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Effect of prostaglandin D_2 on histamine-induced weals in human skin

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1 The effect of prostaglandin D_2 (PGD_2) on weal response to intradermal injection of histamine was examined in normal volunteers.

2 Co-injection of variable amounts of PGD_2 with a fixed dose of histamine (2.5 nmol) was made so that the molar ratio of histamine: PGD_2 covered the range 15 to 1215. There was no significant effect of PGD_2 on weal area.

3 PGD_2, present at a 50 times lower concentration than histamine, had no effect on either the dose-response curves for the histamine-induced weal area or the forearm weal volume response to 2.5 nmol histamine.

4 It is concluded that PGD_2, when present at the relative amounts found *in vitro* and *in vivo*, has no effect on the weal response to histamine in man.

Introduction

Prostaglandin D_2 (PGD_2) is the major prostaglandin produced when human mast cells degranulate (Lewis *et al.*, 1982) and in man is able to cause bronchoconstriction (Hardy *et al.*, 1984), vasodilatation (Heavey *et al.*, 1984), and weal formation (Soter *et al.*, 1983). It has therefore been proposed as an important inflammatory mediator.

Immunological challenge of human mast cells or lung parenchyma *in vitro* leads to the release of between 30 and 600 times more histamine than PGD_2 (Schulman *et al.*, 1981; Lewis *et al.*, 1982). *In vivo*, concentrations of histamine in blood draining urticated skin are 271 to 1242 times higher than those of PGD_2 (Heavey *et al.*, 1985). PGD_2 and histamine are approximately equipotent in the production of weals in human skin (Soter *et al.*, 1983). It is therefore unlikely that PGD_2 contributes significantly through direct vascular effects to the weal formation seen following immunological mast cell degranulation. However, PGD_2 is able to potentiate vascular permeability induced by histamine in rat skin (Flower *et al.*, 1976); therefore, we have investigated its ability to potentiate histamine-induced weals in man.

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Methods

Subjects

A total of 18 subjects (2 female), aged 22–40 years was examined. All gave written, informed consent and the study was approved by the Ethics Committee of Hammersmith Hospital and Royal Postgraduate Medical School. No drugs, with the exception of the oral contraceptive, were taken for at least 3 days before any study.

Intradermal injections

All intradermal injections were made in a volume of either 50 or 20 μl as stated, with a 27 gauge needle. Each weal was formed by a single injection, appropriate stock concentrations of histamine and PGD_2 mixtures being made beforehand. For measurement of weal volume, injections were made on the volar aspect of the forearm, all other injections being made in the back.

Weal area and volume

Weals were measured 10 min after injection. A ball-point pen was used to draw around any raised or indurated area and the image transferred to plain

paper with clear adhesive tape. The area was subsequently measured with a digitising pad and calculated according to the trapezoid rule. To estimate weal thickness, the skin was drawn up in a fold between the jaws of a micrometer screw gauge. The gauge was closed with a ratchet operating at fixed tension. Skin fold thickness was also measured before injection and weal thickness taken as half the difference between the 10 min and pre-injection values. Volume was calculated as area × thickness. Measurements were made by an observer who was unaware of the nature of each injection. During measurements of skin thickness the micrometer gauge was not visible to the observer.

Protocols

(a) *Variable ratio PGD_2: histamine* A total of 8 × 50 μl injections was given to each of 9 subjects. Injections were given in randomised order, 4 each side of the midline. A fixed dose of 2.5 nmol histamine was combined with variable amounts of PGD_2 so that the molar ratio of histamine: PGD_2 covered the range 15 to 1215 (167 to 2.06 pmol PGD_2). Control injections of saline, histamine alone (2.5 nmol), and PGD_2 alone (167 pmol) were made.

(b) *Dose-response to histamine* A total of 10 × 20 μl injections were given to each of 8 subjects. Histamine was injected in doses of 0.33, 1.0, 3.0 and 9.0 nmol with or without PGD_2 present at a 50 times lower dose (6.6, 20, 60, 180 pmol). Saline and PGD_2 alone (180 pmol) were included as controls. Order of injection was randomised, but corresponding injections of histamine alone or histamine with PGD_2 were made at the same level either side of the midline.

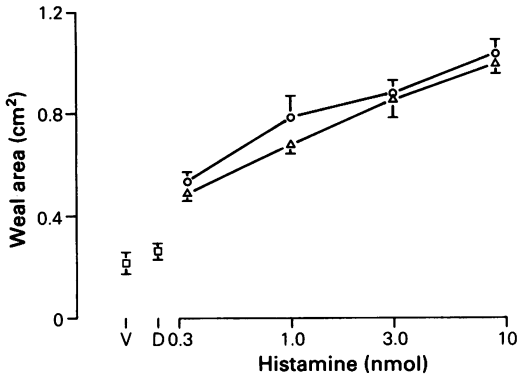

Figure 2 Dose-response curves for the weal area induced by histamine alone (Δ) and histamine plus a 50 fold lower concentration of prostaglandin D_2 (PGD_2; O). V = vehicle (saline), D = PGD_2 (180 pmol). Injections were made in a volume of 20 μl to reduce the control weal areas. Each point represents the mean and vertical lines indicate s.e.mean; $n = 8$.

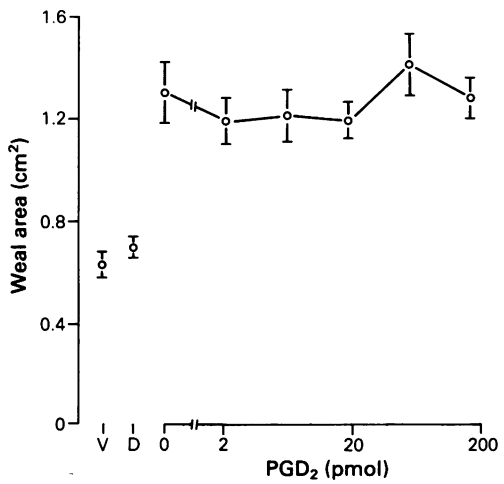

Figure 1 Effect on weal area of variable amounts of prostaglandin D_2 (PGD_2) coinjected with 2.5 nmol histamine. V = vehicle (saline), D = PGD_2 (167 pmol). Each point represents the mean and vertical lines indicate s.e.mean; $n = 9$. There is neither an overall effect of PGD_2 on the histamine-induced weal area (analysis of variance), nor a significant effect at any single ratio of PGD_2 to histamine (paired t test).

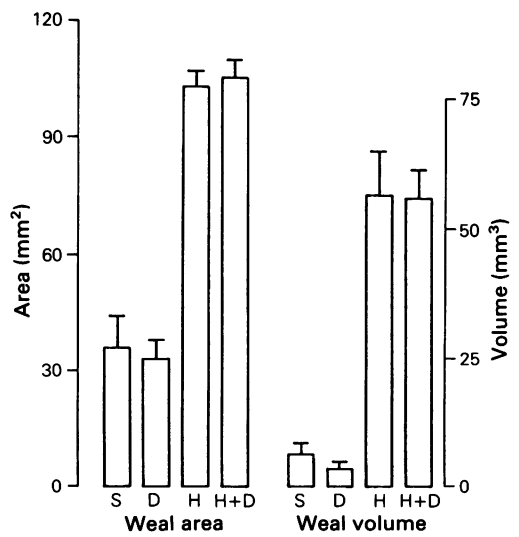

Figure 3 Forearm weal areas and volumes produced by 2.5 nmol histamine alone (H) and in the presence of 50 pmol prostaglandin D_2 (PGD_2) (H + D). V = vehicle (saline), D = PGD_2 (50 pmol). Columns represent mean values with s.e.mean bars; $n = 12$.

(c) *Weal volume* Four 50 μl injections were made in each of 12 subjects. Histamine was injected either alone (2.5 nmol) or in combination with PGD_2 at a concentration (50 pmol) 50 times less than that of histamine. Saline and PGD_2 alone (50 pmol) were included as controls. One control and one histamine-containing injection were made in each forearm, corresponding injections being made at the same distance from the elbow.

Statistics

Weal areas and volumes were compared by the paired, two tailed t test. Analysis of variance was used to determine whether there was an overall effect of PGD_2 in the variable dose study. Significance was taken as $P < 0.05$.

Drugs

Histamine was injected as the acid phosphate (British Pharmacopoeia). PGD_2 was supplied as a sterile solid (Glaxo Group Research, Herts) and stored at − 80°C as a 60 μM solution in sterile isotonic saline. Concentration of PGD_2 was checked by gas chromatography/mass spectrometry (Waddell *et al.*, 1984). All dilutions of histamine and PGD_2 were freshly made in sterile isotonic saline.

Results

Injection into the skin of the back of 2.5 nmol histamine in a 50 μl volume produced a weal of mean area 1.30 ± 0.12 cm^2 (± s.e.mean). The weal area was not affected by co-injection of PGD_2 in amounts ranging from 2.06 to 167 pmol representing molar ratios histamine: PGD_2 of between 1215 and 15 to 1 (Figure 1).

Weal area produced by histamine injected in a 20 μl volume over the dose range 0.33 to 9.0 nmol was not affected when PGD_2 was present at a 50 times lower concentration (Figure 2).

In forearm skin neither weal area nor volume produced by 2.5 nmol histamine was affected by the presence of PGD_2 at a concentration 50 times lower than that of histamine (Figure 3).

Discussion

In our first study we examined the effect of PGD_2 on a single dose of histamine (2.5 nmol) which lies on the linear part of the histamine weal dose-response curve. Variable amounts of PGD_2 were used to cover the range of ratios found *in vivo* and *in vitro*. No significant effect of PGD_2 was found either overall or at any particular ratio. There was a small increase in weal size at a histamine: PGD_2 ratio of 45:1 and so the effect of a similar ratio on the histamine dose-response curve was studied. There was no effect of PGD_2 on histamine-induced weal formation at any part of the curve. A well recognized interaction of inflammatory mediators in skin is the potentiation of bradykinin-induced weals by the vasodilator PGE_2. This is due almost entirely to an increase in weal thickness rather than area (Basran *et al.*, 1982). We therefore examined the effect of PGD_2 on histamine-induced weal area and volume in the forearm but again found no potentiation.

It is concluded therefore that, when present in the relative amount seen following mast cell degranulation, PGD_2 is unable to potentiate histamine-induced weal formation in human skin. The discrepancy between this result and that found in rats may be because a different method, extravasation of radiolabelled albumin, was used to assess permeability, or because species differences exist in the permeability response to PGD_2. Such differences certainly exist in the vasoconstrictor or vasodilator responses to PGD_2 (Wasserman *et al.*, 1977; Chapnick *et al.*, 1978; Fletcher & Ramwell, 1980; Wechsung & Houvenaghel, 1981). There has been a study showing that PGD_2 can potentiate histamine-induced bronchoconstriction in man (Fuller *et al.*, 1984). If extrapolation can be made from skin to mucosal permeability, our results suggest that any potentiation of histamine-induced bronchoconstriction by PGD_2 is more likely to occur at bronchial smooth muscle than through enhanced mucosal oedema.

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