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Elimination of prostacyclin (PGI_2) and 6-oxo-$PGF_{1\alpha}$ in anaesthetized dogs

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The plasma concentration of 6-oxo-$PGF_{1\alpha}$ was measured by radioimmunoassay after constant rate infusion of 6-oxo-$PGF_{1\alpha}$ or prostacyclin (PGI_2) into anaesthetized dogs. A steady-state plasma concentration was rapidly attained with both compounds. After termination of the infusions, the concentration of 6-oxo-$PGF_{1\alpha}$ declined according to a bi-exponential process. The steady-state plasma concentrations of 6-oxo-$PGF_{1\alpha}$ obtained after infusion of 6-oxo-$PGF_{1\alpha}$ and PGI_2 were approximately 10 times higher than the corresponding steady-state level of $PGF_{2\alpha}$ measured after infusion of $PGF_{2\alpha}$ into the same dogs. The data presented suggest that PGI_2 and 6-oxo-$PGF_{1\alpha}$ are eliminated more slowly than $PGF_{2\alpha}$, probably because they are not taken up and metabolized in the lungs as rapidly as $PGF_{2\alpha}$.

Prostacyclin (PGI_2) is the major product of arachidonic acid metabolism in vascular tissue in vitro (Moncada et al 1976); it is a potent inhibitor of platelet aggregation (Moncada et al 1976) and also causes vasodilatation (Armstrong et al 1977, 1978; Dusting et al 1978a). It has been proposed that prostacyclin plays a physiological role in reducing platelet clumping onto blood vessel walls (Moncada & Vane 1978). Damage to vascular endothelium may disrupt this mechanism and lead to a thrombogenic state.

"Classical" prostaglandins (PGE_2 and $PGF_{2\alpha}$) are rapidly metabolized in vivo; Hamberg & Samuelsson (1971) demonstrated that only 3% of a bolus intravenous injection of tritiated PGE_2 remained unchanged in the plasma after 90 s. The initial metabolic step is the oxidation of the 15-hydroxyl group to the corresponding ketone, a reaction catalysed by the enzyme 15-hydroxy-prostaglandin dehydrogenase (PGDH) (Änggård & Samuelsson 1964, 1966; Hamberg & Samuelsson 1971). The lung contains a high concentration of PGDH (Änggård & Samuelsson 1964) and more than 95% of infused PGE_2 is inactivated in one passage through the pulmonary circulation (Ferreira & Vane 1967). The further transformations which occur are saturation of the 13,14-double bond (by the enzyme prostaglandin Δ^{13}-reductase), β- and ω-oxidations of the side chains. However, Dusting et al (1978b) demonstrated that PGI_2 is not inactivated in the passage across the lungs in anaesthetized dogs; but a single passage through other organs (e.g. liver) removed up to 80% of biological activity. Elimination of PGI_2-like activity from the circulation occurred much more rapidly than non-enzymatic degradation of PGI_2 to 6-oxo-$PGF_{1\alpha}$.

We have now compared the rates of elimination of prostacyclin, 6-oxo-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ after constant rate infusions into dogs.

MATERIAL AND METHODS

Three dogs received indomethacin (10 mg kg^{-1}) subcutaneously 16 h before and intravenously immediately on completion of surgery. The dogs were anaesthetized initially with thiopentone (25–30 mg kg^{-1} i.v.); then maintained with chloralose (50–80 mg kg^{-1} i.v.) and supplemented as required. After intubation the dogs were artificially ventilated with room air using a CFP model 5255 positive pressure respiratory pump (rate 18–20 cycles min^{-1}). Arterial blood gas tensions were measured hourly (Corning 175 Automatic pH-Blood-gas system), Po_2, Pco_2 and pH being maintained in the range 93–110 mm Hg, 30–40 mm Hg and 7·3–7·5 pH units respectively. A heating pad was used to maintain rectal temperature at 37–39 °C.

Systemic arterial pressure was recorded from a catheter inserted into a femoral artery. Heart rate was recorded from the ECG (standard lead II). One hour after the injection of the last dose of indomethacin, 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and prostacyclin were separately infused for 10 min at a rate of 1 μg kg^{-1} min^{-1} via a cannula inserted into a femoral vein. The sequence of the infusions in all three dogs was, 6-oxo-$PGF_{1\alpha}$ followed by $PGF_{2\alpha}$ and then PGI_2; the interval between infusions was at least 90 min. Blood (2·5 ml) was removed via a cannula in the contralateral femoral artery at frequent intervals

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before, during and after the infusion into tubes containing EDTA (final concentration 1·7 mg ml^{-1}) and indomethacin (final concentration 20 μg ml^{-1}) to prevent further synthesis of prostaglandins. The samples were rapidly centrifuged to remove blood cells including platelets, and were stored frozen until analyses could be completed.

Extraction and chromatography

Plasma samples (1 ml) were extracted by a procedure similar to that described by Frölich (1976). Approximately 2,000 counts min^{-1} of the appropriate tritiated prostaglandin (either ^{3}H-6-oxo-$PGF_{1\alpha}$ or ^{3}H-$PGF_{2\alpha}$) was added to the plasma followed by cold acetone (3 ml). After shaking the sample, it was centrifuged and the precipitate discarded. Neutral lipids were removed by extraction with hexane (5 ml). The aqueous acetone layer was acidified to pH 4 by addition of 0·5 M citric acid, and the prostaglandins extracted with chloroform (2 × 2·5 ml). The combined chloroform layers were taken to dryness under nitrogen. The residue was dissolved in chloroform-methanol (2:1 v/v; 100 μl) and this solution was quantitatively applied to a lane of a Quantum LQD t.l.c. plate (Pierce Chemical Co.). Unlabelled prostaglandins were applied to separate lanes as standards. After development in the organic phase of ethyl acetate, trimethyl pentane, acetic acid, water (110:50:20:100, v/v/v/v) the standards were visualized by spraying with phosphomolybdic acid in ethanol. This t.l.c. procedure clearly resolves the different bis-enoic prostaglandins and separates the 15-oxo metabolites from their parent prostaglandins. The zones corresponding to the prostaglandin to be assayed were removed from the plate, and the prostaglandin was extracted from the silica gel with 2 × 0·5 ml radioimmunoassay buffer (50 mM Tris buffer at pH 7·5 containing 0·1% gelatin). Aliquots (0·2 ml) of this solution were counted in a liquid scintillation counter to enable estimation of recovery and further aliquots (0·1 ml) were analysed for prostaglandin content by radioimmunoassay.

Radioimmunoassay

Prostaglandin $F_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ were measured by radio-immunoassays (Salmon & Amy 1973; Salmon 1978). Each assay was relatively specific and exhibited only a low cross-reaction with the corresponding 15-oxo metabolites. Prostacyclin quantitatively degrades to 6-oxo-$PGF_{1\alpha}$ during the extraction procedure and was therefore measured as 6-oxo-$PGF_{1\alpha}$.

Aliquots (0·1 ml) of the sample extracts in buffer and dilutions (1:5, 1:10) were mixed with dilution of the appropriate antiserum and approximately 4000 counts min^{-1} of the [^{3}H]prostaglandin. ^{3}H-$PGF_{2\alpha}$ 160 Ci mmol was purchased from the Radiochemical Centre, Amersham and ^{3}H-6-oxo-$PGF_{1\alpha}$ was prepared by incubation of [^{3}H]arachidonic acid (80 Ci $mmol^{-1}$) with ram seminal vesicle microsomes (Salmon 1978). Free and bound prostaglandins were separated by the addition of dextran coated charcoal. The total supernatant was added to 10 ml of scintillation fluid and the radioactivity was determined.

RESULTS AND DISCUSSION

The plasma concentration of 6-oxo-$PGF_{1\alpha}$ following infusion of PGI_2 must derive from either unchanged PGI_2 (which degrades to 6-oxo-$PGF_{1\alpha}$ during extraction procedure) or 6-oxo-$PGF_{1\alpha}$ itself, which may be produced in vivo by non-enzymatic degradation of PGI_2. Any metabolites of PGI_2 containing a 15-oxo-group would decompose to the corresponding derivative of 6-oxo-$PGF_{1\alpha}$; these compounds resolved from 6-oxo-$PGF_{1\alpha}$ during t.l.c. and also exhibit low cross-reactivity in the radioimmunoassay for 6-oxo-$PGF_{1\alpha}$ (Salmon 1978) and would not, therefore, contribute to the measured concentration of 6-oxo-$PGF_{1\alpha}$.

Each prostaglandin rapidly reached a steady-state plasma concentration (Fig. 1). The steady-state concentration of 6-oxo-$PGF_{1\alpha}$ was higher after infusion of 6-oxo-$PGF_{1\alpha}$ than after infusion of prostacyclin in 2 of the 3 dogs but similar in the third (Table 1). The steady state plasma concentration of $PGF_{2\alpha}$ was approximately 10 times lower than the corresponding concentration of 6-oxo-$PGF_{1\alpha}$ reached after infusion of either prostacyclin or 6-oxo-$PGF_{1\alpha}$ (Table 1).

After termination of the infusions the plasma concentration of each prostaglandin declined according to a bi-exponential process. The initial, rapid component (α-phase) which reflects the rate of elimination had a half-life of 0·7–1·6 min for $PGI_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ and 0·5 to 1·4 min for $PGF_{2\alpha}$ (see Table 1). The half-life of the slower component (β-phase), attributed to release of the prostaglandins from sites of distribution was 10·0–19·0 min for PGI_2 and 6-oxo-$PGF_{1\alpha}$ but was less than 10 min for $PGF_{2\alpha}$ (Table 1).

The concentration of 6-oxo-$PGF_{1\alpha}$ measured after infusion of PGI_2 parallelled the decrease in blood pressure (Fig. 2). Neither $PGF_{2\alpha}$ nor 6-oxo-$PGF_{1\alpha}$ modified the blood pressure at the doses employed.

The shorter half-life of $PGF_{2\alpha}$ compared with 6-

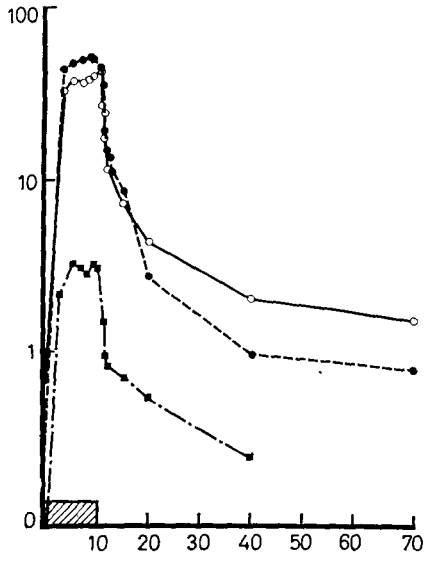

FIG. 1. Concentration of 6-oxo-$PGF_{1\alpha}$ in plasma after infusion of PGI_2 and 6-oxo-$PGF_{1\alpha}$ into dog III at 1 μg min^{-1} kg^{-1} for 10 min. Plasma concentrations of $PGF_{2\alpha}$ obtained after infusion of $PGF_{2\alpha}$ at the same rate into dog III are also plotted. The hatched bar indicates period of the infusion. ○——○ 6-oxo-$PGF_{1\alpha}$ measured after infusion of PGI_2; ●- - - -● 6-oxo-$PGF_{1\alpha}$ measured after infusion of 6-oxo-$PGF_{1\alpha}$ ■— · —■ $PGF_{2\alpha}$ measured after infusion of $PGF_{2\alpha}$. Ordinate: concentration of prostaglandin (ng ml^{-1}). Abscissa: time after commencement of infusion (min).

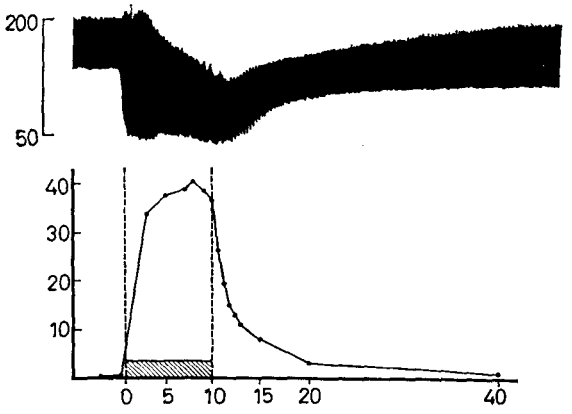

FIG. 2. Comparison of the concentration of 6-oxo-$PGF_{1\alpha}$ detected in plasma with the blood pressure response to an infusion of PGI_2 into dog III at 1 μg min^{-1} kg^{-1} for 10 min. The hatched bar indicates the period of infusion. Upper ordinate: blood pressure (mm Hg). Lower ordinate: concentration of 6-oxo-$PGF_{1\alpha}$ (ng ml^{-1}). Abscissa: time after commencement of infusion (min).

oxo-$PGF_{1\alpha}$ and PGI_2 suggests that $PGF_{2\alpha}$ is eliminated more rapidly than the other compounds. This is also indicated by the fact that the steady-state concentration obtained after infusion of $PGF_{2\alpha}$ is markedly lower than that of 6-oxo-$PGF_{1\alpha}$ obtained after infusions of either PGI_2 or 6-oxo-$PGF_{1\alpha}$. In fact, the differences in the rates of elimination probably reflect differences in the rates of metabolism since the rates of excretion are likely to be comparable.

Differences in rates of metabolism were previously described by Dusting et al (1978b) who demonstrated that unlike the 'classical' prostaglandins (PGE_2 and $PGF_{2\alpha}$) PGI_2 is not rapidly inactivated during passage across the lungs. The hypotensive effects of prostacyclin in rats, rabbits and dogs are similar whether infused into the venous or arterial side of the circulation (Armstrong et al 1977, 1978; Dusting et al 1978b).

In two dogs the steady-state plasma concentration of 6-oxo-$PGF_{1\alpha}$ after infusion of PGI_2 was lower than that obtained after infusion of 6-oxo-$PGF_{1\alpha}$ which suggests that PGI_2 is metabolized more rapidly than 6-oxo-$PGF_{1\alpha}$. The apparently lower metabolic conversion of 6-oxo-$PGF_{1\alpha}$ can be explained by decreased oxidation of the 15-hydroxyl group since 6-oxo-$PGF_{1\alpha}$ is a poor substrate for PGDH in vitro (Sun et al 1978). However, PGI_2 was

Table 1. Steady-state plasma concentrations and $t_{\frac{1}{2}}$ values after infusion of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGI_2 into 3 dogs.

	Dog I (Beagle male 16 kg)			Dog II (Mongrel male 17 kg)			Dog III (Beagle female 9 kg)		
	6-oxo-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGI_2*	6-oxo-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGI_2*	6-oxo-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGI_2*
Mean steady-state plasma concentration (ng ml^{-1})	44·0	2·4	29·2	80·2	6·3	63·9	39·5	3·1	38·5
$t_{\frac{1}{2}}$ α phase (min)	0·8	0·5	0·7	1·6	1·4	1·5	1·2	1·1	1·2
$t_{\frac{1}{2}}$ β phase (min)	19·0	9·9	14·0	13·0	ND	12·8	10·0	ND	14·2

* The concentration of 6-oxo-$PGF_{1\alpha}$ after infusion of PGI_2 is recorded.
ND = Not determined (insufficient data).

a relatively good substrate for PGDH (Sun et al 1978). It is likely that the slow metabolic conversion of prostacyclin in vivo is due to a low uptake into the lungs. Indeed, Bito et al (1977) have reported that pulmonary inactivation of prostaglandins is dependent on an active uptake mechanism which can, for example, distinguish between PGA_1 and $PGF_{2\alpha}$.

Even though prostacyclin and 6-oxo-$PGF_{1\alpha}$ are not metabolized as rapidly as $PGF_{2\alpha}$ they are eliminated rapidly. Dusting et al (1978b) showed that the liver was a major site of inactivation of prostacyclin and the liver does contain high concentrations of enzymes which catalyse β- and ω-oxdiations of prostaglandins. Pace-Asciak et al (1977) demonstrated that the major urinary metabolites of 6-oxo-$PGF_{1\alpha}$ in rats were dinor-6-oxo-$PGF_{1\alpha}$ and dinor-ω-1-hydroxy-6-oxo-$PGF_{1\alpha}$ but that a significant amount (ca 30%) was excreted unchanged.

In conclusion, the data show that prostacyclin and 6-oxo-$PGF_{1\alpha}$ are not as rapidly metabolized as the classical prostaglandin, $PGF_{2\alpha}$. This finding supports the hypothesis that prostacyclin is a circulating hormone (Gryglewski et al 1978; Moncada et al 1978).

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REFERENCES

Änggård, E., Samuelsson, B. (1964) J. Biol. Chem. 239: 4097–4102

Änggård, E., Samuelsson, B. (1966) Ark. Kemi. 25: 293–300

Armstrong, J. M., Chapple, D., Dusting, G. J., Hughes, R., Moncada, S., Vane, J. R. (1977) Br. J. Pharmacol. 61: 136P

Armstrong, J. M., Lattimer, N., Moncada, S., Vane, J. R. (1978) Ibid. 62: 125–130

Bito, L. Z., Baroody, R. A., Reitz, M. E. (1977) Am. J. Physiol. 232: E382–387

Dusting, G. J., Moncada, S., Vane, J. R. (1978a) Eur. J. Pharmacol. 49: 65–72

Dusting, G. J., Moncada, S., Vane, J. R. (1978b) Br. J. Pharmacol. 62: 414–415P

Ferreira, S. H., Vane, J. R. (1967) Nature (London) 216: 868–873

Frölich, J. C. (1976) in: Ramwell, P. W. (ed.) The Prostaglandins, Plenum Press, New York. Vol. 3, pp. 1–39

Gryglewski, R. J., Korbut, R., Ocetkiewicz, A. (1978) Nature (London) 273: 765–767

Hamberg, M., Samuelsson, B. (1971) J. Biol. Chem. 246: 6713–6721

Moncada, S., Gryglewski, R. J., Bunting, S., Vane, J. R. (1976) Nature (London), 263: 663–665

Moncada, S., Korbut, R., Bunting, S., Vane, J. R. (1978) Ibid. 273: 767–768

Moncada, S., Vane, J. R. (1978) Br. Med. Bull. 34: 129–135

Pace-Asciak, C. R., Carrara, M. C., Domazet, Z. (1977) Biochem. Biophys. Res. Commun. 78: 115–121

Salmon, J. A. (1978) Prostaglandins 15: 383–387

Salmon, J. A., Amy, J-J. (1973) Ibid. 4: 523–533

Sun, F. F., McGuire, J. C., Taylor, B. M. (1978) Ibid. 15: 724