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RECIRCULATION OF PROSTACYCLIN (PGI_2) IN THE DOG

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1 The inactivation of prostacyclin (PGI_2) in the circulation of anaesthetized dogs has been studied by the blood-bathed organ bioassay technique.

2 Spiral strips of bovine coronary and rabbit coeliac or mesenteric artery detected concentrations of PGI_2 of 2 to 5 ng/ml. These tissues were insensitive to concentrations at least 200 fold higher of 15-oxo-PGI_2 and 6-oxo-$PGF_{1\alpha}$.

3 PGI_2 assayed on bovine coronary artery, rabbit coeliac artery or rat stomach strip, had a half life in blood of 3.0 ± 0.3 min, indicating non-enzymatic degradation.

4 No disappearance could be detected by bovine coronary artery when PGI_2 was infused across the lungs (0.1 to 0.5 $\mu g\ kg^{-1}\ min^{-1}$). However, PGI_2 was partially inactivated in passage through vascular beds of hindquarters and liver.

5 Of PGI_2 infused into the aorta 35 to 65% escaped inactivation in one complete circulation. Therefore, endogenous PGI_2 released from the lungs may function as a circulating hormone.

Introduction

The powerful vasodilator effects of prostacyclin (PGI_2) have been demonstrated in rats, rabbits (Armstrong, Lattimer, Moncada & Vane, 1978) and dogs (Armstrong, Chapple, Dusting, Hughes, Moncada & Vane, 1977; Dusting, Moncada & Vane, 1978a). Although PGI_2 is unstable (Moncada, Gryglewski, Bunting & Vane, 1976) and its degradation product (6-oxo-$PGF_{1\alpha}$) is much less active (Armstrong, *et al.*, 1977; 1978), the hypotensive effects of PGI_2 *in vivo* were of similar duration to those of prostaglandin E_2 (PGE_2, Armstrong, *et al.*, 1978). Whereas the vasodepressor potency of PGE_2 is much less intravenously than when given into the arterial side of the circulation, PGI_2 is of similar potency by these two routes (Armstrong *et al.*, 1977; 1978). Prostaglandins of the E series are largely inactivated in passage through the lungs (Ferreira & Vane, 1967) and Armstrong, *et al.*, (1978) suggested that prostacyclin escapes pulmonary inactivation.

Recently, Gryglewski, Korbut & Ocetkiewicz (1978) and Moncada, Korbut, Bunting & Vane (1978) have shown that PGI_2 generated in the lungs is continuously released into arterial blood and functions as a circulating hormone. Using bioassay tissues such as bovine coronary artery (Kulkarni, Roberts & Needleman, 1976) which responds characteristically to PGI_2 (Dusting, Moncada & Vane, 1977), we have now examined the stability of PGI_2 in blood, and the fate of circulating PGI_2 in several vascular beds of the dog. Some of these findings have been reported to the British Pharmacological Society (Dusting, Moncada & Vane, 1978b).

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Methods

Experimental animals

Mongrel as well as beagle dogs (8.5 to 27 kg) of either sex were anaesthetized with intravenous thiopentone (25 to 30 mg/kg); anaesthesia was then maintained with chloralose (50 to 80 mg/kg i.v.), supplemented when necessary by additional chloralose (10 mg/kg) or pentobarbitone (2 to 4 mg/kg). Dogs were ventilated artificially by means of CFP model 5255 positive pressure respiratory pump (rate 18 to 20/min), the inspiratory pressure being adjusted to maintain arterial Pco_2 in the range 30 to 38 mmHg; arterial blood pH and Po_2 (measured hourly) were in the range 7.3 to 7.5 pH units and 80 to 110 mmHg respectively. A heating pad was used to maintain rectal temperature at 37 to 39°C. A slow (10 to 30 ml/h)

intravenous infusion of dextran ('Rheomacrodex'; Pharmacia) was maintained throughout the experiments.

Polyethylene cannulae were tied into the carotid or femoral artery and the external jugular vein for removal and replacement of blood. Systemic arterial blood pressure (1 mmHg = 1.333 mbar) was recorded by a transducer (Elcomatic) connected to a cannula in a femoral or brachial (experiments involving infusion into hindquarters) artery. Heparin (1,000 iu/kg) was injected intravenously.

Bioassay tissues

The assay tissues were superfused at 10 ml/min, initially with Krebs solution containing indomethacin (1 μg/ml), and 2 to 2.5 h later with blood from the dog (Vane, 1964). The blood was collected in a reservoir and returned continuously by gravity into the jugular vein. The bioassay organs included gastrointestinal tissues previously used (Ferreira & Vane, 1967) for detection of 'classical' prostaglandins (rat stomach strip, RSS; rat colon, RC) and spiral strips of rabbit aorta (RbA) and rabbit coeliac (RbCA) or mesenteric (RbMA) arteries. In addition we included bovine coronary artery (BCA) and rabbit transverse stomach strip (RbSS) which have been used to distinguish between PGI_2 and PGE_2 in Krebs solution (Dusting *et al.*, 1977; Moncada, Mugridge & Whittle, 1977). Three to five of these tissues were arranged in a single cascade. They were calibrated by the infusion of test substances into the stream of blood (i.b.b.) after it had left the animal or by intravenous or intra-arterial infusions.

Degradation of prostacyclin in blood

An incubation circuit (Ferreira & Vane, 1967) was made of a length of silicone tubing of 3 mm internal bore and of 50 ml capacity and kept at 37°C by a water bath. Blood from an artery first passed through this tubing and then superfused three bioassay tissues (RbCA, BCA and RSS). In six experiments PGI_2 was infused for 3 to 5 min at different points in the circuit so that it was in contact with the circulating blood for 1 to 5 min before being assayed. The responses of the assay tissues were then compared with those produced by infusions given i.b.b. close to the tissues so that there was no delay.

Disappearance of prostacyclin in one circulation through vascular beds

To measure the disappearance in a particular vascular bed, PGI_2 was infused for up to 15 min into the arterial inflow of the vascular bed. The plateau responses of the assay tissues produced by these infusions were compared with responses produced by similar infusions into the blood leaving the vascular bed. For example, if an arterial input infusion of 1 μg kg^{-1} min^{-1} induced a relaxation of BCA similar to that produced by 0.5 μg kg^{-1} min^{-1} given into the venous output there must have been a continuous disappearance of 50% of PGI_2 in the vascular bed under study.

Disappearance of PGI_2 in the lung was measured by comparison of infusions made into the superior vena cava with those made into the base of the ascending aorta or the left ventricle. In these experiments blood for assay was taken from the femoral artery.

To study the disappearance of PGI_2 in the liver or the hindquarters, infusions were made into the portal vein or the abdominal aorta above the iliac bifurcation, respectively and were compared with those made into the superior vena cava.

The disappearance of PGI_2 in the complete circulation was measured by making infusions into the ascending aorta via a coaxial polyethylene catheter. The cardiac output was sampled for assay just above the aortic valves whereas infusions were made through the second catheter, the tip of which was 1 cm downstream. In this way, the infused substance mixed with the total cardiac output and was exposed to a complete circulation before reaching the site of blood sampling. These infusions were compared with those made into the superior vena cava.

Drugs

The following drugs were used: angiotensin II amide (Hypertensin, Ciba), indomethacin (Indocid, Merck, Sharpe & Dohme), (−)-noradrenaline bitartrate (Levophed, Winthrop), prostaglandin E_2 (Cambrian Chemicals). Prostacyclin was obtained as the sodium salt and 6-oxo-$PGF_{1\alpha}$ as the free acid (Johnson, Lincoln, Thompson, Nidy, Mizsak & Axen, 1977; Whittaker, 1977). Prostacyclin was prepared daily as a stock solution in 1 M Tris buffer (pH 8.4 at 5°C) kept at 0°C. 15-oxo-PGI_2 was obtained by base hydrolysis of the methyl ester (Axen, 1978). Prostaglandin solutions were diluted in 50 mM Tris buffer (pH 7.9 at 5°C) immediately before use and kept on ice. Doses of drugs are expressed in terms of the base.

Results

Bioassay of prostacyclin (PGI_2), 15-oxo-PGI_2 and 6-oxo-$PGF_{1\alpha}$

PGI_2 (2 to 40 ng/ml) caused a dose-dependent relaxation of the arterial strips of RbCA, RbMA and BCA. RbA and gastrointestinal tissues were generally less

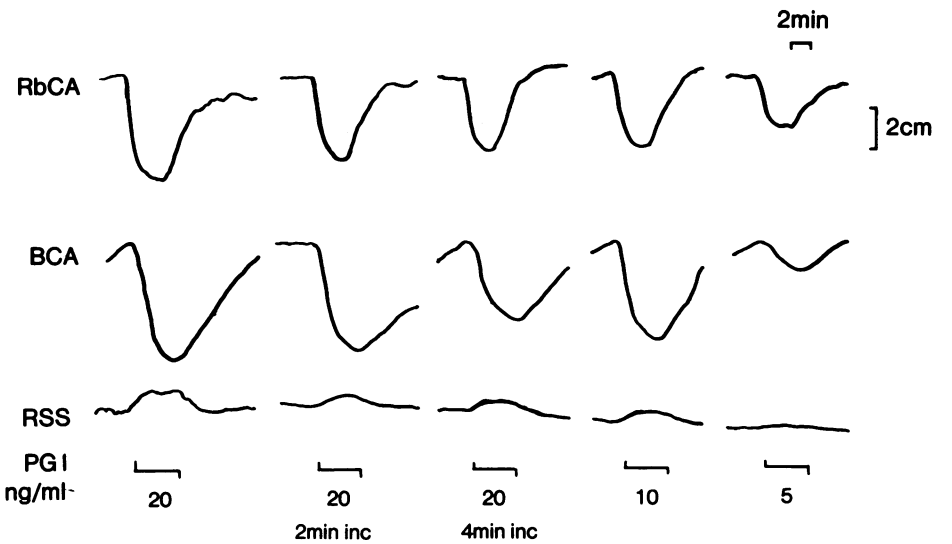

Figure 1 Degradation of prostacyclin (PGI_2) in blood. Spiral strips of rabbit coeliac artery (RbCA) and bovine coronary artery (BCA) and a rat stomach strip (RSS) were bathed in arterial blood from a dog. The response of all bioassay tissues to PGI_2 was diminished by passing it through an incubation coil so it was in contact with the blood for 2 to 4 min (inc) before being assayed. In this case the half-life of PGI_2 was 3.1 min.

sensitive to PGI_2. RSS, RbSS and RC were contracted by PGI_2 (threshold generally above 5 ng/ml) although PGI_2 (10 to 40 ng/ml) reduced spontaneous activity or relaxed 2 out of 8 RCs.

The potency of 15-oxo-PGI_2 on these bioassay tissues was less than 1/200th that of PGI_2, and RSS was more sensitive to 15-oxo-PGI_2 than RbCA or BCA. Similarly, 6-oxo-$PGF_{1\alpha}$ had no effect on the bioassay tissues up to 0.1 μg/ml, and higher concentrations (0.2 to 100 μg/ml) contracted RSS and RC without affecting RbCA or BCA.

Table 1 Degradation of prostacyclin in blood at 37°C

Bioassay tissue	*pH*	*Concentration (ng/ml)*	*$T_{\frac{1}{2}}$ (min)*
RSS	7.42	100	3.8
BCA	7.32	80	2.5
RbCA	7.37	40	2.3
RbCA	7.52	40	4.0
RbCA	7.37	20	3.1
RbCA	7.37	20	2.1
Mean	7.40	50	3.0
s.e. mean	0.03	13	0.3

RSS, rat stomach strip; BCA, bovine coronary artery; RbCA, rabbit coeliac artery.

Degradation of prostacyclin in blood

Incubation of PGI_2 (20 to 100 ng/ml) with blood for 1 to 5 min resulted in loss of activity on all three tissues (Figure 1). The incubation time required for the activity on one of these tissues to be reduced to that of one half the infusion rate i.b.b. (i.e. half-life) was calculated for each experiment (Table 1). There was no obvious correlation between the half-life and blood pH or initial PGI_2 concentration. The mean half-life was 3.0 ± 0.3 min which is similar to that for PGI_2 in aqueous solution (2.8 min), as calculated by extrapolation of the data of Cho & Allen (1978) to comparable conditions of ionic strength, temperature, pH and concentration. Therefore, the inactivation of PGI_2 in blood is probably by a chemical rather than enzymatic mechanism.

Response of bioassay tissues during intravenous infusion of prostacyclin

PGI_2 (0.1 to 0.5 μg kg^{-1} min^{-1}) infused intravenously, reduced blood pressure within 30 s. The hypotension was dose-dependent and was accom-

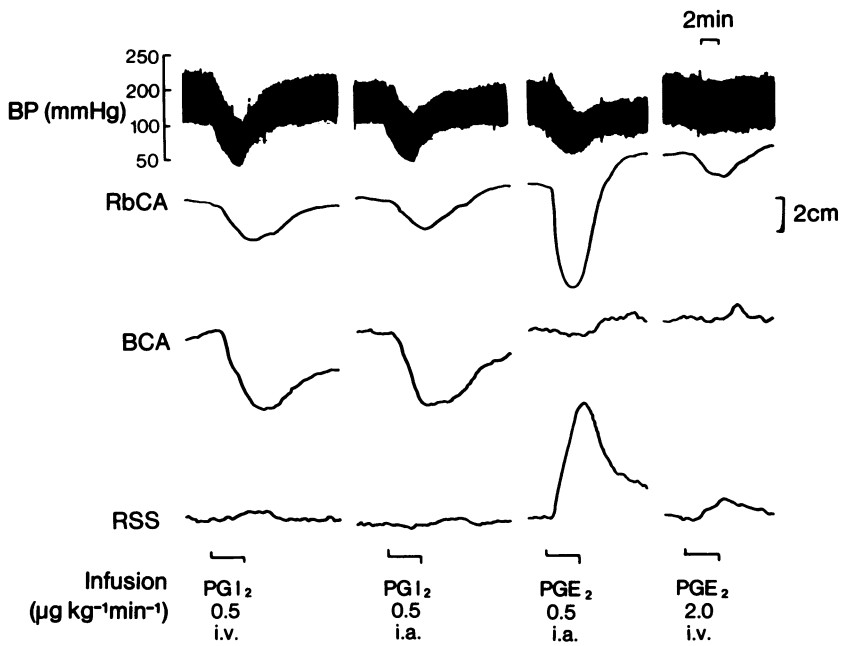

Figure 2 Passage of prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) across the lungs. Bioassay tissues (as in Figure 1) were bathed in arterial blood. PGI_2 infused intravenously (i.v.) caused similar effects on the bioassay tissues and blood pressure (BP) as infusion into the root of the aorta (i.a.), indicating that PGI_2 did not disappear across the lungs. In contrast, more than 75% of PGE_2 was inactivated in passage through the pulmonary circulation.

panied by either tachycardia or bradycardia. With continued infusion the depressor effect was not always maintained. BCA relaxed to a steady state after 10 to 15 min of PGI_2 infusion, but RbCA and RbMA, after initial relaxation, contracted progressively despite continued infusion. RbA, RbSS, RSS and RC contracted during and after PGI_2 infusion. These effects could not be reproduced by i.b.b. infusion of PGI_2 alone, but were closely mimicked by superimposing i.b.b. infusion of noradrenaline (1 to 4 ng/ml) or angiotensin II (1 to 2 ng/ml) or both during i.b.b. infusion of PGI_2 (5 to 20 ng/ml). Therefore, hypotension induced by PGI_2 may be accompanied by circulation of pressor hormones such as catecholamines and angiotensin II. We selected BCA as the most suitable tissue for *in vivo* bioassay of PGI_2 since it was least sensitive to catecholamines and angiotensin II, and was generally the only tissue that responded to intravenous infusion of PGI_2 at the lowest rates.

Disappearance of prostacyclin in vascular beds

Lungs Infusion of PGI_2 into the aorta (0.1 to 0.5 $\mu g\ kg^{-1}\ min^{-1}$) reduced blood pressure and affected all bioassay tissues to a similar extent as intravenous infusions at the same rate (Figure 2). In 8 dogs intra-aortic and intravenous infusions of PGI_2 caused similar steady-state relaxations of BCA. Intra-aortic infusion of PGE_2 (0.2 to 0.5 $\mu g\ kg^{-1}\ min^{-1}$), which had a weaker hypotensive effect than PGI_2, contracted BCA and RSS and relaxed RbCA. In contrast to PGI_2, intravenous infusion of PGE_2 at higher rates had a negligible hypotensive effect and much weaker effects on the bioassay tissues (Figure 2), indicating extensive pulmonary inactivation of this prostaglandin.

Liver Infusions of PGI_2 into the portal vein were made at higher rates (0.2 to 1.0 $\mu g\ kg^{-1}\ min^{-1}$) in order to match the steady state relaxations of BCA produced by intravenous infusion, indicating inactivation of PGI_2 on passage through the liver. A typical record of BCA relaxations during intra-portal infusion is shown in Figure 3. In this experiment BCA relaxation induced by intravenous infusion at 0.25 $\mu g\ kg^{-1}\ min^{-1}$ could be matched only by intra-portal

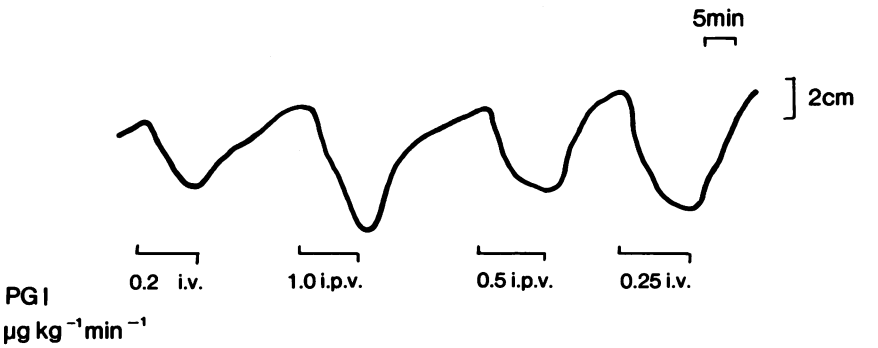

Figure 3 Disappearance of prostacyclin (PGI_2) in passage across the liver. The record from a bovine coronary artery bathed in arterial blood. PGI_2 was infused via a catheter in a femoral vein (i.v.) or in the portal vein (i.p.v.). Relaxations induced by PGI_2 infused i.v. could only be matched by i.p.v. infusions at higher rates indicating inactivation of PGI_2 in the liver.

infusion at a rate of between 2 and 4 fold higher. In 2 dogs it was estimated that the liver removed 73 and 75% of infused PGI_2 in one passage.

Hindquarters PGI_2, infused into the abdominal aorta (0.2 to 1.0 μg kg^{-1} min^{-1}) above the iliac bifurcation, was inactivated in the circulation of the hindquarters. By matching BCA relaxations induced by alternate infusions into the abdominal aorta and intravenously it was estimated that in 4 dogs the hindquarters removed 43, 44, 43 and 61% of PGI_2 present in the aortic blood.

Recirculation of infused prostacyclin

Infusion of PGI_2 (0.2 to 0.6 μg kg^{-1} min^{-1}) into the aorta distal to the point from which blood was withdrawn for bioassay relaxed BCA, although intravenous infusions at the same rate which caused similar hypotension had greater effects on BCA. Therefore a substantial proportion of infused PGI_2 recirculates. In 3 dogs 35, 50 and 65% of PGI_2 infused into the aorta was inactivated in one complete circulation.

Discussion

In rats, rabbits (Armstrong *et al.*, 1978) and dogs (Armstrong *et al.*, 1977) the hypotensive effects of PGI_2 were similar whether it was infused intravenously or into the arterial side of the circulation. In contrast, PGE_2 is a much weaker vasodepressor by the intravenous route because it is extensively inactivated by the lungs (Ferreira & Vane, 1967). We have now obtained direct evidence that PGI_2 is not inactivated in passage across the lungs: relaxations of bovine coronary arteries induced by infusing PGI_2 into arterial blood are not diminished by infusing it through the pulmonary circulation. Moreover, neither the metabolic product of PGI_2 dehydrogenation (Sun, McGuire & Taylor, 1978), 15-oxo-PGI_2, nor of spontaneous hydration, 6-oxo-$PGF_{1\alpha}$, has significant activity on the bioassay tissues.

In other vascular beds the inactivation of PGI_2 in a single passage is comparable to that of PGE_2 (Ferreira & Vane, 1967). Thus the hindquarters and particularly the liver remove some of the PGI_2 which reaches those beds and the inactivation mechanisms for PGE_2 and PGI_2 may be similarly dependent on 15-hydroxy-dehydrogenase. In the lungs, the difference in removal probably reflects differences in uptake, for PGI_2 is a good substrate for prostaglandin dehydrogenase (Sun *et al.*, 1978). Bito, Baroody & Reetz (1977) have shown that pulmonary inactivation of prostaglandins is initially dependent on an active uptake mechanism which can distinguish between PGA_1 and $PGF_{2\alpha}$. Presumably, PGI_2 is not a substrate for this uptake mechanism.

Our results support the suggestion that PGI_2 may be a circulating hormone since about 50% of PGI_2 released from the lungs will reach venous blood. Indeed, Moncada *et al.* (1978) found evidence that both arterial and venous blood contain PGI_2, but that the circulating level is higher in the arterial side of the circulation.

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