Incubation of human neutrophils with the chemotactic peptide F-met-leu-phe induced uptake of extracellular oxygen and production of both superoxide anion ($\text{O}_2^-$) and chemiluminescence in a concentration-dependent fashion. F-met-met-met also stimulated these cell functions, but it was less potent than F-met-leu-phe, whereas the nonformylated met-met-met had little effect. Pretreatment of neutrophils with cytochalasin B produced a twofold to fivefold increase in $\text{O}_2^-$ release and luminescence and a fourfold to sevenfold increase in the lysosomal enzyme release induced by the peptides. In contrast, the $\text{O}_2^-$ release, chemiluminescence, and enzyme release stimulated by phorbol myristate acetate (PMA) were not significantly affected by the presence of cytochalasin. Addition of calcium ion ($\text{Ca}^{2+}$), but not addition of magnesium ion ($\text{Mg}^{2+}$), to cells in cation-free buffer markedly enhanced the $\text{O}_2^-$ release, chemiluminescence, and oxygen consumption induced by F-met-leu-phe. $\text{Ca}^{2+}$ caused no enhancement of the $\text{O}_2^-$ release and luminescence induced by PMA. The enhancement of peptide-stimulated oxidative metabolism by $\text{Ca}^{2+}$ is compatible with the concept that changes in intracellular $\text{Ca}^{2+}$ concentrations are involved in activation of the enzyme responsible for the respiratory burst. The peptide concentrations required to stimulate oxidative metabolism were 10–50 times those needed for chemotaxis. Thus, at the origin of a gradient of chemotactic factors (that is, at sites of inflammation), these factors could stimulate production of reactive oxygen metabolites, which might contribute to the tissue damage and perhaps microbicidal activity mediated by neutrophils.

CERTAIN SMALL synthetic N-formylmethionyl peptides are capable of affecting the biologic functions of phagocytic cells. They are chemotactic for polymorphonuclear neutrophils and peritoneal macrophages, and they induce degranulation of basophils and degranulation of neutrophils pretreated with cytochalasin B. In solution they inhibit phagocytosis, but if placed on latex beads they enhance ingestion of the beads. When given intravenously they produce neutropenia, perhaps as a consequence of changes in the neutrophil membrane surface charge and subsequent cell aggregation.

Some agents capable of inducing chemotaxis can trigger in phagocytic cells a burst of oxidative metabolism like that seen with phagocytosis. During this burst,
oxygen is taken up from the extracellular medium and converted to superoxide anion (O$_2^{-}$),$^{10}$ which leads to the generation of hydrogen peroxide and hydroxyl radical.$^{11}$ Certain N-formylmethionyl chemotactic peptides can stimulate neutrophils and macrophages to generate chemiluminescence,$^{12}$ a phenomenon initiated by the generation of oxygen free radicals. These highly reactive metabolic products of oxygen are involved in the microbicidal activity of phagocytic cells.$^{11,13}$

We report here that formylated methionyl tripeptides can stimulate oxygen uptake and production of O$_2$ and chemiluminescence by human neutrophils and that this stimulation is enhanced by extracellular Ca$^{2+}$. The relative potencies of these peptides in stimulating oxidative metabolism correlate well with their abilities to bind to specific receptors on human neutrophils$^{14}$ and to affect other cell functions.

**MATERIALS AND METHODS**

**Neutrophils**

Human neutrophils were separated to 96%-99% purity by centrifugation of heparinized venous blood through a Ficoll-Hypaque gradient, as previously described.$^{13}$ For experiments examining the effects of cytochalasin B, the cells were washed twice and resuspended in Krebs-Ringer phosphate buffer containing dextrose at 2 mg/ml (KRP-D); for experiments exploring the effects of divalent cations, neutrophils were washed twice and resuspended in 0.01-M sodium phosphate, pH 7.4, containing NaCl at 7.4 mg/ml and dextrose at 2 mg/ml (PBS-D). CaCl$_2$ and MgCl$_2$ were used as the sources of divalent cations.

**Stimuli**

F-met-leu-phe, F-met-met-met, and met-met-met were purchased from Andrulis Research, Bethesda, Md. F-met-leu-phe was also purchased from Vega-Fox Biochemicals, Tucson, Ariz. The two different F-met-leu-phe preparations gave identical results. The peptides were dissolved in physiologic saline at a concentration of 10$^{-4}$ M. Phorbol myristate acetate (PMA, Consolidated Midland, Brewster, N.Y.) was dissolved in dimethylsulfoxide (DMSO, grade I, Sigma Chemical, St. Louis, Mo.) at a concentration of 1 mg/ml.

**Assays**

O$_2$ was quantitated as superoxide-dismutase-inhibitable reduction of ferricytochrome c by a modification of the method of Babior et al.$^{13}$ In experiments with cytochalasin B, neutrophils were preincubated at 2.5 x 10$^6$ cells/ml for 15 min at 37°C with cytochalasin B at 5 μg/ml (Aldrich Chemical, Milwaukee, Wisc., dissolved in DMSO at a concentration of 1 mg/ml). Control cells were preincubated with an equal concentration of DMSO (0.5%). One milliliter of cells was then added to each assay tube containing 0.1 ml of 1.2-mM ferricytochrome c (type III, Sigma Chemical) in PBS-D and 0.4 ml of KRP-D buffer containing peptides or PMA. The final assay volume was 1.5 ml, and the final cytochrome c concentration was 0.08 mM. The incubation time with peptides was 10 min and with PMA was 20 min. The reaction was stopped by plunging the tubes into melting ice. After centrifugation, the supernatant fluids were assayed spectrophotometrically, and the absorbance was converted to nanomoles of cytochrome c reduced.$^{13}$

Chemiluminescence was measured in a liquid scintillation spectrometer at ambient temperature.$^{13}$ Cells (5 x 10$^6$/ml) were preincubated at 37°C for 15 min. One milliliter of cells was then added to a siliconized scintillation vial containing 0.5 ml of buffer and appropriate stimuli and cations. Luminescence induced by peptides was followed by continuous counting at 30-sec intervals; with PMA, counts were obtained at 1- or 2-min intervals. Background chemiluminescence of the empty scintillation vials (1300-2000 cpm) was subtracted from the peak values obtained with neutrophils, and the data are presented as Δcpm.

Oxygen consumption was measured with a Clark oxygen electrode (Yellow Springs Instrument, Yellow Springs, Ohio) during stirring at 37°C. The rate of oxygen consumption was calculated from the
linear trace initiated by addition of peptide to the incubation medium. Incubation conditions were those used for the O2 assay, except that the cell number was 5 x 10^6 in a final volume of 3 ml, and cytochrome c was omitted.

Cell supernatants were assayed for β-glucuronidase by the method of Fishman,\(^2\) for lysozyme by the method given in the Worthington enzyme manual,\(^3\) and for lactate dehydrogenase by the method of Wacker et al.\(^4\) Preincubation and incubation conditions used for the neutrophils prior to measurement of enzyme release were identical to the conditions described for the O2 assay, except that ferricytochrome c was replaced with buffer. The averaged results of duplicate reaction mixtures were expressed as percentages of total enzyme activity released from an equal number of neutrophils lysed by treatment with 1% Triton X-100 (Eastman Organic Chemicals, Rochester, N.Y.).

The binding of radiolabeled peptide was studied by a modification of previously published methods.\(^1\)\(^,\)\(^4\) F-met-leu-[\(^3\)H]phe (specific activity 56.9 Ci/m mole, New England Nuclear, North Billerica, Mass.) at a final concentration of 3.4 x 10^-6 M was incubated with 5 x 10^6 neutrophils in a total reaction volume of 1.035 ml for 60 min at 0°C. The reaction was assayed in triplicate. When the effect of cytochalasin B was examined, the buffer used was KRP-D; for experiments with Ca\(^2+\), PBS-D was the buffer. After the incubation, the cells were centrifuged, the supernatants were discarded, and the cell pellets were washed twice with ice-cold PBS. The pellets were dissolved in a small volume of 30% H2O2 that was then mixed with an aqueous scintillation mixture, and radioactivity was determined in a scintillation spectrometer. Nonspecific binding was defined as the amount of binding not inhibited by 2.4 x 10^-6 M unlabeled F-met-leu-phe; this value was subtracted from the total amount of binding to calculate specific binding.\(^1\) The labeled and unlabeled F-met-leu-phe preparations at 10^-6 M stimulated \(\Delta\)O2 release to the same extent.

**RESULTS**

Incubation of normal human neutrophils with varying concentrations of F-met-leu-phe induced the cells to generate O2 in a concentration-dependent fashion (Table 1). In other biologic systems the formylated peptide F-met-met-met is less active than F-met-leu-phe but more active than nonformylated met-met-met.\(^2\)\(^,\)\(^3\)\(^,\)\(^5\)\(^,\)\(^1\) Table 1 shows that F-met-met-met stimulated normal neutrophils to produce O2 but was less potent than F-met-leu-phe. The nonformylated peptide had no definite effect at comparable concentrations.

Pretreatment of cells with cytochalasin B resulted in marked enhancement of the O2 release stimulated by formylated peptides, and a concentration-dependent

<table>
<thead>
<tr>
<th>Table 1. Release of O2 by Human Neutrophils Stimulated by Chemotactic Peptides and PMA</th>
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<tbody>
<tr>
<td><strong>Agent</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>F-met-leu-phe, 10^-6 M</td>
</tr>
<tr>
<td>F-met-leu-phe, 10^-7 M</td>
</tr>
<tr>
<td>F-met-leu-phe, 10^-8 M</td>
</tr>
<tr>
<td>F-met-met-met, 10^-6 M</td>
</tr>
<tr>
<td>F-met-met-met, 10^-7 M</td>
</tr>
<tr>
<td>F-met-met-met, 10^-8 M</td>
</tr>
<tr>
<td>met-met-met, 10^-6 M</td>
</tr>
<tr>
<td>met-met-met, 10^-8 M</td>
</tr>
<tr>
<td>PMA, 3 ng/ml</td>
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</tbody>
</table>

\*O2 was quantitated by its capacity to reduce ferricytochrome c. Superoxide dismutase (17 μg/ml) eliminated cytochrome reduction. Results are expressed as means ± SEM of averages of duplicate determinations; the number of experiments is given in parentheses. Cells were preincubated with cytochalasin B at 5 μg/ml (+ Cytochalasin B) or with an equal volume of its solvent, DMSO (- Cytochalasin B). Incubation was for 10 min with peptides and 20 min with PMA.

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effect was noted over the range of peptide concentrations studied (Table 1). In contrast, PMA-stimulated \( \text{O}_2^- \) release was not affected by cytochalasin B pretreatment at the lowest consistently stimulatory concentrations of PMA (3–30 ng/ml).

Despite the greater extent of peptide-stimulated \( \text{O}_2^- \) release from cells pretreated with cytochalasin B, when cytochrome c reduction was monitored continuously in a recording spectrophotometer at 37°C it was noted that pretreatment of cells with cytochalasin B did not increase the rate of release. As expected, therefore, \( \text{O}_2^- \) production stimulated by \( 10^{-7} \cdot M \) F-met-leu-phe took longer to reach completion with cytochalasin-treated cells than with cells not preincubated with cytochalasin (means of 7.4 min and 3.5 min, respectively, \( n = 6 \)). Cytochalasin pretreatment had no effect on the rate of \( \text{O}_2^- \) release stimulated by PMA nor on the time at which \( \text{O}_2^- \) production was complete (11–18 min).

In the experiments reported here, cells were prewarmed at 37°C before addition of the peptide. A predictable increase in the rate of \( \text{O}_2^- \) release was noted with prewarmed cells, as compared with cells kept on ice. However, the practical importance of prewarming was emphasized by the finding that total \( \text{O}_2^- \) release from cells kept on ice up to the time of mixing with \( 10^{-6} \cdot M \) F-met-leu-phe was only 15% as much as that achieved with prewarmed cells, after subtraction of the baseline values obtained without stimulation (four paired experiments). If cytochalasin B was present with the cells, F-met-leu-phe-stimulated \( \text{O}_2^- \) release from unwarmed cells was 83% as great as that from prewarmed cells (\( n = 4 \)). If the cells were preincubated with \( 10^{-6} \cdot M \) F-met-leu-phe for 15 min at 0°C and cytochrome c was added to begin the reaction at 37°C, \( \text{O}_2^- \) release was only 6% of that achieved when the reaction was begun by mixing prewarmed components (three paired experiments). The extent of \( \text{O}_2^- \) produced by PMA was not affected by preincubation of the cells at 37°C.

Table 2 summarizes the effects of the chemotactic peptides on neutrophil luminescence. Incubation with F-met-leu-phe caused normal neutrophils to luminesce in a concentration-dependent manner. Chemiluminescence was stimulated less by F-met-met-met, and in the absence of pretreatment with cytochalasin B there was no definite stimulation by met-met-met. Pretreatment of cells with
cytochalasin B resulted in a twofold to fivefold augmentation in the chemiluminescence response to each of the three peptides. PMA effectively induced neutrophils to generate luminescence, but as was seen with $O_2^-$ production, pretreatment with cytochalasin B did not enhance the chemiluminescence of these cells. As noted previously with cells stimulated by zymosan phagocytosis or PMA, superoxide dismutase (17 μg/ml) markedly reduced the chemiluminescence stimulated by F-met-leu-phe, with or without prior treatment with cytochalasin B (means of 85% and 82% inhibition, respectively, $n = 5$). Heat denaturation of the superoxide dismutase removed its inhibitory effect.

Because the oxidative metabolic events stimulated by peptides and those induced by PMA were affected differently by cytochalasin B pretreatment of cells, we explored the effect of cytochalasin B on the release of lysosomal enzymes stimulated by these two agents. Degranulation of neutrophils induced by F-met-leu-phe and PMA also was affected differently by pretreatment of the cells with cytochalasin B. F-met-leu-phe and PMA produced only slight release of the granular enzyme $\beta$-glucuronidase (Fig. 1). Whereas the tripeptide caused a slight but significant release of lysozyme ($p < 0.01$ with $10^{-6} M$, $p < 0.02$ with $10^{-7} M$, paired $t$ test), PMA induced a pronounced release of this enzyme from normal cells. Pretreatment of cells with cytochalasin B had no definite effect on release of granular enzymes in the absence of a stimulus (Fig. 1). However, as shown by others with rabbit neutrophils, human cells pretreated with cytochalasin B and then exposed to F-met-leu-phe actively released both lysozyme and $\beta$-glucuronidase. The effects of cytochalasin B treatment on PMA-induced enzyme release were not statistically significant ($p < 0.1$, paired $t$ test). Release of the cytoplasmic enzyme lactate dehydrogenase was negligible with the peptides or PMA (2.0%–3.7%), with or without pretreatment with cytochalasin B.

The influence of extracellular Ca$^{++}$ and Mg$^{++}$ on peptide-induced $O_2^-$ release was explored in experiments summarized in Fig. 2. In the absence of Ca$^{++}$ and Mg$^{++}$, $O_2^-$-dependent cytochrome reduction stimulated by $10^{-7} M$ F-met-leu-phe was 0.67 nmoles/min. Addition of increasing concentrations of Ca$^{++}$ to the incubation medium increased $O_2^-$ production in a concentration-dependent fashion. Mg$^{++}$ could not substitute for Ca$^{++}$, and, in fact, it was slightly inhibitory.
Enhancement of \( \text{O}_2 \) release by \( \text{Ca}^{++} \) was demonstrated with all three peptides studied (Table 3). In contrast, 1-mM \( \text{Ca}^{++} \) had no significant influence on the total amount of \( \text{O}_2 \) produced in response to PMA.

Table 4 shows the influence of divalent cations on neutrophil chemiluminescence. F-met-leu-phe at \( 10^{-6} \) M tripled the response of resting cells in the absence of extracellular divalent cations. \( \text{Mg}^{++} \) had no effect on this luminescence, but \( \text{Ca}^{++} \) had a significant effect.

**Table 3. Enhancement by \( \text{Ca}^{++} \) of Peptide-Induced \( \text{O}_2 \) Release**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Ferricytochrome c Reduction† (nmoles/2.5 x 10⁶ neutrophils)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- ( \text{Ca}^{++} )</td>
</tr>
<tr>
<td>None</td>
<td>1.4 ± 0.7 (6)</td>
</tr>
<tr>
<td>F-met-leu-phe, 10⁻⁷ M</td>
<td>29.6 ± 6.0 (10)</td>
</tr>
<tr>
<td>F-met-met-met, 10⁻⁷ M</td>
<td>12.9 ± 1.3 (5)</td>
</tr>
<tr>
<td>PMA, 3 ng/ml</td>
<td>0.9 ± 0.6 (3)</td>
</tr>
<tr>
<td></td>
<td>85.6 ± 1.4 (4)</td>
</tr>
</tbody>
</table>

†Means ± SEM. The number of experiments, done in duplicate, is given in parentheses.

**Table 4. Effects of \( \text{Ca}^{++} \) and \( \text{Mg}^{++} \) on Chemiluminescence Stimulated by F-met-leu-phe or PMA**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Chemiluminescence† (Δcpm x 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Cation</td>
</tr>
<tr>
<td>None</td>
<td>1.5 ± 0.2 (6)</td>
</tr>
<tr>
<td>F-met-leu-phe, 10⁻⁸ M</td>
<td>4.4 ± 0.7 (6)</td>
</tr>
<tr>
<td>PMA, 30 ng/ml</td>
<td>14.5 ± 2.2 (4)</td>
</tr>
</tbody>
</table>

†Means ± SEM of peak values after subtraction of the background obtained with empty vials. The number of experiments, done in duplicate, is given in parentheses.
consistently increased it \((p < 0.02, \text{paired } t \text{ test})\). Neither \(\text{Ca}^{++}\) nor \(\text{Mg}^{++}\) increased the neutrophil luminescence stimulated by PMA.

Since the observed effects of calcium and cytochalasin B in our system could reflect enhanced release of \(\text{O}_2\) from the cells but not necessarily enhanced generation of the anion, we measured oxygen consumption. Resting neutrophils consumed \(0.6 \pm 0.1 \text{ nmoles/min/}2.5 \times 10^6 \text{ cells (mean } \pm \text{ SEM, five experiments).}\) Addition of \(10^{-5}M\) F-met-leu-phe increased the rate of oxygen consumption 10-fold \((6.3 \pm 1.3 \text{ nmoles/min}).\) Addition of \(1-\text{mM Ca}^{++}\) to the medium further increased the rate of peptide-induced oxygen consumption by 79\% \((11.3 \pm 1.8 \text{ nmoles/min, } p < 0.005, \text{paired } t \text{ test}).\) F-met-met-met also induced uptake of oxygen but was less effective than F-met-leu-phe. Cytochalasin B treatment had no effect on the rate of oxygen consumption with either peptide \(10^{-6} \text{ M or } 10^{-7} \text{ M (10 experiments).}\)

The effects of cytochalasin B and \(\text{Ca}^{++}\) on specific binding of F-met-leu-[\(^{3}\text{H}\)]phe to neutrophils were also examined in preliminary experiments in two laboratories. Cytochalasin had no effect, and \(\text{Ca}^{++}\) (1mM) had no effect or reduced binding of the labeled peptide to cells (six experiments with each agent).

**DISCUSSION**

A number of soluble agents, including some capable of inducing neutrophil chemotaxis or degranulation, can trigger a burst of oxidative metabolism in phagocytic cells.\(^9\) The fact that certain synthetic formylated peptides are strongly chemotactic and can provoke lysosomal enzyme secretion prompted us to examine their effect on the neutrophil respiratory burst. We found that two of these peptides, F-met-leu-phe and F-met-met-met, stimulated neutrophils to take up oxygen and produce \(\text{O}_2\) and chemiluminescence and that the nonformylated peptide met-met-met had little, if any, effect. The relative abilities of these peptides to evoke such an oxidative response correlated well with their relative capacities to bind to receptors on human neutrophils\(^{14}\) and to induce chemotaxis and degranulation of cytochalasin-B-treated cells.\(^{2,3}\)

We have confirmed with human neutrophils the findings of Showell, Becker, and colleagues with rabbit neutrophils\(^{2,3}\) that these peptides cause exocytosis of \(\beta\)-glucuronidase and lysozyme in the presence of cytochalasin B. The peptides also induced human neutrophils to release small but significant amounts of lysozyme in the absence of cytochalasin B, but pretreatment with cytochalasin markedly enhanced the release caused by both these enzymes.

PMA is known to be a powerful stimulant of neutrophil oxidative metabolism\(^{30}\) and to promote selective degranulation of neutrophil-specific granules.\(^{10,21,22}\) In contrast to our findings with the chemotactic peptides, pretreatment of cells with cytochalasin B did not alter PMA-induced metabolism or significantly affect PMA-induced release of lysosomal enzymes.

Whereas chemotaxis\(^{23-26}\) and phagocytosis\(^{27}\) both require extracellular \(\text{Ca}^{++}\) and \(\text{Mg}^{++}\) for optimal activity, degranulation appears to require only \(\text{Ca}^{++}\).\(^{2,21,26,28-30}\) Showell et al.\(^{31}\) recently reported that \(\text{Ca}^{++}\) enhanced the lysosomal enzyme secretion induced by F-met-leu-phe, and Siraganian and Hook\(^{32}\) showed that \(\text{Ca}^{++}\) enhanced the release of histamine from basophils stimulated by F-met-phosphate. Chemotactic factors have been shown to promote \(\text{Ca}^{++}\) fluxes or redistribution of
intracellular Ca\(^{2+}\); and F-met-leu-phe has been reported to produce a net increase in the intracellular Ca\(^{2+}\) pool. We report here that extracellular Ca\(^{2+}\) increased by twofold to fourfold the ability of the formylated peptides to trigger oxidative metabolism. These findings raise the possibility that the capacity of chemotactic factors to initiate neutrophil movement, degranulation, and respiration derives, at least in part, from their ability to induce Ca\(^{2+}\) transport across the plasma membrane of this cell.

Our finding that Ca\(^{2+}\) enhances the oxidative metabolism of human neutrophils stimulated by chemotactic peptides extends previous studies pointing to a role for Ca\(^{2+}\) in the neutrophil respiratory burst. The divalent cation ionophore A23187, which transfers Ca\(^{2+}\) across biologic membranes,\(^{24,34}\) can in the presence of extracellular Ca\(^{2+}\) trigger neutrophil oxygen uptake,\(^{35,36}\) activation of the hexose monophosphate (HMP) shunt,\(^{35,36}\) and generation of \(\text{H}_2\text{O}_2\) and \(\text{O}_2^\cdot\)\(^{37,38}\) Preincubation of neutrophils with ethylene glycol tetraacetic acid to remove Ca\(^{2+}\) has been shown to inhibit stimulation of the HMP shunt by adherence of cells to fixed aggregated IgG.\(^{39}\) Finally, Cohen and Chovaniec\(^{40}\) have recently reported that Ca\(^{2+}\) is required for induction of \(\text{O}_2\) release by digitonin-stimulated guinea pig neutrophils. Unlike our results with human neutrophils, they found that Mg\(^{2+}\) decreased the requirement for Ca\(^{2+}\).

Goldstein and colleagues\(^{10}\) reported that cytochalasin B enhanced the release of \(\text{O}_2\) from cells stimulated with opsonized zymosan, with IgG aggregates, or with C5a. They suggested that cytochalasin might facilitate diffusion of \(\text{O}_2\) into the extracellular medium. We found that cytochalasin B pretreatment permitted greater \(\text{O}_2\) release after stimulation by formylated peptides without affecting oxygen uptake. F-met-leu-phe has been found to induce vacuolization in human neutrophils.\(^{41}\) If cytochalasin B prevents induction of vacuole formation by F-met-leu-phe, as it does with other stimuli,\(^{42}\) it could facilitate recovery of \(\text{O}_2\) by inhibiting internalization and subsequent intracellular destruction of the anion.

The results obtained with PMA as stimulus differed from those obtained with the synthetic peptides: (1) Extracellular Ca\(^{2+}\) had no apparent effect on the capacity of PMA to initiate the respiratory burst. (2) Pretreatment with cytochalasin B did not augment the generation of \(\text{O}_2\) or chemiluminescence stimulated by PMA. (3) PMA caused a marked release of lysozyme from cells not previously treated with cytochalasin B, as reported by others,\(^{43,51,22}\) and cytochalasin B did not significantly enhance this release. In contrast, F-met-leu-phe provoked relatively little enzyme release from untreated cells, and degranulation was markedly enhanced by cytochalasin B. In addition, it has previously been shown that in contrast to the results with F-met-leu-phe,\(^{31}\) degranulation induced by PMA apparently is not influenced by extracellular Ca\(^{2+}\).\(^{28}\) Moreover, whereas the peptides are strongly chemotactic, PMA is only weakly so.\(^{43}\) These discrepant results do not preclude a role for Ca\(^{2+}\) in degranulation and the burst of oxidative metabolism stimulated by PMA, since it is possible that PMA can promote redistribution of Ca\(^{2+}\) within intracellular pools. However, they do suggest that PMA initiates these responses through a different mechanism than that triggered by the chemotactic peptides.

Prewarming the cells before exposure to F-met-leu-phe to begin the reaction increased by sevenfold the total amount of \(\text{O}_2\) released, as compared with cells kept at \(4^\circ\text{C}\) before exposure to the peptide. \(\text{O}_2\) release was complete in this system by a
NEUTROPHIL STIMULATION BY PEPTIDES

mean of 3.5 min. Thus, during the interval required for unwarmed cells to reach 37°C, interaction of F-met-leu-phe with the neutrophil membrane may have desensitized the cell to further interaction with the peptide under temperature conditions unfavorable to the respiratory burst. (Cytochrome reduction is very slight at 4°C as compared with 37°C). In agreement with this possibility, preincubation of the cells at 4°C with F-met-leu-phe allowed only 6% as much O₂ release as was achieved when the peptide was added to prewarmed cells. Bass et al.44 reported that F-met-phe and F-met-leu-phe, even at concentrations higher than 10⁻⁶ M, affected the respiratory burst in human neutrophils only slightly, if at all. One possibility for this discrepancy in findings could have been the admixture of peptide and cells at 4°C in their system.

In previously published work with human neutrophils, the equilibrium dissociation constant for binding F-met-leu-[³H]phe was approximately 10⁻⁸ M, which is five times higher than the concentration of peptide that gave half the maximal chemotactic response.14 Binding studies with rabbit neutrophils showed a similar relationship.18 Thus, only 10%–20% of the receptors need be occupied for efficient chemotaxis, indicating that "spare receptors" exist that could be important for the sensing of a gradient of chemotactic factors. In the present study, vigorous oxidative metabolic activity was elicited by F-met-leu-phe at a concentration of 10⁻⁷ M, and this should give approximately 90% receptor binding. The requirement for substantially greater receptor occupancy for the stimulation of oxidative metabolism than for chemotaxis might have important biologic significance, since if toxic oxygen radicals were released at sites distant from the origin of the chemotactic gradient, widespread tissue damage could ensue. At the site of inflammation, where the concentration of chemotactic factors would be high, released oxygen radicals could mediate inflammatory tissue damage and perhaps play a role in protecting the host against infection.

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Stimulation of neutrophil oxidative metabolism by chemotactic peptides: influence of calcium ion concentration and cytochalasin B and comparison with stimulation by phorbol myristate acetate

JE Lehmeyer, R Snyderman and RB Jr Johnston