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# The role of cationic transport in the respiratory burst activation in granulocytes

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The neutrophil responds to a single stimulus at its surface by undergoing a series of events such as aggregation, superoxide anion generation and degranulation [1, 6, 7]. It is widely accepted that the cell surface and the plasma membrane play a key role in these responses. A number of molecular and functional changes in the surface membrane are associated with phagocytosis, stimulated movement, secretion and respiration. These include alteration in $Na^+$ and $K^+$ fluxes, changes of transmembrane potential, modifications of $Ca^{++}$ fluxes, mobilization of $Ca^{++}$ from intramembranous stores, changes in phospholipid turnover, protein phosphorilation and arachidonic acid cascade [1, 6, 7, 9, 11, 12, 14, 15].

Previous studies on membrane transport mechanism showed that the chemotactic factor N-Formyl-Methionyl-Leucyl-Phenylalanine (FMLP) induces a rapid and dramatic increase in the rate of $Na^+$ influx across the neutrophil plasma membrane [6]. This selectively increased permeability to $Na^+$ leads to a depolarization of the membrane which is followed by an enhancement of $K^+$ influx and $Na^+$ efflux. These ionic changes are drived by the activation of the $Na^+-K^+$ pump [1, 3–7, 9, 11, 15]. Beside the effect on the $Na^+-K^+$ pump FMLP influences $Ca^{++}$ movement across neutrophil membrane, enhances membrane permeability to $Ca^{++}$ and some time later increases the intracellular exchangeable calcium pool [8].

The consequent cationic membrane transport precedes the onset of $O_2^-$ generation by several seconds [4, 9, 11, 13, 15]. However, despite many investigations it is not clear whether a direct correlation exists between cationic membrane transport and superoxide generation.

In this study we present data on the effect of $Na^+-K^+$ pump inhibition by Ouabain and changes of external cationic concentrations on the respiratory burst activation of human blood neutrophils.

## Materials and Methods

Cell suspensions containig 98% neutrophils were prepared from heparinized venous blood of healthy donors using standard separation techniques [2]. The cells were suspended in Krebs-Ringer Phosphate-dextrose (KRPD) buffer (pH = = 7.28). After seperation more than 95%

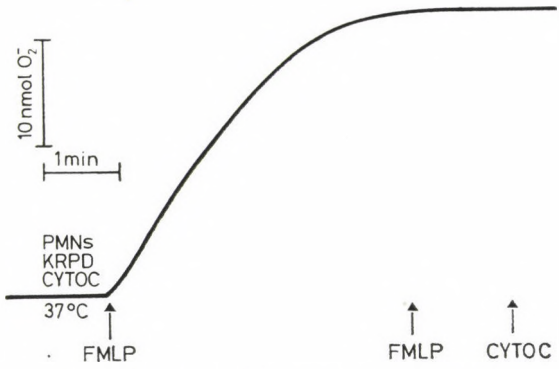


FIG. 1. PMNs: Polymorfonuclear granulocytes, $2\times10^6$ cells; FMLP: N-Formyl-Methionyl-Leucyl-Phenylalanine, $10^{-7}$ M; CYTO C: Ferricytochrome c, $8\times10^{-5}$ M; KRPD: Krebs-Ringer Phosphate-dextrose buffer, pH = 7.28

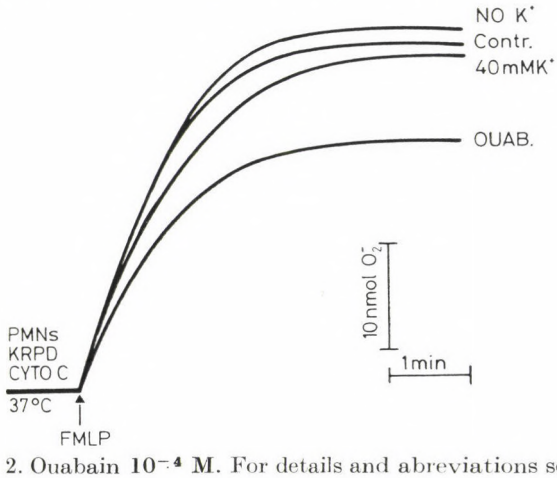


FIG. 2. Ouabain $10^{-4}$ M. For details and abreviations see 1. fig.

of the cells remained viable as checked by Tripan blue exclusion. Production of $O_2^-$ was assayed by recording ferricytochrome c (0.08 mM) reduction monitored continously at 550 nm in Specord model 40 spectrophotometer. All experiment were carried out at 37 °C with cell count of 2 million.

## Results and Discussion

In Fig. 1. a representative experiment of the time course of $O_2^-$ generation of neutrophils stimulated with FMLP is shown. After 10 min equilibration $10^{-7}$ M FMLP was added to the cell suspension which resulted in a rapid reduction of the cytochrome c reaching a plato after 5 min. The second stimulus caused no more increase of the concentration of ferrocytochrome c suggesting that the reaction proceeds according to all-or-nothing principle. No change was observed by adding more ferricytochrome c which indicated that the substrate was not consumed completely in the reaction mixture.

There is evidence for the existence of Ouabain sensitive $Na^+$-$K^+$ pump

in the granulocyte membrane [4, 9, 11]. In our previous studies a complete inhibition of $Na^+$-$K^+$-ATPase activity could be reached with Ouabain using $10^{-4}$ M concentration.

Fig. 2. shows that when experiments were performed with granulocytes preincubated with $10^{-4}$ M Ouabain for 10 min a decrease in the $O_2^-$ generation could be observed. This indicated the possible role of the $Na^+$-$K^+$ pump in the respiratory burst activation. However, when all of the $K^+$ was removed from the suspension no significant change was observed in generation of the $O_2^-$ compared to the control. In absence of the external $K^+$ not only the pump activity is inhibited but membrane hyperpolarization also develops [4].

The effect of membrane depolarization induced by high external $K^+$ concentration on $O_2^-$ production has extensively been studied but results are contradicting [1, 3–5, 9, 11, 15]. The membrane depolarization induced by 40 mM $K^+$ in our system did not result in a respiratory burst activation or increased $O_2^-$ generation to FMLP, indicating that activation of the respiratory burst in granulocytes does not relate directly to changes of membrane potential.

Among cations $Ca^{++}$ plays a key role in most of the leukocyte functions. To investigate whether this regulatory function relates to extracellular or membrane bound $Ca^{++}$ pool all the $Ca^{++}$ was removed from the suspending medium. We found that, in the absence of extracellular $Ca^{++}$ the $O_2^-$ generation did not differ significantly from the control (Fig. 3).

Other workers have demonstrated that the absence of or decrease in extracellular $Na^+$ cause a decrease in effector leukocyte functions [1, 7, 9]. The replacement of extracellular NaCl with choline chloride induced no alteration in $O_2^-$ generation of neutrophil stimulated with FMLP but substitution with equimolar saccharose the $O_2^-$ production was markedly depressed.

Our data suggest that changes in the concentration of extracellular cations have no direct influence on $O_2^-$ generation of leukocytes stimulated

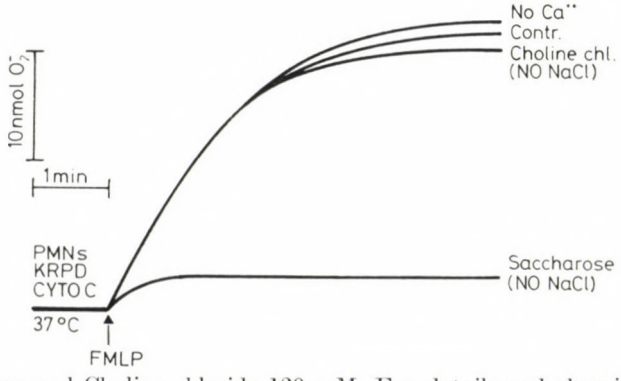


FIG. 3. Saccharose and Choline chloride 120 mM. For details and abreviations see 1. fig.

TABLE I

Effect of Ouabain and various extracellular ionic composition on $O_2^-$ generation by FMLP stimulated PMNs

| Experimental conditions | $O_2^-$ generation (%) Mean ± S.E. | n |
|---|---|---|
| Control | 100 | |
| $10^{-4}$ M Ouabain | 72.9±5 | 23 |
| NO $K^+$ | 101±6.8 | 15 |
| 40 mM $K^+$ | 97.1±3.6 | 9 |
| NO $Ca^{++}$ | 107±16 | 5 |
| Saccharose (NaCl substitution) | 17±0.5 | 8 |
| Choline chloride (NaCl Subst.) | 96.6±18 | 7 |

with FMLP (Table I.) [1, 6, 7]. The moderate inhibition caused by Ouabain may have been due to a non-specific membrane effect. However, replacement of extracellular NaCl with saccharose decreased significantly the $O_2^-$ generation in granulocytes suggesting the role of extra and/or intracellular $Cl^-$ in the metabolic activity of phagocytic cells.

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