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Calcium dependent modulation of histamine release from mast cells by sodium and potassium

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Abstract

The inhibition of sodium–potassium ATPase by ouabain or potassium-deprivation led to a potentiation of histamine release induced by antigen or compound 48/80 from rat mast cells. The fullest potentiation required 30 min preincubation of cells with ouabain or without potassium before adding the triggering agent. The potentiation was observed provided that the calcium concentration was less than 0.5 m*M*. The effect of sodium–potassium ATPase blockade was reversed slowly by washing out ouabain or by adding potassium. Metabolic inhibition with deoxyglucose and dinitrophenol-blocked histamine release observed under all conditions described.

Histamine release from rat mast cells was also elicited when the cells were incubated in sodium-deficient media where sodium was replaced by sucrose. Potassium-deprivation potentiated this secretion process previously shown to be dependent upon metabolic energy and sensitive to millimolar calcium concentrations.

Introduction

The increase of cytosolic calcium is currently considered to be the main event allowing histamine release from mast cells and is now well characterized. In contrast the role of monovalent cations in this secretion process remains unclear. The relationship between $^{22}Na^{+}$ uptake by, and histamine release from, rat mast cells exposed to compound 48/80 has been studied by SLORACH and UVNÄS [1]. Whereas histamine release was complete after 30 sec, Na uptake was not maximal until after 10 min. The changes in membrane potential of rat mast cells under the effect of compound 48/80 or ATP was measured with a microelectrode [2] or a fluorescent dye [3] and demonstrated that depolarization of the mast cell membrane was dissociated from degranulation. In contrast, recent studies with IgE-stimulated rat basophilic leukaemia cells [4, 5] allowed the conclusion that the immunological degranulation was associated with changes in the membrane potential in these cells. However, the ionic nature of these membrane potential changes is not resolved. They might be associated with an increased calcium influx as well as with an increased sodium influx [5].

Some other experiments suggested a critical role for sodium and potassium in the regulation of histamine release in rat mast cells. COCHRANE and DOUGLAS [6] showed that histamine release was elicited when the cells were exposed for 10 min to sodium-deficient media where all NaCl had been replaced by KCl, RbCl, glucose, sucrose, mannitol or Tris, provided the calcium concentration was less than about 0.5 m*M*. This response was inhibited by dinitrophenol combined with glucose deprivation. It was concluded that sodium-lack may mobilize calcium from a cellular site, possibly the inner face of the plasma membrane. Such an effect could result from the operation of a sodium–calcium counter transport mechanism [6]. Recent results by PEARCE and WHITE [7] also supported the presence in the rat mast cell of such a sodium–calcium antiporter allowing calcium efflux. We recently proposed [8] the involvement of sodium–potassium ATPase in the control of histamine secretion induced from rat mast cell by antigen [9] and by compound 48–80 [10]. The inhibition of sodium–potassium ATPase by ouabain or potassium deprivation led to a potentiation of histamine release provided the extracellular calcium concentration was less than 0.5 m*M*. In the present study we show that the mechanism of histamine release observed before in a sodium-deficient medium [6] might be similar

to that elicited by antigen or compound 48/80 in which sodium–potassium ATPase is inhibited.

Materials and methods

Male Wistar rats (250–350 g) were killed by decapitation and exsanguinated. Eight ml of buffered-salt solution (containing (m*M*): NaCl, 137; KCl, 2.7; $MgCl_2$, 1; glucose, 5.6; HEPES, 10; pH, 7.4), were injected into the peritoneal cavity and the abdomen was gently massaged for 2 min. The cell suspension was collected and centrifuged at 220 *g* for 2 min. In some of the experiments, mast cells were purified up to 95% using a gradient of bovine serum albumin (30 and 40%). The pellet was collected in the appropriate medium and washed twice. The tonicity of sodium-deficient media (0, 10 or 20 m*M*) was corrected by adding sucrose (274, 254 or 234 m*M*). Cells (50,000 to 80,000/ml) were incubated at 37°C in a final volume of 600 μl. Histamine release was induced by adding compound 48/80 1 min before stopping the reaction with 1 ml of ice-cold buffer and cooling the tubes in iced water. The tubes were centrifuged at 220 *g* for 2 min at 4°C and the histamine content of the supernatant was determined according to the fluorometric method of SHORE et al. without extraction. No analytical interference was observed for any of the compounds used in our conditions.

Results are expressed in percent of the total histamine content of the cells. Results obtained with compound 48/80 were corrected for spontaneous release (absence of inducer), which ranged from 5 to 7% of total histamine content. Compound 48/80 was obtained from Sigma, ouabain (g-strophantin) from Boehringer–Mannheim and HEPES (2-[4-2-hydroxyethyl]-1-piperazinyl-ethane-sulfonic acid) from Merck. All other chemicals were of analytical grade.

Results and discussion

The Table shows that histamine release induced by compound 48/80 was potentiated when mast cells were preincubated for 45 min with ouabain provided that the calcium concentration in the medium was less than 0.5 m*M*.

Cells washed in the absence of calcium respond poorly but significantly to compound 48/80. This response reached a maximum value when cells were pretreated with ouabain. The control effect of compound 48/80 was increased by the presence of calcium up to 5×10^{-4} *M*, higher calcium concentrations leading to a decrease of the triggered histamine release whether cells were pretreated with ouabain or not. The ouabain pretreatment did not lead to significant histamine release in the absence of triggering agent. However, metabolic inhibition performed with dinitrophenol and replacing glucose by deoxyglucose abolished secretion elicited by compound 48/80 in control cells as well as in mast cells pretreated with ouabain. These data show that the effect of ouabain was not linked to some unspecific alteration of mast cells. The

Table

Effect of metabolic inhibition on histamine release induced by compound 48/80 from peritoneal rat mast cells in the presence of various concentrations of $CaCl_2$.

$CaCl_2$ (*M*)	Histamine release (% of total)		
	Control cells	Ouabain-treated cells	
	Glucose	Glucose	Deoxyglucose and DNP
0	7	37	1
1×10^{-5}	10	34	2.5
5×10^{-5}	22	34	3.5
1×10^{-4}	26	32	4.5
5×10^{-4}	30	26	3
1×10^{-3}	17	13	5
5×10^{-3}	6.5	5	4

Rat peritoneal cells were preincubated for 45 min in the absence (control) or presence of 5×10^{-4} *M* ouabain in calcium-free saline buffers containing either glucose (5.6 m*M*) or deoxyglucose (5 m*M*) and DNP (dinitrophenol, 0.1 m*M*). Calcium chloride was added and histamine secretion was induced by incubation with compound 48/80 (0.2 μg/ml) for 1 min.

effect of ouabain was dose-dependent in a concentration range similar to that shown to be effective in inhibiting the activity of rat sodium–potassium ATPase. Ouabain is well known to be less potent in the rat than in other animal species. Moreover the dose-effect curve of ouabain was shifted to the left by decreasing the potassium concentration in the medium. The potentiation of histamine release was also correlated to the preincubation time and reached a maximum after 30 min. The ouabain effect was reversed by washing but the complete reversion was time-dependent, requiring 45 to 60 min. Its action decreases the active influx of potassium and the active efflux of sodium, thus slowly leading to a decrease of cytosolic potassium and to an increase of cytosolic sodium. These modified sodium and potassium gradients seem to facilitate the triggering of mast cells by compound 48/80 [10] and by antigen [9], namely decreasing the requirement for extracellular calcium.

Figure 1 shows results obtained using potassium-deprivation instead of ouabain in order to inhibit sodium–potassium ATPase. Control experiment contained 2.7 m*M* KCl. When histamine secretion was elicited by compound 48/80 in the presence of low calcium concentrations the preincubation of cells with a decreased con-

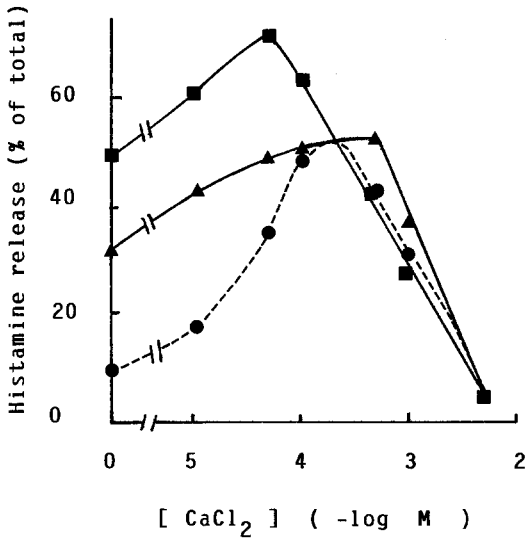

Figure 1
Effect of various concentrations of $CaCl_2$ on histamine release induced by compound 48/80 from peritoneal rat mast cells preincubated for 45 min in a calcium-free saline buffer in the absence of KCl (■) or in the presence of 0.5 (▲) or 2.7 (●) m*M* KCl. After preincubation cells were centrifuged for 2 min at 220 *g* and resuspended in a balanced saline medium containing 2.7 m*M* KCl. Indicated concentrations of $CaCl_2$ were added and histamine secretion was induced 1 min later by adding 0.2 μg/ml compound 48/80.

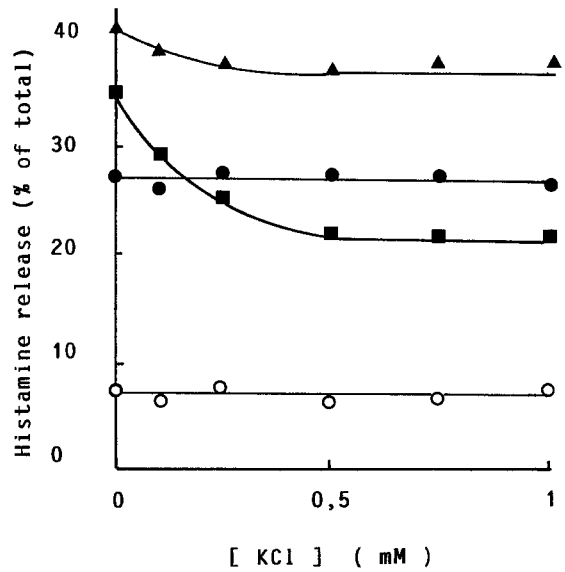

Figure 2
Effect of varying the potassium concentration on histamine release from purified peritoneal rat mast cells induced by sodium deprivation. Purified mast cells were incubated for 45 min at 37°C in calcium-free media containing the indicated concentrations of KCl in the absence of NaCl (●) or in the presence of 10 (▲), 20 (■) or 137 (○) m*M* NaCl. Isotonicity was maintained with sucrose.

centration of KCl or in the absence of KCl led to a potentiation of the induced release. In the illustrated experiment, cells preincubated in potassium-deprived medium were complemented which potassium just before adding the secretagogue. This result confirms the dose-dependence of potassium deprivation and the time-dependent reversion of the potentiating effect of potassium-deprivation [10].

Some major features of histamine secretion induced by compound 48/80 or antigen in conditions where sodium–potassium ATPase was inhibited [9, 10 and above results], were similar to those determined in the case of histamine release by exocytosis on reduction of extracellular sodium [6]. In both cases optimal secretions were observed in calcium-free media but were in fact calcium-requiring as evidenced by the inhibitory effect of EDTA, EGTA and lanthanum. It might be proposed that sodium-lack, as well as potassium-lack, inhibited sodium–potassium ATPase. However, why did sodium deprivation lead to a 'spontaneous' exocytosis whereas potassium-lack only led to a potentiation of evoked secretion by conventional secretagogues? A preliminary experiment in an attempt to resolve this problem is shown on Fig. 2. In the presence of normal sodium concentrations in the incubation medium, potassium-deprivation did not modify spontaneous release under the experimental conditions used. When no sodium was present (sucrose medium), the efficient histamine release was not significantly modified by potassium-deprivation. Similarly potassium-deprivation did not modify secretion induced by compound 48/80 in the presence of maximal ouabain concentrations [10]. However, when 10 or 20 m*M* sodium were present in the medium, potassium-deprivation potentiated histamine release (Fig. 2). This result suggests that mast cell exocytosis observed on reduction of extracellular sodium might also involve sodium–potassium ATPase. Related modifications of cytosolic monovalent cations might be different under the two sets of experimental conditions used. The inhibition of sodium–potassium ATPase in the presence of normal extracellular sodium level led to a decrease of intracellular potassium parallel to an

increase of intracellular sodium. In a sodium-deficient medium both cytosolic and potassium levels should decrease allowing exocytosis, through a not yet known, but energy and calcium-dependent, mechanism.

Acknowledgments

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