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Lanthanides are transported by ionophore A23187 and mimic calcium in the histamine secretion process

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1 Ionophore A23187 induced histamine release from peritoneal rat mast cells in the presence of lanthanum or terbium as it did in the presence of calcium.

2 Low concentrations of lanthanides (10^{-5} to 2×10^{-4} M) were more efficient than similar concentrations of calcium.

3 The effect of low concentrations of calcium and lanthanides were additive.

4 Increasing the concentration of lanthanides above 10^{-3} M decreased histamine release. This decrease was partly reversed by calcium.

5 Calmodulin inhibitors, phenothiazines, R24571 and mepacrine, inhibited the histamine release induced by either calcium or lanthanides.

6 Zn^{2+}, a calmodulin inhibitor transported by A23187, inhibited more potently the calcium-dependent histamine release.

7 Lanthanides decrease histamine release induced by 48/80 in the absence of added calcium.

8 These data show that ionophore A23187 can transport lanthanides across the plasma membrane of mast cells, allowing the trivalent cations to substitute for calcium in the activation of calmodulin or calmoduline-like proteins.

Introduction

The use of terbium was recently proposed as a probe for studying the interaction of calcium with calmodulin *in vitro* (Kilhoffer, Demaille & Gérard, 1980). Terbium and calcium possess similar radii and coordination numbers. Indeed terbium effectively substitutes for calcium *in vitro* in the activation of calmodulin as judged by several criteria: intrinsic fluorescence spectra, altered mobilities on polyacrylamide gel electrophoresis, formation of a stable complex with troponin I or calcineurin and stimulation of phosphodiesterase (Wallace, Tallant, Dockter & Cheung, 1982). However, the usefulness of terbium or other lanthanides as probes of calmodulin has not been shown at the level of intact cells or tissue, whereas changes in the intracellular concentration of calcium ions provide the control for many physiological processes including secretion. The intervention of calmodulin in the release of histamine from rat mast cells has been suggested, in view of the inhibition of release by calmodulin inhibitors (Landry, Amellal & Ruckstuhl, 1980; Douglas & Nemeth, 1982). Foreman, Mongar & Gomperts (1973) showed that ionophore A23187, which selectively increases the permeability of mast cell membrane to calcium, induced histamine release. Lanthanum is widely used as an inhibitor of calcium translocation across a variety of subcellular and cellular membranes and has been shown to form complexes with A23187 (Pfeiffer, Reed & Lardy, 1974). The present paper describes the effects of terbium and lanthanum and compares them with those of calcium on the release of histamine from peritoneal rat mast cells in the presence of ionophore A23187, and the inhibition of this secretion process by calmodulin inhibitors.

Methods

Histamine release

Male Wistar rats weighing from 280 to 350 g were killed by decapitation and exsanguinated. Eight ml of calcium-free buffer was injected into the peritoneal cavity. The body was gently massaged for 2 min and the peritoneal fluid collected and centrifuged for 2 min at 220 *g*. The pellet was washed twice with

calcium-free buffer and finally resuspended in the presence of calcium chloride or lanthanum chloride. The cellular suspension (0.6 ml final volume, containing 40,000 to 50,000 mast cells) was preincubated for 10 min at 37°C. Secretion was elicited with ionophore A23187 and terminated 10 min later by addition of 1 ml of ice-cold buffer, cooled in iced water and centrifuged at 220 *g* for 2 min at 4°C. Supernatants were collected and histamine concentrations measured according to the fluorimetric method of Shore, Burkhalter & Cohn (1959). The spontaneous release of histamine, determined in the absence of ionophore, was 3 to 7% of the total cellular histamine content measured for each sample after treatment of the cell suspension with trichloracetic acid. In the presence of lanthanides (10^{-3} M) the spontaneous release reached 10%. No analytical interference of drugs with histamine measurement was observed under the conditions used. All the determinations were performed in duplicate.

Chemicals and buffers

R24571 [1-[*bis*-(*p*-chlorophenyl) methyl] -3- ([2,4 dichloro- β (2,4 dichlorobenzyl oxy) phenethyl] imidazoliumchloride] was a gift from Janssen Pharmaceutica; mepacrine (quinacrine hydrochloride) from Laboratoires Sobio; and chlorpromazine from Laboratoires Specia. Ionophore A23187 was obtained from Boehringer-Mannheim compound 48/80 from Sigma, HEPES [2-[4- (2-hydroxyethyl) -1-piperazinyl] -ethansulphonic acid] from Merck.

Table 1 Histamine release induced by ionophore A23187 or compound 48/80 in the presence of calcium or lanthanides

	% Histamine release by: *Ionophore A23187*	*Compound 48/80*
Control	4.8 ± 2.1	27.6 ± 2.4
Ca^{2+} (μM)		
100	13.2 ± 2.5	54.6 ± 5.3
500	47.3 ± 5.0	70.5 ± 3.9
1,000	62.4 ± 6.5	59.2 ± 4.4
Tb^{3+} (μM)		
100	18.4 ± 1.2	25.4 ± 4.5
500	49.2 ± 2.1	20.8 ± 5.1
1,000	46.4 ± 7.0	9.2 ± 1.4
La^{3+} (μM)		
100	27.7 ± 4.5	24.5 ± 6.4
500	40.9 ± 2.3	22.0 ± 5.0
1,000	49.4 ± 4.7	13.4 ± 2.1

Rat peritoneal cells were incubated (10 min, 37°C) with 10^{-6} M ionophore A23187 or 0.2 μg ml^{-1} compound 48/80 (Sigma) in the absence (control) or presence of calcium chloride, terbium chloride or lanthanum chloride. Values are means ± s.e.mean for 10 (A23187) or 5 (48/80) experiments.

All other chemicals were of analytical grade. Calcium-free buffer contained (mM): NaCl 137, KCl 2.7, MgCl 1, glucose 5.6, and HEPES 10, pH 7.2

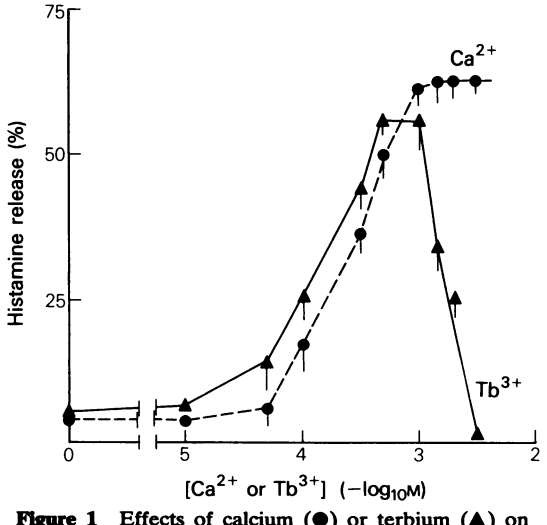

Figure 1 Effects of calcium (●) or terbium (▲) on histamine release from rat mast cells induced by 10^{-6} M A23187. Values are means for six experiments performed in duplicate; vertical lines indicate s.e.mean.

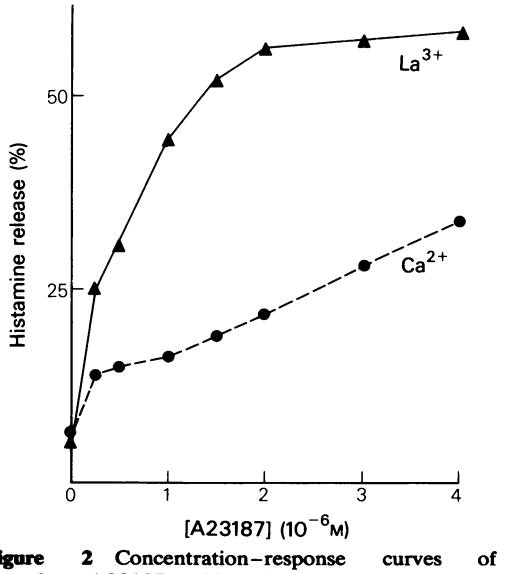

Figure 2 Concentration–response curves of ionophore A23187 on histamine release from rat mast cells in the presence of 10^{-4} M calcium (●) or 10^{-4} M lanthanum (▲). Values are means of 2 experiments performed in duplicate.

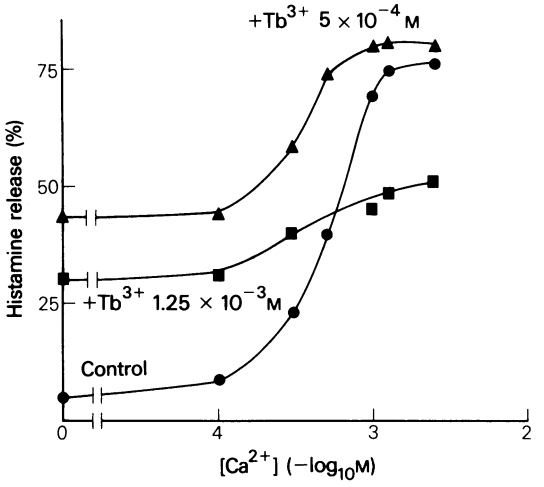

Figure 3 Effects of calcium on histamine release from rat mast cells induced by ionophore A23187 in the presence of 5×10^{-4} M (▲) or 1.25×10^{-3} M (■) terbium. Control experiments (●) were performed simultaneously in the absence of terbium. Cells were preincubated for 10 min at 37°C in the presence of ions. Histamine release was induced by addition of 10^{-6} M A23187. Values are means of 2 experiments performed in duplicate.

Results

Rat peritoneal cells were recovered in a saline buffer devoid of calcium, and incubated with increasing amounts of calcium or terbium chloride. Figure 1 shows that both terbium and calcium were able to activate histamine release in the presence of A23187. Maximal releases were observed in the presence of 1 mM of cations. Increasing the concentration of terbium inhibited histamine release. Calcium itself did not inhibit the release over this concentration-range. Similar results were observed with lanthanum (Table 1). Figure 2 shows that the effect of both calcium and lanthanum are dependent on the concentration of ionophore and clearly confirm the greater efficiency of low concentrations of lanthanides as compared with calcium, as suggested in Figure 1 and Table 1. Figure 3 shows that the effect of low concentrations of calcium and terbium were additive. Calcium partly reversed the inhibitory effect of high terbium concentrations. When compound 48/80 was used to induce release (Table 1), low concentrations of lanthanides did not allow an increase of histamine release as observed in the case of calcium. Moreover high concentrations of lanthanides inhibited the basal release induced by 48/80 in the absence of added cations. Figure 4 shows similar patterns of inhibition of A23187-induced histamine by chlorpromazine, mepacrine and R24571 in the presence of either

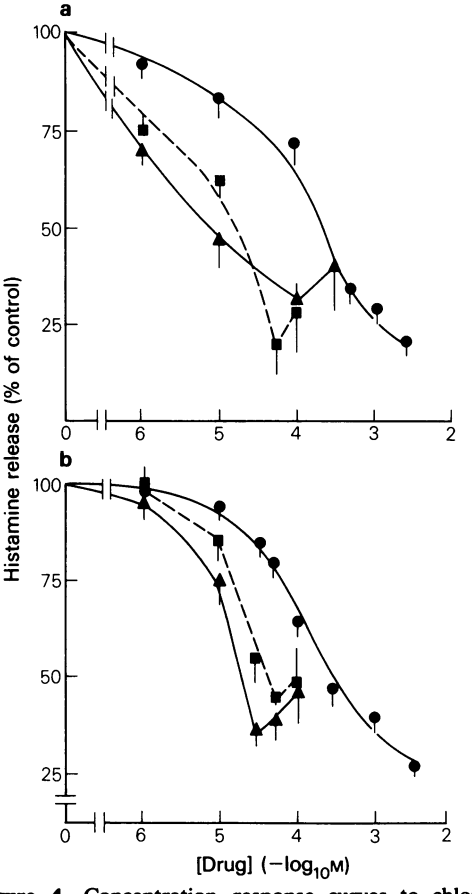

Figure 4 Concentration–response curves to chlorpromazine (▲) R24571 (■) and mepacrine (●) on the A23187-induced histamine release from rat mast cells in the presence of 10^{-4} M lanthanum (a) or 10^{-3} M calcium (b). Histamine release was induced with 10^{-6} M A23187; control releases of histamine (absence of inhibitors) were 30% (lanthanum) and 72% (calcium) of total cellular content. Values are means of 5 (calcium) or 3 (lanthanum) experiments performed in duplicate; vertical lines show s.e.mean.

calcium or lanthanum. High concentrations of chlorpromazine and of R24571 were less effective in inhibiting histamine release than smaller concentrations as previously reported by Church & Gradidge (1980) with phenothiazines. Figure 5 shows the inhibitory effect of Zn^{2+}. This effect was less potent when lanthanum was used instead of calcium to induce histamine release.

Discussion

The rat mast cell is considered as one of the best documented models for calcium-mediated exocytosis

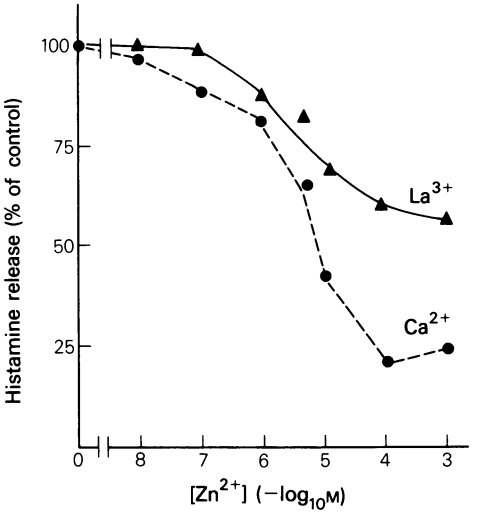

Figure 5 Concentration–response curves to zinc on the A23187-induced histamine release from rat mast cells in the presence of 10^{-4} M lanthanum (▲) or 10^{-3} M calcium (●). Histamine release was induced with 2×10^{-6} M, A23187. The control release of histamine (absence of zinc) was 72.5% (calcium) and 53.2% (lanthanum) of total cellular content. Values are means of 2 experiments performed in duplicate.

(for a review, see Foreman, 1981). The mast cell responds to secretagogues such as antigens and compound 48/80 which act through receptors in the plasma membrane to promote calcium influx or to mobilize cellular calcium respectively. Moreover the mast cell readily responds to calcium itself when treated with the ionophore A23187 (Foreman & Mongar, 1973).

Several studies performed *in vitro* with purified calmodulin indicated that terbium, a trivalent lanthanide, can mimic calcium in binding to the protein (Kilhoffer *et al.*, 1980a; Kilhoffer, Gerard & Demaille 1980b) allowing the activation of calmodulin-sensitive enzymes (Wallace *et al.*, 1982). Lanthanides are generally considered as blockers of calcium influx in cells (for a review, see Martin & Richardson 1979). Consequently their potency in activating calmodulin has not been considered in intact cells. Indeed we show that ionophore A23187 might promote the influx of lanthanides in mast cells, inducing histamine release. The lanthanide-dependent release is sensitive to calmodulin inhibitors. This result strongly suggests that lanthanides mimic calcium at the level of calmodulin or calmodulin-like proteins involved in the secretory process. However, the possibility of a displacement of intracellular calcium stores by lanthanides cannot be excluded. The biphasic effect of lanthanides on histamine release (Figure 1) was quite similar to the effect of terbium on the activation of phosphodiesterase activity by calmodulin *in vitro*. Indeed, high concentrations of lanthanides might precipitate proteins (Kilhoffer *et al.*, 1980) and aggregate mast cells (Foreman & Mongar, 1973). However we have shown that calcium can partly restore histamine release in the presence of high lanthanide concentrations (Figure 2).

The transport by A23187 across phospholipid vesicular membranes has been shown by Hunt, Tipping & Belmont (1978) for the lanthanide, praseodymium. Pfeiffer *et al.* (194) showed that A23187 can form complexes with lanthanum. Ionophore A23187 (A) transports calcium through the formation of the neutral complex A_2Ca. The study of Pfeiffer *et al.* (1974) suggested the possibility of various complexes with lanthanum: A_1La, A_2La and A_2La_3. A23187 releases the cation on the inner side of the plasma membrane and mediates the proton antiport required to maintain electroneutrality across the membrane. The secretion of histamine by lanthanides in the presence of A23187 might be interpreted as a displacement by lanthanides of calcium bound on the surface of mast cells. However, several observations showed that this possibility can be excluded: mast cells extensively washed with EDTA responded similarly to A23187 and lanthanides compared with unwashed cells (result not shown). Moreover the release observed with small concentrations of lanthanides was higher than the release in the presence of similar concentrations of added calcium. This observation also suggested that the active complexes, in the conditions used, might be A_1La or A_2La_3 instead of A_2La which should produce a similar effect to A_2Ca, supposing similar kinetic behaviour of calcium and lanthanides complexes. A greater affinity of intracellular calcium targets or of A23187 for lanthanides might also be possible.

Foreman & Mongar (1972, 1973) and Pearce & White (1981) showed the actions of lanthanum on anaphylactic histamine release. In the concentration range, 10^{-9} to 10^{-6} M, lanthanum inhibited the calcium-dependent component of release but was without effect on the component which was independent of calcium. At concentrations of 10^{-5} M and higher, lanthanum induced a release of histamine in the absence of antigen (Foreman & Mongar, 1973). We did not observe such a release in the absence of ionophore A23187. The spontaneous release in the presence of highest lanthanide concentrations reached 10% instead of 3 to 7% in the absence of the cations. Similar observations were made by Pearce & White (1981). Indeed we have clearly established the dependency of the lanthanide-induced release upon the concentration of A23187 (Figure 3).

Zinc prevents histamine release from rat mast cells

(Hogberg & Uvnäs, 1957) and from human basophils (Marone, Findlay & Lichtenstein, 1981). Brewer, Aster, Knutsen & Kruckeberg (1979) showed that zinc inhibition of calmodulin provides a rational molecular mechanism for the diverse cellular inhibitory effects of zinc, as well as for zinc's antagonism of calcium effects. Moreover, Pfeiffer & Lardy (1976) showed the ability of A23187 to complex zinc was higher than its ability to complex calcium. Indeed the property of A23187 to transport zinc could also be suggested from the data of Marone *et al.* (1981), showing a better inhibitory potency of zinc with respect to A23187-induced histamine release than with immunologically induced release. The inhibitory potencies of zinc on mast cell histamine release induced by A23187 may be relevant to both the competition with calcium and lanthanides at the level of the ionophore and the inhibition of intracellular calmodulin or calmodulin-like proteins. The observation of the different inhibitory potencies of zinc on histamine release in the presence of either calcium or lanthanum offers another argument to exclude the hypothesis of an effect of lanthanum through the displacement of extracellular calcium stores.

The inhibitory effect of some calmodulin inhibitors (phenothiazines, imipramine and pimozide), distal to the calcium entry or mobilization, was demonstrated in mast cells by Douglas & Nemeth (1982), showing that ^{45}Ca uptake in response to A23187 was not affected by these drugs when the cells were deprived of metabolic energy. However the selectivity of these drugs in inhibiting calmodulin has been discussed (Norman, Drummond & Moser, 1979; Landry, Amellal & Ruckstuhl 1981; Weiss, Prozialeck & Wallace, 1982). In the present paper we show that R24571, a calmodulin inhibitor devoided of affinity for membrane receptors (Van Belle, 1981) inhibits mast cells histamine release induced by A23187. The antimalarial drug mepacrine, is as potent as chlorpromazine in its anticalmodulin activity (Volpi, Sha'afi, Epstein, Andrenyak & Feinstein, 1981) but offers the advantage of low liposolubility (Weiss *et al.*, 1982). Figure 3 shows that mepacrine inhibited A23187-induced histamine release as previously shown in the case of the IgE-mediated histamine release from rat basophilic leukaemia cells (McGivney, Morita, Crews, Hirata, Axelrod & Siraganian 1981). These inhibitory effects of R24571 and mepacrine support the observation of Douglas & Nemeth (1982) who used less selective calmodulin inhibitors.

In conclusion, lanthanides are useful not only for inhibiting cellular calcium influx, as currently claimed, but can also be used as probes of intracellular calcium binding proteins, when introduced into the cell by ionophore A23187.

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