0022-1767/83/1304-1495$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 130, No. 4, April 1983
Printed in U.S.A.

Anti-Inflammatory Effects of Prostaglandin E_1: *In Vivo* Modulation of the Formyl Peptide Chemotactic Receptor on the Rat Neutrophil[1]

JOSEPH C. FANTONE, WAYNE A. MARASCO, LAURIE J. ELGAS, AND PETER A. WARD

From the Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109

The local generation of chemotactic factors at sites of acute inflammation is thought to be responsible for the recruitment of polymorphonuclear leukocytes (PMN) from the intravascular compartment to the site of tissue injury (1). Many of these potent phlogistic mediators have also been shown to activate PMN resulting in degranulation of lysosomal granules with release of hydrolytic enzymes into the extracellular environment and the generation of superoxide anion (O_2^-) and other toxic metabolites (reviewed in Reference 2). Several *in vitro* studies have shown that treatment of PMN with specific prostaglandins will inhibit both soluble mediator- and particulate material-induced neutrophil lysosomal enzyme release and chemotaxis (3, 4). *In vivo* studies have demonstrated potent anti-inflammatory activity of prostaglandins of the E series (5–7). Systemic administration of prostaglandin E_1 (PGE_1)[2] or its stable analog 15-(S)-15-methyl-PGE_1 (15-M-PGE_1) will inhibit neutrophil dependent immune complex tissue injury in a dose-dependent manner (8). In addition, i.v. infusion of PGE_1 into humans has been shown to inhibit formyl-methionyl-leucyl-phenylalanine- (FMLP) induced lysosomal enzyme release from PMN (9). This study was undertaken to examine the effects of *in vivo* systemic treatment of rats with 15-M-PGE_1 on FMLP-induced neutrophil lysosomal enzyme release and O_2^- secretion. As we will show, the modulation of FMLP-induced neutrophil function is correlated with a decrease in binding affinity for formyl-methionyl-leucyl-(^{3}H)phenylalanine ((^{3}H)-FMLP) to its specific receptor on the neutrophil plasma membrane. The data suggest a novel mechanism for the anti-inflammatory effects of prostaglandins of the E series.

MATERIALS AND METHODS

Materials. FMLP, cytochrome *c* (type III), superoxide dismutase (SOD), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, oyster glycogen, and cytochalasin B were purchased from (Sigma Chemical Co., St. Louis, MO). Radiolabeled (^{3}H)-FMLP (47.6 μCi/nmol) was obtained from (New England Nuclear, Boston MA). Prostaglandins were a generous gift of Dr. John Pike, Upjohn Co. (Kalamazoo, Mich.)

Cells. Retired breeder Sprague-Dawley rats were injected with 30 ml sterile 0.1% oyster glycogen in saline and sacrificed 4 to 6 hr later with ether. At the time of glycogen instillation, groups of three or four animals received subcutaneous injections of either 15-M-PGE_1 (1 mg/kg) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (1 mg/kg). Control animals did not receive prostaglandin treatment. The peritoneal cavities were opened surgically, and the cells were obtained by repeated lavage with saline containing 10 U/ml of sodium heparin. For all experiments, cells from three or four similarly treated rats were pooled. Microscopic analysis showed a >95% population of PMN.

Lysosomal enzyme release. FMLP-induced lysosomal enzyme release was performed by incubating 7.5×10^6 cells/ml in the presence of 5 μg/ml of cytochalasin B at various concentrations of FMLP for 5 min at 37°C. The reaction was terminated by placing the tubes in an ice bath followed by centrifugation. *N*-Acetyl-β-D-glucosaminidase activity was assayed in the supernatant by measuring the release of *p*-nitrophenol from its substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (10). All assays were performed in duplicate or triplicate, and the data were expressed as percent maximum enzyme release ± standard error of the mean (SEM).

O_2^- production. Four hundred microliters of cells (5×10^6/ml) were incubated with 200 μl of a 0.23-mmol solution of ferricytochrome *c*, cytochalasin B (5 μg/ml), 15 μl of the appropriate dilution of peptide, and 25 μl of Hanks' buffer or a 1-mg/ml solution of SOD (11). Mixtures were incubated at 37°C for 20 min, and the reaction mixture was stopped by the addition of 425 μl of Hanks' buffer to tubes that had previously received 25 μl of SOD, and 400 μl of Hanks' buffer plus 25 μl of SOD to tubes that did not contain SOD. The tubes were centrifuged at 4°C at 700 × *g* for 5 min, and the adsorbence of the supernatant was read at 550 nm. The amount of O_2^- produced was calculated from the difference in adsorbence of the samples with and without SOD. This difference was divided by the extinction coefficient for the change between ferricytochrome *c* and ferrocytochrome *c*. The data are expressed as mean values from triplicate samples ± SEM.

Measurement of (^{3}H)-FMLP binding. Studies of (^{3}H)-FMLP to the rat neutrophils were performed at 24°C as described (12). The binding assays were performed by incubating 100 μl of cells (2×10^7 cells/ml) and 5 μl of radiolabeled peptide in 12 × 75-mm glass test tubes. After incubation for 20 min at 24°C, 2 ml of Hanks' buffer were added to the tube, the mixture was vortexed, and the cells were harvested by a glass fiber vacuum filtration method (13). Filters were washed with an additional 8 ml of buffer, dried, and quantitated for cell-bound radioactivity by liquid scintillation. Nonspecific binding was determined by the amount of (^{3}H)-FMLP bound in the presence of a 1000-fold molar excess of nonradiolabeled FMLP and was <10% of total binding. Specific binding refers to total binding minus nonspecific binding. Each data point was performed in triplicate, and the SEM was consistently less than ± 5%. The number of binding sites per cell and the dissociation constant (K_D) were calculated by Scatchard analysis (14).

Statistics. Student's *t*-test was used to compare the biologic responses of neutrophils isolated from prostaglandin-treated and nontreated animals. Analysis of covariance techniques were used to compare the differences in binding affinity (K_D) of rat neutrophils for FMLP between neutrophils isolated from prostaglandin-treated and nontreated animals.

RESULTS AND DISCUSSION

The effect of systemic prostaglandin treatment on FMLP-induced neutrophil lysosomal enzyme release is shown in Figure 1. Treatment of rats with 15-M-PGE_1 (1 mg/kg) resulted in a significant decrease in *N*-acetyl-β-D-glucosaminidase release over a range of concentrations of FMLP (10^{-10} to 10^{-5} M) compared with control animals. At a concentration of 10^{-5} M FMLP, 15-M-PGE_1-treated animals released 11.7 ± 0.1% of their total glucosaminidase, whereas control animals released 17.4 ± 2.1%. This represents approximately a 33% decrease ($p < 0.01$) in response to FMLP in 15-M-PGE_1-treated animals. There was no significant difference in FMLP-induced lysosomal enzyme release from rat PMN isolated from $PGF_{2\alpha}$-treated animals compared with controls. This dose of 15-M-PGE_1 was used because previous studies have shown that it will inhibit immune complex-induced vasculitis in the rat (8).

Rat neutrophils isolated from prostaglandin-treated animals were tested for their ability to release O_2^- after stimulation with FMLP. Neutrophils from rats treated with 15-M-PGE_1 (1 mg/kg)

Received for publication October 12, 1982.
Accepted for publication November 29, 1982.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported in part by National Institutes of Health Grants HL28442 and HL00905.
[2] Abbreviations used in this paper: FMLP, formyl-methionyl-leucyl-phenylalanine; (^{3}H)-FMLP, formyl-methionyl-leucyl-(^{3}H)phenylalanine; PGE_1, prostaglandin E_1; 15-M-PGE_1, 15(S)-15-methyl-prostaglandin E_1; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SOD, superoxide dismutase.

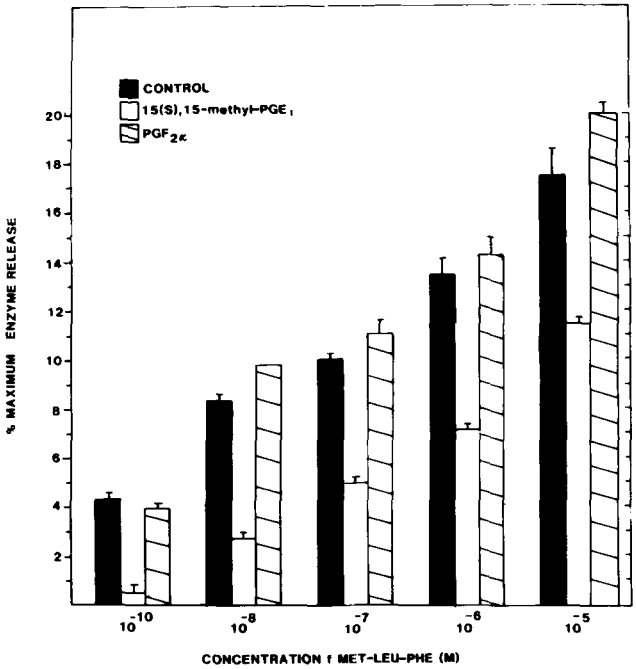

Figure 1. Effect of *in vivo* treatment of rats with 15-M-PGE_1 (1 mg/kg) and $PGF_{2\alpha}$ (1 mg/kg) on FMLP-induced lysosomal enzyme release from peritoneal neutrophils. PMN were harvested from the peritoneal cavity of rats 6 hr after i.p. injection with 0.1% glycogen (*Materials and Methods*). Prostaglandins were administered subcutaneously at the time of glycogen administration. The release of *N*-acetyl-glucosaminidase from rat PMN was determined over a range of concentrations of FMLP in the presence of cytochalasin (5 μg/ml). The data are expressed as percent maximum enzyme release ± SEM.

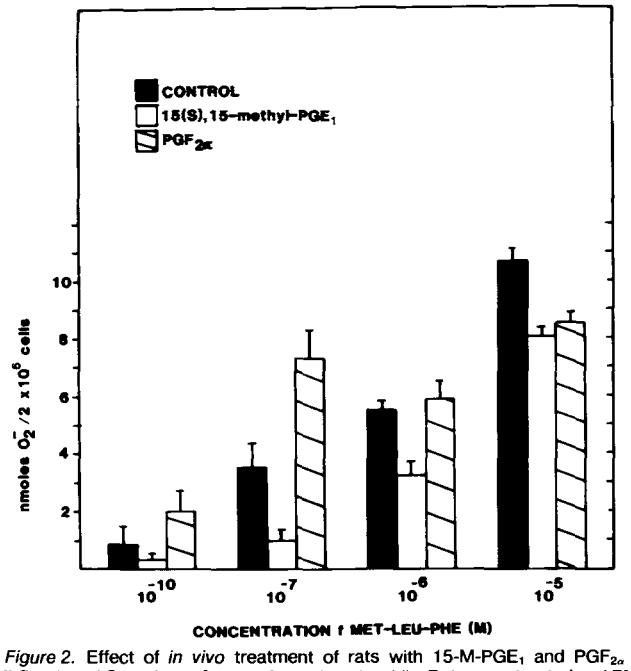

Figure 2. Effect of *in vivo* treatment of rats with 15-M-PGE_1 and $PGF_{2\alpha}$ on FMLP-induced O_2^- release from peritoneal neutrophils. Rats were treated and PMN were harvested as described in Figure 1 and in *Materials and Methods*. The release of O_2^- from rat PMN was determined over a range of concentrations of FMLP in the presence of cytochalasin B. The data are expressed as mean values ± SEM.

showed a significant decrease in their ability to secrete O_2^- into the extracellular environment compared with PMN from nontreated animals over a broad range of concentration of FMLP (10^{-10} to 10^{-5} M) (Fig. 2). At concentrations of 10^{-5} M FMLP, 15-M-PGE_1-treated animals generated 8.1 ± 0.3 nmol of $O_2^-/2 \times 10^6$ cells compared with 10.6 ± 0.5 nmol of O_2^- by PMN from nonprostaglandin-treated animals. This represents a 23.6% decrease ($p < 0.05$) in O_2^- production by PMN isolated from 15-M-PGE_1-treated animals. Overall, secretion of O_2^- by neutrophils from $PGF_{2\alpha}$-treated animals was similar to that of control animals, although there was more variability in the response of these cells to FMLP compared with control and 15-M-PGE_1-treated cells.

The effect of prostaglandin treatment of rats on (^{3}H)-FMLP binding to the formyl peptide receptor on the neutrophil membrane is shown in Table I. Neutrophils isolated from rats treated with 15-M-PGE_1 showed a decrease in binding affinity for FMLP to its receptor in three separate experiments. Scatchard analysis of binding of FMLP to 15-M-PGE_1-treated neutrophils indicated a consistent twofold to threefold increase in dissociation constant (K_D) compared with controls. A single population of formyl peptide receptors was observed on neutrophils isolated from both control and 15-M-PGE_1-treated animals. Although 15-M-PGE_1 treatment altered the binding affinity of (^{3}H)-FMLP to its neutrophil receptor, the total number of receptor sites per cell in prostaglandin-treated animals was similar to control animals in each of the three experiments.

We have demonstrated that systemic administration of 15-M-PGE_1 to rats at a dose that inhibits immune complex-induced vasculitis results in the inhibition of FMLP-induced lysosomal enzyme release and O_2^- production by rat PMN. The suppression of FMLP-induced lysosomal enzyme release and O_2^- production is correlated with an observed decreased binding affinity for FMLP to its receptor on PMN isolated from 15-M-PGE_1-treated animals compared with controls. These observations are consistent with previous *in vitro* data in which prostaglandins of the E series have been shown to inhibit neutrophil chemotaxis, phagocytosis, release of lysosomal enzymes, and O_2^- production in response to a variety of soluble and particulate stimuli (3, 4). In addition, *in vivo* studies have demonstrated suppression of neutrophil-dependent inflammatory reactions by systemic administration of prostaglandins of the E series to rats (5, 8) and inhibition of FMLP-induced lysosomal enzyme release from PMN after i.v. infusion of PGE_1 to humans (9). Previous *in vitro* studies have correlated the ability of PGE_1 to inhibit PMN function with their capacity to elevate intracellular cyclic adenosine monophosphate levels (3, 4).

This study represents the first evidence that *in vivo* treatment of animals with prostaglandins of the E series at concentrations that inhibit inflammatory responses will directly alter the ability of a ligand to bind to its receptor on the neutrophil membrane, specifically, the chemotactic formyl peptide FMLP to the formyl peptide receptor. This alteration in the binding of a chemotactic factor to its receptor on the neutrophil may contribute to the anti-inflammatory effects of prostaglandins of the E series.

The mechanism by which *in vivo* administration of 15-M-PGE_1 alters the binding of FMLP to its receptor on the PMN cell membrane is not known. Recent *in vitro* evidence has demon-

TABLE I

Effect of 15-(S)-15-M-PGE_1 treatment of rats on binding of FMLP to the rat neutrophil formyl-peptide receptor[a]

Expt.	Treatment				
	Control		15(S)15-M-PGE_1		
	Sites/-cell	K_D	Sites/-cell	K_D	P value
1	84,382	8.65×10^{-8} M	115,274	1.73×10^{-7} M	<0.005
2	81,868	3.5×10^{-8} M	78,958	6.66×10^{-8} M	<0.005
3	46,100	1.54×10^{-8} M	43,573	4.38×10^{-8} M	<0.005

[a] Neutrophils were harvested from the peritoneal cavity of rats 6 hr after i.p. instillation of 30 ml of 0.1% glycogen. 15-M-PGE_1 (1 mg/kg) was administered subcutaneously to specific animals at the time of glycogen instillation. The binding of (^{3}H)-FMLP and the determination of the number of binding sites per cell and dissociation constant (K_D) are described in *Materials and Methods*. P values comparing K_D values for neutrophils isolated from 15-M-PGE_1-treated to nontreated control animals were calculated by analysis of covariance techniques.

strated that modulation of human platelet fibrinogen receptors can occur by cyclooxygenase products of arachidonic acid metabolism (15). A similar modulation of Fc and Con A receptors on murine macrophages (16) has also been reported. In addition, prostacyclin treatment of human platelets *in vitro* will inhibit thrombin-induced exposure of the receptor for factor VIII/von Willebrand Factor (17). These data suggest that prostaglandins may alter cell function by modulating the functional characteristics of cell membrane associated receptors.

Recent evidence has suggested that prostaglandins of the E series can modulate proliferation and differentiation of several cell lines *in vitro* (18). PGE_1 at concentrations between 10^{-6} and 10^{-8} M significantly inhibit clonal expansion of granulocyte-macrophage colony-forming units derived from human bone marrow. Because the half-life of circulating PMN is approximately 6 hr, it is possible that the alteration in the formyl peptide receptor affinity for FMLP is the result of the effects of PGE_1 on the end stages of neutrophil differentiation. In addition, the possibility that a secondary mediator(s), such as corticosteroids, is responsible for the altered binding of FMLP to PMN cannot be excluded. A recent *in vitro* study has demonstrated inhibition of (^{3}H)-FMLP to its receptor on the PMN cell membrane after treatment of PMN with relatively high doses of corticosteroids (19). This decreased binding is the result of a decrease in the association rate constant for ligand-receptor interaction. In these studies the total number of receptor sites and the dissociation rate were unchanged. However, it is unlikely that concentrations of corticosteroid used in this study would occur *in vivo* in the absence of exogenous administration.

Several authors have reported the presence of two populations of formyl peptide receptors on both rabbit and human neutrophil cell membranes (20–23). However, the functional significance of these two receptors of differing binding affinity is not known. It has been speculated that the two populations of receptors may modulate different biologic functions with chemotaxis mediated by those receptors of high binding affinity and O_2^- production and lysosomal enzyme secretion mediated by the lower affinity receptors. Our data with the rat neutrophil differ from the results obtained with the human neutrophil in that the neutrophils recovered from rats treated with PGE_1 show a decreased binding affinity for FMLP and a decrease in the ability to secrete lysosomal enzymes in response to the oligopeptide. Fletcher *et al.* (22) suggest that a second population of low affinity receptors on human neutrophils may originate from specific granule membranes. Our results offer a different mechanism by which a lower affinity receptor can be expressed, because with PGE_1 treatment we observe a decrease in receptor affinity when a diminution in enzyme secretion and O_2^- production is also observed. Additional studies are required to determine whether similar mechanisms of expression of lysosomal granule membrane receptors reported with human neutrophils occur in the rat. Our findings of a single population of formyl peptide receptors on the intact rat neutrophil membrane is consistent with the observation that on intact human neutrophils only a single population of low affinity formyl peptide receptors is observed. However, the occurrence of two populations of receptors on intact rabbit neutrophils reported by Mackin *et al.* (23) may represent species differences and is currently under investigation.

We conclude that the anti-inflammatory effect of systemic administration of prostaglandins of the E series *in vivo* may be mediated by alterations of neutrophil functional response to chemotactic peptides. The data indicate that the inhibition may be due in part to alterations in the neutrophil membrane that ultimately result in a decreased binding affinity for FMLP to its specific receptor. Additional studies are necessary to determine the precise mechanism(s) by which PGE_1 modulates this ligand-receptor interaction.

REFERENCES

1. Ward, P. A. 1980. Chemotaxis. *In* Textbook of Immunology, Vol. 1. C. W. Parker, ed. W. B. Saunders, Philadelphia. P. 272.
2. Roos, D. 1980. The metabolic response to phagocytosis. *In* The Cell Biology of Inflammation. G. Weissman, ed. Elsevier/North Holland, Biomedical Press. P. 337.
3. Zurier, R. B., G. Weissmann, S. Hoffstein, S. Kammerman, and H. J. Tai. 1974. Mechanism of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists and agents which effect microtubule function. J. Clin. Invest. 53:297.
4. Rivkin, I., J. Rosenblatt, and E. L. Becker. 1975. The role of cyclic AMP in the chemotactic responsiveness and spontaneous motility of rabbit peritoneal neutrophils. J. Immunol. 115:1126.
5. Zurier, R. B., and F. Quagliata. 1971. Effects of prostaglandin E_1 on adjuvant arthritis. Nature 234:304.
6. Fantone, J. C., S. L. Kunkel, P. A. Ward, and R. B. Zurier. 1980. Suppression by prostaglandin E_1 of vascular permeability induced by vasoactive inflammatory mediators. J. Immunol. 125:2591.
7. Zurier, R. B., I. Damjanov, D. M. Sayadoff, and N. Rothfield. 1977. Prostaglandin E_1 treatment of NZB/NZW F_1 hybrid mice. II. Prevention of glomerulonephritis. Arthritis Rheum. 20:1449.
8. Kunkel, S. L., R. T. Thrall, R. G. Kunkel, P. A. Ward, and R. B. Zurier. 1979. Suppression of immune complex vasculitis by prostaglandins. J. Clin. Invest. 64:1525.
9. Fantone, J. C., S. L. Kunkel, P. A. Ward, and R. B. Zurier. 1981. Suppression of human polymorphonuclear leukocyte function after intravenous infusion of PGE_1. Prostaglandins Med. 2:195.
10. Woollen, J. W., R. Heyworth, and P. G. Walker. 1961. Studies on glucosaminidase. Part III. Testicular *N*-acetyl-β-glucosaminidase and *N*-acetyl-β-galactosaminidase. Biochem. J. 78:111.
11. Babior, B. M., R. S. Kipnes, and J. T. Curnutte. 1973. Biological defense mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. J. Clin. Invest. 52:741.
12. Marasco, W. A., J. C. Fantone, R. J. Freer, and P. A. Ward. Characterization of the rat neutrophil formyl peptide receptor. Amer. J. Pathol. In press.
13. Vitkauskas, G. G., H. J. Showell, and E. L. Becker. 1980. Specific binding of synthetic chemotactic peptides to rabbit peritoneal neutrophils: effects of dissociability of bound peptide, receptor activity and subsequent biologic responsiveness (deactivation). Mol. Immunol. 17:171.
14. Scatchard, G. 1949. The attractions of proteins for molecules and ions. Ann. N.Y. Acad. Sci. 51:660.
15. Bennett, J. S., G. Vilaire, and F. W. Burch. 1981. A role for prostaglandins and thromboxanes in the exposure of platelet fibrinogen receptors. J. Clin. Invest. 68:981.
16. Razin, E., and A. Globerson. 1979. The effect of various prostaglandins on plasma membrane receptors and function of mouse macrophage. Adv. Exp. Med. Biol. 114:415.
17. Fujimota, T., S. Ohara, and J. Hawiger. 1982. Thrombin-induced exposure and prostacyclin Inhibition of the receptor for factor VIII/von Willebrand Factor on human platelets. J. Clin. Invest. 69:1212.
18. Pelas, L. M., H. E. Bromeyer, B. D. Clarkson, and M. A. S. Moore. 1980. Abnormal responsiveness of granulocyte-macrophage committed colony-forming cells from patients with chronic myeloid leukemia to inhibition by prostaglandin E_1. Cancer Res. 40:2512.
19. Skubitz, K. M., P. R. Craddock, D. E. Hammerschmidt, and J. T. August. 1981. Corticosteroids block binding of chemotactic peptide to its receptor on granulocytes and cause disaggregation of granulocyte aggregates *in vitro*. J. Clin. Invest. 68:13.
20. Liao, C. S., and R. J. Freer. 1980. Cryptic receptors for chemotactic peptides in rabbit neutrophils. Biochem. Biophys. Res. Commun. 93:566.
21. Koo, C., R. J. Lefkowitz, and R. Snyderman. 1982. The oligopeptide chemotactic factor receptor on human polymorphonuclear leukocyte membranes exist in two affinity. Biochem. Biophys. Res. Commun. 106:442.
22. Fletcher, M. P., B. E. Seligmann, and J. I. Gallin. 1982. Correlation of human neutrophils secretion, chemoattractant receptor mobilization, and enhanced functional capacity. J. Immunol. 128:941.
23. Mackin, W. M., C. K. Huang, and E. L. Becker. 1982. The formyl peptide chemotactic receptor on rabbit peritoneal neutrophils. I. Evidence for two binding sites but different affinities. J. Immunol. 129:1608.