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Cutaneous reactions to intradermal prostaglandins

PEARL CRUNKHORN AND A. L. WILLIS*

Department of Pharmacology, Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey

Summary

1. The effects of intradermally injected prostaglandins (PGs) E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ have been examined in the rat and in man.

2. PGE_1 and 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 $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were only slightly active even at a dose of 1 μg.

3. Prior administration of mepyramine and methysergide, or depletion of skin mast cell amines with compound 48/80, indicated that PGE_2 exerted its permeability effect in the rat by a release of mast cell amines.

4. Nanogramme doses of PGE_1 and PGE_2 or microgramme doses of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ injected intradermally into the human forearm induced weal and flare responses.

5. It is concluded that prostaglandins E_1 and 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.

* Present address: Department of Pharmacology, The Royal College of Surgeons of England, Lincoln's Inn Fields, London W.C.2.

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 (PGE_2) to 5 μg ($PGF_{1\alpha}$ and $PGF_{2\alpha}$). 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 E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ and A_1 (Upjohn), pontamine blue 6BX (Edward Gurr), testicular hylauronidase (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 E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ administered intradermally in the rat

The effects of these prostaglandins are shown in Table 1. Both PGE_1 and PGE_2 in a dose of 100 ng induced a marked increase in local vascular permeability. This effect was not equalled even by microgramme doses of $PGF_{1\alpha}$ and $PGF_{2\alpha}$. Although there was variability in the response obtained with PGE_2 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 E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were established and the threshold for PGE_2 was shown to be of the order of 1 ng (Table 1).

The means of all the responses to PGE_2 (100 ng), histamine (1 μg) bradykinin (1 μg) 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 μg) 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 1. *Comparison of the potency of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in producing increases in capillary permeability when given intradermally to rats*

Dose (ng)	0·25	1·0	10	100	500	1,000
PGE_2	0	9·5±0·68 (+)	13·6±0·46 (+)	14·8±0·02 (+++)	16·0±0·84 (++++)	16·6±0·04 (+++)
PGE_1	–	–	–	14·4±0·24 (+++)	15·4±0·50 (+++)	17·2±0·20 (++++)
$PGF_{2\alpha}$	–	–	–	2·8±1·57 (+)	2·8±1·71 (+)	4·0±1·70 (+)
$PGF_{1\alpha}$	–	–	–	1·8±1·07 (+)	2·4±1·50 (+)	1·0±0·99 (+)

Mean diameter of the response (mm) ±S.E.; five animals/group. Intensity of the extravasation of blue dye indicated by + =very pale to ++++=very strong.

TABLE 2. *Development of local vascular permeability to PGE_2 compared with histamine*

	PGE_2 (100 ng)	Histamine (1 μg)
5 min	16·6±0·67 (+++)	16·0±0·83 (++)
10 min	14·4±0·69 (+)	13·8±0·76 (+)
15 min	11·6±0·92 (+)	12·2±0·80 (+)
20 min	10·0±0·00 (+)	10·0±0·00 (+)
30 min	0	0

Mean diameter of response (mm) ±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

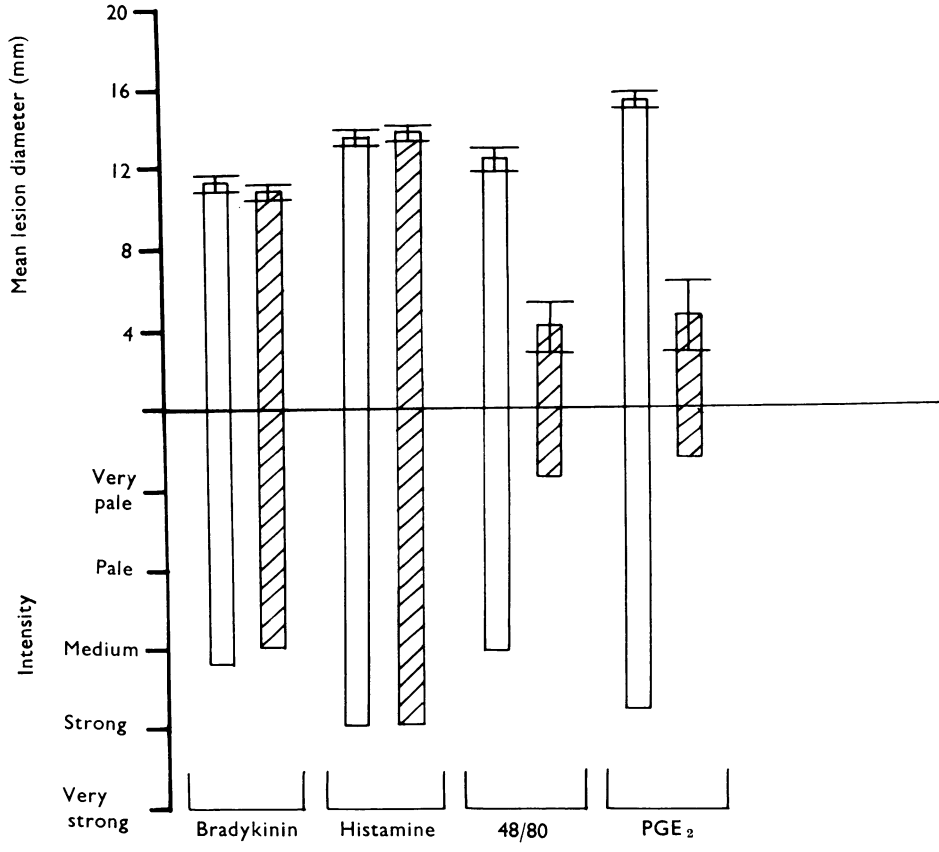

FIG. 1. Reduction, by pretreatment with compound 48/80, of cutaneous responses to intradermal PGE_2 (100 ng) or 48/80 (25 ng), while responses to histamine (1 μg) or bradykinin (1 μg) remain unaltered. In five rats, mast cells on one side only of the abdominal skin were depleted by subcutaneous infiltration of compound 48/80 (20 μg/ml) 24 h previously. The histogram shows mean diameter (mm) and intensity of responses obtained in the depleted area of skin (▨) compared with those obtained in the untreated area (□).

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 PGA_1, indicating that this prostaglandin also acts via histamine release.

Effect of intradermal prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ 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 PGE_1 and PGE_2 (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

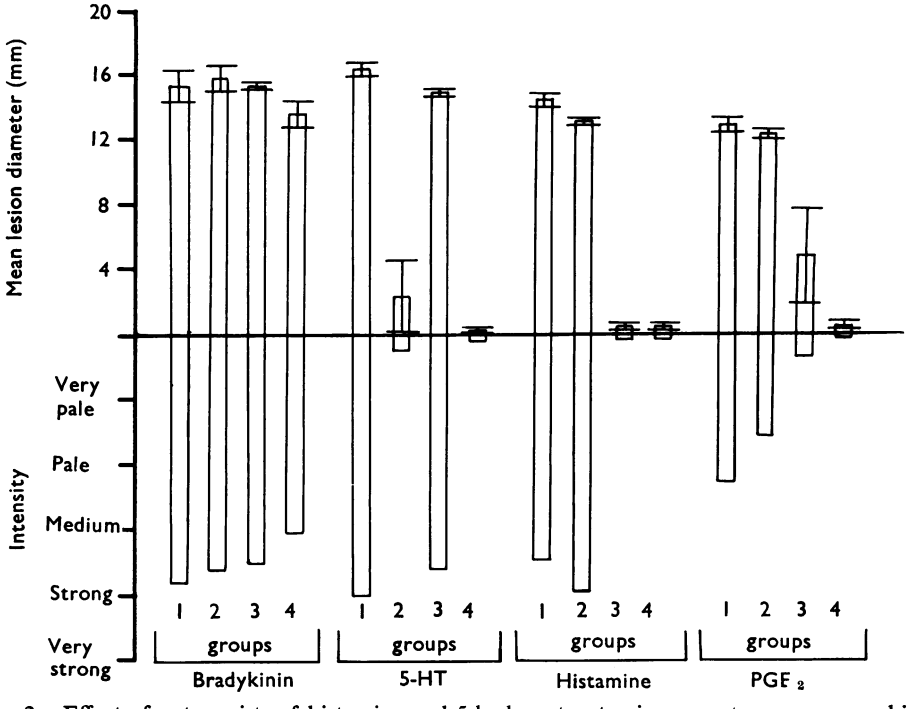

FIG. 2. Effect of antagonists of histamine and 5-hydroxytryptamine on cutaneous permeability responses to bradykinin (1 μg), 5-hydroxytryptamine (100 ng), histamine (1 μg) and PGE_2 (100 ng) injected intradermally at the same time in each rat. Eight animals (group 1) received no treatment. Five (group 2) received mepyramine maleate, 2·5 mg/kg intravenously, and five (group 3) were given methysergide bimaleate, 2·5 mg/kg intravenously. Three animals (group 4) received both antagonists together.

TABLE 3. *Effect of mepyramine on responses to intradermal injections of PGE_1 and PGE_2 in duplicate in five control rats and five treated with mepyramine 2·5 mg/kg*

Control		Mepyramine	
E_2 100 ng	E_1 100 ng	E_2 100 ng	E_1 100 ng
16·2±0·31 (+++)	16·4±0·12 (+++)	14·7±0·22 (+)	14·6±0·00 (+)

Mean diameter of response (mm) ±S.E. Intensity of the extravasation of blue dye indicated by + = very pale to ++++ = very strong.

was pronounced, while in two others the vasodilation and pseudopodia were most striking (Fig. 3). In all subjects PGE_1 and PGE_2 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 PGE_1 or PGE_2 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 $PGF_{1\alpha}$ and/or $PGF_{2\alpha}$ 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 $PGF_{1\alpha}$ (500 ng) mixed with PGE_1 (50 ng) and another was given $PGF_{2\alpha}$ (500 ng) with PGE_2 (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 PGE_1 induces increased local vascular permeability in the skin of the guinea-pig (Horton, 1963) and rat (Kaley & Weiner, 1968) and that

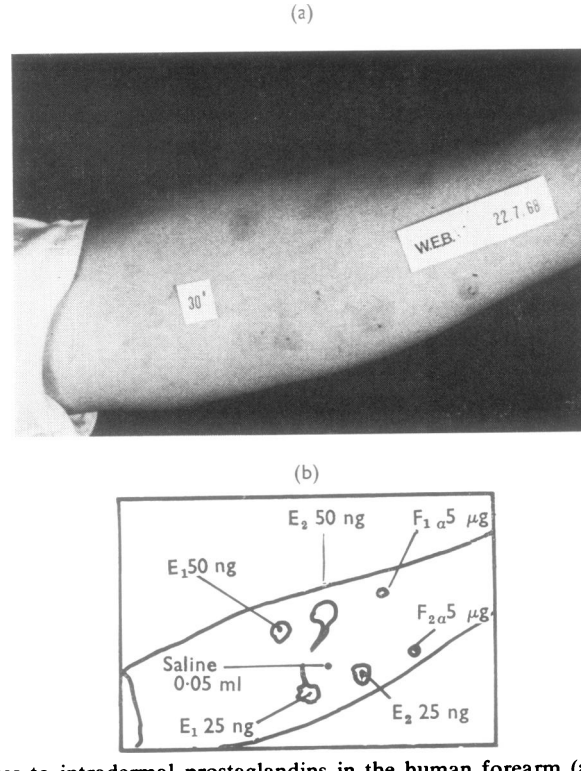

FIG. 3. Responses to intradermal prostaglandins in the human forearm (a). PGE_1 and PGE_2 were effective in doses of 25 ng and 50 ng, but responses to $PGF_{1\alpha}$ and $PGF_{2\alpha}$, given in much larger doses (5 μg), were considerably smaller. Each dose was contained in 0·05 ml of 0·9% NaCl solution. (b) Tracing from a colour photograph taken 30 min after injection.

when infused intra-arterially it induces oedema and vasodilation in the human forearm (Bergström, Carlson, Ekelund & Orö, 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_3 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_1 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_1 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 PGE_1 and PGE_2 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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