Leucocyte migration and prostaglandin E_1

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The role of prostaglandins in the accumulation of leucocytes one of the fundamental symptoms of an inflammatory response, has received scant attention and the results obtained are inconsistent. Thus, while Kaley & Weiner (1971) found prostaglandin E_1 (PGE_1) to be chemotactic to rabbit polymorphonuclear leucocytes in vitro, Arora et al (1970) and Sondergaard & Wolf-Jurgensen (1972), respectively, failed to observe leucocyte migration when PGE_1 was injected either into rat or human skin. Also, in studies in vitro, while Higgs et al (1975) found PGE_1 to be chemotactic to rabbit polymorphonuclear leucocytes (PMNL) at 10 ng ml^{-1}, Walker et al (1976), using fresh solutions of PGE_1, failed to attract PMNL from rat peritoneum or from rabbit or human blood.

The present experiments were performed to assess the in vivo chemotactic activity of PGE_1. The method was similar to that of Moncada et al (1973), in which the activity of PGs is measured by their ability to reverse the inhibitory effect of indomethacin in carrageenan inflammation in rat paws. Since PGE_1 was active in this test, it's potency in causing cell migration when injected either alone or mixed with other mediators of inflammation into the skin of rats and the peritoneal cavity of mice was assessed.

Male Wistar rats (120–150 g) in groups of 10, were injected subcutaneously in one paw with sterile solution of 0·9% NaCl (saline), PGE_1, carrageenan alone or mixed with PGE_1. Except for one of the two groups injected with carrageenan alone, and one of the two groups receiving PGE_1 alone all other rats received indomethacin (20 mg kg^{-1}) orally, 30 min before the subplantar injections, to suppress endogenous prostaglandin formation. At various times after subcutaneous injection, rats were killed and the amputated paws examined histologically and the number of leucocytes from random high power fields (perivascular) counted from two 5 μm sections stained with iron haemotoxylin and eosin. At least 3 fields were counted for each section and the values from both sections were averaged to obtain a mean cell count for each paw.

In intradermal studies, rats in groups of 6 were anaesthetized with ether and a range of doses of PGE_1 (10 ng–10 μg), histamine (10 ng–10 μg), bradykinin (100 ng–8 μg) and of 5-hydroxytryptamine (10 ng–10 μg) in sterile saline were injected in 0·1 ml volume either alone or as mixtures (as given in the result) into areas of the skin at the back which was shaved 24 h before. Animals were killed 45 min later, the skin removed and the cell counts were then made as described for subcutaneous studies.

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In other studies, male or female albino mice (18–23 g) in groups of 10 were injected intraperitoneally in volumes of 0·1 ml each, with sterile solutions of PGE_1, histamine, bradykinin and 5-HT either alone or as mixtures (as specified in the results) in doses similar to those used in the intradermal studies. 45 min after the injection, the animals were killed, bled from the throat and 2 ml of saline injected into the peritoneal cavities. After the abdomen had been massaged for 1 min, it was opened and the available fluid withdrawn. The total number of leucocytes in the fluid was counted using a Neubauer counting chamber. All samples containing blood were discarded; this accounted for the loss of about 30% of samples.

Earlier attempts to obtain differential leucocyte counts in subplantar, intradermal and intraperitoneal studies were abandoned after it was found that 80–90% of the migrated cells were PMNL.

Results obtained with PGE_1 in rat paw experiments are given in Fig. 1 and Table 1.

Fig. 1 shows that subplantar injections of PGE_1 (0·5 μg/paw) resulted in a mild but significant ($P < 0.01$) migration of leucocytes at 1, 2 and 4 h after the injection, when compared with saline-treated groups, which also showed some accumulation of cells. PGE_1 effects in normal rats were not different (not shown in the figure) from that of indomethacin-treated rats. Carrageenan (1 mg/paw) produced a massive migration of leucocytes which reached a peak in 8–24 h. Fig. 1 also demonstrates the effect of indomethacin (20 mg kg^{-1} oral) in causing a significant ($P < 0.01$) reduction in carrageenan-induced cell migration at 2, 4 and 8 h when compared with the groups receiving carrageenan alone. Injections of PGE_1 (0·5 μg/paw) together with carrageenan to indomethacin-treated rats reversed the effects of indomethacin.

Table 1 gives the effects of various doses of PGE_1. PGE_1 (0·1 μg/paw) caused significant ($P < 0.01$) although moderate migration of leucocytes at 1, 2 and 4 h after the injection. However, when compared with the carrageenan control group, this dose was insufficient to antagonize completely the inhibitory effect of indomethacin which still caused a significant ($P < 0.01$) reduction in the accumulation of cells at 4 and 8 h after carrageenan. Increasing the dose of PGE_1 to 2·5 μg/paw only produced significant ($P < 0.01$) migration of a similar magnitude to that produced by this agent at smaller doses.

Intradermal studies in the rat showed that histamine (100 ng–10 μg), bradykinin (10 ng–8 μg) and 5-HT (10 ng–10 μg) over a range of doses failed to produce significant cell migration. PGE_1 gave variable results in some experiments giving a modest but significant ($P < 0.05$) accumulation of leucocytes independent of

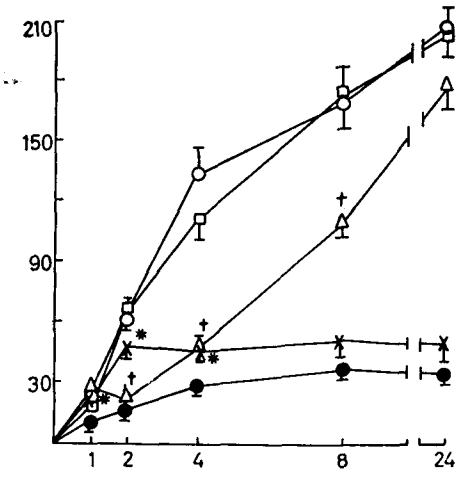

FIG. 1. Number of leucocytes present per high power field (perivascular) (ordinate) at 1, 2, 4, 8 and 24 h after the subcutaneous injections of agents into the rat hind paw. Saline (10 ml kg^{-1}) or indomethacin (20 mg kg^{-1}) was given orally 30 min: before the subcutaneous injections. The values are the mean ± s.e.m. of 10 rats. Abscissa: time (h).
●——● Indomethacin oral + saline 0·1 ml/paw.
×——× Indomethacin oral + PGE_1 0·5 μg/paw.
○——○ Saline oral + carrageenan 1 mg/paw.
△——△ Indomethacin oral + carrageenan 1 mg/paw.
□——□ Indomethacin oral + carrageenan 1 mg/paw + PGE_1 0·5 μg/paw.
* Significant at $P < 0.01$ when compared with saline-injected paws.
† Significant at $P < 0.01$ when compared with carrageenan response in saline-predosed rats.

the dose. Thus, while saline injections into the skin produced migration of 10 ± 1·2 (mean ± s.e.m.) cells, PGE_1 (1000 ng/site) caused 16·1 ± 2·0 (mean ± s.e.m.) leucocytes to migrate to the site of injection, which result was significant at $P < 0.05$ level. When PGE_1 was injected in various doses with other mediators, the effects of the combined injections were not greater than the sum of effects of the agents obtained when they were given separately.

Intraperitoneal injections in the mouse of a wide range of doses (similar to those used in intradermal experiments) of histamine, bradykinin and 5-HT failed to cause a significant ($P > 0.05$) migration of leucocytes. PGE_1 (10 ng–10 μg) was also inactive. Moreover, when PGE_1 was given in various doses, mixed with various doses of other mediators, it failed to produce a response which was greater than that produced by the sum of the effects of PGE_1 and the agent obtained when these two were given separately.

The results show that the subcutaneous injections of PGE_1 in doses of 0·1, 0·5 and 2·5 μg/paw caused moderate though significant ($P < 0.01$) migration of leucocytes in rat paws at 1, 2 and 4 h after the injections, the effects were however not dose dependent. As shown

Table 1. Number of leucocytes present per high power field (perivascular) at 1, 2, 4, 8 and 24 h after the subcutaneous injection of agents into the rat hind paw. Saline (10 ml kg^{-1}) or indomethacin (20 mg kg^{-1}) was given orally to the rats, 30 min: before the subcutaneous injections into the paws. The values are the mean ± s.e.m. of 10 rats.

Pretreatment	Agent injected/paw	Number of leucocytes per high power field with time (h) 1	2	4	8	24
Indomethacin	Saline (0·1 ml)	10 ±1·2	17·2 ±1·9	31·8 ±3·0	42·0 ±4·0	36·1 ±3·1
Indomethacin	PGE_1 (0·1 μg)	20·7* ±2·1	44·4* ±3·5	50·9* ±4·4	45·0 ±3·0	36·1 ±3·1
Indomethacin	PGE_1 (2·5 μg)	26·0* ±1·7	41·0* ±4·4	47·9* ±2·4	48·4 ±6·0	45·6 ±4·6
Saline	Carageenan (1 mg)	18·4 ±1·0	36·5 ±2·4	142·9 ±8·2	198·3 ±7·3	189·4 ±9·8
Indomethacin	Carrageenan (1 mg)	14·6 ±1·3	22·0† ±2·2	59·1† ±5·5	92·0† ±4·3	130·9† ±11·6
Indomethacin	Carrageenan (1 mg) + PGE_1 (0·1 μg)	21·7 ±2·0	46·7 ±3·8	69·1† ±5·4	127·1† ±11·6	156·6 ±11·1
Indomethacin	Carrageenan (1 mg) + PGE_1 (2·5 μg)	22·4 ±1·1	49·5 ±4·4	168·5 ±11·0	188·1 ±13·3	189·3 ±15·4

* $P < 0.01$ in comparison with subcutaneous saline injected rats.
† $P < 0.01$ in comparison with subcutaneous carrageenan injected rats predosed with saline.

by others (DiRosa et al 1971) carrageenan injections into rat paws produced massive migration of leucocytes with the maximum response occurring between 8 and 24 h. While these workers reported that a significant proportion of the cells were of mononuclear types in the later stages of this reaction, the present results agree with the work of Zurier et al (1973), who found that in carrageenan air bleb exudate obtained at 9, 8 and 27 h, 82–94% were PMNL.

Moncada et al (1973), and Lewis et al (1975), showed that PGE_1 (0·1–0·5 μg/paw) not only reversed the effects of indomethacin but also was able to potentiate the increase in paw volume induced by carrageenan. PGE_1 in the present work, even in dose of 2·5 μg/paw, was able only to restore the cellular response to carrageenan which was inhibited by indomethacin. The intradermal and intraperitoneal tests show that PGE_1 possesses neither a direct consistent chemotactic activity, nor does it interact with other mediators to cause leucocyte migration; such an interaction has been shown for PGE_1 in pain and oedema (Ferreira et al 1974; Morley & Williams 1973). In the above studies, cells were counted 45 min after PGE_1 injections as Spector & Willoughby (1964) had shown that a true chemotactic agent would be active within this period.

Thus the results are a further contribution to the evidence that PGE_1 is not a potent leucotactic agent. However, it was effective in reversing the inhibitory effect of indomethacin on cell migration induced by carrageenan in rat paws.

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Labetalol possesses β-adrenoceptor agonist action on the rat isolated uterus

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Labetalol has been described as an antagonist at α- and β-adrenoceptors (Brittain & Levy 1976), which has a lack of intrinsic sympathomimetic activity on β-adrenoceptors, (Farmer et al 1972). It is 6–10 times less potent than phentolamine in blocking α-adrenoceptors, 1·5–3 times less potent than propranolol in blocking β-adrenoceptors, and hence, 4–8 times more potent at β- than at α-adrenoceptors. This profile of labetalol is unique and has provided an agent which has been used successfully in clinical trials in the treatment of hypertension (Prichard & Boakes 1976), and recent observations on its use in the treatment of hypertension in pregnancy appear encouraging (Michael 1979; Lamming & Symonds 1979). Labetalol given to normotensive late pregnant rats prolonged the duration of gestation and the parturient process (Whalley 1977). Since the uterus contains both excitatory α-adrenoceptors and inhibitory β-adrenoceptors (Tothill 1967), it is possible that labetalol may alter uterine function by interfering with these adrenoceptors. This study investigates the effect of labetalol on the in vitro uterus of the non-pregnant rat.

Virgin Sprague-Dawley rats, 200–250 g, in natural oestrus were used. The stage of the oestrous cycle was determined by microscopic examination of the vaginal smear. Whole uterine horns were mounted in a 20 ml organ bath containing Krebs solution at 37 °C bubbled with 5% CO_2 in oxygen. A resting tension of 0·5 g was applied to each tissue and isometric contractions recorded on a pen recorder. Under these conditions a spontaneously contracting uterus was obtained. The effect of labetalol on spontaneous activity was studied in the presence and absence of the β-adrenoceptor blocking agent (±)-propranolol. Labetalol was compared with isoprenaline.

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The drugs used were: labetalol hydrochloride (Glaxo-Allenburys Ltd), (−)-isoprenaline-(+)-bitartate (Sigma), (±)-propranolol hydrochloride (ICI).

Labetalol was found to produce dose-dependent reductions in spontaneous activity, the effect of $2·7 \times 10^{-7}$ M labetalol, which often produced near or complete inhibition, being shown in Fig. 1.

The reduction in uterine activity occurred gradually particularly with the lower doses of labetalol taking up to 10–15 min before a constant reduction was obtained. The effect of labetalol at all doses used was difficult to reverse by washing. When (±)-propranolol ($2·3 \times 10^{-7}$M) was added to the bath during near maximal inhibition by labetalol there was a partial reversal of the inhibitory effects with a return to spontaneous activity (Fig. 1). In contrast, this concentration of (±)-propranolol often produced a full reversal of the inhibitory effect produced by isoprenaline. In the presence of this concentration of (±)-propranolol full inhibition could now be obtained only with a much higher concentration of labetalol or isoprenaline.

Isoprenaline also produced dose-dependent reductions in uterine activity with maximal inhibition being obtained with $1·95 \times 10^{-8}$ M. In contrast to labetalol, all concentrations of isoprenaline produced a rapid

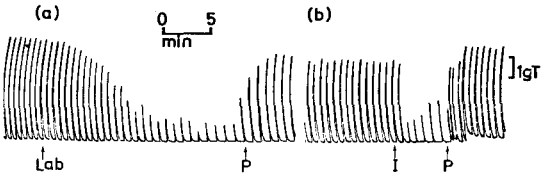

FIG. 1. Effect of (a) labetalol (L), $2·7 \times 10^{-7}$ M, and (b) isoprenaline (I), $0·95 \times 10^{-4}$ M on the spontaneously contracting rat isolated uterus. The effect of (±)-propranolol (P), $2·3 \times 10^{-7}$ M, after adding labetalol or isoprenaline is also shown.