

Membrane Potential Changes in Polymorphonuclear Leukocytes of Patients with Chronic Granulomatous Disease*

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ABSTRACT

The activation of NADPH oxidase on the plasma membranes of polymorphonuclear leukocytes (PMNL) follows the change of membrane potential. It is thought that the activation of NADPH oxidase is disturbed in the PMNL of patients with chronic granulomatous disease (CGD). The change of membrane potential was examined in the PMNL of normal subjects and patients with CGD, with a lipophilic probe, di-O-C₅(3). There was a disturbance in phorbol myristate acetate (PMA) and formyl-methionyl-leucyl-phenylalanine (FMLP)-induced depolarization but not in Ca ionophore A23187-induced depolarization of the PMNL of patients with CGD. A23187-induced depolarization was not always associated with O₂⁻ release in normal PMNL. A23187 differed from PMA and FMLP in several aspects of its action on depolarization. It is concluded that only depolarization which couples with O₂⁻ release is disturbed in the PMNL of patients with CGD. Increase in extracellular potassium (K) caused PMNL of both normal subjects and CGD patients to depolarize but made them not to release O₂⁻. Otherwise, A23187-induced depolarization was disturbed in PMNL of CGD patients with higher extracellular K concentrations. These findings help to confirm the theory that K efflux on the plasma membrane is disturbed in the PMNL of patients with CGD.

INTRODUCTION

Chronic granulomatous disease (CGD) is the most common disorder due to phagocytic dysfunction²⁾. It is thought that oxidative killing is defective because of a reduction in the production of active oxygens. In the PMNL of patients with CGD, either activation or activity of NADPH oxidase on the plasma membrane is so disturbed that O₂⁻ release is markedly reduced¹⁾. The activation of NADPH oxidase follows the mobilization of Ca ion⁷⁾, the change of membrane potential⁹⁾ and several other events. The precise mechanisms of activation of NADPH oxidase and its disturbance in the PMNL of patients with CGD are unknown despite vigorous research³⁾. In this study, the change of membrane potential with the use of

a lipophilic fluorescent probe, di-O-C₅(3), was compared in normal subjects and patients with CGD.

MATERIALS AND METHODS

Phorbol myristate acetate (PMA), cytochalasin (cyt.) A, D, concanavalin A (con. A), ferricytochrome C type III and superoxide dismutase were purchased from Sigma Chemical Co., N-formyl-methionyl-leucyl-phenylalanine (FMLP) from Protein Research Foundation, N-ethyl maleimide (NEM), NiCl₂, MnCl₂ and ethylene diamine tetraacetic acid (EDTA) from Nakarai Chemical Co., Di-O-C₅(3) from Nippon Kan-koh Shikiso Kenkyusho, and A23187 from Calbiochem Behring Co.

PMA, cyt. A, D and A23187 were dissolved in dimethyl sulfoxide and di-O-C₅(3) in ethanol

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as stock solutions.

PMNL were isolated from the venous blood of three patients with CGD and from normal volunteers by the method described previously⁵. Informed written consent was obtained from these subjects. PMNL were kept suspended in Dulbecco's phosphate buffered saline (PBS) at 4°C until use.

O₂⁻ release from PMNL was measured by the reduction of ferricytochrome C continuously, with a dual wave length spectrophotometer (Hitachi 557)⁵. The reaction mixture contained 5 × 10⁵ PMNL, 0.1 mM cytochrome C and 1 ml Dulbecco's PBS with or without 10 μg superoxide dismutase.

Fluorometric assay of membrane potential was done with di-O-C₈(3). A standard assay solution contained 1 ml Dulbecco's PBS, 0.25 μM di-O-C₈(3) and 2 × 10⁵ PMNL at 37°C. The fluorescence was recorded with a fluorometer (Hitachi MPF-4) with the excitation wave length set at 460 nm and the fluorescence wave length at 510 nm. The results were recorded as relative intensities of fluorescence.

RESULTS

The change of membrane potential of PMNL after stimulation by PMA was compared in normal subjects and patients with CGD (Fig. 1). In fig. 1 the upward shift indicates hyperpolarization

(downward shift), but PMNL from patients with CGD did not. Of the three patients with CGD, only patient C.I. was a female and was shown by a study of the family to have autosomal recessive transmission⁶. The disturbance of depolarization in this patient was similar to that in the other two patients. PMNL from the mother of patient C.F., a carrier of an X-linked recessive trait, showed about the half depolarization of the controls. The same phenomena were seen when FMLP was employed as the stimulating agent. A23187, known to induce O₂⁻ release from the PMNL of normal subjects¹⁰, depolarized PMNL from patients with CGD almost the same degree. Since depolarization induced by A23187 was quite different from that induced by FMLP or PMA, the effect of A23187 was studied.

The association of O₂⁻ release with depolarization was investigated. The concentration of A23187 which induced O₂⁻ release was more than 1 × 10⁻⁶M and higher than that which induced depolarization (more than 2 × 10⁻⁸M) (Fig. 2). This fact suggests that O₂⁻ release may not be associated with depolarization induced by A23187.

It is thought that the action of A23187 depends on the extracellular Ca ion. To make a Ca-free solution, Ca was removed from Dul-

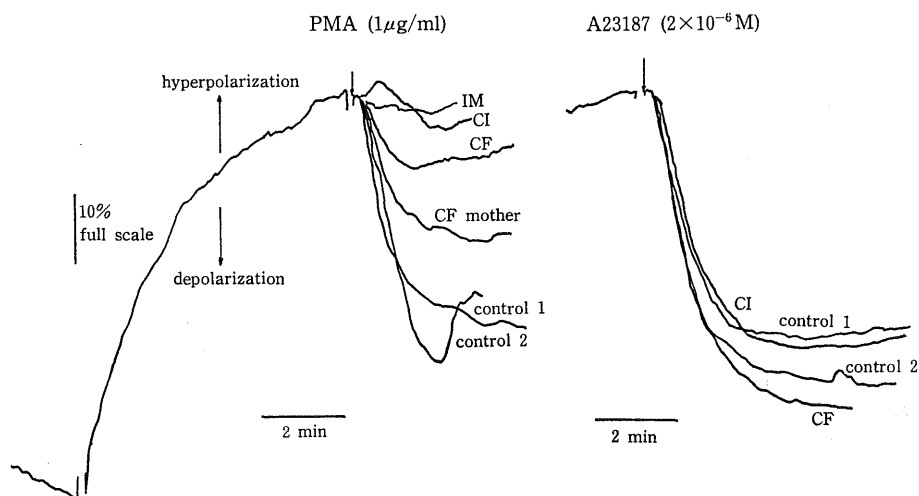


Fig. 1. Changes of membrane potential of PMNL of patients with CGD stimulated by PMA or A23187. PMNL (2×10^5) were added to 1 ml of Dulbecco's PBS containing 0.25 μM di-O-C₈(3). After the fluorescence reached a plateau, stimulating agents such as PMA and A23187 were added. Patients IM and CF had X-linked recessive transmission and CI had autosomal recessive transmission.

becco's PBS and 1 mM EDTA was added. In this Ca-free solution, the resting potential shifted to depolarization (Fig. 3). Thus, this reaction system using di-O-C₅(3) depends on extracellular Ca. In the absence of Ca, A23187 did not induce depolarization of PMNL membranes,

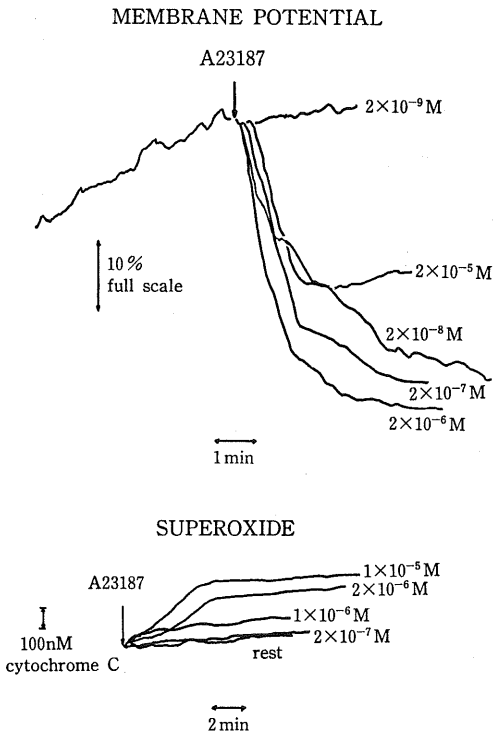


Fig. 2. Relation between membrane potential and O₂⁻ release of PMNL after stimulation by A23187. Change of membrane potential was induced by more than 2 × 10⁻⁸ M of A23187, while O₂⁻ release was induced by more than 2 × 10⁻⁶ M.

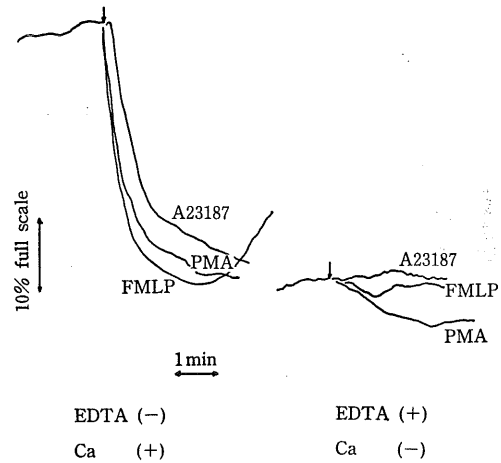


Fig. 3. Effect of Ca on membrane potential of PMNL. The Ca-free condition was obtained by the removal of CaCl₂ from Dulbecco's PBS and the addition of 1 mM of EDTA.

but PMA and FMLP did, slightly. Accordingly, the action of A23187 also depends on extracellular Ca.

The action of A23187 was also examined from another point of view. Pretreatment of PMNL with NEM (0.1 mM) and cyt. A (0.02 mM), which are known to be sulfhydryl (SH) reagents, inhibited FMLP- or PMA-induced, but not A23187-induced depolarization (Fig. 4). Pretreatment of PMNL with 1 mM Ni and Mn, which pass through the Ca channel, inhibited A23187-induced depolarization, but did not inhibit PMA or FMLP-induced depolarization. X537A is known to be a Ca ionophore and induces O₂⁻ release from PMNL⁹. Neither Ni

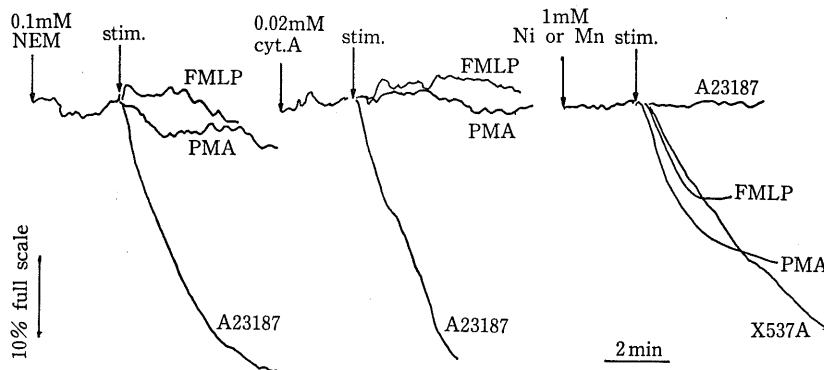


Fig. 4. Effect of SH reagents (NEM, cyt. A), and Mn on membrane potential. After peak fluorescence was attained, SH reagents, Ni and Mn were added. Two min later, PMNL were stimulated by agents.

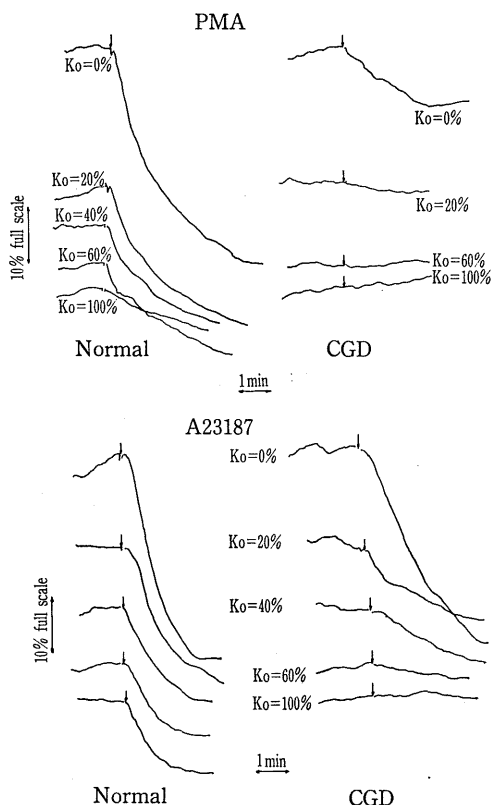


Fig. 5. Changes of membrane potential with increasing concentrations of extracellular K when the percentage of K in the total monovalent cation pool in Dulbecco's PBS was increased from 0% to 100%. The resting potential and depolarization after the addition of stimulating agents were recorded in the PMNL of normal subjects and patients with CGD.

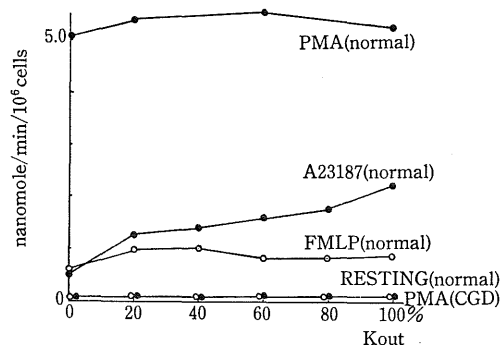


Fig. 6. Changes of O_2^- release with increasing concentrations of extracellular K. The concentration of K in Dulbecco's PBS was changed as fig. 5, and O_2^- release was measured in the PMNL of normal subjects and patients with CGD.

nor Mn inhibited X537A-induced depolarization. In the several points described above, A23187 differed from PMA and FMLP. In an attempt to elucidate the disturbance of depolarization in the PMNL of patients with CGD, the changes of resting potential and depolarization were studied when extracellular K levels ranges from 0% to 100% of the total monovalent cations (Fig. 5). In this system, Na ion was exchanged for K in various proportions. Increased extracellular K depolarized the resting potential of the PMNL of both normal subjects and patients with CGD. PMA-induced depolarization of normal PMNL at all K concentrations, but not PMNL from patients with CGD. Similar results were obtained when FMLP was used. On the other hand, an increase in extracellular K inhibited A23187-induced depolarization in PMNL from patients with CGD, but no such inhibition occurred in normal PMNL. Higher concentrations of extracellular K made conspicuous the difference between CGD and normal PMNL in A23187-induced depolarization.

In a study to determine whether or not depolarization induces O_2^- release, O_2^- release from PMNL was measured when extracellular K was increased (Fig. 6). PMA and FMLP-induced O_2^- release at every concentration of K. However, the higher the concentration of extracellular K, the more O_2^- was released when A23187 was used. O_2^- release was not detected after "passive depolarization" when extracellular K was increased and the resting potential shifted to depolarization without any stimulating agents. In PMNL from patients with CGD, O_2^- release was not seen at any concentration of K and with any stimulating agents.

DISCUSSION

The finding that FMLP and PMA-induced depolarization was disturbed in the PMNL of patients with CGD is consistent with the early report of Seligmann et al.⁹⁾ They showed that A23187-induced depolarization was slightly reduced in the PMNL of patients with CGD, but our results did not show much difference. A23187-induced depolarization in the PMNL of patients with CGD was almost the same as that in normal PMNL. An increase in extracellular K inhibited A23187-induced depolarization in the PMNL of patients with CGD. This

shows that K efflux may be disturbed at higher concentrations of K, in accordance with Lehler's assumption⁹.

The action of A23187 was different from that of PMA and FMLP in several aspects. 1. It depended largely on extracellular Ca, and its action was blocked by Ni and Mn. 2. SH reagents did not inhibit A23187-induced depolarization. 3. Lower concentrations which did not induce O₂⁻ release, induced depolarization. 4. It induced depolarization in the PMNL of patients with CGD without O₂⁻ release. These facts indicate that A23187-induced depolarization is not always coupled with O₂⁻ release.

The PMNL of patients with CGD depolarized normally in response to the increase of extracellular K. However, this passive depolarization did not induce O₂⁻ release in the PMNL of normal subjects or patients with CGD. It was supposed that only depolarization which couples with O₂⁻ release is disturbed in the PMNL of patients with CGD. The relation between depolarization and the activation of NADPH oxidase remains unknown and must be clarified by further studies.

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