1. Introduction

A number of metabolic changes occur when phagocytic cells (i.e., polymorphonuclear neutrophils (PMN)) interact with certain soluble and particulate stimuli (reviewed [1–4]). These metabolic changes include an increase in oxygen consumption, stimulation of hexose monophosphate shunt activity, superoxide anion production (O₂⁻), hydrogen peroxide production and chemiluminescence. It has been proposed that some of these stimulants act at specific receptor sites on the plasma membrane of the PMN [5–7].

Less information is available concerning the effects of similar metabolic stimuli on the alveolar macrophage [3]. Furthermore, the stoichiometry of the various reactions in the alveolar macrophage, particularly the proportion of the increase in oxygen consumption, that results in the formation of O₂⁻ and H₂O₂ has not been established [8,9].

One effective stimulus in the PMN are the soluble N-formyl methionyl peptides [5,10–12]. Attention has been drawn not only to the metabolic but also to the biologic actions of these peptides on phagocytic cells since they are similar in structure to the N-formyl methionyl peptides produced by bacteria. For example, the peptide, N-formyl methionyl phenylalanine (FMP), has also been shown to induce enzyme release in PMN [12] and to stimulate chemotaxis in PMN and guinea pig alveolar macrophages [13]. Here, the effects of FMP on oxygen consumption and superoxide anion production in guinea pig alveolar macrophages are compared to concanavalin A (Con A). Con A is a plant lectin shown to be a reversible soluble stimulus for O₂ consumption in alveolar macrophages [14].

2. Materials and methods

Alveolar macrophages were isolated as in [13]. Cells were suspended in Hanks' buffered salt solution (HBSS) at 2.5–5.0 × 10⁶ cells/ml and stored on ice until used. Cell viability was checked by trypan blue exclusion and was > 90%. Macrophage purity was determined by non-specific esterase staining [15] and ranged from 85–95%. Oxygen consumption was measured using a Yellow Springs Instrument Co. oxygen electrode in a 1.2 ml glass chamber from Gilson Medical Electronics. The electronic apparatus for the oxygen measurements was designed and built by the Johnson Research Foundation (Philadelphia, PA).

Superoxide anion production was monitored continuously using a Shimadzu Spectronic 210 UV recording spectrophotometer by measuring the rate of ferricytochrome c (75 μM, type III, Sigma) reduction at 550 nm. An ε = 18.5 mM (reduced minus oxidized) was used for ferricytochrome c [16]. Catalase, superoxide dismutase (SOD), antimycin A (suspended to 10 mg/ml in either dimethylsulfoxide (DMSO) or absolute ethanol), FMP, Con A and α-methyl mannoside (α-MM) were purchased from Sigma.

3. Results

3.1. Stimulation of O₂ consumption

The stimulation of oxygen consumption by FMP is
Fig. 1. Oxygen consumption of guinea pig alveolar macrophages. Macrophages were suspended in Hanks' buffered salt solution at 2.5 x 10^6 cells/ml in a stirred 1.2 ml glass oxygen electrode chamber. FMP was added as 12 µl of a 10^-4 M solution in water and antimycin A was added as 1.2 µl of a 10 mg/ml solution in absolute ethanol (or DMSO). The lower trace represents the stimulation by FMP of oxygen consumption; the upper trace represents the inhibitory action of antimycin A. The figure is representative of > 6 expt.

shown in fig.1 (lower curve). The initial portion of the curve (before adding the FMP) is the rate for unstimulated cells and equaled 1.9 nmol O_2 . 2.5 X 10^6 cells^-1 . min^-1 . The addition of 10^-6 M FMP resulted in a rapid increase (with 15–30 s) in oxygen consumption to 8.6 nmol . 2.5 x 10^6 cells^-1 . min^-1 . Preliminary experiments indicated this to be the optimal concentration of FMP. After 10–20 min, the rate returned to control values.

The effect of antimycin A, an inhibitor of mitochondrial respiration, is shown in the upper trace of fig.1. Antimycin A completely blocked baseline O_2 consumption and significantly diminished (≈ 50%) FMP-stimulated oxygen consumption. The level of inhibition ranged from 30–50% in 6 experiments; the addition of an equal amount of solvent had no effect.

Con A (250 µg/ml) also stimulated O_2 consumption. However, initial kinetics (lower trace of fig.2A) were characterized by a lag of ~ 2 min before the respiratory rate increased. The stimulation of O_2 consumption by Con A was abolished by α-MM, a competitive inhibitor for Con A binding sites on plasma membranes (upper trace of fig.2A). When similar experiments were repeated using FMP, no effect could be demonstrated with the same concentration of α-MM (fig.2B). The stimulatory effects of Con A and FMP on O_2 consumption were additive (data not shown).

3.2. Stimulation of O_2 production

As shown in fig.3 (bottom trace), FMP stimulated O_2 production with similar kinetics as oxygen consumption. Prior to the addition of FMP, there was no O_2 formation. The addition of 10^-6 M FMP, however, induced O_2 formation within 15–30 s and increased to a maximal rate of 2.8 nmol O_2 . 0.5 x 10^6 cells^-1 . min^-1.
Fig. 3. Superoxide anion production of guinea pig alveolar macrophages. Macrophages were suspended in HBSS at 5 x 10^5 cells/ml in a 1 ml plastic cuvette with 75 μM ferricytochrome c. The A_550 was continuously recorded. FMP was added as 10 μl of a 10^-4 M solution in water, antimycin A was added as 1 μl of a 10 mg/ml solution in absolute ethanol (or DMSO), and SOD was added as 5 μl of 10 units/μl solution in water. (A) represents the response to FMP; (B) represents the inhibitory effects of antimycin A; (C) represents the inhibition of absorbance changes by SOD. The trace is representative of > 6 expt.

and diminished to negligible rates after 10–20 min. As illustrated in the upper trace of fig.3, the same experiment was repeated in the presence of 50 units of superoxide dismutase (SOD). In this case, there was no O_2^- production indicating complete removal of O_2^- by SOD. The addition of 10 μg/ml antimycin A prior to the addition of FMP also resulted in a marked inhibition (80%) of O_2^- production (middle trace of fig.3). The inhibition by antimycin A was 40–80% in 6 experiments.

Con A also stimulated O_2^- formation, again with a significant delay (fig.4, middle trace); the stimulation was also inhibited by α-MM. However, α-MM did not inhibit O_2^- formation induced by FMP (data not shown). The additive effects of Con A and FMP stimulation on O_2^- formation are shown in fig.4. Con A (250 μg/ml) resulted in O_2^- formation which could be further stimulated by the addition of 10^-6 M FMP. However, cells that were stimulated by 10^-6 M FMP did not respond further to the addition of FMP over a broad concentration range (10^-8–10^-5 M). The addition of 400 units of catalase did not alter either oxygen consumption or O_2^- production.

4. Discussion

Addition of FMP to guinea pig alveolar macrophages stimulated both oxygen consumption and superoxide anion production within 15–20 s and lasted for 20 min. In 6 experiments, stimulation of oxygen consumption by FMP gave initial rates of 8.2 ± 1.4 nmol O_2^- . 2.5 x 10^6 cells^-1 . min^-1 while the initial rates for O_2^- production were 2.8 ± 0.4 nmol O_2^- . 0.5 x 10^6 cells^-1 . min^-1. When the respiration of resting macrophages (control) was subtracted and the measurements were corrected for comparable cell concentration (e.g., 2.5 x 10^6 cells/ml), the rate of O_2^- consumption was ~50% (6.4 nmol O_2^-/min) of the O_2^- production (13.9 nmol O_2^-/min). This could be explained by dismutation of two O_2^- to give H_2O_2 and O_2 thereby regenerating 1 O_2 for 2 molecules of O_2 originally consumed. Since the ferricytochrome c method for measuring O_2^- converts all of the O_2^- to
O$_2$ before dismutation can occur, this might explain why only half as much oxygen is consumed as compared to O$_2^-$ formed. This possibility is supported by the finding that the addition of 75 $\mu$M ferricytochrome c with 10 $\mu$g antimycin A/ml completely blocked stimulation of oxygen consumption by FMP. That Con A and FMP probably act at different sites on the cell membrane is based on the observations that:

(i) Each stimulant had different kinetics for the stimulation of both oxygen consumption and O$_2^-$ production;

(ii) $\alpha$-MM completely blocked Con A stimulation but had no effect on the FMP response;

(iii) There was an additive effect when both agents were added to alveolar macrophages.

Antimycin A was observed to be inhibitory for both events. This is in apparent conflict with reports suggesting that inhibitors of mitochondrial respiration do not influence O$_2$ consumption or O$_2^-$ production in phagocytic cells [1-4]. The finding in this study, however, does not prove a direct effect by antimycin A on the O$_2^-$ generating system but may indicate that general metabolic regulation (e.g., energy state of the cell) is involved in the 'respiratory burst'.

The new findings indicate that:

(1) FMP can stimulate guinea pig alveolar macrophages to produce O$_2^-$, a potential microbiocidal agent;

(2) There are different receptors on the alveolar macrophage membrane for FMP and Con A. Since FMP is similar in structure to products of bacterial metabolism, its ability to stimulate these oxygen reactions in alveolar macrophages may be physiologically important.

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References