of nicotinamide cofactors (all 5 mM) on conversion of PGI_2 to 6-keto PGE_1 by rabbit kidney supernatant, measured by the loss of radioactivity method. $n = 8-12$.

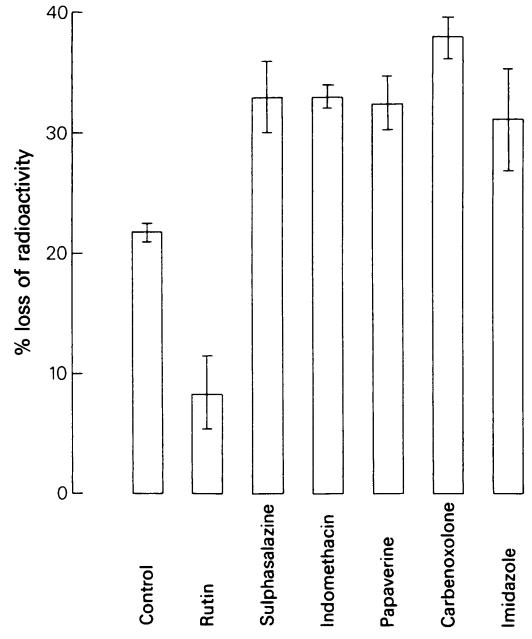

Figure 4 Effect of drugs on conversion of PGI_2 to 6-keto PGE_1 measured by the loss of radioactivity method. Results show percentage reduction in radioactivity following incubation at 37°C compared with a sample extracted on ice at time zero. Concentration of each drug was 50 μM, $n = 8$.

Organ distribution of PG-9HDH

Conversion of PGI_2 to 6-keto PGE_1 was detected in whole rabbit kidney and cortex but not in medulla (Figure 4). No formation of 6-keto PGE_1 was observed in high speed supernatants of rat or guinea-pig colon or liver (data not shown).

Effect on rabbit renal PG-9HDH

Sulphasalazine, indomethacin, papaverine, carbenoxolone and imidazole potentiated 6-keto PGE_1 formation estimated by the loss of radioactivity method by 44.0–81.0% Rutin inhibited rabbit renal PG-9HDH activity by 62.0 ± 15.0, $n = 8$.

Discussion

Prostacyclin is converted to 6-keto PGE_1 by PG-9HDH. This reaction was first demonstrated in the

perfused rabbit liver (Wong *et al.*, 1980b) and has since been shown to occur in human platelets (Wong *et al.*, 1980a; Hoult, Lofts & Moore, 1981) and homogenates of rat (Gans & Wong, 1981), rabbit (McGiff, Spokas & Wong, 1982) and pig (Chang & Tai, 1982) kidney. Renal PG-9HDH from different species show different substrate specificity. For example rabbit kidney converts $PGF_{2\alpha}$ directly to PGE_2 (Moore & Hoult, 1978) whilst rat kidney preferentially oxidizes 13,14-dihydro-15-keto $PGF_{2\alpha}$ to its corresponding PGE derivative and guinea-pig kidney lacks this enzyme (Hoult & Moore, 1977). Human platelet PG-9HDH may utilize 6-keto $PGF_{1\alpha}$ but not $PGF_{2\alpha}$ or its metabolites as substrate (Wong *et al.*, 1980a). Most authors have used 6-keto $PGF_{1\alpha}$ and not PGI_2 as substrate, despite reports that at least one source of PG-9HDH, in intact platelets, converts PGI_2 but not 6-keto $PGF_{1\alpha}$ to 6-keto PGE_1 (Hoult *et al.*, 1981). In these experiments we have studied the metabolism of both PGI_2 and 6-keto $PGF_{1\alpha}$ in renal high speed supernatants of three species using biological and radiochemical techniques.

Biological activity of PGI_2 incubated with rat kidney supernatant declined rapidly at a rate similar to that observed in phosphate buffer. No conversion of PGI_2 to 6-keto PGE_1 was detected by the loss of radioactivity method. These results are in agreement with those of Yaun, Tai & Tai (1980) who failed to observe 6-keto PGE_1 synthesis from 6-keto $PGF_{1\alpha}$ by rat kidney using a radoimmunoassay technique. In contrast, Gans & Wong (1982) demonstrated 6-keto PGE_1 formation in renal homogenates of normotensive and hypertensive rats using a loss of radioactivity method similar to that used in this study. The reasons for these discordant results are not clear but may be due to differences in animal age or strain or the use of assay procedures.

PGI_2 was converted to a biologically active metabolite by guinea-pig kidney. Several lines of evidence suggest that this metabolite was not 6-keto PGE_1. For instance, no biological activity was detected after acidification and extraction of guinea-pig kidney supernatant incubated with PGI_2. Furthermore, no reduction in extracted radioactivity was observed in such incubations and 6-keto $PGF_{1\alpha}$ was not converted to biologically active metabolites. The identity of the biologically active metabolite of PGI_2 remains unknown. It is possible that guinea-pig kidney metabolizes PGI_2 via 15-PGDH and prostaglandin $\Delta 13$ reductase to 13,14 dihydro PGI_2 which does exhibit anti-aggregatory and vasodilator activity (Whittle, Moncada & Vane, 1981). A similar sequence of enzymatic steps converts $PGF_{2\alpha}$ to 13,14 dihydro $PGF_{2\alpha}$ in guinea-pig kidney (Hoult & Moore, 1977). In the absence of authentic 6-keto $PGF_{1\alpha}$ metabolites, it was not possible to identify this metabolite.

In rabbit kidney, both PGI_2 and 6-keto $PGF_{1\alpha}$ were converted enzymatically to a metabolite with chromatographic and biological properties indentical to that of 6-keto PGE_1. Synthesis of 6-keto PGE_1 occurred in rabbit kidney cortex but not medulla or rat or guinea-pig colon and liver. Surprisingly, inclusion of oxidized nicotinamide cofactors (NAD^+ or $NADP^+$) not only failed to stimulate but actually inhibited PG-9HDH activity possibly by increasing conversion of PGI_2 to its 15-keto metabolite thereby reducing the amount of available precursor.

The results obtained in this study may be complicated by the rapid inactivation of newly formed 6-keto PGE_1 in renal supernatants. 6-keto PGE_1 assayed on the rat stomach strip was substantially inactivated after 60 min incubation in guinea-pig and rabbit kidney supernatant whereas little or no breakdown occurred in rat kidney. These results are also of interest since other authors have failed to detect pulmonary metabolism of this prostaglandin *in vivo* (Quilley, Wong & McGiff, 1979; Hyman & Kadowitz, 1980). Thus 6-keto PGE_1 like PGI_2 may not be a substrate for the pulmonary uptake mechanism *in vivo*.

We have also determined the effect of several drugs on synthesis of 6-keto PGE_1 from PGI_2 by rabbit kidney cortex. Sulphasalazine and carbenoxolone elevated 6-keto PGE_1 formation presumably by inhibiting renal 15-PGDH and increasing the amount of available PGI_2 precursor. Papaverine, although not an inhibitor of 15-PGDH, also stimulated 6-keto PGE_1 synthesis probably by direct activation of PG-9HDH as shown previously (Moore, 1979). Quercetin 3β rutinoside (rutin) was a potent inhibitor of 6-keto PGE_1 formation and may prove a useful experimental tool to study the physiological and pathological role of this prostaglandin in the body.

The pharmacological activity of 6-keto PGE_1 has been intensively studied in the last few years. Like PGI_2, 6-keto PGE_1 inhibits platelet aggregation (Miller, Aiken, Shebuski & Gorman, 1980), reduces blood ressure in rats (Quilley et al., 1979), dogs (Nandiwada, Hyman, Feigen & Kadowitz, 1980) and cats (Lippton, Chapnick, Hyman & Kadowitz, 1980), decreases airway resistance (Spannhake, Levin, Hyman & Kadowitz, 1981) and contracts gastrointestinal smooth muscle (Whittle *et al.*, 1981, Griffiths, Lofts & Moore, 1982). However, it has yet to be conclusively determined whether 6-keto PGE_1 occurs naturally *in vivo*. Recently, we have shown spontaneous release of a 6-keto PGE_1-like substance from non-aggregating human platelets (Lofts & Moore, 1982) which was considerably reduced, compared with age- and sex-matched controls, in platelets from patients with diabetes mellitus (Moore, Lofts, Keen & Salter, 1982). This deficiency may, at least in part, underly the elevated platelet reactivity and increased risk of thrombotic disease

observed in diabetes. Moreover, Clive, Leone, MacIntyre, Brown, Salzman & Stoff (1981) have demonstrated abnormally high amounts of 6-keto PGE_1 in plasma from patients with Barrter's syndrome, which may contribute to the platelet hypoaggregability common in this condition. Thus, 6-keto PGE_1 may play a part in regulating platelet reactivity in health and disease. Furthermore, if 6-keto PGE_1 is synthesized in the kidney *in vivo* then it may have a role to play in regulating vascular resistance and renin release in this organ. Before the possible physiological and pathological roles of 6-keto PGE_1 can be fully elucidated it is important to determine which organs have the capability for the synthesis and breakdown of this prostaglandin and to characterize further the enzyme responsible. Such experiments are now under way in this laboratory.

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