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PROSTAGLANDIN E_2, PROSTAGLANDIN I_2 AND THE VASCULAR CHANGES OF INFLAMMATION

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1 Plasma exudation and blood flow changes induced by intradermal injection of prostaglandins E_2 (PGE_2), I_2 (PGI_2), D_2 (PGD_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) were measured in rabbit dorsal skin by the use of [^{131}I]-albumin and ^{133}Xe.

2 Little plasma exudation was produced by any of the prostaglandins when injected alone.

3 Both PGE_2 and PGI_2 were potent at increasing blood flow, whereas $PGF_{2\alpha}$ and PGD_2 produced an increase only at high doses.

4 All of the prostaglandins studied potentiated the plasma exudation induced by bradykinin. PGE_2 and PGI_2 had similar potent potentiating activity, whereas PGD_2 and $PGF_{2\alpha}$ had activity at doses too high to be of biological significance.

5 Intradermal injections of arachidonate alone resulted in little plasma exudation but produced an increase in blood flow. Arachidonate potentiated bradykinin-induced plasma exudation.

6 Locally-injected indomethacin had no effect on basal blood flow and little effect on the exudation produced by bradykinin, but indomethacin did inhibit the vasodilatation and exudation potentiation produced by arachidonate.

7 PGE_2 and PGI_2 had similar potency in producing marked potentiation of plasma exudation induced by intradermal injection of zymosan.

8 In the reaction to zymosan, it is concluded that vasodilatation is the result of the release of arachidonate, which is subsequently converted to either PGE_2 or PGI_2. These substances regulate the plasma exudation induced by independently-released vascular permeability-increasing mediators.

Introduction

Prostaglandins were originally thought to be of importance in inflammation because of their ability to increase vascular permeability either by acting directly on vessel walls (Kaley & Weiner, 1968), or indirectly by releasing histamine from mast cells (Crunkhorn & Willis, 1971). These conclusions were drawn from observations on rat skin, but observations in human skin (Juhlin & Michaëlsson, 1969), guinea-pig (Williams & Morley, 1973) and rabbit skin (Williams, 1976a) suggested that prostaglandins were poor at increasing vascular permeability. Initially it was difficult to reconcile this lack of activity with the observation (Vane, 1971) that aspirin-like compounds with their long-established ability to suppress inflammatory oedema, inhibited prostaglandin synthesis *in vitro*. A similar apparent paradox existed concerning the relevance of prostaglandins to pain, until it was observed that prostaglandins were able to potentiate pain responses (Ferreira, 1972). It was then found in the guinea-pig (Williams & Morley, 1973), the rat (Moncada, Ferreira & Vane, 1973) and in the rabbit (Williams, 1976a) that prostaglandins produced a striking potentiation of the exudation responses induced by other mediators such as histamine and bradykinin. It was suggested (Williams & Morley, 1973) that exudation potentiation might be a consequence of the potent vasodilator action of certain prostaglandins (Bergström, Duner, von Euler, Pernow & Sjövall, 1959) and subsequently a good correlation between vasodilatation and exudation potentiation was observed (Williams, 1976a).

From these observations on the vascular responses to exogenous agents, a two mediator hypothesis was proposed (Williams, 1977), i.e. that inflammation involved the separate production of vasodilator mediators (such as prostaglandins) and permeability-increasing mediators (such as histamine and bradykinin). This was supported by an analysis of the vascular changes involved in the non-allergic response of rabbit skin to intradermal injection of materials such as *Bordetella pertussis* vaccine (Williams & Peck, 1977). In this model it was shown that indomethacin

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specifically inhibited the production of vasodilator exudation-potentiating mediator, without affecting the level of the permeability increasing mediator. This suggests that the permeability-increasing mediator is not a product of the cyclo-oxygenase step of arachidonate metabolism, and later work has shown that this mediator may be produced entirely separately from arachidonate metabolism (Williams, 1978).

This paper is concerned with the mode of action of prostaglandins in inflammation: the probable initiating event in causing prostaglandin production, and the type of prostaglandins most likely to be involved.

Methods

Animals

Male New Zealand White rabbits of approximately 3.5 kg body weight were used in all experiments. The hair on the skin of the back was closely clipped before use, and only animals having skin of even appearance were used. Skin in the phase of rapid hair growth was found to give inconsistent measurements of blood flow, probably because of the number of blood vessels associated with hair follicles, and was therefore avoided for such measurements.

Intravenous injections

An injection of 2 ml of Evans blue dye (2.5% w/v in saline), to which was added (approximately 15 μCi/kg body weight) [^{131}I]-human serum albumin (Code IB. 17P, The Radiochemical Centre, Amersham), was administered into the marginal ear vein via an indwelling cannula. A short-acting anaesthetic, methohexitone sodium (1% w/v in saline, approximately 10 mg/kg), was then administered via the same cannula. Intradermal injections then followed immediately.

Intradermal injections

Test solutions were made up in sterile 0.9% w/v NaCl solution (saline) and kept on ice before use. Immediately before injection, 20 to 60 μl of ^{133}Xe dissolved in saline (Code XAS.120P, The Radiochemical Centre, Amersham) was added to each ml of test solution to give a final activity of 50 to 100 μCi/ml. Solutions were then taken up into 1 ml disposable syringes and fitted with 12 × 0.4 mm disposable needles. Injections (0.1 ml volumes) were administered rapidly in random block order according to a fixed site pattern, balanced to account for intersite variation. Six replicates were used per treatment, and up to nine treatments per experiment. Following injections, three 0.1 ml injections were administered into paraffin oil in three 2 ml polycarbonate tubes and capped immediately. These samples provided the initial ^{133}Xe activity for clearance calculations.

In experiments where the time course of vascular changes was measured following injection of zymosan, the zymosan was injected intradermally at different intervals before the intravenous injection of labelled albumin.

Preparation of skin samples

After a 20 or 30 min interval (see the section on calculation of results), each animal was killed by an excess dose of concentrated pentobarbitone sodium via the indwelling intravenous cannula. Three 1 ml blood samples were then taken by cardiac puncture. The skin encompassing all the injection sites was removed and placed on a sheet of 10 mm thick aluminium. Injection sites were removed with a 16 mm diameter steel punch, the discs of skin were inserted into 2 ml polycarbonate tubes, and the volumes made up with paraffin oil before capping.

The samples were then counted in fixed order in an automatic γ-counter (Nuclear Enterprises, NE8311) equipped with a teleprinter with punched tape output.

Calculation of results

Counts were background subtracted and cross channel corrected (cross-over ^{133}Xe to ^{131}I negligible, ^{131}I to ^{133}Xe approximately 12%). Skin ^{131}I counts were expressed in terms of a volume of blood plasma by dividing skin sample counts by the count of a unit volume of plasma. Since the ratio of the count of 1 ml of whole blood to count of 1 ml of plasma showed little variation between individual rabbits (ratio: 1.43 ± 0.01, n = 6), this ratio was used routinely in calculations, obviating the necessity of preparing and counting plasma samples in every experiment.

The amount of plasma albumin in each skin sample, in terms of a volume of blood plasma was calculated from:

$$\text{skin plasma volume} = \frac{\text{skin sample count/min}}{\text{1 ml whole blood count/min}} \times \frac{1000}{1.43}\,\mu\text{l}$$

Sites injected with saline provided controls which approximated intravascular plasma volume.

When plasma exudation alone was measured, it was found that the number of intradermal injections per rabbit could be increased to 12 treatments ×6 replicates. In this case an isotope accumulation of 30 min was used. The interval between the last intrader-

mal injection and killing the animal was in excess of the duration of increased permeability induced by intradermal injections of low molecular weight inflammatory substances, such as bradykinin (Williams & Morley, 1974).

Blood flow changes produced by intradermal injections of agents were calculated as percentage increase (or decrease) of ^{133}Xe washout over that in the saline-injected control sites. For short clearance periods this could be treated as a mono-exponential process (Williams, 1976b). Results were calculated from the formula:

$$\text{blood flow change} = \frac{(\ln Xe_s - \ln Xe_a)}{(\ln Xe_i - \ln Xe_s)} \times 100\%;$$

where: Xe_s = count/min saline-injected skin, Xe_a = count/min agent-injected skin, and Xe_i = count/min in 0.1 ml volume of injection fluid.

Short clearance periods would be preferred when treating clearance as a mono-exponential process but this is limited by the effect of variation in injection time. In practice, a 20 min clearance period for up to a maximum of 54 rapid injections produced consistent results.

Results

Blood flow changes induced by prostaglandins

Figure 1 shows the dose-response curve of increased blood flow produced by intradermal injections of prostaglandin I_2. Responses to single doses of PGE_2, $PGF_{2\alpha}$, PGD, and 6-oxo-$PGF_{1\alpha}$ (the stable product of the spontaneous breakdown of PGI_2) have been included for comparison. PGI_2 was potent at increasing blood flow; significant changes being produced by doses of 1 ng. In most experiments PGE_2 was more active at the doses tested, but since measurements were made over a period of 20 min, the longer duration of action of PGE_2 (Peck & Williams, 1978) may influence the ratio obtained. In μg doses, PGD_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ produced some increase in blood flow.

Also shown in Figure 1 are the determinations of plasma exudation measured in the same experiment. It is clear that little plasma exudation was produced by any of the prostaglandins over the dose-range tested. In a previous study (Williams & Peck, 1977) it was shown that the unstable endoperoxide intermediate, PGG_2, also had little activity at inducing plasma exudation.

Potentiation of bradykinin-induced plasma exudation by prostaglandins

Figure 2 shows the plasma exudation produced by

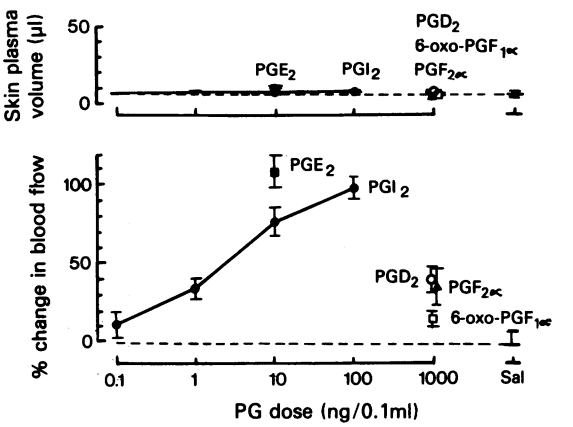

Figure 1 Plasma exudation (measured as $[^{131}I]$-albumin accumulation over a 20 min period) and increase in blood flow (measured by ^{133}Xe washout over a 20 min period) produced by increasing doses of prostaglandin I_2 (PGI_2, ●). Responses to single doses of PGE_2 (■), PGD_2 (○), $PGF_{2\alpha}$ (▲), and 6-oxo-$PGF_{1\alpha}$ (□) have been included for comparison. PGI_2 was stored at −20°C in saline containing 50 mM Tris buffer at pH 9.5. All the solutions used in this experiment were made up finally in saline containing 2.5 mM Tris buffer at pH 7.4. The saline/buffer controls are represented as dashed lines. Points represent the mean of six replicate injections in the rabbit; vertical lines show s.e. mean.

an intradermal injection of the permeability-increasing substances, bradykinin, and the potentiation of exudation produced by mixtures of bradykinin and prostaglandins. Both PGE_2 and PGI_2 were potent in potentiating exudation, whereas potentiation produced by PGD_2 and $PGF_{2\alpha}$ was only achieved with high doses. These results are consistent with the proposition (Williams, 1976a; Williams & Peck, 1977) that potentiation of plasma exudation is dependent on the vasodilator activity of prostaglandins. PGI_2 was more potent than PGE_2 at higher doses (100 ng). The lower potency of PGI_2 found in an earlier study (Peck & Williams, 1978) was due to suboptimal storage conditions of a small quantity of the unstable prostaglandin. At 10 ng, where PGE_2 was more active than PGI_2 in increasing blood flow, the prostaglandins were equi-active in potentiating plasma exudation. This may indicate that an additional component (more pronounced with PGI_2) is involved in potentiation of exudation apart from vasodilatation. Alternatively, the discrepancy may be due to a difference in the time course of vasodilatation produced by PGI_2 and PGE_2; PGI_2 being most active at the time when bradykinin produced peak permeability.

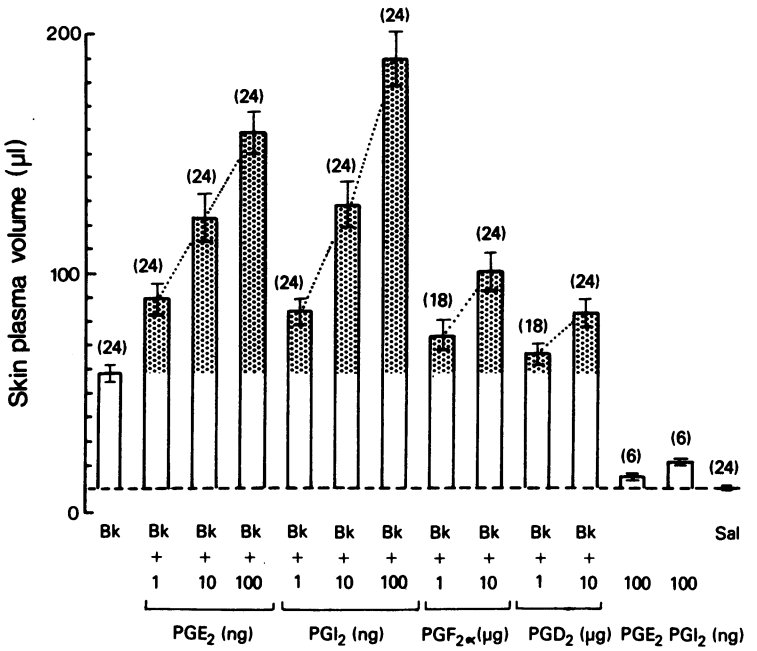

Figure 2 Potentiation of bradykinin-induced plasma exudation by prostaglandin E_2 (PGE_2), PGI_2, $PGF_{2\alpha}$ and PGD_2 measured over a 30 min period in rabbit skin. Bradykinin (Bk) was injected at a dose of 0.5 µg/0.1 ml, and at the same dose mixed with the doses of the prostaglandins shown. Columns represent the mean responses of *n* replicate injections (pooled results from 4 rabbits); vertical lines shown s.e. mean.

The effect of indomethacin on normal blood flow and on arachidonate-induced increased blood flow

Figure 3 shows that intradermal injection of arachidonate (1 µg/site) produced an increase in blood flow. The increase was inhibited by addition of indomethacin to arachidonate solutions before injection; in four similar experiments to the one shown, 1 µg of indomethacin produced an 87.1 ± 10.5% inhibition of the increased flow induced by 1 µg of arachidonate. These results suggest that arachidonate is converted to a vasodilator substance via the cyclo-oxygenase enzyme pathway in the skin, the probable dilator substance being either PGE_2 or PGI_2.

In spite of the ability of indomethacin to inhibit increased blood flow induced by exogenous arachidonate, indomethacin failed to affect basal blood flow when injected intradermally at doses from 0.01 µg to 1 µg. This indicates that the production of vasodilator substances, such as PGI_2, from endogenous arachidonate via cyclo-oxygenase, is low under normal conditions (unless exactly balanced by the production of an arachidonate-derived vasoconstrictor, which is unlikely).

Potentiation of bradykinin-induced plasma exudation by arachidonate and the effect of indomethacin

Figure 4 shows the plasma exudation induced by an intradermal injection of bradykinin. Mixtures of bradykinin with increasing doses of indomethacin produced similar responses to bradykinin alone. At the highest dose of indomethacin, inhibition was observed in some experiments but the effect was always small; in six experiments 1 µg of indomethacin produced a 9.3 ± 3.1% inhibition of the exudation induced by 1 µg of bradykinin. These results indicate that the release of cyclo-oxygenase products contributes little to the exudation response to bradykinin.

Figure 4 shows that a 1 µg dose of arachidonate produced little exudation when injected into skin. However, as shown in a previous study (Ikeda, Tanaka & Katori, 1975), arachidonate potentiated exudation responses to bradykinin when mixed with the peptide before injection. This potentiation, unlike that produced by prostaglandins (Williams & Peck, 1977), was inhibited when indomethacin was added to the mixtures, indicating that the effect was due

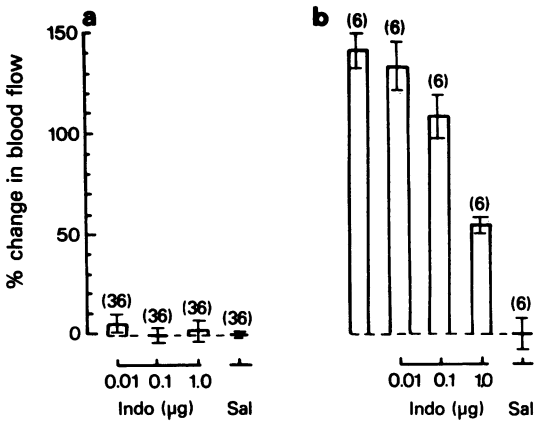

Figure 3 The effects of indomethacin on normal blood flow and on arachidonate-induced increased blood flow in rabbit skin measured over a 20 min period. (a) Effect of increasing doses of indomethacin (Indo) on normal blood flow. This is the pool of identical experiments in 6 rabbits; no significant effect of indomethacin was produced in any one rabbit; (b) an experiment showing increased blood flow produced by an intradermal injection of arachidonate (1 μg/0.1 ml), and the inhibitory effect produced by mixing the arachidonate with increasing doses of indomethacin. Columns represent means, n as shown; vertical lines show s.e. mean.

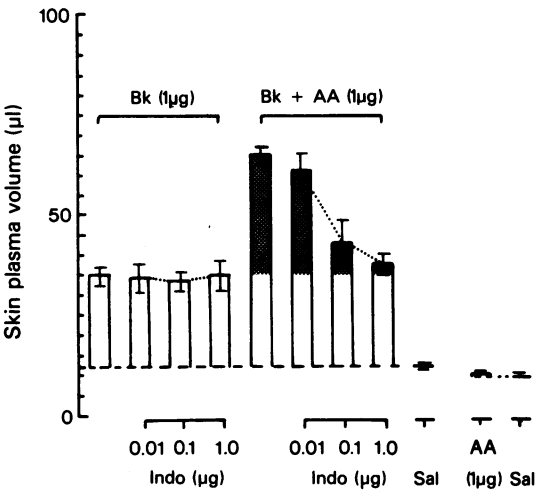

Figure 4 Potentiation of bradykinin-induced exudation by arachidonate measured over a 30 min period. Indomethacin (Indo) had no significant effect on exudation when mixed with bradykinin (Bk, 1 μg) before injection, but indomethacin inhibited exudation potentiation when added to mixtures of bradykinin (1 μg/0.1 ml) and arachidonate (AA, 1 μg/0.1 ml). Arachidonate alone produced little plasma exudation; (this was measured in a separate experiment). Columns represent means of (n = 6) replicate injections; vertical lines show s.e. mean.

to conversion of arachidonate to a vasodilator prostaglandin in the skin.

Potentiation of zymosan-induced oedema by prostaglandin I_2

Figure 5 shows the time course of changes in rate of plasma exudation following an intradermal injection of zymosan. Similar responses were produced by *Bordetella pertussis* and carrageenan. Time courses were measured by injection of zymosan intradermally at various intervals before an intravenous injection of [^{131}I]-albumin.

Some of the sites injected with zymosan were given a further injection of PGI$_2$, administered immediately after the intravenous [^{131}I]-albumin injection. This produced a potentiated response whose time-course differed from that of zymosan alone. It has been proposed (Williams & Peck, 1977) that exudation in the inflammatory reaction to materials such as zymosan is due to the combined action of two endogenous mediators, a permeability increasing mediator and a vasodilator. Since in this experiment the PGI$_2$ dose used was in excess of any endogenous dilator generated, the time-course of the potentiated exudation response should reflect the changes in level of permeability-increasing mediator. For the first 30 min following injection of zymosan, exudation was insignificant. However, PGI$_2$ produced potentiation, indicating the generation of endogenous permeability-increasing mediator in the absence of endogenous vasodilator. This situation was analogous to that observed when the plasma-derived permeability factor (PAPA) was injected into skin. This produced acute exudation only when a vasodilator prostaglandin was added (Williams, 1978). At 30 to 60 min after injection of zymosan, the generation of permeability-increasing mediator reached its peak, but exudation at this time was only moderate, because the generation of vasodilator was not well established. The peak of generation of endogenous vasodilator was apparently at around 60 to 90 min corresponding to the peak of the exudation response to zymosan. After this time the generation of permeability-increasing mediator appeared to decline at a faster rate than that of the endogenous vasodilator.

Discussion

In the rabbit, as previously found in the guinea-pig (Williams & Morley, 1973), intradermal injection of prostaglandins produced little increase in vascular permeability to plasma albumin and consequently little plasma exudation. A similar situation has been described in man (Juhlin & Michaëlsson, 1969). However, intradermal injections of PGE$_2$ and PGI$_2$ were

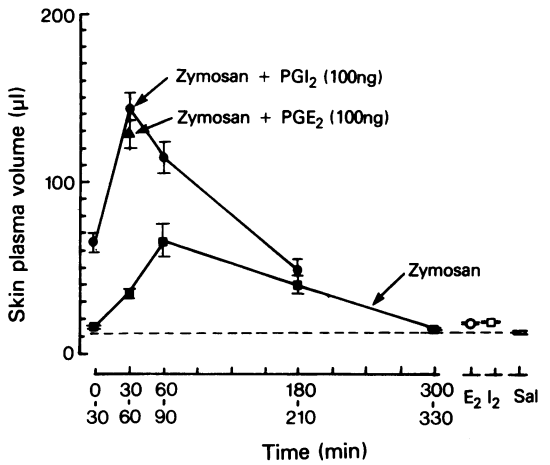

Figure 5 Potentiation of zymosan-induced exudation by prostaglandin I_2 (PGI_2). Zymosan (100 µg/0.1 ml) was injected at various intervals before an intravenous injection of labelled albumin; exudation was then measured over a further period of 30 min. Potentiation was produced by injecting PGI_2 (and PGE_2 for comparison, at one interval) at the start of the measurement period; saline/buffer injections were administered to sites not receiving prostaglandins. Responses to prostaglandin injected into sites previously injected with saline 60 min before are shown for comparison. The abscissa scale shows the period following zymosan injection during which exudation was measured. Points represent means for 6 replicate injections (vertical lines show s.e. mean) and are labelled: (■) first injection zymosan/second injection saline + buffer; (●) zymosan/PGI_2 (100 ng/0.1 ml); (▲) zymosan/PGE_2 (100 ng/0.1 ml); (○) saline/PGE_2 (100 ng/0.1 ml); (□) saline/PGI_2 (100 ng/0.1 ml); and dashed line represents saline/saline + buffer with a 60 min interval.

found to increase blood flow in rabbit skin as measured by ^{133}Xe clearance. This is consistent with the long-established vasodilator activity of PGE_2 in skin, and the recently reported vasodepressor activity of PGI_2 (Armstrong, Lattimer, Moncada & Vane, 1978).

PGE_2 and PGI_2 were both potent in potentiating plasma exudation induced by substances which increased vascular permeability, such as bradykinin. It is considered that this important phenomenon is dependent on the potent vasodilator activity of these prostaglandins (Williams, 1976a; Johnston, Hay & Movat, 1976; Williams & Peck, 1977), although an additional direct modulating effect on vessel wall permeability is a possibility.

In spite of the previous proposals of the importance of $PGF_{2\alpha}$ as an anti-inflammatory substance (Willoughby, Giroud, Di Rosa & Velo, 1972) and PGD_2 as a pro-inflammatory substance (Flower, Harvey & Kingston, 1976), in rabbit skin both these substances produced pro-inflammatory effects, namely vasodilatation and exudation potentiation, but only at doses too high to be of biological importance.

Locally-injected indomethacin had no effect on normal blood flow and little effect on bradykinin-induced plasma exudation. However, indomethacin did inhibit both the vasodilatation and exudation potentiation produced by the prostaglandin precursor, arachidonate. Like its metabolites, arachidonate was a poor inducer of plasma exudation when injected alone.

In the model inflammatory reaction studied, i.e. the non-allergic response to an intradermal injection of zymosan, plasma exudation was shown to be potentiated by local injections of PGI_2 and PGE_2. Williams & Peck (1977) proposed that the observed plasma exudation in this type of reaction (and certain allergic reactions) is the result of the combined action of two types of chemical mediator, a vascular permeability-increasing mediator and a vasodilator mediator. All our results strongly suggest that the permeability-increasing mediator is not a product of arachidonate metabolism, and recent work (Williams, 1978) has revealed that the likely substance in our model is a non-lipid plasma derivative with a molecular weight in the region of 20,000 (Jose, Peck, Robinson & Williams, 1978). It is significant that this factor (PAPA) produced little plasma exudation in the absence of prostaglandin, but a large acute (0 to 30 min) response when a vasodilator prostaglandin was added (Williams, 1978).

By comparison of the time course of the exudation response to zymosan with the time course of the potentiated response produced by PGI_2, the changes in rates of production of the two types of mediator may be deduced. This indicates that the generation of permeability mediator is well established within 30 min, peaks at 30 to 60 min and declines to low levels by 200 min. On the other hand, the generation of vasodilator begins between 30 and 60 min, peaks at 60 to 90 min and declines at a lower rate than that of the other mediator.

That the vasodilator involved in these reactions is a prostaglandin is supported by the observation that indomethacin specifically inhibited the production of vasodilator without affecting the other mediator (Williams & Peck, 1977). The time-course of generation of vasodilator deduced from the present experiments is supported by two previous observations: firstly, the measurements of blood flow changes during the reaction (Williams & Peck, 1977); secondly, the time course of the potentiated response produced when excess exogenous permeability-increasing substance (bradykinin) was injected throughout the reaction to indicate changes in levels of endogenous vasodilator (Williams, 1977).

Since basal blood flow was not influenced by indomethacin, it seems unlikely that vasodilator prostaglandins, such as PGI_2, are generated normally in rabbit skin in quantities sufficient to influence normal vascular tone. However, following an inflammatory stimulus, the release of arachidonate, possibly with a concomitant stimulation of cyclo-oxygenase enzyme activity, would result in vasodilatation due either to PGE_2 or PGI_2 production. The ratio of PGE_2 to PGI_2 may vary in different phases of reactions but either substance could account for the vasodilatation and exudation potentiation observed.

There is evidence that PGI_2 may be produced under conditions relevant to inflammatory reactions. Firstly, carrageenan-induced granuloma tissue in the rat has been reported to produce 6-oxo-$PGF_{1\alpha}$ *in vitro* (Chang, Murota, Matsuo & Tsurufuji, 1976); secondly, anaphylactic reactions induced in guinea-pig lung *in vitro* resulted in 6-oxo-$PGF_{1\alpha}$ as a major product (Dawson, Boot, Cockerill, Mallen & Osborne, 1976); thirdly, 6-oxo-$PGF_{1\alpha}$ has been reported to be produced by macrophages *in vitro* (Humes, Bonney, Pelus, Dahlgren, Sadowski, Kuehl & Davies, 1977).

Prostaglandin E_2 may be produced enzymatically under certain conditions, but the concomitant production of $PGF_{2\alpha}$ often reported, suggests that the unstable intermediate PGG_2 may sometimes be released by the cell and that this substance may spontaneously convert to PGE_2 together with other prostaglandins.

The source of endogenous prostaglandin in the non-allergic model inflammatory reaction described here, was thought to be the polymorphonuclear leucocyte. However, histological examination of the injected skin sites revealed that few such cells were present at the peak of vasodilator production. Since prostaglandin production is a general property of cells, it seems possible that no single cell type is involved. Thus, prostaglandin production may be a response of the tissue as a whole to an injurious, or potentially injurious, stimulus.

Whatever their source and the intricacies leading up to their release, the generation of prostaglandin, either PGE_2 or PGI_2, is likely to be of considerable importance in mediating vasodilatation in inflammation. Further, the quantity of inflammatory exudate appears to be largely governed by the level of vasodilator substance produced when generated concomitantly with substances which increase vessel wall permeability.

The production of these two types of mediators (vasodilators and permeability-increasing mediators) should be regarded as components of a protective system, increasing blood supply to an inflamed tissue with increased metabolic requirements, allowing access for haematogenous cells, and supplying plasma exudate containing substances such as antibodies and complement components.

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