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SOME STUDIES ON THE RELEASE OF HISTAMINE FROM MAST CELLS STIMULATED WITH POLYLYSINE

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1 Polylysine is an extremely potent releaser of histamine from rat peritoneal mast cells. Isolated mesenteric mast cells of the rat also respond to the secretagogue but guinea-pig mesenteric cells are unreactive.

2 The release does not require the presence of extracellular calcium ions but shows some dependence on internal stores of the cation.

3 The effect of polylysine is blocked by extremes of temperature and by metabolic inhibitors.

4 The release is very rapid and is virtually complete within 10 s of adding the inducer.

5 The release is unaffected by the anti-allergic drug, doxantrazole, but is inhibited by theophylline and disodium cromoglycate. The latter compounds are effective in both the presence and absence of added calcium. This result is discussed in terms of the postulated effect of the drugs on calcium transport.

Introduction

Immediate, type I, hypersensitivity reactions are characterized by the immunologically-induced release of histamine and other mediators from mast cells and basophil leucocytes (Kazimierczak & Diamant, 1978). These mediators act on distinct effector cells to produce the symptoms of allergy and anaphylaxis. Histamine may also be selectively released from mast cells by a variety of drugs and other chemical compounds (Kazimierczak & Diamant, 1978). Of these, polybasic substances such as compound 48/80 and peptide 401 (the mast cell degranulating peptide from bee venom) are remarkably potent liberators of the amine. Recent work in our laboratories (Atkinson, Ennis & Pearce, 1979; Ennis & Pearce, 1979; Pearce, Atkinson & Ennis, 1979a; Pearce, Atkinson, Ennis, Truneh, Weston & White, 1979b; Ennis, Atkinson & Pearce, 1980a; Ennis, Truneh, White & Pearce, 1980b) has shown that studies with these secretagogues may provide new information both about the mechanism of histamine release and the mode of action of anti-allergic drugs which interfere with this process. As part of this investigation, we now wish to describe some of the characteristics of the histamine release induced by polylysine. Previous work (Padawer, 1970; Baxter & Adamik, 1978) has shown that this compound is effective in promoting histamine secretion from and degranulation of mast cells but the mechanism of the process has not been studied in detail.

Methods

Mixed peritoneal cells were recovered from male and female Lister-hooded rats (150 to 250 g) by lavage with modified Tyrode solution as previously described (Atkinson *et al.*, 1979). The buffer had the composition (mM): NaCl 137, glucose 5.6, KCl 2.7, NaH_2PO_4 0.4, $CaCl_2$ 1.8, $MgCl_2$ 1 and N-2-hydroxyethyl piperazine -N′-2 ethane sulphonic acid (HEPES) 10. The pH of the solution was adjusted to 7.4 before use. Isolated rat (Lister-hooded, 150 to 250 g) and guinea-pig (Dunkin-Hartley, approx. 400 g) mesenteric cells were obtained by dispersion of the tissue with the enzyme collagenase as recently reported (Pearce & Ennis, 1980). Peritoneal and mesenteric mast cells were thereafter processed in the same way.

In simple release experiments, aliquots (1 ml) of cells were allowed to equilibrate (5 min) at the stated temperatures in a metabolic shaker with gentle mechanical agitation. A solution (10 µl) of the releasing agent was then added, secretion allowed to proceed for a further 10 min and the reaction terminated by the addition of ice-cold Tyrode solution (2 ml). Cells and supernatants were recovered by centrifugation (4°C, 2 min, 150 *g*). The cell pellets were resuspended in Tyrode solution (3 ml) and allowed to stand in a boiling water bath (10 min) to release residual histamine. Individual supernatants were treated similarly. Histamine was then estimated as before (Atkinson *et al.*, 1979). Histamine release was expressed as a

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percentage of the total cellular histamine content and was calculated as the ratio:

$$\frac{\text{histamine in supernatant}}{\text{histamine in supernatant} + \text{residual histamine in cells}} \times 100.$$

All values were corrected for the spontaneous release (approx. 5%) occurring in the absence of inducer. In some experiments, involving test drugs, histamine secretion was conveniently expressed as a percentage of the unblocked release (that occurring in the absence of the inhibitor) and was given by the ratio:

$$\frac{\text{induced histamine release in the presence of inhibitor}}{\text{induced histamine release in the absence of inhibitor}} \times 100.$$

To determine the dependence of the release on extracellular calcium, divalent cations were omitted from the incubation medium and replaced by ethylenediamine tetraacetic acid (EDTA, 10^{-4} M). The chelating agent was used in all experiments involving the absence of added calcium. To examine the requirement for intracellular calcium, cells were incubated in EDTA solution for the lengths of time noted and recovered by centrifugation (room temperature, 2 min, 150 *g*). They were then suspended in the appropriate incubation medium (1 ml) and a solution (10 µl) of the releasing agent was added as required. Secretion was estimated as before.

To determine the kinetics of the release process, aliquots (0.5 ml) of cells were stimulated with polylysine and the reaction terminated after fixed periods of time by the addition of ice-cold buffer (4.5 ml). This procedure rapidly cooled the sample and diluted the secretagogue to essentially ineffective levels. Histamine release was then determined as above.

The effect of various inhibitors was examined in the presence and absence of calcium. To investigate the metabolic requirements for histamine release, cells were preincubated (20 min) in the absence of glucose but in the presence of 2-deoxyglucose (Sigma, 5 mM) or Antimycin A (Sigma, 10^{-6} M). They were then challenged. Similarly, cells were pretreated (5 min) with theophylline (Sigma) and doxantrazole (a gift from Dr L.G. Garland of the Wellcome Research Laboratories, Beckenham) before stimulation. Disodium cromoglycate (generously provided by Dr D. Loveday, Fisons, Loughborough) was active without preincubation and was added to the cells simultaneously with the releasing agent.

Poly-D-lysine (Type IB, mol. wt. 70,000 and Type VIIB, mol. wt. 70,000), poly-DL-lysine (Type VIIB, mol wt. 64,000) and poly-L-lysine (Type IB, mol wt. 70,000; Type II, mol. wt. 3000; Type V, mol. wt. 17,000; Type VI, mol. wt. 13,000 and Type VIIB, mol. wt. 60,000) were purchased from Sigma, London. Dextran (6% w/v in saline, mol. wt. 110,000) was obtained from Fisons, Loughborough and phosphatidyl serine from Lipid Products, Redhill, Surrey.

Results

Histamine release induced by polylysine

All the samples of polylysine tested induced a comparable dose-dependent release of histamine from rat peritoneal mast cells. The samples were roughly equipotent on a weight basis. Poly-DL-lysine (Sigma Type VIIB, mol. wt. 64,000) was selected as representative and used in all subsequent experiments.

Dose-response curves for the secretagogue in the presence and absence of calcium are shown in Figure 1. The release did not require the presence of exogenous calcium and, as previously found for compound 48/80 and peptide 401 (Atkinson *et al.*, 1979), the cation in fact antagonized the secretion produced by suboptimal concentrations of inducer (see also Table 2). The release was totally independent of the presence or absence of magnesium ions (1 mM). Rat isolated mesenteric cells also responded to polylysine (Figure 2) but less strongly than the peritoneal cells. The releases induced by the secretagogue at concentrations of 10 µg/ml (31.5 ± 4.4 and 67.4 ± 4.0, means ± s.e. mean, $n = 4$) and 1 µg/ml (13.3 ± 1.4 and 29.5 ± 4.7, means ± s.e. mean, $n = 4$) were significantly lower ($P < 0.01$ and $P < 0.05$, respectively) for the tissue cells in both cases. Guinea-pig cells were almost unreactive (Figure 2). Rat peritoneal cells were used throughout the remainder of the present work and, unless otherwise stated, all experiments were carried out in the presence of calcium ions.

Requirement for intracellular calcium

Peritoneal mast cells incubated for up to 45 min in the presence of calcium showed a progressive decrease in responsiveness to subsequent challenge with polylysine, the final level of secretion being approx. 60% of the initial value (Figure 3). However, cells incubated in EDTA became more markedly desensitized ($P \approx 0.05$, paired t test) over the same period, the final secretion being approx. 40% of the starting value. Cells pretreated with EDTA for 45 min showed a significantly ($P \approx 0.05$, paired t test) enhanced reactivity

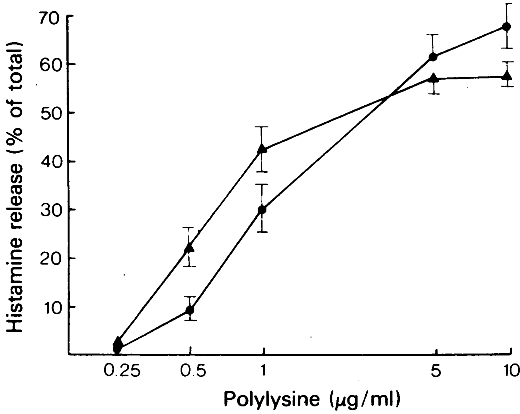

Figure 1 Log dose-response curves for the release of histamine from rat peritoneal mast cells stimulated with polylysine in the presence (●) and absence (▲) of calcium. The points are the means from four experiments and the vertical bars show s.e. mean. The release induced by polylysine at a concentration of 0.5 μg/ml was significantly greater ($P < 0.05$) in the absence than in the presence of calcium. All other pairs of values were not significantly different ($P > 0.05$).

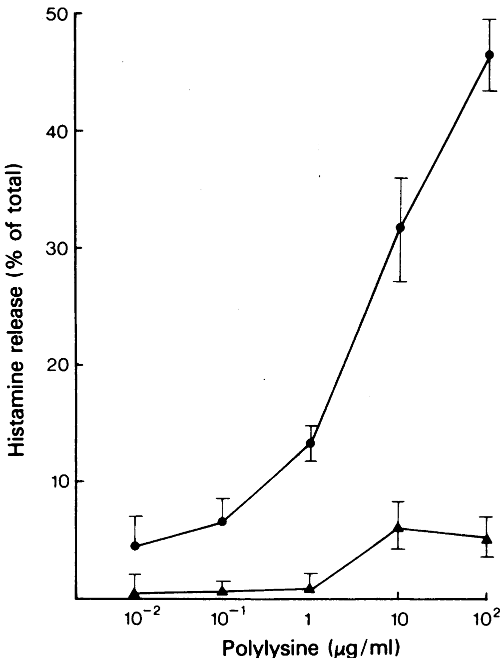

Figure 2 Log dose-response curves for the release of histamine from isolated rat mesenteric (●) and guinea-pig mesenteric (▲) mast cells stimulated with polylysine. The points are the means from four (rat) or three (guinea-pig) experiments and the vertical bars show s.e. mean.

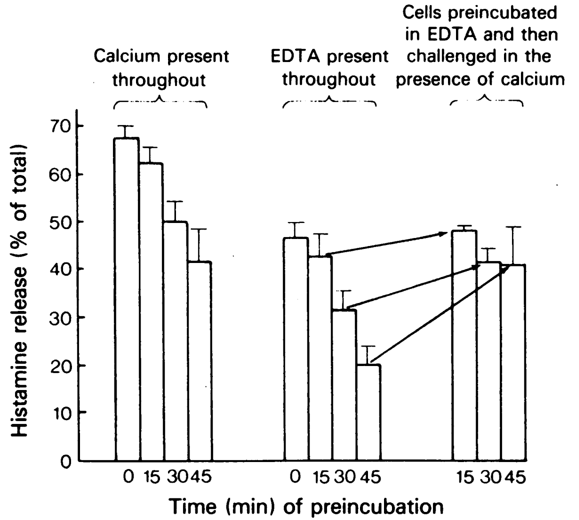

Figure 3 Effect of calcium-depletion on histamine-release induced by polylysine. Cells were preincubated in the presence of calcium or EDTA before challenge. Samples of the cells treated with the chelating agent were also recovered by centrifugation and transferred (→) to a calcium-containing medium. The cells were then stimulated with polylysine (10 μg/ml). Columns represent the means from four experiments and vertical bars show s.e. mean.

if resuspended in a medium containing calcium before challenge and did not differ in responsiveness from cells maintained in the presence of the cation for the same period (Figure 3).

Temperature dependence of the release process

Histamine release induced by polylysine was comparable at 25°C and 37°C but markedly depressed at 0°C and 45°C. The spontaneous secretion of histamine was unaffected over this temperature range.

Kinetics of histamine release

Histamine release induced by polylysine was extremely rapid and was virtually complete within 10 s of adding the inducer (Figure 4). The release was equally rapid in the absence of added calcium.

Effect of metabolic inhibition

The secretion evoked by polylysine was only slightly inhibited by the omission of glucose from the medium but this effect was more marked in the absence of extracellular calcium. The release was significantly

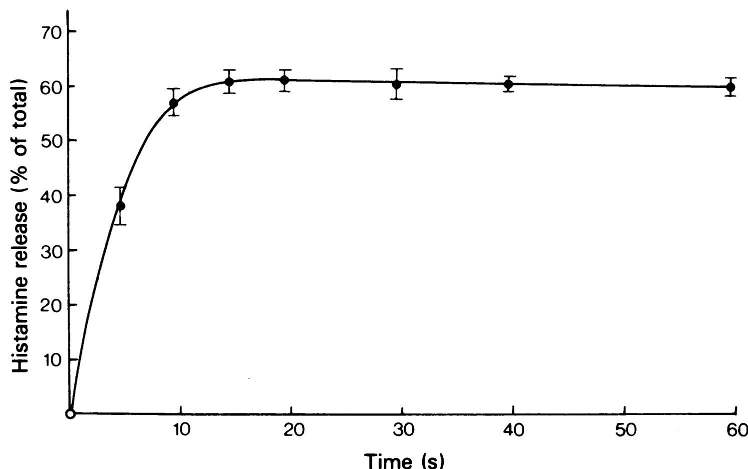

Figure 4 Kinetics of histamine release induced by polylysine (5 μg/ml). The points are the means from four experiments and the vertical bars show s.e. mean.

Table 1 Effect of theophylline on histamine release induced by polylysine

Theophylline (mM)	*Histamine release (% unblocked release) induced by polylysine at a concentration of:* 5 μg/ml (+)Calcium	5 μg/ml (−)Calcium	2.5 μg/ml (+)Calcium	2.5 μg/ml (−)Calcium	1 μg/ml (−)Calcium
1.25	71.9 ± 6.5	86.7 ± 6.3	76.3 ± 11.9	84.0 ± 0.6	74.8 ± 10.5
2.5	68.8 ± 7.1	81.2 ± 3.6	66.5 ± 10.6	79.9 ± 2.2	57.0 ± 7.8
5	59.2 ± 10.5	69.8 ± 7.0	59.5 ± 8.9	62.7 ± 2.1	37.6 ± 10.7
10	49.1 ± 8.6	42.0 ± 7.1	50.4 ± 4.6	39.4 ± 1.0	12.7 ± 11.0
20	25.2 ± 2.4	5.2 ± 1.0	30.4 ± 7.4	7.3 ± 2.6	4.0 ± 2.3

Unblocked releases (% of total histamine) were (+) calcium: 5 μg/ml, 44.3 ± 5.7 and 2.5 μg/ml, 30.9 ± 7.4 and (−) calcium: 5 μg/ml, 55.3 ± 2.4; 2.5 μg/ml, 51.1 ± 1.1 and 1 μg/ml, 26.5 ± 7.2. All values are means ± s.e. mean for three experiments.

Table 2 Effect of doxantrazole on histamine release induced by dextran and polylysine

Releaser		*Histamine release (% of total)* (−)Doxantrazole	(+)Doxantrazole
Dextran		32.9 ± 4.4	3.1 ± 1.2
Polylysine (+)calcium	2.5 μg/ml	48.7 ± 0.3	49.5 ± 0.8
	1 μg/ml	24.2 ± 2.4	25.7 ± 3.4
Polylysine (−)calcium	2.5 μg/ml	60.5 ± 1.2	60.8 ± 2.8
	1 μg/ml	54.2 ± 3.8	52.6 ± 1.3
	0.5 μg/ml	29.4 ± 4.3	29.2 ± 5.4

Cells were preincubated (5 min) with doxantrazole (500 μM) and stimulated with dextran (12 mg/ml) and phosphatidyl serine (15 μg/ml) or with the stated concentrations of polylysine. All values are means ± s.e. mean for four experiments. The unblocked releases at concentrations of polylysine of 2.5 and 1 μg/ml were significantly greater ($P < 0.001$ in both cases) in the absence of calcium than in its presence.

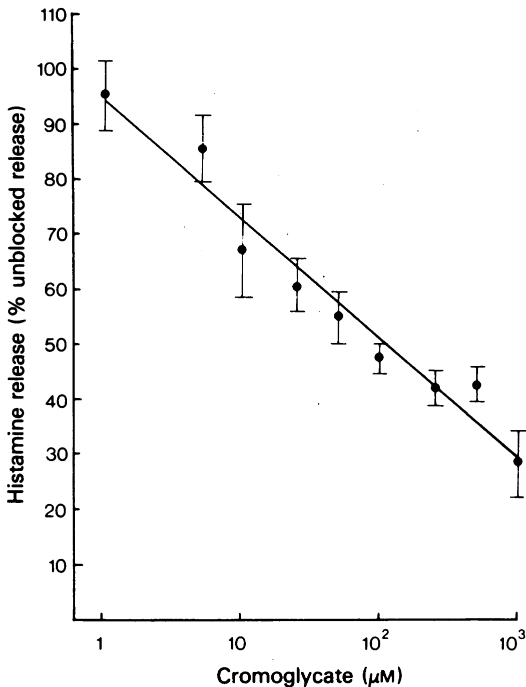

Figure 5 Effect of cromoglycate on histamine-release induced by polylysine (1 μg/ml) in the absence of calcium. Unblocked release (% total histamine) was 26.0 ± 3.0. The points are the means from six experiments and vertical bars show s.e. mean.

depressed by the inhibitor of glycolysis, 2-deoxyglucose and abolished by antimycin A which blocks oxidative phosphorylation.

Effect of theopylline, cromoglycate and doxantrazole

The effect of theophylline on the histamine release produced by various concentrations of polylysine in the presence and absence of calcium is shown in Table 1. The drug produced a dose-dependent inhibition of secretion under all the conditions tested. There was no obvious difference between the activity of the xanthine in the presence and absence of calcium but, like compound 48/80 and peptide 401 (Ennis *et al*., 1980a), the compound was most active against the lowest concentration of secretagogue. Cromoglycate was similarly most effective against suboptimal concentrations of releaser and some representative data in the absence of calcium are shown in Figure 5. In contrast, doxantrazole at concentrations up to 500 μM was inactive in preventing the release induced by polylysine but virtually abolished the secretion evoked by dextran (Table 2).

Discussion

In the present work, as in the earlier study by Baxter & Adamik (1978), polylysine was found to be an extremely potent liberator of histamine from rat peritoneal mast cells. Samples of mol. wt. 3000 to 70,000 were equipotent on a weight basis and the release was independent of the configuration of the amino acid, comparable effects being produced by polymers of the D, L or mixed DL forms of the compound. The process was non-cytolytic, being blocked by extremes of temperature (0°C and 45°C) and by the addition of metabolic inhibitors. Polylysine (mol. wt. 64,000) was maximally active at a concentration of approx. 10^{-7} M compared to concentrations of approx. 10^{-6} M for compound 48/80 (based on the molecular weight of the trimer) and approx. 2×10^{-7} M for peptide 401 (Atkinson *et al*., 1979). The latter two agents are among the most effective histamine-releasers known. Polylysine also evoked secretion from rat mesenteric mast cells but higher concentrations were required than for the peritoneal cells. Guinea-pig mesenteric cells were almost unreactive. Virtually identical behaviour was observed with compound 48/80 and peptide 401, confirming that there are marked inter- and intra-species differences in the sensitivity of mast cells to various secretagogues (Befus, Pearce, Gauldie, Horsewood, Goodacre, Cole, Heatley & Bienenstock, 1979; Pearce & Ennis, 1980).

The release induced by polylysine further resembled that evoked by compound 48/80 and peptide 401 in being largely independent of external calcium. Under these conditions, the latter secretagogues act by mobilizing internal stores of the cation (Cochrane & Douglas, 1974; Atkinson *et al*., 1979; Ennis *et al*., 1980b). Experiments to demonstrate this effect for polylysine (Figure 3) were much less convincing than those previously carried out by us for compound 48/80 and peptide 401 (Atkinson *et al*., 1979; Ennis *et al*., 1980b). Depletion of intracellular stores of calcium by preincubation with chelating agents led to a decreased sensitivity to polylysine which was partially reversed by resuspending the cells in a medium containing calcium before challenge. However, prolonged incubation in the presence of calcium also produced some reduction in response, rendering the results more difficult to interpret. Nevertheless, if an increased concentration of free calcium in the cytosol is the obligatory signal for selective histamine release (Kazimierczak & Diamant, 1978) it is reasonable to assume that polylysine acts in the absence of added calcium by mobilizing internal reservoirs of the cation. Similar conclusions were reached by Baxter & Adamik (1978) who also showed that polylysine was an effective secretagogue in the absence of exogenous calcium.

Secretion evoked by polylysine was extremely rapid

being virtually complete within 10 s in both the presence and absence of extracellular calcium. Comparable results have been obtained with compound 48/80 (Fantozzi, Masini, Blandina, Mannaioni & Bani-Sacchi, 1978) and such kinetic behaviour appears to be characteristic of basic inducers.

The effects of theophylline, cromoglycate and doxantrazole were of particular interest. These drugs are claimed to prevent the entry of external calcium into the mast cell and thus block exocytosis (Foreman, Mongar, Gomperts & Garland, 1975; Foreman, Hallett & Mongar, 1977). However, theophylline and cromoglycate inhibited the secretion induced by polylysine in both the presence and absence of added calcium. As with compound 48/80 and peptide 401 (Pearce *et al.*, 1979b; Ennis *et al.*, 1980a), the drugs were maximally active against suboptimal concentrations of releaser. The effectiveness of the compounds in the absence of extracellular calcium cannot be explained in terms of their ability to prevent movement of external ion into the cell and suggests that they may have an alternative mode of action. As previously discussed (Ennis & Pearce, 1979; Pearce *et al.*, 1979b; Ennis *et al.*, 1980a) the drugs might activate membrane pumps to extrude calcium from the cytosol, have an effect on microtubule assembly or exert an as yet ill-defined stabilizing effect on the mast cell membrane. In contrast, doxantrazole was ineffective in preventing histamine secretion evoked by polylysine but was highly active against dextran-induced release. The mechanism of action of this compound probably differs then from that of cromoglycate and theophylline and the present results are consistent with (but do not prove) its postulated effect on calcium transport.

In conclusion, polylysine was found to be an extremely potent releaser of histamine from rat peritoneal mast cells. Its action resembled that of the other basic secretagogues, compound 48/80 and peptide 401. The reasons for the great effectiveness of the polycationic inducers remains obscure and the necessary structural requirements for the activation of exocytosis have yet to be determined. Nevertheless, the basic releasers are important and useful tools in investigating both the mechanism of mediator release and the mode of action of anti-allergic drugs.

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