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INOSITOL 1,4,5-TRISPHOSPHATE-INDUCED Ca^{2+} RELEASE FROM PERMEABILIZED MASTOCYTOMA CELLS

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SUMMARY: The effect of inositol 1,4,5-trisphosphate (IP_3) on Ca^{2+} release in the transformed murine mast cells, mastocytoma P-815 cells permeabilized with digitonin was studied. Ca^{2+} was sequestered by intracellular organelles in the presence of ATP until the medium free Ca^{2+} concentration was lowered to a new steady-state level. The subsequent addition of IP_3 caused a rapid Ca^{2+} release, which was followed by a slow re-uptake of Ca^{2+}. Fifty percent of the sequestered Ca^{2+} was released by 10 μM IP_3. Maximal Ca^{2+} release occurred at 10 μM and half maximal activity was at 1.3 μM. These results indicate that IP_3 may function as a messenger of intracellular Ca^{2+} mobilization in mastocytoma cells.

It is generally acknowledged that an elevation of cytoplasmic free Ca^{2+} concentration is essential for stimulus-secretion coupling in many cells and this increased intracellular Ca^{2+} then activates various cellular metabolic pathways (1). On the basis of work in various labolatories it has been suggested that mobilization of Ca^{2+} from intracellular store sites such as endoplasmic reticulum provides at least part of the Ca^{2+} responsible for this increment (2,3). Since, in many cells, the rapid event following agonist-receptor interaction is the enhanced hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), the concept has been proposed that IP_3 may mediate mobilization of cellular Ca^{2+} (4). Recently, this hypothesis was supported by observations that IP_3 directly releases Ca^{2+} from non-mitochondrial pool(s) in permeabilized cells as well as microsomes derived from several tissues (5).

The transformed murine mast cells, mastocytoma (P-815 cells) contain much lower amounts of secretory granule contents than those found in normal mast

ABBREVIATIONS: PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; Mops, 4-morpholinepropanesulfonic acid.

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cells (6), and were observed to release much less histamine than mast cells upon stimulation with either antigen , A23187 or compound 48/80 (7). Since the function of IP_3 to release Ca^{2+} has been considered to be mainly involved in stimulus- response coupling (5), it was of interest to investigate whether IP_3 is able to act as a Ca^{2+} mobilizing agent in these mastocytoma cells which do not manifest responsiveness against external stimuli. Therefore, we have used digitonin- permeabilized mastocytoma cells to examine this possibility. The results demonstrate that IP_3 can serve as a Ca^{2+} releaser acting on intracellular store(s) in this cell.

MATERIALS AND METHODS

A cloned cell line of transformed murine mast cell, mastocytoma P-815, derived from the Dunn-Potter's mastocytoma (8) were provided by Drs. A. Ichiyama and H. Hasegawa, Department of Biochemistry, Hamamatsu Medical College and carried in DBA/mice (9). The mastocytoma cells were harvested from the abdominal cavity of the tumor-bearing mice on the 7th day after transplantation. Purity of cell preparations was over 95 % and the viability was over 90 % as assesed by toluidine blue staining. The cells were then washed two times with 20 mM Mops-Tris (pH 7.2), 100 mM KCl, 100 mM sucrose at 4 °C and used for measurement of Ca^{2+} transport.

Ca^{2+} movement was measured using Quin 2 (free acid form; Dojindo Lab., Kumamoto, Japan) as an indicator of free Ca^{2+} concentration in the suspension medium. The standard 2-ml reaction media contained 100 mM KCl, 100 mM sucrose, 2 mM $MgCl_2$, 30 µg/ml creatine phosphokinase, 7.5 mM creatine phosphate, 1 mM ATP, 1 µg/ml of oligomycin, 1 mM NaN_3, 100 µM Quin 2, 2.5 mg/ml of mastocytoma cells and 20 mM Mops-Tris (pH 7.2). The required free Ca^{2+} in the medium was obtained by estimation of known amounts of Ca^{2+} added to the medium essentially by the method of Tsien et al. (10), using a Quin 2-Ca^{2+} dissociation constant of 115 nM (10). Following the method of Murphy et al. (11), Ca^{2+} uptake was started by addition of 80 µg/ml of digitonin (Wako Pure Chemicals, Osaka) to permeabilize the cells and changes in Quin 2-Ca^{2+} fluorescence were measured using FS501S fluorescence spectrophotometer (Union Giken, Japan) with excitation at 340 nm and emission at 490 nm. Data from this instrument was processed by a Sord M223 microcomputer. The cell suspension was continuously stirred in the cuvet chamber maintained at 37 °C with a Haake D3 and G temperature control unit.

Bovine brain D-myo-inositol trisphosphate (IP_3) was a product of Sigma, St. Louis MO. and Ca^{2+} ionophore A23187 was purchased from Calbiochem.

RESULTS AND DISCUSSION

In order to examine the effects of IP_3 on the release of Ca^{2+} from intracellular pool(s) of mastocytomas, their plasma membranes were permeabilized by the digitonin treatment described by Murphy et. al. (11). Fig. 1 shows the movement of Ca^{2+} measured with Quin 2 after permeabilization of mastocytoma

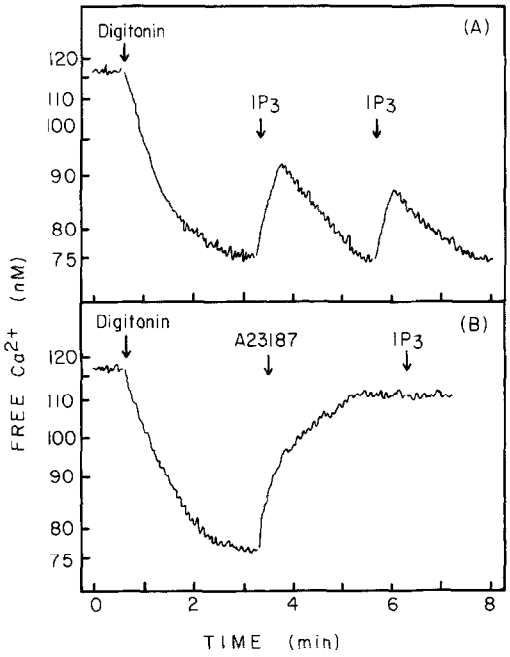

Fig. 1: Ca^{2+} movement of digitonin-permeabilized mastocytoma cells measured with Quin 2. Isolated mastocytomas were incubated at 2.5 mg of cell protein/ml in the standard reaction medium with ATP and ATP-regenerating system as described in Materials and Methods. Reaction was started by addition of 80 µg/ml of digitonin and the changes in medium free Ca^{2+} concentration were followed by measuring the fluorescence of the Quin 2-Ca^{2+} complex. At steady state, 10 µM inositol 1,4,5-trisphosphate (IP_3) [A] or 200 nM of Ca^{2+} ionophore A23187 (A23187) [B] was added. The free Ca^{2+} concentration was calculated as described in Materials and Methods.

cells. In the presence of ATP and an ATP-regenerating system, addition of digitonin (80 µg/ml) to the cell suspension caused a rapid, transient decrease of Quin 2-Ca^{2+} fluorescence (Fig. 1A) which was due to the net reduction in Ca^{2+} concentration by sequestration into the newly exposed intracellular Ca^{2+} pools. Cells were incubated in the presence of mitochondrial inhibitors (oligomycin and NaN_3), and Ca^{2+} would be thus sequestered into non-mitochondrial pool(s), probably endoplasmic reticulum (2, 12). A steady-state free Ca^{2+} of approx. 75 nM was reached within several minutes. Under these conditions, addition of IP_3 induced the rapid release of Ca^{2+} which then slowly reaccumulated until the original steady-state free Ca^{2+} was restored (Fig. 1A). A second addition of IP_3 produced a smaller increase in Ca^{2+} concentration, suggesting the presence of desensitization mechanism as reported for insulinoma cells (13). IP_3 did not increase Quin 2 fluorescence in the control cells

without permeabilization treatment with digitonin (data not shown). It was also shown that the time course of the IP_3-induced release of Ca^{2+} differed markedly from the release profile observed in the case of the addition of calcium ionophore A23187 (Fig. 1B). IP_3 produced the reuptake phenomenon, whereas the ionophore irreversibly released the sequestered Ca^{2+} with no indication of further Ca^{2+} release by addition of IP_3 after ionophore treatment (Fig. 1B). The reason for the transient nature of the IP_3 effect is not clear, but it could be a result of rapid hydrolysis of added IP_3 by endogenous phosphatases (14).

The concentration dependence of IP_3-induced Ca^{2+} release in digitonin-permeabilized mastocytoma is shown in Fig. 2. The maximal Ca^{2+} released, calibrated with a pulse addition of Ca^{2+}, was about 1.0 nmol of Ca^{2+}/mg protein. Since the permeabilized mastocytoma cells were able to sequester approx. 2.0 nmol of Ca^{2+}/mg protein in the presence of ATP and ATP-regenerating system, the maximal amount of Ca^{2+} released with IP_3 represented about 50 % of the total sequestered Ca^{2+}. Half-maximal and maximal amounts of Ca^{2+} release were obtained at IP_3 concentration of 1.3 and 10 μM, respectively. These values are

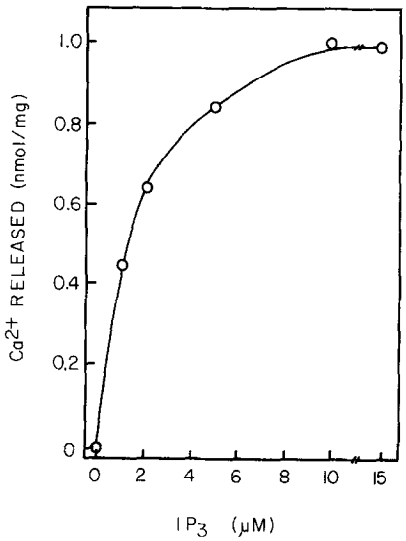

Fig. 2: Concentration dependence of IP_3-induced Ca^{2+} release. The amount of Ca^{2+} released in response to various concentrations of added IP_3 was determined from experiments similar to that shown in Fig. 1. The responses to IP_3 were calibrated by additions of known aliquots of Ca^{2+} to give equivalent fluorescence.

somewhat higher than those reported for permeabilized hepatocytes (15) and neutrophils (12), but a similar degree of diminished sensitivity to IP_3 also was observed with insulinoma microsomes (13) and permeabilized pancreatic islet cells (16).

The results obtained in the present investigation have demonstrated that IP_3 causes a rapid release of Ca^{2+} from intracellular store(s) in digitonin-permeabilized mastocytomas. Since Ca^{2+} was preloaded together with ATP in the presence of mitochondrial inhibitors, the source of released Ca^{2+} may be a non-mitochondrial site, such as the endoplasmic reticulum which actively accumulates Ca^{2+} (2). Furthermore, the IP_3-induced Ca^{2+} release in the permeabilized mastocytoma cells is sufficiently consistent with the role of IP_3 observed in other cell types (12-16), with respect to the rapid and transient nature to support the second messenger function suggested for IP_3 (5).

IP_3-induced Ca^{2+} release has been reported mostly for cells which actively respond to external stimuli (5), and the action of IP_3 has been considered to be closely coupled to stimulus-response coupling in the cell (17). However, the mastocytoma cells used in the present study appear to be inert with respect to stimulus-secretion coupling. They contain a very low level of histamine and do not undergo an active histamine secretion as seen in mast cells (6). At present, although the precise role of IP_3 to release Ca^{2+} in mastocytomas is not specified, further studies with this tumor cell may provide a new understanding of the role of IP_3 within the cell.

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