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INHIBITION OF THE NEUTROPHIL OXIDATIVE RESPONSE TO A CHEMOTACTIC PEPTIDE BY INHIBITORS OF ARACHIDONIC ACID OXYGENATION

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SUMMARY: N-formylmethionylphenylalanine stimulates a short burst of antimycin A-insensitive O_2 uptake, O_2^- production and hexosemonophosphate shunt oxidation of glucose by guinea pig peritoneal neutrophils. The stimulated oxidative metabolism, as well as release of lysosomal enzymes ± cytochalasin B, are inhibited by 5,8,11,14-eicosatetraynoic acid (ID_{50} 1.5 x 10^{-5} M). High concentrations of indomethacin inhibit the peptide-stimulated oxidations (ID_{50} 1.6 x 10^{-4} M) while acetylsalicylic acid (2.5 x 10^{-3} M) does not. Digitonin-stimulated oxidative metabolism and enzyme release are not inhibited by 5,8,11,14-eicosatetraynoic acid or indomethacin at concentrations that depress effects of the N-formylated peptide.

INTRODUCTION

N-formylated peptides are potent chemotactic stimuli which bind in a highly specific manner to receptor sites on the neutrophil plasma membrane and induce release of lysosomal enzymes (1). Evidence exists which suggests they may produce their effects by stimulating an influx of extracellular Ca^{2+} and/or changes in membrane bound Ca^{2+} (2). A recent report suggests that release of lysosomal enzymes from cytochalasin B-treated rabbit neutrophils stimulated by N-formylmethionylleucylphenylalanine may involve the production of oxygenated metabolites of arachidonic acid (3). Neutrophils have been shown to produce prostaglandins, thromboxane B_2 and hydroxylated, unsaturated fatty acids (4,5) and the release of some of these increases when the cells encounter phagocytizable particles.

Neutrophils respond to particles and a variety of soluble stimuli, such as certain detergents, with an "oxidative burst" thought to be produced by activation of a membrane bound NADPH oxidase (6). Activation of this enzyme

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ABBREVIATIONS: N-formylmethionylphenylalanine = FMP
5,8,11,14-eicosatetraynoic acid = ETYA

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results in increased O_2 uptake, O_2^- generation and stimulation of the hexosemonophosphate shunt by the $NADP^+$ produced (6,7). This report presents data which show that certain inhibitors of arachidonic acid oxygenation produce a relatively selective depression of the oxidative response of neutrophils to the chemotactic peptide N-formylmethionylphenylalanine (FMP).

EXPERIMENTAL PROCEDURE

Guinea pig peritoneal leukocytes were collected 12-14 hr after injection of a neutral, 2% casein solution, washed twice in 0.154 M NaCl and suspended in Krebs Ringer phosphate medium, pH 7.4, containing 5.5 mM glucose. Oxygen uptake (8) and [^{14}C]-glucose oxidation (7) were measured by the methods referred to. Superoxide release was monitored continuously as superoxide dismutase-inhibitable reduction of ferricytochrome C (9). Stock solutions of ETYA were made up in 100% ethanol and stored at -20° under nitrogen. Arachidonic acid was prepared in 50% ethanol and neutralized with HCl. Indomethacin was prepared in 20 mM Na_2CO_3 and sonicated to achieve solubilization. Acetylsalicylic acid was brought to pH 7.4 with triethanolamine base.

N-formylated peptides, type III cytochrome C, digitonin, indomethacin and superoxide dismutase were obtained from Sigma Chemical Co. Glucose-1- and -6- [^{14}C] were from New England Nuclear. ETYA was a gift from Hoffman LaRoche and acetylsalicylic acid was from Merck and Co. Antimycin A was a gift from Dr. Henry A. Lardy, University of Wisconsin. The data presented are typical of experiments repeated 3 or more times and yielding very similar results.

RESULTS

Figures 1 and 2 show the stimulation of O_2 uptake and O_2^- generation observed when neutrophils are exposed to optimally effective concentrations of FMP or digitonin. FMP induces a rapid burst of O_2 uptake and O_2^- generation which terminates after 2-3 minutes, before available O_2 or cytochrome C become limiting. In contrast, digitonin produces a longer lasting stimulation which terminates when anaerobiosis or complete reduction of cytochrome C are reached. While the basal rate of neutrophil O_2 uptake is partially inhibited by antimycin A, the rates stimulated by FMP or digitonin are unaffected by this inhibitor of mitochondrial electron transport. Reduction of cytochrome C is completely abolished by 10 μg/ml of superoxide dismutase (Fig. 2), indicating that O_2^- is the reducing agent responsible. A second addition of FMP, after the initial oxidative burst is complete, produces no further effect, although the cells are still capable of an oxidative response to digitonin (Fig. 1).

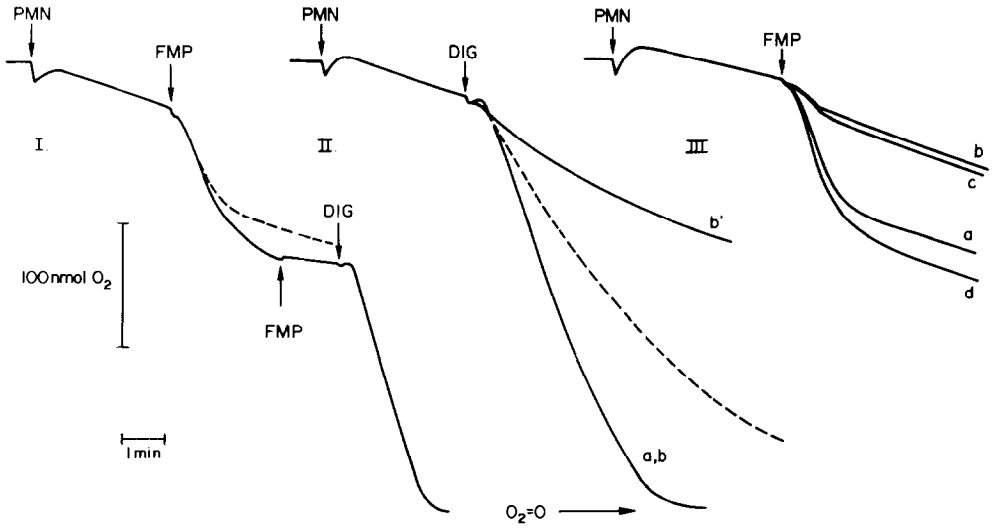

Figure 1. Stimulation of neutrophil (PMN) oxygen uptake by FMP or digitonin (DIG) and effects of inhibitors on the oxygen burst. FMP ($5x10^{-6}$ M) or digitonin (20 µg/ml) were added as indicated. Dashed lines, no added Ca^{2+}. II. a, digitonin alone; b, + $2x10^{-5}$ M ETYA and b', $1.2x10^{-4}$ M ETYA 2 min before digitonin. III. a, FMP alone; b, + $2.5x10^{-5}$ M ETYA; c, + $2x10^{-4}$ M indomethacin; and d, + $2.5x10^{-3}$ M acetylsalicyclic acid, each added 2 min before FMP.

Both FMP and digitonin stimulate the oxidation of glucose by the hexosemonophosphate shunt, increasing the ratio of 1-C/6-C glucose oxidation from 30 ± 2 to 127 ± 16 and 230 ± 13, respectively, after 15 minutes. Stimulation of all three oxidative parameters by either FMP or digitonin is depressed in the absence of added Ca^{2+} (Fig. 1 and 2), which supports the suggested role for Ca^{2+} in activation of the NADPH oxidase (9). The concentration of FMP necessary to produce half maximal stimulation of oxidative metabolism, 4×10^{-7} M, compares favorably to those stimulating half maximal chemotaxis (4×10^{-7} M) (1), enzyme release (2×10^{-6} M) (1) or chemiluminescence (4×10^{-7} M) (10). Thus, activation of all these parameters by FMP may be mediated by interaction of the peptide with a single receptor (1).

Pretreatment of neutrophils with ETYA, an inhibitor of cyclooxygenase and lipoxygenase, results in a potent inhibition of both the FMP-stimulated O_2 uptake (Fig. 1) and the O_2^- release (Fig. 2) with an ID_{50} of 1.5×10^{-5} M. ETYA has no effect on the digitonin-induced oxidations at levels which com-

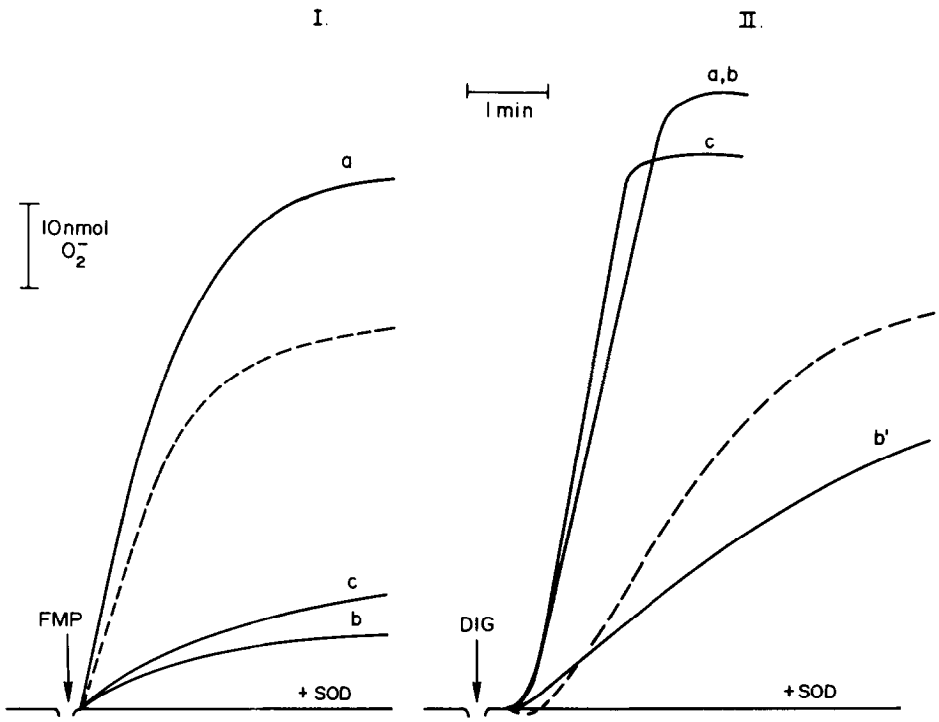

Figure 2. Superoxide release from neutrophils stimulated by FMP or digitonin. I. + $5x10^{-6}$ M FMP : a, FMP alone; b, + $3.3x10^{-5}$ M ETYA; c, + $3x10^{-4}$ M indomethacin each added 1 min before FMP. II. + 10 ug/ml digitonin (DIG) : a, digitonin alone; b, + $3.3x10^{-5}$ M ETYA; b', + $1.7x10^{-4}$ M ETYA; c, + $4x10^{-4}$ M indomethacin, each added 1 min before digitonin. + SOD, + 10 µg/ml superoxide dismutase added 1 min before stimuli. Dashed lines, no added Ca^{2+}.

pletely inhibit stimulation by FMP, but does produce significant inhibition at much higher concentrations (Fig. 1 and 2). Acetylsalicylic acid, a cyclooxygenase inhibitor, does not decrease the oxidative response to either stimulus and potentiates slightly the response to FMP (Fig. 1). Indomethacin, another cyclooxygenase inhibitor, depresses the FMP-induced oxidative responses without effect on the stimulation by digitonin (Fig. 1 and 2). The concentration of indomethacin necessary to inhibit the FMP-induced responses, however, ID_{50} 1.6×10^{-4} M, is well above that necessary to inhibit neutrophil cyclooxygenase (4). Stimulation of the hexosemonophosphate shunt by FMP is inhibited by ETYA and indomethacin in a similar fashion.

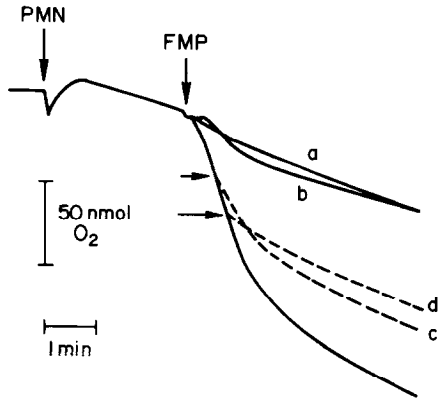

Figure 3. Effects of varying the time of addition of ETYA on inhibition of FMP-stimulated O_2 uptake. FMP (5×10^{-6} M) was added at the arrow. ETYA (4×10^{-5} M) was added 2 min prior to FMP, trace a; simultaneously with FMP, b; 36 sec, c, or 50 sec, d, after FMP.

Figure 3 shows an experiment designed to determine if ETYA can inhibit the FMP-induced oxidative response once it is activated by the peptide. Maximally effective inhibition of oxygen uptake is achieved when ETYA is added prior to or simultaneously with FMP, but inhibition is still seen when ETYA is added after the peptide, although it is less effective.

In other experiments, we have shown that 2×10^{-5} M ETYA significantly inhibits FMP-stimulated release of acid phosphatase, β-glucuronidase and β-N-acetylglucosaminidase from guinea pig neutrophils incubated with or without cytochalasin B (data not shown). These findings are consistent with the recent observations of others employing rabbit neutrophils (3). Digitonin produces only a small release of lysosomal enzymes which is neither potentiated by cytochalasin B nor inhibited by ETYA.

DISCUSSION

ETYA inhibits the FMP-stimulated NADPH oxidase activity and enzyme release from guinea pig neutrophils at concentrations which have been shown to inhibit cyclooxygenase and lipoxygenase in leukocytes (11). This is consistent with a role for one or more oxygenated metabolites of arachidonic acid in the regulation of these activities. However, both ETYA and indome-

thacin might be expected to have effects on cell membranes and a more direct inhibitory effect of these agents on the NADPH oxidase is possible. Inhibition of FMP-stimulated O_2 uptake and O_2^- release by ETYA after activation by the peptide is consistent with this latter suggestion. The inhibition of the neutrophil oxidative burst by ETYA seems relatively selective for oxidase activation through the FMP receptor, however, suggesting that a unique FMP-initiated event is being affected. ETYA might disrupt the coupling mechanism between the FMP receptor and oxidase, but it does not inhibit binding of the N-formylated peptides to their receptors in rabbit neutrophils (H. Showell, personal communication).

If an oxygenated metabolite of arachidonic acid does play a role in regulating the NADPH oxidase activity stimulated by FMP, it is most likely produced by a lipoxygenase since neither acetylsalicylic acid nor mefenamic acid inhibit the FMP-induced oxidations. Depression of the FMP-induced oxidative burst by indomethacin could be explained either by inhibition of the formation of lipoxygenase products as reported with high concentrations of this agent in lymphocytes (11) or by inhibition of neutrophil phospholipase A_2 (12). The Ca^{2+} fluxes shown to occur when N-formylated peptides bind to their receptors could activate a phospholipase A_2 to provide arachidonic acid as substrate for a lipoxygenase. Such a mechanism has been suggested to explain activation of prostaglandin or thromboxane production in several cell types exposed to ionophores which transport Ca^{2+} across cell membranes (13). Direct studies of the effects of purified lipoxygenase metabolites of arachidonic acid on neutrophil oxidative metabolism and enzyme release are needed to test the hypothesis that one or more of these molecules play a regulatory role in neutrophil function.

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