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SODIUM-POTASSIUM ATPase, CALCIUM, AND IMMUNOLOGICAL HISTAMINE RELEASE

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Digitalis glycosides and potassium-deprivation cause an increase in the release of secretory products, neurotransmitters and hormones, from most tissues (for review see refs. 1,2). Such an increase of secretion linked to sodium-potassium ATPase inhibition has not yet been observed in the immunological release of mediators from rat mast cells (3,4) or human basophils (5). Moreover, ouabain produced an inhibition of histamine secretion elicited by antigen in passively-sensitized guinea pig lung (6,7). We recently showed that the inhibition of sodium-potassium ATPase strongly potentiated mast cell histamine release induced by compound 48/80 (8). The present work shows that immunological histamine release might also be regulated by sodium-potassium ATPase activity. The potentiation of release observed in response to sodium potassium ATPase inhibition is dependent on extracellular calcium concentrations.

Material and Methods

Male Wistar rats (220 to 230 g) were sensitized by an intraperitoneal injection of 20 µg ovalbumin in 0.3 ml of 2 % $Al(OH)_3$ suspension (9). A second injection of 20 µg ovalbumin in saline was performed 26 to 40 days later. Rats were killed 4 days after this booster challenge by decapitation and exsanguinated. Tris buffer, (NaCl 112 mM, KCl 5 mM, $CaCl_2$ 0.9 mM, glucose 5.6 mM and trishydroxymethylaminomethane 25 mM-HCl, pH 7.2) was injected (10 ml) in the peritoneal cavity. The peritoneal fluid was collected and centrifuged for 2 min at 220 g. Cells were washed twice in Tris buffer devoid of calcium and/or potassium (see legends). Cells were preincubated at 37°C in the same buffer for 30 min (KCl experiments) or 60 min (ouabain experiments). Then calcium was added if required. Secretion process was induced 5 min later adding ovalbumin. After 5 min, the tubes were cooled in iced water and centrifuged for 7 min at 220 g. Histamine was measured in the supernatants as previously described (9). Spontaneous release of histamine was determined simultaneously in the absence of antigen stimulation, allowing to calculate the antigen-dependent histamine release expressed as a percentage of total cellular histamine content (% antigen-induced histamine release). Ouabain (g-strophantin) was obtained from Boehringer - Mannheim (FRG), lanthanum chloride from Fluka (CH), ovalbumin grade V from Sigma (USA).

Results and discussion

The effect of sodium-potassium ATPase inhibition was studied on the antigen-induced

histamine release from sensitized rat peritoneal mast cells. Fig. 1a shows that in the presence of calcium, ouabain up to 10^{-3}M fails to produce any significant modification of histamine release. However, in a calcium-free medium, the very low release obtained in response to antigen was markedly increased by ouabain from 10^{-5} to 10^{-3}M. A 45 min preincubation of cells with digitalis glycoside was required to observe a full potentiation effect (not shown). The potent doses of ouabain in these experiments were similar to those usually reported in secretion studies, whereas smaller concentrations (10^{-7} to 10^{-5}M) were usually effective in the case of catecholamine secretion from adrenal medullary cells (2,10). Fig. 1b shows that in the absence of extracellular calcium, the slight release observed in response to antigen was markedly potentiated by ouabain. As in the presence of calcium, a bell-shaped curve was obtained. A maximum release was obtained with 10^{-6} g/ml ovalbumin, with mast cells from booster challenged rats (present method of sensitization), whereas 10^{-4} g/ml was necessary with cells from animals sensitized by a single injection of antigen (9).

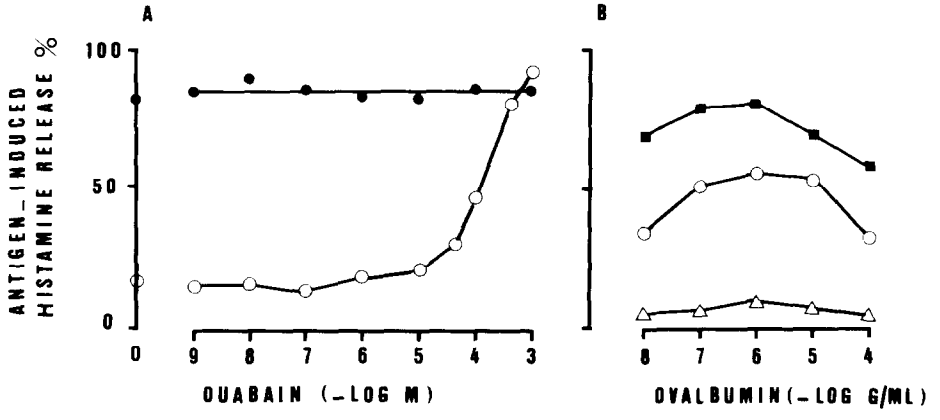

Fig. 1 : Effect of ouabain on antigen-induced histamine release from sensitized rat peritoneal mast cells. A. Dose response curve of ouabain on histamine release induced by 10^{-6} g/ml ovalbumin in the presence (●) or absence (○) of extracellular calcium (0.9 mM). B. Dose response curve of ovalbumin in a calcium free medium in the presence (○) or absence (△) of ouabain ($5x10^{-4}$M). Simultaneous controls (■) were performed without ouabain in the presence of calcium (0.9 mM).

Ouabain and other digitalis glycosides are generally considered as selective inhibitors of sodium-potassium ATPase. However, other cellular targets might exist for these drugs (for a discussion see ref. 11). Consequently, potassium-deprivation was used as another tool to inhibit sodium-potassium ATPase activity. Fig. 2 shows that omitting potassium in the assay medium mimicked the effect of ouabain on the release of histamine induced by antigen. Potassium deprivation slightly stimulated the secretion induced by antigen in the presence of calcium and allowed to observe a potent secretion in the absence of calcium (fig. 2a). The antigen-induced curve was maintained in the absence of extracellular potassium and calcium (fig.2b).

Fig. 3a shows that in the absence of sodium-potassium ATPase blockade, the release of histamine induced by allergen was calcium-dependent from $5x10^{-5}$ to $5x10^{-4}$M. In the presence of ouabain, the release was maximum even in the absence of added calcium. However, fig. 3b shows that lanthanum concentrations from 10^{-8} to 10^{-6}M were able to inhibit the release linked to added calcium (experiments in the presence of calcium, without ouabain) but also the release observed in the presence of ouabain without added calcium. Lanthanum concentrations higher than 10^{-6}M led to multiphasic phenomena previously reported by Pearce and White (12).

Low concentrations of lanthanum displace calcium ions from membranous outer sites and inhibit calcium influx. Consequently the present results suggest that some minute calcium influx might be involved in the potentiating effect of sodium-potassium ATPase blockade of antigen-induced histamine release from rat peritoneal mast cells.

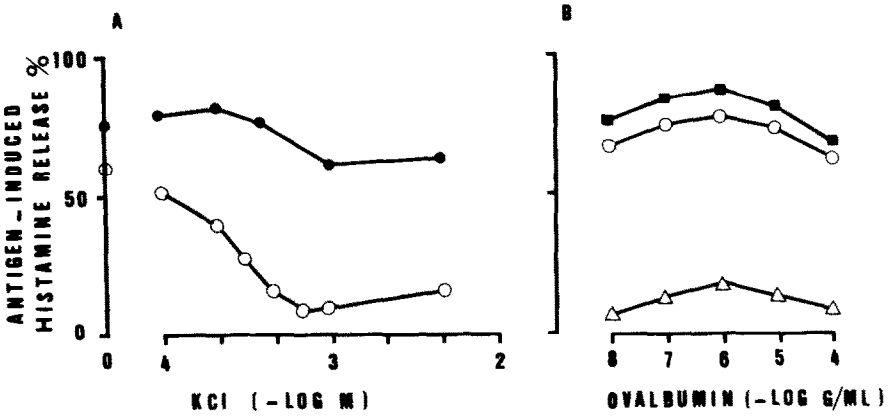

Fig. 2 : A. Effect of potassium concentrations on antigen (ovalbumin 10^{-6} g/ml)-induced histamine release, in the presence (●) or absence (○) of calcium (0.9 mM). B. Dose response curve of antigen (ovalbumin) in calcium-free medium in presence (△) or absence (○) of potassium. Simultaneous controls (■) were performed in the presence of calcium (0.9 mM) and potassium (5 mM).

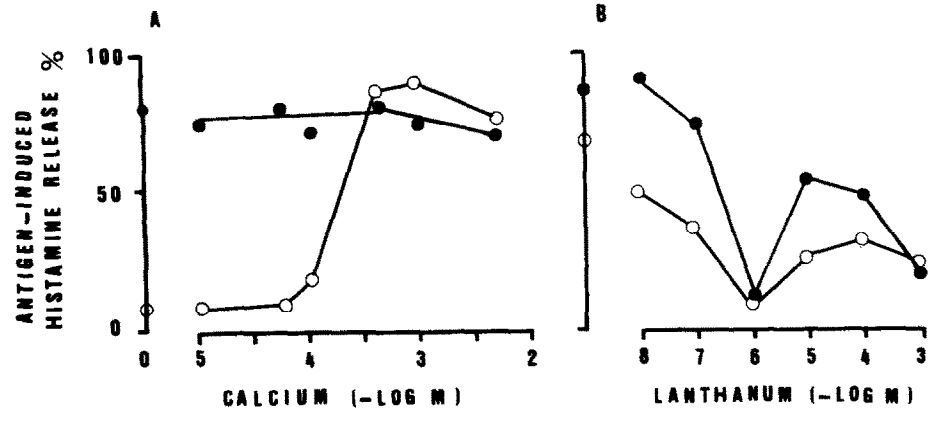

Fig. 3 : Effect of calcium and lanthanum on antigen (ovalbumin 10^{-6} g/ml)-induced histamine release. A. Effect of calcium concentrations in the presence (●) or absence (○) of 5×10^{-4} M ouabain. B. Effect of lanthanum concentrations in the presence of calcium (0.9 mM) without ouabain (●), and in the presence of ouabain (5×10^{-4} M) without calcium (○).

In conclusion, sodium-potassium ATPase appears to be involved in the regulation of immunological release of mast cell mediators in a way very similar to that of most secretory processes. The failure of other workers to observe an effect of ouabain on histamine release from isolated cells (3-5) was linked to the use of high calcium levels in their assay medium. Moreover, we suggest that the inhibition of histamine release by ouabain observed in sensitized guinea pig lung (6,7) might not be related to a reactivity of lung mast cells similar to that of renin secretory cells (13,14), but might rather be linked to an effect of ouabain on lung sympathetic fibers leading to catecholamine secretion (15,16). Catecholamine

would then act on lung mast cells to inhibit secretion process (17). We suggest that sodium-potassium ATPase has now to be taken into account in the regulation of calcium-dependent stimulus-secretion coupling in mast cells, together with cyclic nucleotides and phospholipid metabolism. Indeed, sodium-potassium ATPase activity and calcium ions seem to be closely intricated in the regulation of secretion. On one hand, the increase of intracellular calcium following a physiological stimulus might lead to the inhibition of sodium-potassium ATPase (18,19). On the other hand, exogenous blockade of sodium-potassium ATPase might decrease extracellular calcium requirement. Several hypothesis may be proposed to take into account this observation. Sodium-potassium ATPase inhibition might increase membranous calcium pool (20). Such bound calcium might be mobilized to trigger secretion. Moreover, the exogenous blockade of sodium-potassium ATPase might induce, through an unknown mechanism, a decrease of calcium-ATPase activity, i.e. of active calcium efflux (21). These two phenomena might be implicated in the increase of cytosolic calcium required to allow secretion.

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