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The time course and magnitude of prostacyclin (PGI_2) production by rat aortic rings incubated in human plasma

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The clinical pharmacology of PGI_2 makes it an attractive candidate for a role in protection against vascular disease [1]. In vessel walls it is the intima that has the greatest PGI_2 synthesising activity [2, 3]. In life the intimal endothelium is bathed in plasma and this may contain factors that stimulate [3–5] or inhibit [6–8] prostanoid synthesis. Chopped aortic rings produce PGI_2 when incubated in Krebs' solution or buffer [9] and are a suitable system for the study of PGI_2 synthesis *in vitro*. However, studies on aortic rings incubated in plasma [4] have been hampered by dependence on bioassay, because PGI_2 is rapidly hydrolysed to 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) which is biologically inactive. Consequently PGI_2 concentrations measured sequentially during an incubation reflect the rate of breakdown as well as synthesis. Even though hydrolysis of PGI_2 is a non-enzymic process its rate varies substantially in platelet-poor plasma (PPP) from different subjects. Thus, in one study [10] half-lives in PPP from healthy subjects varied from 8.9 to 23.3 min at 37°, probably because of variable stabilization of PGI_2 by albumin [10–12].

To circumvent these problems we measured prostanoid synthesis by rat aortic rings incubated with PPP at 37°, using a modified radioimmunoassay (RIA) for 6-oxo-$PGF_{1\alpha}$ [13, 14]. Interference in the RIA by plasma protein [14] was avoided by diluting all samples by 20-fold or greater before assay. The diluted samples were temporarily acidified to ensure that all PGI_2 was hydrolysed. 6-oxo-$PGF_{1\alpha}$ measured in this way reflects only the synthesis of PGI_2 and not its breakdown during incubation. In addition, the concentration of PGI_2 present as such was determined by bioassay and by a modification of a 6-oxo-$PGF_{1\alpha}$ RIA.

Materials and methods

Preparation of aortic rings. Male CD rats (Charles River, Margate, U.K.) 250–350 g were stunned and killed by cervical dislocation. The aorta was dissected rapidly from the heart to the aortic bifurcation and rinsed in ice cold balanced salt solution. A 5 cm length immediately distal to the origin of the left subclavian artery was chopped into 1 mm rings with a McIlwain tissue chopper. The fifty rings produced were divided into two groups of 25, alternate rings being allotted to each group. Each group of rings was stored in 5 ml balanced salt solution (Gey's solution, Gibco, Uxbridge, U.K) on ice for less than 30 minutes until the start of the incubation with PPP.

PPP. Venous blood was drawn from healthy male subjects and added to 3.8% trisodium citrate (10 vol. blood: 1 vol. citrate) in plastic vials. It was spun immediately at 1000 *g* at 4° for 20 min and the supernatant plasma separated and stored at −20° until required.

Incubations. PPP was thawed at room temperature and 2 ml added to a polypropylene incubation vial in a shaking water bath at 37°. The pH of the plasma was maintained at pH 7.4–7.6 by gassing with 5% CO_2 in O_2. At zero time a group of 25 rings was added to the plasma. At 4, 8, 15, 30, 45 and 60 min a 100 μl aliquot of incubate was removed and added to 2.5 μl of 1M NaOH on ice to adjust the pH to greater than 10. The samples were then stored at −20° and assayed within 24 hr.

Radioimmunoassay. The 4 and 8 min samples were diluted 20-fold with Tris buffer (50 mM) pH 8.5; the 15–60 min samples were similarly diluted 100-fold. One portion (250 μl) of the diluted sample was then acidified with 5 μl of 2M HCl. After 20 min (allowing conversion of all PGI_2 present to 6-oxo-$PGF_{1\alpha}$) the pH was restored to 8.5 with 5 μl of 2M NaOH. Another portion (250 μl) of the diluted sample was added to 10 μl of 1M NaCl (non hydrolysed sample). This method allows quantitation of both 6-oxo-$PGF_{1\alpha}$ and PGI_2. The total 6-oxo-$PGF_{1\alpha}$ content was that determined in the hydrolysed sample. The PGI_2 concentration was calculated from the difference in 6-oxo-$PGF_{1\alpha}$ content between the hydrolysed and non-hydrolysed samples. The full methodological details and assay validation will be published elsewhere [14].

Bioassay. Platelet-rich plasma (PRP) was prepared from citrated venous blood prepared as above. This was immediately centrifuged at 400 *g* at 20° for 7.5 min. Undiluted incubate (5–20 μl) was added to 250 μl of PRP in the cuvette of a Payton aggregometer at 37° one min before the addition of adenosine diphosphate (Sigma Chemical Co., London, U.K.) sufficient to produce secondary aggregation. Inhibition of aggregation by the incubate was compared with inhibition produced by known amounts of standard PGI_2 (a generous gift from Wellcome Research Laboratories, Beckenham, U.K.). Each sample was bracketed between two standards producing respectively a slightly greater and a slightly smaller effect than the unknown. The concentration of PGI_2-like activity in the incubate was then calculated by interpolation.

Results and discussion

The time-course of appearance of PGI_2 in PPP incubated with aortic rings at 37° was measured by RIA and bioassay (Fig. 1). There was good agreement between the two methods. PGI_2 concentration reached a peak at 15 min and then declined. This can be explained by rapid early synthesis of PGI_2 that is not sustained, the falling part of the curve being due to hydrolysis. As predicted, 6-oxo-$PGF_{1\alpha}$ (Fig. 2, upper curve) rose asymptotically, approaching a plateau in 30–60 min. PGI_2 measured by bioassay on the same samples (Fig. 2, lower curve) agreed with 6-oxo-$PGF_{1\alpha}$ only at 4 min; at later times a discrepancy between the two curves occurred that progressively widened. Bioassay is thus not an acceptable way of estimating the extent of PGI_2 production for incubation periods longer than 4 min. It does, however, provide an accurate measure of the rate of synthesis up to this time and studies that employ short incubation times are thus valid.

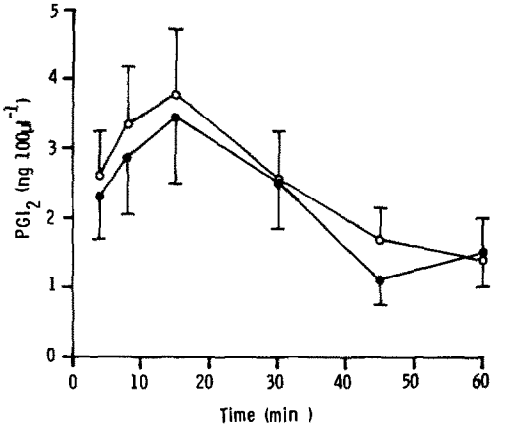

Fig. 1. PGI_2 concentration (ng/100 µl aliquot) in PPP incubated with duplicate groups of 25 aortic rings. Seven incubations were performed, aliquots being withdrawn from each incubation at 6 times and each aliquot being assayed by RIA (●) and by platelet bioassay (○). Each point is the mean ± S.E.

The initial rate of production of 6-oxo-$PGF_{1\alpha}$ (expressed per mg blotted weight of tissue) was 0.63 (± 0.09) ng/mg/min (mean ± S.E., $n = 10$). This is similar to rates previously observed in buffer or salt solutions: Bunting and co-workers [9] found a rate of 0.2–1.0 ng/mg/min at 22° for rabbit aortic rings in buffer, and Peskar and co-workers [15] a rate of 0.28 ng/mg/min by rat aorta in Krebs' solution at 37°. These rates are substantial relative to the potency of PGI_2 [1, 16]. Our finding of a similar rate of production of PGI_2 in plasma, the normal physiological milieu of endothelium, is consistent with a role for PGI_2 in the local response to vascular injury.

The absolute values of PGI_2 and 6-oxo-$PGF_{1\alpha}$ produced by aortic rings varied considerably from rat to rat, as indicated by standard errors of the order of 15% of the mean ($n = 10$). Greater consistency was observed when prostanoid production by one group of rings was compared with that produced by a paired group of rings taken from the same rat (Fig. 3). This is important in the design of subsequent experiments to compare the effects of plasmas from patients with different diseases, and to determine the effects of drugs on PGI_2 synthesis *in vitro*.

In summary, the time course and magnitude of PGI_2 production by rat aortic rings incubated at 37° in PPP have been determined by RIA and bioassay. For incubation periods longer than 4 min only measurement of 6-oxo-$PGF_{1\alpha}$ by RIA gave an adequate reflection of the extent of PGI_2 synthesis. The initial rate of synthesis was 0.63 (± 0.09) ng/mg/min and was maintained for 8 min but then declined, the concentration of 6-oxo-$PGF_{1\alpha}$ approaching a plateau in 30–60 min. There was considerable variation from rat to rat in the amount of prostanoid produced, but prostanoid production by paired rings from the same rat was very similar, providing a method for comparison of the effects of different drugs and plasmas on PGI_2 synthesis.

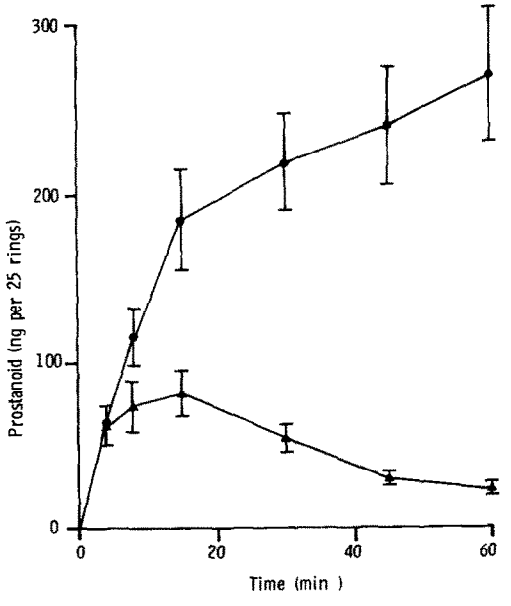

Fig. 2. 6-Oxo-$PGF_{1\alpha}$ production (●) in PPP incubated with 25 aortic rings, compared with PGI_2 production measured by bioassay (▲). Each point is the mean ± S.E. of 10 determinations, each on a different rat.

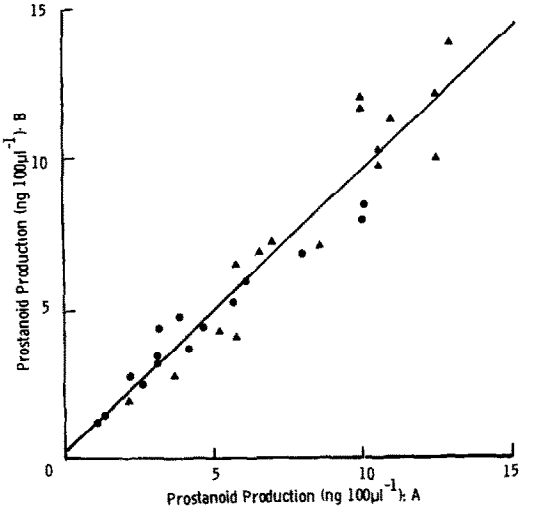

Fig. 3. Prostanoid production in paired incubations from 5 rat aortas: correlation of prostanoid produced by 25 aortic rings (A) with that produced by 25 paired rings from the same rat (B). ▲, 6-oxo-$PGF_{1\alpha}$; ●, PGI_2 (determined by bioassay). The line is the least squares regression line $y = 0.93 \times 0.003$ ($r = 0.96$).

Department of Clinical Pharmacology
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0HS
U.K.

JAMES M. RITTER*
MARGARET A. ORCHARD
IAN A. BLAIR
PETER J. LEWIS

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* Author to whom correspondence should be addressed.