Br. J. Pharmac. (1983), **80**, 395–402

Conversion of prostacyclin to 6 oxo prostaglandin E_1 by rat, rabbit, guinea-pig and human platelets

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1 The enzymatic catabolism of prostacyclin (PGI_2) to 6 oxo prostaglandin E_1 (6 oxo PGE_1) was studied in platelet-rich and platelet-poor-plasma of rat, rabbit, guinea-pig and man.

2 Rat, rabbit and human platelets convert PGI_2 to a product with biological activity and thin layer chromatographic mobility identical to that of authentic 6 oxo PGE_1. Platelets from these species also converted 9β-[^{3}H]-PGI_2 to non-radioactive 6 oxo PGE_1 as shown by the progressive loss of extracted radioactivity following incubation. Formation of 6 oxo PGE_1 was inhibited by the flavonoid drugs, rutin and naringenin.

3 Guinea-pig platelets did not convert PGI_2 to 6 oxo PGE_1.

4 Rat, rabbit and guinea-pig platelets do not spontaneously release a 6 oxo PGE_1-like substance when incubated at 37°C in the absence of added PGI_2 or aggregating agents.

5 The relevance of these findings to the possible physiological and pathophysiological roles of 6 oxo PGE_1 in the regulation of platelet function is discussed.

Introduction

Prostacyclin (PGI_2) is enzymatically converted to 6 oxo prostaglandin E_1 (6 oxo PGE_1) by a prostaglandin 9-hydroxydehydrogenase (PG-9HDH) enzyme found in man (Hassid, & Dunn, 1982), rat (Gans & Wong, 1981), rabbit (Griffiths & Moore, 1983b) and pig (Chang & Tai, 1982) kidney, rabbit liver (Wong, Lee, & Weiss & McGiff, 1980) and human platelets (Hoult, Lofts & Moore, 1981). Unlike other metabolites of PGI_2, 6 oxo PGE_1 has considerable vasodilator (Quilley, Wong & McGiff, 1979), anti-aggregatory (Miller, Aiken, Shebuski & Gorman, 1980) and bronchodilator (Spannhake, Levin, Hyman & Kadowitz, 1981) activity, is a potent spasmogen on gastrointestinal smooth muscle (Griffiths, Lofts & Moore, 1982), induces renin secretion (McGiff, Spokas & Wong, 1982) and inhibits noradrenaline release from sympathetic nerve endings (Griffiths & Moore, 1983a). Although there is little doubt that 6 oxo PGE_1 has potent biological activity in many assay systems, its biosynthesis from PGI_2 *in vivo* has yet to be demonstrated conclusively. Jackson, Goodwin, Fitzgerald, Oates & Branch (1982) have shown the presence of 6 oxo PGE_1 in human plasma at a concentration (about 30 pg ml^{-1}) in excess of the normal plasma concentration of the chemical hydrolysis product of prostacyclin namely 6 oxo prostaglandin $F_{1\alpha}$ (6 oxo $PGF_{1\alpha}$, about 3 pg ml^{-1}; Blair, Barrow, Waddel, Lewis & Dollery, 1982). Furthermore, large amounts of 6 oxo PGE_1 (> 100 pg ml^{-1}) have been detected in plasma of patients with Bartter's syndrome, a condition characterized clinically by hyperaldosteronism and defective platelet aggregation (Stoff, Clive, Leone, MacIntyre, Brown & Salzman, 1983).

The possibility that 6 oxo PGE_1 may have a role to play in the regulation of platelet reactivity is also suggested by recent work from this laboratory. Human platelets both convert PGI_2 to 6 oxo PGE_1 (Wong, Lee, Chao, Reiss & McGiff, 1980; Hoult, *et al.*, 1981) and spontaneously release a 6 oxo PGE_1-like substance when incubated in plasma at 37°C in the absence of an aggregating agent (Lofts & Moore, 1982). In order to determine whether platelets from other species share these properties we have studied the spontaneous release of 6 oxo PGE_1 and its formation from added PGI_2 by platelets from rat, rabbit and guinea-pig.

Methods

Preparation of plasma

Male rats (Wistar, 200–250 g), rabbits (New Zealand White, 1.5–2.5 kg) and guinea-pigs (Dunkin-Hartley, 400–500 g) were used in this study. Human

(from male volunteers aged 20–30 years) and rabbit blood (both 20 ml) were obtained by venepuncture of the antecubital and marginal ear vein respectively. Guinea-pig blood (10 ml) was obtained from pentobarbitone-anaesthetized animals (40 mg kg^{-1} i.p.) by cardiac puncture while rats were anaesthetized with ether and blood (10 ml) removed by inserting a fine needle into the abdominal aorta. Blood from all species was anticoagulated (1:9 v/v) with 3.8% (w/v) trisodium citrate and centrifuged twice at room temperature (200 *g* for 20 min, 1,000 g for 10 min) to prepare platelet-rich-plasma (PRP) and platelet-poor plasma (PPP) respectively. Both PRP and PPP were stored at room temperature until required. All human volunteers denied taking aspirin-like drugs in the two week period before the experiment.

Conversion of prostacyclin to 6 oxo PGE_1: extraction, chromatography and bioassay

Human, rat, rabbit or guinea-pig PRP or PPP (1.0 ml) were incubated at 37°C with PGI_2 (0.1–1.0 μg ml^{-1}). At timed intervals, aliquots (1–50 μl) were removed and bioassayed without further extraction ('live assay') for spasmogenic activity on the rat isolated stomach strip preparation and for anti-aggregatory activity against ADP-induced human platelet aggregation as described previously (Hoult & Moore, 1977; Griffiths & Moore, 1983a, b). The rat stomach strip was chosen since 6 oxo PGE_1 is approximately 3 times more potent than PGI_2 on this preparation (Griffiths, Lofts & Moore, 1982).

In some experiments, aliquots (0.2 ml) of the incubation mixture were removed on ice and at timed intervals at 37°C and extracted twice into 0.8 ml ethyl acetate after acidification to pH 3.4 with 1N formic acid. The combined ethyl acetate phase was evaporated to dryness at 30°C and the residue resuspended in 25 μl Krebs solution for immediate bioassay or alternatively in 20 μl methanol for thin layer chromatography. In the latter case, 5–10 μl extracts were applied to plastic-backed, silica gel coated thin layer chromatography sheets (Kodak Ltd., type 13181) together with 5–10 μg authentic prostaglandin standards and chromatography carried out in a Shandon Southern tank containing 100 ml of solvent F6 (ethyl acetate:acetone:acetic acid, 90/10/1, v/v). After development to a distance of 10 cm the chromatogram was cut into 10 × 1 cm sections from the origin to the solvent front and each section eluted twice into 1 ml methanol. The combined phase was evaporated to dryness at 30°C under a stream of air and the residue resuspended in 25 μl Krebs solution for bioassay as described above. The R_F values of prostaglandin standards in solvent F6 are as follows: $PGF_{2\alpha}$ 0.15, 6 oxo $PGF_{1\alpha}$ 0.19, 6 oxo PGE_1 0.25, PGE_2 0.38, PGD_2 0.41 (all means of 6 observations).

Conversion of prostacyclin to 6 oxo PGE_1; loss of radioactivity method

Some incubations contained in addition 0.05 μCi [9β-^{3}H]-PGI_2. Aliquots of the incubation were removed at timed intervals at 37°C, acidified and extracted as above. The dried residues were resuspended in 200 μl methanol of which 20 μl were transferred to scintillation vials containing 7 ml scintillant and counted in a Beckman LS 230 liquid scintillation counter. Counts obtained were corrected for background and quenching as appropriate and compared with those of the zero time sample (removed on ice). The reduction in extracted radioactivity following incubation reflects conversion of radioactive PGI_2 to non-radioactive 6 oxo PGE_1 (or its metabolites) (Hoult & Moore, 1977; Griffiths & Moore, 1983 a, b).

Spontaneous release of 6 oxo PGE_1 from platelets

The methods used to assess the spontaneous release of 6 oxo PGE_1 from non-aggregating platelets have been described in detail elsewhere (Lofts & Moore, 1982). Briefly, PRP (1.5 ml) was incubated at 37°C in the absence of added PGI_2. Aliquots (50–100 μl) were transferred without extraction to organ baths containing rat isolated stomach strip preparations bathed in Krebs solution containing a mixture of antagonists to prevent the action of other spasmogens. Aliquots (0.1 ml) were removed at the same time, transferred to cuvettes and aggregation to ADP (5–10 μM) determined with a Payton dual channel aggregometer (Model 300BD). The loss of aggregability to ADP and appearance of spasmogenic activity on the rat stomach strip was taken to indicate the appearance of 6 oxo PGE_1 in the incubation mixture. The presence of 6 oxo PGE_1 in the incubation was checked by thin layer chromatography followed by bioassay of the eluted 6 oxo PGE_1 zone of the chromatogram.

Statistics

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

Materials

Prostacyclin sodium salt was obtained from Wellcome Ltd. and stored in 0.01 N NaOH (pH 12) at

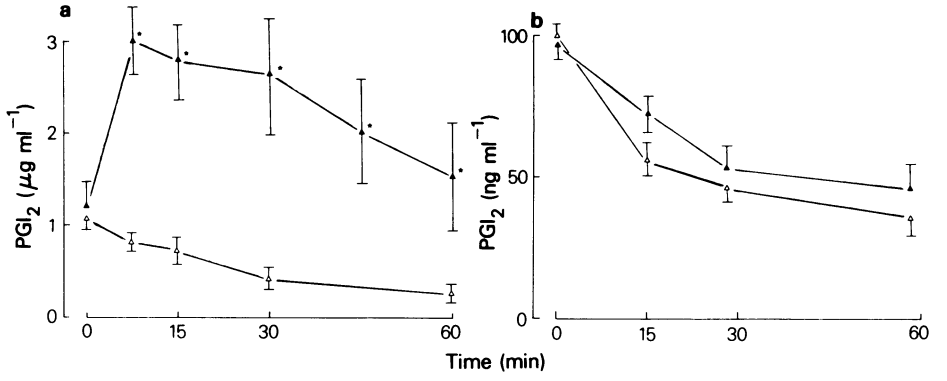

Figure 1 Rat PRP (▲) or PPP (△) was incubated at 37°C with PGI_2 (0.1–1.0 μg ml^{-1}). Aliquots were removed at timed intervals and bioassayed for spasmogenic activity on the rat stomach strip (a) or for anti-aggregatory activity against ADP-induced human platelet aggregation (b). Results shown mean values; vertical lines are s.e.mean; $n = 6$; $^*P < 0.01$.

−20°C. 6 oxo $PGF_{1\alpha}$ (Wellcome Ltd.) and 6 oxo PGE_1 (Upjohn Co.), PGE_2, PGD_2 and $PGF_{2\alpha}$ (Sigma Ltd.) were dissolved in methanol at −20°C. [9β-^{3}H]-PGI_2 methyl ester (sp. act. 12 Ci $mmol^{-1}$) was purchased from New England Nuclear. ADP was obtained from Sigma Ltd. All organic reagents were analytical grade.

Results

Rat

Incubation of rat PRP (but no PPP) with PGI_2 at 37°C resulted in a time-dependent increase in spasmogenic activity on the rat stomach strip which peaked at 7.5 min (3.03 ± 0.31 μg PGI_2 equiv ml^{-1}, $n = 6$ cf. 1.26 ± 0.25 μg PGI_2 equiv ml^{-1}, $n = 8$ in zero time samples, $P < 0.01$) and declined thereafter to 2.16 ± 0.6 μg PGI_2 equiv ml^{-1}, $n = 6$ after 60 min incubation. In contrast, the anti-aggregatory activity of PGI_2 declined at a similar rate following incubation in either rat PRP ($t_{\frac{1}{2}} = 43.4 \pm 6.2$ min, $n = 6$) or PPP ($t_{\frac{1}{2}} = 33.0 \pm 7.1$ min, $n = 6$). In each case the rate of decline of anti-aggregatory activity was considerably slower than in incubation containing 50 mM Tris-HCl buffer, pH 7.4 ($t_{\frac{1}{2}} = 3.6 \pm 0.4$ min, $n = 6$). These results are shown in Figure 1.

Anti-aggregatory activity was detected after acidification and extraction of incubations containing rat PRP and PGI_2 (Figure 2). Following thin layer chromatography in solvent F6, biological activity was confined to a zone which co-chromatographed with authentic 6 oxo PGE_1. No such activity was detected on any other part of the chromatogram (inset to Figure 2). The loss of radioactivity method also revealed the conversion of PGI_2 to a non-radioactive prostaglandin of the E series (Figure 3). After 30 min incubation at 37°C, extracted radioactivity had declined (compared with radioactivity in samples extracted at zero time) by $34.1 \pm 2.1\%$, $n = 8$. No such loss of radioactivity was detected in incubations containing either rat PPP or 50 mM Tris-HCl buffer. The loss of extractable radioactivity following incubation of PGI_2 with rat PRP was abolished by the inclusion

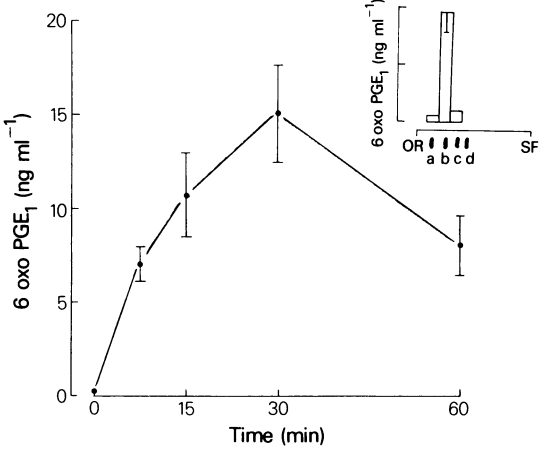

Figure 2 Anti-aggregatory activity of extracted rat PRP incubated with PGI_2 (1 μg ml^{-1}) at 37°C for 60 min. Results are expressed as ng 6 oxo PGE_1 equiv ml^{-1} PRP, $n = 6$ and shown mean values; vertical lines are s.e.mean. Figures are not corrected for losses which may occur on extraction. Inset: Distribution of anti-aggregatory activity following thin layer chromatography in solvent F6. Incubation contained 1 ml rat PRP, 1 μg PGI_2 and were at 37°C for 60 min. OR = origin, SF = solvent front; (a) = 6 oxo $PGF_{1\alpha}$, (b) = 6 oxo PGE_1, (c) = PGE_1, (d) = PGD_2. Results are mean with vertical line showing s.e.mean, $n = 6$.

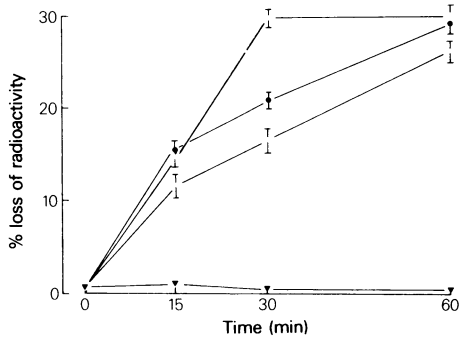

Figure 3 Conversion of [9β-^{3}H]-PGI_2 to 6 oxo PGE_1 estimated by the loss of radioactivity method. Incubations contained PGI_2 (1 μg ml^{-1}), [9β-^{3}H]-PGI_2 (0.05 μCi) and were at 37°C for 60 min. Rat (○), rabbit (●), guinea-pig (▼) and human (□) PRP. No loss of radioactivity was detected in incubations containing 50 mM Tris-HCl buffer, or rat, rabbit, guinea-pig or human PPP (data not shown). Results show mean values; vertical lines are s.e.mean; $n = 6-8$.

of rutin (50 μM) and reduced by the inclusion of naringenin (50 μM, 59.0 ± 3.1% inhibition, $n = 8$, after 60 min incubation) into the incubation mixture. Both rutin and naringenin are potent and selective inhibitors of the enzyme PG-9HDH (Griffiths, Lofts & Moore, 1983).

These results support the possibility that rat platelets contain a PG-9HDH enzyme which converts PGI_2 to 6 oxo PGE_1. However, unlike human platelets we could find no evidence for the spontaneous release of a 6 oxo PGE_1 like substance from non-aggregating rat platelets. No increase in spasmogenic activity on the rat stomach strip was observed when rat PRP was incubated without PGI_2 (94.2 ± 25.0 ng PGI_2 equiv ml^{-1}, $n = 7$ after 60 min, cf. 95 ± 28.0 ng PGI_2 equiv ml^{-1}, $n = 7$ at zero time). Similarly, platelet reactivity to a standard concentration of ADP (2 μM) which produced 70–80% maximal aggregation of rat platelets, was unchanged in rat PRP incubated at 37°C for up to 60 min (aggregation was 109 ± 1.5%, $n = 6$, of the response obtained at zero time). To confirm the lack of spontaneous release of a 6 oxo PGE_1-like substance from rat platelets, aliquots of the incubation were removed, acidified, extracted and subjected to thin layer chromatography. No spasmogenic or anti-aggregatory activity was detected on any part of the chromatogram (data not shown). The extraction efficiency for 6 oxo PGE_1 (1 μg ml^{-1}) added to rat PRP was 58.6 ± 5.7%, $n = 8$ and thus the limit of detection of 6 oxo PGE_1 in incubated rat PRP was about 1 ng ml^{-1} measured by the anti-aggregatory assay. The extraction efficiency of the same concentration of 6 oxo PGE_1 added to rabbit PRP (54.7 ± 7.9%, $n = 8$), guinea-pig PRP (63.6 ± 6.8%, $n = 8$), human PRP (54.8 ± 8.9%, $n = 8$) and 50 mM Tris-HCl buffer (60.0 ± 5.5%, $n = 8$) was similar to that observed in rat PRP.

To check the possibility that rat platelets incubated without PGI_2 have the capacity to release a 6 oxo PGE_1 like substance but are insensitive to its inhibitory effect we have compared the anti-aggregatory potency of 6 oxo PGE_1 and PGI_2 using rat PRP. The concentrations required for 50% inhibition of ADP-induced rat platelet aggregation were 24.0 ± 2.0 ng ml^{-1}, and 3.1 ± 0.07 ng ml^{-1}, both $n = 5$, respectively.

Rabbit

An increase in spasmogenic activity on the rat stomach strip was also observed following incubation of rabbit PRP (but not PPP) with PGI_2. Biological

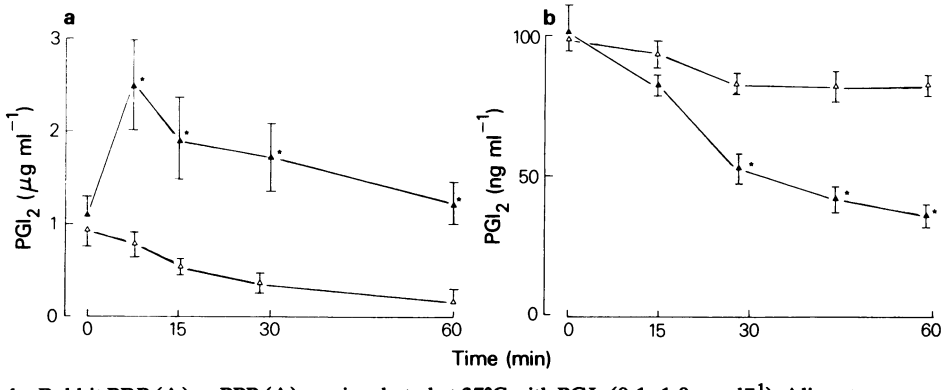

Figure 4 Rabbit PRP (▲) or PPP (△) was incubated at 37°C with PGI_2 (0.1–1.0 μg ml^{-1}). Aliquots were removed at timed intervals and bioassayed for spasmogenic activity on the rat stomach strip (a) and for anti-aggregatory activity against ADP-induced human platelet aggregation (b). Results show mean values; vertical lines are s.e.mean; $n = 6$; $P < 0.01$.

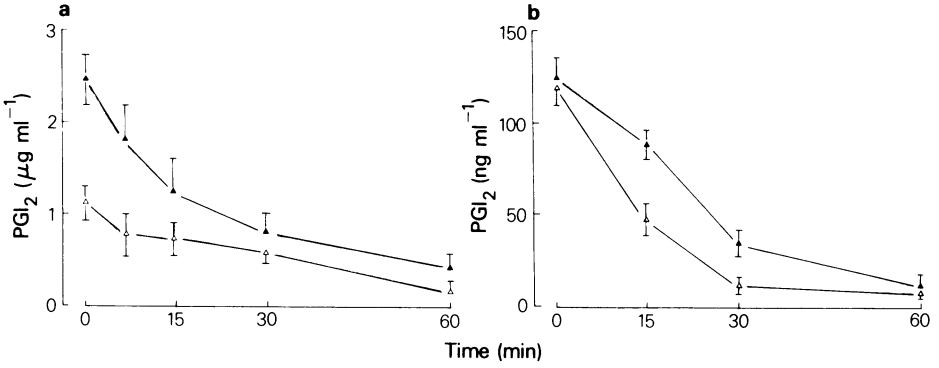

Figure 5 Guinea-pig PRP (▲) or PPP (△) was incubated at 37°C with PGI_2 (0.1–1.0 $\mu g\,ml^{-1}$). Aliquots were removed at timed intervals and bioassayed for spasmogenic activity on the rat stomach strip (a) and for anti-agreggatory activity against ADP-induced human platelet aggregation (b). Although guinea-pig PRP had little or no spasmogenic effect on the rat stomach strip (see text), small volumes potentiated the contractile effect of added PGI_2 thus accounting for the abnormally high spasmogenic activity observed in these experiments. Results show mean values; vertical lines are s.e.mean; $n = 6$.

activity peaked at 7.5 min ($2.5 \pm 0.5\,\mu g\,PGI_2$ equiv ml^{-1}, $n = 6$ cf. $1.1 \pm 0.2\,\mu g\,PGI_2$ equiv ml^{-1}, $n = 6$ in zero time samples, $P < 0.01$) and declined thereafter to $1.21 \pm 0.25\,\mu g\,PGI_2$ equiv ml^{-1}, $n = 5$ at 60 min incubation (Figure 4a). The anti-aggregatory activity of PGI_2 incubated in rabbit PRP ($t_{\frac{1}{2}} = 39.4 \pm 5.2$ min, $n = 6$) was significantly prolonged ($P < 0.001$) compared with activity following incubation in 50 mM Tris-HCl buffer. Interestingly, the anti-aggregatory effect of PGI_2 was even more prolonged following incubation in rabbit PPP (Figure 4b). Acidification, extraction and thin layer chromatography of aliquots of rabbit PRP incubated with PGI_2 for 30 min revealed the presence of anti-aggregatory activity (equivalent to 45.1 ± 17.9 ng 6 oxo PGE_1 equiv ml^{-1}, $n = 8$) only in a zone which co-chromatographed with authentic 6 oxo PGE_1 and pro-aggregatory activity in a zone close to the solvent front ($R_F = 0.9$–1.0). Incorporation of [9β-^{3}H]-PGI_2 into the incubation mixture resulted in a time-dependent loss of extractable radioactivity in PRP but not PPP (Figure 3). After 30 min incubation, extractable radioactivity had declined by $21.1 \pm 3.9\%$, $n = 8$. Rutin (50 μM) prevented and naringenin (50 μM) reduced ($43.2 \pm 2.1\%$ inhibition, $n = 8$, at 60 min) the reduction in extractable radioactivity.

As with rat PRP, we were unable to demonstrate the spontaneous release of a 6 oxo PGE_1-like substance from non-aggregating rabbit platelets. No increase in spasmogenic activity on the rat stomach strip was observed when rabbit PRP was incubated at 37°C for 60 min (28.2 ± 9.9 ng PGI_2 equiv ml^{-1}, $n = 7$, cf. 23.2 ± 1.7 ng PGI_2 equiv ml^{-1}, $n = 6$ at zero time). In addition, platelet aggregability to ADP (7 μM) failed to decline over the same period ($95.0 \pm 4.4\%$, $n = 6$ of control aggregation at zero time). In experiments in which rabbit PRP was kept at 37°C for 60 min and aliquots extracted and subjected to chromatography, small amounts of anti-aggregatory activity (6.0 ± 1.1 ng 6 oxo PGE_1 equiv ml^{-1}, $n = 6$) were detected in the 6 oxo PGE_1 zone. No such biological activity occured in any other part of the chromatogram.

Both PGI_2 and 6 oxo PGE_1 potently inhibited ADP-induced rabbit platelet aggregation. The concentrations required for 50% inhibition were 0.63 ± 0.02 ng ml^{-1} and 14.3 ± 1.8 ng ml^{-1} respectively (both $n = 6$).

Guinea-pig

In contrast to platelets from rat and rabbit, guinea-pig PRP did not convert PGI_2 to 6 oxo PGE_1 as shown by the absence of an increase in contractile activity on the rat stomach strip following incubation with exogenous PGI_2 for up to 60 min (Figure 5). In incubations containing radiolabelled PGI_2, no loss of radioactivity was detected for up to 60 min at which time the experiment was terminated (Figure 3). Extraction and chromatography of guinea-pig PRP incubated for up to 60 min failed to reveal any anti-aggregatory activity in the 6 oxo PGE_1 zone of the chromatogram. Incubation of PGI_2 in guinea-pig PRP or PPP resulted in the prolongation of the anti-aggregatory effect ($t_{\frac{1}{2}} = 19.8 \pm 1.6$ min, $n = 5$ and 21.3 ± 1.2 min, $n = 5$ for PRP and PPP respectively).

Guinea-pig platelets did not spontaneously release a 6 oxo PGE_1 like substance. As with rat and rabbit PRP, no change in spasmogenic activity on the rat stomach strip was observed following incubation for up to 60 min without added PGI_2 (15.3 ± 2.4 ng PGI_2

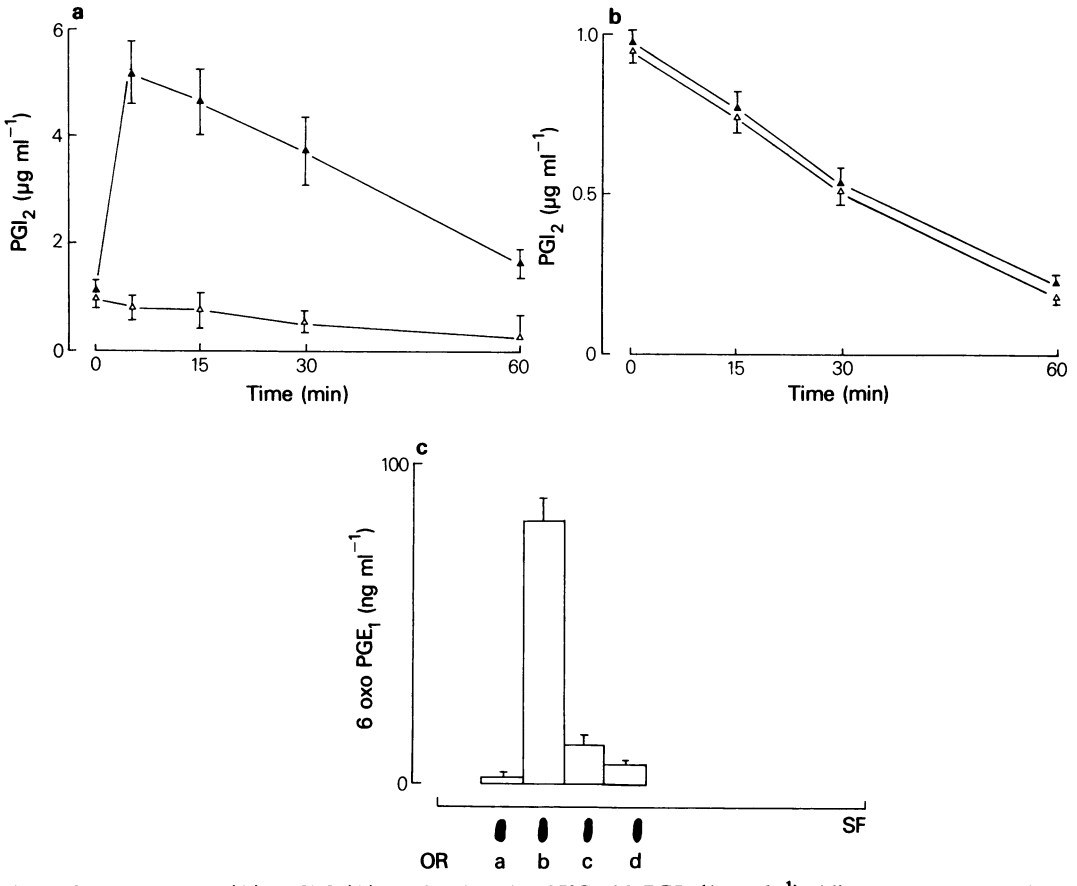

Figure 6 Human PRP (▲) or PPP (△) was incubated at 37°C with PGI_2 (1 μg ml^{-1}). Aliquots were removed at timed intervals and bioassayed for spasmogenic activity on the rat stomach strip (a) and for anti-aggregatory activity against ADP-induced human platelet aggregation (b). The distribution of anti-aggregatory activity following thin layer chromatography in solvent F6 is shown in C. OR = origin, SF = solvent front, (a) = 6 oxo $PGF_{1\alpha}$, (b) = 6 oxo PGE_1, (c) = PGE_1, (d) = PGD_2. Results show mean values; vertical lines are s.e.mean; $n = 6-8$.

equiv ml^{-1}, $n = 6$ cf. 14.6 ± 3.5 ng PGI_2 equiv ml^{-1}, $n = 6$ in zero time samples). Furthermore, platelet reactivity to ADP (10 μM) remained constant on incubation for 60 min at 37°C (99.0 ± 2.9% of control aggregation at zero time, $n = 6$). No anti-aggregatory activity was detected on any part of the chromatogram following thin layer chromatography of extracts of guinea-pig PRP incubated without additions for 60 min. Both PGI_2 and 6 oxo PGE_1 inhibited ADP-induced guinea-pig platelet aggregation ($IC_{50} = 1.9 \pm 0.35$ ng ml^{-1} and 46.2 ± 8.1 ng ml^{-1}, both $n = 6$ respectively).

Human

In a previous communication to the British Pharmacological Society, we reported the conversion of PGI_2 to 6 oxo PGE_1 by human PRP (Hoult *et al.*, 1981). We can now confirm and extend these findings. Spasmogenic activity on the rat stomach strip increased rapidly following incubation of human PRP (but not PPP) with PGI_2 at 37°C. Peak contractile effect occurred at 5 min (5.1 ± 0.7 μg PGI_2 equiv ml^{-1}, $n = 8$ cf. 1.3 ± 0.5 μg PGI_2 equiv ml^{-1}, $n = 8$ at zero time, $P < 0.01$) and gradually declined to reach a lowest value of 1.9 ± 0.3 μg equiv ml^{-1}, $n = 8$ after 60 min incubation (Figure 6a). The loss of radioactivity method also demonstrated the conversion of PGI_2 to an E type prostaglandin by human PRP (Figure 3). Anti-aggregatory activity of PGI_2 was prolonged following incubation in human PRP ($t_{\frac{1}{4}} = 35.5 \pm 4.3$ min, $n = 8$) and PPP ($t_{\frac{1}{4}} = 34.8 \pm 3.4$ min, $n = 8$) as shown in Figure 6b. Extraction and chromatography of aliquots of human PRP incubated with PGI_2 for 30 min demonstrated the presence of both anti-aggregatory and spas-

mogenic activity in a zone which co-chromatographed with authentic 6 oxo PGE_1 (Figure 6c).

Discussion

We show here that platelets from rats and rabbits but not guinea-pigs convert PGI_2 to a stable prostaglandin with biological activity and thin layer chromatographic mobility identical to that of authentic 6 oxo PGE_1. In these experiments we have used a novel loss of radioactivity method to extend our previously published observation that human platelets convert PGI_2 to a 6 oxo PGE_1-like substance (Hoult *et al.*, 1981). The percentage conversion as determined by the loss of radioactivity method or estimated from the increase in biological activity on the rat stomach strip is approximately 25–30% in platelet-rich-plasma from rats, rabbits and man. As yet we do not know whether the failure of guinea-pig platelets to convert PGI_2 to 6 oxo PGE_1 represents a deficiency of the intracellular PG-9HDH enzyme within platelets of this species or alternatively lack of uptake of precursor PGI_2 across the platelet membrane. We are not aware of any other attempts to measure guinea-pig platelet PG-9HDH activity using either $PGF_{2\alpha}$ or 13, 14 dihydro 15 oxo $PGF_{2\alpha}$ which are known to be substrates for this enzyme in other organs. Attempts to separate PGI_2 uptake into the platelet from its enzymatic conversion to 6 oxo PGE_1 by preparing cytosolic (100,000 *g*) supernatants of platelets from the four species used in this study are now in progress in this laboratory.

In this study we have attempted to demonstrate conversion of PGI_2 to 6 oxo PGE_1 by parallel bioassay on the rat stomach strip and inhibition of ADP-induced human platelet aggregation coupled with a chemical loss of radioactivity method. It is clear that in 3 of the 4 species, anti-aggregatory activity of PGI_2 incubated in PRP was not significantly different from activity incubated in PPP. Thus, this particular bioassay is incapable of detecting conversion of PGI_2 to 6 oxo PGE_1 which may explain the failure of Doe, Moncada, Vane & Whittle (1982) to demonstrate this reaction in incubated human PRP. In their study, these authors relied exclusively on assay of anti-aggregatory activity. Other authors have also failed to detect 6 oxo PGE_1 synthesis from PGI_2 in incubated human plasma (Mikhailidis, Mikhailidis, Wollard & Dandona, 1982) but this was perhaps not surprising since in their experiments prostacyclin was incubated in plasma devoid of platelets. The enzyme responsible for 6 oxo PGE_1 formation is known to occur in platelets but not in plasma (Wong *et al.*, 1980; Hoult *et al.*, 1981). Determination of the anti-aggregatory effect of PGI_2 incubated in plasma does however fulfil a useful purpose by measuring the stabilization of PGI_2 which occurs when it binds to plasma proteins.

The flavonoid derivatives, rutin and naringenin have previously been shown to inhibit rabbit renal PG-9HDH (Griffiths *et al.*, 1983). Of the two drugs, naringenin was approximately twice as potent as rutin as an inhibitor of this enzyme. In the present study, rutin (50 μM) completely inhibited conversion of PGI_2 to 6 oxo PGE_1 by rat and rabbit PRP whilst naringenin produced only a partial inhibition of this enzyme activity at a similar concentration. Rutin (and to a lesser extent naringenin) may thus be useful experimental tools to study the *in vivo* formation of 6 oxo PGE_1 by platelets in these species.

Human platelets spontaneously release an anti-aggregatory stable prostaglandin tentatively identified as 6 oxo PGE_1 (Lofts & Moore, 1982). We were disappointed to find that similar spontaneous release of 6 oxo PGE_1 could not be demonstrated from non-aggregating rat, rabbit and guinea-pig platelets even though rat and rabbit platelets share with human platelets the capacity to synthesize 6 oxo PGE_1 from added PGI_2. Both PGI_2 and 6 oxo PGE_1 potently inhibited ADP-induced platelet aggregation in all species studied. The potency ratio (PGI_2:6 oxo PGE_1) varied from 7.7 (rat) through 22.8 (rabbit) to 24.3 (guinea pig). We (Griffiths *et al.*, 1982) and others (Miller *et al.*, 1980) have previously reported that PGI_2 has 18–20 times the activity of 6 oxo PGE_1 as an inhibitor of ADP-induced human platelet aggregation. Clearly if 6 oxo PGE_1 release had occurred from incubated rat, rabbit or guinea-pig PRP we would have expected to detect a time-dependent loss of platelet reactivity to ADP. Although data from such 'live assay' experiments did not show spontaneous release of 6 oxo PGE_1 from platelets, small amounts of anti-aggregatory activity were detected following thin layer chromatography of rabbit PRP extracts in a zone that corresponded to authentic 6 oxo PGE_1. It is thus possible that rabbit platelets do release a 6 oxo PGE_1-like substance although obviously in much smaller quantities than human platelets.

The physiological relevance of the formation of 6 oxo PGE_1 by platelets has not been elucidated. As a potent inhibitor of platelet function and a vasodilator, the formation of stable 6 oxo PGE_1 if it should occur *in vivo* may be of importance for the maintenance of cardiovascular homeostasis. Elevated plasma levels of 6 oxo PGE_1 follow intravenous administration of PGI_2 in the dog (Taylor, Shebuski & Sun, 1983) but not in man (Jackson *et al.*, 1982). These results do not rule out the possibility of conversion of endogenous PGI_2 synthesized locally by the blood vessel wall to 6 oxo PGE_1. Our finding that platelets from rats and rabbits also convert PGI_2 to

6 oxo PGE_1 will allow experiments to be carried out to explore this possibility.

We would like to thank the MRC for financial support, Dr J. O'Grady (Wellcome Ltd.) and Dr J.E. Pike (Upjohn Co.) for gifts of prostaglandins.

References

BLAIR, I.A., BARROW, S.E., WADDEL, K.A., LEWIS, P.J. & DOLLERY, C.T. (1982). Prostacyclin is not a circulating hormone in man. *Prostaglandins*, **23**, 579–589.

CHANG, D.G.-B. & TAI, H.H. (1982). A radioimmunoassay for 6 keto PGE_1. *Prostaglandins Leuk. Med.*, **8**, 11–20.

DOE, S., MONCADA, S., VANE, J.R. & WHITTLE, B.J.R. (1982). Action of human platelets on the duration of the anti-aggregatory action of prostacyclin. *Br. J. Pharmac.*, **75**, 166P.

GANS, K.R. & WONG, P.Y.-K. (1981). Renal 9-hydroxyprostaglandin dehydrogenase (9-OH PGDH) in spontaneously hypertensive (SHR) and New Zealand genetically hypertensive rats. *Fedn. Proc.*, **40**, 681.

GRIFFITHS, R.J. & MOORE, P.K. (1983a). Effect of 6 keto prostaglandin E_1 on sympathetic neurotransmission in the vas deferens. *J. Pharm. Pharmac.*, **35**, 184–186.

GRIFFITHS, R.J. & MOORE, P.K. (1983b). Formation of 6 keto prostaglandin E_1 in mammalian kidneys *Br. J. Pharmac.*, **79**, 149–155.

GRIFFITHS, R.J., LOFTS, F.J. & MOORE, P.K. (1983). The effect of some flavone drugs on the conversion of prostacyclin to 6 oxo prostaglandin E_1. *Biochem. Pharmac.*,, (in press).

GRIFFITHS, R.J., LOFTS, F.J. & MOORE, P.K. (1982). Effect of 6 keto PGE_1 on gastrointestinal and reproductive smooth muscle in vitro. *Br. J. Pharmac.*, **77**, 491P.

HASSID, A. & DUNN, M.J. (1982). Biosynthesis and metabolism of prostaglandins in human kidney in vitro. In *Prostaglandins and the Kidney*. ed. Dunn, M.J., Patrono, C. & Cinotti, G.A., pp. 3–15. New York: Plenum Press.

HOULT, J.R.S. & MOORE, P.K. (1977). Pathways of prostaglandin $F_{2\alpha}$ in mammalian kidneys. *Br. J. Pharmac.*, **61**, 615–626.

HOULT, J.R.S., LOFTS, F.J. & MOORE, P.K. (1981). Stability of prostacyclin in plasma and its transformation by platelets to a stable spasmogenic product. *Br. J. Pharmac.*, **73**, 218P.

LOFTS, F.J. & MOORE, P.K. (1982). Release of a 6 oxo prostaglandin E_1 like substance from human platelets. *Clin. Sci.*, **64**, 63–68.

JACKSON, E.K., GOODWIN, R.P., FITZGERALD, G.A., OATES, J.A. & BRANCH, R.A. (1982). Assessment of the extent to which exogenous prostaglandin I_2 is converted to 6 keto prostaglandin E_1 in human subjects. *J. Pharmac. exp. Ther.*, **221**, 183–187.

McGIFF, J.C., SPOKAS, E.G. & WONG, P.Y-K. (1982). Stimulation of renin release by 6 oxo prostaglandin E_1 and prostacyclin. *Br. J. Pharmac.*, **75**, 137–144.

MIKHAILIDIS, D.P., MIKHAILIDIS, A.M., WOLLARD, M.L. & DANDONA, P. (1982). Protection of prostacyclin-like activity in human plasma – a non-enzymatic mechanism?, *Clin. Sci.*, **62**, 177–181.

MILLER, O.V., AIKEN, J.W., SHEBUSKI, R.J. & GORMAN, R.R. (1980). 6 keto prostaglandin E_1 is not equipotent to prostacyclin (PGI_2) as an anti-aggregatory agent. *Prostaglandins*, **20**, 391–400.

QUILLEY, C.P., WONG, P.Y-K. & McGIFF, J.A. (1979). Hypotensive and renovascular actions of 6 keto prostaglandin E_1, a metabolite of prostacyclin. *Eur. J. Pharmac.*, **57**, 273–276.

SPANNHAKE, E.W., LEVIN, G.L., HYMAN, A.L. & KADOWITZ, P.J. (1981). 6 keto PGE_1 exhibits more potent bronchodilatory activity in the cat than its precursor, PGI_2. *Prostaglandins*, **21**, 266–275.

STOFF, J.S., CLIVE, D.M., LEONE, D., MacINTYRE, D.E., BROWN, R.S. & SALZMAN, E. (1983). The role of arachidonic acid metabolites in the pathophysiology of Barrter's syndrome. In *Prostaglandins and the Kidney*. ed. Dunn, M.J., Patrono, C. & Cinotti, G.A., pp. 353–363. New York: Plenum Press.

TAYLOR, B.M., SHEBUSKI, R.J. & SUN, F.F. (1983). Circulating prostacyclin metabolites in the dog. *J. Pharmac. exp. Ther.*, **224**, 692–698.

WONG, P.Y-K., LEE, W.H., CHAO, P.H-W., REISS, R.F. & McGIFF, J.C. (1980). Metabolism of prostacyclin by 9-hydroxyprostaglandin dehydrogenase in human platelets. *J. biol. Chem.*, **255**, 9021–9024.

WONG, P.Y-K., LEE, W.H. & REISS, R.F. & McGIFF, J.C. (1980). Metabolism of prostacyclin (PGI_2) by purified 9-hydroxyprostaglandin dehydrogenase (9-OH PGDH) of rabbit liver and human platelets. *Fedn. Proc.*, **75**, 137–144.

(Received May 27, 1983. Revised June 23, 1983.)