*Br. J. Pharmac.* (1985), **85**, 819–826

# Transmembrane sodium and potassium gradients modulate histamine secretion induced by ionophore A23187

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**1** Histamine secretion was induced from rat peritoneal mast cells by calcium ionophore A23187 in the presence of various extracellular calcium concentrations. Transmembrane sodium and potassium gradients were altered by cold pretreatment of mast cells or through the inhibition of sodium-potassium ATPase by the use of ouabain or potassium-deprivation.

**2** Such pretreatments led to a parallel shift to the left of the extracellular calcium concentration-histamine secretion curve, i.e. to an apparent decrease of extracellular calcium requirement for the ionophore-induced histamine release.

**3** These effects were fully reversed by warming mast cells, by washing out ouabain or by adding potassium. Metabolic inhibition of mast cells prevented the ionophore-induced secretion in all the experimental conditions described. Secretion observed in the absence of added calcium was inhibited by short term treatment of cells with $5 \times 10^{-6}$ M EGTA or EDTA provided magnesium was absent from the assay medium.

**4** Data show that ionophore A23187 was able to induce secretion in the presence of micromolar concentrations of extracellular calcium, when the efficiency of the ionophore was not decreased by extracellular magnesium and when transmembrane sodium and potassium gradients were altered.

## Introduction

Histamine secretion may be achieved from mast cells by the anaphylactic reaction or by a variety of chemical compounds. The exocytosis process involves an increased concentration of free calcium in the cytosol (reviews: Foreman, 1981; Pearce, 1982) allowing the activation of calmodulin (Douglas & Nemeth, 1982; Amellal & Landry, 1983) and of other calcium-binding proteins. Although the optimal release of histamine requires the presence of extracellular calcium ions, a significant secretion is observed in the absence of added calcium with compound 48/80 (Uvnäs & Thon, 1961) antigen (Foreman & Mongar, 1972) and most secretagogues (Ennis *et al.*, 1980; Pearce *et al.*, 1981). Such observations suggest that the increase of cytosolic calcium might be derived from either extra- or intracellular sources according to the experimental conditions. The secretion observed in calcium-free media was enhanced by brief pretreatment of the cells with EDTA but was abolished by prolonged exposure to chelating agents (Ennis *et al.*, 1980; Pearce *et al.*, 1981). Pearce (1982) suggested that calcium reservoirs deep within the cell membrane were the most likely location of the intracellular calcium stores involved in histamine secretion elicited in calcium-free media. We recently observed that the inhibition of mast cell sodium-potassium ATPase, in the presence of low calcium concentrations, potentiated the immunological histamine release (Frossard *et al.*, 1983) and the release induced by compound 48/80 (Amellal *et al.*, 1984; Binck *et al.*, 1985). These potentiations were inhibited by EGTA and lanthanum, observations that are in agreement with the involvement of an increase of putative membranous calcium stores. However, the possibility that some minute influx of calcium, sufficient to induce exocytosis, might occur from the extracellular medium was not excluded (Amellal *et al.*, 1984). Our concern in the present paper was to check this hypothesis using ionophore A23187 as a triggering agent of mast cell secretion, considered to bypass the plasma membrane-dependent steps of the regulation of exocytosis.

## Methods

Male Wistar rats weighing 250 to 300 g were killed by stunning and bled. Eight ml of buffered salt solution containing (mM): NaCl 137, KCl 2.7, $MgCl_2$ 1, $NaH_2PO_4$ 0.4, glucose 5.6 and HEPES 10 mM-NaOH pH 7.4 were injected into the peritoneal cavity. Alternatively, KCl and/or $MgCl_2$ were omitted from the buffer (see legends). The body was gently massaged for 2 min and the peritoneal fluid collected and centrifuged for 2 min at 220 *g*. The pellet was resuspended in saline buffer and washed twice. Cell suspensions, containing 80,000 to 100,000 mast cells $ml^{-1}$, were preincubated at 37°C in the appropriate medium (see preincubation time in legends). Histamine secretion was usually induced by the addition of $10^{-6}$ M ionophore A23187. The incubation was terminated 10 min later by adding 1 ml of ice-cold buffer. Tubes were cooled immediately in iced water and centrifuged for 2 min at + 4°C. Supernatants were collected and histamine concentrations determined in duplicate according to the fluorimetric method of Shore *et al.* (1959) omitting the extraction procedure. Results were expressed as percentages of total histamine content measured for each batch of cells after treatment of the cell suspension with trichloracetic acid. The compounds used did not interfer with the histamine assay under the conditions of our experiments. HEPES (2-(4-2 - hydroxyethyl)-l-piperazinyl)-ethanesulphonic acid) was obtained from Merck. EDTA (ethylenediamine tetraacetic acid disodium salt dihydrate) and EGTA (ethyleneglycol *bis* (amino-2-ethylether) N N N'N'-tetraacetic acid) were from Prolabo. Ionophore A23187 and ouabain (g-strophanthin) were from Boehringer-Mannheim. Ionophore A23187 ($5 \times 10^{-3}$ M) was disolved in dimethylsulphoxide just before use and subsequent dilutions performed in appropriate saline buffers. Dimethylsulphoxide did not modify secretion under our conditions. Saline buffers were prepared with deionized double-distilled water. True concentration of calcium was measured by flame emission and plasma emission spectroscopy in 'calcium-free' buffers, corresponding to zero added calcium in assays. This calcium concentration was $1.4 \pm 0.5 \times 10^{-5}$ M.

## Results

Rat peritoneal mast cells were recovered in a saline buffer devoid of calcium and preincubated for 40 min at 37°C in the presence or absence of ouabain. Cells were supplemented with various concentrations of calcium. Figure 1 shows that ouabain led to a shift to the left of the calcium concentration-effect curve of histamine secretion induced by ionophore A23187. A large potentiation by ouabain of histamine release was

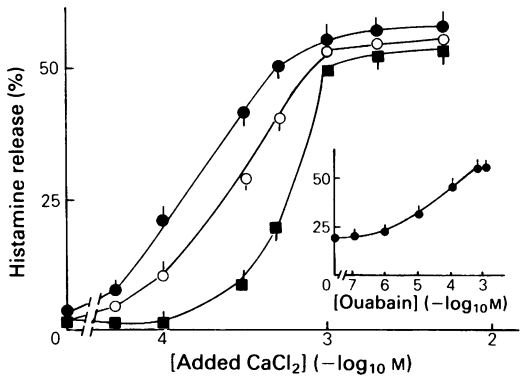


**Figure 1** Effect of ouabain on mast cell histamine release induced by $10^{-6}$ M ionophore A23187: variation with calcium concentration. Peritoneal rat mast cells were collected in a calcium-free buffer and preincubated at 37°C for 40 min in the absence of ouabain (■) or in the presence of $10^{-5}$ M (○) or $5 \times 10^{-4}$ M (●) ouabain. Calcium was added and the secretion was initiated 5 min later with $10^{-6}$ M ionophore A23187. Insert: concentration-response curve to ouabain in the presence of $5 \times 10^{-4}$ M added calcium. Values are the means of 3 experiments; s.e.means shown by vertical lines.

observed in the presence of $5 \times 10^{-5}$ M to $7 \times 10^{-4}$ M added calcium. In the absence of added calcium, corresponding to $1.4 \times 10^{-5}$ M calcium in the medium (see methods), or in the presence of high calcium concentrations (from $10^{-3}$ M) the effect of ouabain was not significant. The concentration of ouabain required to produce half maximum potentiation of histamine release in the presence of $5 \times 10^{-4}$ M added calcium was $2.5 \times 10^{-5}$ M (Figure 1, insert). A 40 min preincubation of peritoneal mast cells with ouabain had to be performed in order to observe a full potentiation effect of the drug (not shown). Passive histamine release, i.e. basal release in the absence of secretagogue, was not significantly modified by ouabain.

Ouabain and related digitalis glycosides are selective inhibitors of ATP hydrolysis and of the active transport of sodium and potassium which are catalysed by sodium-potassium ATPase. However other targets may exist for these drugs. The absence of extracellular potassium also inhibits the enzyme. Consequently peritoneal rat mast cells were preincubated in a calcium and potassium-free buffer before the addition of different doses of calcium and ionophore A23187 to trigger secretion. Figure 2 shows that potassium deprivation also led to a shift to the left of the calcium curve. The shift was dependent upon KCl concentration from 1.5 to 0.2 mM (Figure 2, insert). As noted before in the case of ouabain, a long preincubation time (45 min) in the absence of or with low doses of potassium was required to observe a full potentia-

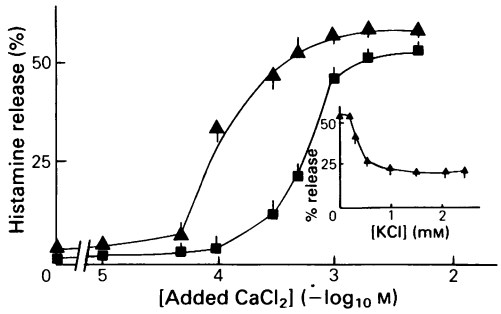


**Figure 2** Effect of potassium-deprivation on mast cell histamine release induced by $10^{-6}$ M ionophore A23187: variation with calcium concentration. Peritoneal rat mast cells were collected in a calcium-free medium and preincubated in the absence (▲) or in the presence of 2.7 mM KCl (■) for 40 min at 37°C. Calcium was added and the secretion was initiated 5 min later with A23187. Values are the means of 3 experiments performed in duplicate; s.e.means shown by vertical lines. Insert: concentration-response curve to potassium in the presence of $5 \times 10^{-4}$ M added calcium (means ± s.e.mean of 4 experiments).

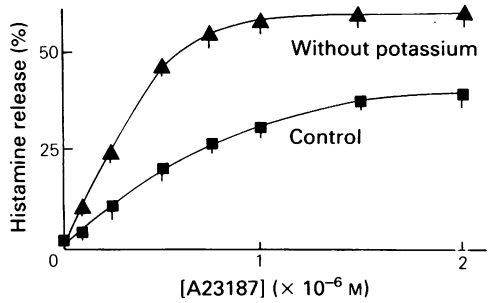


**Figure 3** Concentration-response curves of calcium ionophore A23187 on the secretion of histamine from rat peritoneal mast cells preincubated at 37°C for 40 min in a balanced salt medium including $5 \times 10^{-4}$ M $CaCl_2$ (control ■) or in a potassium-deprived medium (▲). Values are the means of 3 experiments with s.e.means shown by vertical lines.

tion effect. Figure 3 shows a similar dependence of secretion upon the dose of ionophore A23187 in the presence or the absence of potassium; the concentrations required to produced half maximum release were 0.5 and $0.3 \times 10^{-6}$ M respectively. Passive histamine release was not modified in the absence of potassium.

Metabolic inhibition with deoxyglucose and dinitrophenol inhibited ionophore-induced histamine release observed in the presence or in the absence of potassium and various calcium concentrations (Table 1).

The selective inhibition of sodium-potassium ATPase leads to an increase of intracellular sodium and a decrease of intracellular potassium. We suggested (Amellal *et al.*, 1984) that such modification might also occur when preincubating mast cells at + 2°C, as previously performed by Garland & Payne (1979). Figure 4a shows that cold pretreatment of mast cells also led to a shift to the left of the calcium concentration-effect curve. A full potentiation of histamine release, in the presence of $5 \times 10^{-4}$ M added calcium, required a 50 min preincubation time. This potentiation was slowly but fully reversible by warming mast cells before the triggering of secretion (Figure 4b). Similarly, the potentiation of histamine release linked to ouabain or potassium deprivation was slowly reversible by washing out ouabain or adding potassium, respectively (not shown).

A major difference between the present results obtained with ionophore A23187 and our previous observations triggering mast cells with antigen (Frossard *et al.*, 1983) or compound 48/80 (Amellal *et al.*, 1984) is seen in the calcium-dependence of secretion when transmembrane cation gradients were impaired. The potentiation of the antigen or 48/80-induced release was already maximum in the absence of added

**Table 1** Effect of metabolic inhibition on histamine secretion induced from peritoneal rat mast cells by ionophore A23187

| *Added $CaCl_2$* (M) | *Histamine release* (%) *Control cells* Glucose | Deoxyglucose DNP | *KCl-deprived cells* Glucose | Deoxyglucose DNP |
|---|---|---|---|---|
| 0 | 1.2 ± 0.4 | 0.9 ± 0.3 | 7.2 ± 2.4 | 1.1 ± 0.4 |
| $5 \times 10^{-5}$ | 6.1 ± 0.4 | 1.5 ± 0.5 | 13.5 ± 2.5 | 1.4 ± 0.2 |
| $10^{-4}$ | 8.5 ± 0.7 | 1.7 ± 0.5 | 19.3 ± 2.7 | 1.0 ± 0.5 |
| $3 \times 10^{-4}$ | 27.3 ± 2.9 | 1.1 ± 0.8 | 36.2 ± 4.9 | 0.4 ± 0.2 |

Mast cells were preincubated for 45 min in a calcium-free buffer in the presence of 2.7 mM KCl (control) or in the absence of KCl. The buffers contained 5.6 mM glucose or 5.6 mM deoxyglucose and 0.1 mM dinitrophenol (DNP). Calcium was added and histamine release was induced 5 min later with $10^{-6}$ M ionophore A23187. Results are the means ± s.e.mean of 4 experiments.

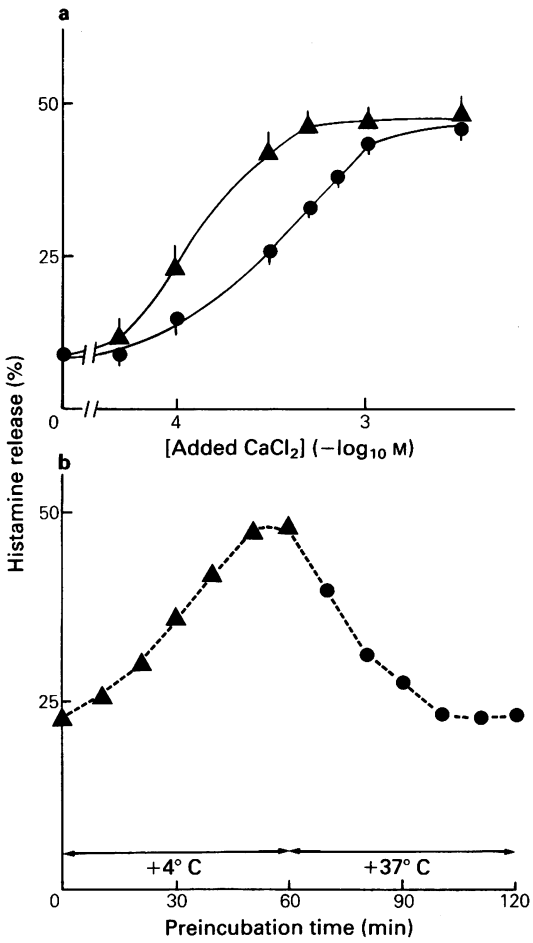


**Figure 4** Effect of cold pretreatment of rat peritoneal mast cells on histamine release induced by ionophore A23187. (a) Mast cells were collected in a calcium-free buffer and preincubated for 60 min at + 2°C (▲) or + 37°C (●). Calcium was added and both sets of tubes were maintained at + 37°C. Secretion was induced 3 min later by adding $10^{-6}$ M ionophore A23187. Values are means ± s.e.mean of 4 experiments. (b) Mast cells were preincubated in a calcium-free buffer at + 2°C for different times (▲). Some batches were incubated for 60 min at + 2°C, and then, for 10 to 60 min at + 37°C (●). $CaCl_2$ ($5 \times 10^{-4}$ M) was added, cells were preincubated at 37°C for 3 min and the secretion of histamine was induced by adding $10^{-6}$ M ionophore A23187. Incubation was stopped 10 min later. Values are means of two experiments performed in duplicate.

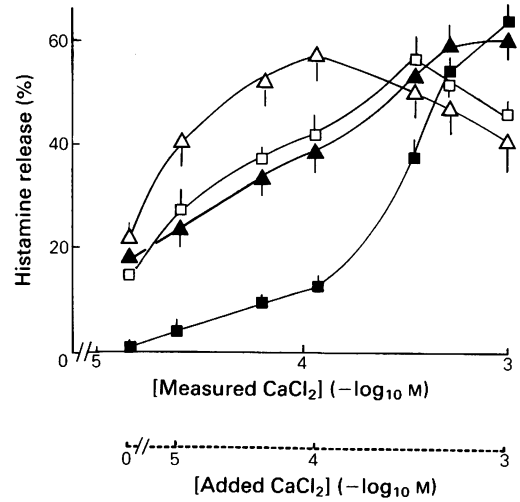


**Figure 5** Effect of potassium- and magnesium-deprivation on histmine release induced by ionophore A23187 according to calcium concentration. Mast cells were collected and washed in a saline buffer containing (mM) NaCl 137, $MgCl_2$ 1, $NaH_2PO_4$ 0.4, glucose 5.6 and HEPES 10 pH 7.4. The pellet was resuspended in the same buffer but without $MgCl_2$. Mast cells were preincubated for 45 min at 37°C in the presence of 2.7 mM KCl and 1 mM $MgCl_2$ (balanced medium, ■), in the presence of 2.7 mM KCl ($MgCl_2$-deprived cells, □) in the presence of 1 mM $MgCl_2$ (KCl-deprived cells, ▲) or in the absence of both KCl and $MgCl_2$ (KCl and $MgCl_2$-deprived cells, △). Calcium was added 5 min before the induction of histamine secretion performed with $10^{-6}$ M ionophore A23187. Incubation was stopped 10 min later. The kinetics of histamine release were similar under these different experimental conditions and were fully completed 1.5 min after the addition of A23187. Values are means of 4 experiments with s.e.means shown by vertical lines.

calcium. In contrast, the observation of a potentiated ionophore-induced secretion required the presence of added calcium irrespective of the method used to modify transmembrane gradients (Figures 1, 2 and 4).

We undertook a new set of experiments in order to clarify this difference since competition between extracellular calcium and magnesium might occur at the level of ionophore A23187. Such a competition was suggested before by Foreman *et al.* (1973) and Di Virgilio & Gomperts (1983). Moreover Pfeiffer & Lardy (1976) showed a similar binding constant of A23187 for calcium and magnesium. Figure 5 shows that the absence of magnesium in the medium, controlled by atomic absorption spectroscopy, led to an increased histamine release in the presence of low calcium concentrations, i.e. up to $3 \times 10^{-4}$ M added calcium. Higher concentrations of calcium led progressively to a decrease of the secretion in the absence of magnesium. Moreover Figure 5 shows that potassium-deprivation performed as described before (Figure 2) but in the absence of magnesium, produced a large potentiation of histamine release in the presence of added calcium concentrations up to

$10^{-4}$ M. Whereas pretreatment of cells by potassium-deprivation, ouabain or cold required a long preincubation time in order to observe a subsequent modification of the induced secretion, the effects of magnesium deprivation were not time-dependent. Moreover the absence of potassium and magnesium did not modify significantly the time course of histamine secretion which was complete 1.5 min after adding the ionophore (not shown).

The secretion from cells in the absence of added calcium was related to the ionophore concentration from 1 to $5 \times 10^{-6}$ M provided potassium and/or magnesium were absent (Figure 6). Metabolic inhibition obtained with deoxyglucose and dinitrophenol decreased almost completely the secretion elicited with $5 \times 10^{-6}$ M ionophore (Figure 6, insert) in the different conditions used, assuming that this high dose of ionophore did not lead to some lytic process. These results confirm that potent histamine secretion could be elicited by the ionophore through an energy-dependent mechanism in a nominally calcium-free media (i.e. about $10^{-5}$ M, see above) provided magnesium and/or potassium were absent.

Further experiments were performed in order to appreciate the involvement of an extracellular or intracellular source of calcium in the secretion process. Figure 7 shows that the short term addition of $5 \times 10^{-6}$ M EDTA or EGTA was sufficient to observe a maximal inhibition when magnesium was absent and with or without potassium deprivation. The low

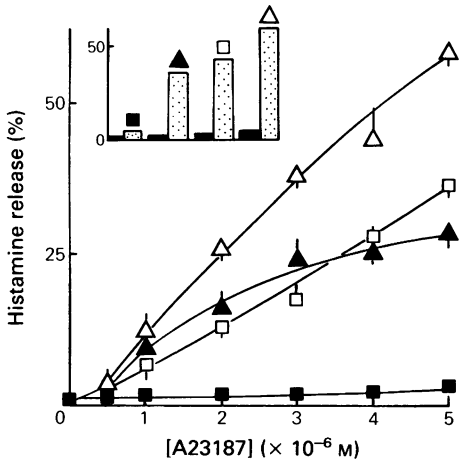


**Figure 6** Effect of various concentrations of ionophore A23187 on the secretion of histamine in the absence of added calcium and in the presence or absence of potassium and magnesium. Mast cells were washed in a calcium- and potassium-free buffer. The pellet was resuspended in the same buffer but without magnesium. Cells were preincubated at 37°C for 45 min in the presence of 2.7 mM KCl and 1 mM $MgCl_2$ (balanced medium, ■), in the presence of 2.7 mM KCl ($MgCl_2$ deprived cells, □), in the presence of 1 mM $MgCl_2$ (KCl deprived cells, ▲) or in the absence of both KCl and $MgCl_2$ (KCl and $MgCl_2$ deprived cells, △). Secretion was initiated by adding ionophore A23187 and was stopped 10 min later. The kinetics of histamine release were similar under the different conditions used and were fully completed 1.5 min after the addition of ionophore. Values are the means of 4 experiments with s.e.means shown by vertical lines. Controls were performed (insert), under similar conditions but in the presence of 0.1 mM dinitrophenol and 5.6 mM deoxyglucose instead of glucose, (black areas) or in the presence of glucose (stippled areas); secretion was reduced with $5 \times 10^{-6}$ M ionophore A23187.

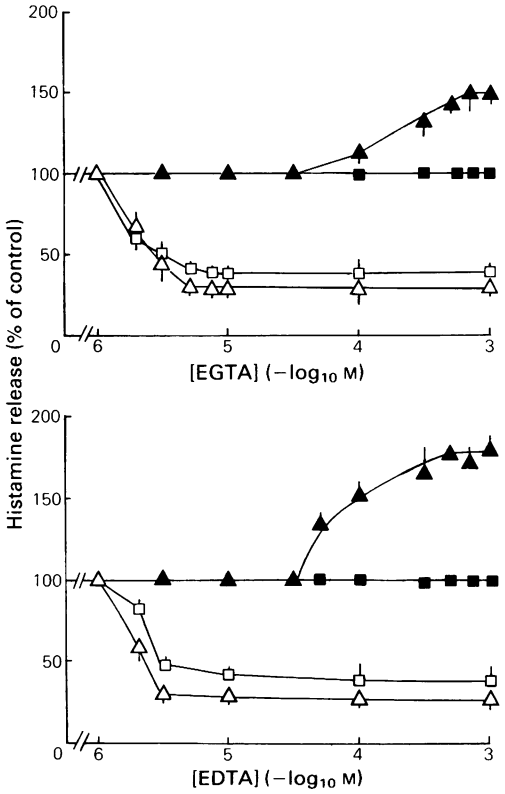


**Figure 7** Effects of EGTA and EDTA on mast cell histamine secretion induced by ionophore A23187 in the absence of added calcium and in the presence or absence of potassium and magnesium. The different batches of peritoneal rat mast cells were prepared as described in Figure 6: (■) balanced medium; (□) $MgCl_2$-deprived cells; (▲) KCl-deprived cells; (△) KCl and $MgCl_2$ deprived cells. EGTA or EDTA was added 1 min before the induction of secretion with $5 \times 10^{-6}$ M ionophore A23187. Values are the means of 4 experiments with s.e.means shown by vertical lines. Histamine secretion in the absence of EGTA or EDTA (controls) was: (■) $3.0 \pm 0.4$; (□) $40.3 \pm 0.7$; (▲) $27.2 \pm 0.9$ and (△) $62 \pm 2.4$% of total histamine content.

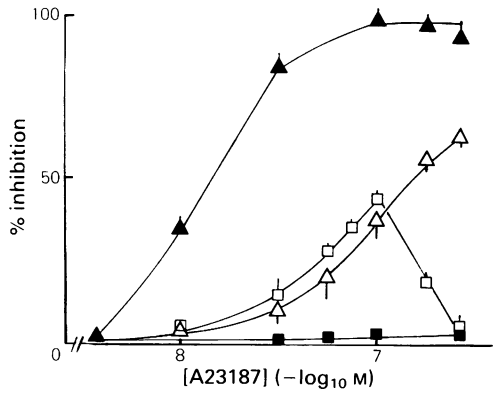


**Figure 8** Effect of preincubation of rat mast cells with low concentrations of ionophore A23187 in the absence of added calcium on histamine secretion induced with $5 \times 10^{-6}$ M ionophore A23187. Peritoneal rat mast cells were prepared as described in Figure 6: (■) balanced medium; (□) $MgCl_2$-deprived cells; (▲) KCl deprived cells; (△) KCl and $MgCl_2$ deprived cells. Mast cells were preincubated for 15 min at 37°C in the corresponding medium. Then the low concentrations of ionophore were added and the reaction was allowed to proceed for 30 min. Ionophore A23187 $5 \times 10^{-6}$ M was then added and incubation was stopped 10 min later. No secretion occurred during the first contact of cells with ionophore, provided the ionophore concentration remained below $5 \times 10^{-7}$ M. Values are the means ± s.e.mean of 4 to 6 experiments. Histamine secretion in the absence of ionophore A23187 during the preincubation period was: (■) 3.3 ± 0.2; (□) 40.6 ± 1.6; (▲) 20.3 ± 1.0 and (△) 49.6 ± 2.2% of total histamine content.

histamine release observed in the presence of both cations was not modified by up to $10^{-3}$ M of the chelating drug. In the presence of magnesium, secretion elicited from potassium-deprived cells was potentiated by high concentrations of chelating drugs with a slightly higher potency in the case of EDTA. These experiments strongly suggest that extracellular calcium corresponding to the so-called calcium-free media could be mobilized by ionophore provided no competition with extracellular magnesium occurred. However these experiments did not allow us to exclude the possibility of the involvement of some intracellular calcium store, as the short term treatment with chelating drugs did not fully abolish histamine secretion. Indeed Figure 8 shows that the pretreatment of cells with low concentrations of ionophore A23187 known to deplete cellular calcium stores (Diamant & Patkar, 1975), produced a decrease in the subsequent histamine secretion induced by a high dose of ionophore. This inhibition was total in the case of potassium-deprived cells in the presence of magnesium. In the absence of extracellular magnesium this inhibitory effect was partial.

## Discussion

The kinetic analysis of histamine release induced by compound 48/80 and the changes in membrane potential indicated that depolarization of mast cells did not initiate histamine secretion (Tasaka *et al.*, 1970; Sugiyama & Utsumi, 1979). Similar experiments performed with antigen-stimulated basophilic leukaemia cells demonstrated an apparent depolarization (Kanner & Metzger, 1983; Sagi-Eisenberg & Pecht, 1983). However these observations appeared to be related to mitochondrial membrane depolarization (Sagi-Eisenberg & Pecht, 1984). In our experiments the impairment of sodium and potassium gradients did not induce histamine release but facilitated the secretagogue-effect of antigen, compound 48/80 or ionophore A23187. In contrast the inhibition of sodium-potassium ATPase in neuronal cells (Vizi *et al.*, 1982) and in chromaffin cells (Pocock, 1983) led both to the release of mediators and to the increase of the evoked secretion. This might represent a major difference between non excitable systems, as mast cells, and excitable secretory cells. The resting plasma membrane potential in excitable cells has been found to be set predominantly by the $K^+$ diffusion potential with a small contribution from the electrogenic mechanism such as sodium-potassium ATPase. In contrast, recent results obtained with the non-excitable Lettré cells (Bashford & Pasternak, 1984) indicated that electrogenic pump(s) can generate membrane potential. In these cells increasing extracellular KCl up to 80 mM did not modify membrane potential whereas ouabain depolarized the membrane. The part played by these two mechanisms in the resting membrane potential of peritoneal rat mast cells should be determined. However, neither the increase of extracellular potassium (Pintado *et al.*, 1984) nor the inhibition of sodium potassium ATPase induced secretion, confirming the absence of voltage-gated calcium channel in peritoneal rat mast cells.

We recently proposed the involvement of sodium-potassium ATPase in the regulation of histamine secretion from mast cell elicited by antigen and compound 48/80 (Frossard *et al.*, 1983; Landry *et al.*, 1983; Amellal *et al.*, 1984). These results suggested that the increase of intracellular sodium and/or the decrease of intracellular potassium might play a role in this regulation, mainly through the control of membranous calcium stores. The present data clearly show that the modification of transmembrane gradients led to a decrease of the requirement for extracellular calcium necessary to the secretion process. Similarly, a shift of the calcium-concentration-effect curve has been described for the action of ouabain on the heart (Salter *et al.*, 1949) and for the effect of dihydroouabain on the papillary muscle (Reiter, 1963; 1981). Micromolar levels of extracellular calcium were

sufficient to allow mast cell secretion with ionophore A23187, through an energy-dependent mechanism, provided magnesium was absent from the medium. The absence of extracellular magnesium was also required to observe the inhibitory effect of EDTA and EGTA. The interference by magnesium with ionophore A23187, EDTA and EGTA have to be considered together with the possibility of a decrease of intracellular magnesium due to the absence of magnesium in the assay medium. Such a decrease of cellular magnesium might modulate secretion as shown in rabbit neutrophils (Di Virgilio & Gomperts, 1983). However, the absence of magnesium did not modify mast cell secretion induced with compound 48/80 (unpublished observation). Thus we consider that the present effects linked to magnesium are predominantly relevant to extracellular events.

The increase of cytosolic calcium allowing the activation of cells is usually considered to be from $10^{-8}$–$10^{-7}$ to $10^{-6}$ M–$10^{-5}$ M. Our results suggest that the modification of sodium and potassium transmembrane gradient facilitates an equilibrium between extracellular and cytosolic calcium levels, whatever the stimulus used, calcium ionophore, compound 48/80 or antigen. Such an equilibrium also implies a decreased efficiency of the different mechanisms allowing the efflux of calcium from the cytosol to the outside of the cell or to intracellular compartments. Alternatively, an increase of the affinity for calcium of intracellular calcium targets, such as calmodulin and protein kinase C, can be suggested.

The depletion of intracellular calcium stores with long term treatments by chelating drugs or with low doses of ionophore (Diamant & Patkar, 1975) led to a decrease of mast cell secretion with greater effect in the presence of magnesium (Figure 8). Further experiments with other secretagogues are required in order to explain this observation.

In conclusion the present results and our previous observations demonstrate that the modification of transmembrane sodium and potassium gradients allows a potent histamine secretion related to a minute influx of calcium.

This work was supported by the CNRS (ATP 0982 immunopharmacologie) and the INSERM (CRE 83-5008). We thank A. Exinger for making the calcium measurements.

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*(Received December 18, 1984.*
*Revised March 27, 1985.*
*Accepted April 5, 1985.)*