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The release of prostaglandin D_2 from human skin *in vivo* and *in vitro* during immediate allergic reactions

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1 The release of prostaglandin D_2 (PGD_2) during immediate allergic reactions in human skin was investigated *in vivo* and *in vitro*.

2 Skin exudates were collected from abraded sites on the thigh of atopic subjects sensitive to *D. pteronyssinus* antigen and from non-atopic control subjects. Challenge with antigen caused the release of PGD_2 and histamine, but not PGE_2, from the skin of the atopic subjects. The molar ratio of histamine to PGD_2 was about 140:1. Control subjects were unresponsive.

3 PGD_2 was released from passively sensitized human skin challenged with antigen *in vitro*. The time course was similar *in vitro* and *in vivo*. The ratio of histamine to PGD_2 *in vitro* was 78:1.

4 The identities of the prostaglandins were confirmed by high performance liquid chromatography and radioimmunoassay to PGD_2 and PGE_2.

5 PGD_2 is the major arachidonic acid cyclo-oxygenase product synthesized by human mast cells. It is pro-inflammatory in human skin but its functions as a mediator in immediate hypersensitivity reactions in human skin are not clear. The results of this study suggest that, relative to histamine, PGD_2 contributes little to the oedema and erythema of immediate reactions in human skin.

Introduction

It is well established that mast cells play a critical role in immediate hypersensitivity reactions. Vasoactive and chemotactic mediators released from activated mast cells in skin are involved in the pathophysiology of urticarias and allergic dermatitis. The cutaneous mast cell is located in the dermis, frequently in small groups around nerves or blood vessels and in greater numbers close to the dermal-epidermal junction (Eady *et al.*, 1979; Cowen *et al.*, 1979). Skin mast cells can be triggered to degranulate by a variety of factors, both exogenous and endogenous. The oedema, erythema and pruritus typical of cutaneous hypersensitivity result from the rapid interaction of the released mediators on the adjacent dermal vessels and nerves.

Stimulated mast cells release a diverse range of inflammatory mediators which include arachidonic acid metabolites. Prostaglandin D_2 (PGD_2) is the predominant cyclo-oxygenase product formed by mast cells isolated from human lung and intestine (Lewis *et al.*, 1982; Holgate *et al.*, 1984; Peters *et al.*, 1984). Leukotriene C_4, a product of the 5-lipoxygenase pathway, is also released by stimulated human lung mast cells but in lower amounts than PGD_2 (Peters *et al.*, 1984). In contrast, purified mast cells from the spleen of a subject with mastocytosis released greatly increased amounts of PGD_2 but not leukotrienes, when stimulated immunologically or with ionophore A23187 (Holgate, 1987). Evidence is accumulating for heterogeneity in human mast cells although it is less well defined than in rodents. Human isolated skin mast cells have not been extensively studied but recent results show that these cells have increased sensitivity for histamine release in response to non-immunological stimulation compared to lung-derived mast cells (Benyon *et al.*, 1987). PGD_2 is released from passively sensitized guinea-pig skin challenged with antigen *in vitro* (Kozuka *et al.*, 1987) and further evidence suggesting that mast cells in human skin generate PGD_2 has come from studies on subjects with urticaria. Venous blood draining thermally challenged skin contained substantially increased concentrations of PGD_2 in both cold (Heavey *et al.*, 1986a) and heat (Koro *et al.*, 1986) urticaria. The time course of release was similar to that of histamine suggesting the mast cell as the common source. Although useful, the estimation of mediator concentrations in blood may not

accurately reflect the relative proportions of the mediators released within the skin.

Intradermal injection of PGD_2 in the rat causes increased vascular permeability and in man produces a dose-related erythema (Flower *et al.*, 1976; Maurice *et al.*, 1987). Intravenous infusion causes vasodilatation (Heavey *et al.*, 1984). However, a comparison of the ratio of histamine to PGD_2 measured in venous blood in patients with urticaria and the relative vasodilator potencies of these compounds when injected into human skin suggests that histamine is the prime mediator of the urticarial wheal and erythema (Heavey *et al.*, 1986a). The significance of PGD_2 release remains obscure and its interaction with other mediators in urticaria needs to be investigated. A more direct measurement of PGD_2 released in skin would be an important contribution for assessment of its function.

In vitro studies have improved our understanding of mediator secretion by mast cells but confirmation that similar mechanisms occur *in vivo* as *in vitro*, and definition of the relationship between mediator release and pharmacological effect are required. The accessibility of skin makes it a useful organ for the *in vivo* investigation of the pharmacology of mast cell mediators in man in normal and diseased states. We have adapted a chamber technique for the collection of exudates to measure directly the release of mediators in immediate allergic reactions in human skin. The method reduces trauma in sampling human skin, allows multiple sampling and has enabled the detection of an increase in PGD_2 release in skin exudates.

Methods

In vivo *experiments*

Experimental protocols were approved by the Institute of Dermatology Ethical Committee and all subjects gave informed consent.

Exudates were collected from human skin by use of a chamber technique similar to that previously described (Camp *et al.*, 1983; Barr *et al.*, 1984). Briefly, areas of skin on the thigh were gently abraded with the edge of a scalpel blade to remove the superficial scale and upper layers of the epidermis to reveal a smooth glistening surface without bleeding. A cylindrical acrylic chamber, 2 cm internal diameter and 1.5 cm length was placed over each abrasion and held in place with cyanoacrylate adhesive. After allowing the adhesive to dry for about 30 s the chambers were filled with Tyrode solution (composition, $mmol\,l^{-1}$: NaCl 137, KCl 2.7, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $MgCl_2$ 0.5, $CaCl_2$ 1.8, glucose 5.6). Each abrasion took 4–5 min to prepare.

Four normal healthy volunteers (3 females, 1 male, mean age 24, range 21 to 25 years) took part in a preliminary experiment to determine the time course of PGD_2 and PGE_2 release in response to the trauma of the abrasion. A single abrasion, 1.8 cm in diameter, on the thigh was prepared as described above. The acrylic chamber was filled with 1 ml buffer. The chamber contents were replaced with a fresh buffer at 20 min intervals until 5 samples had been collected at 100 min. Each 20 min chamber sample was analysed for PGD_2 and PGE_2.

Antigen challenge was performed on five subjects (3 females, 2 males, mean age 37 years, range 24 to 62 years) who were strongly skin prick test positive to *D. pteronyssinus* antigen (5000 units ml^{-1}). Three 1 cm square abrasions were prepared on the thigh skin of each of the 5 atopic subjects. The abrasions were each initially washed twice for 5 min with 2 ml of Tyrode solution to remove prostaglandins evoked during preparation of the sites. Each chamber was then filled with 1 ml Tyrode solution. A 0.2 ml aliquot of the chamber fluid was removed for analysis at 5 min intervals and immediately replaced with 0.2 ml fresh Tyrode solution to maintain the volume in the chamber at 1 ml. At 25 min, after 5 aliquots had been removed, the skin was challenged by addition of 200 and 1000 units of *D. pteronyssinus* antigen to the replacement solution for chambers 1 and 2 respectively. Chamber 3, the control site, received Tyrode solution alone. Five further aliquots of 0.2 ml were collected from each chamber at 5 min intervals and replaced with Tyrode solution alone. All aliquots of chamber fluid were analysed for PGD_2 and PGE_2.

A control group consisted of seven volunteers (3 females, 4 males, mean age 31 years, range 24 to 60) who gave negative reactions to skin prick tests with *D. pteronyssinus* antigen. A single abrasion was prepared on the thigh of each volunteer in the control group and aliquots of chamber fluid were collected at 5 min intervals as described above. Antigen, 1000 units, was added at 25 min to the chamber contents.

Sites were examined for wheal and flare reactions following antigen challenge. Flare was measured 10 min after challenge by outlining the edge with a marker pen. When the chamber had been removed at the end of the sampling period, the flare edge was traced onto clear plastic film and the area calculated from the weight of the image cut from a photocopy of the tracing.

In vitro *studies*

Circumcision samples of neonatal human foreskin were collected in medium 199 containing ampho-

tericin B 2.5 $\mu g\,ml^{-1}$ and kanamycin 100 $\mu g\,ml^{-1}$ at 5°C. The skin was cleansed of subcutaneous tissue and cut into 0.5–1.0 mm thick by 10 mm long slices with a hand microtome. The skin slices were passively sensitized by incubation for 90 min at 37°C in 20% serum obtained from a subject who gave a strongly positive immediate wheal and flare skin reaction to *D. farinae* antigen.

The sensitized skin slices were washed 3 times with ice-cold Tyrode solution and distributed to give 25 slices in each of two tubes containing 3.75 ml Tyrode solution on ice. The tubes were preincubated at 37°C for 2 min and *D. farinae* antigen added to one tube to give a final concentration of 1000 units ml^{-1} in a total volume of 4 ml. The control incubation received Tyrode solution alone. Aliquots (0.2 ml) of supernatant were removed from each tube at intervals to 20 min and analysed for PGD_2. The dry weight of skin was determined following freeze drying and the residual histamine released by resuspending the slices in Tyrode solution and heating to 100°C for 5 min.

Adult skin, obtained from plastic surgery was processed in an identical way and was passively sensitized and challenged with *D. farinae* antigen for 20 min. An aliquot of the supernatant was assayed directly for PGD_2 and PGE_2, the remaining supernatant was extracted and chromatographed for precise identification of the immunoreactive material.

Radioimmunoassay of prostaglandins D_2 and E_2

PGD_2 and PGE_2 were analysed by radioimmunoassay (RIA) as their methyloximate derivatives by methods similar to those previously described (Kelly *et al.*, 1986; Koro *et al.*, 1986). Briefly, each 0.2 ml sample was mixed with 0.2 ml of methyloximating reagent (methoxyamine hydrochloride 0.12 mM, sodium acetate 1.0 M and ethanol 10% v/v, pH 5.6–5.8) and incubated either at 60°C for 30 min or at room temperature overnight. The reaction mixture was diluted with 0.4 ml of assay buffer (0.05 M Tris-HCl buffer pH 7.4 containing EDTA 0.3 $g\,l^{-1}$, sodium azide 0.5 $g\,l^{-1}$ and γ-globulin, 5 $g\,l^{-1}$) and serially diluted. Each RIA tube contained 0.1 ml of diluted sample, 7000 d.p.m. [^{3}H]-PGD_2 or 5000 d.p.m. [^{3}H]-PGE_2 in 0.1 ml assay buffer, and 0.1 ml of the respective antiserum. The antisera dilutions used were 1:16,000 for the PGD_2 assay and 1:60,000 for PGE_2. Standard curves covered the range 2–250 pg prostaglandin. The assay tubes were incubated overnight at 4°C and the bound tracer precipitated with 0.8 ml of 25% w/v polyethylene glycol 6000 in 0.05 M sodium phosphate buffer, pH 7.0 for 10 min. Tubes were centrifuged at 1400 *g* for 30 min, the supernatant removed by aspiration and the pellets resuspended and counted in 1 ml of Picofluor 30 or Optiphase-RIA scintillant.

Cross reactivity of the PGD_2 and PGE_2 antisera for other prostanoids was as previously reported. In addition, cross reactivity of the anti-PGD_2 methyloxime antiserum for PGD_1 methyloxime was 71% and the anti-PGE_2 methyloxime antiserum against PGE_1 methyloxime 29%. The interassay and intra-assay coefficients of variation for the PGD_2 RIA were 7.1% ($n = 12$) and 7.5% ($n = 30$) respectively and for PGE_2 19% ($n = 7$) and 9.2% ($n = 30$).

Histamine assay

Histamine was bioassayed on atropinized guinea-pig ileum (Lawrence *et al.*, 1981). Release from skin slices was calculated as previously described (Kozuka *et al.*, 1987).

Identification of the immunoreactive prostaglandins

The identity of the immunoreactive material from *in vitro* incubations of passively sensitized adult skin slices challenged with antigen was confirmed by purification of the material by high performance liquid chromatography (h.p.l.c.) in two systems.

For reverse phase chromatography, 1 ml aliquots of supernatant were directly injected onto a C18 Guard Pak cartridge mounted on the h.p.l.c. injector valve. The cartridge was washed with 3 ml 10% v/v methanol:water and then switched to elute bound material onto the main analytical column. The analytical column, LiChrospher 100 CH-18 125 × 4 mm, was eluted with methanol:water:acetic acid, 61:39:0.1 v/v at 1 ml min^{-1} and 0.3 min fractions collected from 4.8 to 9.9 min. Fractions were taken to dryness under vacuum and analysed for immunoreactivity after derivatization.

Aliquots of skin incubation supernatants for normal phase h.p.l.c. were first extracted on C18 Sep-Pak cartridges as previously described (Koro *et al.*, 1986). The lipid extracts were then chromatographed on a LiChrosorb Si60 column, 250 × 4 mm, eluted with chlorobutane:acetonitrile:methanol:acetic acid 60:40:5:0.3 at 1.5 ml min^{-1} (Barr *et al.*, 1984). Fractions, 0.5 min, were collected, taken to dryness and analysed for immunoreactivity.

Materials

D. pteronyssinus and *D. farinae* antigens were supplied by Pharmacia, Sweden. The PGD_2 and PGE_2 methoximate antisera were a gift from Dr R.W. Kelly of the M.R.C. Reproductive Biology Unity, Edinburgh. Standard prostaglandins were purchased

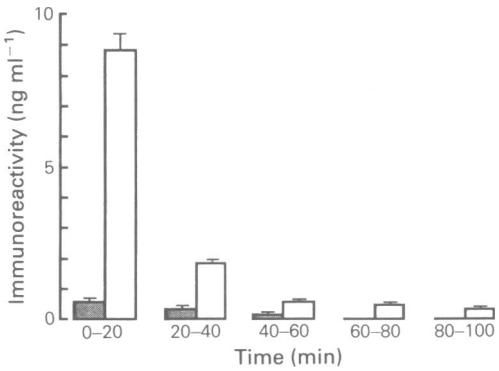

Figure 1 Time course of prostaglandin D_2 (PGD_2) and PGE_2 release in response to abrading human skin. Each site was 1 cm^2. Stippled columns PGD_2; open columns PGE_2.

from the Sigma Chemical Co. Poole; radiolabelled PGD_2 and PGE_2 from Amersham International, Amersham; h.p.l.c. solvents from Fisons, Loughborough; Guard Pak and Sep Pak cartridges from Millipore (UK) Ltd, Harrow, and h.p.l.c. columns and reagents from BDH Chemicals Ltd, Poole.

Results

Time course of prostaglandin release from skin abrasions

Immediately after abrading the skin there was a rapid but transient release of prostaglandins into the chamber fluids as shown in the preliminary time course experiment (Figure 1). Release of PGE_2 during the first 20 min was about 15 times that of PGD_2, 8.9 ± 1.1 and 0.59 ± 0.14 ng per chamber respectively. The rate of prostaglandin release declined rapidly and after 60 min PGE_2 accumulated in the chambers at a constant rate of about 0.3 ng per 20 min collection period. PGE_2 could still be detected at 180 min after preparing the abrasions. PGD_2 release declined in a similar manner and after 60 min was usually below the detection limit of 80 pg per 20 min sample.

Prostaglandin D_2 release in vivo

In the *in vivo* antigen challenge experiments the abraded sites were washed twice for a total time of 10 min to remove most of the prostaglandins generated in response to the abrasion. Although care was taken to prepare uniform abrasions there was variation in the release of prostaglandins from the three sites on each subject. The coefficients of variation for prostaglandin release during the 25 min prior to challenge were calculated for each subject. The mean coefficients of variation for the 5 subjects were 34% and 35% for PGD_2 and PGE_2 respectively.

In four of the 5 atopic subjects who were skin prick test positive to *D. pteronyssinus* antigen, evoked PGD_2 release occurred on addition of antigen to the skin chambers (Table 1). The amount of PGD_2 produced varied widely between the 4 responsive subjects, ranging from 342 to 2791 pg in the 25 min after addition of 1000 units of antigen. The higher antigen dose evoked the same or greater release of PGD_2 than the lower dose. Time courses for PGD_2 and PGE_2 release for subject 2 are shown in Figure 2a,b. Release of PGD_2 was rapid, extrapolation of the curves indicates a lag of about 2 min from addition of antigen to the release of PGD_2. The PGD_2 was still accumulating in the chambers at 25 min after challenge but the rate of increase was declining. There was no evidence for specific release of PGE_2 by antigen in any of the 5 atopic subjects. The same data are shown normalized in Figure 2c,d to take account of the variation in the basal release of prostaglandins from the three sites. Although there was a quantitative difference between sites they were qualitatively similar.

Table 1 The total release of prostaglandin D_2 (PGD_2) and PGE_2 during the 25 min following challenge with 0, 200 and 1000 units of *D. pteronyssinus* (D.p.) antigen for each of the 5 atopic subjects

Treatment (units D.p.)	*PGD_2* (pg) 0	200	1000	*PGE_2* (pg) 0	200	1000	*Flare* (cm^2) 0	200	1000
Subject									
1	86	412	411	170	−100	180	10	31	30
2	70	518	1263	360	330	310	0	11	28
3	56	1283	2791	1050	580	140	0	2	12
4	68	301	342	320	310	370	0	43	30
5	256	106	181	1300	1410	1120	0	21	32
Mean	107	524	997	640	506	424	2	22	26

Flare around each site was measured at 10 min.
A negative value indicates a decline in the prostaglandin concentration in the chamber.

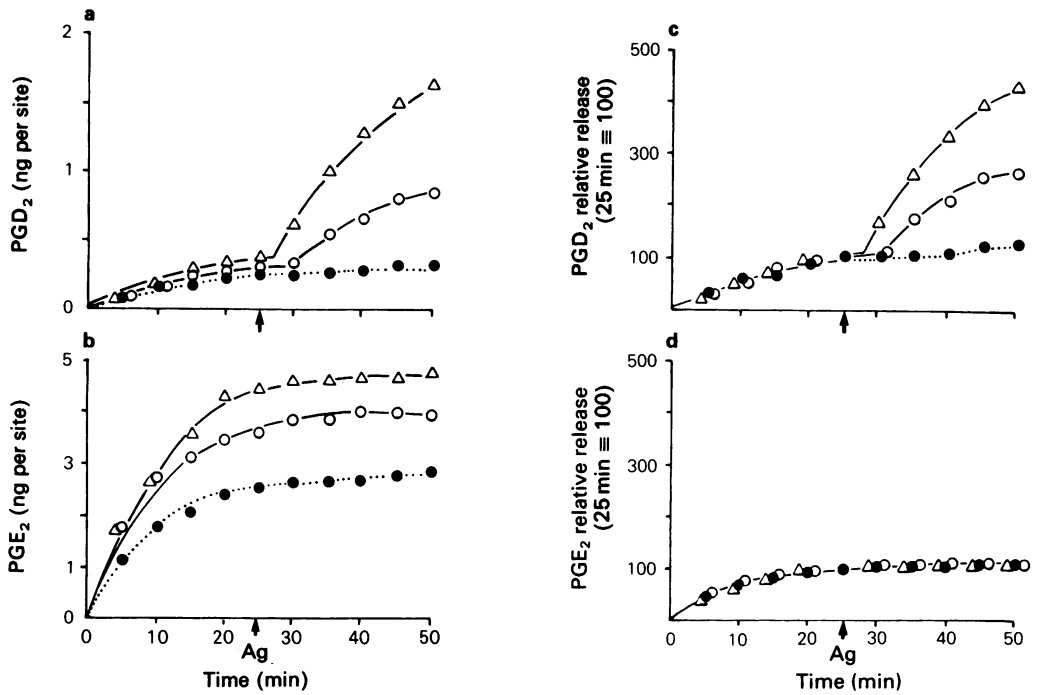

Figure 2 Release of prostaglandin D_2 (PGD_2) and PGE_2 *in vivo* in response to *D. pteronyssinus* antigen challenge. Data for subject 2 are shown. The times indicated are from completion of a 10 min wash of the abraded sites. Antigen, 200 units (○) and 1000 units (△) was added after the fifth sample had been taken at 25 min. The control site (●) had buffer alone. PGD_2 (a) PGE_2 (b) release expressed as ng per site. As there is variation in preparing skin abrasions the same data have been normalised by expressing the amount of PGD_2 (c) and PGE_2 (d) in each chamber at each time point as a percentage of the chambers content at 25 min.

The atopic subjects responded to antigen with a flare reaction which was measured 10 min after challenge and whealing which was confirmed after removal of the chambers at the end of the experiment (Table 1). There was no correlation between the area of flare around the chamber and the amount of PGD_2 recovered. Subject 5 had a good clinical reaction with a well defined flare and whealing but there was no stimulation of PGD_2 release at either of the challenged sites.

Table 2 Histamine release (ng per site) from abraded human skin challenged by *D. farinae* antigen

Time (min)	+ *Antigen*	− *Antigen*
5 min Wash	2.7 ± 0.6	2.3 ± 0.6
0–10	15.0 ± 2.3	1.7 ± 0.4
10–20	10.5 ± 1.1	1.6 ± 0.4
20–30	5.6 ± 1.4	1.5 ± 0.4

Abraded sites were washed for 5 min before addition of antigen (1000 units) for 10 min.
Values are means ± s.e. mean, $n = 5$.

None of the 7 control volunteers (skin prick test negative to antigen) gave a positive clinical response to 1000 units of antigen added to a chamber. PGD_2 was measurable in 3 of the subjects (detection limit 80 pg ml^{-1} chamber buffer) and in this group there was no evidence for stimulated release by antigen; the mean increase in PGD_2 at 25 min after challenge was 112 pg compared to 997 pg for the 5 atopic subjects. The lack of detectable PGD_2 for the remaining 4 control volunteers implies that any specific stimulation of PGD_2 release by antigen must have been minimal (< 80 pg).

Histamine release in vivo

Antigen-evoked histamine release from human skin *in vivo* was measured by a similar procedure but as abrading skin did not stimulate significant histamine release, the challenge with antigen was at 5 min after

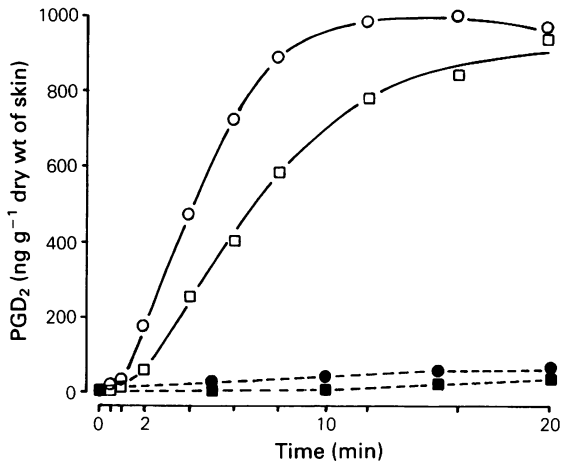

Figure 3 The release *in vitro* of prostaglandin D_2 (PGD_2) from passively sensitized human neonatal skin in response to challenge with 1000 units ml^{-1} *D. farinae* antigen (○□); control incubation (●■). Results of two separate experiments are shown.

preparing the abrasions. Most histamine was released within 10 min of challenge with diminishing amounts recovered thereafter (Table 2). There was no release from unchallenged control sites.

Release of prostaglandin D_2 and histamine by antigen in vitro

Antigen challenge of passively sensitized human neonatal skin *in vitro* released PGD_2 (Figure 3). Generation of PGD_2 was complete within 20 min. The maximum release of PGD_2 in two experiments was 2.7 and 2.5 nmol g^{-1} dry weight of skin. There was no significant release of PGD_2 from the unchallenged skin slices. Histamine release in the same experiments was 271 and 148 nmol g^{-1} dry weight of skin respectively. The molar ratios of histamine: PGD_2 were 99:1 and 57:1.

The identity of the prostaglandins released in vitro *from human skin*

The identities of the immunoreactive products in the PGD_2 amd PGE_2 radioimmunoassays obtained from *in vitro* incubations of passively sensitized adult human skin with *D. farinae* antigen were confirmed by h.p.l.c. using 2 systems.

H.p.l.c. on a silica column demonstrated that the immunoreactive material in the PGD_2 and PGE_2 assays cochromatographed with authentic tritium-labelled PGD_2 (elution time 3.7 min) and PGE_2 (5.4 min) respectively. Chromatography in this system separated PGD_2 and PGE_2 from the other major eicosanoids; arachidonic acid, hydroxy-eicosatetraenoic acids, 13,14-dihydro-15-keto-PGE_2, leukotriene B_4, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, thromboxane B_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$.

Further identification was by reverse phase h.p.l.c. which clearly separated PGE_2 (7.1 min) from PGE_1 (8.3 min) and just separated PGD_2 (7.4 min) from PGD_1 (7.7 min). Immunoreactivities in the PGD and PGE assays cochromatographed with authentic PGD_2 and PGE_2 respectively. The chromatography indicated that neither PGD_1 nor PGE_1 were released in significant amounts from passively sensitized human skin.

Discussion

Previous studies have demonstrated the release of histamine into exudates of human skin in allergic reactions (Dunsky & Zweiman, 1978; Dorsch *et al.*, 1982) and cold urticaria (Misch *et al.*, 1982; Heavey *et al.*, 1986a). In this study we have directly demonstrated release of PGD_2 in human skin during immediate hypersensitivity reactions. The time course for the recovery of PGD_2 in skin exudates was similar to that of histamine implying that both mediators were derived from the same source, probably the cutaneous mast cell. The release of PGD_2 in conjunction with histamine was also shown *in vitro* with passively sensitized neonatal skin challenged with antigen. The time courses for PGD_2 and histamine releases *in vivo* and *in vitro* were similar. The identity of the immunoreactive material released by stimulated adult skin *in vitro* was confirmed by h.p.l.c.

Antigen stimulated the *in vivo* release of PGD_2 in 4 of the 5 atopic subjects. The amount of PGD_2 released varied widely and there was no correlation with the extent of erythema. The subject who developed the largest area of erythema in response to antigen showed no stimulated release of PGD_2. Oedema of the abraded skin was visible through the Tyrode solution in the chambers within minutes of adding antigen. In one subject the oedema extended beyond the outer edge of the plastic chamber. The area of oedema was not measured because the chamber glued to the skin may have restricted its spread.

The ratio of histamine to PGD_2 recovered in the skin exudates was not measured in the same experiment but a comparison between experiments gave a molar ratio of 140:1. Although collecting exudates is the most direct method for sampling skin, the levels of the mediators detected are only estimates of their true concentration within the skin. Metabolism of PGD_2 during collection of the samples did not appear to be significant. The half life of PGD_2 in plasma is reported to be 4.2 h (Barrow *et al.*, 1984)

and in initial experiments there was complete recovery of PGD_2 added to chambers on the skin. The molar ratio of histamine to PGD_2 found in the *in vitro* experiments was 78:1.

Injection of PGD_2 into human skin causes a long-lasting erythema and an increase in blood flow but relatively little oedema (Flower *et al.*, 1976; Soter *et al.*, 1983; Maurice *et al.*, 1987). Although PGD_2 potentiates the increase in vascular permeability due to histamine in rat skin (Flower *et al.*, 1976) there is no evidence for the potentiation of the wheal (Barnes & Heavey, 1986) erythema, or increased blood flow (Maurice *et al.*, 1987) due to histamine injection in human skin. The relative proportions of histamine and PGD_2 found in this study are similar to the ratio in blood draining whealed skin in immediate cold and heat urticaria (Heavey *et al.*, 1986a; Koro *et al.*, 1986) and we conclude that PGD_2 has at best a minor role in immediate allergic reactions in skin.

Sampling skin by any method causes some trauma which stimulates the release and metabolism of arachidonic acid. The failure of previous studies (Misch *et al.*, 1982; Heavey *et al.*, 1986b) to detect increased amounts of PGD_2 in suction blister fluid from whealed skin of subjects with cold urticaria was probably due to the high background levels of prostaglandins released when the blisters were raised. In the present study, using a chamber technique, the background release of PGD_2 was minimized by washing and equilibrating the abraded sites before challenge with antigen. The cellular sources of the prostaglandins released *in vivo* immediately after abrasion cannot be defined but *in vitro* studies suggest that PGE_2, but not PGD_2, is synthesized by the epidermal cells (Hammarström *et al.*, 1979). Abrasion may cause release of some PGD_2 from the superficial mast cells but there was no evidence for histamine release, and clinically there was no whealing and only a small amount of erythema surrounding freshly prepared abraded skin. It is unlikely that there was significant activation of mast cells during preparation of the sites but it cannot be excluded that the mediators released whilst abrading the skin may subsequently modify the responses of the mast cells.

There was no evidence for the stimulated release of PGE_2 either directly or indirectly as a secondary mediator generated in response to the mast cell products within 25 min of initiation of the immediate reaction. The identities of the prostaglandins measured by RIA as PGD_2 and PGE_2 were substantiated by prior h.p.l.c. purification.

The significance of PGD_2 release, the mechanisms for mast cell activation and the role of other mast cell mediators and how they interact in immediate hypersensitivity reactions is still to be fully explained. Human skin may be a useful organ for investigation of some of these points and may provide data of relevance to pulmonary hypersensitivity.

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