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Non cytotoxic guinea-pig mesenteric mast cell stimulation by protamine[1]

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Abstract

Protamine stimulates guinea-mesenteric mast cells in a concentration-dependent manner, both histamine release and mast cell degranulation being correlated. Mast cell stimulation is blocked by 2,4-DNP (0.03 m*M*), low (0 °C) and high (45 °C) temperature. The inhibitory effect by 2,4-DNP is reversed by glucose (5.0 m*M*), while incubation at 37° reverses that by low and high temperature. Lack of calcium from the incubation medium does not influence mast cell stimulation by protamine. However calcium chelation with EDTA (2.0 m*M*) or EGTA (2.0 m*M*) blocks mast cell stimulation. Addition of calcium (0.9 m*M*) reverses this inhibition. These observations indicate that guinea-pig mast cell stimulation by protamine is a nonlytic, energy and calcium dependent process, similar to anaphylaxis, but different from that of other basic compounds which induce mast cell lysis.

Introduction

Protamine is a polycationic peptide commonly used after several surgical procedures because of its capacity to reverse the anti-coagulant activity of heparin [1, 2]. There are indirect evidences that the adverse reactions to protamine reported clinically could be caused by the release of histamine [3]. Basic compounds stimulate rat mast cells by a non-lytic process similar to anaphylaxis. In the guinea-pig however they cause mast cell lysis [4]. We have shown that in the rat protamine also induces a non-lytic degranulation [5]. In the present paper evidences of non-lytic guinea-pig mast cell stimulation by protamine are presented.

Materials and methods

Guinea-Pigs (300 g) of either sex were killed by a blow on the head and bled from the jugular veins. The mesentery was dissected away from the small intestine and divided. The pieces were pre-incubated in Tyrode (136 m*M* NaCl, 2 m*M* KCl, 0.9 m*M* CaCl, 0.8 m*M* $MgSO_4$, 50 m*M* Tris-HCl buffer, pH 7.3) for 20 min and incubated for 30 min more after addition of protamine (Grade II, Sigma). When an inhibitory effect was studied, the pieces were pre-incubated in the presence of the inhibitor (2,4-dinitrophenol Grade II, Sigma, and p-hydroxymercuribenzoate, Sigma) for 30 min. In the experiments to evaluate the role of calcium the pieces were pre-incubated in Tyrode containing 2 m*M* EDTA (Merck) without calcium and magnesium or in Tyrode containing 2 m*M* EGTA (Sigma) without calcium. All experiments were performed at 37 °C, except when indicated.

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The histamine was assayed according the fluorimetric method of Shore et al. [6]. Histamine release was expressed as a percentage of the total and was corrected for release occurring in the absence of protamine (about 2%). In order to count mast cells the mesentery was fixed in a 1% lead subacetate solution containing 50% ethanol and 3% acetic acid for 20 min and stained in a 0.1% toluidine blue solution containing 1% acetic acid for 10 min. To evaluate degranulation mast cells were counted 100 microscopical fields in each piece (400×magnification). The results are expressed as a percentage of mast cells, controls being considered as 100%.

Results

Histamine release and mast cell degranulation

Incubation of guinea pig mesentery with protamine induced histamine release and mast cell degranulation. This mast cell stimulation was concentration-dependent, 400 μg/ml of protamine causing about 60% of histamine release and reduction in mast cell number (Fig. 1). At this concentration of protamine 20% histamine release and mast cell degranulation occurred after 1 min incubation, most of the secretion having occurred by 4 min (Fig. 2).

Varying the pH from 6.5 to 8.5 did not influence histamine release or mast cell degranulation by protamine at 400 μg/ml (Fig. 3), at 800 or 1200 μg/ml.

Inhibition of histamine release and mast cell degranulation

Histamine release and mast cell degranulation by protamine (400 μg/ml) was inhibited by 2,4-dini-

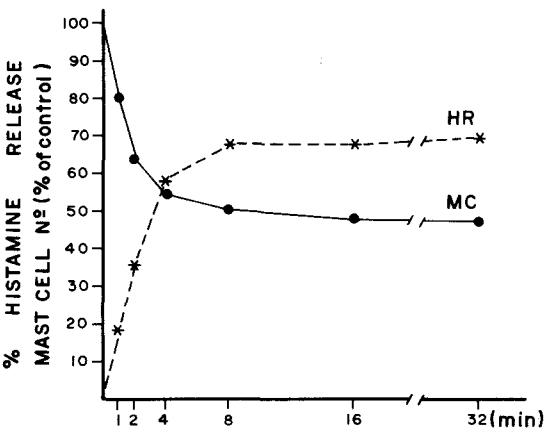

Figure 2
Pieces of mesentery were incubated (37 °C) in the presence of protamine (400 μg/ml). All Values are means, $n=6$. MC = mast cell degranulation; HR = histamine release.

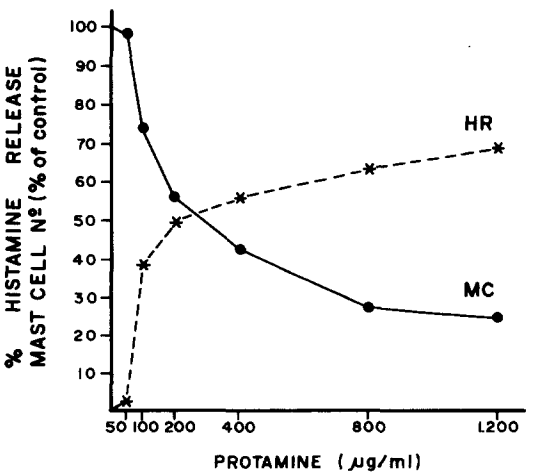

Figure 1
Pieces of mesentery were incubated (37 °C) for 30 min. All values are means, $n=8$. MC = mast cell degranulation; HR = histamine release.

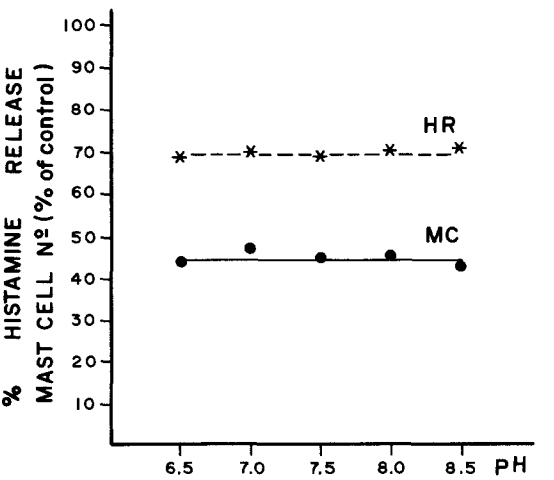

Figure 3
Pieces of mesentery were incubated (37 °C) in the presence of protamine (400 μg/ml) for 30 min. All values are means, $n=8$. MC = mast cell degranulation; HR = histamine release.

Table 1
Effect of metabolic inhibitors and temperature on mast cell degranulation and histamine release by protamine (400 μg/ml).

Additions to medium	% MC degran.	% inhib.	% HR	% inhib.	
–	70.6	–	65.3	–	(6)
DNP (0.03 m*M*)	35.1	50.7	24.4	66.2	(6)
DNP (0.03 m*M* + Glucose (5.0 m*M*)	68.2	–	66.5	–	(6)
–	60.3	–	51.5	–	(4)
Hg benz (0.3 m*M*)	34.2	46.7	28.7	54.5	(4)
37 °C → 37 °C	61.0	–	62.0	–	(8)
45 °C → 45 °C	11.2	81.5	3.6	95.2	(8)
0° → 0°	14.3	76.7	4.2	93.5	(8)
45 °C → 37 °C	60.4	–	56.0	–	(8)
0° → 37 °C	66.3	–	62.3	–	(8)

Pieces of mesentery were pre-incubated in the presence of inhibitors for 30 min and incubated for 30 min more with protamine. In the experiments with temperature the pieces were pre-incubated at 45 °C or 0 °C for 30 min. After protamine was added the pieces were kept 30 min more at 0 °C, 45 °C or 37 °C. Controls did not receive protamine and were incubated at 37 °C. All values are means. ( ) no. of animals. HR = histamine release, DNP = 2,4-dinitrofenol, HG benz = Hydroxymercuribenzoate. MC = Mast cells.

Table 2
Effect of calcium on mast cell degranulation and histamine release by protamine (400 μg/ml).

EDTA	Calcium	% MC degran.	% inhib.	% HR	% inhib.
–	+	58.5 (8)	–	53.6 (4)	–
–	–	56.4	–	48.9	–
+	+	53.2	–	61.3	–
+	–	5.0	91.5	12.5	76.7

Pieces of mesentery were pre-treated with EDTA (2.0 m*M*) in a Tyrode solution without calcium and magnesium for 3 hours. Pieces not submitted to pre-treatment with EDTA were kept in Tyrode solution without calcium and magnesium. The pieces were incubated with protamine for 30 min with or without calcium. All values are means, ( ) = no. of animals. HR = histamine release. MC = Mast cells.

trofenol and p-hidroxymercuribenzoate. Glucose reversed the effect of DNP (Table 1). Low (0 °C) and high temperature (45 °C) also inhibited histamine release and mast cell degranulation. Subsequent incubation at 37 °C fully reversed this inhibition (Table 1).

Effect of calcium and magnesium

Lack of calcium from the incubation medium did not influence histamine release or mast cell degranulation by protamine. Pretreatment with 2.0 m*M* EDTA inhibited almost completely mast cell secretory response, and when 0.9 m*M* calcium was added to the medium this response was restored (Table 2). Lack of magnesium or addition to EDTA quelated mesentery pieces did not influence the histamine release or mast cell degranulation even in the absence of calcium. Pretreatment with 2.0 m*M* of EGTA (3 hours) also inhibited histamine release and mast cell degranulation.

Discussion

The present results show that protamine produces a dose-dependent guinea-pig mesenteric mast cell stimulation, histamine release and mast cell degranulation being correlated. Protamine induced morphological mast cell changes are characterized by disappearance of the cytoplasmic granules which are never found scattered around the cell, thus being similar to those caused by antigen and differing from other basic compounds [4].
Guinea pig mast cell stimulation by protamine is a process very similar to that induced by anaphylaxis. In both mast cells are stimulated within a few seconds after exposure, the processes being complete in about 8 min [7], the pH range of maximal action is equivalent [7, 8] and are blocked by metabolic inhibitors and by changes in temperature, while other basic compounds are potentiated with metabolic inhibitors and not blocked by temperature [9, 10, 11]. Unlike anaphylaxis [8] and ionophore A23187 [12] guinea pigs mast cell stimulation by protamine is not dependent on external calcium. However, cal-

cium is necessary for the action of protamine since pre-treatment with EDTA or EGTA blocks the stimulating effect. In conclusion, we show that protamine stimulates guinea-pig mast cells through a non-lytic, energy-requiring, calcium-dependent process, similar to anaphylaxis, but different from that of other basic compounds [4, 13].

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