Br. J. Pharmac. (1981), **72**, 341–347

EFFECT OF LANTHANIDE IONS ON HISTAMINE SECRETION FROM RAT PERITONEAL MAST CELLS

F.L. PEARCE & J.R. WHITE

Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ

1 Ions of the lanthanide series (lanthanum-lutetium) inhibit histamine release induced by allergen and anti-IgE in the presence of extracellular calcium. The inhibition is dose-dependent in the range 10^{-6} to 10^{-9} M and there is no marked difference in potency between the lanthanides.

2 The response to lanthanum is biphasic and higher concentrations (10^{-4} M) potentiate the release. Maximal concentrations (10^{-3} M) again abolish secretion.

3 The effect of concanavalin A is weakly antagonized by lanthanum but strongly inhibited by higher lanthanides.

4 Inhibition of histamine release evoked by basic agents is markedly dependent on the ionic radius of the lanthanide. In the presence of extracellular calcium, dysprosium is the most effective inhibitor. Similar results are observed with dextran. In the absence of calcium, there is a regular increase in inhibition with decreasing ionic radius.

5 Inhibition of release in the presence of calcium is immediate and does not require preincubation with the lanthanide. The antagonism due to lanthanum is competitive and the pA_2 values vary with the secretagogue. In contrast, the inhibitory effect in the absence of extracellular calcium increases progressively with time.

6 These results are discussed in terms of the calcium-pools important in histamine release and the mode of action of different secretagogues.

Introduction

As in other secretory systems, histamine release from the mast cell is triggered by an elevated level of free calcium in the cytosol (Berridge, 1975). This calcium may be derived from intra- or extracellular sources according to the experimental conditions and nature of the secretagogue (Baxter & Adamik, 1978; Atkinson, Ennis & Pearce, 1979; Ennis & Pearce, 1979; Pearce, Atkinson, Ennis, Truneh, Weston & White, 1979; Ennis, Pearce, Truneh & White, 1980; Ennis, Pearce & Weston, 1980; Pearce, Ennis, Truneh & White, 1981). Binding of the cation to regulatory sites in the membrane may also modulate the uptake and mobilization of available calcium (Ennis *et al.*, 1980; Pearce *et al.*, 1981).

Lanthanum ions, and to a lesser extent other members of the lanthanide series, have been used in the study of a variety of calcium-dependent processes (Weiss, 1974; Martin & Richardson, 1979). Lanthanum has a similar ionic radius to calcium and is able to displace competitively the divalent cation from superficial sites in cell membranes. By virtue of its higher valency, lanthanum binds to these sites with greater affinity than calcium and blocks subsequent movements of calcium across the membrane. Lanthanum ions thus act as specific calcium-antagonists and have been used to differentiate the calcium-pools important in the functional response of nerve and muscle (Takata, Pickard, Lettvin & Moore, 1966; Weiss & Goodman, 1969; Weiss, 1970; Goodman & Weiss, 1971; Heuser & Miledi, 1971; Langer & Frank, 1972). The ion has also been used in the investigation of secretory processes (Borowicz, 1972; Russell & Thorn, 1972; Matthews, Legros, Grau, Nordmann & Dreifuss, 1973), including the anaphylactic release of histamine from the mast cell (Foreman & Mongar, 1973). We here describe the effects of different lanthanides on histamine release induced by a variety of secretagogues.

Methods

Mixed peritoneal cells were recovered from male and female Lister-hooded rats (150 to 250 g) by lavage with modified Tyrode solution having the composition (mM): NaCl 137, glucose 5.6, KCl 2.7, $CaCl_2$ 1 and N-2-hydroxyethyl piperazine-*N'*-2 ethane sulphonic acid (HEPES) 10. The pH of the solution was adjusted to 7.2 before use. Calcium was omitted from the medium, without the addition of chelating agents,

0007-1188/81/020341-07 $01.00

where specifically stated. In some experiments, rats were sensitized by a single subcutaneous injection of L3 larvae (4000) of the nematode *Nippostrongylus brasiliensis*. Cells were used 21 to 30 days later and secretory allergen prepared as previously described (Pearce *et al.*, 1979). Larvae were generously provided by Dr B. M. Ogilvie, National Institute for Medical Research, London.

In simple release experiments, aliquots of cells (to a final volume of 1 ml) were suspended in prewarmed buffer or allowed to equilibrate (5 min or as noted, 37°C) in the stated media in a metabolic shaker with gentle mechanical agitation. A solution of the releasing agent was then added in a minimum volume, secretion allowed to proceed for a further 10 min and the reaction terminated by addition of ice-cold Tyrode solution (2 ml). Cells and supernatants were recovered by centrifugation (2 min, 4°C, 150 *g*) and histamine determined as previously described (Atkinson *et al.*, 1979). Histamine release was expressed as a percentage of the total cellular content of the amine 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 occurring in the absence of inducer. This normally amounted to approx. 5% of the total histamine but was elevated to approx. 10% at the highest concentration (10^{-3} M) of lanthanide used. At lower concentrations, none of the lanthanides produced any increase in the spontaneous secretion. In some experiments, histamine secretion was conveniently expressed as a percentage of the unblocked release (that occurring in the absence of the lanthanide inhibitor) and was given by the ratio:

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

Compound 48/80 was a gift from Dr A. N. Payne of the Wellcome Research Laboratories, Beckenham and peptide 401 (the MCD-peptide from bee venom) was generously provided by Dr A. J. Garman of the Chemistry Department, University College London. Concanavalin A (Sigma), dextran (6% w/v in saline, mol. wt. 110,000, Fisons), phosphatidyl serine (Lipid Products) and sheep antiserum to rat IgE (Miles) were purchased as indicated. The chlorides of lanthanum (La), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), dysprosium (Dy), erbium (Er), ytterbium (Yb) and lutetium (Lu) were obtained from BDH Chemicals, Koch-Light or Lancaster Synthesis.

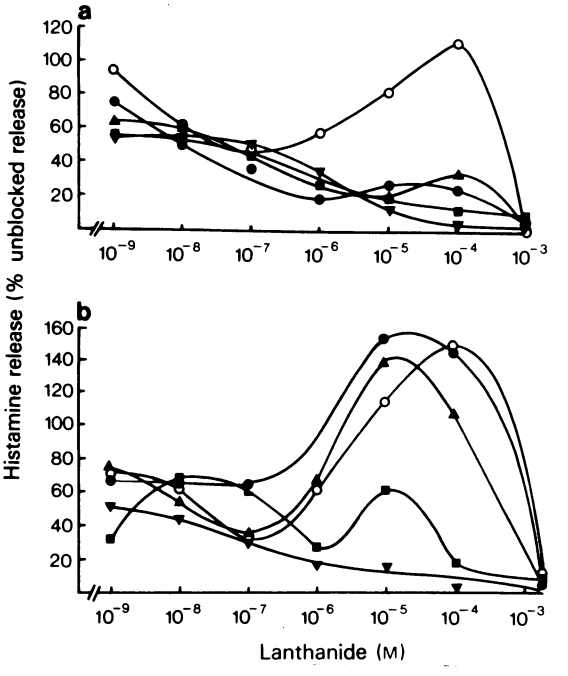

Figure 1 Effect of lanthanum (○), praseodymium (▲), neodymium (●), dysprosium (■), and lutetium (▼) on histamine release induced by (a) allergen (20 worm equivalents/ml) and (b) anti-IgE (400 fold dilution). Cells were preincubated (5 min, 37°C) with the lanthanide in the presence of calcium (1 mM) and then challenged. The points are the means from three experiments; s.e. means are omitted for clarity but did not exceed ±15% of the unblocked release. Unblocked releases (% total histamine) were (a) 34.2 ± 1.7 and (b) 36.6 ± 2.9.

The salts were dissolved in buffer immediately before use.

Results

Effect of lanthanides on IgE-directed ligands

The effect of lanthanides on histamine release induced by allergen and anti-IgE is shown in Figure 1. The noted ions (10^{-9} to 10^{-6} M) produced dose-dependent inhibition of the secretion evoked by both agonists. Similar results were obtained with samarium, europium, erbium and ytterbium. There was no marked difference in potency between the various lanthanides and no obvious dependence on ionic radius. The effect of lanthanum was biphasic: at higher concentrations (10^{-4} M) the ion potentiated the release. Secretion due to anti-IgE was also enhanced by praseodymium and neodymium (10^{-4} to 10^{-5} M). At the

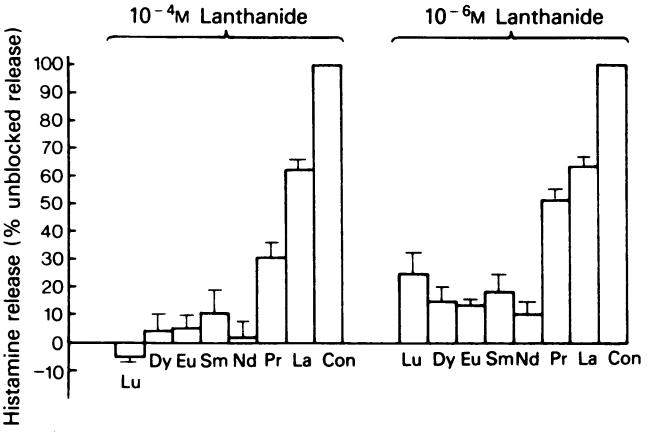

Figure 2 Effect of lanthanides on histamine release induced by concanavalin A (20 μg/ml). Cells were preincubated (5 min, 37°C) with the lanthanide in the presence of calcium (1 mM) and then challenged. Columns represent the means from four experiments and vertical bars show s.e. mean. The unblocked release (% total histamine) was 15.4 ± 1.1.

highest concentration tested (10^{-3} M), all of the lanthanides totally abolished the release produced by both secretagogues. The effect of concanavalin A was weakly antagonized by lanthanum but strongly inhibited by the higher lanthanides (Figure 2). There was again no obvious dependence on ionic radius and no potentiation of release was observed under any conditions.

Effect of lanthanides on basic agents and dextran

Peptide 401 is a potent releaser of histamine in both the presence and absence of extracellular calcium (Atkinson *et al.*, 1979; Ennis *et al.*, 1980; Pearce *et al.*, 1981). As shown in Figure 3, secretion under both conditions was blocked by lanthanum (10^{-3} to 10^{-9} M). Other lanthanides also produced dose-dependent inhibition and, in contrast to the IgE-directed ligands, the extent of the antagonism was now markedly dependent on ionic radius. Some representative data in the presence of a fixed concentration (10^{-6} M) of inhibitor is shown in Figure 4; very similar graphs can be drawn for other concentrations. In the presence of calcium, the inhibitory effect progressively increased on passing from lanthanum to dysprosium and thereafter decreased (Figure 4a). In the absence of calcium, there was a steady increase in inhibition with decreasing ionic radius along the series lanthanum to lutetium (Figure 4b). Virtually identical results were obtained with compound 48/80, although lanthanum itself was rather less active in the absence of calcium.

Histamine release induced by dextran is totally dependent on the presence of phosphatidyl serine and extracellular calcium (Ennis *et al.*, 1980; Pearce *et al.*, 1981). This release was blocked by lanthanide ions in identical manner to the basic agents, with dysprosium being the most effective inhibitor. This inhibition may be due to interaction between the lanthanide and phosphatidyl serine since the lipid prevented elution of the ion from the cell (see also below).

Time-course of the inhibition

The time-course of the inhibition produced by lanthanum was markedly dependent on the presence or absence of calcium in the incubation medium. In the presence of extracellular calcium, lanthanum was fully active without preincubation. The ion produced an immediate inhibition of release which did not increase

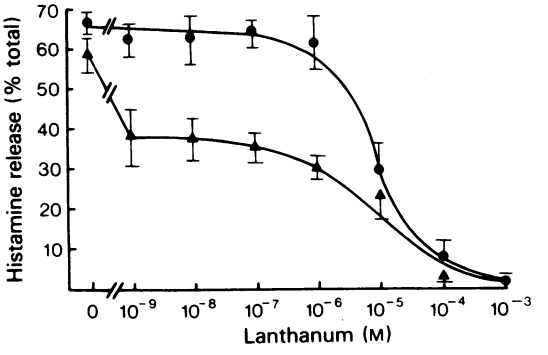

Figure 3 Effect of lanthanum on histamine release induced by peptide 401 (1 μg/ml) in the presence (●) and absence (▲) of calcium (1 mM). Cells were preincubated (5 min, 37°C) with lanthanum in the stated media and then challenged. The points are the means from four experiments and vertical bars show s.e. mean.

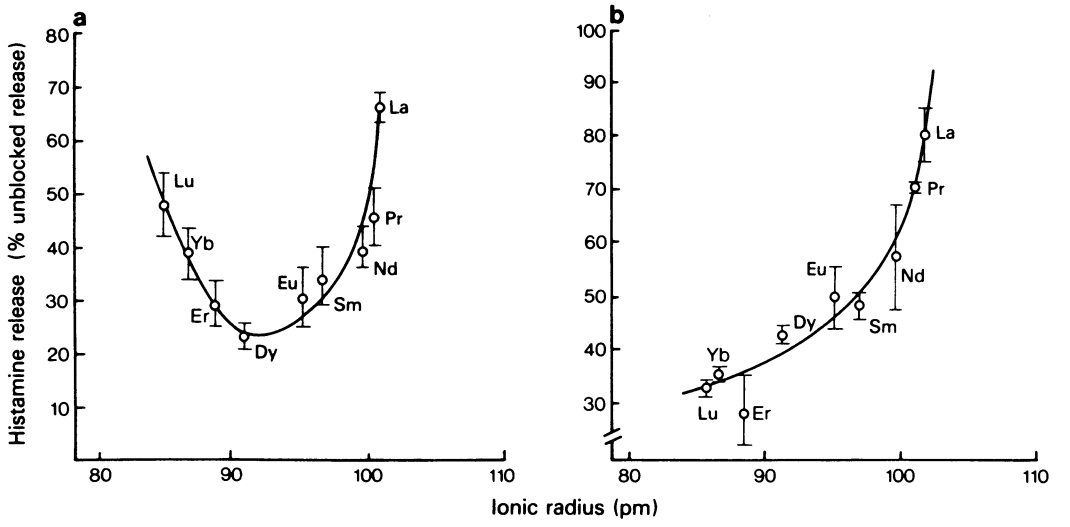

Figure 4 Effect of ionic radius on inhibition by lanthanides (10^{-6} M) of histamine release produced by peptide 401 (1 μg/ml) in (a) the presence and (b) the absence of calcium (1 mM). Cells were preincubated (5 min, 37 C) with the lanthanide and then challenged. The points are the means from four (presence of calcium) or three (absence of calcium) experiments and vertical bars show s.e. mean. Unblocked releases (% total histamine) were (a) 69.8 ± 3.7 and (b) 42.9 ± 2.7. Ionic radii were taken from Weast (1978).

with time. A representative experiment for peptide 401 is shown in Figure 5a. Similar results were obtained for all of the other secretagogues mentioned above, except anti-IgE which was not tested. In the absence of added calcium, the release due to peptide 401 was immediately inhibited only by high concentrations of lanthanum whereas lower concentrations produced a progressive effect (Figure 5b). Similar results were obtained with compound 48/80 and with other lanthanides, although the ions of smallest radius tended to produce an immediate, partial inhibition which then steadily increased with time. The inhibitory effect of lutetium in calcium-free media was reversed by washing the cells with buffer. The latter were then fully responsive if resuspended in media with or without added calcium (Table 1). Lanthanum was also readily eluted from the cells but dysprosium retained some inhibitory effect.

Nature of the inhibition

To define the nature of the antagonism due to lanthanum, the effect of the inhibitor on the release produced by allergen, anti-IgE and compound 48/80 was examined as a function of the concentration of added calcium (Figure 6). Increasing amounts of lanthanum produced graded, parallel displacements of the concentration-response curves for calcium. Schild plots

Table 1 Elution of lutetium from treated mast cells

First medium	*Second medium*		
Lutetium	*Calcium*	*Lutetium*	*Histamine release (%)*
−	+	−	85.0 ± 2.8
+	+	−	76.7 ± 4.5
−	−	−	65.7 ± 5.0
+	−	−	60.2 ± 11.0
+	−	+	18.9 ± 5.8
+	+	+	13.5 ± 2.1

Cells were preincubated (10 min, 37°C) in a calcium-free medium containing lutetium (10^{-5} M) as noted. They were recovered by centrifugation, washed once in calcium-free medium and resuspended in media with or without calcium (10^{-3} M) and lutetium (10^{-5} M) as noted. They were then challenged with peptide 401 (1 μg/ml). Values are means ±s.e. mean for four experiments.

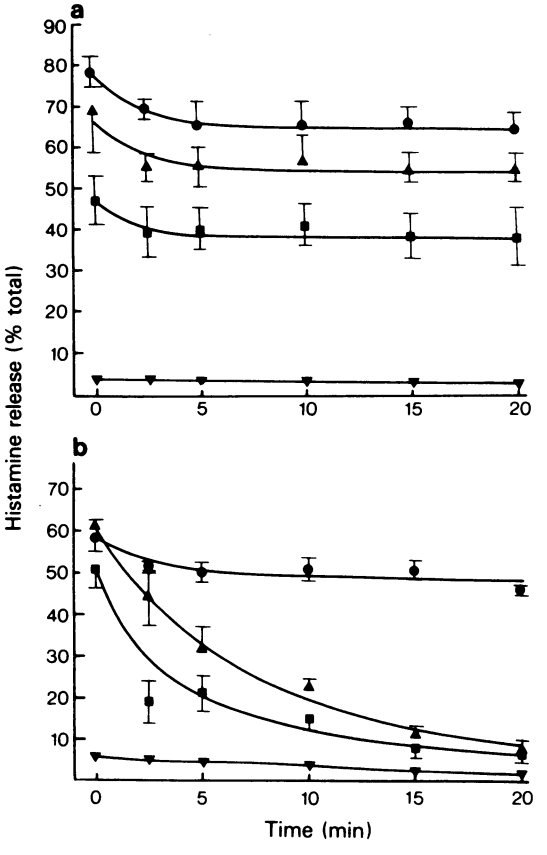

Figure 5 Time-course of the inhibition by lanthanum of histamine release induced by peptide 401 (1 μg/ml) in (a) the presence and (b) the absence of calcium (1 mM). Cells were suspended in prewarmed buffer containing lanthanum at a concentration of 0 (●), 10^{-6} M (▲), 10^{-5} M (■), and 10^{-4} M (▼). They were then challenged after the noted times and secretion assessed after a further 10 min. The points are the means from four experiments and vertical bars show s.e. mean.

yielded straight lines with slopes approximating to unity for all three secretagogues (Figure 7), consistent with the competitive antagonism of binding of calcium to a cellular receptor (Arunlakshana & Schild, 1959). The pA_2 values calculated from these plots were 7.7 for allergen, 7.6 for anti-IgE and 6.7 for compound 48/80.

Discussion

The present study confirms that lanthanum can competitively antagonize the binding of calcium to superficial receptors on the mast cell membrane. This binding presumably constitutes the first event in the inter-

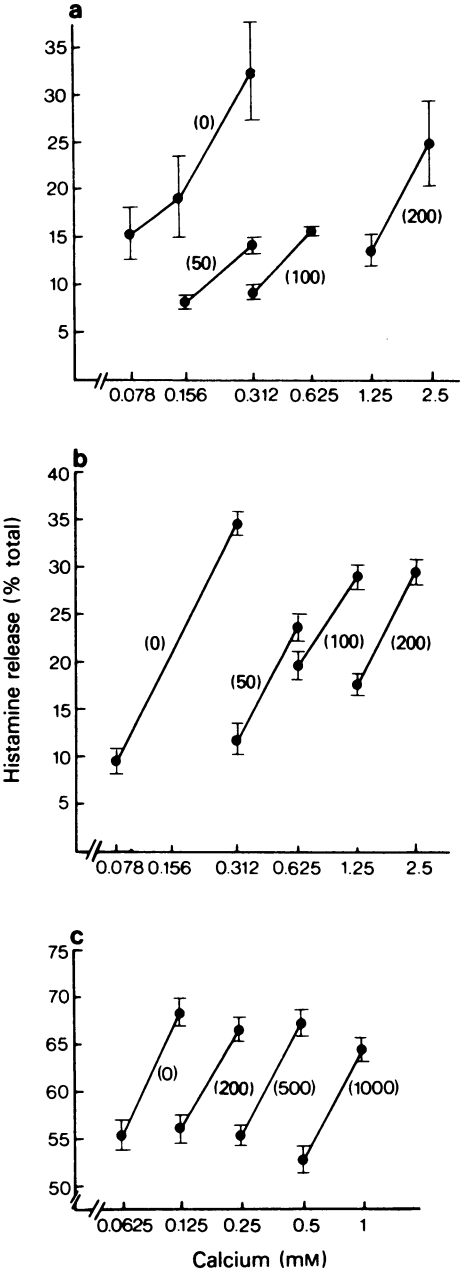

Figure 6 Effect of lanthanum on the concentration-response curves for calcium in histamine release induced by (a) allergen (20 worm equivalents/ml), (b) anti-IgE (400 fold dilution), and (c) compound 48/80 (1 μg/ml). Cells were preincubated (5 min, 37°C) with calcium and the stated concentrations of lanthanum (nM, given in parentheses) and then challenged. The points are the means from three or four experiments and vertical bars show s.e. mean.

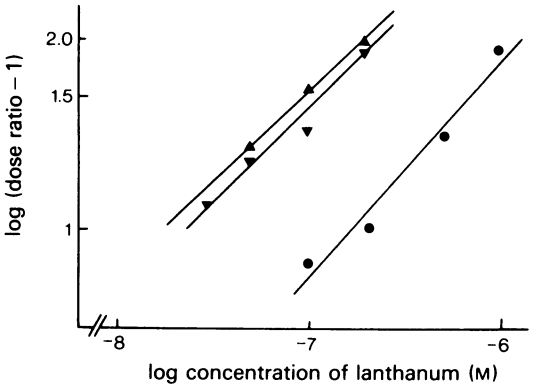

Figure 7 Schild plots for the antagonism by lanthanum of responses to calcium evoked by allergen (▲), anti-IgE (▼), and compound 48/80 (●). The dose-ratio was calculated from the concentrations of calcium required to produce the same effect in the presence and absence of lanthanum. The slopes of the lines, as determined by regression analysis, were 0.94 (allergen), 0.93 (anti-IgE), and 1.10 (compound 48/80).

nalization of the ion following activation of calcium-channels by anaphylactic and other stimuli (Grosman & Diamant, 1974; Ennis *et al.*, 1980; Pearce *et al.*, 1981). The pA_2 value for the antagonism was virtually identical for histamine release evoked by allergen or anti-IgE, which both cross-link reaginic antibody fixed to the cell surface but was appreciably smaller for compound 48/80. This indicates that different calcium-receptors and channels may be involved according to the nature of the secretagogue. The pA_2 value found for allergen in the present work (7.7) is very similar to that (7.6) previously reported by Foreman & Mongar (1973).

At particular concentrations, lanthanum enhanced rather than inhibited the release produced by allergen and anti-IgE. Lanthanum also promotes a limited increase in secretory activity in other systems and, under these conditions, binding of the ion to superficial membrane sites may initiate the release of sequestered calcium from more deeply buried cellular stores (Heuser & Miledi, 1971; Borowicz, 1972; Matthews *et al.*, 1973). A different action must, however, be proposed for basic agents in the absence of extracellular calcium (see below).

The effect of higher lanthanides further distinguished the release produced by various secretagogues. In the presence of added calcium, there was no marked difference between the inhibitory effects of the ions on release induced by allergen and anti-IgE. In contrast, lanthanum was the least effective inhibitor of secretion produced by concanavalin A, dextran, and basic agents. Inhibition of the latter was markedly dependent on the ionic radius and dysprosium was most effective. The difference between concanavalin A and the indicated IgE-directed ligands supports recent suggestions (Shores & Mongar, 1980) that the lectin may not act entirely by cross-linking antibody fixed to the cell surface.

The inhibition of the basic agents in the absence of extracellular calcium is of particular interest. Under these conditions the releasers act by mobilizing firmly bound stores of calcium, probably from reservoirs in the cell membrane (Atkinson *et al.*, 1979; Pearce *et al.*, 1979; Ennis *et al.*, 1980; Pearce *et al.*, 1981). In contrast to the immediate antagonism observed in the presence of added calcium, the inhibition now steadily increased with time and with decreasing ionic radius of the lanthanide. This suggests that the ion may progressively penetrate into less accessible sites in the membrane. Two possibilities then exist. Most simply, the lanthanide may displace into the extracellular environment the bound calcium which is normally mobilized by the test secretagogues. Such an action is unlikely, however, since the inhibitor was rapidly eluted on washing the cells. The latter were then fully responsive to the basic inducers without further reintroduction of calcium into the medium. If available stores had been depleted by the lanthanide, these would then have to be promptly replenished from within the cell. Alternatively, binding of the inhibitor might stabilize the membrane and prevent the subsequent mobilization of the sequestered stores utilized in the initiation of exocytosis. We have previously demonstrated the existence of such regulatory binding sites for calcium in the mast cell membrane (Ennis *et al.*, 1980; Pearce *et al.*, 1981) and precisely such a role has been proposed for lanthanum in other systems (Weiss & Goodman, 1969; Weiss, 1974). The lanthanides thus provide important additional tools in delineating the calcium-pools involved in histamine secretion and additionally emphasize differences in the detailed mode of action of diverse seretagogues.

This work was supported by a grant from the Wellcome Trust.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother*, **14**, 48–58.

ATKINSON, G., ENNIS, M. & PEARCE, F.L. (1979). The effect of alkaline earth cations on the release of histamine from rat peritoneal mast cells treated with compound 48/80 and peptide 401. *Br. J. Pharmac.*, **65**, 395–402.

BAXTER, J.H. & ADAMIK, R. (1978). Differences in require-

ments and actions of various histamine-releasing agents. *Biochem. Pharmac.*, **27**, 497–503.

BERRIDGE, M.J. (1975). The interaction of cyclic nucleotides and calcium in the control of cellular activity. *Adv. cyclic Nuc. Res.*, **6**, 1–97.

BOROWICZ, J.L. (1972). Effect of lanthanum on catecholamine release from adrenal medulla. *Life Sci.*, **11**, 959–964.

ENNIS, M. & PEARCE, F.L. (1979). Effect of cyclic AMP on histamine release induced by compound 48/80. *Int. Archs Allergy appl. Immun.*, **60**, 461–464.

ENNIS, M., PEARCE, F.L., TRUNEH, A. & WHITE, J.R. (1980). Calcium pools involved in histamine release from rat mast cells. *Int. Archs. Allergy appl. Immun.*, **62**, 467–471.

ENNIS, M., PEARCE, F.L. & WESTON, P.M. (1980). Some studies on the release of histamine from mast cells stimulated with polylysine. *Br. J. Pharmac.*, **70**, 329–334.

FOREMAN, J.C. & MONGAR, J.L. (1973). The action of lanthanum and manganese on anaphylactic histamine secretion. *Br. J. Pharmac.*, **48**, 527–537.

GOODMAN, F.R. & WEISS, G.B. (1971). Dissociation by lanthanum of smooth muscle responses to potassium and acetylcholine. *Am. J. Physiol.*, **220**, 759–766.

GROSMAN, N. & DIAMANT, B. (1974). Studies on the role of calcium in the anaphylactic histamine release from isolated rat mast cells. *Acta pharmac. tox.*, **35**, 284–292.

HEUSER, J. & MILEDI, R. (1971). Effect of lanthanum ions on function and structure of frog neuromuscular junctions. *Proc. R. Soc. B.*, **179**, 247–260.

LANGER, G.A. & FRANK, J.S. (1972). Lanthanum in heart cell culture. Effect on calcium exchange correlated with its localization. *J. cell Biol.*, **54**, 441–455.

MARTIN, R.B. & RICHARDSON, F.S. (1979). Lanthanides as probes for calcium in biological systems. *Q. Rev. Biophys.*, **12**, 181–209.

MATTHEWS, E.K., LEGROS, J.L., GRAU, J.D., NORDMANN, J.J. & DREIFUSS, J.J. (1973). Release of neurohypophysial hormones by exocytosis. *Nature New Biol.*, **241**, 86–88.

PEARCE, F.L., ATKINSON, G., ENNIS, M., TRUNEH, A., WESTON, P.M. & WHITE, J.R. (1979). Effect of anti-allergic compounds on histamine release induced by basic agents, antigen and the calcium ionophore A23187. In *The Mast Cell: Its Role in Health and Disease*. ed. Pepys, J. & Edwards, A.M. pp. 69–75. Tunbridge Wells: Pitman Medical Publishing Co.

PEARCE, F.L., ENNIS, M., TRUNEH, A. & WHITE, J.R. (1981). Role of intra- and extracellular calcium in histamine release from rat peritoneal mast cells. *Agents & Actions* (in press).

RUSSELL, J.T. & THORN, N.A. (1972). Effects of lanthanum and of oligomycin and cyanide on release of vasopressin from isolated rat neurohypophyses. *Acta Physiol. scand.*, **84**, 33–34A.

SHORES, A.J. & MONGAR, J.L. (1980). Modulation of histamine secretion from concanavalin A-activated rat mast cells by phosphatidyl serine, calcium, cAMP, pH and metabolic inhibitors. *Agents & Actions*, **10**, 131–137.

TAKATA, M., PICKARD, W.F., LETTVIN, J.Y. & MOORE, J.W. (1966). Ionic conductance changes in lobster axon membrane when lanthanum is substituted for calcium. *J. gen. Physiol.*, **50**, 461–471.

WEAST, R.C. (1978). *CRC Handbook of Chemistry and Physics*. Florida: Chemical Rubber Publishing Co.

WEISS, G.B. (1970). On the site of action of lanthanum in frog sartorius muscle. *J. Pharmac. exp. Ther.*, **174**, 517–526.

WEISS, G.B. (1974). Cellular pharmacology of lanthanum. *A. Rev. Pharmac.*, **14**, 343–354.

WEISS, G.B. & GOODMAN, F.R. (1969). Effects of lanthanum on contraction, calcium distribution and Ca^{45} movements in intestinal smooth muscle. *J. Pharmac. exp. Ther.*, **169**, 46–55.

(Received June 16, 1980.)