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INFLAMMATORY EFFECTS OF PROSTAGLANDIN D_2 IN RAT AND HUMAN SKIN

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1 Intradermal injection of prostaglandin (PG) D_1 and D_2 in the human forearm produced a long-lasting dose-related erythema. When compared with prostaglandin E_1 or E_2 the order of potency for erythema production was $PGE_1 > PGE_2 > PGD_2 > PGD_1$.

2 In rat skin, prostaglandin D_2 but not D_1 caused an increase in vascular permeability as quantitated by the Evans blue method and the ^{125}I-albumin extravasation technique. Prostaglandin E_2 was 3–5 times more potent than prostaglandin D_2.

3 Prostaglandin D_2 (10 ng) potentiated the increase in vascular permeability in rat skin produced by histamine, but not that produced by bradykinin.

4 Prostaglandin D_2 (10, 20 and 50 ng) did not elicit oedema or hyperalgesia in the rat paw oedema test, but potentiated carrageenan-induced oedema; hyperalgesia was potentiated by doses of 100 ng and above.

Introduction

There is increasing evidence for the involvement of prostaglandins of the E and F series in the inflammatory process. Whereas the E prostaglandins are pro-inflammatory, the F prostaglandins have anti-inflammatory properties.

Intradermal injections of prostaglandin E_1 produce a long-lasting erythema (Solomon, Juhlin & Kirschbaum, 1968; Juhlin & Michaelson, 1969) and hyperalgesia (Ferreira, 1972) and potentiate the increase in vascular permeability (Williams & Morley, 1973; Moncada, Ferreira & Vane, 1973; Thomas & West, 1974), pain (Ferreira, 1972) and itching (Greaves & McDonald-Gibson, 1973) produced by bradykinin or histamine. Increased amounts of E prostaglandin are found in inflamed skin (Sondergaard & Greaves, 1970; Greaves, Sondergaard & McDonald-Gibson, 1971; Angaard & Jonsson, 1971; Hamberg & Jonsson, 1973), in ocular (Eakins, Whitelocke, Perkins, Bennet & Ungar, 1972) and in many other types of inflammation (Ferreira, Flower, Moncada & Vane, 1975).

Prostaglandins of the F series antagonize some effects of the E prostaglandins (Crunkhorn & Willis, 1971). Prostaglandin $F_{2\alpha}$ is a veno-constrictor (Sweet, Kadowitz & Brody, 1971) and is found in increased amounts in the later stages of inflammation (Velo, Dunn, Giroud, Timsit & Willoughby, 1973), suggesting that it is involved in the termination of the inflammatory reaction.

The 'endoperoxide' intermediate (prostaglandin H_2) in prostaglandin biosynthesis may break down to form prostaglandin D in addition to prostaglandins E and F (Granström, Lands & Samuelsson, 1968) and formation of all three prostaglandins by skin homogenates has been observed (Nugteren & Hazelhof, 1973; Kingston, 1975, unpublished observation). In view of the involvement of E and F prostaglandins in the inflammatory process, we have also studied the action of prostaglandins D_1 and D_2.

Methods

Intradermal injections in man and measurements of erythema

Double blind studies were carried out in 5 male volunteers who gave their informed consent. Prostaglandin solutions (in pyrogen-free sterile 0.9% w/v NaCl solution, saline) were injected intradermally in a volume of 0.1 ml into the volar surface of the human forearm.

Erythema was measured 30 min after injection by covering the skin with a clear plastic sheet and tracing the contours of the intense erythema on to the plastic. A photocopy of the area of response was made and the area representing erythema was cut out and weighed.

Measurement of vascular permeability changes in rat skin

Prostaglandins D_1, D_2, E_1, E_2, bradykinin or histamine were injected (in 0.1 ml sterile saline) intradermally

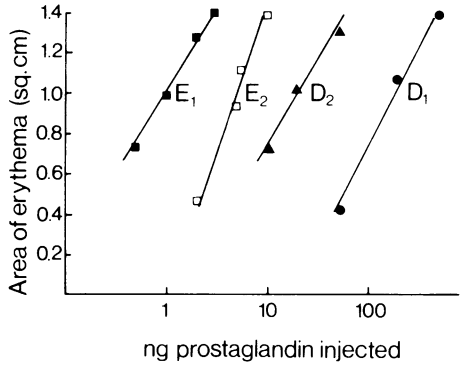

Figure 1 Dose related erythema in the human forearm 30 min after intradermal injection of prostaglandin E_1 (■), prostaglandin E_2 (□), prostaglandin D_2 (▲), prostaglandin D_1 (●).

either alone or as a mixture into the (shaved) abdominal skin of Olac rats (150–200 g). Control injections of saline were also made in each animal. The effects on vascular permeability were quantitated by the Evans blue method and by measurement of extravasation of ^{125}I-albumin.

Evans blue elution

Five minutes prior to intradermal injection, each rat was injected intravenously with Evans blue (40 mg/kg). Rats were killed 30 min after intradermal injection. The skin was removed and discs of skin (which included the whole of the lesion) were excised with a 15 mm punch. The skin was frozen in liquid nitrogen and disintegrated by hammering in a cooled stainless steel mortar. The Evans blue was then extracted in 4 ml of formamide and the absorbance at 600 nm measured, as described by Rees, Okino & Rocha e Silva (1971).

Measurement of extravasation of ^{125}I-albumin

Immediately prior to intradermal injection of inflammatory agents 5 μCi of ^{125}I-albumin was injected into a tail vein. Evans blue (40 mg/kg) was also injected to visualize the sites of increased vascular permeability. After 20 min the animals were killed and the blue areas excised with a punch as already described. The excised skin was then wrapped in a single layer of 'Parafilm' and the radioactivity measured in a gamma counter. The radioactivity in 10 μl of blood was measured as a reference.

Measurement of carrageenan-induced oedema and hyperalgesia in the rat paw

Oedema and hyperalgesia were measured in male Olac rats (130–150 g) after injection of either carrageenan

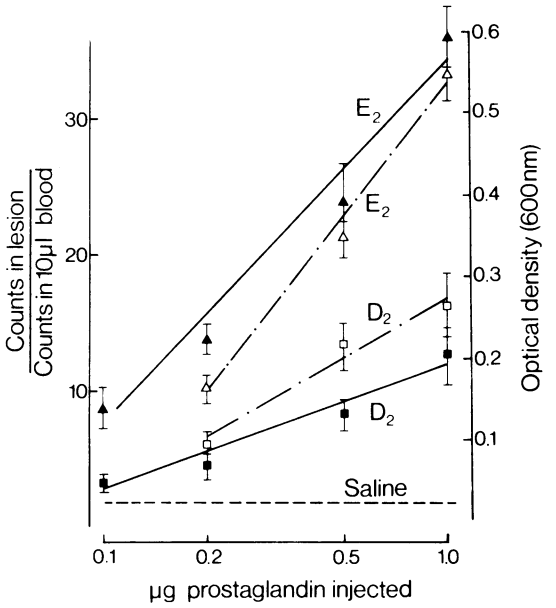

Figure 2 Increased vascular permeability elicited by intradermal injection of Prostaglandin E_2 or D_2 in rat skin as measured by 2 assay methods:

(a) Evans blue elution (——·——, right ordinate) from lesions produced by intradermal injection of prostaglandin E_2 (△), prostaglandin D_2 (□).

(b) Extravasation of ^{125}I-albumin (——, left ordinate) elicited by intradermal injection of prostaglandin E_2 (▲), prostaglandin D_2 (■).

Results expressed as mean ± s.e. mean. (——), represents effect of intradermal injection of saline (0.1 ml).

or prostaglandin D_2 alone, and the results compared with those obtained with a mixture of carrageenan and prostaglandin D_2. The rats were treated with indomethacin (10 mg/kg) 30 min prior to carrageenan injection to abolish endogenous prostaglandin release. Injections (0.1 ml) of prostaglandin E_2, D_2 and/or carrageenan (0.5% in saline) were made into one of the hind paws of a rat. The contralateral (control) paw was injected with 0.1 ml saline. The oedema was determined by mercury displacement manometry. Hyperalgesia was measured by applying an increasing pressure to the paw and measuring the time taken for the animal to react by withdrawal of the paw (Randal & Selitto, 1957). The oedema and hyperalgesia elicited by inflammatory agents was in each case compared with that produced by saline in the contralateral paw.

Materials

Evans blue was obtained from Phase Separations; ^{125}I-albumin from the Radiochemical Centre, Amersham;

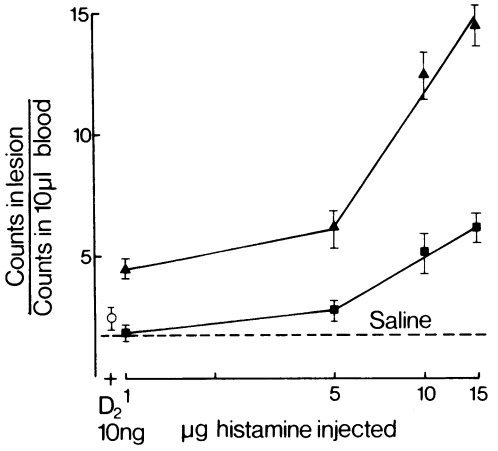

Figure 3 Effect of prostaglandin D_2 on the ^{125}I-albumin extravasation in rat skin elicited by histamine (■), 10 ng prostaglandin D_2 (○), histamine + 10 ng prostaglandin D_2 (▲). Results are expressed mean ± s.e. mean. (——), represents effect of intradermal injection of saline (0.1 ml).

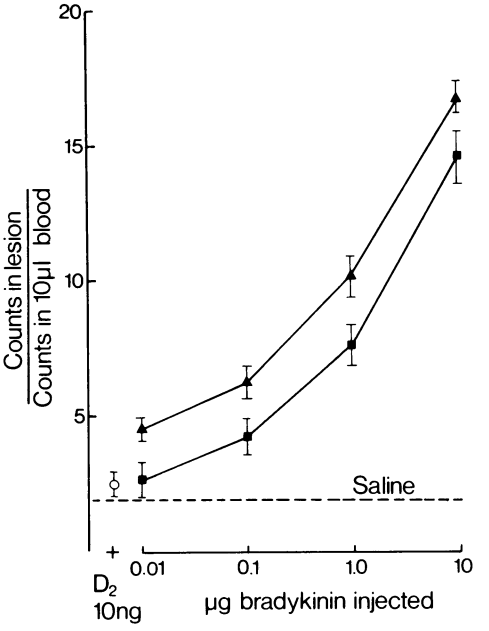

Figure 4 Effect of 10 ng prostaglandin D_2 on the increased vascular permeability in rat skin produced by intradermally injected bradykinin. Results are expressed (mean ± s.e. mean). Effects of bradykinin (■), 10 ng prostaglandin D_2 (○), bradykinin + 10 ng prostaglandin D_2 (▲). Bradykinin solution contained 2 µg of bradykinin potentiating peptide 5A. (——), represents effect of intradermal injection of saline (0.1 ml).

bradykinin from Schwarz/Mann; histamine acid phosphate from B.D.H.; sodium carrageenan from Marine Colloids; and indomethacin from Merck, Sharpe & Dohme. Prostaglandins E_1, E_2, $F_{2\alpha}$, D_1 and D_2 were pure as judged by thin layer chromatography.

Results

Effects of intradermal injection of D prostaglandin in man

In human skin, intradermal prostaglandin D_1 or D_2 produced long-lasting erythema with relatively little oedema. The erythema was dose-related and was maximal some 30 min after injection. For prostaglandin D_1, the threshold dose was about 50 ng, and for prostaglandin D_2 it was 2–10 ng. Increased vasodilatation was still visible 4 h after injection of 500 ng prostaglandin D_1 and 6 h after the same dose of prostaglandin D_2.

The results obtained for prostaglandins E_1, E_2, D_1 and D_2 were qualitatively similar in each trial but there was considerable inter-subject variation in the size of erythema elicited by a given dose of prostaglandin. For this reason the results in an individual subject are shown in Figure 1. Prostaglandin E_1, E_2, D_2 and D_1 produced fairly parallel dose response curves. The potency ratio $E_1:E_2:D_2:D_1$, was 1:5:25:300. No hyperalgesia was evident at the sites of prostaglandin D_1 or D_2 injection up to doses of 500 ng.

Effects of D prostaglandins on rat skin

Intradermal injection of prostaglandin D_2 into rat skin produced an increase in vascular permeability with doses as low as 10 ng. Prostaglandin D_1 at doses up to 10 µg did not increase vascular permeability. The dose response curves for prostaglandins E_2 and D_2 on vascular permeability were not parallel. Prostaglandin E_2 was 3–5 times more potent than prostaglandin D_2. Both the Evans blue method and the ^{125}I-albumin method gave similar results (Figure 2).

Actions of PGD_2 on cutaneous responses to histamine and bradykinin in the rat

In the rat skin, the increase in vascular permeability induced by histamine was potentiated, in excess of simple summation, by simultaneous administration of 10 ng of prostaglandin D_2 (Figure 3). Prostaglandin D_2 (10–100 ng) also slightly enhanced the increased permeability produced by bradykinin (Figure 4), but this enhancement could have been due to simple

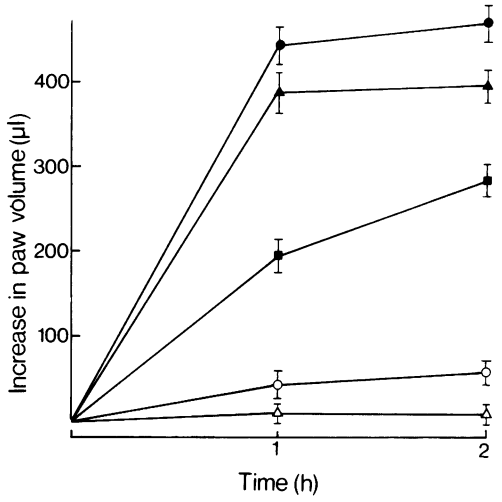

Figure 5 Potentiation by prostaglandins E_2 and D_2 of the rat paw swelling induced by carrageenan. Increase in paw volume (mean ± s.e. mean) elicited by 0.5% carrageenan (■), 0.5 ng prostaglandin E_2 (○), carrageenan + 0.5 ng prostaglandin E_2 (●), 10 ng prostaglandin D_2 (△), carrageenan + 10 ng prostaglandin D_2 (▲). In each case the oedema produced by the test agent was calculated by subtraction of the effect of saline injection in the contralateral paw.

summation of the effects of prostaglandin D_2 and bradykinin.

Action of prostaglandin D_2 on the carrageenan-induced oedema and hyperalgesia in the rat paw

Injection of 10, 20 and 50 ng prostaglandin D_2 alone into the rat paw produced neither oedema nor hyperalgesia. Oedema produced by carrageenan was potentiated by about 100% at 1 h after simultaneous injection of 10 ng prostaglandin D_2 (Figure 5 and Table 1). A similar effect was produced by 0.5 ng prostaglandin E_2. Prostaglandin D_2 (100 ng) elicited a slight hyperalgesia, and potentiated carrageenan induced hyperalgesia by about 100% at 1 h (Table 1).

Discussion

E prostaglandins elicit inflammatory responses in the skin including vasodilatation (Juhlin & Michaelsson, 1969; Solomon, Juhlin & Kirschbaum, 1968) increased vascular permeability (Kaley & Weiner, 1971) and hyperalgesia (Ferreira, 1972). Two important characteristics of the inflammatory properties of E prostaglandins are the long duration of action (Juhlin & Michaelsson, 1969; Ferreira, 1972), and their ability to potentiate the effects of other mediators of inflammation (Ferreira, 1972; Williams & Morley, 1973; Ferreira, Moncada & Vane, 1973; Greaves & McDonald-Gibson, 1973; Thomas & West, 1974). We have now shown that the inflammatory effects of prostaglandin D_2 show the same two characteristics. Both prostaglandins D_1 and D_2 produce a long-lasting erythema in the human skin. Like the E prostaglandins (Juhlin & Michaelsson, 1969; Ferreira, 1972) they produce vasodilatation more effectively than oedema. It is of interest that for the production of erythema, prostaglandin E_1 is more potent than E_2, whereas prostaglandin D_1 is less potent than D_2. No hyperalgesia was noted at the sites of intradermal injections of up to 500 ng of prostaglandins D_1 or D_2. In addition to eliciting the inflammatory response when given alone, prostaglandins D_2 and E_2 potentiate (in much lower concentrations) the inflammatory effects of histamine and carrageenan. Thus it appears that these prostaglandins could promote inflammation at two levels: at low concentrations they potentiate the effects of other inflammatory mediators, and at higher concentrations they additionally produce direct inflammatory effects. The importance of the potentiating ability of these prostaglandins is highlighted by the fact that even very low concentrations increase by 100% the vascular permeability produced by histamine or carrageenan.

Unlike prostaglandin E_2, prostaglandin D_2 does not potentiate the increased vascular permeability induced by bradykinin. This suggests an important difference in the inflammatory actions of the two prostaglandins. Since the peak of histamine production precedes that of bradykinin in the inflammatory reaction (Di Rosa, Giroud & Willoughby, 1971),

Table 1 Threshold doses of prostaglandins D_2 and E_2 for production of oedema and hyperalgesia in the rat paw.

Threshold dose	*PGE_2 (ng)*	*PGD_2 (ng)*	*Dose Ratio E_2:D_2*
For oedema alone	5.0	100	1:20
For potentiation of carrageenan-induced oedema	0.5	10	1:20
For hyperalgesia alone	10	100	1:10
For potentiation of carrageenan-induced hyperalgesia	10	100	1:10

prostaglandin D_2 could be important in modulating the effects of histamine in the early stage.

The amounts of prostaglandin D_2 or E_2 which potentiated the carrageenan-induced hyperalgesia in the rat paw were similar to those which produced a direct effect on cutaneous vasculature, and rather higher than those needed to potentiate the oedema.

Prostaglandin D_2 was in all its actions less potent than prostaglandin E_2. It would be of interest to determine the factors involved *in vivo* in the selective breakdown of the endoperoxide intermediate to prostaglandin D, E or F. It is possible that altering the ratio of prostaglandins exerts a fine control of the inflammatory process. Nugteren & Hazelhof (1973) reported that the formation of prostaglandin D is stimulated by a factor present in the cytosol and that in many tissues D prostaglandins are the main products of the endoperoxide breakdown. These authors also suggested that the D prostaglandins were devoid of biological activity. However, prostaglandin D_2 potently inhibits platelet aggregation (Mills & MacFarlane, 1974) and acts on a number of smooth muscle preparations (Horton & Hones, 1974; Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975).

The presence of prostaglandin D_2 in inflammatory exudates has not yet been demonstrated and awaits the development of a sensitive assay for prostaglandin D_2.

We conclude that prostaglandin D_2 appears to possess similar inflammatory properties to the E prostaglandins, but is less potent, and should be considered as a potential mediator of the inflammatory reaction.

References

ÄNGAÅRD, E. & JONSSON, C.E. (1971). Efflux of prostaglandins in lymph from scalded tissue. *Acta physiol. scand.*, **81**, 440–447

CRUNKHORN, P. & WILLIS, A.L. (1971). Interaction between prostaglandins E and F given intradermally in the rat. *Br. J. Pharmac.*, **41**, 507–512.

DI ROSA, M., GIROUD, J.P. & WILLOUGHBY, D.A. (1971). Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. Path.*, **104**, 13.

EAKINS, K.E., WHITELOCKE, R.A.F., PERKINS, E.S., BENNET, A. & UNGAR, W.G. (1972). Release of prostaglandins in ocular inflammation in the rabbit. *Nature, New Biol.*, **239**, 248–249.

FERREIRA, S.H. (1972) Prostaglandins, aspirin like drugs and analgesia. *Nature, New Biol.*, **240**, 200–203.

FERREIRA, S.H., FLOWER, R.J., MONCADA, S. & VANE, J.R. (1975). Inhibition of prostaglandin biosynthesis as the mechanism of action of non-steroid anti-inflammatory drugs. *Exerpta Medica* (In press).

GRANSTRÖM, E. LANDS, W.E.M. & SAMUELSSON, B. (1968). Biosynthesis of 9α, 15-dihydroxy-11-ketoprost-13-enoic acid. *J. biol. Chem.*, **243**, 4104–4108.

GREAVES, M.W. & McDONALD-GIBSON, W. (1973) Itch: Role of prostaglandins. *Brit. Med. J.*, **3**, 608–609.

GREAVES, M.W., SÖNDERGAARD, J. & McDONALD-GIBSON, W. (1971). Recovery of prostaglandins in human cutaneous inflammation. *Brit. Med. J.*, **2**, 258–260.

HAMBERG, M., HEDQVIST, P., STRANDBERG, K., SVENSSON, J. & SAMUELSSON, B. (1975). Prostaglandin endoperoxides iv. Effects on smooth muscle. *Life Sci.*, **16**, 451–462.

HAMBERG, M. & JONSSON, C.E. (1973). Increased synthesis of prostaglandins in the guinea pig following scalding injury. *Acta physiol. scand.*, **87**, 240–245.

HORTON, E.W. & JONES, R.L. (1974). Biological activity of prostaglandin D_2 on smooth muscle. *Br. J. Pharmac.*, **52**, 110–111P.

JUHLIN, S. & MICHAELSSON, G. (1969). Cutaneous vascular reactions to prostaglandins in healthy subjects and in patients with urticaria and atopic dermatitis. *Acta Derm. Vener. (Stockholm)*, **49**, 251–261.

KALEY, G.D. & WEINER, R. (1971). Prostaglandin E_1, a potential mediator of the inflammatory response. *Ann. N.Y. Acad. Sci.*, **180**, 338–350.

MILLS, D.C.B. & MacFARLANE, D.E. (1974). Stimulation of human platelet adenyl cyclase by prostaglandin D_2. *Thrombosis Research*, **5**, 401–412.

MONCADA, S., FERREIRA, S.H. & VANE, J.R. (1973). Prostaglandins, aspirin like drugs and the oedema of inflammation. *Nature, Lond.*, **246**, 217–218.

NUGTEREN, D.H. & HAZELHOF, E. (1973). Isolation and properties of intermediates in prostaglandin biosynthesis. *Bioch. Bioph. Acta*, **326**, 448–461.

RANDAL, L.O. & SELLITO, J.J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Arch. Int. Pharmacodyn.*, **61**, 409–419.

REES, M.W., OKINO, L. & ROCHA e SILVA, M. (1971). Comparative pharmacological actions of bradykinin and related kinins of larger molecular weight. *Biochem. Pharmac.*, **20**, 2935–2946.

SÖNDERGAARD, J. & GREAVES, M.W. (1970). Pharmacological studies in inflammation due to exposure to ultraviolet radiation. *J. Path.*, **101**, 93–97.

SOLOMON, L.M., JUHLIN, L. & KIRSCHBAUM, M.M. (1968). Prostaglandins on cutaneous vasculature. *J. Invest. Derm.*, **51**, 280–282.

SWEET, C.S., KADOWITZ, P.J. & BRODY, M.J. (1971). A hypertensive response to infusion of prostaglandin $F_{2\alpha}$ into the vertebral artery of the conscious dog. *Eur. J. Pharmac.*, **16**, 229–232.

THOMAS, G. & WEST, G.B. (1974). Prostaglandins, Kinin and inflammation in the rat. *Br. J. Pharmac.*, **50**, 231–235.

VELO, G.P., DUNN, C.J., GIROUD, J.P., TIMSIT, J.& WILLOUGHBY, D.A. (1973). Distribution of prostaglandins in inflammatory exudate. *J. Path.*, **111**, 149–158.

WILLIAMS, T.J. & MORLEY, H. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature, Lond.*, **246**, 215–217.

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