Stimulus Specificity of Prostaglandin Inhibition of Rabbit Polymorphonuclear Leukocyte Lysosomal Enzyme Release and Superoxide Anion Production

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Prostaglandins (PGs) of the E series and PGI_2 have been shown to inhibit acute inflammatory reactions *in vivo* and polymorphonuclear leukocyte (PMN), chemotaxis, lysosomal enzyme release, and superoxide anion (O_2^-) production *in vitro*. This inhibition of neutrophil stimulation by PGEs and PGI_2 has been correlated with their ability to increase intracellular cyclic adenosine monophosphate (cAMP) levels. However, the mechanism(s) by which PGEs and PGI_2 alter the complex biochemical and biophysical events associated with stimulus–response coupling in the neutrophil are not clear. It is reported here that both PGEs and PGI_2 in micromolar concentrations inhibit formyl-methionyl-leucyl-phenylalanine (FMLP)- and zymosan-induced lysosmal enzyme secretion and superoxide anion production in a dose-dependent manner. No preincubation time of PMNs with the prostaglandins is required for inhibition. Addition of PGEs 10 seconds or later after FMLP stimulation does not alter the biologic response of the neutrophils to the stimulus, suggesting that the prostaglandin inhibition effects early events associated with stimulus–response coupling in the neutrophil. Prostaglandin inhibition of lysosomal enzyme release by the calcium ionophore A23187 was overcome by increasing the extracellular ionophore and/or calcium concentration, suggesting that PGs may modulate intracellular free calcium levels in a manner similar to that observed with platelets. Inhibition of phorbol myristate acetate (PMA)-induced neutrophil lysosomal enzyme secretion by PGEs and PGI_2 was overcome by increasing concentrations of PMA. However, neither PGEs nor PGI_2 altered O_2^- production by PMA-treated neutrophils. These data indicate a dissociation between PMA-stimulated O_2^- production and lysosomal enzyme release. These findings are consistent with the hypothesis that inhibition of neutrophil stimulation by PGEs and PGI_2 is a result of increased intracellular cyclic AMP levels and modulation of calcium-dependent events. In addition, the data indicate that there are at least two mechanisms by which PMNs can be stimulated to produce O_2^-, one inhibited by PGEs and PGI_2 and a second independent of prostaglandin modulation. (Am J Pathol 1984, 115:9–16)

A NUMBER of phagocytic and soluble stimuli have been shown to induce secretion of lysosomal enzymes and superoxide (O_2^-) from polymorphonuclear leukocytes (PMNs).[1-10] Both lysosomal enzymes and oxygen metabolites derived from O_2^- have been shown to cause cell and tissue injury in a number of different *in vivo* and *in vitro* systems and are believed to play an important role in neutrophil-mediated tissue injury.[11,12] Several studies have demonstrated suppression of neutrophil chemotaxis, lysosomal enzyme release, and superoxide production *in vitro* by prostaglandins (PGs) of the E series, PGEs.[13-16] This has been correlated with the ability of PGEs to increase intracellular cyclic adenosine monophosphate (cAMP) levels in the neutrophil. Recently, PGEs have also been shown to inhibit acute inflammatory reactions and edema formation *in vivo*.[17-19] Neutrophils isolated from rats treated systemically with PGE_1 show decreased secretion of O_2^- and lysosomal enzymes after stimulation with formyl-methionyl-leucyl-phenylalanine (FMLP).[20] In addition, PMNs isolated from human patients treated intravenously with PGE_1 secrete decreased quantities of lysosomal enzymes when stimulated with FMLP.[21] In an effort to increase our understanding of the effects of PGE inhibition of

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Table 1—Effect of Prostaglandins on FMLP-, Zymosan-, PMA-, and Ionophore A23187-Induced Lysosomal Enzyme Release From Rabbit Neutrophils

Treatment	Percent maximum enzyme release ± SEM	
	Glucosaminidase	Lysozyme
Experiment 1		
FMLP (10^{-8} M)	73.4 ± 0.0	64.8 ± 3.9
FMLP + 15-M-PGE_1 (10 μM)	46.5 ± 1.6	43.1 ± 1.9
FMLP + 15-M-PGE_1 (1 μM)	66.1 ± 1.0	56.8 ± 3.9
FMLP + 15-M-PGE_1 (0.1 μM)	72.7 ± 0.6	68.6 ± 0.0
FMLP + $PGF_{2\alpha}$ (10 μM)	73.8 ± 1.1	68.6 ± 0.0
Experiment 2		
Zymosan alone (1 mg/ml)	14.4 ± 1.0	41.5 ± 6.5
Zymosan + 15-M-PGE_1 (10 μM)	9.7 ± 2.4	15.3 ± 1.6
Zymosan + PGE_2 (10 μM)	11.6 ± 0.4	11.9 ± 1.1
Zymosan + PGI_2 (10 μM)	8.0 ± 0.1	16.9 ± 3.1
Zymosan + $PGF_{2\alpha}$ (10 μM)	20.9 ± 0.3	33.9 ± 4.6
Zymosan + TxB_2 (10 μM)	15.1 ± 1.5	35.4 ± 3.1
Experiment 3		
PMA (10 ng/ml)	–	37.0 ± 5.1
PMA + PGE_2 (10 μM)	–	13.0 ± 1.1
PM + PGE_2 (1 μM)	–	14.0 ± 1.6
PMA + PGI_2 (10 μM)	–	0.5 ± 6.9
PMA + PGI_2 (1 μM)	–	13.5 ± 4.7
Experiment 4		
PMA 50 ng/ml	–	33.6 ± 4.3
PMA 5 ng/ml	–	33.6 ± 0.9
PMA 0.5 ng/ml	–	24.1 ± 3.3
PMA 50 ng/ml + 15-M-PGE_1 (100 μM)	–	30.2 ± 1.3
PMA 5 ng/ml + 15-M-PGE_1 (100 μM)	–	10.5 ± 1.3
PMA 0.5 ng/ml + 15-M-PGE_1 (100 μM)	–	7.2 ± 2.0
PMA 5 ng/ml + 15-M-PGE_1 (10 μM)	–	12.0 ± 1.7
PMA 5 ng/ml + $PGF_{2\alpha}$ (10 μM)	–	29.3 ± 1.7
Experiment 5		
A23187		
10^{-5} M	65.6 ± 0.5	104.5 ± 2.3
10^{-6} M	50.9 ± 0.8	70.4 ± 4.5
10^{-7} M	17.0 ± 0.7	40.9 ± 4.6
10^{-8} M	9.1 ± 1.0	18.2 ± 2.3
A23187		
10^{-5} M + 15-M-PGE_1 (10 μM)	64.6 ± 1.2	97.7 ± 2.3
10^{-6} M + 15-M-PGE_1 (10 μM)	36.0 ± 0.2	72.7 ± 2.3
10^{-7} M + 15-M-PGE_1 (10 μM)	−2.8 ± 0.4	−6.8 ± 0.0
A23187		
10^{-7} M + PGE_2 (10 μM)	4.4 ± 1.4	20.4 ± 2.3
10^{-7} M + $PGF_{2\alpha}$ (10 μM)	18.3 ± 2.0	70.4 ± 9.1
10^{-7} M + TXB_2 (10 μM)	18.5 ± 1.0	63.6 ± 2.3

Data represent mean values ± SEM. Cells were preincubated with prostaglandins at 37 C for 5 minutes prior to the addition of stimulus. Each experiment shown is a representative example from at least three separate experiments.

neutrophil function, a comprehensive study was initiated to examine PGE-mediated inhibition of neutrophil activation by particulate stimuli (opsonized zymosan) and three soluble stimuli, the chemotactic formyl peptide FMLP, phorbol myristate acetate (PMA), and the calcium ionophore A23187. This study demonstrates marked differences both temporally and quantitatively in the ability of PGE and PGI_2 to modulate rabbit neutrophil activation by these four agents.

Materials and Methods

Neutrophil Preparation

Neutrophils were obtained from rabbit peritoneal cavities 16–18 hours after injection of 200 ml of a 0.1% solution of oyster glycogen in 0.9% saline as previously described.[2] Neutrophils were suspended in Hanks' balanced salt solution (HBSS) and stored on ice. Cells were equilibrated at 37 C for 10 minutes in a shaking water bath prior to the initiation of all assays.

Chemicals

All chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo) unless otherwise noted. PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2, 15-S-15-methyl-PGE_1 (15-M-PGE_1) (a stable analog of PGE_1), and thromboxane B_2 (TxB_2) were the generous gift of Dr. John Pike, of the Upjohn Co. (Kalamazoo, Mich). The PGs and TxB_2 were prepared in stock solutions in ethanol (10 mg/ml). Cytochalasin B (5 mg/ml), PMA (1 mg/ml), A23187 (10^{-2} M) and FMLP (10^{-2} M) were prepared and stored in stock solutions of dimethyl sulfoxide at −20 C.

Lysosomal Enzyme Release

Lysosomal enzyme release was performed by incubating 7.5×10^6 cells/ml in the presence of 5 μg/ml of cytochalasin B at varying concentrations of FMLP or A23187 for 5 minutes at 37 C. PMA-induced lysosomal enzyme release was performed at a similar cell concentration both in the absence or presence of cytochalasin B, and the cells were incubated for 20 minutes at 37 C. Zymosan-induced lysosomal enzyme release was performed in the absence of cytochalasin B at a concentration of 1 mg/ml zymosan and incubated at 37 C for a specified time. The reactions were 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-nitrophenyl from its substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide.[22] Lysozyme activity was assayed in the supernatant by determining the rate of lysis of *Micrococcus lysodeikticus*.[23] All assays were performed in triplicate, and the data are expressed as percent maximum enzyme release.

Superoxide Anion Production

The amount of superoxide anion produced was determined by the reduction of ferricytochrome C to

ferrocytochrome C by stimulated cells as previously described.[2,10] Two million cells were incubated in the presence of 5 μg/ml cytochalasin B, 0.23 mM ferricytochrome C, and varying concentrations of FMLP for 10 minutes at 37 C. PMA- and zymosan-induced O_2^- production was performed in the absence of cytochalasin B, and the cells were incubated for 10 minutes and 45 minutes at 37 C, respectively. The amount of O_2^- produced was calculated from the difference in adsorbance between samples of cells that received SOD prior to activation and those receiving SOD after activation. This difference was divided by the extinction coefficient for the change between ferricytochrome C and ferrocytochrome C to determine nmoles O_2^- produced per 2 × 10^6 cells. The data are expressed as mean values from triplicate samples ± standard error of the mean (SEM).

Statistical Analysis

The Student *t* test (two-tailed analysis) was used to compare the biologic response of PG-treated rabbit neutrophils to nontreated control cells.

Results

Effects of Prostaglandins on Rabbit Neutrophil Lysosomal Enzyme Release

The effects of PG treatment on FMLP-induced lysosomal enzyme release is shown in Table 1 (Experiment 1). Rabbit neutrophils pretreated with 15-methyl-PGE_1 for 5 minutes at 37 C showed inhibition of both N-acetyl-glucosaminidase and lysosyme secretion at concentrations of between 1 and 10 μM. $PGF_{2\alpha}$ did not alter FMLP-induced enzyme release. This range of inhibition of lysosomal enzyme secretion by PGE_1 is consistent with previously published results.[13-16] There was no effect of preincubation time on the ability of 15-M-PGE_1 to inhibit FMLP-induced lysosomal enzyme release. When FMLP (10^{-8} M) and 15-M-PGE_1 (10^{-4} M) were added simultaneously, glucosaminidase secretion was inhibited by 54.6% ($P < 0.01$) and lysozyme secretion by 42.4% ($P < 0.01$). A similar degree of inhibition of FMLP-induced lysosomal enzyme secretion was observed when PMNs were incubated with 15-M-PGE_1 for up to 120 minutes prior to stimulation with FMLP. Addition of 15-M-PGE_1 10 seconds or later after FMLP stimulation did not alter lysosomal enzyme secretion. The ability of 15-M-PGE_1 to inhibit FMLP lysosomal enzyme release was diminished but not totally abrogated at higher concentrations of FMLP (Figure 1). When rabbit neutrophils incubated with

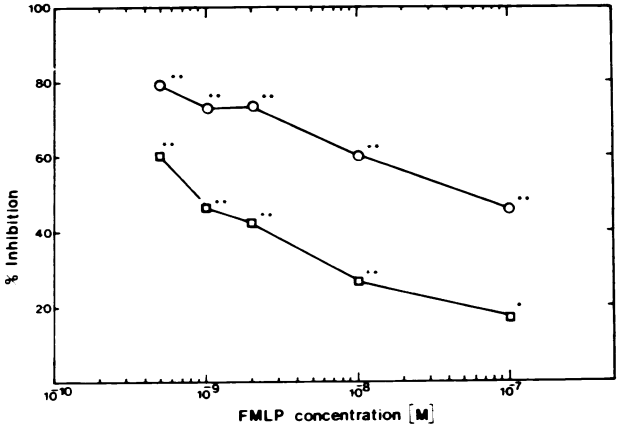

Figure 1 — Inhibition of rabbit neutrophil FMLP-induced N-acetylglucosaminidase release and O_2^- production by 15-M-PGE_1. Rabbit neutrophils (2 × 10^6 cells/ml) in HBSS were equilibrated at 37 C for 10 minutes, incubated in the presence or absence of 15-M-PGE_1 (30 μM) at 37 C for 5 minutes, and stimulated with varying concentrations of FMLP in the presence of cytochalasin B for an additional 5 minutes at 37 C. Percent maximal N-acetyl-glucosaminidase release and O_2^- production were determined (see Materials and Methods). The percent inhibition of enzyme release and O_2^- production by 15-M-PGE_1, compared with non-prostaglandin-treatment controls was determined. The data are the results from a single experiment that is representative of three separate experiments. The maximum percent enzyme release and O_2^- production varied between 40% and 70% and 12 and 24 nmol/10^6 cells, respectively, depending on the experiment. -O-, percent inhibition of O_2^- production; -□-, percent inhibition of N-acetyl-glucosaminidase secretion. * $P < 0.05$. ** $P < 0.01$.

15-M-PGE_1 (30 μM) were stimulated with FMLP at a concentration of 2 × 10^{-9} M, there was 42.1% ($P < 0.01$) inhibition of N-acetyl-glucosaminidase release. However, at a concentration of 10^{-7} M FMLP, 15-M-PGE_1 treatment inhibited neutrophil lysosomal enzyme release by 17.7% ($P < 0.05$).

15-M-PGE_1 also inhibited opsonized zymosan-induced lysosomal enzyme release, as shown in Table 1 (Experiment 2). In the absence of cytochalasin B, rabbit neutrophils were preincubated with 15-M-PGE_1, PGE_2, or PGI_2 at a concentration of 10 μM for 5 minutes at 37 C prior to addition of opsonized zymosan. Inhibition of zymosan-induced glucosaminidase and lysozyme secretion by rabbit neutrophils was observed after a 30-minute incubation. $PGF_{2\alpha}$ and TxB_2 had minimal inhibitory effect on enzyme secretion.

PMA activates neutrophils by binding to specific receptors on the neutrophil membrane[24,25] and will cause a dose-dependent release of lysosomal enzymes. Contents of the secondary granules are secreted to a greater degree than primary granule contents.[6] When neutrophils are pretreated with PGE_2 or PGI_2 at concentrations between 1 and 10 μM, there is inhibition of PMA-induced lysozyme secretion (Table 1, Experiment 3). 15-M-PGE_1 showed inhibitory activity similar to PGE_2, while $PGF_{2\alpha}$ was not effective in inhibiting PMA-induced lysosomal enzyme secre-

Table 2—Effect of Extracellular Calcium Concentration on 15-M-PGE_1 Inhibition of A23187-Induced N-Acetyl-glucosaminidase Release From Rabbit Neutrophils

	Calcium concentration			
	0.1 mM	0.5 mM	1 mM	5 mM
A23187 (10^{-6} M)	37.5 ± 1.3	49.1 ± 0.7	51.8 ± 0.2	45.9 ± 0.3
A23187 (10^{-6} M) + 15-M-PGE_1 (10^{-5} M)	28.9 ± 0.2	40.7 ± 1.2	45.4 ± 0.1	42.4 ± 0.5
% Inhibition	23.0%	17.1%	12.4%	7.6%
P value	<0.01	<0.01	<0.01	<0.01

Data represent percent maximum enzyme release (mean ± SEM). Cells were preincubated with 15-M-PGE_1 at 37 C for 5 minutes prior to addition of A23187.

tion (Table 1, Experiment 4). The inhibition of PMA-induced neutrophil lysozyme secretion by PGEs was overcome by increasing the concentration of PMA. When rabbit PMNs were preincubated at 37 C for 5 minutes in the presence of 15-M-PGE_1 (10 μM) (Table 1, Experiment 4), 12.0% of the total lysozyme was released into the supernatant after a 20-minute incubation with PMA (5 ng/ml), compared with 33.6% for nontreated controls. This represents an inhibition of 62.3% ($P < 0.01$) of PMA-stimulated lysozyme secretion. However, at a PMA concentration of 50 ng/ml, 15-M-PGE_1 (100 μM) had no inhibitory effect on lysozyme secretion.

The calcium ionophore A23187 causes a dose-dependent release of lysosomal enzymes from rabbit neutrophils (Table 1, Experiment 5).[2,9] Pretreatment of neutrophils with either 15-M-PGE (10 μM) or PGE_2 (10 μM) resulted in inhibition of both primary and secondary granule enzyme release induced by A23187 (10^{-7} M). Neither $PGF_{2\alpha}$ nor TxB_2 inhibited A23187-induced lysosomal enzyme release and at lower concentrations of A23187 appeared to selectively enhance lysozyme release. The inhibition of A23187-induced lysosomal enzyme release by 15-M-PGE_1 was overcome by increasing concentrations of A23187 or increasing extracellular calcium concentrations (Table 2). 15-M-PGE_1 (10μM) inhibited A23187 (10^{-6} M)-induced lysosomal enzyme secretion by 23% at a calcium concentration of 0.1 mM. However, when the calcium concentration was increased to 5 mM, 15-M-PGE_1 inhibited A23187-induced lysosomal enzyme release by 7.6%.

Effect of Prostaglandins on Rabbit Neutrophil Superoxide Anion Production

The effects of prostaglandins on FMLP induced O_2^- production by rabbit PMNs at concentrations similar to those that inhibited lysosomal enzyme release are shown in Table 3. Preincubation of rabbit PMNs with 15-M-PGE_1 inhibited FMLP-induced superoxide anion production in a dose-dependent manner, while $PGF_{2\alpha}$ showed only minimal inhibition of FMLP induced O_2^- production. Inhibition of FMLP O_2^- production by 15-M-PGE_1 was less at higher concentrations of FMLP (Figure 1). At a concentration of 2×10^{-9} M FMLP, 15-M-PGE_1 (30 μM) treatment inhibited neutrophil O_2^- production by 74.7% ($P < 0.01$). However, at a concentration of 10^{-7} M FMLP, 15-M-PGE_1 treatment resulted in 46.3% inhibition ($P < 0.01$) of O_2^- release. This FMLP dose-dependent effect on 15-M-PGE_1 inhibition of O_2^- production by rabbit neutrophils is similar to that observed with FMLP-induced lysosomal enzyme. Similar to the

Table 3—Effect of Prostaglandins on FMLP-, PMA-, and Zymosan-Induced O_2^- Release From Rabbit Neutrophils

Treatment	O_2^- (nmol) produced/ 2×10^6 cells ± SEM
Experiment 1	
HBSS	2.2 ± 0.1
FMLP (10^{-7} M)	24.6 ± 0.6
FMLP + 15-S-15-M-PGE_1 (100 μM)	6.9 ± 0.4
FMLP + 15-S-15-M-PGE_1 (10 μM)	15.9 ± 0.5
FMLP + 15-S-15-M-PGE_1 (1 μM)	20.4 ± 0.4
FMLP + $PGF_{2\alpha}$ (100 μM)	18.4 ± 0.7
FMLP + $PGF_{2\alpha}$ (10 μM)	25.8 ± 0.6
Experiment 2	
HBSS	0.4 ± 0.2
Zymosan (1 mg/ml)	9.3 ± 1.0
Zymosan + 15-S-15-M-PGE_1 (100 μM)	5.5 ± 0.5
Zymosan + 15-S-15-M-PGE_1 (30 μM)	6.3 ± 0.7
Zymosan + 15-S-15-M-PGE_1 (3 μM)	6.6 ± 0.2
Zymosan + $PGF_{2\alpha}$ (30 μM)	9.8 ± 0.9
Experiment 3	
HBSS	2.1 ± 0.1
PMA (20 ng/ml)	39.9 ± 1.8
PMA + 15-S-15-M-PGE_1 (100 μM)	39.5 ± 1.8
PMA + 15-S-15-M-PGE_1 (10 μM)	35.9 ± 3.6
PMA + $PGF_{2\alpha}$ (10 μM)	33.2 ± 2.7
Experiment 4	
HBSS	1.2 ± 0.2
PMA (20 ng/ml)	37.1 ± 1.1
PMA + 15-S-15-M-PGE_1 (100 μM)	38.2 ± 0.1
PMA + PGE_2 (100 μM)	37.9 ± 0.3
PMA + PGI_2 (100 μM)	37.8 ± 0.9
PMA + $PGF_{2\alpha}$ (100 μM)	38.1 ± 0.1
PMA + TxB_2 (100 μM)	37.9 ± 0.3

Each experiment shown is a representative example from at least three separate experiments. Cells were preincubated with prostaglandins at 37 C for 5 minutes prior to addition of stimulus.

effects of 15-M-PGE_1 on lysosomal enzyme release, preincubation of PMNs with 15-M-PGE_1 was not required for inhibition of FMLP-induced O_2^- secretion. When 15-M-PGE_1 was added 10 seconds or later after FMLP stimulation of PMNs, minimal inhibition of O_2^- secretion was observed (data not shown). These data indicate that the inhibitory effects of 15-M-PGE_1 on FMLP-induced O_2^- and lysosomal enzyme secretion are rapid and must occur prior to or concurrent with FMLP stimulation of the rabbit neutrophil.

15-M-PGE_1 also inhibited zymosan-induced O_2^- secretion in a dose-dependent manner (Table 3, Experiment 2). When rabbit neutrophils were preincubated at 37 C for 5 minutes in the presence of 15-M-PGE_1 (30 μM), there was 32.2% inhibition ($P < 0.02$) of zymosan-induced O_2^- secretion. $PGF_{2\alpha}$ at a concentration of 30 μM did not significantly alter zymosan-induced O_2^- production. There was no preincubation time required for the inhibition of zymosan-induced enzyme release and O_2^- secretion by 15-M-PGE_1. When 15-M-PGE_1 was added at different times after the addition of opsonized zymosan to PMNs, there was inhibition of subsequent enzyme release and O_2^- secretion. In separate experiments, when 15-M-PGE_1 (10^{-5} M) was added 10 minutes after zymosan addition to PMNs, there was 54.7% ($P < 0.01$) inhibition of glucosaminidase and 27.3% ($P < 0.01$) inhibition of O_2^- secretion, compared with control cells, at 45 minutes and 60 minutes, respectively. These data indicate that 15-M-PGE_1 will inhibit neutrophil activation by opsonized zymosan even after an initial period of stimulation. The difference between the degree of inhibition of zymosan-induced O_2^- production and lysosomal enzyme secretion by 15-M-PGE_1 is the result of using different cell preparations for the experiments and does not represent an increased sensitivity of lysosomal enzyme secretion to PGE_1 inhibition.

In contrast to the inhibitory effects of 15-M-PGE_1, PGE_2, and PGI_2 on PMA-induced lysosomal enzyme secretion, pretreatment of rabbit PMNs with up to 10^{-4} M 15-M-PGE_1, PGE_2, or PGI_2 did not inhibit PMA-induced O_2^- secretion (Table 3, Experiments 3 and 4). The failure to demonstrate inhibition of PMN O_2^- production did not appear to be related to the concentration of PMA, because there was no shift in the dose-response curve between cells treated with 15-M-PGE_1 (10^{-4} M) and control cells (Figure 2). These data suggest a dissociation between the regulatory control of PMA-induced O_2^- secretion (resistant to inhibition by PGEs) and PMA-induced lysosomal enzyme secretion (sensitive to inhibition by PGEs at low PMA concentration).

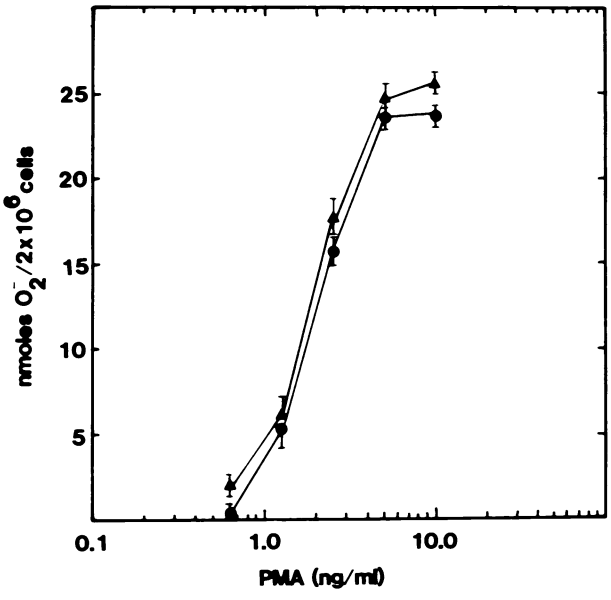

Figure 2—Inhibition of rabbit neutrophil PMA-induced O_2^- production. Rabbit neutrophils (10^6 cells) in HBSS were equilibrated at 37 C for 10 minutes, incubated in the presence of 15-M-PGE, (10^{-4} M) at 37 C for 5 minutes, and stimulated with varying concentrations of PMA for an additional 10 minutes at 37 C. O_2^- production was determined (see Materials and Methods). The data are the results from a single representative experiment. In this experiment FMLP (10^{-7} M)-treated cells produced 14.8 ± 0.2 nmol of $O_2^-/2 \times 10^6$, while cells pretreated with 15-M-PGE, (10^{-4} M) followed by FMLP stimulation produced 6.5 ± 0.2 nmol of O_2^- (56.1% inhibition, $P < 0.01$). -▲-, control cells; -●-, 15-M-PGE_1-treated cells.

Discussion

The ability of PGEs and PGI_2 to modulate PMN functional responses to phagocytic and chemotactic stimuli has been previously described and correlated with increased intracellular levels of cyclic AMP.[13-16] However, there is a paucity of data concerning the mechanism(s) by which PGEs modulate the various biochemical processes involved in neutrophil activation. The experiments described in this paper confirm that PGEs and PGI_2 at concentrations previously shown to increase intracellular cAMP levels will inhibit lysosomal enzyme release and superoxide anion production by FMLP and opsonized zymosan.[13-16] In addition, PGEs and PGI_2 were observed to inhibit both PMA- and A23187-induced lysozyme secretion. However, while relatively high concentrations of FMLP (10^{-7} M) resulted in less inhibition of lysosomal enzyme release by 15-M-PGE_1 the inhibition of PMA- and A23187-induced lysosomal enzyme secretion was completely overcome at higher concentrations of these stimuli. Increasing calcium concentrations also decreased the inhibition of A23187 enzyme release by 15-M-PGE_1.

Recent kinetic studies have shown that the initiation of FMLP-induced O_2^- production and lysosomal

enzyme secretion occurs within 10 seconds after exposure of the human neutrophils to stimulus.[26] This is consistent with our observations that treatment of rabbit PMNs with 15-M-PGE_1 10 seconds or later after FMLP stimulation had no inhibitory effect on O_2^- and lysosomal enzyme secretion. This suggests that the inhibitory effect of PGEs and PGI_2 on FMLP stimulation may be the result of modulation of the early biochemical events following ligand–receptor binding.

Previous studies using human and rabbit PMNs have shown that the degree of lysosomal enzyme release induced by chemotactic factors is enhanced in the presence of extracellular calcium.[27,28] Additional studies have demonstrated that increased intracellular cAMP levels in rat mast cells will inhibit IgE-induced calcium uptake and subsequent histamine release.[29] Other *in vitro* studies suggest that PGE_1 and PGI_2 inhibition of platelet aggregation is in part the result of increased intracellular cyclic AMP levels that result in the activation of a calcium pump that sequesters free calcium in the dense tubular system of the platelet.[30,31] The data presented here would be consistent with the hypothesis that PGEs inhibit lysosomal enzyme secretion by modulating the availability of calcium within different compartments of the neutrophil. Whether a similar cyclic AMP-dependent mechanism occurs in the neutrophil requires additional study.

Recent reports describe a specific receptor for phorbol esters on the human neutrophil.[24,25] The data presented here suggest that the biochemical events following PMA receptor binding are regulated in a manner different from that of the formyl peptide receptor. Not only could the inhibition of lysosomal enzyme secretion be overcome by increasing concentrations of PMA, but neither PGEs nor PGI_2 significantly inhibited PMA induced O_2^- secretion. Other authors have shown that PMA-induced neutrophil aggregation, lysosomal enzyme release, and O_2^- production are relatively insensitive to extracellular calcium.[32,33] This may explain the relative lack of effect of PGEs and PGI_2 on modulating PMA-induced neutrophil activation and support the hypothesis that PGEs and PGI_2 alter neutrophil function by increasing intracellular cAMP and the availability of calcium.

An alternative explanation for these observations would be that PGEs and PGI_2 modulate FMLP- and zymosan-induced neutrophil activation at a step prior to activation of a PMA-sensitive membrane-associated protein kinase. A calcium-activated phospholipid-dependent protein kinase which is activated by unsaturated diacylglycerol has been characterized in the human platelet.[34,35] A recent report has demonstrated that PMA can substitute for unsaturated diacylglycerol, activating protein kinase C without inducing phosphotidylinositol turnover.[36] In addition, the phorbol diester receptor present in rat brain has been shown to copurify with protein kinase C.[37] Although a cAMP-dependent protein kinase capable of phosphorylating lipomodulin, a phospholipase inhibitory protein, has been described in the rabbit neutrophil,[38] the characterization of a PMA-activated protein kinase C in the rabbit neutrophil remains to be determined.

The observation of the dissociation between lysosomal enzyme inhibition and the failure of prostaglandins to inhibit superoxide anion production induced by PMA is in contrast to a recent preliminary report that demonstrates a lack of inhibition of both O_2^- production and lysosomal enzyme release by prostaglandin-treated human neutrophils.[39] However, dose-response curves are not reported, and it is possible that a similar inhibition of lysosomal enzyme release would occur at low concentrations of PMA. A dissociation between O_2^- and lysosomal enzyme secretion has also been reported following activation of the rabbit neutrophils with the calcium ionophore A23187,[2,9] supporting the hypothesis that lysosomal enzyme release and O_2^- are independently regulated functions. Alternatively, it is possible that PMA, being a hydrophobic molecule capable of altering the fluidity and hydration of phospholipid bilayers,[40] may become a part of the neutrophil cell membrane independent of receptor binding and alter the biophysical characteristics of the cell membrane, which could result in activation of the NADPH oxidase system.

The ability of PGEs to inhibit FMLP-induced O_2^- production and lysosomal enzyme secretion without prior incubation is consistent with previous reports that demonstrate a rapid increase (within 60 seconds) in intracellular cAMP levels after exposure of human and rabbit PMNs to PGEs or PGI_2.[13,14] However, the observations do not exclude the possibility that PGEs may have alternative effects on PMN cell membranes that may alter the ability of specific stimuli to activate the cells. We have recently observed that glycogen-elicited peritoneal neutrophils isolated from rats treated with 15-M-PGE_1, compared with nontreated controls, show a decreased binding affinity of FMLP to the formyl peptide receptor.[20] Additional studies have also demonstrated altered binding of specific ligands to their receptors on PGE- and PGI_2-treated platelets and macrophages.[41,42] Although a decrease in binding affinity of a chemotactic stimulus such as

FMLP to its receptor could play a significant role in modulating neutrophil recruitment and activation *in vivo*, it would not account for the inhibition of neutrophil lysosomal enzyme release and O_2^- at relatively high concentrations of FMLP. Therefore, at relatively high doses of chemotactic stimulus, elevations in cAMP may be the primary mechanism of neutrophil modulation by PGEs and PGI_2.

The observations presented here expand our understanding of the mechanism of PGE and PGI_2 modulation of PMN activation. The data demonstrate a dissociation between phorbol-ester-induced lysosomal enzyme release and superoxide anion production. Additional studies examining the effects of PGEs on cell membrane function may provide insight into the mechanisms by which PGEs and PGI_2 modulate the inflammatory response.

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