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TIME-DEPENDENT STIMULATORY AND INHIBITORY EFFECTS OF PROSTAGLANDIN E_1 ON EXUDATIVE AND TISSUE COMPONENTS OF GRANULOMATOUS INFLAMMATION IN RATS

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1 The effects of prostaglandin (PGE_1), following local administration during different phases of developing sponge-induced granulomata, were studied in normal and essential fatty acid deficient (EFAD) rats.

2 In normal rats, a single dose of 1 μg PGE_1 on implantation (day 1) increased exudate production without altering total leucocyte counts after 6 h and stimulated granulomatous tissue formation after 8 days.

3 Repeated daily administration of the same dose of PGE_1 on days 1 to 3 had no effect, while administration on days 4 to 7 (i.e. when tissue growth is already in progress) inhibited granuloma formation.

4 In EFAD rats, which are known to produce only very small amounts of endogenous prostaglandins, acute (6 h) exudate formation was unaffected by 0.05 μg PGE_1. However, early stimulatory and later inhibitory effects of 0.05 μg PGE_1 per day were obtained on the granulomatous tissue, similar to those obtained with the 20 fold higher dose in normal rats.

5 The early stimulatory action of PGE_1 on granulomatous tissue formation was enhanced, in normal rats, by concomitant administration of 10 μg theophylline. This latter compound did not influence the later inhibitory effect of PGE_1.

6 These results indicate that PGE_1 exerts either pro- or anti-inflammatory actions on the proliferative (tissue) component of the inflammatory process, depending on the time of administration. While the stimulatory effect following early administration may have been secondary to an initial cyclic adenosine 3′,5′-monophosphate-mediated, vascular response, such a mechanism is unlikely to have been responsible for the later anti-inflammatory action of PGE_1.

7 The implications of these results are discussed in relation to the postulated negative-feedback role of endogenous PGE in chronic inflammation.

Introduction

It is now widely accepted that prostaglandins, particularly those of the E series, contribute to the development of a variety of acute inflammatory conditions, both clinical and experimental (Flower, 1977; Moncada & Vane, 1977). However, under chronic inflammatory conditions, particularly those involving the superimposition of characteristics associated with acute inflammation, the role of prostaglandins is less clear (Bonta & Parnham, 1978b). Some of this confusion is avoided when one considers effects of prostaglandins on 'vascular' components of inflammation (i.e. vasodilatation, increased capillary permeability, diapedesis of leucocytes) separately from their effects on 'tissue' components (i.e. cell proliferation, connective tissue formation).

With regard to vascular components, prostaglandins of the E series (PGEs) are potent vasodilators (see Moncada & Vane, 1977) and this action may be responsible for their enhancement of increased vascular permeability induced by other agents (Williams & Peck, 1977). PGEs also appear to increase diapedesis of leucocytes, though this may not be a directional chemotactic effect (Higgs, McCall & Youlten, 1975; Walker, Smith & Ford-Hutchinson, 1976; Shibuya, Masuda & Izawa, 1976; McClatchey & Snyderman, 1976). In contrast to these pro-inflammatory effects of PGEs, their action on tissue components appear to be mostly inhibitory. Thus, PGEs inhibit fibroblast growth *in vitro* (Johnson & Pastan, 1971; Ko, Page & Narayanan, 1977) and inhibit collagen

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synthesis by bone (Raisz & Koolemans-Beynen, 1974) and gingival fibroblasts *in vitro* (Ko, Cooper & Page, 1976), though stimulation of collagen synthesis by PGE_1 has been observed with embryonic tissue *in vitro* (Blumenkrantz & Søndergaard, 1972).

Indications of inhibitory effects of prostaglandins on tissue components *in vivo* have also been obtained with experimental models of granulomatous inflammation. DiPasquale, Rassaert, Richter, Welaj & Tripp (1973) observed inhibition of granuloma formation after impregnating sponges with very high amounts of PGE_2. More recently, we found that in essential fatty acid deficient (EFAD) rats, which produce only very small amounts of endogenous prostaglandins due to a lack of precursors, granuloma formation was enhanced (Bonta, Parnham & Adolfs, 1977). This enhancement was associated with increased collagen synthesis (Parnham, Shoshan, Bonta & Neiman-Wollner, 1977). These studies suggested that endogenous prostaglandins may exert a suppressive action on tissue components of inflammation.

The present study was carried out to investigate the effects of low doses of PGE_1 on vascular and tissue components of granulomatous inflammation *in vivo*. PGE_1 was used because it has been shown, by several authors, to be slightly more potent than PGE_2 both in stimulating intracellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP) and in producing anti-inflammatory effects *in vitro* (Parker, 1972; Lichtenstein, 1974; Smith, 1977). Furthermore, while PGE_2 is the more important endogenous prostaglandin of the E series released during inflammation in the rat (Youlten & McCall, 1976), the pharmacology of PGE_1 as an anti-inflammatory agent *in vivo* has been more extensively studied (Zurier & Quagliata, 1971; Zurier & Ballas, 1973; Zurier, Hoffstein & Weissmann, 1973; Nusbickel, 1976; Bonta, Parnham & van Vliet, 1978). Since experimental granulomata become more sensitive to the vascular permeability increasing effects of exogenous PGE_1 and PGE_2 as the inflammatory process becomes increasingly chronic (Chang & Tsurufuji, 1976), we treated the granulomata with PGE_1 at different times to investigate possible changes in the effects of PGE_1 on developing granulomata. The effects of PGE_1 were also studied in EFAD rats, which are highly sensitive to the acute pro-inflammatory actions of PGE (Denko, 1974; Bonta, Bult, v.d. Ven & Noordhoek, 1976). Finally, to investigate the possible mediation of the PGE_1 effects by cyclic AMP, we administered PGE_1 in combination with theophylline, which prevents the breakdown of cyclic AMP by inhibition of cyclic AMP phosphodiesterase (Weinryb, Chasin, Free, Harris, Goldenberg, Michel, Paik, Phillips, Samaniego & Hess, 1972). Some of the results described in the present paper have been communicated to the British Pharmacological Society (Bonta & Parnham, 1978a).

Methods

Animals

Male Wistar rats (TNO, Central Breeding Institute, Zeist, The Netherlands), weighing 180 to 250 g were used in all experiments. EFA deficiency was induced by feeding rats on the same diet (Hope Farms, Woerden, The Netherlands) as described previously (Vincent, Zijlstra & Bonta, 1975). EFAD rats were used for experiments when their body weights lay within the same range as the normal rats (Bonta, Chrispijn, Noordhoek & Vincent, 1974). After implantation of sponges, rats were caged separately until killed. Normal animals were caged in Makrolon cages containing sawdust and received standard laboratory food and water *ad libitum*. EFAD rats were caged in Makrolon cages containing Sol 'Speedi-Dri' (Metallochemie, Ramondt, Holland) and received EFAD diet and water *ad libitum* (Bonta *et al.*, 1977).

Sponge implantation and assessment of effects

Polyether sponges were implanted, together with attached cannulae (permitting local injection of drugs at different times), as described elsewhere (Bonta, Adolfs & Parnham, 1979). Briefly, a polyethylene cannula was tied into the centre of each of the sponges, which were then implanted sub-cutaneously into the backs of rats (2 sponges per rat) and the cannulae were brought out at the back of the neck. Carrageenin (2%) was injected directly in a volume of 1 ml into each sponge before closing the incision. One group of rats was killed with chloroform 6 h after sponge implantation and the remaining animals were all killed in a similar manner 8 days after sponge implantation. The cannulae were pulled out of all the sponges and the tissue capsules surrounding the 8 day sponge implants were removed, dried at 80°C for 24 h and weighed. Sponges removed after 6 h were each initially washed in 5 ml heparinized saline in separate test tubes. A piece of wire was then inserted through each sponge which was then centrifuged over the appropriate saline-containing test tube (Higgs, Harvey, Ferreira & Vane, 1976). The volume of exudate thus collected was recorded and total leucocytes counted in a Coulter counter, both parameters being determined from pooled samples from both sponges per rat. Because of the surrounding granulomatous tissue and the finite fluid-retaining capacity of the sponge, the method used in the present study is not suited to the study of prolonged exudate production. Thus, no data are presented on exudate

volumes or total cell counts beyond the 6 h values. Differential cell counts were performed by light microscopy on 6 h exudates from 2 control rats by counting 500 cells per slide after staining the preparations with haematoxylin and eosin.

Drug administration

Animals were divided into groups, each consisting of 5 rats, according to the timing of drug administration. Three treatment periods were used: (1) PGE_1, and/or theophylline, was dissolved in the 2% carrageenin solution which was injected directly into the sponges on implantation. Controls were rats with sponges receiving carrageenin only. No further treatment was given before removal of sponges after 6 h or 8 days. (2) PGE_1 or an equivalent volume of saline (control animals) was injected daily, through the cannulae, on days 2 and 3, following initial treatment on implantation (day 1), as described above. No further drug treatment was given before sponges were removed on day 8. (This treatment regime was used only with PGE_1 in normal animals. Since no effects were observed, it was not considered necessary to repeat this regime either in EFAD rats or in combination with theophylline.) (3) PGE_1, and/or theophylline (0.5 ml), or an equivalent volume of saline (control animals) was injected daily, through the cannulae on days 4 to 7, inclusive, and sponges were removed on day 8. In every case each of the 2 sponges implanted in the rat received the same treatment. The doses used are recorded in Table 1 and in the figure legends.

Statistical analysis

Significance of differences from control values was determined by one-tailed Mann-Whitney U test throughout.

Drugs

Prostaglandin E_1 was obtained from Unilever, Vlaardingen, The Netherlands; theophylline from Merck A.G., Darmstadt, W. Germany; carrageenin sodium (Viscarin) from Marine Colloids, Springfield, N.J., U.S.A.

Results

Acute effects of prostaglandin E_1

The effects of PGE_1 on exudate volume and total leucocyte count, 6 h after injection with 2% carrageenin into sponges implanted in normal and EFAD rats, are shown in Table 1. PGE_1 (1 μg/sponge) significantly increased exudate volume in normal rats, but the lower dose in EFAD rats, although increasing exudate volume slightly, did not produce a significant effect. Total leucocyte counts did not show any appreciable changes in either type of rat. These leucocytes consisted of >95% neutrophils in normal rats, as determined by differential cell counting.

Effects of prostaglandin E_1 on granulomatous inflammation

PGE_1 (1 μg/sponge), administered on implantation, markedly enhanced the subsequent production of granuloma, as measured on day 8 in normal rats (Figure 1b), and this effect was even more significant with the very low dose of PGE_1 (0.05 μg/sponge) which was injected into sponges in EFAD rats (Figure 1a). However, when PGE_1 treatment was continued on days 2 and 3, this enhancement of granuloma formation disappeared in normal rats (Figure 1b). In contrast, administration of PGE_1 on days 4 to 7 (when connective tissue growth is already in progress) inhibited the growth of granuloma (Figure 1b), even at the low dose of 0.05 μg/sponge in EFAD rats (Figure 1a).

Effects of prostaglandin E_1 in combination with theophylline

In the series of experiments involving the combined drug treatment, PGE_1 (1 μg/sponge) did not pro-

Table 1 Effects of prostaglandin E_1 (PGE_1) on acute (6 h) inflammation induced in normal and essential fatty acid deficient (EFAD) rats by carrageenin-impregnated sponges

	Normal rats		*EFAD rats*	
	Carrageenin only	*PGE_1 (1 μg/sponge)*	*Carrageenin only*	*PGE_1 (0.05 μg/sponge)*
Exudate (ml)	0.58 ± 0.06	0.94 ± 0.10*	0.90 ± 0.05	1.22 ± 0.16
Total leucocytes ($\times 10^8$)	0.81 ± 0.12	0.65 ± 0.05	0.50 ± 0.03	0.53 ± 0.03

Values given are means ± s.e. mean of 5 observations (each observation being obtained by pooling exudates from 2 sponges per rat).
*$P < 0.025$.

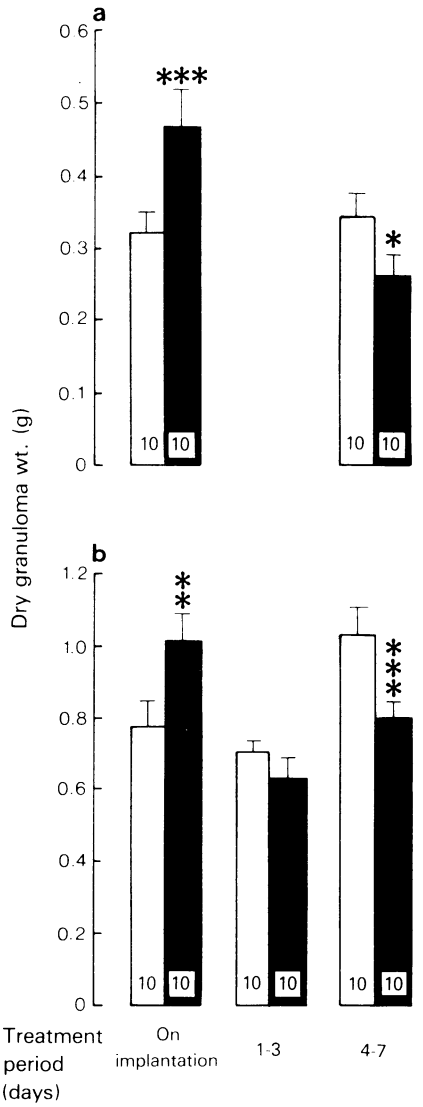

Figure 1 Effects of prostaglandin E_1 (PGE_1) administered locally at different times, on sponge-induced granuloma formation after 8 days in (a) essential fatty acid deficient (EFAD) and (b) normal rats. Data shown are the means of the number of observations shown at the base of the columns; vertical lines show s.e. mean. Open columns are control values and solid columns are the results from PGE_1-treated sponge implants. Data from EFAD rats were obtained with PGE_1 0.05 μg sponge and from normal rats with PGE_1 1 μg sponge. $^*P < 0.05$; $^{**}P < 0.025$; $^{***}P < 0.01$.

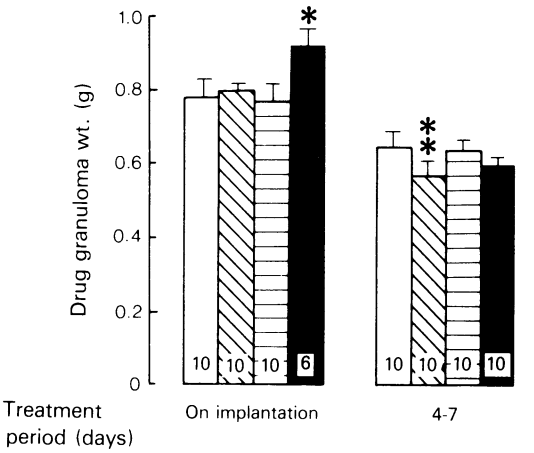

Figure 2 Effects of prostaglandin E_1 (PGE_1) (1 μg/sponge, daily), theophylline (10 μg/sponge, daily) and the combination of both, administered at different times, on granuloma formation, 8 days after sponge implantation into normal rats. Data shown are the means of the number of observations shown at the base of the columns; vertical lines show s.e. mean. Open columns are control values, obliquely hatched columns are results from rats with PGE_1-treated sponges, horizontally hatched columns are results from rats with theophylline-treated sponges and filled columns are results from rats with sponges treated with a combination of PGE_1 and theophylline. $^*P < 0.05$; $^{**}P < 0.025$.

duce the significant increase in granuloma weight (measured on day 8), following treatment on implantation (Figure 2), as it had done in the earlier experiment (Figure 1b). However, when PGE_1 was injected together with theophylline (10 μg/sponge) on implantation, granuloma weight on day 8 was significantly increased, despite the lack of effect of theophylline alone (Figure 2). In fact, the response to the combined treatment was so great that the granulomata grew through the skin in 2 rats, which had to be killed before day 8 because of the wounds. The increased mean dry granuloma weight, derived from the values obtained with the 3 remaining rats, as shown in Figure 2, is, thus, probably an underestimate of the true situation.

The inhibitory effect of PGE_1 (1 μg/sponge), administered on days 4 to 7, on granuloma formation (Figure 1b) was confirmed in this series of experiments (Figure 2). However, with this later treatment period, theophylline failed to potentiate the inhibitory effect of PGE_1 (Figure 2); if anything, the action of PGE_1 was partially reversed. Theophylline itself, administered on days 4 to 7, failed to exert any effect on granuloma formation (Figure 2).

Discussion

The data presented here indicate that increased local concentrations of prostaglandin E_1, in the acute

phase of granulomatous inflammation, stimulate both the early vascular (exudative) component and the subsequent tissue component of the inflammatory response. As the process becomes more chronic, sensitivity to PGE_1 alters, so that once the granuloma tissue has become established, increased local concentrations of PGE_1 inhibit tissue formation.

The stimulation of acute (6 h) exudate formation by PGE_1 confirms the well-documented potentiating effect of PGE on oedema production and increasing vascular permeability (see Moncada & Vane, 1977), an action which appears to be secondary to the vasodilator effect of PGE (Williams & Peck, 1977). The failure of the PGE_1 to produce a significant increase in acute exudation in EFAD rats was probably the result of too low a dose for this particular effect to be observed under the experimental conditions used. Previous studies, with other models of acute inflammation, have shown that EFAD rats are particularly sensitive to the oedema-potentiating effects of PGE (Denko, 1974; Bonta *et al.*, 1976). The role of PGE in enhancing vascular permeability during granulomatous inflammation is open to question (see Bonta & Parnham, 1978b) and Chang, Murota & Tsurufuji (1976) have suggested that this action of PGE is unimportant after the acute phase.

The possible chemotactic effects of PGE have been the subject of considerable discussion (see Walker *et al.*, 1976). Shibuya *et al.* (1976) have suggested that these compounds simply stimulate random migration of polymorphonuclear leucocytes (PMNLs), though PGE_2 may be chemotactic for monocytes in man (McClatchey & Snyderman, 1976). Certainly, our results on the acute effects of PGE_1 (at which stage neutrophil PMNLs are the major migrating leucocytes) do not indicate a chemotactic action of PGE_1 in the rat, though it should be noted that PGE_1 does not appear to be a major product during acute inflammation in the rat (Youlten & McCall, 1976).

The most striking effects of PGE_1, observed in the present investigation, were those on granuloma (tissue) formation, the parameter for the study of which the sponge implant model is particularly suited. While PGE_1, administered on implantation, enhanced 8 day granuloma formation in EFAD rats and in the first experiment in normal rats, the lack of a significant effect of PGE_1 in the second group of normal rats (Figure 2) may be attributable to differences in the sensitivity of the groups of rats to PGE_1. Certainly the amount of granuloma tissue formed is subject to considerable variation (as reflected by the values in saline-treated groups in Figures 1 and 2), which may be related to changes in circulating corticosteroids (Parnham, Adolfs & Bonta, 1977). Furthermore, in subsequent experiments (unpublished) we have observed more marked effects of PGE_1 (1 μg/sponge) on granuloma formation when using smaller sponges. Thus, it is possible that the larger sponges used in the present study did not provide optimal conditions for observing effects of PGE_1. Nevertheless, it seems likely that the stimulation of 8 day granuloma formation, which was observed following treatment with PGE_1 on implantation, was secondary to the acute vascular effects of the prostaglandin. Obviously, when injected at such an early stage, the PGE_1 would have only exerted actions on skin, though, by being trapped in the sponge, it may have remained at the site for longer than following a simple subcutaneous injection. The vasodilatation induced by PGE_1 and its potentiation of the increase in vascular permeability induced by other endogenous mediators would both have provided a more intense 'trigger' to the subsequent development of the granuloma. The involvement of the vascular actions of PGE_1 in this enhancement of granuloma formation is also suggested by the studies in which PGE_1 was administered in combination with theophylline. That theophylline, an inhibitor of cyclic AMP phosphodiesterase (Weinryb *et al.*, 1972), enhanced the action of PGE_1, a stimulator of adenylate cyclase (Butcher & Baird, 1968), to the extent that the skin above the granulomata was eroded in two out of five rats, clearly suggested the mediation of the effect by cyclic AMP. In this context, both the vasodilator and vascular permeability enhancing actions of PGE appear to be related to increases in intracellular cyclic AMP (see Kahn & Brachet, 1976). Whether the increased granuloma formation, after treatment with PGE_1 on implantation, can be wholly attributed to the vasodilatation induced by PGE_1 cannot be decided from our data. However, it is worth noting that the stimulation of granuloma formation was also achieved in EFAD rats with only 0.05 μg PGE_1/sponge. At this low dose, the most prominent effect of PGE_1 would indeed be vasodilatation, although the dose was apparently too low to enhance exudate production in these rats.

Prostaglandin E_1 causes vasodilatation in the granuloma vasculature throughout the first 10 days of sponge-induced granulomatous inflammation (De Leve, Parnham & Saxena, unpublished observations). Nevertheless, this pro-inflammatory action is gradually overcome by a granuloma-inhibiting action, during the development of the granuloma, as shown by the results of the present investigation. The anti-inflammatory action of PGE_1 was clearly reproducible, since it was obtained in 3 different series of experiments. Swingle & Shideman (1972) have shown that granulation tissue formation is detectable 3 to 4 days after implantation of a foreign body. Thus, the inhibitory effect which we observed, following PGE_1 administration on days 4 to 7, is likely to have been due to a direct action of the prostaglandin on the developing granuloma. The inhibitory effect of

PGE on fibroblast growth *in vitro* is well known and appears to be mediated by stimulation of adenylate cyclase (Johnson & Pastan, 1971; Ko *et al.*, 1977). However, the inhibitory effect of PGE_1, administered on days 4 to 7, in the present investigation, was not enhanced by theophylline, suggesting that the PGE_1 was not acting through increasing intracellular cyclic AMP. Several prostaglandins have been shown to alter the production of connective tissue constituents, such as collagen, by fibroblasts *in vitro* (Ko *et al.*, 1976; Desmukh & Sawyer, 1977). The stimulatory effects of PGE on glycosaminoglycan synthesis have been related to increases in cyclic AMP (Castor, 1975; Peters, Peskar & Schönhöfer, 1977) but, while the inhibition of bone collagen synthesis by PGE_1 and PGE_2 (Raisz & Koolemans-Beynen, 1974) may be associated with increased cyclic AMP levels (Yu, Wells, Ryan & Lloyd, 1976), these two actions may not be related in other tissues (Desmukh & Sawyer, 1977). An effect of prostaglandins on collagen metabolism in sponge-induced granulation tissue was suggested by the finding that, in EFAD rats, granuloma formation was enhanced, while protaglandin levels in exudates were markedly reduced (Bonta *et al.*, 1977) and that the increased tissue formation was associated with an increase in collagen synthesis (Parnham *et al.*, 1977). It is possible that the inhibition of granuloma formation by PGE_1, observed in the present study, was due to an effect on collagen synthesis and/or metabolism.

The low dose at which PGE_1 was effective in inhibiting granuloma formation is particularly noteworthy. DiPasquale *et al.* (1973) have reported anti-imflammatory effects of PGE_2 on granuloma formation, but these authors used the massive dose of 1 mg. We have also observed that PGE_2 exerts similar effects to those described here for PGE_1 (Parnham, Bonta & Adolfs, unpublished observations) using a dose of 2 μg, i.e. 500 fold less than that used by DiPasquale *et al.* (1973). In EFAD rats we found PGE_1 to be effective even at 0.05 μg (present data). Since estimations of exudate volumes in day 8 sponge-induced granulomata indicated that at least 2 to 3 ml of fluid is present at this time (Bonta & Parnham, 1978a), the local concentration of PGE_1 which was achieved after administration to normal rats on days 4 to 7 would have been <0.5 μg/ml. This value compares favourably with levels of PGE in synovial fluid from rheumatoid arthritis patients (up to 60 ng/ml) as determined by bioassay (Higgs, Vane, Hart & Wojtulewski, 1974; Swinson, Bennett & Hamilton, 1976). Radioimmunoassay measurements have indicated somewhat lower levels in such synovial fluids (Robinson & Levine, 1974). In exudates from carrageenin-induced pouch granulomata, levels of PGE, determined by radioimmunoassay, have been reported to be relatively high (up to 8 ng/ml) during the first 3 days of inflammation, but decline thereafter (Chang, Murota & Tsurufuji, 1975; Ohuchi, Sato & Tsurufuji, 1976). Nevertheless, the capacity of the granulomata to convert arachidonic acid to PGE_2 increases with time (P.C. Bragt & I.L. Bonta, unpublished observations). Moreover, when measured by bioassay, levels of PGE-like material in exudates of carrageenin-induced inflammation of rats reach around 100 ng/ml after 24 h (Willis, 1969; McCall & Youlten, 1974) and are still as high as 30 ng/ml after 8 days (Bonta *et al.*, 1977). Thus, a modulatory role of PGE in chronic inflammation is conceivable. It should also be noted that the present study was carried out with PGE_1. The major endogenous PG produced during inflammation in the rat is PGE_2 (Youlten & McCall, 1976). In a study completed during the preparation of the present manuscript, we observed that PGE_2 produces similar effects to those described here for PGE_1 and the data on PGE_2 will be presented elsewhere.

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