Cutaneous reactions to intradermal prostaglandins

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Summary

1. The effects of intradermally injected prostaglandins (PGs) \(\text{E}_1, \text{E}_2, \text{F}_{1\alpha}\) and \(\text{F}_{2\alpha}\) have been examined in the rat and in man.
2. \(\text{PGE}_1\) and \(\text{PGE}_2\) caused an increase in local vascular permeability in rat skin; their potency was comparable with that of other putative mediators of inflammation (histamine, bradykinin, and 5-hydroxytryptamine), but \(\text{PGF}_{1\alpha}\) and \(\text{PGF}_{2\alpha}\) were only slightly active even at a dose of 1 \(\mu\)g.
3. Prior administration of mepyramine and methysergide, or depletion of skin mast cell amines with compound 48/80, indicated that \(\text{PGE}_2\) exerted its permeability effect in the rat by a release of mast cell amines.
4. Nanogramme doses of \(\text{PGE}_1\) and \(\text{PGE}_2\) or microgramme doses of \(\text{PGF}_{1\alpha}\) and \(\text{PGF}_{2\alpha}\) injected intradermally into the human forearm induced weal and flare responses.
5. It is concluded that prostaglandins \(\text{E}_1\) and \(\text{E}_2\) can act as intermediates in the production of hyperaemia and oedema resulting from cell damage in the rat and man.

Introduction

Identification of E-type prostaglandins in rat inflammatory exudate has recently been reported (Willis, 1969). The significance of this observation would be enhanced if it could be shown that prostaglandins could induce signs of inflammation in this species and also in man. This paper describes their effects on local vascular permeability in skin. Photographs obtained during this study have been exhibited in a demonstration to the British Pharmacological Society (Crunkhorn & Willis, 1969).

Methods

Female Wistar rats of 130–140 g were used throughout. The abdominal fur was clipped 24 h before the intradermal injections. The animals were anaesthetized with the ultra-short acting barbiturate methohexitone sodium (Brietal, Lilly, 40 mg/kg intraperitoneal) before clipping or intradermal injection because without anaesthetic the cutaneous responses were much smaller and with ether anaesthesia they were more variable.

**Intradermal injections.** Pontamine blue 6BX (100 mg/kg) was injected intravenously 30 min before a series of intradermal injections was made into the clipped abdominal skin, using a 30 gauge × 1.27 cm needle. Drug doses were contained in 0.1 ml for all intradermal injections, prepared in Tyrode solution and adjusted to pH 7.4. Forty-five minutes after the intradermal injections, the rats were killed and blueing was examined from the underside of the abdominal skin. The degree of vascular permeability was estimated by measuring the mean diameter of each blue reaction site and its intensity was scored on a five point scale.

**Depletion of mast cell amines.** The skin area to be depleted was infiltrated subcutaneously with the histamine releasing compound 48/80 (20 µg/ml) mixed with testicular hyaluronidase (50 µg/ml) 24 h before intradermal challenge, according to the method of Brocklehurst, Humphrey & Perry (1955).

**Administration of antagonists.** Mepyramine maleate (2.5 mg/kg) and/or methysergide bimaleate (2.5 mg/kg) were administered intravenously 30 min before intradermal injection.

**Intradermal injections in man.** After cleaning the injection site with 70% ethanol, the prostaglandins (in 0.05 ml of sterile pyrogen-free 0.9% NaCl solution) were injected into the inner surface of the forearm. Doses of the prostaglandins administered ranged from 5 ng (PGE₂) to 5 µg (PGF₁α and PGF₂α). In some cases mixtures of E and F-type prostaglandins were also given. Five volunteers were used in this study (two female and three male). Responses were recorded by serial colour photographs taken up to 90 min after injection of the prostaglandins.

**Drugs.** The materials used were synthetic bradykinin (Sandoz), compound 48/80 (Burroughs Wellcome), PGs E₁, E₂, F₁α, F₂α, and A₁ (Upjohn), pontamine blue 6BX (Edward Gurr), testicular hyaluronidase (Rondase: Evans), histamine acid phosphate, 5-hydroxytryptamine creatinine monosulphate, mepyramine maleate and methysergide bimaleate. Doses of histamine and 5-hydroxytryptamine are expressed in terms of the base; doses of the antagonists refer to the salt.

Prostaglandin solutions were freshly prepared from 100 µg/ml stock solutions in 95% ethanol. Aliquots were evaporated to dryness under a stream of cold air and dilutions were made with freshly prepared Tyrode solution. For administration to man the dilutions were made with sterile pyrogen-free 0.9% NaCl solution, and the final solution for injection was passed through a bacterial filter (Millipore) into sterile rubber capped vials.

**Results**

**Effect of prostaglandins E₁, E₂, F₁α, and F₂α administered intradermally in the rat**

The effects of these prostaglandins are shown in Table 1. Both PGE₁ and PGE₂ in a dose of 100 ng induced a marked increase in local vascular permeability. This effect was not equalled even by microgramme doses of PGF₁α and PGF₂α. Although there was variability in the response obtained with PGE₂ on different occasions, a dose of 100 ng always gave a measurable response and was used throughout this series of experiments.

Dose-response relationships to PGs E₁, E₂, F₁α and F₂α were established and the threshold for PGE₂ was shown to be of the order of 1 ng (Table 1).
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The means of all the responses to PGE\(_2\) (100 ng), histamine (1 \(\mu\)g) bradykinin (1 \(\mu\)g) and 5-HT (100 ng), obtained throughout these experiments were calculated. Only 5-HT (zone diameter = 15-08 mm \(\pm\) 1.84 S.E.) was more potent than PGE\(_2\) (14.1 mm \(\pm\) 2.38 S.E.), while histamine (10.78 mm \(\pm\) 5.26 S.E.) and bradykinin (13-64 mm \(\pm\) 2.07 S.E.) were required in doses 10 times greater. The blueing was intense in all cases.

Development and duration of the permeability changes. Matching intradermal injections of PGE\(_2\) (100 ng) and histamine (1 \(\mu\)g) were given in the same rat at intervals of 5, 10, 15, 20 and 30 min before the intravenous pontamine blue. Results obtained in five animals are given in Table 2. The blueing reaction induced by both drugs took a parallel course; it reached its maximum within 5 min, had largely subsided by 20 min, and was completely absent after 30 minutes.

Mechanism by which prostaglandin induces increased vascular permeability

The possibility that PGE\(_2\) was acting indirectly, by release of mast cell amines was examined by injecting the prostaglandin either after depletion of mast cell amines with compound 48/80, or after administration of mepyramine and methysergide.

(i) Effect of destroying mast cells. Errors due to variation between rats were minimized by obtaining control and test responses in the same rat. This was achieved by a local administration of compound 48/80 to only one half of the abdominal skin, and injection of bradykinin, histamine, 5-HT and compound 48/80, into both halves of the skin, that is, the normal and the depleted areas. Results from this experiment are shown in Fig. 1.

<table>
<thead>
<tr>
<th>Table 1. Comparison of the potency of prostaglandins (E_1), (E_2), (F_{1a}) and (F_{2a}) in producing increases in capillary permeability when given intradermally to rats</th>
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<td>Dose (ng)</td>
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Mean of diameter of the response (mm) \(\pm\) S.E.; five animals/group. Intensity of the extravasation of blue dye indicated by + = very pale to ++++= very strong.

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<th>Table 2. Development of local vascular permeability to PGE(_2) compared with histamine</th>
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<td>PG(_E) (100 ng)</td>
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Mean of diameter of response (mm) \(\pm\) S.E. in five animals. Intensity of extravasation of blue dye indicated by + = very pale to ++++= very strong.
Prior destruction of the mast cells by compound 48/80 did not diminish the increase in permeability induced directly by histamine or bradykinin, but it greatly reduced responses to PGE$_2$ and to further doses of compound 48/80.

(ii) *Use of specific antagonists of histamine and 5-hydroxytryptamine.* The permeability reactions to PGE$_2$, histamine, 5-HT and bradykinin were measured in groups of animals which had received mepyramine (2.5 mg/kg) and/or methysergide (2.5 mg/kg; Fig. 2). The response to PGE$_2$ was greatly reduced by mepyramine and completely suppressed by methysergide. When PGE$_1$ and PGE$_2$ were compared (Table 3), the responses to both prostaglandins were similarly diminished in intensity by mepyramine, but not in area.

**Effect of intradermal PGA$_1$ in the rat**

In a preliminary experiment, PGA$_1$ was injected in doses of 2 µg, 1 µg, 500 ng, 100 ng, and 10 ng into each of four rats. Only doses of 100 ng or above gave a blueing reaction. The responses were small and sometimes there was a white
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centre at the site of injection. In two animals which had previously received mepyramine (2.5 mg/kg) no blueing reaction was obtained with any of these doses of PGA1, indicating that this prostaglandin also acts via histamine release.

**Effect of intradermal prostaglandins E₁, E₂, F₁₀, and F₂₀ in man**

The prostaglandins were injected into the inner surface of the forearm in a volume of 0.05 ml in doses similar to those used for the rats. In all five subjects, both PGE₁ and PGE₂ (in the range 25–100 ng) induced a marked cutaneous reaction which began soon after injection and had largely subsided at 90 minutes. There was an initial weal which was most pronounced after 15 min and a diffuse redness, with 'pseudopodia', most evident after about 30 minutes. In two of the subjects oedema...
was pronounced, while in two others the vasodilation and pseudopodia were most striking (Fig. 3). In all subjects PGE₁ and PGE₂ appeared to be about equipotent, including one subject who received doses of 5, 10, 20 and 50 ng. After 15–30 min the saline control injection site was only apparent as a small white mark, but at all the sites which had received PGE₁ or PGE₂ there was swelling with a reddish fringe. Pseudopodia were present only at sites which had received 50 ng or more. None of the subjects complained of any discomfort except for the very slight initial stinging of the injections. All the subjects experienced warmth and three of them felt a slight itching in the injected area.

Four subjects received PGF₁α and/or PGF₂α in doses of 500 ng or 5 μg. The reactions were more localized and more prolonged than those produced by the much smaller doses of E-type prostaglandins. In order to ascertain whether there was any interaction between E and F prostaglandins, one subject received PGF₁α (500 ng) mixed with PGE₁ (50 ng) and another was given PGF₂α (500 ng) with PGE₂ (50 ng). Without circulating dye, changes in intensity of the reaction could not be assessed accurately, but there was certainly no marked potentiation of either response when PGE and PGF were given together.

Discussion

It has been reported that PGE₁ induces increased local vascular permeability in the skin of the guinea-pig (Horton, 1963) and rat (Kaley & Weiner, 1968) and that

![Fig. 3. Responses to intradermal prostaglandins in the human forearm (a). PGE₁ and PGE₂ were effective in doses of 25 ng and 50 ng, but responses to PGF₁α and PGF₂α, given in much larger doses (5 μg), were considerably smaller. Each dose was contained in 0·05 ml of 0·9% NaCl solution. (b) Tracing from a colour photograph taken 30 min after injection.]
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when infused intra-arterially it induces oedema and vasodilation in the human forearm (Bergström, Carlson, Ekelund & Örö, 1965). In 1961 Ambache observed a strong but delayed flare in man after intradermal injection of chromatographically purified Irin, which is now known to contain PGE$_2$ and PGF$_{2\alpha}$. Our experiments extend these findings.

Prostaglandins E$_2$ and F$_{3\alpha}$ were not available, but from the results in this paper it appears that prostaglandins of the E-type are potent inducers of permeability while those of the F-type are not; that is, a ketone group at position 9 is essential for activity. Furthermore, the low potency of PGA$_1$ suggests that the hydroxyl group at position 11 is also necessary.

During acute inflammation engendered in the rat by subcutaneous injection of carrageenin, PGE$_2$ is released (Willis, 1969a, b and unpublished results). Our results show that potencies of PGE$_1$ and PGE$_2$ in the rat compare well with those of other putative mediators of inflammation (5-HT, histamine and bradykinin) strongly suggesting a role for PGE$_2$ as an inflammatory mediator in this species. The high potency of the E-type prostaglandins in inducing erythematous weals also suggests that they could be involved in the aetiology of some human skin reactions. It is very likely that prostaglandins contribute to the swelling from bee stings, for bee venom contains phospholipase A (Högberg & Uvnäs, 1957) which is known to induce production of prostaglandins (Bartels, Vogt & Wille, 1968).

The ability of compound 48/80 and of PGE$_2$ to induce increases in local vascular permeability was greatly reduced after destruction of the mast cells by pretreatment with 48/80 (Fig. 1). Compound 48/80 releases histamine and 5-HT from rat mast cells (Moran, Uvnäs & Westerholm, 1962) and this result suggested that PGE$_2$ acted similarly. A subsequent experiment (Fig. 2) showed that mepyramine greatly reduced permeability changes induced by PGE$_2$ and that complete suppression was obtained when methysergide was also given; methysergide alone only slightly reduced the permeability response to PGE$_2$. This experiment provided further evidence that PGE$_2$ acts by release of mast cell amines and indicated that the histamine-releasing effect was predominant. Blueing induced by PGE$_3$ and PGA$_1$ was greatly reduced or abolished by mepyramine and so the mode of action postulated for PGE$_2$ appears to be common to the prostaglandins in general.

The histamine-releasing action of prostaglandins, proposed here, has been observed in vitro by Cabut, Vincenzi & Paoletti (1967), who showed that rat mast cells were disrupted, with release of histamine and heparin, by concentrations of PGE$_1$ of the order of 50 ng/ml. In our experiments, local concentrations of PGE$_1$ and PGE$_2$ were similar and histological examination of the skin sites which had received PGE$_1$ or PGE$_2$ showed that mast cell granules had been extruded.

Our experiments and conclusions differ in detail from those of Kaley & Weiner (1968) who used only PGE$_1$ in their studies with rat skin. They found that the increase in local vascular permeability (shown by extravasation of Evans blue) occurred more slowly with PGE$_1$ than with histamine, 5-HT or bradykinin, although the increased permeability had developed fully 15 min after injection. However, in our experiments with PGE$_2$ and pontamine blue, the time course of blueing was almost identical for prostaglandin and for histamine, reaching a peak within 5 min (Table 2). Kaley & Weiner (1968) concluded that PGE$_1$ did not act entirely via histamine release for they found that PGE$_2$ still elicited a reaction after chronic pretreatment with compound 48/80. They did not state, however, whether test
doses of 48/80 were given intradermally to ascertain that mast cell depletion was complete, and no work with antagonists was reported.

It is concluded that PGE₁ and PGE₂ now deserve to be considered as potential mediators of skin reactions in the rat and in man. They appear to act in the rat by release of mast cell amines, and therefore might be regarded as physiological "trigger substances" in the release of mast cell histamine. This role has previously been suggested for phospholipase A (Högberg & Uvnäs, 1957; Änggård, Bergquist, Högberg, Johansson, Thon & Uvnäs, 1963) which is present on the surface of mast cells (Uvnäs, 1968) and is known to be capable of inducing production of prostaglandins (Bartels et al., 1968). E-type prostaglandins are released with histamine from perfused guinea-pig lung during anaphylactic shock (Piper & Vane, 1969); we envisage a similar pattern of events in the skin of the rat and perhaps also in man.

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REFERENCES


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