fMLP-induced respiratory burst and the intracellular Ca$^{2+}$ signal are not interrelated in neutrophils

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Abstract—Reactive oxygen intermediate (ROI) production and the development of the intracellular (IC) Ca$^{2+}$ ([Ca$^{2+}$]$_i$) signal by formyl-Met-Leu-Phe (fMLP) stimuli were investigated in neutrophils. When the concentration was varied between 2.3 nM–2.3 µM, ROI production and the [Ca$^{2+}$]$_i$ signal showed different fMLP concentration dependencies. ROI production increased continuously with increasing fMLP concentrations, while the [Ca$^{2+}$]$_i$ signal responses reached a plateau around 230 nM fMLP. Moreover, when a consecutive, 2.3 µM fMLP stimulus was applied 10 min after the first fMLP stimulus, the intensity of the ROI production and that of the [Ca$^{2+}$]$_i$ signal showed a variable dependence on the fMLP concentration of the first stimulus. An initial fMLP dose of 2.3 nM and 23 nM sensitized the cells regarding their ROI production and [Ca$^{2+}$]$_i$ signals. After a first fMLP stimulus of 230 nM, the second stimulus produced an increased [Ca$^{2+}$]$_i$ signal, while no ROI production could be activated. A strong fMLP stimulus of 2.3 µM desensitized the cells regarding both [Ca$^{2+}$]$_i$ signal and ROI production. However, even in these desensitized cells, a high level of ROI production could be evoked by other stimuli like PMA or opsonized zymosan. The differences observed between the fMLP concentration dependence of ROI production and the [Ca$^{2+}$]$_i$ signal strongly suggest that these two phenomena are not interrelated.

Key words: Consecutive stimuli; ROI production; Ca$^{2+}$ signal; fMLP; signal transduction.

ABBREVIATIONS

ADA adenosine deaminase
[Ca$^{2+}$]$_i$ intracellular Ca$^{2+}$ concentration
CB cytochalasin B
EC extracellular

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EGTA  ethylene-glycol-bis-(β-aminoethyl-ether)-N,N,N′,N′-tetraacetate
fMLP  formyl-methionyl-leucyl-phenylalanine
IC  intracellular
INDO-1  1-[2-amino-5-(6-carboxyindol-2-il)-phenoxy]-2-(2′-amino-5′methylphenoxy)ethan-N,N,N′,N′-tetraacetic acid, fluorescent, intracellular Ca$^{2+}$ indicator
INDO-1-AM  1-[2-amino-5-(6-carboxyindol-2-il)-phenoxy]-2-(2′-amino-5′methylphenoxy)ethan-N,N,N′,N′-tetraacetic acid pentaacetoxy-methyl ester
LDCL  luminol-dependent chemiluminescence
O.Z.  opsonized zymosan
PKC  protein kinase C
PMA  phorbol-myristate-acetate
PMNL  polymorphonuclear leukocyte
ROI  reactive oxygen intermediate
TEO  theophylline

INTRODUCTION

Phagocytes migrate towards a continuously increasing concentration of chemotactic agent(s) [1]. The effect of increasing concentration of chemotactic agents on the cell functions can be modeled by the administration of consecutive stimuli [2]. Subsequent stimulation of cells may cause both up-regulation through priming and down-regulation by desensitization of cellular responses [3].

A great deal of work has been done regarding the desensitization processes of phagocytes. Desensitization of polymorphonuclear leukocytes (PMNLs) was investigated by using formyl-Met-Leu-Phe (fMLP) pretreatment at 15°C [4] and at 4°C [5]. It was found that binding of fMLP to its neutrophil surface receptor was followed by an association of the ligand-receptor complex to the cytoskeleton, and this association occurred concomitant with desensitization of the cells in respect to activation of NADPH oxidase [4]. When cells were pretreated with 100 nm fMLP, desensitization of the 100 nm fMLP-induced NADPH oxidase response in human neutrophils was attributed partly to a rapid internalization of the receptor-ligand complex and partly to changes in receptor phosphorylation, but not to an effect on the oxidase itself [5–7].

Activation of PMNLs by fMLP is accompanied by changes in intracellular (IC) Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) [8]. Data from the laboratory of Nowak et al. [9] showed that fMLP in 100 nm concentration, even when applied three times in 5-min intervals, could elicit Ca$^{2+}$ transients, although with gradually decreasing intensities. In contrast, Lundqvist et al. [4] found that 10 min after a 100 nM fMLP pretreatment at 15°C, no [Ca$^{2+}$]$_i$ signal could be activated upon an fMLP