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Prostaglandins and leucotaxis

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Freshly prepared solutions of prostaglandin E_1 exhibit chemotactic activity against rabbit polymorphonuclear leucocytes harvested from the peritoneal cavity but are devoid of such activity either against similar cells obtained from the rat or against polymorphonuclear leucocytes obtained from the blood of the rabbit, rat and man. Some implications of these findings with respect to the development of inflammatory responses and the mode of action of non-steroidal acidic anti-inflammatory drugs are discussed.

Many types of acute inflammatory responses, including irritant-induced paw oedemas and cutaneous Arthus reactions, are characterized by an initial increase in vascular permeability which is intensified and maintained by the migration of circulating leucocytes into the inflamed site. The role of the prostaglandins in the development of these responses may be two fold. They not only enhance the impaired vascular permeability by potentiating the effects of other mediators (Moncada, Ferreira & Vane, 1973; Williams & Morley, 1973) but could sustain the inflammatory reaction by acting as leucotactic factors (Kaley & Weiner, 1971a). It has been proposed that the main source of the prostaglandins in the later phases of immunogenic uveitis, carrageenan-induced pleurisy and various paw oedemas is the polymorphonuclear leucocyte which releases E-type prostaglandins during phagocytosis (Movat, Macmorine & Takeuchi, 1971; Eakins, Whitelocke & others, 1972; Higgs & Youlten, 1972). The anti-inflammatory actions of conventional non-steroidal acidic anti-inflammatory drugs, such as aspirin, phenybutazone and indomethacin, become explicable on the basis of a primary interference with the biosynthesis of PGs *in vivo* (Vane, 1973).

A crucial stage in this chain of events is the ability of the prostaglandins to function as effective leucotactic agents. It has been reported that prostaglandin E_1 (PGE_1), but not other prostaglandins (A_1, E_2 and $F_{2\alpha}$), is chemotactic towards rabbit peritoneal polymorphonuclear leucocytes *in vitro*. The earlier observations were obtained using a concentration of 1 μg ml^{-1} (Kaley & Weiner, 1971a; McCall & Youlten, 1973) but more recently it has been shown that the prostaglandin is chemotactic at concentrations down to 10ng ml^{-1} (Higgs, McCall & Youlten, 1975). This finding is of much more relevance to inflammation *in vivo* since it occurred at concentrations below those of the total prostaglandins found in the carrageenan-induced air bleb in the rat (McCall & Youlten, 1974). There are some observations which throw doubt on the real or potential importance of PGE_1 as a leucotactic agent in species other than the rabbit. Firstly, the local injection of PGE_1 into areas of rat and human skin failed to cause an increased emigration of leucocytes (Arora, Lahiri & Sanyal, 1970; Søndergaard & Wolf-Jürgensen, 1972). Secondly, freshly prepared solutions of the prostaglandin, at a concentration of 1 μg ml^{-1}, did not exhibit chemotactic activity against either human (Turner, Campbell & Lynn, 1975) or rat peripheral leucocytes *in vitro* (Ford-Hutchinson Smith & Walker, 1976a) although such activity could be detected in aged solutions, presumably being due to chemical changes which occur during storage. We have therefore studied the effects of freshly prepared solutions of PGE_1 on the chemotaxis of polymorphonuclear leucocytes separated from the blood of the rabbit, rat and man and from the peritoneal exudates formed after the intraperitoneal injection of glycogen into the rabbit and rat.

MATERIALS AND METHODS

Blood samples, obtained either by cardiac puncture from male albino Wistar rats (Oxfordshire Laboratory Animal Colonies, Ltd) 250–400 g, from the ear of New Zealand white rabbits (Buxted OLAC, Great Totease Farm, Sussex) 1·5 to 3·0 kg, or by venepuncture from normal healthy subjects were collected, using 3 % (w/v) sodium citrate as anticoagulant, and the leucocytes separated by the procedures described by Henson (1971) and Broder, Tackaberry & others (1974). The method for the production of a sterile peritonitis and the harvesting of the peritoneal polymorphonuclear leucocytes in the rat and rabbit was that described by Higgs & others (1975). The chemotactic experiments were performed using disposable polystyrene chambers

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(Adaps, Inc, Dedham, Mass., U.S.A.) and Millipore filters with 8 μm pore size (Millipore Corp., Bedford, Mass., U.S.A.). The techniques for the assembly, staining and counting of the cells were as described previously (Walker, Badcock & others, 1975) except that a standard incubation time of 3 h was employed. Directed migration (chemotaxis) of the leucocytes was measured as the difference in the number of cells which had migrated to the lower surface of the filter when the prostaglandin was in the lower chamber only compared to the results obtained when it was added in equal concentrations to both chambers (random migration, see Keller, Hess & Cottier, 1974; Higgs & others, 1975). Solutions of PGE_1, a gift from Dr K. Crowshaw (May and Baker, Ltd, Dagenham), were prepared according to Bennett, Eley & Stockley (1975) and diluted with appropriate amounts of Medium 199 (Wellcome Reagents, Ltd, Beckenham). The separative procedures for both peripheral and peritoneal leucocytes ensured the preparation of suspensions containing at least 95% polymorphonuclear leucocytes.

RESULTS

The results with rabbit polymorphonuclear leucocytes obtained from sterile peritoneal exudates, given in Table 1, show that freshly prepared solutions of PGE_1 produced a significant increase in directed migration (chemotaxis) *in vitro* at a concentration of 1 μg ml^{-1} but not at lower concentrations. In contrast the chemotactic effect of the 1 μg ml^{-1} solution was not observed (Table 2) when polymorphonuclear leucocytes separated from either rabbit, human or rat blood or harvested from the peritoneal exudates of the rat were used as the test system.

Table 1. *Chemotactic effect of PGE_1 on rabbit peritoneal polymorphonuclear leucocytes.* Results given as number of cells per high power field on the lower surface of the filter, ten fields being counted in each experiment. Each figure is the mean with s.d. of at least three chambers. For the measurement of random migration PGE_1 was placed in both compartments of the Boyden chambers, for directed migration (chemotaxis) it was in the lower chamber only.

Prostaglandin concn (μg ml^{-1})	Random migration	Directed migration
0·01	16 ± 4	25 ± 8
0·1	17 ± 5	30 ± 15
1·0	16 ± 2	40 ± 7*

* Significant difference ($P < 0.05$) between results of random and direct migration experiments.

Table 2. *Lack of chemotactic effect of PGE_1 (1 μg ml^{-1}) on peripheral polymorphonuclear leucocytes from the rabbit, man and rat and on peritoneal leucocytes from the rat. Results given as in Table 1.*

Cell source	Exp. No.	Number of cells: Random migration	Number of cells: Directed migration
Rabbit peripheral	1	25 ± 12	27 ± 6
	2	20 ± 3	24 ± 8
	3	12 ± 3	14 ± 2
Human peripheral	1	23 ± 3	22 ± 2
	2	10 ± 2	10 ± 1
Rat peripheral	1	31 ± 12	36 ± 8
	2	9 ± 5	7 ± 4
	3	10 ± 5	9 ± 4
Rat peritoneal	1	23 ± 3	22 ± 2
	2	20 ± 4	22 ± 2
	3	26 ± 2	26 ± 4

DISCUSSION

The results of the present work show that the chemotactic effect of freshly prepared solutions (1 μg ml^{-1}) of PGE_1 *in vitro* is restricted to rabbit peritoneal polymorphonuclear leucocytes. The effect does not occur with similar cells in the rat and PGE_1 is inactive towards circulating polymorphonuclear leucocytes in the rabbit, rat and man. When such activity has been seen it appears to have been due to some chemical change in the prostaglandin solutions on storage (Ford-Hutchison & others, 1976a).

These findings imply that the leucotactic properties of PGE_1 *in vitro* are of no importance in the development of inflammatory exudates *in vivo* since they are limited to one leucocyte preparation in only one of the species studied. Furthermore the chemotactic activity of PGE_1 towards the rabbit peritoneal leucocytes is very modest compared to the effects of other chemotactic factors in serum (Kaley & Weiner, 1971b). Thus the association between prostaglandin formation and leucocyte emigration in developing inflammatory exudates *in vivo* becomes suspect. Strong support for this view has been provided by the results of experiments on the exudates formed in implanted porous sponges in the rat (Walker, Smith & Ford-Hutchinson, 1976). The administration of an anti-inflammatory fraction from human plasma did not affect the prostaglandin content of the exudate but almost completely inhibited polymorphonuclear leucocyte migration whereas the administration of a specific inhibitor of prostaglandin biosynthesis, 5, 8, 11, 14-eicosate-

traynoic acid, produced the reverse effects. Thus not only are the two phenomena, i.e. the presence of prostaglandins and leucocyte emigration, not causally connected but the migrated leucocytes cannot be the main source of the prostaglandins in the sponge exudate. Conventional anti-inflammatory drugs, such as indomethacin, were found to affect both the prostaglandin content and leucocyte emigration by independent mechanisms (Ford-Hutchinson, Smith & Walker, 1976b). However there is no correlation between anti-inflammatory activity and a decrease in the prostaglandin content of the rat sponge exudate since this latter parameter was not affected by the human plasma fraction, which exerts a wide variety of anti-inflammatory effects in animal models (Smith & Ford-Hutchinson, 1975), but was significantly reduced by paracetamol, which is devoid of either experimental or clinical anti-inflammatory effects (Vane, 1973).

It is concluded that prostaglandin E_1 does not act as a chemotactic agent in the development of inflammatory exudates *in vivo*. A correlation between the anti-prostaglandin synthetase activity and the inhibitory action of conventional anti-inflammatory drugs on leucocyte migration *in vivo* cannot be justified unless leucotactic products, other than PGE_1 are formed from the fatty acid precursors by prostaglandin synthetase. It is known that the prostaglandin cyclo-oxygenase system converts arachidonic acid into prostaglandin endoperoxides (PGG_2 and PGH_2) which are then converted to both prostaglandins and thromboxanes (Hamberg, Svensson & Samuelsson, 1975). One of the more stable thromboxanes (TxB_2) has recently been reported to be weakly leucotactic towards rat polymorphonuclear leucocytes *in vivo* and mouse peritoneal leucocytes *in vitro* at concentrations ranging from 0·5 to 2 $\mu g\,ml^{-1}$ (Boot, Dawson & Kitchen, 1976). No information is yet available about the possible leucotactic properties of the short-lived prostaglandin endoperoxides and thromboxanes. A further enzyme system, soluble lipoxygenase, also present in blood platelets, transforms arachidonic acid into 12-L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (HETE) and this product has been claimed to be a potent mediator of neutrophil chemotaxis (Turner, Tainer & Lynn, 1975). The soluble lipoxygenase activity however, unlike that of the cyclo-oxygenase, is not inhibited by indomethacin.

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