Direct measurements of the dextran-dependent calcium uptake by rat peritoneal mast cells

Ronit Sagi-Eisenberg, Carmi Geller-Bernstein*, Ziva Ben-Neriah+ and Israel Pecht

*Department of Chemical Immunology, The Weizmann Institute of Science, *Pediatric Allergy Unit, Kaplan Hospital* and *+Department of Pediatrics, Kaplan Hospital, Rehovot 76100, Israel*

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The metallochromic indicator murexide has been used to monitor calcium concentration changes during the dextran-induced, phosphatidylserine-dependent degranulation of rat peritoneal mast cells. The dextran-induced Ca^{2+}-uptake showed an absolute dependence on the presence of phosphatidylserine. The extent of Ca^{2+}-uptake increased with phosphatidylserine in a concentration-dependent manner. At 25°C the half-life of the uptake process equalled 35 ± 5 s. Exposure of the mast cells to dextran in the presence of Ca^{2+}, but in the absence of phosphatidylserine, desensitized the cells. The subsequent addition of phosphatidylserine failed to restore the Ca^{2+}-uptake activity. However, the Ca^{2+}-ionophore A23187 did promote Ca^{2+} uptake by the cells without PS.

Calcium uptake *Dextran induction (Rat peritoneal mast cells)* *Phosphatidylserine* *A 23187 ionophore* *Murexide*

1. INTRODUCTION

In common with many secretory processes, the induced degranulation of mast cells is believed to be triggered by a transiently increased level of free calcium in the cytosol. These calcium ions may be derived from either extra- or intracellular sources depending on the nature of the stimulating agent. The anaphylactic release of histamine induced by antigen, dextran or by concanavalin A depends on the presence of external calcium [1] while positively charged secretion inducers such as compound 48/80 can mobilize intracellular stores of calcium [2]. It has been suggested that the anaphylactic release is preceded by a transient increase in the permeability of the membrane to Ca^{2+}, allowing their influx down the concentrations gradient. Measurements of calcium fluxes employing $^{45}Ca^{2+}$ as a tracer have indeed revealed that the process of degranulation is accompanied by an increased uptake of ^{45}Ca [3]. The interpretation of those measurements is, however, problematic since not all of the cell-associated ^{45}Ca necessarily represents the result of net calcium influx [3]. An increased accumulation of ^{45}Ca may represent an increase in the total cellular calcium or merely an increase in the exchangeable calcium. In the case of the phytohemagglutinin-induced mitosis of lymphocytes for instance, the process is accompanied by an early increase in ^{45}Ca uptake, yet an increase in total cell calcium is not essential for mitogenesis and only an increase in ^{45}Ca exchange occurs [4]. It was therefore of particular interest to determine if the reported [3] increased accumulation of ^{45}Ca by mast cells is the result of an increase in total or exchangeable calcium.

Calcium indicators such as murexide allow a fast and continuous spectrophotometric monitoring of Ca^{2+} concentrations [5]. They therefore make it possible to measure directly net changes in the calcium concentrations of the medium overcoming the problem of exchange. This method has been successfully applied to mast cells allowing to monitor the ionophore A23187-induced Ca^{2+} uptake [6]. We have, therefore, extended the use of this method to study the Ca^{2+} fluxes which accompany the dextran-induced degranulation of mast cells. This polysaccharide induces histamine release by interacting with a glucoside binding component on the cells' membrane, most probably

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associated with the IgE receptor [7]. The process has been extensively studied and was found to depend on the presence of external Ca^{2+} and phosphatidylserine [8,9]. We here describe the Ca^{2+}-fluxes which accompany the interaction of dextran with its cell membrane receptor and discuss the correlation existing between the secretory process and the uptake of calcium.

2. EXPERIMENTAL

2.1. *Preparation of cells*

Male Wistar rats (400–500 g) were the source for peritoneal mast cells. The rats were sacrificed by cervical dislocation and 40 ml of Tyrode solution (137 mM NaCl, 2.7 mM KCl, 10 mM HEPES, 0.4 mM NaH_2PO_4, 1.0 mM $MgCl_2$ and 5.6 mM glucose, pH 7.4) containing heparin 40 μg/ml (5000 units/ml, Evans Medical) were injected intraperitoneally. The peritoneum has been massaged for about 1 min and the fluid withdrawn through an abdominal incision. The peritoneal washings were pooled, washed and separated from other cells by centrifugation through Ficoll-BSA [10]. About 2–3 × 10^6 cells (80% mast cells), resuspended in Tyrode were used per assay in a final vol. of 3 ml.

2.2. *Determination of Ca^{2+} concentrations*

Ca^{2+} concentrations were monitored spectrophotometrically using the calcium indicator murexide. The absorbance changes were measured at 540–507 nm using a thermostated (25°C) dual-wavelength Aminco DW2 spectrophotometer and a stirred cuvette. The addition of $CaCl_2$ produced a decrease in the absorbance at 540 nm due to the formation of the Ca^{2+}–murexide complex (K_d = 1–3 mM) [5], $\Delta\epsilon$ = 0.22 $mM^{-1}.cm^{-1}$.

2.3. *Materials*

The dextran used had a molecular mass of 70000 and was supplied as a 6% solution in saline (Pharmacia). Murexide was supplied by Sigma.

3. RESULTS

3.1. *Dextran-induced Ca^{2+} fluxes monitored by murexide*

The direct observation of dextran-induced change in the medium Ca^{2+} concentration of a mast cell suspension is illustrated in fig.1. The addition of dextran at a concentration of 12 mg/ml to cells pre-equilibrated with calcium (0.8 mM), murexide (50 μM) and PS (10 μg/ml) caused an increase in the 540 nm absorbance. This increase reflects a decrease in the Ca^{2+}–murexide complex concentration [5]; i.e., a reduction of the free Ca^{2+} concentration in the medium.

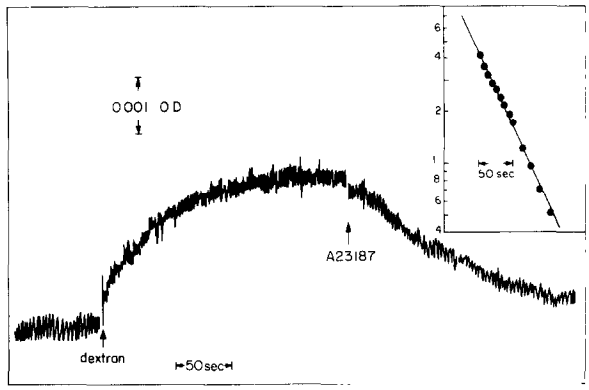

Fig.1. Dextran-induced changes in the external calcium ion concentrations during degranulation. A suspension of purified mast cells (3 × 10^6/assay) was preincubated with Ca^{2+} (0.8 mM), phosphatidylserine (10 μg/ml) and murexide (50 μM). Dextran was added (12 mg/ml) and the changes in the absorbance were monitored as described in section 2. The addition of the calcium ionophore A23187 (7 μM) after the uptake reaction had reached saturation was followed by a calcium efflux from the cells. Insert: the first order plot of the uptake reaction, k = 0.0198 s^{-1}.

This process has an apparent first-order pattern with a half-life of 35 ± 5 s at 25°C, approaching termination within 2 min (fig.1, insert). The decrease in the extracellular Ca^{2+} concentration as estimated from the maximal increase in the measured absorbance was 21 ± 4 μM. The subsequent addition of the calcium ionophore A23187 caused an efflux of calcium back into the external medium (fig.1). Titration with lower concentrations of dextran up to 12 mg/ml failed to produce any significant changes in the external Ca^{2+} concentration.

3.2. *Dextran-induced desensitization*

The addition of dextran, again at a 12 mg/ml concentration, but to a mast cells suspension equilibrated with Ca^{2+} and murexide in the absence of PS, failed to induce any significant

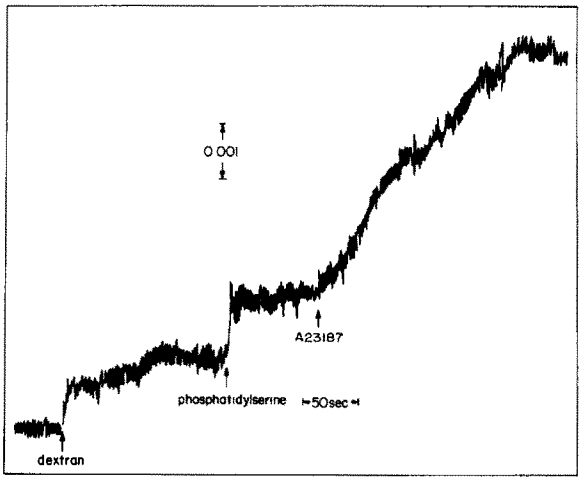

Fig.2. Monitoring calcium concentrations in the absence of phosphatidylserine. The cells were preincubated in the presence of calcium and murexide as described in fig.1, except that phosphatidylserine was omitted from the medium. The subsequent additions of dextran (12 mg/ml) and phosphatidylserine (10 μg/ml) did not cause any significant change in the external calcium ions concentration. The addition of the calcium ionophore A23187 (7 μM) was followed by calcium uptake.

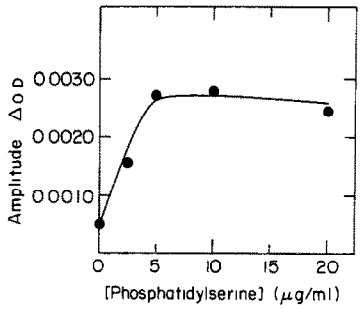

Fig.3. Effect on dextran-induced Ca^{2+} concentration changes of different concentrations of phosphatidylserine. The dextran-induced decrease in the external Ca^{2+} concentration was monitored as described in fig.1 in the presence of various phosphatidylserine concentrations. The data were analysed as described in fig.1 and the amplitudes and apparent rate constants of these processes were calculated. The amplitudes depended on the phosphatidylserine concentration while the apparent rate constants remained the same as described in fig.1.

change in the external Ca^{2+} concentration (fig.2). Moreover, the subsequent addition of PS to the cells which had previously been exposed to dextran did not restore the effect of calcium uptake (fig.2). The small increase in absorbance which follows the addition of PS (fig.2) reports a slight decrease in the external Ca^{2+} concentration resulting from the Ca^{2+} binding capacity of PS itself. This change was also observed in control experiments in the absence of cells. However, the addition of the Ca^{2+}-ionophore A23187 to cells which were desensitized by dextran did promote an influx of Ca^{2+} into the cells (fig.2).

3.3. *The effect of PS on the dextran-induced Ca^{2+}-uptake*

Experiments in which cells pre-incubated for 5 to 10 min with Ca^{2+} and murexide were also supplemented with increasing concentrations of PS, showed that the extent of dextran-induced decrease in external Ca^{2+} concentration depended on the PS concentration, up to a limiting level (fig.3). The higher the PS concentration in the reaction mixture, the larger was the reduction in external Ca^{2+} concentration as figured by the increased change in the measured absorbance. The kinetic pattern and the specific rates under the varying PS concentrations were not affected (not shown).

4. DISCUSSION

The results presented here show that the dextran-induced degranulation of mast cells is accompanied by a concomitant decrease in the extracellular concentration of Ca^{2+}. This process shows an absolute dependence on the presence of PS and the extent of degranulation increases with increasing PS concentrations. Moreover, the subsequent addition of PS to cells which have previously been exposed to dextran and Ca^{2+} in PS absence, fails to restore the dextran-induced decrease in the extracellular Ca^{2+} concentrations. Thus, the dextran-induced degranulation of mast cells and changes in extracellular Ca^{2+} concentrations are well correlated. Both share a similar time course [11] and a similar dependence on PS [12]. Furthermore, under the conditions which were reported to desensitize the cells, namely, exposure to dextran and Ca^{2+} in the absence of PS [13], no significant change in the external Ca^{2+} concentration can be detected.

The observed decrease in the external Ca^{2+} con-

centration can be interpreted either as a cellular Ca^{2+} uptake process or as binding of Ca^{2+} to newly exposed sites resulting from exocytosis. The fact that this process can be reversed by the Ca^{2+} ionophore A23187 implies that the observed decrease does in fact result from a net uptake of Ca^{2+} by the cells. Still, calcium binding to normally inaccessible intracellular components is most probably involved since calculations based on the internal volume of 1 μl for 10^6 mast cells [3] and the observed Ca^{2+} uptake of 20 nmol/10^6 cells indicate that Ca^{2+} is present in the cells at about 20-times its concentration in the external medium. The cells' mitochondria are most probably the internal organelles responsible for the accumulation of nearly all the Ca^{2+} taken up by the cells (unpublished). Thus, the ionophore-induced Ca^{2+} efflux which follows the accumulation step apparently results from ionophore-induced release of Ca^{2+} from the mitochondria, similar to the ionophore-produced effect reported for isolated rat liver hepatocytes [14] and bovine spermatozoa [15].

In summary, the presented data show that an increase in the total cell calcium is closely associated with the degranulation of mast cells induced by secretagogues that require the presence of extracellular Ca^{2+}. The dependency of the secretory process on PS derives from the important role this lipid plays in the mechanism of Ca^{2+} uptake, a mechanism which can be by-passed by the calcium ionophore A23187.

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