STIMULATION OF THE OXIDATIVE METABOLISM OF POLYMORPHONUCLEAR LEUCOCYTES BY THE CALCIUM IONOPHORE A23187

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1. Introduction

Phagocytosis by polymorphonuclear leucocytes is accompanied by a wide variety of metabolic alterations, among them an increase in oxygen consumption, in the oxidation of glucose via the hexose monophosphate shunt and in the activity of the H_2O_2–halide–myeloperoxidase antibacterial system. The increase in oxygen uptake and shunt activity can be detected within a few seconds after the addition of phagocytosable particles [1]. In addition, several agents thought to interact with the PMN cell surface membrane, e.q. concanavalin A [2], phorbol myristate acetate [3], phospholipase C [4], endotoxin and detergents [5] provoke these oxidative changes. Thus we and others have proposed that contact between a phagocytic particle and the surface of the PMN may generate a chemical signal or signals which in turn mediate the characteristic metabolic accompaniments of phagocytosis [1,6]. We have previously investigated the role of cAMP as a chemical signal. Cyclic AMP does not play such a role in phagocytosis in human PMNL [6].

In the present communication we report initial studies on the role of Ca^{2+} in phagocytosis using the divalent cation-specific ionophore A23187. This antibiotic appears to act as a freely mobile carrier to equilibrate divalent cation concentrations across membranes via an electroneutral process [7,8]. We have shown that guinea pig PMNL undergo the increased oxidative metabolism characteristic of phagocytosis when treated with A23187 within a specific concentration range of calcium.

Abbreviations:
cAMP, cyclic AMP: adenosine 3′,5′-cyclic monophosphate
cGMP, cyclic GMP: guanosine 3′,5′-cyclic monophosphate
PMNL: polymorphonuclear leucocytes
HMP shunt: hexose monosphosphate shunt.

2. Methods

Leucocytes were isolated from the peritoneal fluid of guinea pigs (400–600 g) which had received 20 ml of 12% sodium caseinate solution intraperitoneally 16 hr previously. The cells were washed twice in the incubation medium, Krebs-Ringer bicarbonate or phosphate buffer without calcium and an aliquot counted in a Coulter counter. Differential counts revealed greater than 90% polymorphonuclear cells in each preparation.

Oxygen uptake was measured at 37°C with a Clark oxygen electrode. Oxidation of glucose, labeled with ^{14}C in the 1 position, to $^{14}CO_2$ was estimated as previously described [9]. The conversion of iodide to a TCA precipitable form was determined according to Pincus and Klebanoff [10]. For the glucose oxidation and iodination experiments the leucocytes were preincubated in the presence or absence of A23187 at the calcium concentrations indicated for 30 min prior to addition of appropriate tracers, and phagocytic particles where indicated, for an additional 30 min incubation. A23187 was dissolved in ethanol, an equal volume of ethanol being added to the control Flasks. Mg^{2+} concentrations in all experiments was 1.25 mM. Zymosan was preopsonized by incubation in 20% pooled guinea pig serum for 30 min at 37°C.

The suspension was centrifuged and the zymosan resuspended in fresh medium without external proteins.

A23187 was a generous gift of Dr R. L. Hamill (Eli Lilly Co., Indianapolis, USA).

3. Results

At a calcium concentration of 10^{-3} M, the addition of the divalent cation ionophore A23187, 10^{-6} to 10^{-5} M, significantly increased the oxygen consumption of guinea pig PMNL (fig. 1). This effect was evident within 60 sec and was dependent on ionophore concentration. The effect was not decreased in the presence of, or after addition of KCN 5.10^{-3} M. Such ionophore-treated cells showed an additional increase in O_2 uptake within 80 sec of the addition of latex particles.

Oxidation of glucose via the HMP shunt, as measured by the conversion of glucose–C1-^{14}C to $^{14}CO_2$, was increased 3–5-fold in the presence of A23187 10^{-6} M for extracellular calcium concentrations of 10^{-4} to 10^{-3} M (fig. 2a). The ionophore had no effect in the absence of Ca^{2+} or at Ca^{2+} concentrations less than 10^{-4} M. The ionophore effect on resting leucocytes was less than that which could be obtained after addition of phagocytic particles.

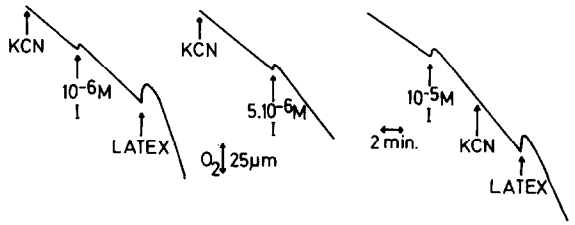

Fig. 1. Stimulation of the rate of oxygen consumption of guinea pig polymorphonuclear leucocytes by the divalent cation ionophore A23187. 2×10^{-7} PMN were suspended in 2 ml Krebs–Ringer-phosphate buffer containing $8 \cdot 10^{-3}$ M glucose and 10^{-3} M calcium. Oxygen uptake was continuously recorded with a Clark electrode at 37°C. Dissolved oxygen was taken to be 0.4 µatom O_2 per ml. KCN = 5.10^{-3} M. I = ionophore.

Similar results were obtained for the activation of the H_2O_2–halide–myeloperoxidase systems as detected by conversion of iodide to a TCA precipitable form (fig. 2b). Basal iodination was very low as would be expected in the absence of protein in the medium.

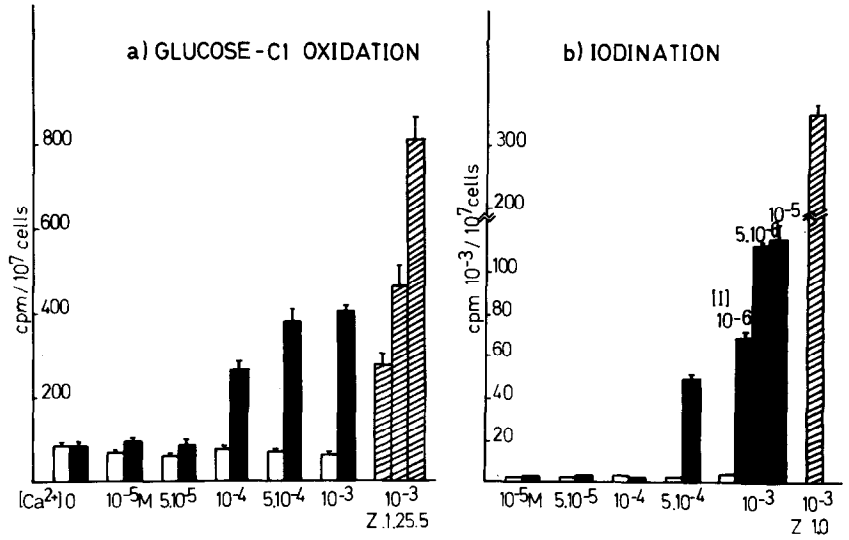

Fig. 2. Effect of the divalent cation ionophore A23187 on glucose–Cl-^{14}C oxidation to $^{14}CO_2$ (a) and iodination (b) at varying calcium concentrations. (a) PMNL $0.8 \cdot 10^7$ per ml were incubated in Krebs–Ringer bicarbonate buffer containing glucose 16 mM and calcium as indicated in a total volume of 2.0 ml. [^{14}C] glucose = 0.5 µCi per ml. (b) PMNL $1.4 \cdot 10^7$ per ml were incubated in Krebs–Ringer bicarbonate buffer containing glucose 8 mM, potassium iodide 2.10^{-5} M and varying calcium concentrations in a total volume of 2 ml. ^{131}I = 125 µCi per ml. Results are expressed as the average and range of duplicate determinations in a representative of 3 experiments. (▭) resting cells, (▨) zymosan, (■) A23187 10^{-6} M.

In the experiment illustrated, the threshold of ionophore response was at 5.10^{-4} M calcium. The stimulatory effect was again dependent on ionophore concentration.

4. Discussion

The role of calcium in the phagocytic process is ill-defined. Both Ca^{2+} and Mg^{2+} are needed for maximal opsonic activity of serum [11]. The effect of divalent cations on ingestion of phagocytic particles depends on the nature of the particle and varies with leucocytes from different species [11–13]. In guinea pig granulocytes opsonized particles dialysed against 1 mM EDTA and phosphate buffered normal saline were ingested in the absence of Ca^{2+} or Mg^{2+}. However, in medium without divalent cations but containing 1 mM EDTA no ingestion took place [11], suggestion that these ions do play a necessary role in phagocytosis.

The very rapid appearance of the metabolic stimulations accompanying phagocytosis and the stimulatory effect of membrane-perturbing agents suggest that these metabolic changes are not dependent on particle uptake but result from particle-cell contact by way of chemical signals. To prove that calcium is such a signal one must be able to show: 1) that induction of calcium movement, in the absence of phagocytic particles, can mimic the metabolic changes, 2) that phagocytosis is associated with changed calcium flux. In this communication we have shown that, within a specific calcium concentration range, the production of calcium flux by the divalent cation ionophore A23187 reproduces the stimulated oxidative activities characteristic of phagocytosis. In animal cells the concentration of calcium ion in the cytosol has been estimated to be from 10^{-8} to 10^{-5} M [14]. Thus at total extracellular calcium concentrations of 10^{-4} M or higher the expected effect of ionophore would be to increase calcium entry into the cell. The ionophore stimulated oxygen consumption rapidly, within 1 min, suggesting that the effects on oxidative metabolism resulted from increased calcium transport across the cell surface membrane.

The observed effects were specific for calcium. A23187 binds Mg^{2+} about as well as Ca^{2+} [15]. However, in our experiments Mg^{2+} concentration was held constant at 1.25 mM and ionophore stimulation occurred only at Ca^{2+} concentrations of 10^{-4} M or higher. This suggests that the stimulatory effects result from Ca^{2+} entry into the cell rather than from a release of Ca^{2+} sequestered intracellularly. It is surprising that such a high external calcium concentration was necessary to observe the ionophore effects. The reason for this is unclear. One possibility, currently being investigated, is that Mg^{2+} and Ca^{2+} compete for transport by the ionophore. This might also explain why ionophore stimulation was quantitatively inferior to that which could be produced by phagocytic particles.

The mechanism of the stimulatory effect of calcium movement is unknown. Calcium may be playing a direct role or acting indirectly via another signal such as cyclic GMP. In several tissues calcium ions appear to be necessary for the action of most if not all agents known to increase cGMP levels [16]. Recently increased cGMP concentrations have been demonstrated during PMNL phagocytosis [17]. Phorbol myristate acetate increases cGMP in several cell types and has also been shown to stimulate the oxidative changes of phagocytosis in human leucocytes [3].

The experiments reported in this paper satisfy the first criterion nevessary to prove the role of Ca^{2+} as one chemical mediator of the phagocytic process. Experiments are now in progress to fulfill the second criterion, the demonstration of increased calcium flux during phagocytosis and to elucidate the mechanism of the Ca^{2+} effect.

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