The Actions of Ca\(^{2+}\) Ionophores on Rat Basophilic (2H3) Cells Are Dependent on Cellular ATP and Hydrolysis of Inositol Phospholipids

A COMPARISON WITH ANTIGEN STIMULATION

(Received for publication, July 30, 1986)

Theresa N. Lo, Wilford Saul, and Michael A. Beaven
From the Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Calcium-specific ionophores are used widely to stimulate Ca\(^{2+}\)-dependent secretion from cells on the assumption that permeabilization of the cell membranes to Ca\(^{2+}\) ions leads to a rise in concentration of cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)), which in turn serves as a signal for secretion. In this way, events that precede mobilization of Ca\(^{2+}\) ions via receptor stimulation are bypassed. One such event is thought to be the rapid hydrolysis of membrane inositol phospholipids to form inositol phosphates and diacylglycerol. Accordingly, rat leukemic basophil (2H3) cells can be stimulated to secrete histamine either with the ionophores or by aggregation of receptors for IgE in the plasma membrane. We find, however, that ionophore A23187 stimulates secretion of histamine only at concentrations (200–1000 nM) that stimulate hydrolysis of membrane inositol phospholipids.

The extent of hydrolysis of inositol phospholipids was 1) dependent on the concentration of ionophore and the presence of external Ca\(^{2+}\) ions and 2) correlated with the magnitude of the secretory response. A similar correlation between secretion and hydrolysis of inositol phospholipids was observed in response to the Ca\(^{2+}\)-specific ionophore, ionomycin. Although this hydrolysis (possibly a consequence of elevated [Ca\(^{2+}\)]\(_{i}\)) was less extensive than that induced by aggregation of receptors, it may govern the secretory response to A23187. The studies revealed one paradox. The rise in [Ca\(^{2+}\)]\(_{i}\) depended on intracellular ATP levels, when either an ionophore or antigen was used as a stimulant irrespective of whether hydrolysis of inositol phospholipids was stimulated or not. The concept of how the ionophores act, therefore, requires critical reevaluation.

The hydrolysis is associated with a rise in cytosolic Ca\(^{2+}\) concentrations and secretion of histamine (6, 7). Stimulation by direct mobilization of Ca\(^{2+}\) ions with the calcium ionophore, A23187, results, on the other hand, in the hydrolysis of less than 5% of inositol phospholipids (5).

Past studies with 2H3 cells have failed to provide convincing evidence that hydrolysis of phosphatidylinositol bisphosphate to inositol 1,4,5-trisphosphate by phospholipase C alone, is responsible for the mobilization of Ca\(^{2+}\) ions in antigen-stimulated 2H3 cells (5, 6). This is in contrast to other secretory cells in which the rise in [Ca\(^{2+}\)]\(_{i}\) is closely correlated with the increases in the intracellular levels of inositol 1,4,5-trisphosphate (8). Release of Ca\(^{2+}\) from intracellular stores by the trisphosphate (9–15) is thought to provide one signal for secretion. An additional synergistic signal is generated through the activation of protein kinase C by diacylglycerol, the other product formed on hydrolysis of the inositol phospholipid (8, 16, 17). The membrane pool of phosphatidylinositol bisphosphate is then replenished by sequential phosphorylation of phosphatidylinositol through the action of ATP-dependent kinases (18–21).

In an attempt to clarify whether the Ca\(^{2+}\) signal was dependent on the production of inositol 1,4,5-trisphosphate, we varied the intracellular ATP content by titration of 2H3 cells with antimycin A in glucose-free medium according to previously published protocols (4, 22). We reasoned that complete depletion of intracellular ATP should suppress the formation of phosphatidylinositol bisphosphate and, as a consequence, the formation of inositol 1,4,5-trisphosphate in stimulated cells. Our approach proved to be less straightforward than anticipated when, in control experiments, we found that the rise in [Ca\(^{2+}\)]\(_{i}\), in response to the Ca\(^{2+}\) ionophore, A23187, and antigen, were equally dependent on intracellular ATP levels. In addition, A23187 itself stimulated small, but significant, hydrolysis of inositol phospholipids. As these findings raised questions about previous assumptions on the actions of the Ca\(^{2+}\) ionophores, we undertook a systematic evaluation of the stimulatory and secretory events in cells stimulated by either antigen or the Ca\(^{2+}\) ionophores, particularly as to the effects of ATP depletion on these events.

MATERIALS AND METHODS AND RESULTS

1 The abbreviations used are: [Ca\(^{2+}\)]\(_{i}\), free Ca\(^{2+}\) concentration in the cytoplasm; EGTA, [ethyleneglycol-bis(2-aminoethyl)glycine]; tetraacetic acid (Miniprint).

2 Portions of this paper (including "Materials and Methods," "Results," Tables I and II, and Figs. 1–5) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-2620, cite the authors, and include a check or money order for $5.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

141
Stimulatory Events Associated with Aggregation of IgE Receptors—In a variety of secretory cells, receptor-mediated hydrolysis of membrane inositol phospholipids requires the activation of a phospholipase C, possibly through the interaction of receptors with a GTP-regulatory protein (29–38), and the hydrolysis of phosphatidylinositol 4,5-bisphosphate to form inositol 1,4,5-trisphosphate and diacylglycerol (8, 16, 17). These two metabolites mediate, respectively, the release of Ca$^{2+}$ ions from ATP-dependent Ca$^{2+}$ stores in the endoplasmic reticulum (9–15) and the activation of protein kinase C (8, 16, 17). The regeneration of phosphatidylinositol 4,5-bisphosphate from phosphatidyl-inositol may lead to a net loss of phosphatidylinositol. Hydrolysis of monophosphatidinositol specifically may occur as a secondary reaction once [Ca$^{2+}$], is elevated and thereby provide an additional source of diacylglycerol at later stages of the response (39, 40).

Additional information is required, however, before the sequence of signals spawned by these reactions can be established. Recent work indicates that inositol 1,4,5-trisphosphate is converted to inositol 1,3,4,5-tetrakisphosphate and thence inositol, 1,3,4-trisphosphate, whose time course of appearance and disappearance does not synchronize with the calcium signal in contrast to that of the 1,4,5-derivative which does show such synchrony (41–43).

With specific reference to the 2H3 cells, stimulation of hydrolysis of inositol phospholipids appears to be a direct consequence of aggregation of IgE receptors. For example, it can be shown with IgE-oligomers of defined multiplicity that the intensity of the stimulatory signals, i.e. the rate of hydrolysis of inositol phospholipids and a rise in [Ca$^{2+}$]i, is related to the size of receptor clusters and numbers of IgE receptors aggregated (6). The calcium signal in 2H3 cells, however, is generated largely through influx of Ca$^{2+}$ ions across the plasma membrane (5). Whether this influx is mediated indirectly through the hydrolysis of inositol phospholipids (6, 7) or directly through IgE-receptor-activated Ca$^{2+}$ ion channels (44) remains an unresolved question. Nevertheless, any manipulation that increases (e.g. with heavy water) or decreases the phosphoinositide response (e.g. temperatures above or below 37 °C or depletion of cellular ATP), inevitably results in analogous changes in the Ca$^{2+}$ signal (6, 45 and this paper).

**Relationship of Receptor-initiated Stimulatory Events to Secretion**—Cultured 2H3 cells can generate profound stimulatory signals. Exposure of 2H3 cells to increasing concentrations of oligomeric preparation of IgE leads to progressive increases in rates of phospholipid hydrolysis and in the intensity of the Ca$^{2+}$ signal until all IgE receptors are occupied, whereas the secretary response reaches a maximum once 20% of the receptors are occupied. Below this level of stimulation, stimulatory and secretory responses are correlated (6).

Although the Ca$^{2+}$ signal is obligatory for antigen-induced secretion (5), it is clearly not the only signal required for secretion as will be discussed later. In regard to the activation of protein kinase C, stimulation of mast cells or the tumor

---

**Stimulatory Events Induced by A23187**—The increase in [Cat$^{2+}$]i—The original studies with A23187 by Foreman and co-workers (54) provided the first convincing evidence that recruitment of Ca$^{2+}$ ions can stimulate exocytosis in mast cells. It has been widely assumed since then that calcium ionsophores intercalate in membrane lipids and act as diffusible Ca$^{2+}$ carriers that permit redistribution of Ca$^{2+}$ ions as dictated by the concentration gradients of free Ca$^{2+}$. The increase in [Ca$^{2+}$]i, could thus be attributed to enhanced permeability of the plasma membrane to high concentration of external Ca$^{2+}$ ions and, possibly, to the release of Ca$^{2+}$ from mitochondrial and other membrane-bound stores of Ca$^{2+}$.

The above model is probably overly simplistic in view of the lack of a Ca$^{2+}$ response in ATP-depleted cells. The rises in [Ca$^{2+}$], in response to antigen and ionophore appear to be equally sensitive to decreases in ATP concentration (Figs. 2 and 3 and Table I). Similarly, others have observed a suppression of enhanced 45Ca$^{2+}$ influx (4) or efflux (22) in response to A23187 or antigen in the same cells and conditions used here for depletion of cellular ATP. It is unlikely, therefore, that our observations reflect artifacts with quin 2-loaded cells. Furthermore, the concentrations of antimycin A (i.e. 4–6 nm in the absence of glucose) that inhibited influx of 45Ca$^{2+}$ by 50% (4) were almost identical to those (3–5 nm) required for 50% suppression of the Ca$^{2+}$ signal in our study.

The studies with A23187 raise another issue. As noted in previous work, the absence of a secretory response to low concentrations of A23187, despite increases in [Ca$^{2+}$], of >1000 nm suggest that the Ca$^{2+}$ signal alone is not a sufficient stimulus for secretion.

**Hydrolysis of Inositol Phospholipids may Provide the Additional Signal(s) for Secretion in Response to A23187**—Exocytosis in 2H3 cells in response to A23187 is more closely related to hydrolysis of the inositol phospholipids than to the Ca$^{2+}$ signal (Fig. 5). We calculate that in 2H3 cells, at least 1–2% breakdown is required to initiate detectable secretion and that 3–5% breakdown is required to promote substantial secretion (i.e. >20% release of histamine). In this situation, the rate of hydrolysis and not the increase in [Ca$^{2+}$], may be the rate-limiting factor for secretion. As high concentrations of A23187 cause hydrolysis in 2H3 cells only in the presence of external Ca$^{2+}$ (Fig. 5), the hydrolysis may be a consequence of the large increases in [Ca$^{2+}$]. From the present data, increases in [Ca$^{2+}$], of 1000 nm or greater appear to be necessary for initiating hydrolysis of the phospholipids independently of that induced by aggregation of IgE receptors.

Previous studies with A23187 in a variety of cells have produced contrary results, but when both radiolabeled inositol lipids and water-soluble metabolites were estimated, it was apparent that the ionophore did stimulate hydrolysis of the phospholipids (see "Discussion" in Ref. 55).

---

whether stimulation by A23187 leads to the formation of just inositol mono- and bisphosphates (e.g. Ref. 55) or inositol trisphosphate as well (e.g. this study), either reaction should yield diacylglycerol which, in fact, is produced in A23187-stimulated mast cells (56). Although A253187 and ionomycin differ with respect to their counter ion specificities (i.e. Ca²⁺/ Mg²⁺ exchange with A23187 and Ca²⁺/H⁺ exchange with ionomycin (57)) both ionophores stimulate breakdown of the inositol phospholipids (Fig. 4).

The Role of ATP in the Generation of Signals—If stimulation of inositol phospholipid turnover requires an escalation of inositol phospholipids (Fig. 4).

In conclusion, it is well established that secretion from mast cells (60) and the 2H3 cells (4, 22) utilizes intracellular ATP. High intracellular levels of ATP are certainly required for the generation of calcium responses in 2H3 cells irrespective of the need for ATP in the formation of the polyphosphoinositides. We suggest that intracellular ATP-dependent binding sites for Ca²⁺ must be functional to promote influx of Ca²⁺ or increase in [Ca²⁺], in response to either antigen or ionophore. Maintenance of basal [Ca²⁺], in contrast, is less dependent on intracellular ATP concentrations and this is disturbed only when ATP concentrations are reduced by >97%. Under normal conditions, the production of ATP in 2H3 cells is sufficient to sustain all events stimulated by antigen (Fig. 1) or A23187 (Table II) without marked decreases in cellular ATP.

Acknowledgments—We thank Dr. James R. Gillette for his careful review of the manuscript. We would like also to acknowledge the useful discussions with Dr. Henry Metzger and Dr. Bastien Gomperts.

REFERENCES

The Actions of Ca²⁺-phospholipases on Rat Mesangial Cells: Subcellular Location and Calcium Influx and Secretory Response

D. A. Kennerly, T. J. Sullivan, and T. B. Parker


Supplemental Material

METHODS

Preparation of HU Cells - The procedures used here were based on those described previously. HU cells were grown in HEPES-buffered rat mesangial cell growth medium. At confluence, the cells were trypsinized and seeded into 24-well plates at a concentration of 10⁶ cells per well. The cells were grown to confluence and then prepared for intracellular measurement of [Ca²⁺].

Human mesangial cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were harvested by trypsinization and seeded onto 60-mm diameter dishes at a density of 10⁵ cells per dish. The cells were grown to confluence and then prepared for intracellular measurement of [Ca²⁺].

Human mesangial cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were harvested by trypsinization and seeded onto 60-mm diameter dishes at a density of 10⁵ cells per dish. The cells were grown to confluence and then prepared for intracellular measurement of [Ca²⁺].

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.

The intracellular concentration of free calcium was measured using the fluorescent dye Fura-2 AM. The dye was added to the culture medium and allowed to stain the cells for 30 minutes. The dye was then washed away and the cells were stimulated with various agents.
with the quin 2 measurements, but after correction for this fluorescence, they appeared to be in excess of 1500 nM. These high concentrations of luminescence, caused a significant stimulation of the phosphorylation of inositol phosphates and to the secretion of histamine. Such results were consistently observed in all experiments with the quin 2 measurements, although in some experiments with the quinone 2 measurements, a small increase in the fluorescence was observed. This increase was not seen in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates and secretion (i.e., Ca\(^{2+}\) release) to prestimulated (basal) values in [Ca\(^{2+}\)] was observed in some experiments. This suggests that the hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that observed with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.

The extent of hydrolysis of [3H]inositol phosphates caused by A23187 was considerably less than that noted with stimulation by antigen or higher stimulation of allergen (Fig. 3). With the highest concentration of A23187 tested, 1000 nM, the release of [3H]inositol phosphates (4.6 ± 0.2% release of [3H]inositol phosphates) was 29% of that (22 ± 1.2% release of [3H]inositol phosphates) noted with 5 PL, a high antigen stimulus. The results were identical in three experiments performed at varying concentrations of A23187 and the threshold concentrations for stimulation of histamine release by A23187 were determined to be 10-11 M for 3H-inositol phosphates and to be 0.71 ± 0.3 units at 100 nM, in the absence of external Ca\(^{2+}\) ions. In the absence of external Ca\(^{2+}\) ions, the release of [3H]inositol phosphates (100 nM A23187) varied from 1.2 ± 0.2% to 1.4 ± 0.2% for all experiments performed at varying concentrations of A23187. This release was significantly different from the maximum release of histamine (75%) obtained in the same experiments. The data from all experiments are shown in Table 1.