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SPECIFIC MODULATION OF THE INTRACELLULAR pH OF RABBIT NEUTROPHILS BY CHEMOTACTIC FACTORS

T.F.P. Molski, P.H. Naccache, M. Volpi, L.M. Wolpert and R.I. Sha'afi

Departments of Physiology and Pathology, University of Connecticut Health Center

Farmington, Connecticut 06032

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SUMMARY: We have monitored the intracellular pH of rabbit neutrophils by following the distribution of the weak acid, 5,5-Dimethyloxazolidine-2, 4-dione. The synthetic formyl-methionyl chemotactic factors were found to induce complex and specific changes in the intracellular pH of the neutrophils, a rapid drop followed by a slower and more sustained rise. These effects are receptor mediated. The relationship of these events to the physiology of the neutrophils is discussed.

INTRODUCTION

Intensive investigations of the biochemical reactions elicited by the interaction of chemotactic factors with polymorphonuclear leukocytes (neutrophils) are under way in a number of laboratories. The aim of these studies is to map the sequence of events that lead from surface binding of soluble extracellular mediators to the expression of the ultimate functions of the neutrophils in particular and various other hormone sensitive cells in general. Membrane related events such as changes in ion fluxes, membrane potential and patterns of protein and lipid methylation have thus been recently described in neutrophils (1-7). We wish now to report the results of experiments in which we have examined the possible involvement of the

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Abbreviations:
Formyl-methionyl-leucyl-phenylalanine = F-Met-Leu-Phe
Methionyl-leucyl-phenylalanine = Met-Leu-Phe
t-butoxycarbonyl-phenylalanyl-leucyl-phenylalanyl-leucyl-phenylalanine = boc-Phe-Leu-Phe-Leu-Phe
5,5-Dimethyloxazolidine-2,4-dione = DMO
Intracellular pH = pH_i
Extracellular pH = pH_e

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intracellular pH of the neutrophils in the initiation or the modulation of their functions. Formyl-Methionyl-Leucyl-Phenylalanine (F-Met-Leu-Phe), a synthetic peptide with potent and well-defined biologic activities, was found to induce specific, time-dependent biphasic changes in the intracellular pH of the neutrophils. The relationship of these events to the functions of the neutrophils will be discussed.

MATERIALS AND METHODS

Rabbit peritoneal neutrophils were collected and handled as previously described (3,4). Magnesium was omitted from the Hank's balanced salt solution in order to minimize spontaneous and chemotactic factor induced cell aggregation. No bovine serum albumin was added to the cell suspensions.

The intracellular pH of the neutrophils was monitored by following the distribution of the weak acid, 5,5-Dimethyloxazolidine-2,4-dione (DMO). This method is based on the assumption that only the uncharged form of DMO is permeable to the plasma membrane. The distribution of DMO is thus pH dependent and the value of the latter parameter can be calculated using Henderson-Hasselbach relationships (13).

Specifically, DMO was dried under N_2, resuspended in Hank's balanced salt solution and added (0.1 μCi/ml, 2 x 10^{-6}M) to thermally equilibrated cell suspensions (10^7 cells/ml). Carbon dioxide (5% CO_2, 95% air) was blown over the cell suspensions during the length of the incubation in order to stabilize the extracellular pH which was adjusted by buffering the pH of the HEPES (N-2-hydroxy ethyl piperazine-N',2'-ethane sulfonic acid) (10 mM) which was added to the Hank's balanced salt solution. Preliminary experiments have shown that DMO equilibrates across rabbit neutrophil membranes in much less than one minute. Consequently the various stimuli tested were added 1 minute after DMO and the distribution of DMO followed with time with the rapid sampling silicone oil method previously described in details (3).

F-Met-Leu-Phe, Met-Leu-Phe and boc-Phe-Leu-Phe-Leu-Phe were generous gifts of Dr. R.J. Freer (Medical College of Virginia, Richmond, Va). DMO was purchased from New England Nuclear, Boston, Mass. All other reagents were analytical grade

RESULTS AND DISCUSSION

The validity of the DMO technique for use with neutrophils (8) and other cell types (9-12) has been repeatedly confirmed since its original description by Waddell and Butler in 1959 (13). In addition the rapidity with which DMO equilibrates across the neutrophil membranes (much less than 1 minute) makes this probe particularly useful for the study of dynamic events.

The dependence of the intracellular pH (pH_i) of the neutrophils on the pH of the extracellular medium (pH_e) is illustrated in Figure 1. A linear

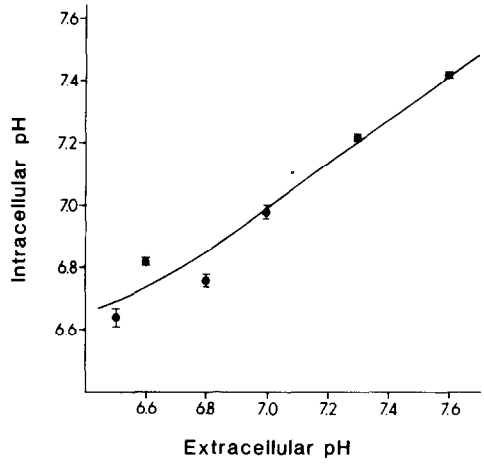

Figure 1: The relationship between the intracellular pH of the neutrophils and the extracellular pH.

relationship between these two variables appears to hold in the middle part of the extracellular pH range tested. The slope of this straight line (as determined from linear regression analysis) is 0.67 indicating intracellular buffering. A curvature of the relationship appears at pH_e less than 6.8. The average pH_i determined at pH_e = 7.0 is 6.981 ± 0.008 (120 determinations), a value in agreement with previously published figures for the neutrophils (8).

With this additional experimental validation of the DMO technique at hand we have examined the effects of F-Met-Leu-Phe on the pH_i of the neutrophils. The time dependency of these effects is illustrated in Figure 2. F-Met-Leu-Phe causes rapid and biphasic changes in the intracellular pH of the neutrophils. Initially there is a rapid drop of about 0.05 pH units in pH_i which is followed by a slower and larger increase of about 0.15 pH units. The maximum drop occurs 0.5 minutes after the addition of F-Met-Leu-Phe, the cross-over point around 2 minutes and the increase subsequently.

The specificity of this chemotactic factor-dependent change in pH_i was ascertained as follows. The effect of the known, binding antagonist boc-Phe-Leu-Phe-Leu-Phe (14) on the chemotactic factor induced changes in pH_i was first examined. As shown in Figure 2, both the decrease and the subsequent increase, although possibly to a lesser extent, in pH_i induced by

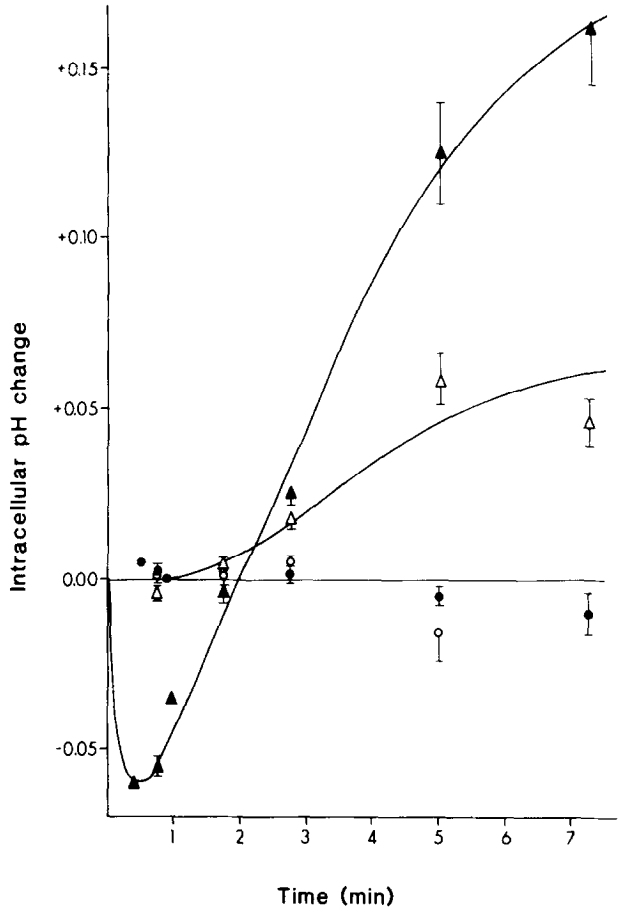

Figure 2: The time course of the effect of the chemotactic factor F-Met-Leu-Phe on the intracellular pH of the neutrophils and its dependency on the presence of the binding antagonist boc-Phe-Leu-Phe-Leu-Phe. The points represent the means and the standard error of the mean of at least three experiments each performed in duplicate. The concentrations of F-Met-Leu-Phe and boc-Phe-Leu-Phe-Leu-Phe were 10^{-9} M and 2×10^{-5} M respectively. Filled circles: no additions; open circles: boc-Phe-Leu-Phe-Leu-Phe; filled triangles: F-Met-Leu-Phe; open triangles: boc-Phe-Leu-Phe-Leu-Phe and F-Met-Leu-Phe.

F-Met-Leu-Phe are inhibited in the presence of boc-Phe-Leu-Phe-Leu-Phe. The latter has no effect of its own on pH_i. In addition, Met-Leu-Phe, a peptide structurally similar but of considerably less biologic activity (15) than F-Met-Leu-Phe was found to have no effect on pH_i at the same concentration as F-Met-Leu-Phe (The changes in pH_i induced by F-Met-Leu-Phe and Met-Leu-Phe were found to be -0.063 ± 0.006 and -0.007 ±0.007 at 0.7 minutes and + 0.161 ± 0.015 and + 0.001 ±0.006 at 7 minutes respectively, average and

standard error of the mean of three experiments each performed in duplicate). The changes in pH_i caused by the addition of F-Met-Leu-Phe thus appear to be specific and receptor mediated.

Reliable dose-response curves have so far proven to be experimentally difficult to obtain. Preliminary experiments suggest that changes in pH_i can be detected at concentrations of F-Met-Leu-Phe as low as 10^{-11}M and that the magnitude of the responses observed saturates around 10^{-9} - 10^{-8}M. F-Met-Leu-Phe thus induces changes in pH_i at the same concentrations at which it exhibits biologic activity (15).

Preliminary experiments with biologically active preparations of C5a, the chemotactic small molecular weight fragment of the fifth component of complement (kindly provided by Mr. H.J. Showell, Department of Pathology, University of Connecticut Health Center) have so far shown that C5a caused an increase in pH_i of similar magnitude and time course as that described above with F-Met-Leu-Phe. Additional work is in progress to further define the effects of C5a.

The role and underlying mechanisms of the chemotactic factors induced pH_i changes remain to be elucidated. The interpretation of the results described above depends in part on the intracellular localization of the DMO. As pointed out by Gillies and Deamer (12) DMO will tend to be excluded from the lysosomes were these to possess acidic interiors. It is thus likely, although unproven at present, that the pH_i monitored in this study is mostly a reflection of cytoplasmic pH. Changes in pH_i will have countless effects on numerous enzymes and metabolic activities as evidenced by the pH changes which trigger the late events of fertilization in sea urchin eggs (16). In addition, dramatic effects of small changes in pH on the behavior of purified preparations of actin and myosin, proteins intrinsically involved in neutrophil functions, have been demonstrated (17). Cellular correlates of these findings have been indirectly described in the acrosomal reaction in echinoderm sperms in which it has been shown that the accompanying actin polymerization is pH dependent (18,19).

The possible involvement of the Na^+/K^+ gradient (20) and anion movements (21) in the maintenance or regulation of the pH_i of the neutrophils is under investigation.

Finally, the demonstration of chemotactic factor induced pH_i changes in neutrophils raises some questions about the previously described membrane potential changes (6,7) as the probes used to monitor the latter parameter are known to be sensitive to pH gradients (22,23).

The involvement of pH changes in signal transduction or the modulation of the activation of other stimuli (e.g. hormones) sensitive cells remains to be carefully examined.

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