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STUDIES ON STIMULUS-RESPONSE COUPLING IN HUMAN NEUTROPHILS

I. ROLE OF MONOVALENT CATIONS IN THE RESPIRATORY AND SECRETORY RESPONSE TO *N*-FORMYLMETHIONYLLEUCYLPHENYLALANINE

VITTORINA DELLA BIANCA, P. BELLAVITE, P. DE TOGNI, R. FUMARULO and F. ROSSI

Instituto di Patologia Generale, Università degli Studi di Padova, sede di Verona, Strada Le Grazie, Verona (Italy)

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Human blood neutrophils suspended in Na^+-free, high-K^+, phosphate-buffered solution exhibit respiratory and secretory responses to *N*-formylmethionylleucylphenylalanine (fMet-Leu-Phe) much higher than those suspended in phosphate-buffered solution containing physiological concentration of K^+ and Na^+. The differences between the responses are very marked at low doses of fMet-Leu-Phe (10^{-9}, 10^{-8} M), progressively decrease at higher doses, and disappear at the maximal stimulatory concentration of the peptide (10^{-6} M). The higher responses of human neutrophils to fMet-Leu-Phe are not dependent on the membrane depolarization, that occurs when the cells are suspended in high-K^+ buffered solution, but on the absence, or on the low concentration, of Na^+ in the suspending medium. In fact: (i) the higher respiratory and secretory responses progressively decrease by substituting K^+ with Na^+ in the suspending solution, without change of the state of depolarization; (ii) the replacement of extracellular Na^+ with choline ions does not affect the transmembrane potential of neutrophils but induces higher respiratory and secretory responses to fMet-Leu-Phe; (iii) the membrane depolarization induced by gramicidin and by ouabain does not result in a higher respiratory response to chemotactic peptide. These results indicate that in human neutrophils Na^+ plays a regulative role in the stimulation of the respiratory burst and in the secretion induced by the chemotactic peptide. This regulation does not influence the maximal responses, but the threshold of the responses. K^+ is also involved at least in the respiratory response, since the effect of the absence of Na^+ is potentiated when the concentration of K^+ of the suspending solution is high. Furthermore, the finding that a very high respiratory burst and the secretion of β-glucuronidase and vitamin B-12-binding protein can be induced by fMet-Leu-Phe in human neutrophils in the absence of external Na^+ indicates that the entry of this cation and the consequent decrease in transmembrane potential are not necessary events for the activation of respiration and secretion by the peptide. The mechanism underlying the effect of the modification of ionic composition of the external medium is discussed in terms of the molecular events triggered by the stimulus at the level of the plasma membrane and of the recognition phenomena at the cell surface, that are common steps for the induction of the respiratory and secretory responses in neutrophils.

Introduction

The interactions with phagocytosable particles and with appropriate membrane-binding factors induce in leucocytes a series of responses such as chemotaxis, endocytosis, secretion and changes in the respiratory metabolism with activation of an NADPH oxidase and production of O_2^- and H_2O_2

[1–12]. The cell surface and the plasma membrane play a key role in these responses and many molecular and functional changes of these structures are associated with phagocytosis and with the stimulation of movement, secretion and respiration [2,4–7,10,11,13–16]. These include, among others modifications of Ca^{2+} fluxes and mobilization from intramembranous stores [17–19], and changes in Na^+ and K^+ fluxes [18,20], transmembrane potential [21–27], phospholipid turnover [28,29], protein phosphorylation [30] and arachidonic acid cascade [10,31–34]. However, the precise nature and the sequence of events triggering the manifold responses remain to be clarified.

During a series of investigations on the role of some of the molecular changes associated with the stimulation of the respiratory metabolism, we found that when guinea pig neutrophils are suspended in Na^+-free, high-K^+ buffered solution [35] they exhibit a spontaneous respiratory burst similar to that induced by phagocytosis or by membrane-perturbing agents. A spontaneous increase in O_2^- production in macrophages suspended in high-K^+ medium has been observed also by others [27].

In an attempt to understand the mechanism of this phenomenon, we have investigated the effect of changes of external Na^+ and K^+ concentrations on the respiratory and secretory responses of human blood neutrophils. This paper reports data showing that these responses to *N*-formylmethionylleucylphenylalanine (fMet-Leu-Phe) are greatly enhanced by the absence or low concentration of Na^+ in the external medium.

Materials and Methods

Reagents. fMet-Leu-Phe, ferricytochrome *c* type VI, catalase, cytochalasin B, choline hydrochloride, gramicidin and valinomycin were purchased from Sigma (U.S.A.). The fluorescent dye 3,3′-dipropylthiocarbocyanine was a generous gift from Dr. Alan Waggoner. Superoxide dismutase was a gift from Dr. J.V. Bannister. Stock solutions of 3,3′-dipropylthiocarbocyanine were made in ethanol and of fMet-Leu-Phe and cytochalasin B in dimethyl sulfoxide (DMSO) and kept frozen at −20°C.

Collection of cells. Leucocyte suspensions containing 85–90% neutrophils were prepared from venous blood of healthy volunteers by employing a standard technique of dextran sedimentation as described by Dunham et al. [20].

Suspending buffered solutions. The solutions employed, buffered at pH 7.4 and containing 5 mM glucose, were as follows:

(1) Krebs-Ringer phosphate: [Na^+] 152 mM; [K^+] 5 mM; [Cl^-] 130 mM; [PO_4^{3-}] 16.6 mM; [Mg^{2+}] 1.22 mM.

(2) Na^+-free, high-K^+ Krebs-Ringer phosphate: [K^+] 157 mM; [Cl^-] 130 mM, [PO_4^{3-}] 16.6 mM; [Mg^{2+}] 1.22 mM.

(3) Krebs-Ringer phosphate containing different concentrations of Na^+ and K^+.

(4) Krebs-Ringer phosphate where Na^+ was totally or partially substituted with choline.

Metabolic studies. O_2 consumption by the cells was measured at 37°C with a Clark oxygen electrode (Yellow Springs Instrument Co., OH) using $2 \cdot 10^7$ neutrophils in 2 ml of appropriate buffered solution containing 5 μg/ml cytochalasin B, 1 mM KCN and 0.5 mM $CaCl_2$ [35]. The O_2^- production was assayed by recording superoxide dismutase-sensitive cytochrome *c* reduction [36].

Secretion assays. $1 \cdot 10^7$ human neutrophils were incubated for 5 min at 37°C in polystyrene tubes in appropriate buffered solutions containing 0.5 mM $CaCl_2$, 1 mM KCN and 5 μg/ml cytochalasin B. Thereafter, fMet-Leu-Phe in 2 μl DMSO was added and the cells incubated at 37°C for a further 5 min. After this time, the cell suspension was rapidly pelleted by centrifugation at $8000 \times g$ for 30 s in a microcentrifuge (Eppendorf). The supernatants were assayed for released β-glucuronidase [37], as a marker of secretion from azurophilic granules, vitamin B-12-binding protein [38], as a marker of secretion from specific granules, and lactate dehydrogenase, as a marker of release of cytosol components.

Membrane potential. The procedure used was mostly that described in Ref. 39 as applied in Refs. 25, 35 and 40. The fluorescent probe used was 3,3′-dipropylthiocarbocyanine at 2 μM concentration. The changes of fluorescence intensity were recorded at 37°C with a spectrofluorimeter (Ciampolini, Italy) with excitation and emission wavelengths of 622 and 660 nm, respectively. All the measurements were performed in the presence

of 550 I.U. catalase and 25 μg superoxide dismutase in order to avoid quenching of the dye by the intermediates of oxygen reduction [40].

Results

Effect of ionic composition of the suspending medium on the respiratory response of human neutrophils to fMet-Leu-Phe

When suspended in high-K^+ Krebs-Ringer phosphate, human neutrophils do not exhibit a spontaneous respiratory burst, as do guinea pig neutrophils [35], but present a marked modification of respiratory response to the chemotactic peptide fMet-Leu-Phe. As shown in Fig. 1, the stimulation of O_2 consumption and O_2^- production by $2 \cdot 10^{-8}$ M fMet-Leu-Phe is much greater in human neutrophils suspended in high-K^+ Krebs-Ringer phosphate than in Krebs-Ringer phosphate. The effects of different concentrations of fMet-Leu-Phe on O_2 consumption are reported in Fig. 2: (1) the respiratory response of human neutrophils suspended in Krebs-Ringer phosphate starts at 10^{-8} M fMet-Leu-Phe, progressively increases at higher concentrations of the stimulant and reaches a maximum value at 10^{-6} M fMet-Leu-Phe; (2) the respiratory response of human neutrophils suspended in high-K^+ Krebs-Ringer phosphate is already present at 10^{-9} M fMet-Leu-Phe and increases with increasing stimulant concentration at a rate much higher than that of human neutrophils suspended in Krebs-Ringer phosphate. At 10^{-6} M fMet-Leu-Phe, the respiratory response of human neutrophils suspended in high-K^+ Krebs-Ringer phosphate is similar to that of neutrophils suspended in Krebs-Ringer phosphate.

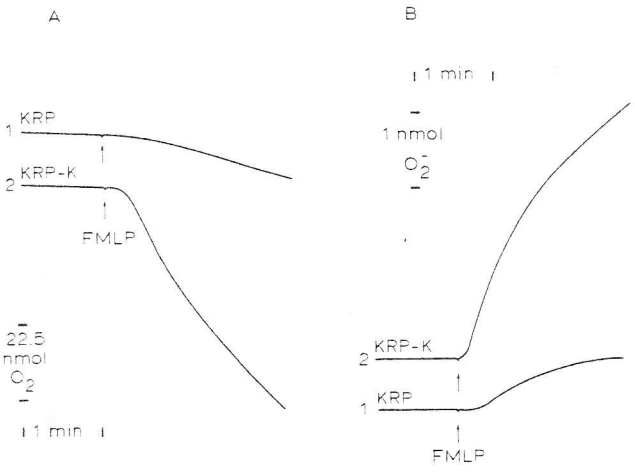

Fig. 1. Effect of ionic composition of the suspending medium on the respiratory response of human neutrophils to $2 \cdot 10^{-8}$ M fMet-Leu-Phe (FMLP). (A) Polargraphic traces of oxygen consumption by $2 \cdot 10^7$ neutrophils. (B) Spectrophotometric recording of O_2^- production by $2 \cdot 10^6$ human neutrophils. (1) KRP, Krebs-Ringer phosphate; (2) KRP-K, high K^+ Krebs-Ringer phosphate.

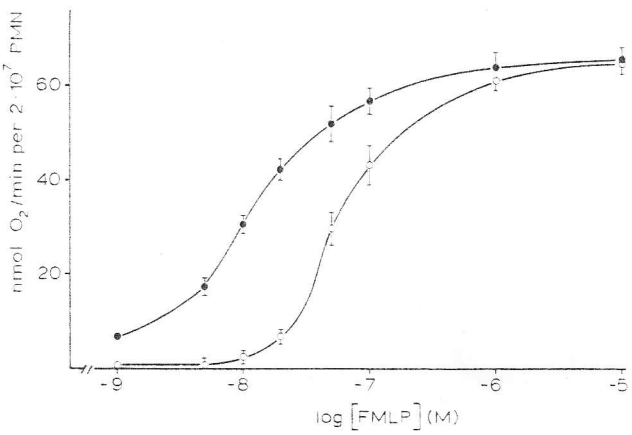

Fig. 2. Respiratory response of $2 \cdot 10^7$ human neutrophils suspended in Krebs-Ringer phosphate and high-K^+ Krebs-Ringer phosphate as a function of different concentrations of fMet-Leu-Phe (FMLP). Each point represent the mean ± S.E. of four experiments. (○——○) Human neutrophils in Krebs-Ringer phosphate; (●——●) human neutrophils in high-K^+ Krebs-Ringer phosphate.

Relationship between the higher rate of respiratory response and transmembrane depolarization

Fig. 3A shows that the suspension of human neutrophils in high-K^+ Krebs-Ringer phosphate induces a marked membrane depolarization. This is indicated by the higher values of fluorescence intensity reached by human neutrophils in high-K^+ Krebs-Ringer phosphate at the end of the equilibrium (baseline fluorescence). Furthermore, the addition of valinomycin (a K^+ ionophore) causes opposite effects in the transmembrane potential: hyperpolarization in cells suspended in Krebs-Ringer phosphate (low-K^+ buffer) and depolarization in cells suspended in high-K^+ Krebs-Ringer phosphate (high-K^+ buffer), as expected when the membrane potential depends on K^+ gradients.

Since a transmembrane depolarization correlates and precedes the onset of the respiratory burst by different stimuli in leucocytes [21,22,

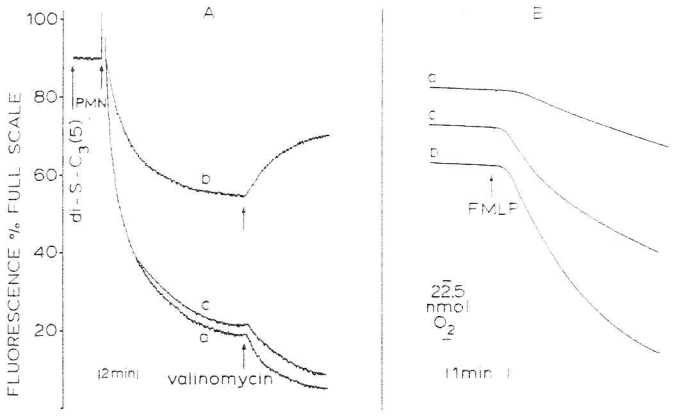

Fig. 3. (A) Transmembrane potential measured as baseline fluorescence of 3,3′-dipropylthiocarbocyanine (di-S-C_3(5)) of human neutrophils (PMN) suspended in Krebs-Ringer phosphate (a) high-K^+ Krebs-Ringer phosphate (b), Krebs-Ringer phosphate containing choline (c) and its variations by 2 μM valinomycin. For the experimental conditions see Materials and Methods. (B) Recording of polarographic assay of the stimulation of O_2 consumption induced by $2 \cdot 10^{-8}$ M fMet-Leu-Phe (FMLP) in $2 \cdot 10^7$ human neutrophils suspended in Krebs-Ringer phosphate (a), high-K^+ Krebs-Ringer phosphate (b) and Krebs-Ringer phosphate containing choline (c).

24–27], it seems reasonable to advance the hypothesis that the high rate of respiratory response to fMet-Leu-Phe exhibited by human neutrophils suspended in high-K^+ Krebs-Ringer phosphate buffer is a consequence of the change in transmembrane potential. The validity of this hypothesis was tested by measuring the activation of the respiration by fMet-Leu-Phe in leucocytes suspended in Krebs-Ringer phosphate buffer, where all Na^+ was substituted with choline (Krebs-Ringer phosphate containing choline). In agreement with previous results [35], the suspension of human neutrophils in this medium does not change the transmembrane potential, meaured as the baseline fluorescence in the presence of 3,3′-dipropylthiocarbocyanine (Fig. 3A), but enhances the respiratory response to fMet-Leu-Phe (Fig. 3B). This result indicates that mechanisms other than the change in membrane potential are involved in the greater response to fMet-Leu-Phe. Indirect evidence showing that a depolarization state does not induce per se a greater responsiveness is supported by the finding that gramicidin, a ionophore that increases both Na^+ and K^+ permeability [41], and ouabain, an inhibitor of the neutrophil Na^+, K^+ pump [20,42], cause in human neutrophils a decrease in the transmembrane potential but do not modify the intensity of their respiratory burst induced by fMet-Leu-Phe (data not shown).

Respiratory responsiveness of human neutrophils as a function of different K^+ and Na^+ concentrations

Fig. 4 shows that the high respiratory response to fMet-Leu-Phe exhibited by human neutrophils in high-K^+ Krebs-Ringer phosphate progressively decreases on substituting Na^+ for K^+. The comparison between the changes in the respiratory response and in the depolarization state caused by the substitution of K^+ with Na^+ in the medium (Table I) shows that the respiratory response to fMet-Leu-Phe decreases without modifications of the depolarization state induced by K^+. These results indicate that the degree of response of human neutrophils to fMet-Leu-Phe is an inverse function of Na^+ concentration in the external medium and that the greatest response occurs in the absence of this cation.

Changes of transmembrane potential induced by fMet-Leu-Phe

Fig. 5 shows the changes of transmembrane potential and the stimulation of O_2 consumption induced by fMet-Leu-Phe in human neutrophils suspended in Krebs-Ringer phosphate and in high-K^+ Krebs-Ringer phosphate. It can be seen that fMet-Leu-Phe induces both a decrease in the

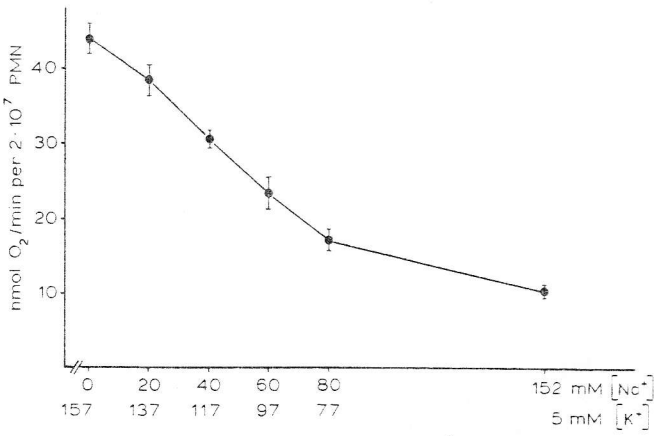

Fig. 4. Respiratory response to $2 \cdot 10^{-8}$ M fMet-Leu-Phe (FMLP) by human neutrophils (PMN) as a function of different K^+ and Na^+ concentrations in the suspending buffered solutions. The values are the mean ± S.E. of six experiments.

TABLE I

RESPIRATORY RESPONSE TO $2 \cdot 10^{-8}$ M fMet-Leu-Phe AND TRANSMEMBRANE POTENTIAL (F_b/F_o) OF HUMAN NEUTROPHILS AS A FUNCTION OF EXTERNAL CONCENTRATION OF Na^+ AND K^+, AND ITS CHANGES BY ADDITION OF 2 μM VALINOMYCIN (F_v/F_b)

F_b is the fluorescence intensity of the leucocyte suspensions at the end of equilibration and F_o that of 3,3'-dipropylthiocarbocyanine solution. The ratio F_b/F_o is a function of transmembrane potential; the lower the value, the higher the transmembrane potential. F_v/F_b, is the ratio of changes of fluorescence of leucocyte suspensions after the addition of 2 μM valinomycin (F_v) to that at the end of equilibration before the addition of valinomycin (Fb). The decrease in this ratio indicates hyperpolarization, an increase depolarization, (a representative experiment).

Conditions of incubation		O_2 consumption (nmol O_2/min per $2 \cdot 10^7$ neutrophils)	F_b/F_o	F_v/F_b
$[K^+]$ (mM)	$[Na^+]$ (mM)			
157	0	44.4	0.45	1.22
137	20	35.5	0.45	1.22
117	40	29.2	0.45	1.22
97	60	16.2	0.41	1.16
77	80	10.1	0.37	1.02
5	152	10.1	0.29	0.72

transmembrane potential and a respiratory burst in human neutrophils suspended in Krebs-Ringer phosphate. In contrast, in neutrophils suspended in high-K^+ Krebs-Ringer phosphate, the peptide induces a higher respiratory response which is not associated with modification of the transmembrane potential. These results indicate that Na^+ is required for the depolarization induced by fMet-Leu-Phe and that the change of membrane potential does not show a positive correlation with the presence and intensity of the burst.

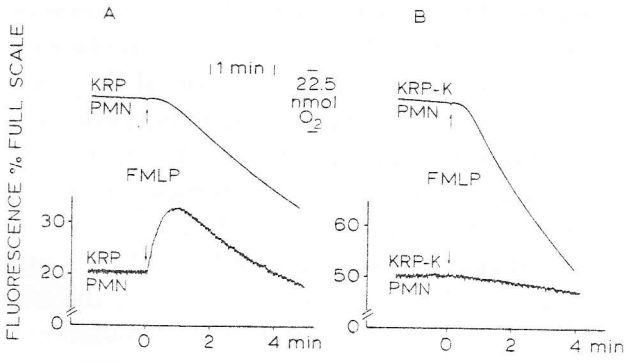

Fig. 5. Stimulation of oxygen consumption (upper curves – polarographic recording) and modification of transmembrane potential (lower curves – spectrofluorimetric traces) induced by $5 \cdot 10^{-8}$ M fMet-Leu-Phe (FMLP) in human neutrophils (PMN) suspended in Krebs-Ringer phosphate (A) and in high-K^+ Krebs-Ringer phosphate (B). For details see Materials and Methods.

Effect of ionic composition of the suspension media on the secretory response to fMet-Leu-Phe

Table II shows that the secretory response to fMet-Leu-Phe is also influenced by the external ionic concentration. The secretion by cytochalasin B-treated human neutrophils of β-glucuronidase, a marker of azurophilic granules, and of vitamin B-12-binding protein, a marker of specific granules, is much greater when the cells are suspended in high-K^+ Krebs-Ringer phosphate.

Fig. 6 depicts the secretory responses as a function of different concentrations of fMet-Leu-Phe. The data show that the stimulation of secretion behaves similarly to that of the respiration reported in Fig. 2. In fact, the differences between the secretion induced in human neutrophils suspended in high-K^+ Krebs-Ringer phosphate and in Krebs-Ringer phosphate are much greater at low doses of fMet-Leu-Phe, progressively decrease at higher doses and disappear at the maximal

TABLE II

SECRETORY RESPONSE TO 10^{-8} M fMet-Leu-Phe OF HUMAN NEUTROPHILS AS A FUNCTION OF EXTERNAL CONCENTRATION OF Na^+ AND K^+

Total cell enzyme activities were: 316 ± 55.3 nmol phenolphthalein/4 h per 10^7 neutrophils for β-glucuronidase; 865 ± 104.0 pg vitamin B-12 bound/10^6 neutrophils for vitamin B-12-binding protein. Data represent the mean $\pm$ S.E. of four experiments. For experimental condtions see Materials and Methods.

Conditions of incubation		Vitamin B-12-binding protein release (pg vitamin B-12 bound/10^6 neutrophils)	β-Glucuronidase release (nmol phenolphthalein/4 h per 10^7 neutrophils)
$[K^+]$ (mM)	$[Na^+]$ (mM)		
5	152	44.7 ± 11.1	4.7 ± 0.3
157	0	162.7 ± 10.7	36.2 ± 3.6

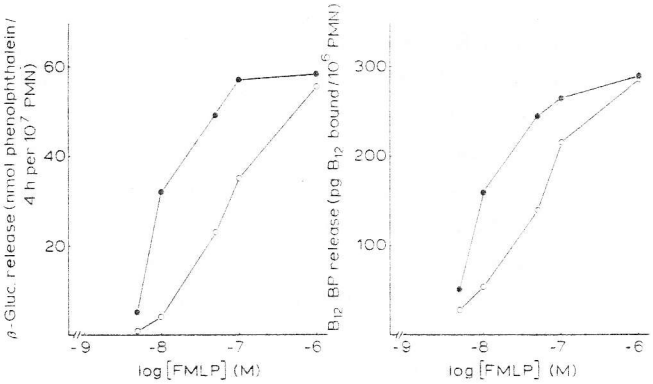

Fig. 6. Secretion of β-glucuronidase and of vitamin B-12-binding protein (B_{12} BP) by human neutrophils (PMN) suspended in Krebs-Ringer phosphate and high-K^+ Krebs-Ringer phosphate as a function of different concentrations of fMet-Leu-Phe (FMLP) (a representative experiment). (●———●) High-K^+ Krebs-Ringer phosphate; (○———○) Krebs-Ringer phosphate.

stimulatory concentration of the peptide, i.e., at 10^{-6} M.

The data of Fig. 7 demonstrate that the enhancement of the secretory response is due to the absence of Na^+ in the suspending media. In fact, by substituting K^+ with Na^+, the secretory activity induced by fMet-Leu-Phe progressively decreases and become similar to that exhibited by human neutrophils suspended in Krebs-Ringer phosphate when the external concentration of Na^+ reaches 40 mM. A greater secretory response to fMet-Leu-Phe has been observed also in human

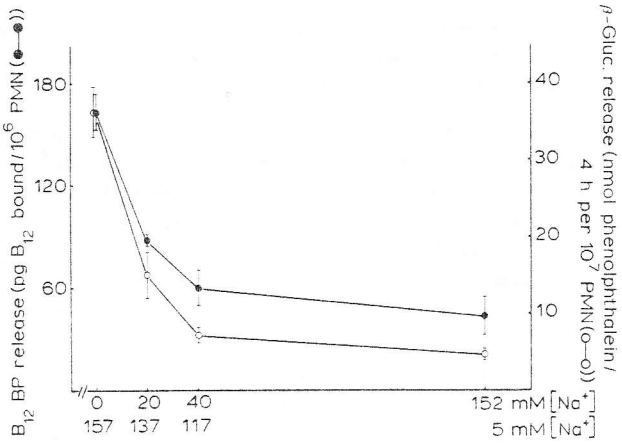

Fig. 7. Secretion of β-glucuronidase and of vitamin B-12-binding protein (B_{12} BP) induced by 10^{-8} M fMet-Leu-Phe (FMLP) in human neutrophils (PMN) as a function of different K^+ and Na^+ concentrations in the suspending buffered solutions. The values are the mean ± S.E. of four experiments.

neutrophils suspended in Krebs-Ringer phosphate where all Na^+ has been substituted with choline.

Discussion

The general opinion that ions are important in the multifold responses of leucocyte to chemotactic factors and to other stimulants is based on a series of experimental observations. The first, given in our laboratory, was that ionophores which transfer monovalent and/or divalent cations through the plasma membrane, are able to induce respiratory and secretory responses [16,43]. Thereafter, many molecular events involving movements of ions at the level of the plasma membrane of phagocytes have been shown to occur during or before the onset of various responses. These include, among others, modification of Ca^{2+} fluxes and mobilization from cell membranes [17–19], changes of Na^+ and K^+ fluxes [18,20], transmembrane potential [21–27], and the Ca^{2+} dependency of the respiratory and of secretory responses [44, 45] to various stimulants.

The results presented here show that human neutrophils suspended in Na^+-free, high-K^+ buffered solutions exhibit respiratory and secretory responses to fMet-Leu-Phe greater than those of neutrophils suspended in medium containing physiological concentration of Na^+ and K^+. The role of Na^+ and K^+ in the various functions of leucocytes is at present not clear. It has been shown [46] that the removal of all Na^+ from the external medium increases the spontaneous motility but depresses the chemotactic response of rabbit neutrophils. Furthermore, the replacement of Na^+ with varying concentration of K^+ causes an increase in chemotactic reactivity, indicating that physiological concentration of K^+ in the external medium enhances the effectiveness of chemotactic factors [46]. The presence of K^+ was also shown to enhance the secretion induced by chemotactic factors and by Ca^{2+} ionophore, and the substitution of Na^+ with K^+ induces a stimulation of the spontaneous secretion and an increase in the Ca^{2+} dependent secretion by fMet-Leu-Phe [47].

Other workers have found that the absence of or decrease in Na^+ cause a decrease in the leucocyte responses. The replacement of extracellular Na^+ with either K^+ or choline ions depresses O_2^-

generation in human neutrophils exposed to concanavalin A or immune complex, and the replacement of Na^+ with choline also decreases the release of β-glucuronidase and of lysozyme [48].

Results in contrast with those reported in this paper have been published by Simchowitz and Spilberg [49] who have found that fMet-Leu-Phe-induced O_2^- generation in human neutrophils requires the presence of Na^+, and by Miles et al. [27] who have shown that in the presence of zymosan, rat alveolar macrophages release less O_2^- when suspended in low-Na^+, high-K^+ buffered solution. These discrepancies could be due to the marked differences in the experimental conditions and to the type of cells employed. As regards the differences with the results presented by Simchowitz and Spilberg, it is worthwhile pointing out that our experiments have been done in the presence of cytochalasin B, which greatly potentiates the respiratory and secretory responses to fMet-Leu-Phe [47,50–52], and also in the presence of cyanide which, by impeding the destruction of the stimulant via H_2O_2 myeloperoxidase released [53,54], assures optimal conditions of stimulation and of response.

The results presented in this paper can be discussed in relation to the relevance of ion changes in the stimulus-response coupling in leucocytes. It has been shown in different laboratories that the interaction between fMet-Leu-Phe and the surface receptors is followed by an influx of Na^+ [18,49,55] which, in turn, would be responsible for the transmembrane depolarization [21,22,24–26]. The finding that a very high respiratory burst can occur in the absence of external Na^+ indicates that the entry of Na^+ and the consequent change of membrane potential are events not necessary for triggering the activation of respiration.

The main point to be discussed is the mechanism by which the changes of ionic composition of the medium influence the responses by the respiratory system and of the secretory apparatus to fMet-Leu-Phe. Since in a high external K^+ concentration the leucocytes are depolarized, it seems, at first sight, that a direct correlation exists between the decrease in transmembrane potential and the greater response to fMet-Leu-Phe. This correlation would be in agreement with the concept that membrane depolarization is involved in leucocyte activation [21,22,24–27]. However, the data presented in this paper indicate that the enhanced responsiveness of neutrophils to fMet-Leu-Phe is not linked to the changes of membrane potential. In fact: (i) the higher response is progressively decreased by substituting K^+ with Na^+ without modification of the state of depolarization; (ii) the replacement of all extracellular Na^+ with choline ion does not affect the transmembrane potential but induces a high response to fMet-Leu-Phe; (iii) the decrease in transmembrane potential induced by gramicidin and by ouabain does not result in a greater respiratory response to fMet-Leu-Phe.

All the data presented in this paper indicate that modification of the responses to the peptide is directly linked to the change of monovalent cations in the medium, mostly to the absence or low concentration of Na^+. However, it is worthwhile pointing out that also the change in the concentration of K^+ is involved at least in the respiratory response, since the effect of the absence of Na^+ is potentiated when the concentration of K^+ is high. In considering the role of the plasma membrane in the stimulus-response coupling, it is likely that the main mechanism responsible for the modification of the responses to fMet-Leu-Phe consists of changes of intramembrane ionic composition of human neutrophils. These produce molecular and structural modifications of membrane components, which result in variation of the intensity of the responses to fMet-Leu-Phe. This variation does not affect the maximal responses, i.e., the O_2 consumption and the secretory activity induced by the maximal stimulatory doses of fMet-Leu-Phe, but the responses to suboptimal concentration of the stimulant, i.e., the threshold of the responses. Thus, as far as the NADPH oxidase is concerned, the higher response is not expressed as an increase in its maximal velocity, but by the activation of a greater number of enzyme molecules in the presence of suboptimal concentrations of the stimulant.

The molecular level and the nature of the events responsible for the higher response of the respiratory system and of the secretory apparatus remain to be elucidated. One possibility is that the movements of Ca^{2+} from and to the extracellular medium or the release of membrane-associated

Ca^{2+} are involved. Another possibility is that the change of the responses is due to modifications of the recognition system, i.e., of the number and/or the properties of the receptor specific for the stimulant. It is know that Na^{+} may play a regulative role in the affinity of surface receptors for various agonists in different cells [56,57].

In the accompanying paper, data are presented on the effect of the changes of ionic composition of the suspending solution on the properties of the surface receptors of fMet-Leu-Phe in human neutrophils.

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