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MEASUREMENT OF MEMBRANE POTENTIAL IN POLYMORPHONUCLEAR LEUKOCYTES AND ITS CHANGES DURING SURFACE STIMULATION

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The membrane potential of guinea pig polymorphonuclear leukocytes has been assessed with two indirect probes, tetraphenylphosphonium (TPP^+) and 3,3′-dipropylthiadicarbocyanine (diS-C_3-(5)). The change in TPP^+ concentration in the medium was measured with a TPP^+-selective electrode. By monitoring differences in accumulation of TPP^+ in media containing low and high potassium concentrations, a resting potential of −58.3 mV was calculated. This potential is composed of a diffusion potential due to the gradient of potassium, established by the Na^+, K^+ pump, and an electrogenic potential. The chemotactic peptide fMet-Leu-Phe elicits a rapid efflux of accumulated TPP^+ (indicative of depolarization) followed by its reaccumulation (indicative of repolarization). In contrast, stimulation with concanavalin A results in a rapid and sustained depolarization without a subsequent repolarization. The results obtained with TPP^+ and diS-C_3-(5) were comparable. Such changes in membrane potential were observed in the absence of extracellular sodium, indicating that an inward movement of sodium is not responsible for the depolarization. Increasing potassium levels, which lead to membrane depolarization, had no effect on the oxidative metabolism in nonstimulated or in fMet-Leu-Phe-stimulated cells. Therefore, it seems unlikely that membrane depolarization per se is the immediate stimulus for the respiratory burst.

Introduction

Following membrane stimulation by particulate or soluble agents, polymorphonuclear leukocytes undergo a large increase in O_2 consumption which has been termed the respiratory burst. This burst of O_2 consumption is important in bacterial killing which depends on the formation of highly reactive products of O_2 metabolism, including superoxide ($O_2^{-}\cdot$) hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) [1]. Although the key event of this respiratory burst appears to be the activation of a plasma membrane-bound NAD(P)H oxidase that is dormant in the resting state, its triggering mechanism is still unknown [2].

It has been suggested recently that changes in transmembrane potential play an important role in the function of phagocytic cells. For example, Gallin and Gallin [3], using intracellular microelectrodes, have reported hyperpolarization of human macrophages in response to chemotactic factors. However, electrophysiologic measurement of membrane potential in polymorphonuclear leukocytes is extremely difficult because the cells are relatively small and because the nucleus occupies most of the intracellular volume. Therefore, most

Abbreviations: diS-C_3-(5), 3,3′-dipropylthiadicarbocyanine iodide; TPP^+, tetraphenylphosphonium; $TPMP^+$, triphenylmethylphosphonium; fMet-Leu-Phe, *N*-formylmethionylleucylphenylalanine.

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of the recent investigations have been made by using the distribution of a fluorescent [4–15,19] or an isotopically labeled lipophilic cation [16–19] between the external medium and the cell. Unfortunately, until now, conflicting data have been published on the resting potential and on the changes in membrane potential upon activation by a variety of chemotactic factors and secretagogues. For example, Jones et al. [11] have measured the resting potential of human granulocytes to be -102 mV, using the fluorescent dye diS-C_3-(5), at the valinomycin null point, which occurred at an extracellular K^+ concentration of 2.7 mM. However, Whitin et al. [7] reported the valinomycin null point to occur at 10 mM K^+, which is indicative of a much lower value of the resting potential. Studies on the distribution of the isotopically labeled lipophilic cation triphenylmethylphosphonium ($TPMP^+$) have yielded lower values of -26.7 mV [16] and -45 mV [19]. In addition, reports on changes in membrane potential during surface stimulation are more complicated. The transient changes (indicative of transient depolarization) in fluorescence upon activation by chemotactic factor (fMet-Leu-Phe), the secretagogue phorbol myristate acetate and concanavalin A were described in studies with cyanine dyes [5–8,10,12,13,15,19] which are in contrast to the findings with $TPMP^+$ by Weissmann et al. [16–18]. They have reported that concanavalin A, antigen-antibody complexes and fMet-Leu-Phe all cause a transient hyperpolarization. On the other hand, a dose-dependent response to concanavalin A has also been described in earlier work with diS-C_3-(5) by Utsumi et al. [4], whose data illustrate that concanavalin A produces a rapid and sustained fluorescence increase compatible with a depolarization.

In a previous paper [20], we developed an electrode sensitive to TPP^+ and showed that this electrode possesses a number of clear advantages over methods currently used for the monitoring of membrane potential in microscopic systems. In a more recent report [21], we showed that the Na^+, K^+ pump contributed to the membrane potential in guinea pig polymorphonuclear leukocytes. In this paper, we examined the response of membrane potential in guinea pig polymorphonuclear leukocytes to fMet-Leu-Phe and concanavalin A using two indirect probes: a lipophilic cation (TPP^+) and a fluorescent, potential-sensitive dye (diS-C_3-(5)). The close correlation between ionic conditions and membrane potential then led us to a study of ionic control of leukocyte activation. Previous studies [17,22] suggested a role for Na^+ in the modulation of stimulus-response coupling in leukocytes. We have therefore examined the effects of external Na^+ on the membrane potential changes and the activation of the respiratory burst.

Experimental Procedure

Chemicals. Tetraphenylphosphonium chloride was obtained from Dojindo Laboratories, Kumamoto. Cyanine dye diS-C_3-(5) was a gift from Nippon Kankoh Shikiso Kenkyusho, Okayama. Chemotactic factor (fMet-Leu-Phe) was obtained from Protein Research Foundation, Osaka. Concanavalin A, gramicidin and cytochrome *c* (type III) were purchased from Sigma Chemical. Co., St. Louis, MO, and ouabain from Merk, Darmstadt. All other materials were of analytical grade and obtained from commercial sources.

Preparation of polymorphonuclear leukocytes. Polymorphonuclear leukocytes were collected as previously described [21] from guinea pig peritoneal cavity 18 h after intraperitoneal injection of 30 ml of sterile 2.0% sodium caseinate/isotonic saline. The exudate was washed once with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline and suspended in this solution. The composition of phosphate-buffered saline was 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4 (pH 7.3). More than 90% of the cells obtained in this manner were polymorphonuclear leukocytes as determined by Wright-stain smears.

Determination of membrane potential by use of the TPP^+ electrode. The accumulation and efflux of TPP^+ were monitored continuously with a TPP^+-selective electrode. The construction and properties of a TPP^+-selective electrode were previously described [20]. Reactions were initiated by adding 100–200 μl of suspended cells to 2.0 ml of a solution containing 5 μM TPP^+ in one of the following mixtures: (i) 'low-K^+ medium,' 137 mM NaCl/2.7 mM KCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4/1.0 mM $CaCl_2$; (ii) 'high-K^+ medium,'

same as low-K^+ medium except that 137 mM KCl was used in place of NaCl; and (iii) 'choline$^+$ medium,' same as low-K^+ medium except that 137 mM choline chloride was used in place of NaCl. Magnesium was omitted from these media in order to minimize spontaneous and stimulus-induced cell aggregation. The temperature of the system was kept constant at 37°C, and the cells were maintained in suspension by agitating with magnetic stirring bar.

The membrane potential, $\Delta\psi$, can be calculated from the shift of the electrode potential provided that TPP^+ is passively distributed in accordance with the Nernst equation. When the electrode potential shifts by ΔE from the baseline, $\Delta\psi$ is given by the following equation [20]:

$$\Delta\psi = 2.3(RT/F)\log(v/V) - 2.3(RT/F)\log[10^{F\Delta E/2.3RT} - 1] \quad (1)$$

where v and V represent the volumes of polymorphonuclear leukocytes and of the medium, respectively, and $2.3RT/F$ is equal to 61 mV at 37°C. The baseline is the electrode potential before addition of the cells. Assuming that $\Delta\psi$ across the plasma membrane is negligible when the cells are suspended in high-K^+ medium [23] and that the first term in Eqn. 1 remains constant, we obtain the following equation:

$$\Delta\psi = 2.3(RT/F)\log[10^{F\Delta E_h/2.3RT} - 1] - 2.3(RT/F)\log[10^{F\Delta E_l/2.3RT} - 1] \quad (2)$$

where ΔE_h and ΔE_l represent ΔE in high-K^+ and low-K^+ medium, respectively. Thus, $\Delta\psi$ can be calculated from ΔE_h and ΔE_l.

Fluorescence measurements. Polymorphonuclear leukocytes ($2 \cdot 10^6$ cells/ml) were suspended in incubation medium at 37°C, and then the fluorescent dye was added to give a final concentration of 2 μM. The medium in a cuvette was mixed gently with a magnetic stirrer throughout the measurements. Fluorescence measurements were made on a Shimazu spectrofluorometer model RF-510 as described previously [4]. Excitation was at 622 nm and emission was recorded at 670 nm.

Measurements of superoxide generation. O_2^- generation by polymorphonuclear leukocytes was monitored continuously as superoxide dismutase-inhibitable cytochrome *c* reduction in a dual-wavelength spectrophotometer (Shimazu UV-300) by a modification of the method of Nakagawara et al. [24]. The cuvette containing cell suspension ($2.0–2.5 \cdot 10^6$ cells/ml) was put into the thermostatically controlled cuvette holder (37°C) of a spectrophotometer, and then ferricytochrome *c* was added to give a final concentration of 50 μM. The reduction of cytochrome *c* was measured at 550 nm with the reference wavelength at 540 nm. The rate of O_2^- generation was calculated from the linear rate of absorbance change and the molar extinction coefficient for this reduction ($19.1 \cdot 10^3$).

Results

Measurement of membrane potential by use of a TPP^+-selective electrode

The time course for uptake of TPP^+ by polymorphonuclear leukocytes suspended in low- and

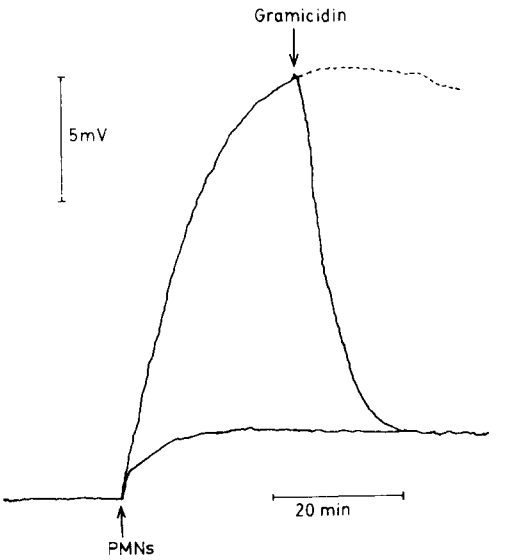

Fig. 1. Time course of the uptake of TPP^+ by guinea pig polymorphonuclear leukocytes (PMNs). Cells ($2 \cdot 10^7$/ml) were suspended in low-K^+ (upper) or high-K^+ (lower) medium at 37°C. TPP^+ accumulation was monitored with a TPP^+ electrode as described in Experimental procedure. As indicated by the arrow, after 25 min of incubation, gramicidin was added to a final concentration of 25 μm/ml. Dashed lines, no added gramicidin. The vertical bar represents a change of 5 mV in the TPP^+ electrode.

high-K^+ media at 37°C is shown in Fig. 1. As previously shown [21], TPP^+ uptake reached a maximum in 30–40 min that gradually declined with time. The uptake of TPP^+ by polymorphonuclear leukocytes suspended in low-K^+ medium was several times greater than that in high-K^+ medium. When polymorphonuclear leukocytes were suspended in low-K^+ medium and gramicidin was then added at 25 min, rapid loss of TPP^+ occurred, and within 20 min the level of accumulation approximated that observed when polymorphonuclear leukocytes were suspended in high-K^+ medium. It is also significant that gramicidin-induced loss of TPP^+ was not observed with cells suspended in high-K^+ medium. This observation is consistent with the assumption that the membrane potential in high-K^+ medium is 0 mV.

Fig. 1 suggests that the resting potential is dependent on the K^+ gradient across the plasma membrane. Thus, the effect of varying external K^+ concentration was examined. When values for membrane potential were plotted against the log of external K^+ concentration, the slope of the plot

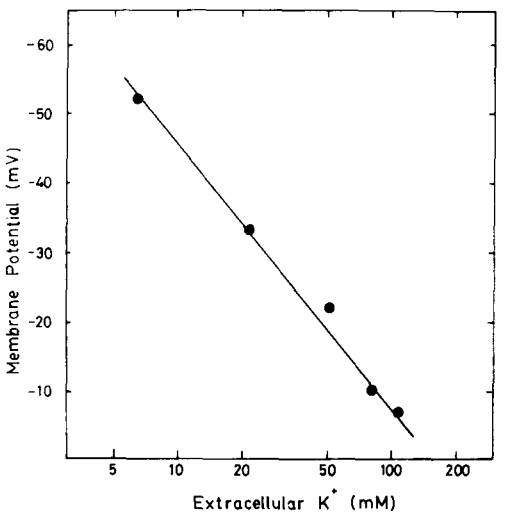

Fig. 2. Effect of extracellular K^+ concentration on TPP^+ accumulation by polymorphonuclear leukocytes. Cells were suspended in phosphate-buffered isotonic solution containing 1.0 mM $CaCl_2$ and various concentrations of choline chloride and KCl ($[choline^+]+[K^+]=141$ mM in all buffers). The membrane potential was calculated from measurements of ΔE at each concentration of KCl and ΔE_h as described in Experimental Procedure. Each point represents the mean of duplicate or triplicate determinations.

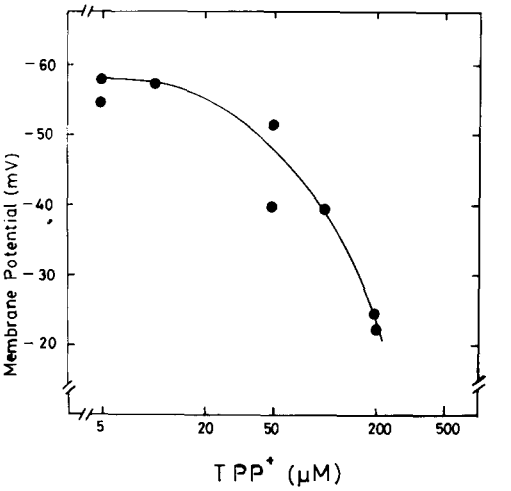

Fig. 3. Effect of TPP^+ concentration on the calculated membrane potential. Experiments were performed as described in Fig. 1, except that TPP^+ concentration was varied. The membrane potential was calculated as described in Experimental Procedure.

indicated a change of 37 mV for a 10-fold increase in K^+ (Fig. 2). In low-K^+ medium (4.2 mM K^+), the estimated membrane potential was -58.3 ± 4.4 mV (mean ± S.E. of nine separate experiments). This value was relatively constant irrespective of cell number tested (data not shown).

Next we have examined how TPP^+ uptake depends on the concentration of TPP^+. Fig. 3 shows the membrane potential calculated at varying concentrations of TPP^+. With increasing concentrations of TPP^+, the estimated membrane potential was reduced. The decrease in the estimated membrane potential found at high TPP^+ concentrations may point to a depolarization, for example, caused by the toxic properties of this compound. This effect, however, is negligibly small if the cation is applied at low concentrations. Since the membrane potential is relatively constant at concentrations lower than 10 μM, its use as a probe of membrane potential within this range appears valid.

Effects of fMet-Leu-Phe and concanavalin A on membrane potential

The effects of fMet-Leu-Phe and concanavalin A on membrane potential of guinea pig polymorphonuclear leukocytes are shown in Fig. 4A and B,

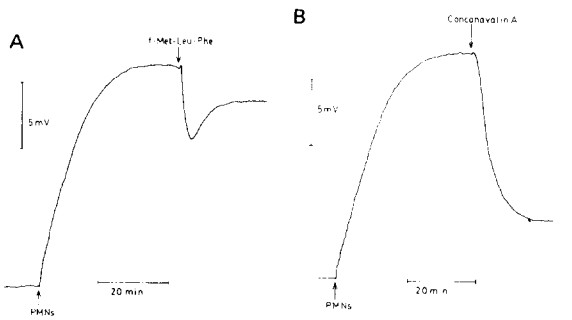

Fig. 4. Effect of fMet-Leu-Phe (A) and concanavalin A (B) on TPP^+ accumulation. Cells ($2 \cdot 10^7$/ml) were suspended in low-K^+ medium at 37°C. At the point indicated by the arrow, fMet-Leu-Phe or concanavalin A was added to a final concentration of $2.5 \cdot 10^{-7}$ M and 75 μg/ml, respectively. The vertical bar represents a change of 5 mV in the TPP^+ electrode. PMNs, polymorphonuclear leukocytes.

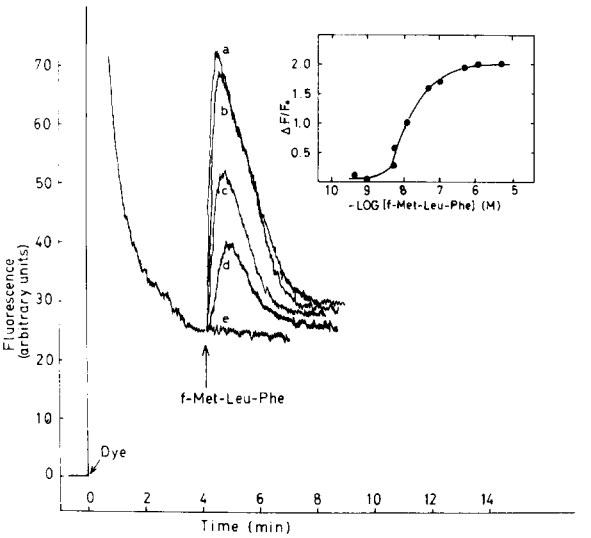

Fig. 5. Time course of the fluorescence changes induced by addition of different concentrations of fMet-Leu-Phe. At the point indicated by the arrow, fMet-Leu-Phe was added: (a) $1 \cdot 10^{-7}$ M, (b) $5 \cdot 10^{-8}$ M, (c) $1 \cdot 10^{-8}$ M, (d) $5 \cdot 10^{-9}$ M, (e) $1 \cdot 10^{-9}$ M. The inset shows a dose-response curve for fMet-Leu-Phe-induced fluorescence changes. The symbols are defined as F, the steady-state fluorescence intensity baseline level before fMet-Leu-Phe addition; ΔF, change of fluorescence level following fMet-Leu-Phe addition.

respectively. After the addition of fMet-Leu-Phe to polymorphonuclear leukocytes, a rapid and transitory change in membrane potential was observed. Addition of fMet-Leu-Phe caused a rapid efflux of TPP^+ which peaked at 2–3 min. This rapid loss of accumulated TPP^+ was followed by its reaccumulation which returned to much the same level of control. In contrast, addition of concanavalin A caused a large efflux of accumulated TPP^+, indicating that a sustained depolarization occurred. This result confirmed previous observations with diS-C_3-(5) by Utsumi et al. [4].

Effects of external Na^+ on the resting potential and the changes in membrane potential during surface stimulation were examined. Substitution of Na^+ with choline$^+$ (i.e., choline $^+$ medium) had little effect on the maximum value of accumulated TPP^+ concentration or the response elicited by fMet-Leu-Phe or concanavalin A (data not shown). Thus, it is unlikely that external Na^+ is responsible for the maintenance of the resting potential or for the stimulus-induced membrane depolarization.

Studies with cyanine dye

As previously reported [4], the fluorescence intensity of diS-C_3-(5) reached a steady state 4–5 min after the addition of the dye to polymorphonuclear leukocytes suspended in low-K^+ medium containing 1.0 mM $MgCl_2$. As shown in Fig. 5, fMet-Leu-Phe caused a rapid, transitory increase in the fluorescence followed by a slower decrease. The maximum intensity in fluorescence occurred within 1 min after the addition of fMet-Leu-Phe. As the concentration of fMet-Leu-Phe was raised, the maximum intensity of fluorescence increased and was saturated at approx. $5 \cdot 10^{-7}$ M. However, the time required for the maximum fluorescence intensity was independent of fMet-Leu-Phe concentration. For a rapid, transitory change in membrane potential such as that in fMet-Leu-Phe-stimulated polymorphonuclear leukocytes, these methods must underestimate the extent of membrane depolarization because the distribution of lipophilic cations used as probes takes a longer time compared to the time course of the true membrane depolarization (possibly it may occur on the millisecond time scale), although diS-C_3-(5) permeates membranes much faster than TPP^+, thus permitting faster detection of membrane potential changes. Nevertheless, the fluorescence

would still accurately change in the direction of membrane depolarization. For this reason the fluorescence change can only be discussed in qualitative terms and the extent of depolarization has not been calculated.

Fig. 6 shows the effect of ouabain on the resting fluorescence level and the changes produced by the addition of fMet-Leu-Phe. Addition of K^+ (5 mM) to polymorphonuclear leukocytes suspended in a K^+-free medium caused a rapid decrease in fluorescence which was reversed by subsequent addition of ouabain (50 μM). This observation is consistent with the idea that the Na^+,K^+ pump contributes to the membrane potential of polymorphonuclear leukocytes, and confirmed previous work obtained with TPP^+ distribution [21]. However, ouabain had no significant effect on the fMet-Leu-Phe-induced fluorescence changes, indicating that the Na^+,K^+ pump is not responsible for the fMet-Leu-Phe-induced membrane potential changes.

The effect of external K^+ on the fMet-Leu-Phe-induced fluorescence changes was examined. Typical traces of the fluorescence response of this cell to fMet-Leu-Phe at different concentrations of external K^+ are shown in Fig. 7A. In Fig. 7B, the initial fluorescence level and the maximum fluorescence intensity produced by the addition of fMet-Leu-Phe ($5 \cdot 10^{-8}$ M) are plotted as a function of external K^+ concentration. We found that membrane depolarization always occurred in the range of K^+ concentration tested, and that the depolarization maximum was independent of external K^+ concentration. Since the resting potential depends on the external K^+ concentration, this indicates that the fMet-Leu-Phe-induced membrane potential change is independent of the resting potential. Moreover, it is surprising that fMet-Leu-Phe further depolarized the membrane even when polymorphonuclear leukocytes were suspended in high-K^+ medium (141 mM K^+) in which the cells would be expected to be depolarized to close to 0 mV. This result indicates that there is not only a depolarization, but also an apparent 'overshoot', and the membrane potential becomes positive inside.

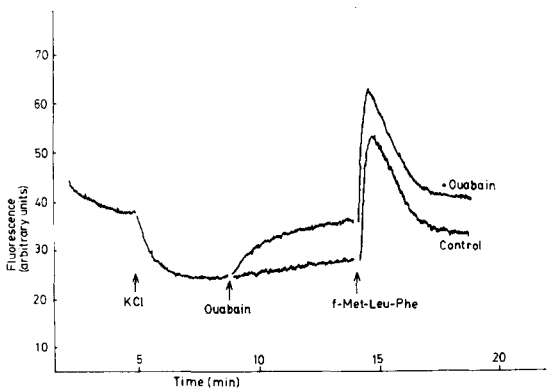

Fig. 6. Effects of ouabain on the resting fluorescence level and the changes induced by the addition of fMet-Leu-Phe. Cells were suspended in K^+-free medium and then KCl (5 mM) was added. At the point indicated, either buffer which is the control or ouabain (50 μM) was added. Finally, fMet-Leu-Phe ($5 \cdot 10^{-8}$ M) was added to both.

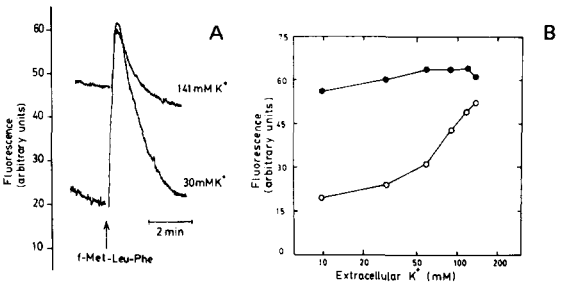

Fig. 7. Effect of external K^+ on the fMet-Leu-Phe-induced fluorescence changes. (A) Cells were suspended in phosphate-buffered saline which contained either 30 mM K^+ (lower) or 141 mM K^+ (upper). At the point indicated by the arrow, fMet-Leu-Phe was added to a final concentration of $5 \cdot 10^{-8}$ M. (B) The initial fluorescence level (○) and the maximum fluorescence intensity (●) induced by the addition of fMet-Leu-Phe ($5 \cdot 10^{-8}$ M) were plotted as a function of external K^+ concentration. The various levels of external K^+ concentration were obtained by replacing NaCl with KCl in phosphate-buffered saline.

Extracellular pH changes following stimulation of fMet-Leu-Phe

Molski et al. [25] have reported rapid and biphasic changes in the intracellular pH of rabbit polymorphonuclear leukocytes following stimulation with fMet-Leu-Phe. As shown in Fig. 8, we also observed that fMet-Leu-Phe caused a transient alkalinization of the external medium. These findings suggest that fMet-Leu-Phe-induced membrane potential change may be effected by the

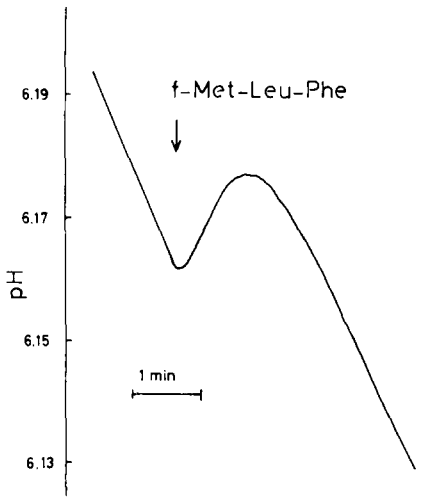

Fig. 8. Extracellular pH changes following stimulation of fMet-Leu-Phe. Cells ($2 \cdot 10^6$/ml) were suspended in nonbuffered salt solution containing 145 mM NaCl, 5 mM KCl and 1 mM $CaCl_2$. At the point indicated by the arrow, fMet-Leu-Phe was added to a final concentration of $5 \cdot 10^{-8}$ M. Extracellular pH changes were monitored with a pH electrode.

movement of H^+ or OH^-. However, both the resting potential and fMet-Leu-Phe-induced membrane potential changes were not influenced by pH variations between pH 6.25 and 7.75 (data not shown). This implies that there is no direct effect of external pH on any component involved in these membrane potential changes. Moreover, this indicates that a proton gradient makes no direct contribution to the resting potential. We cannot deduce as yet whether these changes in membrane potential and in pH occur simultaneously or sequentially.

Membrane potential changes and superoxide generation

Since changes in membrane potential precede the onset of the respiratory burst and of other functional responses, it has been suggested that membrane potential changes are involved in the mechanism triggering the activation of the respiratory burst [7,16]. If depolarization of the cell membrane leads to the activation of the respiratory burst, an increase in the external K^+ concentration should also cause the activation of the respiratory burst because increases in the external K^+ concentration cause membrane depolarization of guinea pig polymorphonuclear leukocytes (see Figs. 2 and 7). Therefore, we examined this possibility by comparing the respiratory activity and the state of membrane potential in polymorphonuclear leukocytes. However, the stimulation of O_2 consumption measured by a Clark-type O_2 electrode and O_2^- generation was not induced by membrane depolarization with increasing external K^+ concentration (data not shown). Thus, membrane depolarization per se is not a sufficient stimulus for the activation of the respiratory burst. In another series of experiments, we studied the effects of varying the K^+ concentration in the medium on O_2^- generation induced by fMet-Leu-Phe. As shown in Fig. 9, increasing external K^+ concentration had no effect on O_2^- generation induced by fMet-Leu-Phe. This result indicates that fMet-Leu-Phe-induced O_2^- generation is not related to the resting potential. Moreover, the data of Fig. 9 show also that external Na^+ is not responsible for the fMet-Leu-Phe-induced O_2^- generation because increasing K^+ concentration in the medium is correlated

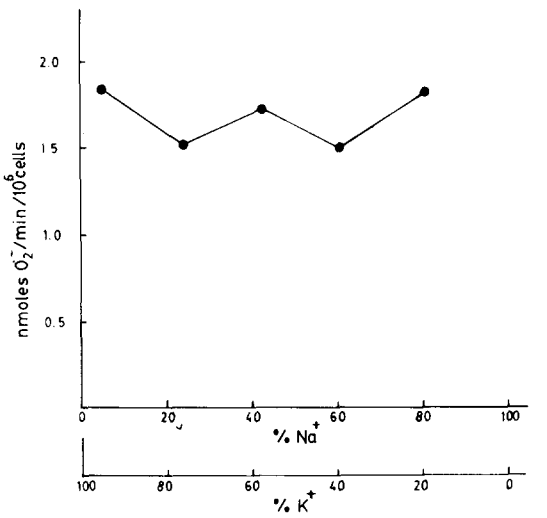

Fig. 9. Effect of external K^+ concentration on the fMet-Leu-Phe-stimulated O_2^- generation. Cells were incubated for 5 min at 37°C in phosphate-buffered saline containing 1.0 mM $CaCl_2$ and then fMet-Leu-Phe was added to a final concentration of $2.5 \cdot 10^{-7}$ M. O_2^- generation was measured as described in Experimental Procedure. The various levels of external K^+ concentration were obtained by replacing NaCl with KCl in phosphate-buffered saline.

with the concomitant decrease in the amount of Na^+.

Discussion

The results obtained with the TPP^+ distribution method indicate that guinea pig polymorphonuclear leukocytes, in physiological saline, have a resting potential of -58.3 ± 4.4 mV and that the resting membrane permeability is predominantly K^+ selective. As shown previously [21] and in Fig. 6, the Na^+,K^+ pump contributes to the membrane potential in guinea pig polymorphonuclear leukocytes. From these results, we can infer that the overall potential consists of a K^+ diffusion potential and an electrogenic potential. The value of the membrane potential estimated in this study is comparable with those reported in other cell types: -52 ± 11 mV [26] and -65 ± 2 mV [27] for lymphocytes, -52 mV [28] and -48 ± 13 mV [29] for human platelets, -66 ± 5 mV [23] for neuroblastoma-glioma hybrid cells and -58 ± 5 mV [30] for rat white adipocytes. However, previous studies [16,19] with the $TPMP^+$ distribution method for human polymorphonuclear leukocytes give considerable lower values than that which we have estimated here. A possible explanation for these results is that the membrane potential can be affected by the lipophilic cations themselves. The results shown in Fig. 3 demonstrate that this is the case. In fact, the value at 50 μM TPP^+ is very close to that reported by Seligmann and Gallin [19]. They obtained a value of -45 ± 2 mV using 50 μM $TPMP^+$ plus 10 μM tetraphenylboron to hasten equilibration. Hence, it appears that TPP^+, if applied at sufficiently low concentrations, can be used as a quantitative probe for the membrane potential in polymorphonuclear leukocytes.

Carbocyanine dyes have been used extensively to monitor membrane potential changes in a wide variety of cells because of the technical simplicity and rapid detection of changes in membrane potential [31]. Furthermore, the advantage of using a carbocyanine dye is that it is possible to follow the time course of its fluorescence changes. However, a critical problem in the use of cyanine dyes is the calibration of the optical signals in terms of 'known' values of the membrane potential. Most workers have used the ingenious calibration method of Hoffman and Laris [32]. In red blood cells or other cells in which the K^+ permeability is low, the ionophore valinomycin may be used to drive the cells close to a K^+ diffusion potential. Varying external K^+ and measuring the changes in fluorescence signal on the addition of valinomycin will give a set of data from which, if the intracellular K^+ concentration is known, the resting potential can be calculated. Therefore, the accuracy of the calibration depended on the use of valinomycin to find the 'valinomycin null-point' and on the value taken for the intracellular K^+ concentration. A small effect of valinomycin would be observed in cells that are already K^+ permeable. In such a case it is difficult to find the null-point accurately. Moreover, a number of studies have shown that the dyes may act as metabolic inhibitors [33] or alter certain plasma membrane permeabilities [34,35]. Thus, we have not employed diS-C_3-(5) to determine the resting potential.

In this study we compared the two probes with respect to changes in membrane potential of polymorphonuclear leukocytes elicited by fMet-Leu-Phe and concanavalin A. Our results suggest that both probes give comparable results and appear to reflect reliably changes in membrane potential. We found that fMet-Leu-Phe causes a rapid depolarization, while concanavalin A induces a rapid and sustained depolarization. At present, we do not have sufficient information to explain in detail the mechanisms that give rise to changes in membrane potential produced by fMet-Leu-Phe and concanavalin A. Jones et al. [12] demonstrated that there is a large decrease in the magnitude of the fMet-Leu-Phe-induced depolarization when external Na^+ is decreased from 155 to 10 mM and the subsequent repolarization is partially inhibited by ouabain. These results were attributed to an Na^+-dependent depolarization, followed by activation of the electrogenic Na^+,K^+ pump by increased cellular Na^+. In contrast to their observations, we found that substitution of Na^+ with $choline^+$ has no significant effect on the fMet-Leu-Phe-induced depolarization. Reasons for the discrepancy between our results and those of Jones et al. [12] are not apparent, other than differences in the cell types used (i.e., guinea pig peritoneal polymorphonuclear leukocytes in the present study and human

peripheral polymorphonuclear leukocytes in the study of Jones et al.).

Naccache et al. [36,37] have used radioactive tracer techniques to measure ion fluxes in rabbit polymorphonuclear leukocytes. Their results show that fMet-Leu-Phe increases the influx of Na^+ and Ca^{2+}. They suggested that the transient increase in membrane permeability to Na^+ and Ca^{2+} may cause the plasma membrane to depolarize. If there is an enhanced Na^+ influx, this may arise as a secondary consequence of membrane depolarization. Furthermore, we observed that substitution of Na^+ by K^+ or choline$^+$ had no significant effect on the fMet-Leu-Phe-induced O_2^- generation and O_2 consumption that are contradictory to previous reports [17,22]. Thus, extracellular Na^+ is not required for the respiratory burst as well as membrane depolarization. These observations indicate that the role of Na^+ in leukocyte activation will have to be reconsidered. Further studies would be needed to resolve the present discrepancy.

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