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Effect of vasoactive peptides on prostacyclin synthesis in man

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1 Bradykinin, angiotensin II, arginine vasopressin (AVP) or des-amino-D-arginine vasopressin (DDAVP) were administered by intravenous infusion to 10 healthy men.

2 The concentration of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), the stable hydrolysis product of prostacyclin (PGI_2), was measured in plasma using gas chromatography/negative ion chemical ionisation mass spectrometry.

3 Dose-related increases in plasma concentrations of 6-oxo-$PGF_{1\alpha}$ occurred during administration of bradykinin (100–3200 ng kg^{-1} min^{-1}). The concentrations of 6-oxo-$PGF_{1\alpha}$ rose from baseline values in the range < 1.0–4.9 pg ml^{-1} to 24.9–47.6 pg ml^{-1} at maximum tolerated infusion rates.

4 There were no changes in the concentrations of 6-oxo-$PGF_{1\alpha}$ during administration of angiotensin II, AVP or DDAVP at infusion rates which caused haemodynamic changes.

Introduction

Prostaglandin I_2 (prostacyclin; PGI_2) is the principal eicosanoid synthesized by blood vessels. It is a vasodilator and the most potent endogenous inhibitor of platelet aggregation known (Moncada & Vane, 1979). Low concentrations of PGI_2 (< 3 pg ml^{-1} measured as its stable hydrolysis product, 6-oxo-$PGF_{1\alpha}$) are present in the circulation of normal subjects (Blair *et al.*, 1982) but local synthesis at sites of endothelial cell damage may be an important protective mechanism against vascular occlusion. We have previously demonstrated that local irritative mechanical or chemical stimuli can cause synthesis of PGI_2 by human veins *in vivo* but concentrations in blood sampled at the site of stimulation are too variable to provide a satisfactory measure of vascular PGI_2 responsiveness (Dollery *et al.*, 1983; Ritter *et al.*, 1983). The object of the present study was to find a more reproducible stimulus of PGI_2 synthesis *in vivo* in man. We have infused a number of vasoactive peptides which stimulate PGI_2 synthesis by isolated tissues and cells (Grodzinska & Gryglewski, 1980; Hong, 1980; Shebuski & Aiken, 1980; Alhenc-Gelas *et al.*, 1982; Hassid & Williams, 1983) or which generate PGI_2-like activity/immunoreactive 6-oxo-$PGF_{1\alpha}$ in the circulation (Mullane & Moncada, 1980; Belch *et al.*, 1982; Greaves & Preston, 1982). These peptides were angiotensin II, arginine vasopressin (AVP), 8-des-amino-D- arginine vasopressin (DDAVP) and bradykinin.

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Methods

Subjects

Ten healthy men, aged 28–39 years and weight 65–86 kg, participated in these studies. Protocols were approved by the Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital. Subjects refrained from caffeine containing products for at least 6 h, and any medication for at least 10 days before study.

Protocols

Subjects were studied after an overnight fast and remained supine throughout. Peptides or saline control were administered by a Braun constant infusion pump via an indwelling venous cannula in one forearm. Blood was sampled from a venous cannula in the opposite arm. Heart rate was monitored using a

Grass Polygraph 7D ECG monitor and blood pressure was measured indirectly at 5 min intervals during the infusions using a Roche Arteriosonde. Once subjects had established basal haemodynamic conditions, saline was infused for 15 min before administration of active compound. In later studies, this control period was reduced to 5 min. Bradykinin, angiotensin II and AVP were each administered to a minimum of 3 subjects at incremental infusion rates in the range 100–3200 ng kg^{-1} min^{-1}, 4–32 ng kg^{-1} min^{-1} and 0.002–0.16 iu kg^{-1} min^{-1}, respectively. Each infusion period lasted 5 min. Three subjects were infused with DDAVP for 15 min at a rate of 400 ng kg^{-1} min^{-1}. An assessment of facial flushing (where appropriate) was made by an independent observer at the end of each infusion period.

During the final min of each infusion, or at 5 min intervals during infusion of DDAVP, blood (ca. 20 ml) was withdrawn for measurement of 6-oxo-$PGF_{1\alpha}$ and in the case of DDAVP, further samples (ca. 5 ml) were taken for measurement of Factor VIII.

Sample collection

Blood for prostaglandin analysis was collected into ice-cold lithium heparin tubes which were centrifuged immediately (1000 *g*; 4°C; 10 min). Plasma (10 ml) was separated and an internal standard of 2 ng [$^2H_4$]-6-oxo-$PGF_{1\alpha}$ was added. Blood samples for Factor VIII analysis were anticoagulated with trisodium citrate (3.8% w/v; 9 vol blood: 1 vol citrate) and centrifuged as above. All plasma samples were stored at − 20°C. Concentrations of 6-oxo-$PGF_{1\alpha}$ were determined by a method based on capillary column gas chromatrography/negative ion chemical ionisation mass spectrometry (GC/NICIMS) as described elsewhere (Blair *et al.*, 1982). Factor VIII activities were measured by a method based on kaolin caphalin clotting time (Denson, 1976).

Statistical analyses

Results are expressed as means ± s.e.mean, where *n* is the number of values. An analysis of variance was used to compare haemodynamic measurements obtained during control infusions with those during infusions of peptide. The relationship between dose and plasma concentrations of 6-oxo-$PGF_{1\alpha}$ was determined using Spearman rank-correlation coefficient. Correlations were considered significant when $P < 0.05$.

Drugs

Bradykinin (RIA Biochemicals Ltd), angiotensin II (Ciba-Geigy Pharmaceuticals), AVP (Pitressin; Parke Davis & Co.) and DDAVP (Desmopressin; Ferring Pharmaceuticals Ltd.) were administered as pyrogen-free sterile preparations in 0.9% w/v NaCl solution (saline).

Results

Plasma concentrations of 6-oxo-$PGF_{1\alpha}$, in samples taken following infusion of saline, were in the range, < 1.0–4.9 pg ml^{-1} (where 1.0 pg ml^{-1} is the limit of detection). Dose-related increases ($P < 0.05$) in the plasma concentrations of 6-oxo-$PGF_{1\alpha}$ occurred during infusions of bradykinin. Typical selected ion monitoring (SIM) chromatograms of 2 plasma extracts assayed for 6-oxo-$PGF_{1\alpha}$ by GC/NICIMS are illustrated in Figure 1. Concentrations rose from baseline values, following saline infusions, in the range < 1.0–4.9 pg ml^{-1} to 24.9–47.6 pg ml^{-1} at maximum tolerated infusion rates (Figure 2).

In a further experiment, 2 subjects were infused with bradykinin (800 ng kg^{-1} min^{-1}) for 20 min to determine the time course of the response. In both subjects, plasma 6-oxo-$PGF_{1\alpha}$ rose to a plateau (30.9 and 9.0 pg ml^{-1}) within 5 min. On cessation of the infusion, plasma 6-oxo-$PGF_{1\alpha}$ concentrations returned to baseline within 5 min.

Cardiovascular responses to bradykinin, shown in figure 3, are typical of a systemic vasodilator and are comparable with previously published data (Fox *et al.*, 1961). Heart rate rose in a dose-related manner ($P < 0.01$); (basal value 67.8 ± 2.1 ($n = 6$) rising to 91.8 ± 2.6 ($n = 5$) beats min^{-1} at 1600 ng kg^{-1} min^{-1}) but the small increase in pulse pressure did not achieve statistical significance (basal value 56.2 ± 5.6 ($n = 6$) rising to 63.2 ± 10.1 ($n = 5$) at 1600 ng kg^{-1} min^{-1}). Symptoms caused by bradykinin infusions were facial flushing, burning sensation, headache and swelling around the eyes and lips. All but one subject tolerated doses up to 1600 ng kg^{-1} min^{-1} and one tolerated a further dose of 3200 ng kg^{-1} min^{-1}.

The vasoconstrictors, angiotensin II and AVP increased systolic blood pressure while DDAVP, which is a vasodilator, reduced diastolic blood pressure (8 ± 1 mmHg) and increased heart rate (26 ± 7 beats-min^{-1}). Factor VIII activity had increased 6 fold by the end of infusions of DDAVP (range 1.4–1.5 iu ml^{-1}, rising to 8.0–8.5 iu ml^{-1}). However, no dose-related increases in the plasma concentrations of 6-oxo-$PGF_{1\alpha}$ occurred. Values were in the range < 1.0–6.3 pg ml^{-1} with all but two of these values lying within the basal range. We have investigated two further vasodilators in single experiments. Intravenous histamine (128 ng kg^{-1} min^{-1}; 20 min) and calcitonin-gene-related peptide (25 ng kg^{-1} min^{-1}; 10 min) caused decreases in diastolic blood pressure of 23 and 19 mmHg and increases in heart rate of 14 and 24 beats min^{-1} respectively. No changes in the plasma concentrations

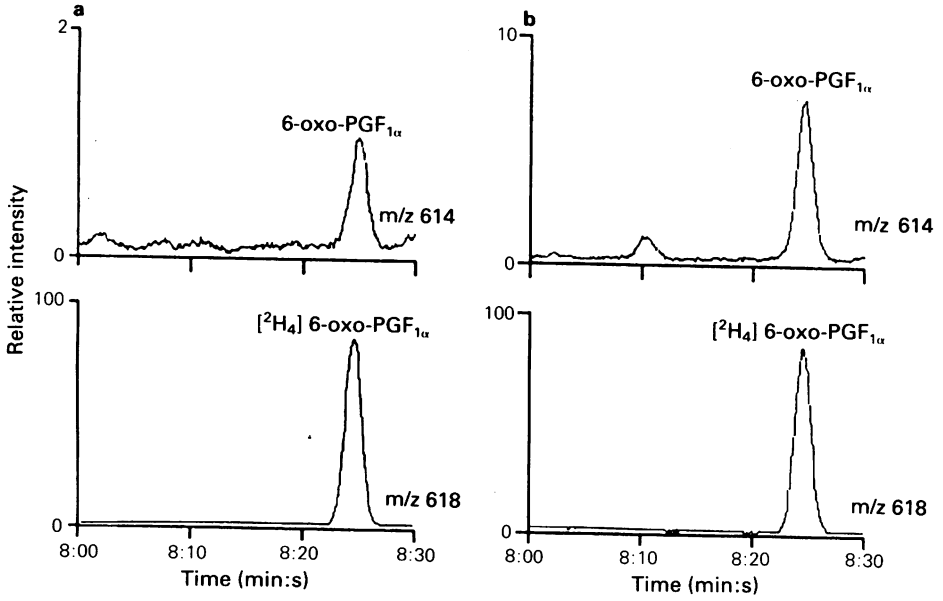

Figure 1 Selected ion chromatrograms of plasma extracts. (a) During infusion of saline; concentration of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) = 1.8 pg ml^{-1}. (b) During infusion of bradykinin (800 ng kg^{-1} min^{-1}); concentration of 6-oxo-$PGF_{1\alpha}$ = 17.1 pg ml^{-1}. Note that the relative ion intensities are scaled 0–100 for the internal standard and 0–2 and 0–10 for 6-oxo-$PGF_{1\alpha}$.

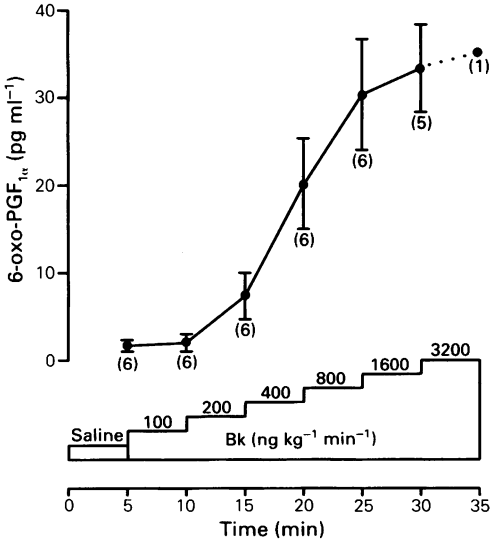

Figure 2 Plasma concentrations of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) during infusions of bradykinin (Bk). Values of baseline samples below the limit of detection of 1.0 pg ml^{-1} were taken as equal to 1.0 pg ml^{-1}. Each point represents the mean, with vertical lines showing s.e.mean, of *n* (number in parentheses) values.

of 6-oxo-$PGF_{1\alpha}$ occurred.

Discussion

In these experiments, PGI_2 synthesis was assessed by measurement of plasma concentrations of 6-oxo-$PGF_{1\alpha}$, in preference to measurement of urinary metabolites, to maximize the endothelial contribution to the measurement. Samples were quantified using an assay based on GC/NICIMS which overcomes many of the problems of specificity associated with measurement of PGI_2 and 6-oxo-$PGF_{1\alpha}$ in blood and plasma by bioassay or radioimmunoassay (Blair *et al.*, 1983).

Bradykinin caused a dose-related increase in PGI_2 synthesis, as reflected in plasma concentrations of 6-oxo-$PGF_{1\alpha}$, during infusions. The increase was rapid and had reached a plateau within 5 min which is consistent with a site of action of bradykinin near the luminal surface of blood vessels rather than in deeper layers. The possibility that bradykinin stimulates synthesis of PGI_2 by leucocytes was excluded by incubation of freshly heparinised whole blood with bradykinin (final concentration 1.5 μg ml^{-1}) at 37°C for 15 min. No 6-oxo-$PGF_{1\alpha}$ was detected in plasma isolated from these samples.

Bradykinin stimulates PGI_2 synthesis by cultured

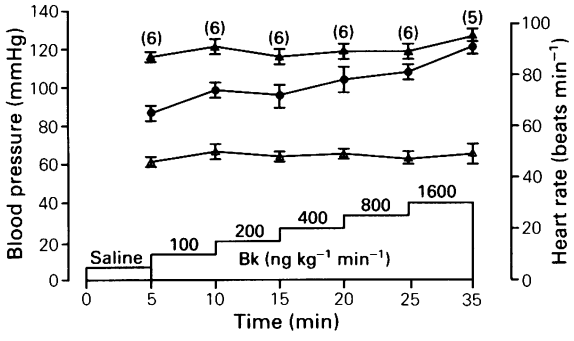

Figure 3 The effect of infusion of cumulative doses of bradykinin (Bk) upon systolic blood pressure (▲), diastolic blood pressure (△) and heart rate (●). Each point represents the mean, with vertical lines showing s.e.mean, of *n* (number in parentheses) values.

human endothelial cells by liberating arachidonate from membrane phospholipids (Hong, 1980; Alhenc-Gelas *et al.*, 1982). However, we considered the possibility that increased plasma concentrations of 6-oxo-$PGF_{1\alpha}$ caused by bradykinin *in vivo* could be due to inhibition of metabolism. The basal rate of PGI_2 synthesis is $0.08\,ng\,kg^{-1}\,min^{-1}$ (FitzGerald *et al.*, 1981). It is therefore unlikely that increases of the magnitude we observed (Figure 2) could be caused by this mechanism. The volume of distribution of 6-oxo-$PGF_{1\alpha}$ is approximately 8 litres in a 70 kg man (Patrono *et al.*, 1982). Even if elimination were totally blocked, accumulation of 6-oxo-$PGF_{1\alpha}$ within 5 min would therefore be no greater than $3.5\,pg\,ml^{-1}$. Furthermore, in our time course experiments, we found that there was no prolongation of the elimination half-life of 6-oxo-$PGF_{1\alpha}$ immediately after bradykinin infusion.

The systemic effects of angiotensin II, AVP and DDAVP are not mediated by increases in circulating PGI_2 but we do not exclude the possibility that these peptides may stimulate vascular PGI_2 synthesis in the muscular media where local concentrations of PGI_2 might control or modulate regional blood flow. The overflow of such PGI_2 into the circulation may be too low to appear as a change in concentrations of 6-oxo-$PGF_{1\alpha}$ in peripheral venous plasma.

Angiotensin II stimulation of PGI_2-like activity has previously been found *in vivo* in the dog using infusion rates of $0.25-1.0\,\mu g\,kg^{-1}\,min^{-1}$ (Mullane & Moncada, 1980) and in isolated animal tissues (Grodzinska & Gryglewski, 1980; Shebuski & Aiken, 1980; Dusting *et al.*, 1982). Immunoreactive 6-oxo-$PGF_{1\alpha}$ has been detected following AVP stimulation of cultured rat vascular smooth muscle cells (Hassid & Williams, 1983). However, cultured human endothelial cells do not produce PGI_2 on treatment with angiotensin II or AVP (Alhenc-Gelas *et al.*, 1982). Furthermore, there is no increase in 2,3-dinor-6-oxo-$PGF_{1\alpha}$ in human urine during activation of the renin-angiotensin system (Watson *et al.*, 1984). The positive findings may reflect species differences or be due to exposure to concentrations of peptide higher than would be tolerated *in vivo* in man.

There has been controversy concerning the proposal that DDAVP stimulates PGI_2 production by vascular endothelial cells *in vivo*. Greaves & Preston (1982) and Belch *et al.* (1982) have reported increases in plasma concentrations of immunoreactive 6-oxo-$PGF_{1\alpha}$ during infusion of DDAVP in normal subjects and patients with mid haemophilia whereas D'Angelo *et al.* (1983) observed no changes. In this study, we adopted a similar protocol for administration of DDAVP and found no elevations in plasma concentrations of 6-oxo-$PGF_{1\alpha}$ during infusions. Since the vasodilator effects of DDAVP are said to be abolished by pretreatment with aspirin (Belch *et al.*, 1982), we considered the possibility that a vasodilator prostanoid other than PGI_2 might be involved. In a single experiment, we measured PGE_2 and PGD_2 in plasma samples but neither were present either during or following infusion (detection limit = $5\,pg\,ml^{-1}$).

The stimulation of PGI_2 by bradykinin appears to be a specific response unrelated to changes in vessel wall diameter since the other vasodilator peptides caused marked decreases in diastolic blood pressure and increases in heart rate but failed to cause any increase in plasma concentrations of 6-oxo-$PGF_{1\alpha}$.

We do not envisage a therapeutic use for bradykinin in the control of vascular disease but this peptide may have a use as a probe of PGI_2 production *in vivo* in pathological states. This approach, however, will require cautious interpretation since the metabolism of bradykinin by endothelium may be altered in disease. We are currently using bradykinin infusions to study cyclo-oxygenase inhibition and recovery *in vivo* (Barrow *et al.*, 1985; Heavey *et al.*, 1985).

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