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COMPARISON OF THE VASODEPRESSOR EFFECTS OF PROSTACYCLIN AND 6-OXO-PROSTAGLANDIN $F_{1\alpha}$ WITH THOSE OF PROSTAGLANDIN E_2 IN RATS AND RABBITS

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1 Vasodepressor effects of prostacyclin (5*z*-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin F_1) and its decomposition product 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) have been compared with those of prostaglandin E_2 (PGE_2) in anaesthetized rats and rabbits.

2 In rats intravenous prostacyclin produced hypotension and was 4–8 times more potent than PGE_2 and about 128 times more potent than 6-oxo-$PGF_{1\alpha}$.

3 In rabbits also, intravenous prostacyclin (less than 2 μg/kg) produced hypotension and was twice as active as PGE_2 and approximately 250 times more active than 6-oxo-$PGF_{1\alpha}$.

4 In rats and rabbits vasodepressor responses induced by prostacyclin were similar in magnitude after either intravenous or intra-aortic administration.

5 Thus, in both species prostacyclin resembles PGE_2 in producing vasodepression but does not lose activity on passage through the lungs. The results emphasize the need to consider prostacyclin in addition to PGE_2 as a major determinant influencing blood pressure.

Introduction

Prostaglandins synthesized in blood vessels could act locally either directly to influence vascular smooth muscle tension or indirectly by modifying responsiveness to other vasoactive substances. Thus, they could contribute to the regulation of peripheral vascular resistance and blood pressure (Vane & McGiff, 1975). Recently, a hitherto unknown prostaglandin has been discovered (Moncada, Gryglewski, Bunting & Vane, 1976). This agent is the major metabolite of arachidonic acid in the arterial walls of a number of species, including man (Bunting, Gryglewski, Moncada & Vane, 1976; Moncada, Higgs & Vane, 1977). This prostaglandin, originally called PGX, was later identified as 5*z*-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin F_1 and renamed as prostacyclin (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976) or PGI_2.

Prostacyclin is a potent inhibitor of platelet aggregation (Moncada *et al.*, 1976). It also relaxes strips of coeliac and mesenteric arteries of the rabbit (Bunting *et al.*, 1976) and strips of bovine coronary arteries (Dusting, Moncada & Vane, 1977). If these effects correlate with those in vascular beds *in vivo*, prostacyclin should lower systemic arterial blood pressure. We therefore determined the effects of prostacyclin and its degradation product 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) (Johnson *et al.*, 1976) *in vivo* in anaesthetized rabbits and rats. Prostaglandin E_2 (PGE_2) previously thought to be the primary depressor product synthesized from arachidonic acid (Cohen, Sztokalo & Hinsch, 1973) is substantially removed during passage through the lung of these species (Ferreira & Vane, 1967; Papanicolaou & Meyer, 1972). Because of this, we have compared intravenous and intra-arterial injections of PGE_2 on blood pressure with injections of prostacyclin and 6-oxo-$PGF_{1\alpha}$.

Methods

Male Wistar rats (245–275 g) and New Zealand white rabbits, (2–2.5 kg) were used. In rats, anaesthesia was induced with chloroform and thereafter maintained with intravenous chloralose (60 mg/kg). In rabbits anaesthesia was achieved initially with halothane and then maintained with an intravenous dose of a mixture of chloralose (40 mg/kg) and pentobarbitone sodium (2 mg/kg).

Arterial pressure was recorded from the cannulated left femoral artery with a Statham pressure transducer and integrated heart rate by means of a cardiotachometer triggered by the arterial pressure wave. Both variables were displayed on a Beckman Dynograph. Each prostaglandin was injected into the left femoral

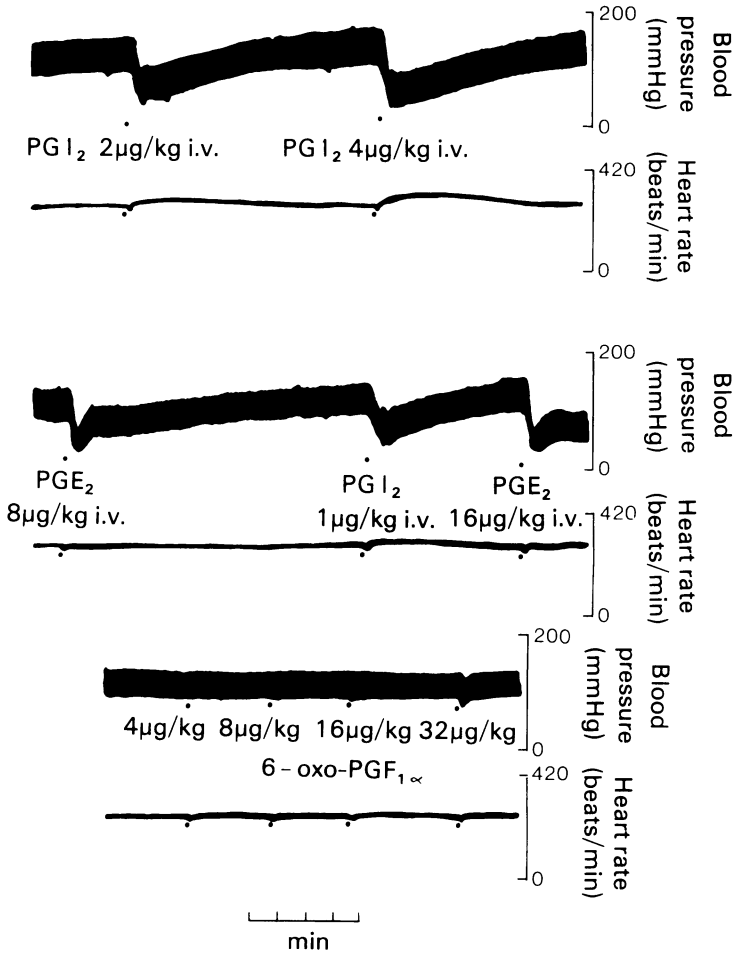

Figure 1 Recordings of blood pressure and heart rate of anaesthetized rats. At the dots the dose of each prostaglandin indicated was injected intravenously. PGI_2 = prostacyclin; PGE_2 = prostaglandin E_2; 6-oxo-$PGF_{1\alpha}$ = 6-oxo-prostaglandin $F_{1\alpha}$.

vein in a logarithmic series of increasing doses and flushed in with 0.1–0.3 ml of 0.9% w/v NaCl solution (saline). Additionally prostacyclin or PGE_2 was injected retrogradely into the aortic arch via a catheter inserted into the left carotid artery to avoid immediate passage through the lungs.

Rats were pithed according to the method of Shipley & Tilden (1947). After a brief period of anaesthesia with chloroform the trachea was cannulated with polyethylene tubing (PP 202) and a stainless steel rod (o.d. 1.59 mm) passed through the left orbit and down the spinal column. Artificial respiration was maintained with a Palmer respiration pump set to deliver a tidal volume of 1 ml/100 g at 70 strokes/minute. Rectal temperature was stabilized at about 38°C by radiant heat. Blood pressure was recorded from the left carotid artery by transducer and displayed together with integrated heart rate on a Beckman Dynograph physiological recorder. Drugs were administered into the cannulated left femoral vein.

Prostacyclin (kindly supplied by Dr N. Whittaker of the Wellcome Research Laboratories) was used as the sodium salt and dissolved in 1 M Tris buffer (pH 8.0) to give a 1 mg/ml solution and stored on ice; subsequent dilutions were made in 50 mM Tris buffer pH 7.6.

PGE_2 (Cambrian Chemicals) and 6-oxo-$PGF_{1\alpha}$ (from Dr N. Whittaker) were made up as stock solutions in ethanol and stored at −20°C. Aliquots of PGE_2 stock were taken, the ethanol removed with

a stream of nitrogen and made up to the required concentration with saline. A similar procedure was used for 6-oxo-$PGF_{1\alpha}$. Pentacynium bismethylsulphate (Presidal, Burroughs Wellcome Co.) was dissolved in saline.

Linear regression analysis was applied to the data using the fall in diastolic blood pressure as a measure of the depressor response. An indication of the duration of the hypotensive effect was obtained by measuring the half life ($T_{\frac{1}{2}}$). This was taken as from the beginning of the response to when it had recovered to half the maximum fall. Differences between means were assessed with Student's t test, significance being accepted with values of probability less than 0.05.

Results

Intravenous administration of prostaglandins to anaesthetized rats

For ten rats, the average (±s.e. mean) basal blood pressure recorded immediately before prostaglandin administration was 146 ± 6 mmHg systolic and 99 ± 5 mmHg diastolic and the mean heart rate was 382 ± 26 beats/minute.

Falls in both systolic and diastolic arterial blood pressure were produced by intravenous administration of prostacyclin (0.125–64 μg/kg) PGE_2 (1.0–64.0 μg/kg) or 6-oxo-$PGF_{1\alpha}$ (16–32 μg/kg), (Figures 1 and 2). The linear portion of the prostacyclin dose-response curve did not differ significantly in parallelism from that for PGE_2. After intravenous injection, prostacyclin was 4–8 times more potent in producing hypotension than PGE_2 and about 128 times more potent than 6-oxo-$PGF_{1\alpha}$. In 3 of 10 animals the depressor response induced by prostacyclin, but not by PGE_2 was followed by a small secondary rise in pressure of between 10–25 mmHg.

Tachycardia (3–66 beats/min) accompanied the hypotension induced by prostacyclin. This effect was probably of reflex origin, for it was reduced in pithed animals given similar intravenous injections of prostacyclin which also caused falls in blood pressure. Responses to PGE_2 were variable, tachycardia (3–20 beats/min) being the usual effect. There was no change in heart rate after 6-oxo-$PGF_{1\alpha}$.

The duration of the hypotension induced by prostacyclin was proportional to the fall in blood pressure and was usually similar to that after PGE_2. Thus, the mean (±s.e. mean) $T_{\frac{1}{2}}$ of a standard fall in blood pressure (20 mmHg) was 0.7 ± 0.5 min for prostacyclin and 0.7 ± 0.1 min for PGE_2. Similarly, after doses of the prostaglandins producing a hypotension of 50–60 mmHg the half lives were 2.2 ± 0.4 min for PGI_2 and 3.2 ± 0.7 min for PGE_2, a difference not significant at the $P < 0.05$ level.

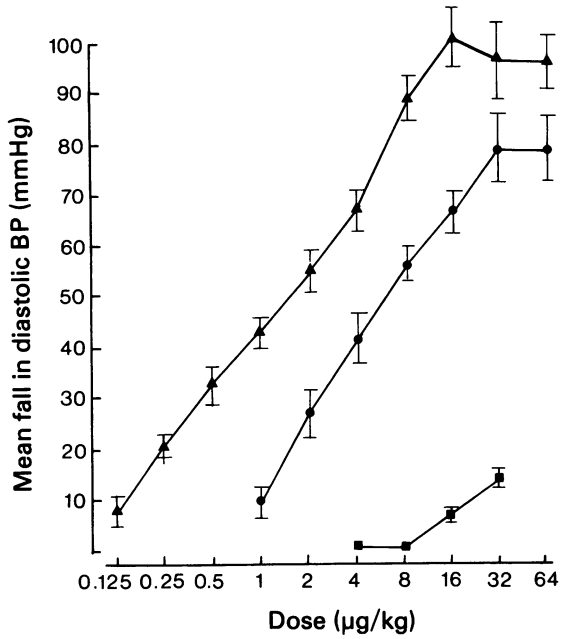

Figure 2 Vasodepressor responses in rats to prostaglandin E_2 (●), prostacyclin ( ) or 6-oxo-prostaglandin $F_{1\alpha}$ (■). Mean falls in blood pressure are plotted against the dose of each agent administered intravenously. Each point is the mean of 4–6 observations. Vertical bars indicate s.e. mean.

Intravenous administration to anaesthetized rabbits

In 9 animals, control blood pressures were 127 ± 5 mmHg systolic and 70 ± 3 mmHg diastolic. Mean heart rates were 289 ± 12 beats/minute. Hypotension occurred after intravenous injection of prostacyclin (0.125–16 μg/kg) or PGE_2 (1–16 μg/kg); 6-oxo-$PGF_{1\alpha}$ (4–32 μg/kg) was relatively inactive (Figure 3). In 4 of 9 animals a slight pressor response (10–30 mmHg) occurred after the fall in blood pressure produced by amounts of prostacyclin greater than 1.0 μg/kg. Low doses (0.5–2.0 μg/kg) but not high doses (4–16 μg/kg) of prostacyclin were more effective in producing hypotension than similar amounts of PGE_2.

As with prostacyclin in rats, tachycardia (3–34 beats/min) accompanied the falls in blood pressure. Changes in heart rate after PGE_2 were variable and 6-oxo-$PGF_{1\alpha}$ was inactive. In animals with their autonomic nerve function blocked with the ganglion

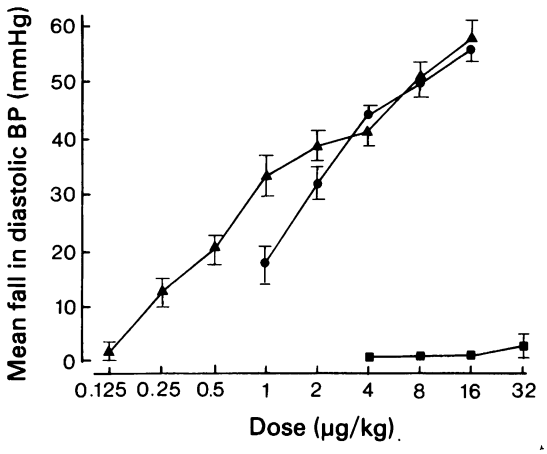

Figure 3 Vasodepressor responses in rabbits to prostaglandin E_2 (●), prostacyclin (▲) or 6-oxo-prostaglandin $F_{1\alpha}$ administered intravenously. Each point is the mean of 6 observations and represents the mean maximum fall in blood pressure. Vertical bars show s.e. mean.

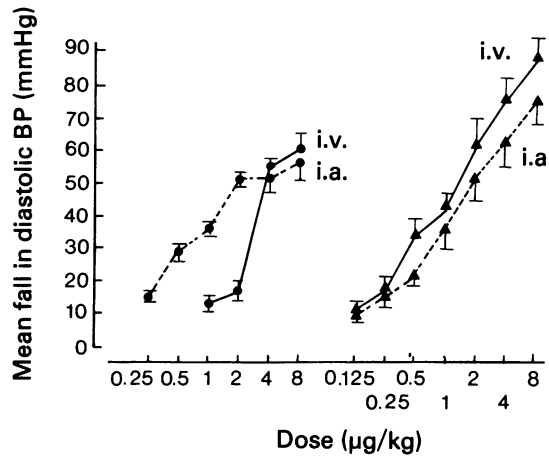

Figure 4 Vasodepressor responses in rats to prostacyclin (▲) or prostaglandin E_2 (●) administered either intravenously (i.v.-complete line) or into the aortic arch (i.a.-dashed line). Each symbol represents the mean observations and shows the maximum fall in the diastolic blood pressure. Vertical bars show s.e. mean. For prostacyclin $n = 6$ and for prostaglandin E_2 $n = 5$ to 10.

blocking agent pentacynium (2 mg/kg i.v.), the tachycardia after prostacyclin was smaller (6–12 beats/min) despite hypotension being evident.

The duration of the hypotension induced by prostacyclin was similar to that for PGE_2. The $T_{\frac{1}{2}}$ of the response after a 30 mmHg fall in blood pressure was 0.6 ± 0.1 min with prostacyclin and 0.8 ± 0.05 min with PGE_2 ($P > 0.05$). For a 50 mmHg response the $T_{\frac{1}{2}}$ was 2.7 ± 0.6 min for prostacyclin and 2.6 ± 0.8 min for PGE_2 ($P > 0.05$).

Comparison of the intra-aortic and intravenous routes of administration to rats or rabbits

The fall in diastolic blood pressure, its duration and the increase in heart rate induced by injections of prostacyclin were similar, given either intravenously or intra-arterially. Figure 4 shows this effect in rats.

Discussion

Prostacyclin is the primary metabolite of arachidonic acid in blood vessels of all species so far tested, including man. (Moncada *et al.*, 1977). We have now shown that prostacyclin is a powerful vasodepressor substance. These results must question the supposition that PGE_2 could be the predominant endogenous prostaglandin responsible for arterial dilatation (Cohen *et al.*, 1973). Of the other metabolites of arachidonic acid, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is pressor in rats *in vivo* (Ducharme & Weeks, 1966; Horton, 1969) and endoperoxides contract vascular tissue *in vitro* (Tuveno, Strandberg, Hamberg & Samuelsson, 1976; Moncada, Needleman, Bunting & Vane, 1976).

Attribution of effects to endogenous formation of PGE_2 has often been based on bioassay with a rat stomach strip and chick rectum. These tissues by themselves do not readily differentiate between PGE_2, prostacyclin or its stable metabolite 6-oxo-$PGF_{1\alpha}$. The cross reactivity with prostaglandins of the E series using radioimmunoassay remains to be clarified.

When exogenous cofactors (e.g. glutathione or hydroquinone) are added to enzymes which metabolize arachidonic acid, there is a substantial shift from prostacyclin to PGE_2 production (Cottee, Flower, Moncada, Salmon & Vane, 1977).

The assumption that the cardiovascular effects of arachidonic acid are due to metabolism to PGE_2 is also difficult to maintain in the light of the potent depressor effects of prostacyclin. In dogs, hypotension after injection of arachidonic acid is delayed as compared with that of PGE_2 or prostacyclin, suggesting that time is needed for conversion of arachidonic acid to an active metabolite. Moreover, bovine coronary artery strips are contracted by PGE_2, PGD_2, $PGF_{2\alpha}$ or endoperoxides but relaxed by arachidonic acid (Dusting *et al.*, 1977; Needleman, Raz & Isakson,

1977; Raz, Isakson, Minkes & Needleman, 1977). Reductions in coronary arterial pressure are produced by prostacyclin but not PGE_2 infused into rabbit hearts *in vitro* (Moncada, Shrör, Ubatuba & Vane, unpublished observations).

In rats, prostacyclin was 4–8 times more potent than PGE_2 in producing hypotension and more than 100 times more active than 6-oxo-$PGF_{1\alpha}$. However, the duration of the hypotension for prostacyclin was similar to that for PGE_2. This occurred despite the fact that prostacyclin is known to be less stable than PGE_2, spontaneously decomposing to its much less active metabolite.

PGE_2 is inactivated during passage through the pulmonary blood vessels (Ferreira & Vane, 1967) although this process can be saturated by high doses (Papanicolaou & Meyer, 1972; Armstrong, Boura, Hamberg & Samuelsson, 1976). Pulmonary inactivation does not occur in rats and rabbits with prostacyclin, for the dose-response curves obtained upon intravenous injection did not differ from those obtained after intra-aortic administration. However, in contrast, substantially lower intra-arterial amounts of PGE_2 (less than 3 μg/kg) in rats are needed for a threshold hypotensive response than are necessary intravenously for a response of similar size (Armstrong *et al.*, 1976; Strand, Miller & McGiff, 1974).

The endoperoxide intermediates prostaglandins G_2 and H_2 are vasodepressor in rats (Armstrong *et al.*, 1976) but the assumption that PGE_2 is the agent responsible for the effect should now be reassessed following the discovery of prostacyclin. Moreover, the role of prostaglandins in the control of blood pressure and the contribution made by abnormal prostaglandin mechanisms to hypertensive disease should take into account that a deficiency of prostacyclin and not PGE_2 could be a major factor causing the elevated tension developed in vascular smooth muscle and augmented vessel responsiveness to stimuli.

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