Cytosol Mg^{2+} modulates Ca^{2+} ionophore induced secretion from rabbit neutrophils

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The influence of extracellular Mg^{2+} on Ca^{2+} ionophore (A23187 and ionomycin) induced secretion and changes in the cytosol pH of rabbit neutrophils suspended in Ca^{2+}-free buffer has been investigated. While extracellular Ca^{2+} is obligatorv for ionomycin induced secretion, we have defined conditions under which A23187 can induce secretion in Ca^{2+}-free media. The different behaviour of these two Ca^{2+} ionophores is discussed on the basis of their different counter cation specificities.

Neutrophil *Ca^{2+} ionophore* *Cytosol pH* *Cytosol Mg^{2+}* *Cytosol Ca^{2+}* *Secretion*

1. INTRODUCTION

The finding that secretion of lysosomal enzymes from cytochalasin B treated neutrophils can be stimulated by the Ca^{2+} ionophores A23187 and ionomycin [1,2] is strong evidence in support of the idea that the cell surface receptors (e.g. for formylmethionyl peptides and anaphylatoxins) act by mobilising Ca^{2+}. The receptor directed ligands such as fMetLeuPhe can cause elevation of cytosol Ca^{2+} and consequent secretion even in the absence of extracellular Ca^{2+}, mobilising Ca^{2+} in these conditions from intracellular sources [3]. An anomalous situation thus exists for the Ca^{2+} ionophores, since measurements using the cytosol Ca^{2+} probe quin2 indicate that they too are able to elevate cytosol Ca^{2+} in the absence of extracellular Ca^{2+}, yet under these conditions they fail to stimulate secretion [4].

We define here conditions under which A23187 (but not ionomycin) is able to stimulate secretion in the absence of extracellular Ca^{2+}. The results are discussed in the light of the differing counter cation specificities of the two ionophores. It is concluded that the rise in cytosol Ca^{2+} due to the ionophores is not sufficient for stimulation unless the cells are also depleted of Mg^{2+}, suggesting that the dependence of secretion on the concentration of cytosol Ca^{2+} is subject to modulation by cytosolic Mg^{2+}.

2. MATERIALS AND METHODS

Neutrophils were obtained from rabbit peritoneal exudates as in [5,6]. The cells were washed twice by centrifugation in a Ca^{2+}-free buffered salt solution (pH 7.4) which comprised (unless otherwise indicated in the figure legends) 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 20 mM Hepes, 5.6 mM glucose and 1 mg ml^{-1} bovine serum albumin. They were finally suspended in the same solution at 10^7 cells ml^{-1}. Secretion experiments were initiated 2 min after addition of 10 μg ml^{-1} cytochalasin B; the cells were then added to equal volumes of solution containing Ca^{2+} ionophores.

Cytosolic Ca^{2+} and pH were monitored with quin2 and bis-carboxy-ethylcarboxyfluorescein (BCECF), respectively [7,8]. Cells (10^8 ml^{-1}) were incubated at 37°C in the presence of 50 μM quin2 in a standard albumin-free buffer containing 1 mM Ca^{2+}. After 15 min the cells were diluted to 2.5×10^7 ml^{-1} with standard buffer containing

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0.1% albumin and 40 min later they were centrifuged and resuspended in an albumin and Ca^{2+}-free buffer and kept at room temperature until the experiment was started. Fluorescence (339–492 nm) was measured in a Perkin-Elmer LS5 fluorimeter equipped with thermostat and magnetic stirring device (Rank Bros., Bottisham, UK). The calibration of quin2 fluorescence was performed by lysing the cells with Triton X-100 (0.1%) at pH 8.67 in the presence of EGTA and Tris (6.6 mM and 40 mM, respectively) in order to give the zero Ca^{2+} fluorescence (F_{min}) and then by adding back excess Ca^{2+} to give F_{max}. Cytosol Ca^{2+} $[Ca^{2+}]_i$ was calculated according to

$$[Ca^{2+}]_i = K_d(F - F_{min})/(F_{max} - F)$$

where K_d is 115 nM and F is the fluorescence of the intracellular indicator before lysing the cells. In our experiments quin2 content was approx. 0.14 nmol/10^6 cells.

The pH indicator (10 μM) was loaded into the cells by a method similar to that used for the quin2. The fluorescence (490–530 nm) was calibrated by lysing the cells at known external pH. No corrections were made to the pH values thus measured to account for the slight shift in the emission peak which BCECF undergoes in the cytoplasm as compared to the extracellular environment [8,9]. Thus our values of pH could be slightly on the low side.

β-Glucuronidase and lactate dehydrogenase were determined as in [10]. All experiments were performed at 37°C.

3. RESULTS

Fig.1 shows, in confirmation of the results of others, that the Ca^{2+} ionophores A23187 and ionomycin are both capable of elevating cytosol Ca^{2+} in neutrophils incubated in Ca^{2+}-free medium. Note that in this experiment we used ionomycin at 2.5 μM, but we were limited to working at 0.1 μM with A23187 due to the intense fluorescence of this compound. The fluorescence change due to the addition of A23187 is abrupt and easily recognised as distinct from that due to change of Ca^{2+} in the cytosol and this has been subtracted from the trace in fig.1. Even at this low concentration a rise in cytosol Ca^{2+} is quite evident.

Fig.2 illustrates the dependence of β-glucuronidase secretion due to the two ionophores on added Mg^{2+} in the absence of added extracellular Ca^{2+} and in the presence of 10 μM EGTA. On chelation of Mg^{2+} with EDTA, A23187 but not ionomycin was capable of supporting secretion. This finding prompted an investigation into the differential effects of the two ionophores, particularly in respect of cellular ionic homeostasis and cytosol pH. In view of earlier demonstrations [11] that ionomycin has the properties of a strict Ca^{2+}/H^+ exchanger

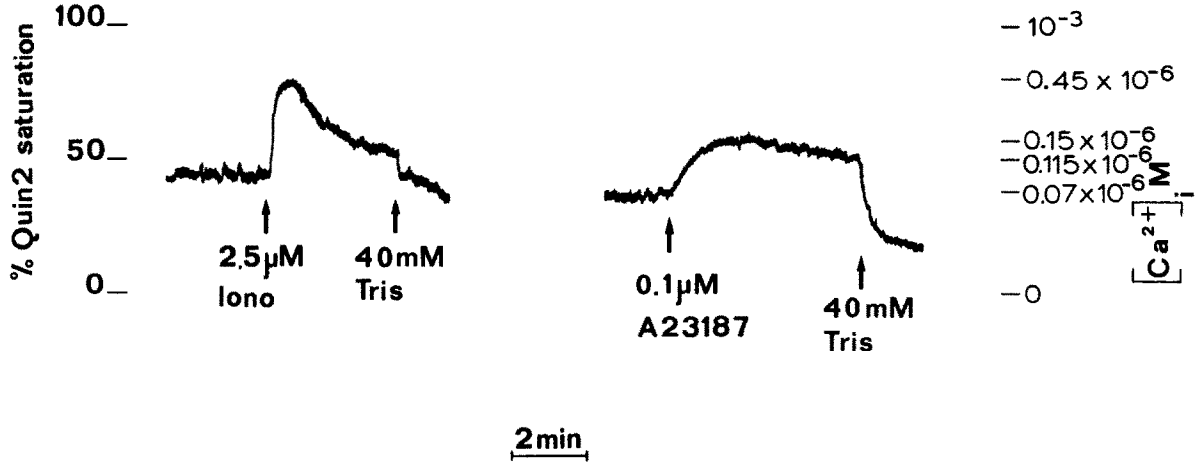

Fig.1. Ionophore induced rise in cytosol Ca^{2+} in the absence of extracellular Ca^{2+}. The incubation medium contained 6.6 mM EGTA, and neither added Ca^{2+} nor albumin. Cell concentration was 5×10^6 ml^{-1}.

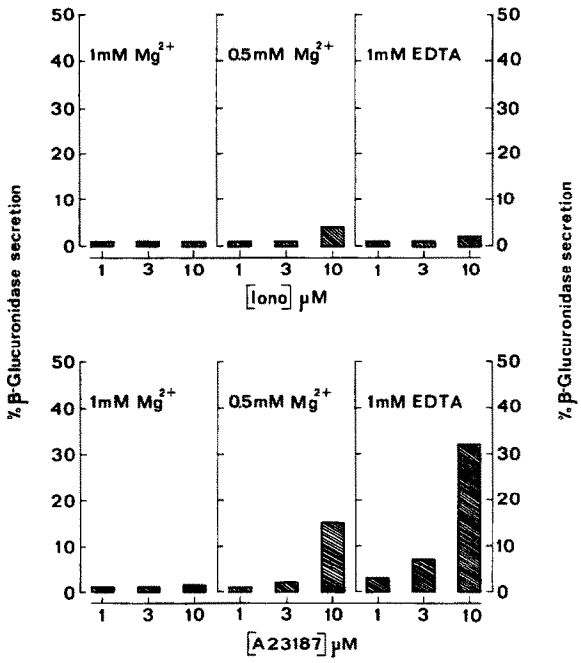

Fig.2. Effect of extracellular Mg^{2+} on Ca^{2+} ionophore induced secretion. β-Glucuronidase secretion was measured after 15 min of incubation in the presence of the ionophores plus 10 μM EGTA. Lactate dehydrogenase release was never more than 4% in the presence of 10 μM ionophore.

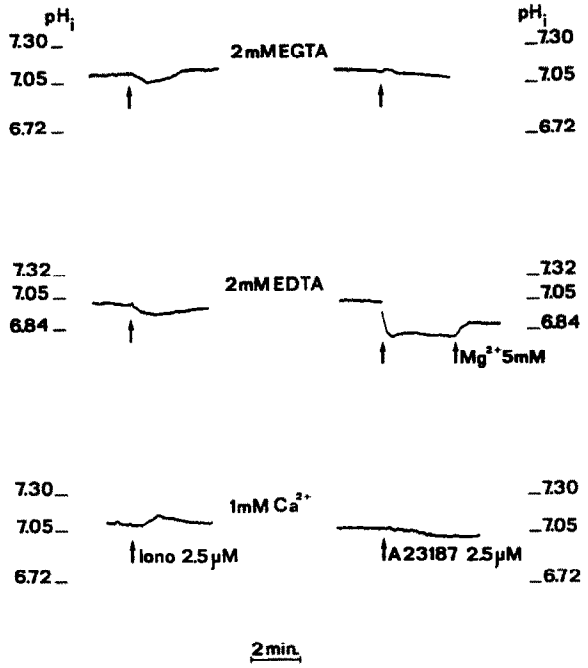

Fig.3. Ca^{2+} ionophore induced cytosol pH changes. Cells (5×10^6 ml^{-1}) were incubated in the standard buffer without albumin.

while A23187 is also able to exchange Ca^{2+} for Mg^{2+} (among the main physiological cations) it appeared likely that the different effects of the two ionophores could be ascribed to their different specificities in respect of the counter cations in the process of electroneutral exchange diffusion.

Fig.3 illustrates the effect on cytosol pH of A23187 and ionomycin, tested under three conditions. In the presence of 2 mM EGTA (upper traces, no added Ca^{2+}), ionomycin caused an initial decrease in cytosol pH which then slowly recovered to the resting level. The reason for this transient acidification is not clear. We would have anticipated a transient alkalinisation in these conditions due to exodus of Ca^{2+} from internal stores in exchange for cytosol H^+ which would then have declined as cytosol Ca^{2+} is exchanged for H^+ across the plasma membrane. In contrast, A23187 has little effect on cytosol pH under these conditions.

When the same experiment was carried out in the presence of 2 mM EDTA (i.e. zero Ca^{2+}, zero Mg^{2+}, middle traces) A23187 caused a drop in cytosol pH (to 6.7) which exceeded by far that due to ionomycin. This could be partially reversed by addition of 5 mM Mg^{2+}. Thus, in conditions under which the Ca^{2+} ionophores can only act to deplete the cells of divalent cations in exchange for H^+, the less specific A23187 has access to a far greater pool of exchangeable intracellular cations (i.e. Mg^{2+} + Ca^{2+}) than has ionomycin (Ca^{2+} alone).

The lower traces show that in the presence of 1.8 mM Ca^{2+} the effect of ionomycin on cytosol pH is reversed as extracellular Ca^{2+} is now exchanged for intracellular H^+. By contrast, A23187 is almost without effect, again suggesting that under these conditions, its preferred counter cation is Mg^{2+}, no H^+.

4. DISCUSSION

It is generally accepted that the Ca^{2+} ionophores stimulate neutrophil and other cellular responses by virtue of their Ca^{2+} carrying activity [12]. Their absolute dependence on extracellular Ca^{2+} has

been taken as the strongest evidence for the role of cytosol Ca^{2+} in cellular activation processes. Yet it has recently been shown that they are also able to elevate cytosol Ca^{2+} in neutrophils in the absence of extracellular Ca^{2+}. Under these conditions no secretion occurs [4].

We offer two explanations for this anomaly:
(i) Ca^{2+} ionophores in the absence of extracellular Ca^{2+} might perturb cytosol in homeostasis in a way which would inhibit the cellular activation process. We cannot of course exclude the possibility that the changes in cytosol pH due to ionomycin could affect secretion; indeed we note that in the presence of external Ca^{2+}, the alkalinisation shows a lag phase of similar duration as that exhibited by secretion (i.e. 30 s) [2]. On the other hand the greatest degree of secretion (by A23187 in the presence of EDTA) was accompanied by the greatest degree of acidification and it is therefore unlikely that pH is a relevant modulator of cell function in these conditions.
(ii) Normal receptor directed activation could cause other changes in addition to an elevation of cytosol Ca^{2+}: the ionophore (in the presence of extracellular Ca^{2+}) could then obviate the need for such changes by elevating cytosol Ca^{2+} above the range normally achieved in the physiological situation as previously shown [4]. Here we provide evidence that this is probably the way which the Ca^{2+} ionophores stimulate secretion. Our observation of different effects of ionomycin and A23187 on secretion and cytosol pH points to a role for cytosol Mg^{2+} as a modulator for Ca^{2+} ionophore induced Ca^{2+} dependent processes. This conclusion is based on the recognition, from experiments with liposomes, that while ionomycin is an obligate Ca^{2+}/H^{+} exchanger A23187 can also transfer Mg^{2+} [11]. Here we show clearly that these ion exchange selectivities also pertain when the ionophores are applied to cells.

In conclusion we show that two extensively used Ca^{2+} ionophores can have very different effects on neutrophil function and ion homeostasis. With A23187 applied in the absence of extracellular Ca^{2+}, secretion can occur at levels of cytosol Ca^{2+} below that normally achieved by the Ca^{2+} ionophores and we suggest that this is because the affinity of putative cytoplasmic binding sites for Ca^{2+} is enhanced by the depletion of Mg^{2+}. This conclusion is in agreement with earlier results from permeabilised adrenal chromaffin cells [13] in which it was shown that Mg^{2+} reduces the apparent affinity of intracellular Ca^{2+} binding sites.

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