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Sodium-potassium ATPase inhibition potentiates compound 48/80-induced histamine secretion from mast cells

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1 The effect of ouabain on the histamine secretion induced by compound 48/80 has been studied using rat peritoneal mast cells.

2 Ouabain did not modify histamine release in the presence of millimolar concentrations of extracellular calcium.

3 However, when mast cells were previously washed with a calcium-free buffer, ouabain strongly potentiated histamine release elicited by compound 48/80.

4 The full potentiation of mast cell secretion by ouabain required 30 min preincubation before adding compound 48/80. It was inhibited by lanthanum and EGTA.

5 Potassium deprivation mimicked the effect of ouabain. A 30 min preincubation time without potassium was also required.

6 Potassium concentrations below 2.7 mM increased the effect of ouabain whereas higher potassium concentrations reversed this effect.

7 The potentiation of compound 48/80-induced histamine release by ouabain or potassium deprivation was not immediately reversed by washing away ouabain or by adding potassium, respectively.

8 The data confirm that sodium-potassium ATPase is involved, through a calcium-dependent process, in the regulation of histamine release from mast cells.

Introduction

Digitalis glycosides cause an increase in the release of secretory products from a variety of tissues. This has been shown for acetylcholine (Elmqvist & Feldman, 1965; Birk & Cohen, 1968; Paton *et al.*, 1971; Meyer & Cooper, 1981; O'Fallon *et al.*, 1981), for catecholamines (Banks, 1967; Nakazato *et al.*, 1978; Reading & Isbir, 1980; Lorenz *et al.*, 1980; Palaty, 1981; Wakade, 1981; Vizi *et al.*, 1982; Pocock, 1983a), for vasopressin (Douglas, 1974) and for insulin (Lowe *et al.*, 1976). These results suggest a critical role for sodium-potassium ATPase in the control of secretion phenomena, the inhibition of the enzyme leading to the increase of secretion. We recently proposed such a role for sodium-potassium ATPase in the antigen-induced histamine release from rat peritoneal mast cells (Frossard *et al.*, 1983). However, these results were in contrast with several earlier findings which suggested that ouabain did not modify histamine secretion from rat mast cells (Fewtrell & Gomperts, 1977; Magro, 1977b) or from human basophils (Magro, 1977a). Our concern in the present experiments was to reconsider the effect of sodium-potassium blockade on histamine secretion from rat peritoneal mast cells using compound 48/80 as a trigger of the secretory process.

Methods

Male Wistar rats weighing 250 to 300 g were killed by stunning and exsanguination. Eight ml of buffered salt solution were injected into the peritoneal cavity. The body was gently massaged for 2 min. The peritoneal fluid was collected and centrifuged for 2 min at 220 *g*. The pellet was resuspended in buffered salt solution (composition mM: NaCl 137, KCl 2.7, $MgCl_2$ 1, $CaCl_2$ 0.5, NaH_2PO_4 0.4, glucose 5.6, HEPES 10, pH 7.4) and washed twice. Alternatively, $CaCl_2$ and/or KCl were omitted from the medium as indicated in legends. Cells (80,000 to 100,000 ml^{-1})

were preincubated at 37°C in the appropriate medium and histamine release was initiated by the addition of compound 48/80 ($0.2\,\mu g\,ml^{-1}$). After 1 min, the incubation was terminated by the addition of 1 ml of ice-cold buffer with the same composition as the incubation medium (histamine secretion was complete after 10 s at 37°C). Tubes were cooled in iced water and centrifuged at 220 *g* for 2 min at +4°C. Each assay was performed in duplicate. Supernatants were collected and histamine concentrations were determined according to the fluorimetric method of Shore *et al.* (1959). The spontaneous histamine released, determined in the absence of compound 48/80 and in the absence or presence of 5×10^{-4} M ouabain was 7% or 12% respectively, of the total histamine content measured for each sample after treatment of the cell suspension with trichloracetic acid. No analytical interference of the compounds used was observed under our conditions.

Lanthanum chloride was obtained from Fluka; g-strophanthin (ouabain) from Boehringer-Mannheim; compound 48/80 from Sigma; HEPES (2 - [4 - (2 - hydroxyethyl] - 1 - piperazinyl) - ethanesulfonic acid) and EGTA (ethylene glycol bis [B-aminoethylether]-N, N, N′, N′-tetraacetic acid) from Merck. All other chemicals were of analytical grade.

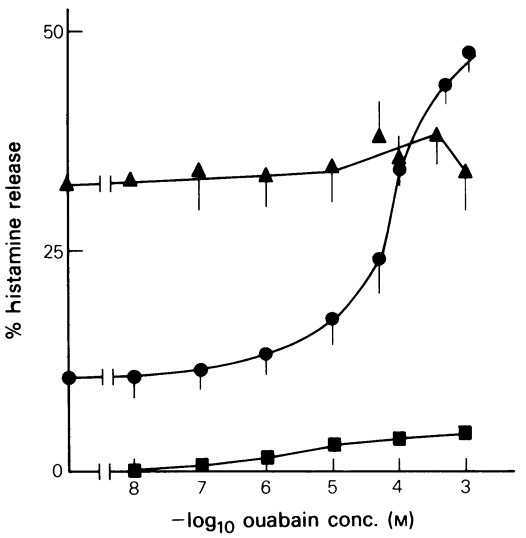

Figure 1 Effects of varying the concentration of ouabain on the histamine release induced by compound 48/80 from peritoneal rat mast cells. Cells were washed twice in the absence (●,■) or presence of 1 mM $CaCl_2$ (▲) and preincubated for 60 min with ouabain. Secretion was induced by adding $0.2\,\mu g\,ml^{-1}$ compound 48/80 (●, ▲). Alternatively controls were performed omitting the secretagogue (■). Values are the means ± s.e.mean of six experiments performed in duplicate.

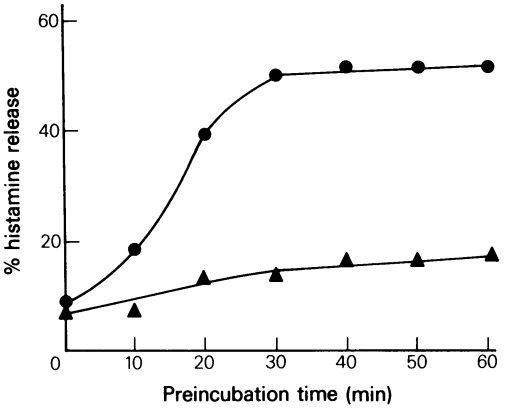

Figure 2 Effects of varying the preincubation times, of peritoneal rat mast cells, with ouabain, on the secretion of histamine induced by compound 48/80. Cells were washed twice, suspended in the buffered saline medium which included 5×10^{-5} M $CaCl_2$, and preincubated at 37°C in the absence (▲) or presence (●) of 5×10^{-4} M ouabain. Secretion was induced by adding $0.2\,\mu g\,ml^{-1}$ compound 48/80. Values are the means of two experiments performed in duplicate.

Results

Potentiation by ouabain of histamine secretion induced by compound 48/80

The effects of compound 48/80 and ouabain were studied on two aliquots of rat peritoneal mast cells from the same animal. Cells were either washed with the buffered salt solution containing calcium or with the same buffer deprived of calcium. Figure 1 shows that in the absence of ouabain, compound 48/80 ($0.2\,\mu g\,ml^{-1}$) induced the release of 11% of the total histamine from cells washed and incubated without calcium. Ouabain caused a dose-dependent (10^{-6} to 10^{-3} M) increase in histamine release under these conditions. This effect required a 30 min preincubation of the cells with ouabain before the addition of compound 48/80 (Figure 2). In the presence of calcium 1 mM, histamine secretion reached 33% of the total content. Ouabain 10^{-6} to 10^{-3} M failed to modify this secretion observed in the presence of calcium 1 mM, even after a preincubation period of up to 1 h. The highest concentrations of ouabain slightly increased the spontaneous histamine release.

Calcium related effect of ouabain

Rat peritoneal mast cells were washed with the calcium-free buffer and then supplemented with various calcium concentrations (Figure 3). In the presence of 5×10^{-4} M ouabain, the secretion of histamine induced by compound 48/80 was maximum in

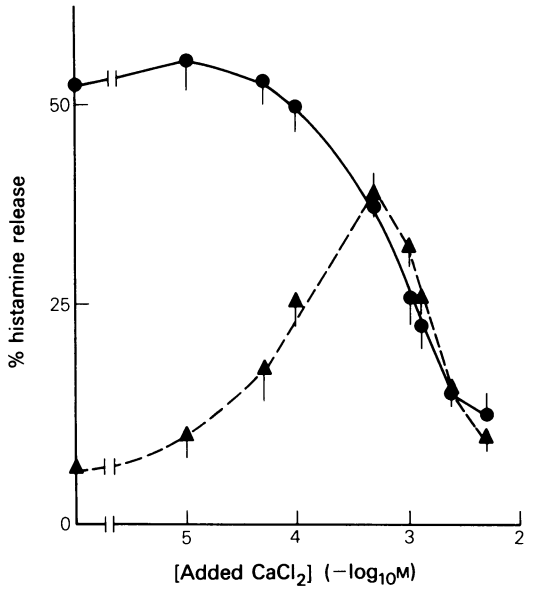

Figure 3 Effects of varying the concentrations of calcium on the secretion of histamine from peritoneal rat mast cells induced by compound 48/80 in the absence (▲) or presence (●) of 5×10^{-4} M ouabain. Cells were collected and washed twice with calcium-free medium, preincubated for 60 min at 37°C before adding $CaCl_2$. Compound 48/80 (0.2 μg ml^{-1}) was added one min later to induce histamine secretion. Values are the means ± s.e.mean of six experiments performed in duplicate.

the absence of added calcium to the incubation medium. Adding calcium up to 5×10^{-5} M did not significantly modify histamine release. Higher concentrations of calcium (from 5×10^{-4} M) led progressively to a decrease in the secretion, reaching similar secretion levels to those observed in the absence of ouabain. When ouabain was omitted (control) histamine secretion induced by compound 48/80 was increased by supplementing cells with calcium concentrations up to 5×10^{-4} M. Higher calcium concentrations led to a decrease in histamine secretion. This decrease in the release of histamine in the presence of high calcium concentrations could be overcome by increasing the concentration of compound 48/80 (Figure 4). Similar observations were observed by Atkinson *et al.* (1979). Cochrane *et al.* (1982) also showed that the addition of ionophore A23187 was able to restore maximum histamine secretion in the presence of the highest calcium concentrations. Altogether these data were in agreement with the suggestion of Atkinson *et al.* (1979), that the decreased effect of 48/80 in the presence of high calcium concentrations might be related to competition between 48/80 and calcium. The use of low calcium concentrations allowed us to observe vigorous histamine secretion using low concentrations of compound 48/80. We usually triggered mast cell secretion with 0.2 μg ml^{-1} 48/80 whereas 1 μg ml^{-1} or even higher concentrations are currently used.

The following experiments were performed in order to determine the significance of results obtained in the absence of added calcium. Figure 5a shows that lanthanum from 10^{-8} to 10^{-5} M inhibited the histamine secretion induced by compound 48/80, both in the presence or absence of ouabain, from mast cells washed and incubated without added calcium. Higher lanthanum concentrations led to multiphasic phenomena previously observed by Pearce & White (1981) and Frossard *et al.* (1983). Similar experiments were performed with EGTA. Figure 5b shows that 50% of the secretion induced in the presence of ouabain was inhibited by 10^{-4} M EGTA. In the absence of ouabain, micromolar concentrations of EGTA slightly increased histamine release. EGTA 10^{-4} M almost completely inhibited the release.

Potassium deprivation and histamine release

Ouabain is well known to be a selective inhibitor of sodium-potassium ATPase. However, other targets might exist for this drug. The activity of sodium-

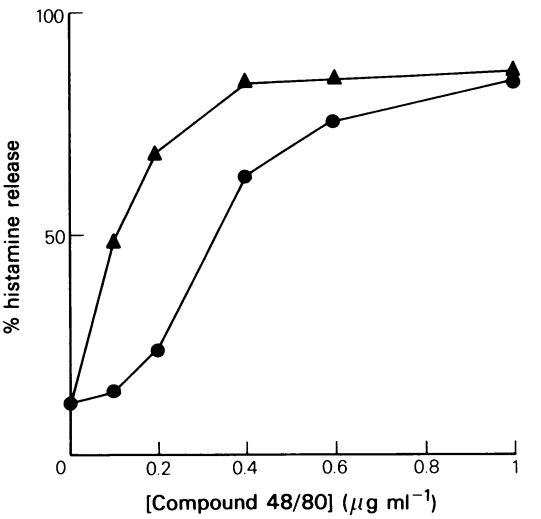

Figure 4 Effects of varying the concentrations of compound 48/80 on the secretion of histamine from peritoneal rat mast cells in the presence of 5×10^{-4} M (▲) or 2×10^{-3} M (●) $CaCl_2$. Cells were collected and washed twice with calcium-free medium, preincubated for 10 min at 37°C before adding $CaCl_2$. Compound 48/80 was added one minute later to induce histamine secretion. Values are the means of two experiments performed in duplicate.

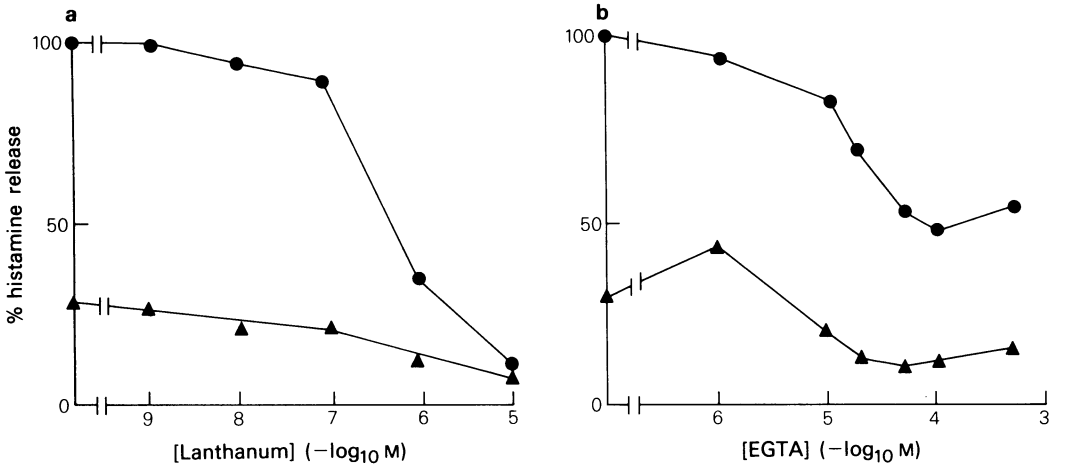

Figure 5 Effects of (a) lanthanum chloride and (b) EGTA on the secretion of histamine from rat peritoneal mast cells in the absence (▲) or presence (●) of 5×10^{-4} M ouabain. (a) Cells were preincubated with (●) or without (▲) ouabain for 45 min at 37°C. Then La^{3+} was added and secretion induced 15 min later with 48/80 (0.2 μg ml^{-1}). The control release of histamine (absence of lanthanum) was 54% of total histamine content. (b) Cells were preincubated for 60 min at 37°C with EGTA. Then ouabain was added when indicated (●) and secretion induced 60 min later with 48/80 (0.2 μg ml^{-1}). The control histamine release (in the absence of EGTA) was 54% of total histamine content. Values are the means of three duplicate experiments.

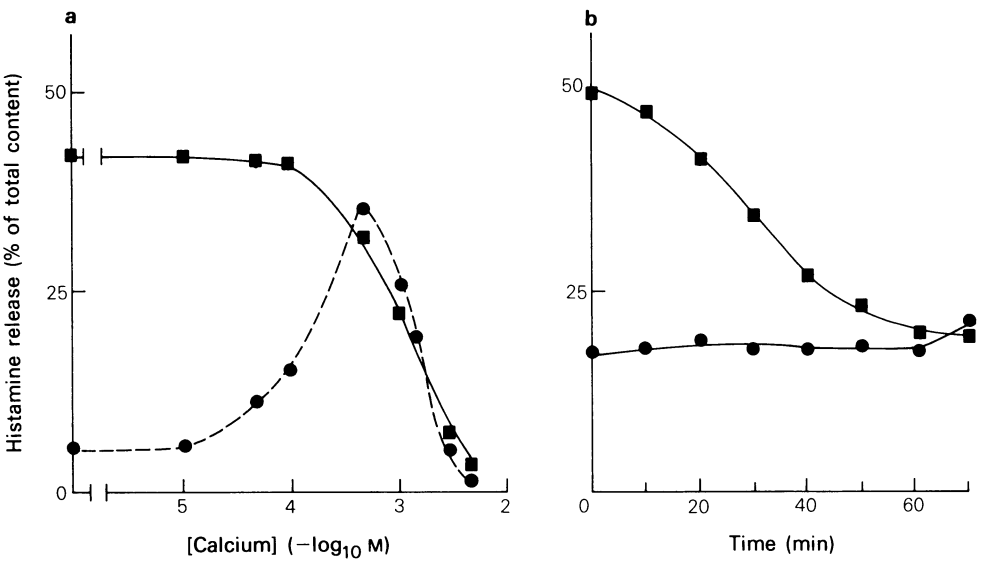

Figure 6 Effect of potassium deprivation on the secretion of histamine from rat peritoneal mast cells induced by compound 48/80 in relation to calcium concentration. (a) Cells were washed twice with calcium-free buffer including 2.7 mM potassium chloride (●) or in the absence of potassium chloride (■). Cells were preincubated for 45 min at 37°C, then calcium chloride was added. Secretion was induced 1 min later by the addition of compound 48/80 (0.2 μg ml^{-1}). Values are the means of three experiments performed in duplicate. (b) Cells were washed twice in the absence of calcium and potassium, resuspended in the presence (●) or the absence (■) of potassium chloride and incubated for 60 min at 37°C. Then, 2.7 mM potassium was added at time zero to cells previously depleted (■) and compound 48/80 (0.2 μg ml^{-1}) added from 10 to 70 min later. Values are means of two duplicated experiments.

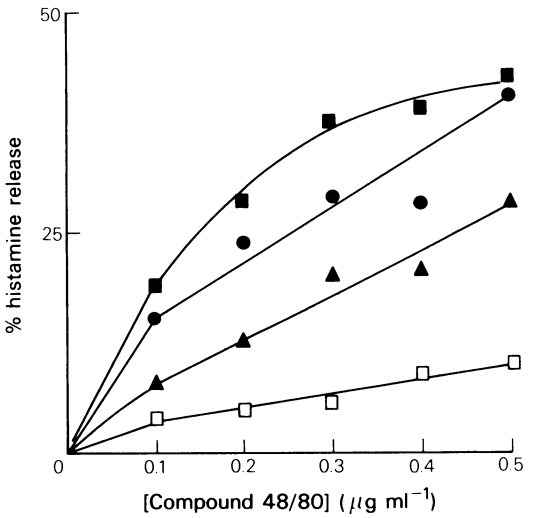

Figure 7 Effects of varying the concentrations of compound 48/80 on the secretion of histamine from rat peritoneal mast cells with the various conditions reported in Figures 1, 3 and 6: cells in calcium-free buffer (□); cells in the presence of 10^{-4} M calcium (▲), cells in calcium-free buffer preincubated for 60 min with 5×10^{-4} M ouabain (●), cells in calcium and potassium-free buffer (■). Values are means of two experiments performed in duplicate.

potassium ATPase can also be inhibited by potassium deprivation. Figure 6a shows that potassium deprivation exactly mimicked the effect of ouabain on the release of histamine induced by compound 48/80. The addition of calcium to the medium was not required in order to observe a maximum secretion. Calcium concentrations over 10^{-5} M decreased the secretion elicited by compound 48/80 in the absence of potassium. As shown in Figure 2 for ouabain, the full effect of potassium deprivation required preincubation of mast cells for 30 min before adding compound 48/80 (results not shown). Moreover, Figure 6b shows that the addition of potassium to cells previously preincubated without potassium did not immediately reverse the effect of potassium deprivation. A full restoration was observed after 60 min. Similarly, the effect of ouabain was not quickly reversed when mast cells were washed and resuspended in the balanced, buffered salt solution (results not shown). Figure 7 indicates that the blockade of sodium-potassium ATPase using ouabain or potassium-free medium potentiated the release of histamine triggered by compound 48/80. The secretion phenomena were dependent on the dose of compound 48/80 whatever the conditions used.

Potency of ouabain in relation to potassium concentrations

Figure 8a shows that the inhibitory effect of potassium was dose-dependent up to 1.5 mM. Potassium did not modify the spontaneous release observed in

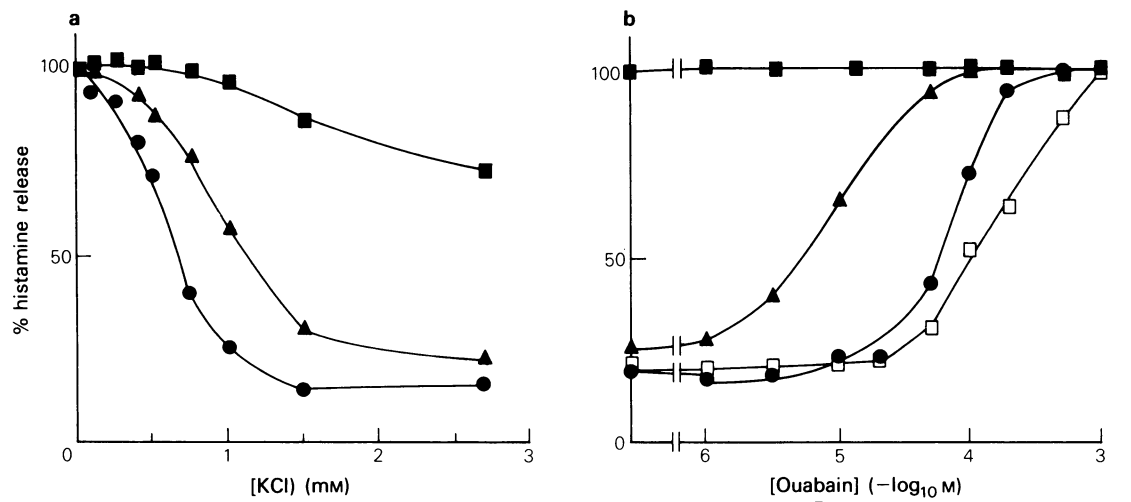

Figure 8 Potency of ouabain in relation to potassium concentrations, on the secretion of histamine from peritoneal mast cells induced by compound 48/80. Cells were washed twice in the absence of calcium and potassium. Potassium and/or ouabain were added. Cells were preincubated for 45 min at 37°C and secretion induced by adding compound 48/80 (0.2 μg ml^{-1}). (a) Various concentrations of potassium were added without ouabain (●) or together with ouabain 10^{-5} M (▲) or 10^{-4} M (■). Values are means of two duplicated experiments. (b) Various concentrations of ouabain were added without (■) or together with 1 mM (▲), 2.7 mM (●) or 5 mM (□) potassium chloride. Values are means of duplicated experiments.

the absence of compound 48/80 under the conditions used. Ouabain added in the presence of low concentrations of potassium prevented the inhibition of the induced histamine release. Similar results are plotted in Figure 8b in relation to the concentration of ouabain, allowing a more clear illustration of the interaction between ouabain and potassium. The concentration-effect curve for ouabain was shifted to the left in the presence of 1 mM potassium (a concentration smaller than the physiological (2.7 mM) potassium level) and shifted to the right in the presence of a higher potassium concentration.

Discussion

This study clearly shows that sodium-potassium ATPase is involved in the regulation of histamine release, from rat mast cells, induced by compound 48/80, confirming our recent observation with antigen triggered mast cells (Frossard *et al.*, 1983). We show in the present paper that a long preincubation time with ouabain or in the absence of potassium was required to observe their potent effects. Washing out ouabain or adding potassium to pretreated cells did not immediately reverse these effects (Figure 6). Sodium potassium ATPase blockade decreases the active influx of potassium and the active efflux of sodium. The time courses of the effects of ouabain and of potassium deprivation and the time courses of their reversal strongly suggest that these effects are linked to modifications of intracellular sodium and/or potassium levels rather than to the inhibition of ATPase activity *per se*. The modification by ouabain or potassium deprivation of compound 48/80-induced histamine secretion in relation to the calcium concentration (Figure 3) were quite similar to the effect seen when preincubating mast cells at 2°C for 30 min (Garland & Payne, 1979). These authors did not comment on the role of sodium and potassium in their experiments. However, cold is usually thought to abolish ionic gradients and might modify mast cell secretion in a similar way to ouabain and potassium deprivation.

The concentrations of ouabain required in the present experiments were rather high but were in good agreement with those needed to inhibit rat sodium-potassium ATPase (Erdman *et al.*, 1980). This low sensitivity of rodent ATPase to ouabain, in comparison to other species, suggested the use of other tools to inhibit the enzyme. The combined effect of ouabain and potassium deprivation and the reversal of the ouabain effect by increasing the concentration of potassium (Figure 6) clearly demonstrate the involvement of sodium-potassium ATPase in the effect of ouabain. Similar results for the effects of combining ouabain and potassium were recently obtained by Pocock (1983a) on catecholamine secretion from bovine adrenal glands. The bovine tissue reacted to smaller ouabain concentrations but was affected by the same concentrations of potassium as referred to for rat mast cells. Perhaps the evidence would be more convincing if the modification of mast cell histamine release by inhibitors of sodium-potassium ATPase were correlated to their efficiency at inhibiting the enzyme activity in mast cells. However, as previously demonstrated by Magro (1977b), we observed that sodium-potassium ATPase represented only a small percentage of the total ATPase activity of mast cell membranes, precluding reliable quantification of the inhibitor's efficiency. This difficulty should be overcome by measuring the inhibition of sodium and potassium fluxes.

The increase in intracellular sodium and/or the decrease in intracellular potassium following ATPase inhibition might potentiate histamine release by promoting an increase in cytosolic calcium. Cytosolic calcium would stimulate calcium-binding proteins, similar to the effect of calmodulin involved in the secretion phenomena (Douglas & Nemeth, 1982; Amellal & Landry, 1983). The increase in cytosolic calcium might be caused by several different mechanisms: (1) an increase in calcium influx from the extracellular medium as has been currently proposed to explain the inotropic effect of ouabain and related digitalis glycosides (review Schwartz, 1976); (2) a decrease in the active efflux of calcium linked to the calcium ATPase as has been recently documented by Pocock (1983b) in the release of catecholamine from adrenal gland; (3) the increase in intracellular sodium might also facilitate the release of calcium from internal stores such as mitochondria (Silbergeld, 1977; Crompton *et al.*, 1978), or endoplasmic reticulum, or from stores embedded into the plasma membrane (Gervais *et al.*, 1977).

The potentiation, through sodium-potassium ATPase inhibition, of histamine release induced by antigen or compound 48/80 was observed in the absence of added calcium or in the presence of low external calcium concentrations. In the absence of added calcium, the fact that the potentiation of histamine release was inhibited by low concentrations of lanthanum or EGTA suggests that some minute influx of calcium might occur from the medium or from some sequestered stores of the plasma membrane. The release from internal stores may not be implicated in those conditions. Pearce & White (1981) suggested that the inhibitory effect of lanthanum in the absence of added calcium might reflect a stabilizing action of the lanthanide on the cell membrane and a subsequent inhibition of the sequestered stores utilized in the initiation of exocytosis. This proposal is in agreement with the hypothesis that sodium-potassium ATPase blockade might lead to an in-

creased pool of membrane-bound calcium (Gervais *et al.*, 1977). Such an increase might fully explain the potentiation observed in mast cells. The lack of potentiation in the presence of millimolar concentrations of external calcium might be due to some competition between external calcium and deeply buried membrane calcium stores. Histamine release in the presence of an optimal extracellular calcium concentration might be due predominantly to calcium from the external environment. Membrane stores, filled as a consequence of sodium-potassium ATPase inhibition, would be mobilized when external concentrations of calcium do not allow a full response to the secretagogue. Alternatively, as suggested by Pearce *et al.* (1981), extracellular calcium might prevent the mobilization of membrane stores.

We conclude that sodium-potassium ATPase might play a part in the regulation of mast cell secretion mainly through the control of membrane calcium stores, modulating the accessibility of calcium to calmodulin-sensitive enzymes involved in secretion. This hypothesis will have to be proved by measuring sodium, potassium and calcium fluxes in the presence and the absence of sodium-potassium ATPase blockade.

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