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The ability of thapsigargin and thapsigargicin to activate cells involved in the inflammatory response

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1 The ability of thapsigargin and thapsigargicin to activate mast cells and leukocytes has been investigated.

2 The thapsigargin-induced histamine release from rat peritoneal mast cells was found to be dependent on the concentration of thapsigargin, the purity of the mast cell preparations, and the number of mast cells in suspension.

3 Thapsigargin induced histamine release from human basophil leukocytes.

4 Thapsigargin induced β-glucuronidase and lysozyme release from human neutrophil leukocytes.

5 Thapsigargin caused a release of histamine from mesentery, lung, and heart mast cells of the rat, but only to a minor extent from the corresponding guinea-pig cells.

6 Thapsigargicin induced histamine release from mesentery, lung, and heart mast cells of the rat at concentrations from 0.1 μM but provoked only a release from the corresponding guinea-pig cells in the concentration-range 0.16 to 1.6 μM.

7 Thapsigargin increased the cytoplasmic free calcium level in intact human blood platelets at concentrations from 3.0 nM.

Introduction

Resin from the umbelliferous plant, *Thapsia garganica*, belonging to the flora of the western Mediterranian countries, has for centuries been used as a counter-irritant for relief of rheumatic pains. Chemical investigations of the roots have led to the isolation and structural elucidation of two major skin irritating constituents: thapsigargin (Tg) and thapsigargicin (Tc) (Christensen & Schaumburg, 1983; Christensen & Norup, 1985) together with some minor constituents (Christensen *et al.*, 1984). Pharmacological studies have revealed that Tg and Tc are very potent histamine releasers from rat peritoneal mast cells (Rasmussen *et al.*, 1978). The secretory response to Tg and Tc can be divided into a two-step reaction, a stimulation phase and a calcium ion-triggering secretory phase (Patkar *et al.*, 1979; Diamant & Patkar 1980). No histamine release is obtained, if calcium is replaced with strontium in the secretory step (Patkar *et al.*, 1979). A number of similarities between the reaction induced by Tg, A 23187, and fluoride have been demonstrated. Thus cells preincubated with these secretagogues respond to the action of calcium whenever the ion is introduced, but become insensitive to the secretory action of compound 48/80 in the absence of calcium. The latter effect is partly counteracted, if strontium (1 mM) is added together with compound 48/80 (Patkar *et al.*, 1979). In contrast to the effect of ionophore A 23187, but like the fluoride ion, Tg does not release histamine from isolated histamine-retaining granules (Patkar *et al.*, 1979).

In addition to a further investigation of the Tg-provoked histamine release from peritoneal rat mast cells we have expanded the studies to include rat mast cells from mesentery, lung, and heart, human basophil and neutrophil leukocytes, human platelets, and also guinea-pig mast cells from mesentery, lung, and heart.

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Methods

Preparation of peritoneal mast cells

Sprague-Dawley rats (300–400 g) were killed by asphyxiation in an atmosphere of nitrous oxide. The peritoneal cavity was opened and washed with saline containing heparin (25 u ml^{-1}) and buffered to pH 7.5 with N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES). After centrifugation for 5 min and resuspension in Tyrode solution, aliquots of cells were incubated in Tyrode solution of the following composition (mM): NaCl 137, glucose 5.6, KCl 2.7, NaH_2PO_4 0.4, and HEPES 20. The pH of the solution was adjusted to 7.5 before use.

Purification of mast cells

Cells from three rats were suspended in 3 ml of Tyrode solution and then layered on to a discontinuous gradient of bovine serum albumin (35% protein, s.g. = 1.095–1.105 at 25°C, Path-o-cyte, Miles Laboratories). The gradients were centrifuged at 600 *g* for 15 min at room temperature. After removal of the upper layers, the mast cells in the pellet were washed twice in Tyrode solution. The yield was approximately 2×10^6 mast cells from 3 rats. After staining with toluidine blue the purity of the cells was determined by counting the number of mast cells and the total number of cells. The purity was about 80%.

In some experiments, purification over human serum albumin was performed as follows: Cells from three rats were suspended in Tyrode solution, layered on to a discontinuous gradient of human serum albumin (lower layer 26% protein, upper layer 20% protein) and centrifuged at 90 *g* for 10 min at room temperature. After removal of the upper layers the pellet was washed twice in Tyrode solution. The yield was approximately 2×10^6 cells, the purity being about 25%.

Determination of histamine secretion from rat peritoneal mast cells

Unless otherwise indicated, cells were suspended in 0.9 ml of Tyrode solution with calcium chloride added to achieve a final concentration of 1.1 mM. The number of cells per sample was standardized to 1×10^5, in the cases of the purified cells by adding Tyrode solution to the obtained cell suspension after counting a representative amount; in the cases of unpurified cells by suspending the obtained cells in appropriate amounts of Tyrode solution assuming the number of peritoneal mast cells to be 2×10^6 per rat. After preincubation for 10 min at 37°C, a solution of drug or anti IgE (1 : 30) in 0.1 ml of Tyrode solution was added and the mixture incubated for a further 10 min at 37°C. The reaction was stopped by addition of 4 ml ice-cold Tyrode solution. Cells and supernatant were separated by centrifugation (5 min, 1000 *g*). The cell pellets were resuspended in Tyrode solution and allowed to stand in a boiling water bath for 5 min to release residual histamine. Histamine was determined spectrofluorimetrically after coupling with *o*-phtaldialdehyde as described by Shore *et al.* (1952), but without the extractions steps (Loeffler *et al.*, 1971). Histamine release was calculated as the ratio 100 × (histamine in supernatant)/(histamine in supernatant + residual histamine in cells). All values were corrected for the spontaneous release occurring in the absence of any histamine releasing agent, which was 11.1 ± 3.0% on average.

Preparation of mesentery, lung, and heart mast cells.

Mast cells were obtained by the method of Pearce and coworkers (Ali & Pearce, 1985; Ennis & Pearce, 1980; Pearce & Ennis, 1980). The animals were anaesthetized with ether and killed by exsanguination. The heart was rapidly removed and flushed free of blood via a cannula inserted into the aorta. The lung was removed and dissected free of major airways. The different tissues were then washed separately in Tyrode solution added calcium chloride to a final concentration of 1 mM. The pH of the Tyrode solution was adjusted to 7.4 before use. The tissue was cut automatically into sections (ca. 1 mm^2) with a McIlwain tissue chopper. The divided tissue was then incubated for a pre-determined optimum period (90 min, rat; 180 min, guinea-pig) in a soluton of collagenase (Sigma Type IA, 160 units ml^{-1}, 25 ml) in buffer supplemented with foetal calf serum (Gibco, 20%). The solution was gassed throughout with a mixture of oxygen (95%) and carbon dioxide (5%). At the end of the incubation, the tissue was disrupted by expression through a syringe and the resulting suspension was filtered through a moistened gauze. Cells were recovered through centrifugation (4°C, 5 min, 150 *g*) and washed sequentially in Tyrode solution containing foetal calf serum (10%) and then in buffer alone. Any remaining debris or clumps of cells were at times removed by passage through a porous plastic filter, and the cells were then divided into aliquots for the experiments.

Determination of histamine secretion from mesentery, lung, and heart mast cells

The histamine release was determined as described above for peritoneal cells except for the assay, which was performed according to Atkinson *et al.* (1979) in a commercial automated apparatus (Technichon Autoanalyser II). Spontaneous histamine release of 1–5% was subtracted from the totals.

Preparation of basophil leukocytes

Blood was drawn by venipuncture from healthy adult volunteers and leukocytes were obtained from the whole blood by dextran sedimentation as described by Lichtenstein & Osler (1964). The whole leukocytes obtained from the dextran sedimentation were washed twice at room temperature in Tyrode solution free from divalent ions and finally resuspended in Tyrode solution either free from calcium or containing calcium 1 mM as required by the experimental protocol. The cells were preincubated for 5 min at 37°C in a volume of 0.9 ml before the addition of either thapsigargin or Tyrode solution as a volume of 0.1 ml. Histamine release following this addition was then allowed to proceed for 45 min at 37°C, and at the end of this incubation, the cells were separated from the supernatant by centrifugation at 1000 *g* for 5 min. Histamine remaining in the cell pellet was released by heating to 100°C for 5 min. Histamine released into the supernatant and that released from the heated pellets was assayed fluorimetrically. Histamine released into the supernatant during incubation has been expressed as a percentage of the total histamine content for each aliquot of cells. Spontaneous release of 2–5% was subtracted from the total release in the presence of Tg.

Forty millilitres of blood provided about 20 samples of cells, each sample containing about 50 ng of histamine corresponding to 5000 cells. The whole leukocyte preparation used for these experiments contained approximately 1% basophils.

Preparation of neutrophil leukocytes

Blood was drawn from healthy, adult volunteers by venipuncture. For every 50 ml of whole blood, 4 ml of EDTA solution 0.1 M was added as an anticoagulant. The 50 ml of blood was diluted with 130 ml of saline (NaCl, 154 mM) to prevent clumping of the cells. Neutrophils were obtained by the method of Böyum (1968) with hypotonic lysis to remove the erythrocytes. The neutrophils were washed twice at room temperature in Tyrode solution free from divalent ions and finally resuspended in Tyrode solution containing cytochalasin B, 10 $\mu g\,ml^{-1}$, with or without calcium as required by the experimental protocol. The cells were distributed into polyethylene tubes with about 5×10^6 cells per tube in a volume of 0.9 ml. The cell suspensions were preincubated at 37°C for 5 min before an addition of either thapsigargin or Tyrode solution as a volume of 0.1 ml. Enzyme release from the neutrophils was allowed to proceed for a further 45 min at 37°C and then the cells were separated from the supernatant by centrifugation at 1000 *g* for 5 min. Enzyme remaining in the pellet was released by mixing the pellet with 1 ml of a solution of Triton X-100, 0.4% v/v. β-Glucuronidase was assayed in the supernatants and the Triton-treated pellets by the method of Talalay *et al.* (1946). β-Glucuronidase released into the supernatant is expressed as a percentage of the total amount of enzyme in each aliquot of cells. Lysozyme was assayed by the method of Boasson (1938).

Spontaneous release of 2–5% was subtracted from the total release in the presence of Tg. Fifty millilitres of blood yielded a total of about 2×10^8 neutrophils. After treatment of the cells with 0.1 M HCl the purity was determined by counting the number of polymorphonuclear cells and the total number of cells. The purity was greater than 95%.

Preparation of platelets

Blood was drawn from healthy, adult volunteers by venipuncture, anticoagulated with EDTA, 0.25 M, in the ratio 1 ml of EDTA to 50 ml of blood, and centrifuged at 700 *g* for 5 min. The supernatant platelet-rich plasma was centrifuged at 700 *g* for 10 min. The resulting pellet was resuspended in a physiological saline buffer to achieve a platelet number of 10^8 cells ml^{-1}. The buffer had the following composition (mM): NaCl 145, KCl 5, $MgSO_4$ 1, Na_2HPO_4 0.5, HEPES 10, glucose 5, and the pH was adjusted to 7.4. Quin-2 acetoxymethylester was added to the platelet suspension to achieve a concentration of 5 μM. The suspension was incubated for 25 min at 37°C and centrifuged at 700 *g* for 10 min in order to remove any extraneous dye. A platelet number of 10^8 cells ml^{-1} was obtained by resuspending the pellet in physiological saline buffer, to which calcium chloride was added to achieve a final concentration of 1 mM. The fluorescence (F) was measured at 37°C with continuous stirring in a Perkin Elmer 204-A spectrophotometer, excitation at 339 nm and emission at 492 nm. The cytoplasmic free calcium level ($[Ca^{2+}]_i$ in nM) was calculated according to the following equation:

$$[Ca^{2+}]_i = 115 \times (F - F_{min})/(F_{max} - F)$$

in which 115 nM is the apparent dissociation constant for Ca^{2+} and Quin-2 (Tsien *et al.*, 1982). The maximal fluorescence (F_{max}) was calculated by adding the cell autofluorescence to the fluorescence of the sonicated cell suspension. The minimal fluorescence (F_{min}) was calculated by adding the cell autofluorescence to the fluorescence of the sonicated cell suspension with added EDTA to achieve a final concentration of 5 mM.

Distribution of ^{45}Ca between modified Tyrode solution and an organic phase in the presence and absence of Tg

Tyrode solution was prepared with $^{45}CaCl_2$ (1.77 Ci g^{-1}Ca) at a final concentration of 55 μM. To 500 μl of

this solution was added 50 μl of a 1.5 mM solution of Tg in dimethyl sulphoxide and to another 500 μl was added 50 μl of dimethyl sulphoxide. The two solutions were extracted with 500 μl of toluene.

K^+ release from red blood cells

A K^+-sensitive electrode filled with sodium chloride, 116 mM and potassium chloride, 2 mM was calibrated in the range 2 to 7 mM external potassium.

Human red blood cells were obtained by superficial venipuncture of a healthy, adult volunteer and anticoagulated with EDTA 0.1 M, pH 7.6 in the ratio 4 ml of EDTA to 50 ml of blood. The red cells were washed three times in calcium-free Tyrode solution by alternate centrifugation and resuspension. The red cells were finally resuspended in either calcium-free Tyrode solution or Tyrode solution containing calcium, 1 mM, at a haematocrit of approximately 7%.

Volumes (2 ml) of red cell suspension were maintained in a bath at 37°C, stirred continuously and the extracellular K^+ concentration, $[K^+]_o$, was continuously monitored with the K^+-sensitive electrode immersed in the suspension of cells.

The cells were allowed to reach thermal equilibrium over a 3 min period and then either A 23187 or thapsigargin was added as a small volume (about 20 μl) of concentrated stock solution to give the final concentration indicated in the results. $[K^+]_o$ was monitored for 3 min after the addition of the drug and the K^+ loss in the first min was calculated. Digitonin, 0.1 mM, was then added to release from the cells all of the remaining potassium and $[K^+]_o$ was again measured with the electrode. The K^+ released in the first min after drug addition was expressed as a percentage of the total cell potassium.

Reagents

Tg and Tc were isolated as previously described (Rasmussen *et al.*, 1978), substance P(SP) was obtained from Beckman, Geneva, Path-o-Cyte and anti IgE from Miles Laboratories Inc. Quin-2 acetoxymethylester was obtained from Amersham, U.K., $^{45}CaCl_2$ from Risø Isotoplaboratoriet, Denmark, cytochalasin B, L-α-phosphatidyl-L-serine (PS), and benzalkonium chloride from Sigma Chemical Co., U.K., and bovine test thrombin (30 u ml^{-1}) from Behringwerke, W. Germany. Stock solutions of the Tg and Tc (10 mg ml^{-1}) were prepared in dimethyl sulphoxide and stored in the refrigerator until required. Aliquots were then diluted in buffer to the desired final concentration. The final concentration of dimethyl sulphoxide (<0.1% v/v) did not affect histamine secretion or cytoplasmatic free calcium level. The PS was dissolved in chloroform. The solvent was evaporated on a boiling water bath and the lipid homogenized in buffer.

Results

Tg induced a dose-dependent release of histamine from rat peritoneal mast cells (Figure 1). Purification of the mast cells made the cells apparently more sensitive to the compound. Furthermore, a 10 fold increment of the cell concentration in the suspension decreased the Tg-induced histamine release (Figure 2). Addition of bovine serum albumin (1 mg ml^{-1}) or phosphatidyl serine (10 μg ml^{-1}) to the incubation medium was found not to influence the Tg-induced release (Figure 3), whereas the release provoked by anti IgE was significantly potentiated, if PS was added (legend to Figure 3). Benzalkonium chloride did not significantly inhibit release provoked by submaximal Tg doses (Figure 4), whereas it produced marked inhibition of release induced by the peptide substance P, a basic histamine releasing agent (Johnson & Erdös, 1973).

Rat mesenteric mast cells, and to a much lesser extent pulmonary and cardiac mast cells from this

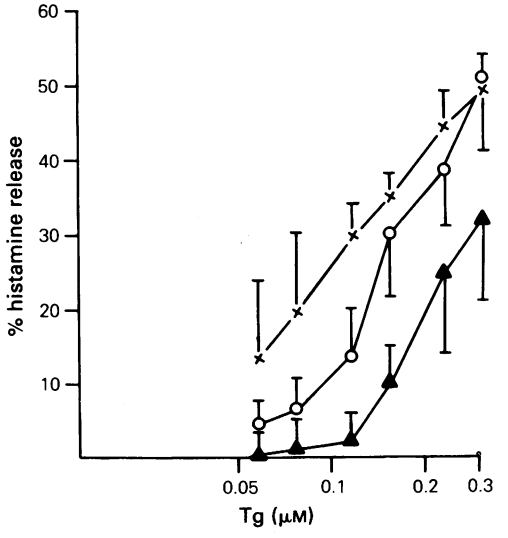

Figure 1 Dose-response curves for thapsigargin (Tg)-induced release of histamine from unpurified rat peritoneal mast cells (▲) and rat peritoneal mast cells purified to about 30% (○) and to about 80% (×). The number of mast cells was standardised to 10^5 per ml of incubation buffer. The points are means from 3 experiments, in which one part of the batch of cells was incubated without purification, while another part of the batch was purified to either a purity of 30% or 80%. Each experiment was performed in duplicate and the vertical bars show s.e. mean.

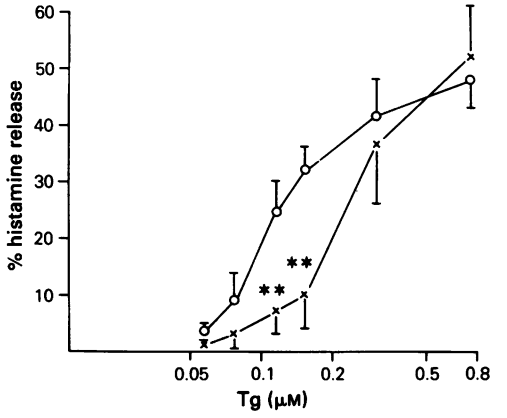

Figure 2 Dose-reponse curves for thapsigargin (Tg)-induced release of histamine from rat peritoneal mast cells purified to about 70% purity in cell suspensions of 22,000 cells ml^{-1} of incubation medium (O) or 220,000 cells ml^{-1} (×). The points are means from 3 experiments, in which one part of the batch of purified cells was incubated in diluted cell suspension and another part in concentrated cell suspension. Each experiment was performed in duplicate and vertical bars show s.e.mean. $**0.01 > P > 0.005$, unpaired t test.

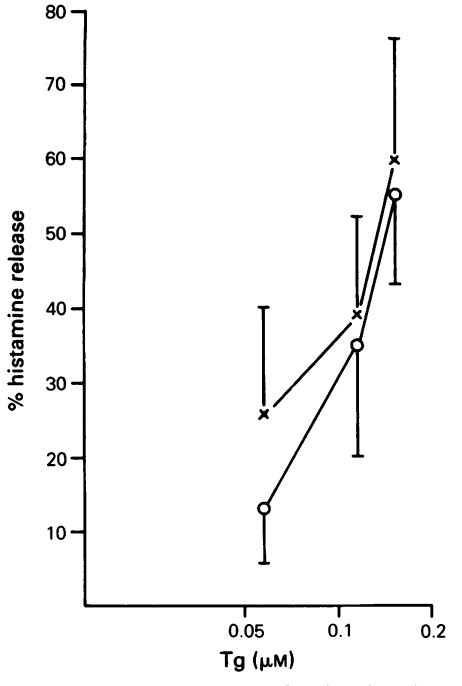

Figure 3 Dose-response curves for thapsigargin (Tg)-induced release of histamine from unpurified rat peritoneal mast cells in the presence of phosphatidyl serine (PS) 10 μg ml^{-1} (O) or in its absence (×). The points are means from 3 experiments, in which one part of the batch of cells was incubated in the presence and another part in the absence of PS. In the absence of PS, anti IgE induced a release of 36.3 ± 11%, whereas the release in the presence of PS was 62.1 ± 11%. Each experiment was performed in duplicate and vertical bars show s.e.mean.

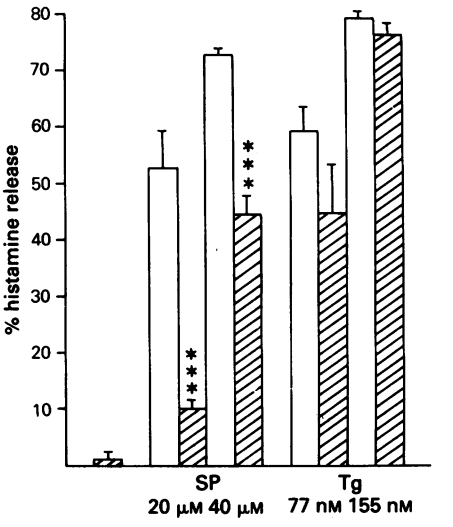

Figure 4 Activation of histamine secretion by thapsigargin (Tg) or substance P (SP) from unpurified rat peritoneal mast cells in the absence (open columns) or presence of benzalkonium chloride 10 μM (hatched columns). The columns represent the mean of 5 experiments, in which one part of the batch of cells was incubated in the absence and another part in the presence of benzalkonium chloride. Each experiment was performed in duplicate and vertical bars show s.e.mean. $***0.005 > P$, unpaired t test.

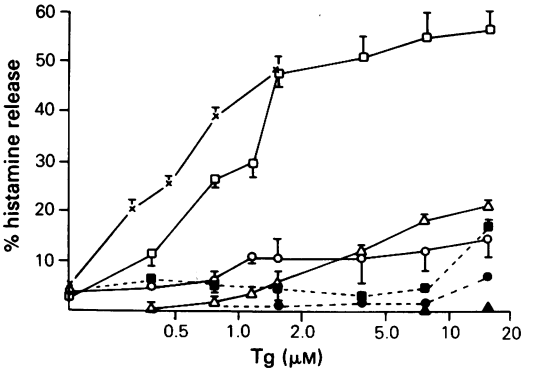

Figure 5 Dose-response curves for thapsigargin (Tg)-induced histamine release from rat (×, O, △, □) or guinea-pig (●, ▲, ■) tissue mast cells. Cells were obtained from heart (O, ●), lung (△, ▲), mesentery (□, ■), and peritoneum (×). The points are the means from 4 experiments, and the vertical bars show s.e.mean.

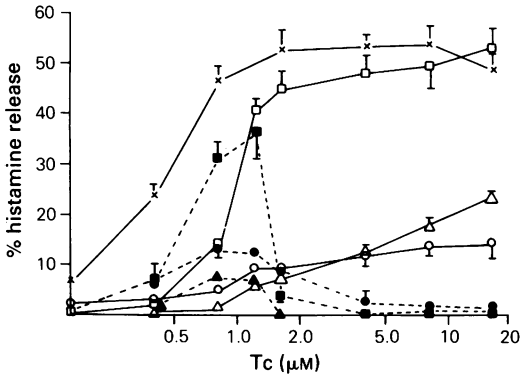

Figure 6 Dose-response curves for thapsigargicin (Tc)-induced histamine release from rat (×, ○, △, □) or guinea-pig (●, ▲, ■) tissue mast cells. Cells were obtained from heart (○, ●), lung (△, ▲), mesentery (□, ■), and peritoneum (×). The points are the means from 4 experiments and the vertical bars show s.e.mean.

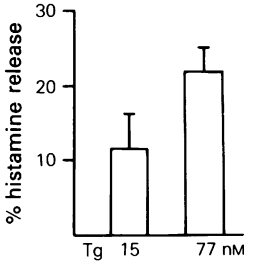

Figure 7 Activation of human basophil leukocytes by thapsigargin (Tg) in the presence of Ca^{2+}, 1 mM. In the absence of Ca^{2+} no release was obtained. The columns are the means from two experiments each performed in duplicate, and the bars show s.e.mean.

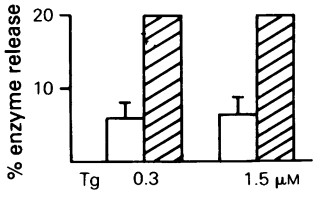

Figure 8 Thapsigargin (Tg)-induced release of β-glucuronidase (open columns) or lysozyme (hatched columns) from human neutrophil leukocytes in the presence of Ca^{2+}, 1 mM, and cytochalasin B. 10 μg ml^{-1}. In the absence of Ca^{2+} no release was obtained. The columns are the means from two experiments each performed in duplicate, and the bars show s.e.mean.

Table 1 Release of potassium from human red blood cells by A 23187 and thapsigargin

Drug and concentration		*K^+ released in first min* (%)	
A 23187, 1 μM + Ca^{2+}, 1 mM		8.1 ± 0.4	($n = 4$)
A 23187, 1 μM + no Ca^{2+}		0.9	($n = 1$)
Thapsigargin, 0.15 μM	Ca^{2+}, 1 mM	0.5	($n = 2$)
Thapsigargin, 0.38 μM	Ca^{2+}, 1 mM	0.0	($n = 2$)
Thapsigargin, 1.5 μM	Ca^{2+}, 1 mM	0.5	($n = 2$)

species, were also found to be sensitive to Tg (Figure 5). In contrast, guinea-pig mast cells were found to be far less sensitive to Tg. Only the mesentery mast cells were found to yield histamine release exceeding 10% and then only at a Tg concentration of 15 μM. Poor solubility of Tg in aqueous media prevents investigations at higher concentrations. Thapsigargicin (Tc) was found to activate rat mast cells in the same concentration range as did Tg and to the same degree. Very surprisingly, however, a totally different picture was obtained with guinea-pig cells. Tc activated the guinea-pig mesenteric mast cells, in particular, in the range 0.16–1.6 μM, whereas no release was observed either at lower or at higher concentrations (Figure 6).

In addition to mast cells, Tg was also found to be able to activate human basophil leukocytes (Figure 7). The release was totally dependent on the presence of calcium ions in the incubation medium. Also human neutrophil leukocytes were activated in a calcium-dependent way. Azurophil and specific granules were both secreted (Figure 8). Tc induced no release of lysozyme from the neutrophils.

Thapsigargin 0.15 to 1.5 μM did not cause potassium release from human red blood cells in the presence of extracellular calcium (1 mM). In contrast, the ionophore A 23187 induced a calcium-dependent release of potassium from red blood cells (Table 1). Analogously, Tg in contrast to A 23187 was not able to

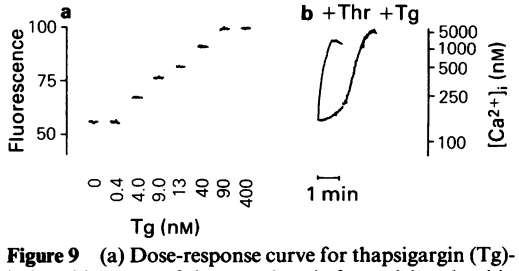

Figure 9 (a) Dose-response curve for thapsigargin (Tg)-induced increase of the cytoplasmic free calcium level in intact human blood platelets. The fluorescence intensities were measured after incubation of the platelets with Tg at 37°C for 4 min. (b) Rise in the cytoplasmic free calcium level in intact human blood platelets induced by thrombin, 0.4 iu ml^{-1} (+ Thr) or Tg, 400 nM (+ Tg).

transfer $^{45}Ca^{2+}$ from an aqueous phase into an organic phase.

Thapsigargin increased the cytoplasmic free calcium level ($[Ca^{2+}]_i$) in human blood platelets in a dose-dependent manner (Figure 9a). A maximal rise in $[Ca^{2+}]_i$ was obtained with a Tg concentration of only 30 nM. The calcium level increase induced by Tg developed considerably more slowly than the increase obtained after addition of thrombin (Figure 9b).

Discussion

The present study has revealed that Tg is able to induce histamine release from a variety of histamine-containing cells, enzyme release from human neutrophil leukocytes, and to increase the cytoplasmic free calcium level in human platelets.

Except for the immunological stimuli very few secretogogues are known to stimulate mast cells, neutrophil and basophil leukocytes, and platelets. The muramyl peptides, which are believed to activate the cells through an interaction with receptors on the cell-surface, constitute one group (Adam & Lederer, 1984). The ionophores form another group. The carboxylic ionophores, e.g. A 23187, are assumed to be dissolved in the cell membranes. At the outer interface a lipid soluble complex is formed between the inorganic ion and the ionophore, which then diffuse through the membrane to the inner interface, where the inorganic ion is exchanged for hydrogen (Pressman, 1976). Tg does not contain a carboxylate group, but at physiological pH the lactone ring will to some extent be opened to give a carboxylate group. Furthermore the number of polar groups make a Tg-Ca^{2+}-complex likely. The hypothesis that Tg might be acting as a Ca^{2+}-ionophore would thus require that Tg is taken up by the cell membrane and that Tg forms a lipid soluble complex with Ca^{2+}. The observed depenence of the activity of Tg on the purity of the mast cell preparation (Figure 1) and on the cell concentration in the incubation medium suggests that Tg is taken up by the cells. However, the inability of Tg to transfer $^{45}Ca^{2+}$ into an organic phase indicates that no lipid soluble complex between Ca^{2+} and Tg is formed. Also, unlike A 23187, Tg is unable to release potassium from red blood cells, which it would be expected to do if it was acting as an ionophore.

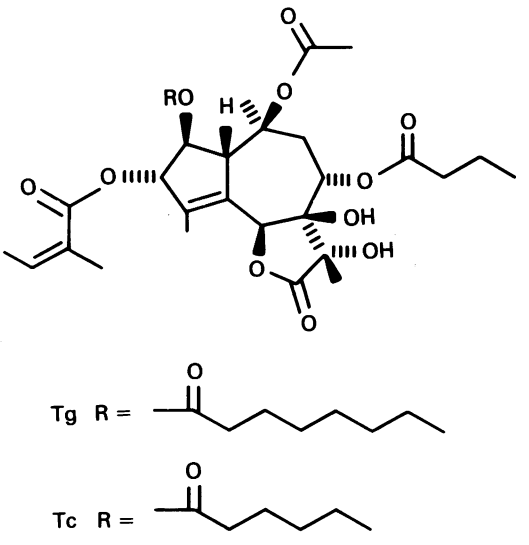

Figure 10 Structures of Thapsigargin (Tg) and Thapsigargicin (Tc).

Comparison of the structures of Tg and Tc reveals that the only difference between the two compounds is the presence of four methylene groups in the acyl group (R) of Tc, whereas six are present in Tg. This difference only causes a minor quantitative difference of the ability of the two compounds to activate purified rat peritoneal mast cells (Rasmussen *et al.*, 1978). No pronounced differences were demonstrated between the ability of the two compounds to release histamine from crude cell suspensions from rat heart, skin or mesentery (Figures 5 and 6). The observed order for sensitivity of the different cell types (mesentery > lung > heart) is similar to that obtained for other histamine releasing agents (Ali & Pearce, 1985). Surprising, however, is the poor ability of Tg to activate the corresponding guinea-pig mast cells, whereas Tc strongly stimulates guinea-pig cells in the concentration range 0.1–1.5 μM. Although a number of other ionophores have bell-shaped dose-response curves (Leung & Pearce, 1984) the total absence of response of the guinea-pig cells at higher concentrations of Tc is surprising. A similar bell-shaped dose response curve is observed for the histamine release from rat peritoneal mast cells induced by palytoxin, which in low concentrations is a histamine releaser, but in high concentrations is an inhibitor for every specific releaser investigated, including palytoxin itself (Chhatwal *et al.*, 1982). The apparent inability of Tg to activate guinea-pig cells might be explained by assuming that the cells are becoming insensitive to the effects of Tg at the same or even a lower concentration than that necessary for activating the cells.

In conclusion, Tg releases histamine in a calcium-dependent manner from a variety of histamine-containing cells from different sources as well as inducing enzyme release from human neutrophils and an increased cytoplasmic free calcium level in human platelets. The action of Tg has been shown to be dependent upon the cell concentration and on the purity of cell preparation. It is possible that Tg is taken

up, therefore, by cells in order to induce cell activation and secretion. There are clear similarities between A 23187, the calcium ionophore, and Tg in their action on the cell types we have studied, but we have been unable to demonstrate any calcium ionophore-like activity with Tg. Tg appears, therefore, to have a novel and, as yet, unknown mechanism of action on mast cells, basophils, neutrophils, and platelets.

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References

ADAM, A. & LEDERER, E. (1984). Muramyl peptides, immunomodulators, sleep factors, and vitamins. *Med. Res. Rev.*, **4**, 111–152.

ALI, H. & PEARCE, F.L. (1985). Isolation and properties of cardiac and other mast cells from the rat and guinea-pig. *Agents and Actions*, (in press).

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.

BOASSON, D.G. (1938). On the bacterial lysis by lysozyme. *J. Immunol.*, **34**, 281–293.

BÖYUM, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. lab. Invest.*, **21**, suppl. **97**, 77–89.

CHHATWAL, G.S., AHNERT-HILGER, G., BERESS, L. & HABERMANN, E. (1982). Polytoxin both induces and inhibits the release of histamine from rat mast cells. *Int. Archs Allergy appl. Immun.*, **68**, 97–100.

CHRISTENSEN, S.B. & NORUP, E. (1985). Absolute configurations of the histamine liberating sesquiterpene lactones thapsigargin and trilobolide. *Tetrahedron Lett.*, **26**, 107–110.

CHRISTENSEN, S.B., NORUP, E., RASMUSSEN, U. & MADSEN, J.Ø. (1984). Structure of histamine releasing guaianolides from *Thapsia* species. *Phytochemistry*, **23**, 1659–1663.

CHRISTENSEN, S.B. & SCHAUMBURG, K. (1983). Stereochemistry and carbon-13 nuclear magnetic resonsance spectroscopy of the histamine-liberating sesquiterpene lactone thapsigargin. *J. org. Chem.*, **48**, 396–399.

DIAMANT, B. & PATKAR, S.A. (1980). The influence of hypertonicity on histamine release from isolated mast cells. *Agents and Actions*, **10**, 140–144.

ENNIS, M. & PEARCE, F.L. (1980). Differential reactivity of isolated mast cells from the rat and guinea-pig. *Eur. J. Pharmac.*, **66**, 339–345.

JOHNSON, A.R. & ERDÖS, E.G. (1973). Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. exp. Biol. Med.*, **142**, 1252–1256.

LEUNG, K.B.P. & PEARCE, F.L. (1984). A comparison of histamine secretion from peritoneal mast cells of the rat and hamster. *Br. J. Pharmac.*, **81**, 693–701.

LICHTENSTEIN, L.M. & OSLER, A.G. (1964). Histamine release from human leukocytes by ragweed pollen antigen. *J. exp. Med.*, **120**, 507–530.

LOEFFLER, L.J., LOVENBERG, W. & SJORDSMA, A. (1971). Effects of dibutyryl 3′,5′-cylcic adenosine monophosphate, phosphodiesterase inhibitors and prostaglandin E, on compound 48/80-induced histamine release from rat peritoneal mast cells *in vitro*. *Biochem. Pharmac.*, **20**, 2287–2297.

PATKAR, S.A., RASMUSSEN, U. & DIAMANT, B. (1979). On the mechanism of histamine release induced by thapsigargin from *Thapsia garganica*. *Agents and Actions*, **9**, 53–57.

PEARCE, F.L. & ENNIS, M. (1980). Isolation and some properties of mast cells from the mesentery of the rat and guinea-pig. *Agents and Actions*, **10**, 124–131.

PRESSMAN, B.C. (1976). Biological applications of ionophores. *A. Rev. Biochem.*, **45**, 501–530.

RASMUSSEN, U., CHRISTENSEN, S.B. & SANDBERG, F. (1978). Thapsigargin and thapsigargicin, two new histamine liberators from *Thapsia garganica*. *Acta Pharmaceut. Suec.*, **15**, 133–140.

SHORE, P.A., BURKHALTER, A. & COHN, V.H. (1952). A method for the fluorimetric assay of histamine in tissues. *J. Pharmac. exp. Ther.*, **127**, 182–186.

TALALAY, P., FISHMAN, W.H. & HUGGINS, C. (1946). Chromogenic substrates. II Phenolphthalein glucuronic acid as a substrate for the assay of glucuronidase activity. *J. biol. Chem.*, **116**, 757–772.

TSIEN, R.Y., POZZAN, T. & RINK, T.J. (1982). Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. *J. cell. Biol.*, **94**, 325–334.

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