ELSEVIER

European Journal of Pharmacology 295 (1996) 243–252

ejp

Some studies on the effects of α-chymotrypsin on mast cells from the rat and other species

Behzad Emadi-Khiav *, Frederick L. Pearce

Department of Chemistry, University College London, 20 Gordon Street, London WC1H OAJ, UK

Received 13 July 1995; revised 25 September 1995; accepted 10 October 1995

Abstract

We have examined the effect of α-chymotrypsin on isolated mast cells from different sources. The enzyme induced a dose-dependent secretion of histamine from purified and non-purified populations of rat peritoneal mast cells. The release was non-cytotoxic and was inhibited by metabolic blockers and extremes of temperature. The process was relatively slow, being essentially complete within 20 min, and was unaffected by phosphatidylserine. A substantial component of the secretion persisted in the absence of extracellular Ca^{2+}. The release was suppressed by extremes of pH and a variety of anti-allergic compounds and serine esterase inhibitors. In addition to the secretion of preformed mediators, α-chymotrypsin also induced the metabolism of arachidonic acid, resulting in the release of prostaglandin D_2 in a dose-related manner from purified rat peritoneal mast cells. α-Chymotrypsin exhibited a marked tissue and species selectivity in its action and tissue mast cells of the rat, guinea pig and human were generally resistant to the enzyme except at cytotoxic concentrations. On the basis of these results, the possible role of endogenous serine esterases in mast cell activation is discussed.

Keywords: Anti-allergic drug; α-Chymotrypsin; Histamine; Mast cell; Serine esterase

1. Introduction

It has been suggested that activation of one or more proteolytic enzymes may constitute the earliest biochemical change that ultimately leads to mediator secretion from the mast cell (Ishizaka and Ishizaka, 1984; Kido et al., 1985). In accordance with this hypothesis, diisopropyl fluorophosphate (DFP), an irreversible inhibitor of serine esterases, blocks IgE-dependent histamine release from chopped guinea-pig lung (Ishizaka and Ishizaka, 1984), rat mast cells (Dvorak et al., 1984) and human lung fragments (Kaliner and Austen, 1974). However, this effect is only seen if the compound is present at the time of Fc_ϵ receptor cross-linking and the inhibition is greatly reduced if the cells are incubated with the drug and washed prior to the addition of the stimulus. This implies that the enzyme in resting cells is present in an inactive proesterase form which is unaffected by DFP. DFP is also ineffective when added after the stimulus, suggesting that IgE-dependent activation of mast cells leads to the priming of serine esterases necessary for histamine secretion.

Further support for a potential role of serine esterases in the initiation of mediator release was provided when α-chymotrypsin (Lagunoff et al., 1975) and rat mast cell chymase (Schick and Austen, 1989) were found to induce mediator secretion from rat mast cells. In addition, Ishizaka and Ishizaka (1984) demonstrated that inhibitors of chymotrypsin and trypsin blocked IgE-mediated histamine release and increased phospholipid metabolism in rat mast cells. They also observed that histamine release induced by chymotrypsin and IgE-directed ligands was inhibited by a number of diverse serine esterase inhibitors. Thus, the above findings suggest that one or more serine esterases is involved in IgE-dependent histamine release. This effect may potentially be mimicked by the addition of exogenous serine esterases. With this in mind, we have now examined the effect of α-chymotrypsin on isolated mast cells from different sources.

* Corresponding author. Tel.: (44) 0171-387-7050×4674; fax: (44) 0171-380-7463.

SSDI 0014-2999(95)00663-X

2. Materials and methods

2.1. Materials

Antimycin A, *N*-acetyl-L-tyrosine ethyl ester (ATEE), 8-bromo cAMP (8-BrcAMP), bovine serum albumin, calcium ionophore A23187, α-chymotrypsin, α-chymotrypsin pretreated with the serine esterase inhibitor DFP or the trypsin inhibitor *N*-tosyl-L-lysine chloromethylketone (TLCK), α-chymotrypsinogen, chymostatin, compound 48/80, 2-deoxy-D-glucose, DFP, dibutyryl cAMP (Bu_2cAMP), isobutyl methylxanthine (IBMX), isatoic anhydride, 2-nitro-4-carboxyphenyl *N*,*N*-diphenyl carbamate (NCDC), phenylmethylsulphonyl fluoride (PMSF), phenylboronic acid, theophylline, *N*-tosyl-L-phenylalanine chloromethylketone (TPCK), TLCK and quercetin were purchased from Sigma. Sheep antiserum to rat IgE (anti-rat IgE) was obtained from ICN Immunobiologicals. Rabbit antiserum to human IgE (anti-human IgE) was obtained from Dako. Salbutamol sulphate was a gift from Glaxo Group Research. Disodium cromoglycate (DSCG) and nedocromil sodium were gifts from Fisons Pharmaceuticals. Prostaglandin D_2 ^{3}H-radioimmunoassay (RIA) kits were obtained from Amersham.

2.2. Isolation and purification of cells

Sprague-Dawley rats (200–500 g) and Dunkin-Hartley guinea pigs (800–1000 g) were used throughout the present study. Rat peritoneal cells were obtained by direct lavage (White and Pearce, 1982) and purified by density gradient centrifugation over Percoll (Mackay and Pearce, 1992). The purity of mast cell samples was determined using a Neubauer haemocytometer, after staining with alcian blue (0.1%, w/v in 0.7 M HCl). Under these conditions, preparations of up to 99% purity are obtained.

Macroscopically normal human tissue was recovered following surgery for bronchial and colonic carcinoma or for circumcision. Colonic mucosa and submucosa/muscle were separated by blunt dissection. Normal human lung parenchyma was dissected free of major airways and blood vessels. Infant foreskins (0–12 years) were obtained following circumcision and were dissected free from underlying fat. Fragments of the tissue, approximately 1 mm^2, were digested with collagenase (160 U/ml) and hyaluronidase (500 U/ml, skin only) in bovine serum albumin-Hepes buffer (pH = 7.4) composed of 137.0 mM NaCl, 5.6 mM glucose, 2.7 mM KCl, 1.0 mM $CaCl_2$, 10.0 mM Hepes, 0.4 mM NaH_2PO_4 and 1.0 mg/ml bovine serum albumin, for 2×90 min at 37°C or longer if after this time substantial tissue remained undigested. Cells were then recovered as previously reported (Ali and Pearce, 1985). Basophil leukocytes were isolated by dextran sedimenation of peripheral blood. Guinea pig and rat tissue fragments were treated as described above for the human.

2.3. Secretion and inhibition of histamine release

In general, isolated mast cells were left to equilibrate in a water bath (37°C) in the Hepes buffer

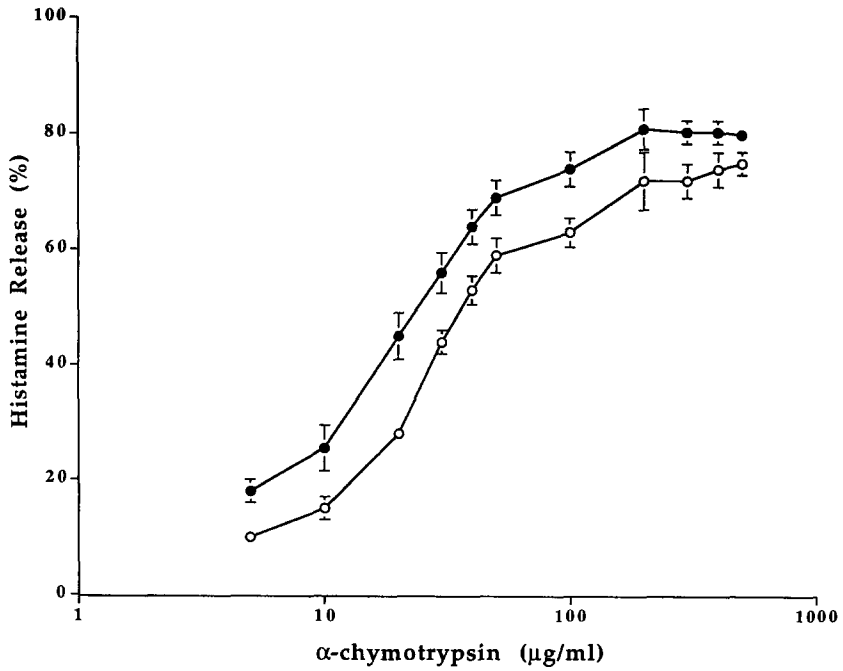

Fig. 1. Effect of α-chymotrypsin on purified (○) and non-purified (●) rat peritoneal mast cells. Values are means ± S.E.M.; $n = 6$.

described above, except that bovine serum albumin was excluded before addition of the releasing agent. The reaction was allowed to proceed for 10 min and was terminated by adding cold Hepes buffer. To determine the dependence of the release on extracellular Ca^{2+}, cells were preincubated with an equal volume of appropriately modified Hepes buffer. This solution contained either Ca^{2+}, no Ca^{2+}, or the Ca^{2+} chelator ethylenediaminetetraacetic acid (EDTA, 0.1 mM), as stated. The reaction was allowed to proceed for 10 min and terminated by addition of Ca^{2+}-free Hepes buffer. In experiments to examine the metabolic requirements for release, cells were preincubated (10 min) with antimycin A (1 μM), to inhibit oxidative phosphorylation, and 2-deoxyglucose (5 mM) to inhibit glycolysis. In other inhibition experiments, cells were preincubated with 8-BrcAMP or Bu_2cAMP (30 min), while disodium cromoglycate and nedocromil sodium were added stimultaneously with the secretory stimulus. Remaining inhibitors were preincubated with the cells for 10 min before challenge.

2.4. Measurement of histamine and prostaglandin D_2 release

Histamine release was measured using an automated spectrofluorometric assay as described previously (Atkinson et al., 1979). Values for histamine release are expressed as a percentage of the total cellular histamine and are corrected for the spontaneous release of histamine that occurred in the absence of any stimulus. For attenuation experiments, results are given as the percentage inhibition of the control release induced by the stimulus alone.

Prostaglandin D_2 release was determined by radioimmuassay using a commercially available kit. The results were expressed as ng prostaglandin $D_2/10^6$ mast cells. All values are given as means ± S.E.M. for the number (n) of experiment performed.

3. Results

3.1. The effect of α-chymotrypsin on rat peritoneal mast cells

α-Chymotrypsin (5–500 μg/ml) induced a dose-dependent secretion of histamine ($\leq$ 80%) from purified and non-purified populations of rat peritoneal mast cells (Fig. 1). Maximal secretion was observed in the presence of physiological concentrations (1 mM) of Ca^{2+}, but a substantial component of the release persisted in the absence of the cation and this was further enhanced by brief pretreatment (5 min) of the cells with EDTA (0.1 mM, Fig. 2).

The release was unaffected by simple omission of glucose from the medium but was essentially abolished by the combined metabolic blockers antimycin A (1 μM) and 2-deoxyglucose (5 mM). Under these conditions, the release from rat peritoneal mast cells stimulated by α-chymotrypsin (500 μg/ml) in normal buffer was 71.0 ± 3.0% and in the presence of the inhibitors was 9.0 ± 1.0% (n = 4).

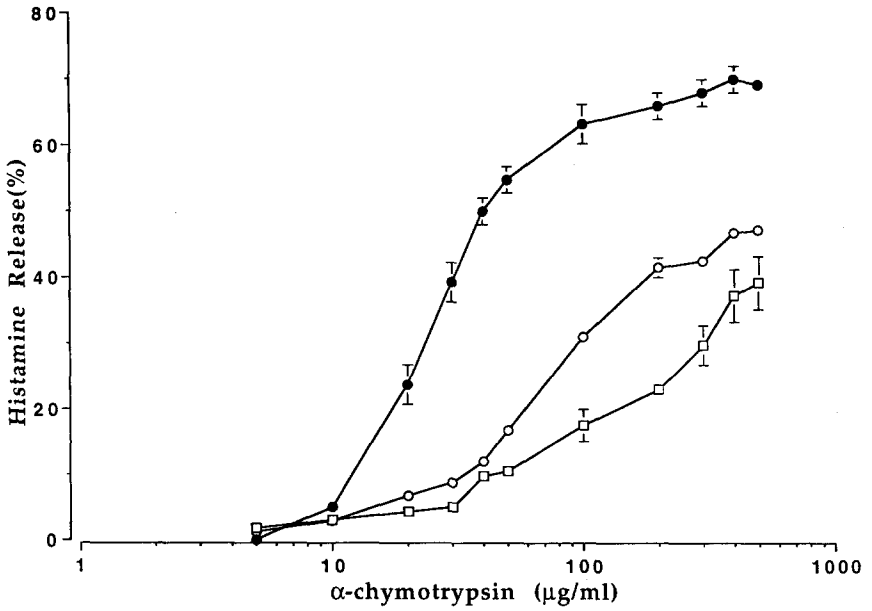

Fig. 2. Calcium dependence of α-chymotrypsin-induced histamine release from rat peritoneal mast cells. Cells were challenged in the presence of calcium (●), absence of calcium (□), and in the presence of EDTA (○). Values are means ± S.E.M.; n = 5.

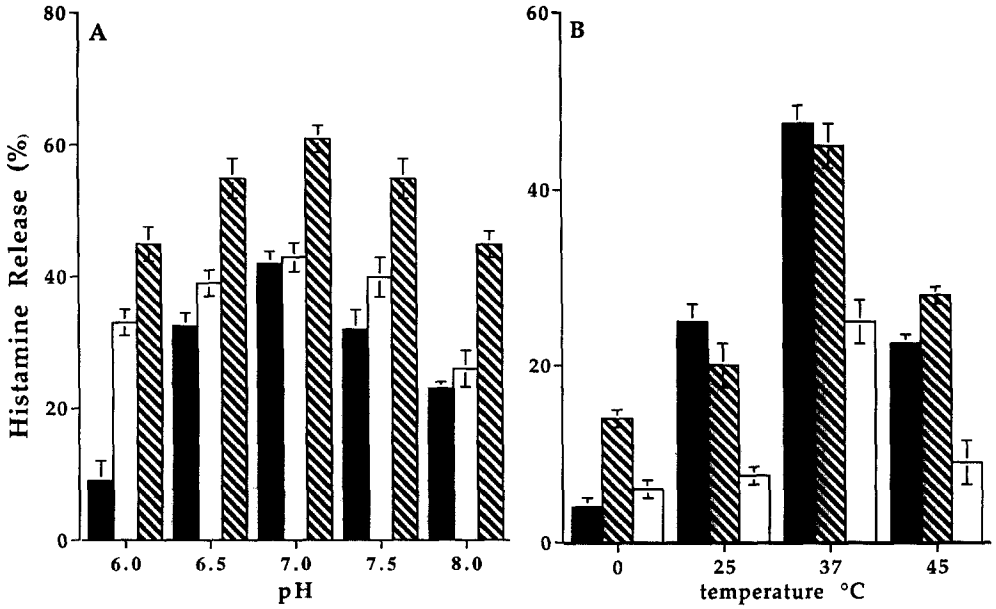

Fig. 3. Effect of pH (A) and temperature (B) on histamine release from rat peritoneal mast cells stimulated with α-chymotrypsin (20 μg/ml, filled columns), compound 48/80 (1 μg/ml, hatched columns) and anti-IgE (1/300 dilution, open columns). Values are means ± S.E.M.; $n = 4$.

The histamine release from rat peritoneal mast cells stimulated by α-chymotrypsin (20 μg/ml) was found to be maximal at physiological pH (7.0–7.5) and was depressed under more alkaline or, particularly, more acidic conditions (Fig. 3A). The releases evoked by anti-IgE (1/300 dilution) and compound 48/80 (1 μg/ml) were similarly seen to be optimal around physiological pH (Fig. 3A). The effect of α-chymotrypsin was again maximal at physiological temperatures (37°C) and markedly suppressed at higher (45°C) and lower values (0, 20°C). Similar results were obtained with compound 48/80 (1 μg/ml) and anti-IgE (1/300 dilution) (Fig. 3B). Histamine release induced by α-chymotrypsin was essentially completed within 20 min, with a half-life of about 40 s (i.e. 50% of the releasable histamine was secreted within this time, data not shown). Histamine release stimulated by α-chymotrypsin was unaffected by addition of exogenous phosphatidylserine (data not shown). In contrast to α-chymotrypsin, its precursor zymogen α-chymotrypsinogen produced a negligible release of histamine (1.0 ± 0.5%, $n = 4$) at the highest concentration tested (500 μg/ml).

Low concentrations of α-chymotrypsin (in the range 3–10 μg/ml), which themselves caused negligible or restricted direct secretion of histamine, were seen to potentiate release induced by anti-IgE (1/300 dilution) (Fig. 4).

α-Chymotrypsin (5–200 μg/ml) produced a dose-dependent release of prostaglandin D_2 from purified rat mast cells. A similar effect was seen with compound 48/80 (0.05–1 μg/ml) and anti-IgE (1/300–1/1000), which were included as positive controls (Table 1). For

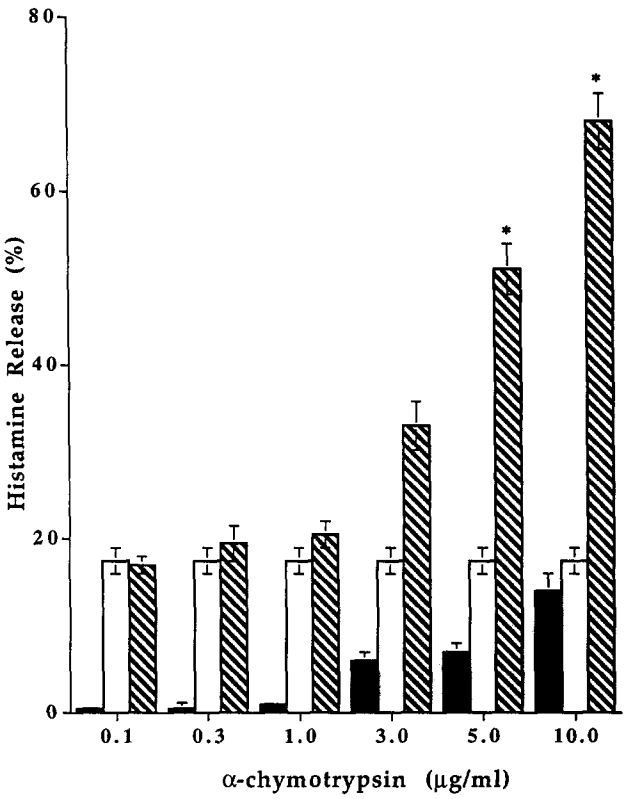

Fig. 4. Effect of anti-rat IgE (1/300 dilution, open columns) on α-chymotrypsin (filled columns) mediated histamine release from isolated rat peritoneal mast cells. Cells were preincubated (10 min) with α-chymotrypsin before addition of IgE. Secretion was allowed to proceed for a further 10 min and the reaction was terminated by addition of cold Hepes buffer. Asterisks show where the histamine release induced by the two agonists together (hatched columns) is significantly ($P < 0.05$) greater than the sum of the releases produced by the agents alone. Values are means ± S.E.M.; $n = 4$.

Table 1
Release of prostaglandin D_2 from purified rat peritoneal mast cells treated with various stimuli

Agonist	Histamine release (%)	Prostaglandin D_2 release (ng/10^6 mast cells)
α-Chymotrypsin		
300 μg/ml	73.0±3.0	71.0±4.0
200 μg/ml	68.0±5.0	53.0±5.0
100 μg/ml	63.0±2.5	36.0±4.0
50 μg/ml	45.0±2.0	35.0±2.0
30 μg/ml	25.0±1.8	27.0±1.0
10 μg/ml	5.0±2.0	11.0±1.0
Compound 48/80		
1.0 μg/ml	71.0±2.0	41.0±1.0
0.5 μg/ml	61.0±3.0	30.0±1.0
0.1 μg/ml	42.0±1.0	7.0±3.0
0.05 μg/ml	12.0±2.0	8.0±2.0
Anti-IgE		
1/100 dilution	18.0±2.0	17.0±2.0
1/300 dilution	15.0±1.0	15.0±1.0
1/1000 dilution	5.0±2.0	14.0±1.0

Purified rat peritoneal mast cells were left to equilibrate (10 min) before addition of the releasing agents. Reaction was allowed to proceed for 10 min and was terminated by addition of cold buffer. In the case of prostaglandin D_2, samples after challenge were centrifuged and supernatants were separated from the cell pellets and snap-frozen in liquid nitrogen. Values are means±S.E.M. for 4 experiments.

a given level of histamine secretion, α-chymotrypsin seemed to induce a rather greater production of prostaglandin D_2 than compound 48/80.

Commercially available α-chymotrypsin which had been pretreated with DFP was ineffective in inducing histamine release from rat mast cells, while material pretreated with TLCK, which inhibits any trypsin in the sample, showed comparable activity to the native enzyme (data not shown).

3.2. The effects of anti-allergic compounds and cAMP-active drugs on histamine release from rat peritoneal mast cells

DSCG and nedocromil sodium produced a comparable dose-dependent inhibition of anti-IgE induced histamine release from rat peritoneal mast cells. However, both drugs exhibited a sharp tachyphylaxis and activity was rapidly lost on preincubation (10 min) with the cells before challenge (data not shown). Representative values for the inhibitions produced by typical concentrations of the drugs, without preincubation, are shown in Table 2. Nedocromil sodium had a limited effect on the release produced by compound 48/80 and α-chymotrypsin, although DSCG was more active against these two stimuli (Table 2).

Quercetin is a naturally occurring flavonoid structurally related to DSCG, but lacking tachyphylactic properties. It was a potent inhibitor of histamine release from rat mast cells stimulated with anti-IgE, compound 48/80 and α-chymotrypsin (Table 2). In contrast, the β-agonist salbutamol was ineffective at inhibiting histamine release from rat peritoneal mast cells stimulated by anti-IgE, compound 48/80 and α-chymotrypsin, but isoprenaline was rather more active against compound 48/80 and α-chymotrypsin (Table 2).

Table 2
Inhibition of histamine release from rat peritoneal mast cells treated with various stimuli

Inhibitor	Inhibition (%) of release induced by		
	α-Chymotrypsin	Compound 48/80	Anti-IgE
DSCG (10 μM)	80.0±3.0	40.0±1.5	48.0±1.2
Nedocromil sodium (10 μM)	31.0±1.0	22.0±2.0	48.0±2.0
Quercetin (100 μM)	89.0±3.0	61.0±2.8	72.0±1.5
Salbutamol (100 μM)	2.5±1.0	4.2±1.8	14.0±2.2
Isoprenaline (100 μM)	25.0±2.5	30.0±3.0	11.0±0.8
Theophylline (1 mM)	81.0±1.6	24.0±3.0	62.0±1.0
IBMX (1 mM)	68.0±2.3	ND	42.0±2.0
8-BrcAMP (1 mM)	78.0±2.9	ND	42.0±2.0
Bu_2cAMP (1 mM)	89.0±2.3	ND	58.0±3.5

Rat peritoneal mast cells were preincubated (10 min or 30 min for cAMP analogues) with the inhibitors and then challenged, except for DSCG and nedocromil sodium which were added stimultaneously with the secretory stimulus. Secretion was allowed to proceed for a further 10 min. Values are means±S.E.M. for 4 experiments. ND denotes not determined.

The phosphodiestrease inhibitors, theophylline and IBMX, effectively blocked the release induced by both anti-IgE and α-chymotrypsin, although theophylline was less active against compound 48/80 (Table 2).

Table 3
Effect of serine esterase inhibitors on histamine release from rat peritoneal mast cells treated with various stimuli

Inhibitor	Inhibition (%) of release induced by		
	Anti-IgE	Compound 48/80	Calcium ionophore
TPCK (10 μM)	75.0±1.5	ND	68.0±2.0
TLCK (10 μM)	12.0±1.0	2.0±1.0	−2.0±1.0
PMSF (100 μM)	18.0±1.8	−1.5±2.0	8.0±2.3
Isatoic anhydride (100 μM)	33.0±3.1	5.0±3.0	3.0±1.2
ATEE (100 μM)	35.0±2.0	−2.5±2.6	0.0±0.5
NCDC (100 μM)	30.0±2.0	−2.8±2.0	0.0±0.5
Chymostatin (100 μM)	31.0±2.1	−4.0±1.5	7.5±2.0
Phenylboronic acid (100 μM)	48.7±5.4	3.3±2.3	3.5±3.0

Rat peritoneal mast cells were preincubated (10 min) with the inhibitors and then stimulated with anti-IgE (1/200 dilution), compound 48/80 (0.5 μg/ml) and calcium ionophore (0.5 μM). Secretion was allowed to proceed for a further 10 min. Values are means±S.E.M. for 4 experiments. ND denotes not determined.

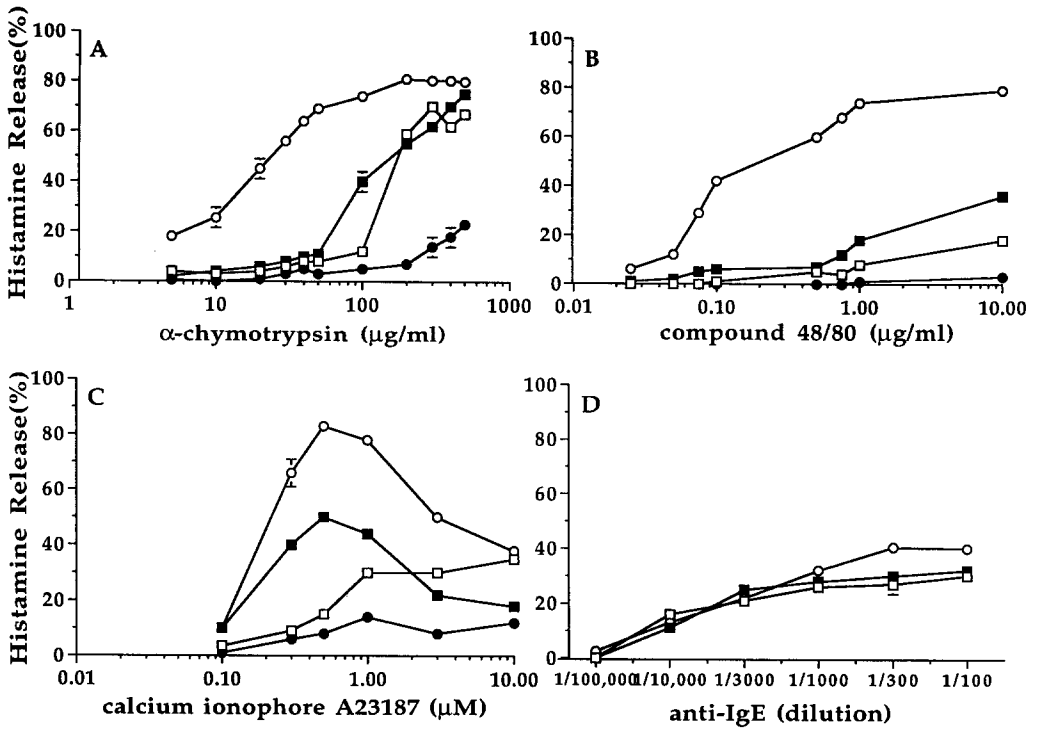

Fig. 5. Functional heterogeneity of rat mast cells from the peritoneal cavity (○), mesentery (■), lung (□) and skin (●) to α-chymotrypsin (A), compound 48/80 (B), calcium ionophore A23187 (C) and anti-IgE (D). Mast cells were left to equilibrate at 37°C in Hepes buffer before addition of the releasing agents. The reaction was allowed to proceed for 10 min and was terminated by addition of cold Hepes buffer. Values are means ± S.E.M; $n = 4–6$.

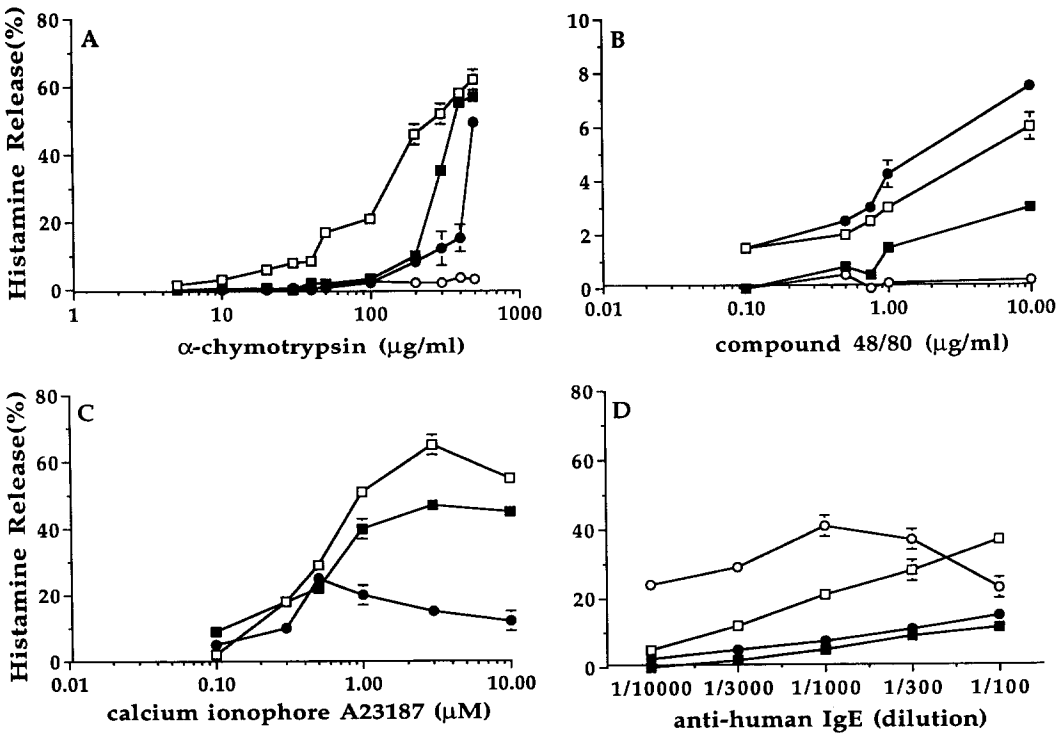

Fig. 6. Functional heterogeneity of human basophils (○) and mast cells from colonic mucosa (■), lung (□) and skin (●) to α-chymotrypsin (A), compound 48/80 (B), calcium ionophore A23187 (C) and anti-human IgE (D). Mast cells were left to equilibrate at 37°C in Hepes buffer before addition of the releasing agents. The reaction was allowed to proceed for 10 min and was terminated by addition of cold Hepes buffer. Values are means ± S.E.M.; $n = 6–8$.

Table 4
Effect of α-chymotrypsin on mast cells from different sources in the presence and absence of the metabolic inhibitors antimycin A (1 μM) and 2-deoxyglucose (5 mM)

Source	Histamine release (%) induced by α-chymotrypsin at a concentration of			
	50 μg/ml		200 μg/ml	
	(−) Inhibitors	(+) Inhibitors	(−) Inhibitors	(+) Inhibitors
Rat				
peritoneum	49.5 ± 0.5	1.0 ± 1.0	70.0 ± 1.5 *	18.5 ± 1.0 *
mesentery	10.5 ± 2.0	5.0 ± 1.5	80.5 ± 3.0 *	74.5 ± 4.0 *
lung	7.0 ± 2.5	2.0 ± 0.5	72.5 ± 2.0 *	71.5 ± 1.0 *
skin	3.0 ± 0.3	1.5 ± 0.5	22.0 ± 1.5 *	18.0 ± 2.0 *
Guinea-pig				
lung	30.0 ± 3.0	11.0 ± 4.0	42.0 ± 3.5	41.0 ± 3.0
mesentery	12.5 ± 2.0	5.0 ± 3.5	18.0 ± 1.5	7.0 ± 3.5
Human				
lung	16.0 ± 4.5	5.0 ± 2.5	31.5 ± 2.5	23.5 ± 2.0
colonic mucosa	3.0 ± 1.0	1.5 ± 0.5	10.0 ± 5.0	7.0 ± 2.5
skin	1.0 ± 1.5	0.8 ± 0.5	9.0 ± 4.0	6.0 ± 3.5
basophils	5.0 ± 2.0	ND	5.0 ± 2.0	ND

Cells were preincubated (10 min) with the inhibitors and then challenged. Secretion was allowed to proceed for a further 10 min. Values are means ± S.E.M. for 4–5 experiments. * Denotes that an enzyme concentration of 500 μg/ml was used. ND denotes not determined.

The cyclic nucleotide analogues 8-BrcAMP and Bu_2cAMP were seen to block histamine release induced by anti-IgE and α-chymotrypsin and in each system the drug was significantly more active against α-chymotrypsin (Table 2).

3.3. The effects of serine esterase inhibitors on histamine release from rat peritoneal mast cells

TPCK, an inhibitor of α-chymotrypsin, potently inhibited histamine release from rat peritoneal mast cells following stimulation by anti-IgE and the calcium ionophore A23187 (Table 3). In contrast, the related trypsin inhibitor TLCK was ineffective (Table 3).

PMSF, which is a general inhibitor of active seryl enzymes, isatoic anhydride, a suicide inhibitor of α-chymotrypsin, ATEE, a chymotryptic substrate, NCDC, again a substrate for α-chymotrypsin, chymostatin, a microbial peptide inhibitor of α-chymotrypsin, and phenylboronic acid, a transition state analogue, all produced a modest inhibition of histamine release from rat peritoneal mast cells stimulated with anti-IgE. However, they were ineffective in inhibiting histamine release stimulated by the calcium ionophore A23187 or compound 48/80 (Table 3).

3.4. The effect of chymotrypsin and various secretagogues on a variety of histaminocytes isolated from different species

As discussed above, α-chymotrypsin induced a dose-dependent release of histamine from rat peritoneal mast cells. However, mast cells from rat mesentery, lung and skin released significant amounts of histamine only at high concentrations of enzyme (Fig. 5A). Moreover, these concentrations were, at least in part, cytotoxic as judged by the effect of metabolic inhibitors (Table 4). The effects of compound 48/80 and calcium ionophore were similarly reduced for the tissue cells, compared to those from the peritoneum, although this difference was less marked for anti-IgE (Fig. 5B–D).

α-Chymotrypsin was effective in releasing histamine in a dose-related manner from isolated guinea-pig lung, but at higher concentrations of α-chymotrypsin (> 200 μg/ml) the release was essentially cytotoxic (Table 4). The effect of α-chymotrypsin was greatly reduced with isolated guinea-pig mesenteric mast cells (Table 4). α-Chymotrypsin was ineffective in releasing histamine from mouse peritoneal mast cells (data not shown).

The effect of α-chymotrypsin on isolated human histaminocytes from a number of different locations was observed (Fig. 6A). These cells are seen to be resistant to the effect of α-chymotrypsin, with the possible exception of the lung, and they released significant amounts of histamine only at cytotoxic concentrations (Table 4). Human basophils and cell preparations from intestine and lung released histamine upon challenge with IgE-directed ligands and the calcium ionophore A23187, but the former preparations were essentially unresponsive to compound 48/80 (Fig. 6B–D). In contrast skin mast cells, and to some extent those from lung, responded significantly to the polyamine (Fig. 6B). Interestingly, low concentrations of α-chymotrypsin potentiated anti-human IgE (1/1000 dilution) mediated histamine release from lung mast cells (Fig. 7).

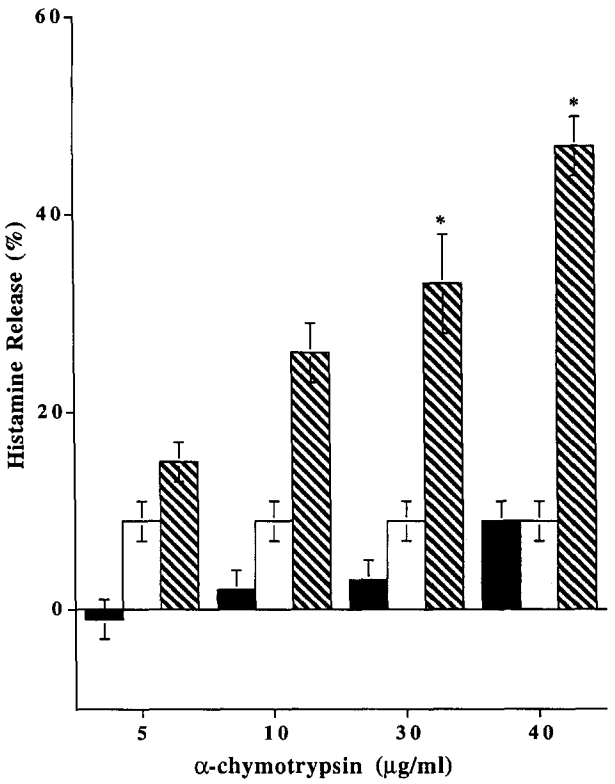

Fig. 7. Effect of anti-human IgE (1/1000 dilution, open columns) on α-chymotrypsin (filled columns) mediated histamine release from human lung mast cells. Cells were pre-incubated (10 min) with α-chymotrypsin before addition of IgE. Secretion was allowed to proceed for a further 10 min and the reaction was terminated by addition of cold Hepes buffer. Asterisks show where the histamine release induced by the two agonists together (hatched columns) is significantly ($P < 0.05$) greater than the sum of the releases produced by the agents alone. Values are means ± S.E.M.; $n = 4$.

4. Discussion

The present study confirms and extends previous observations (Cockroft and Gomperts, 1979; Nials et al., 1983) that α-chymotrypsin induces a dose-dependent secretion of histamine from both purified and non-purified populations of rat peritoneal mast cells. The effect of the enzyme in the former case excludes the possibility that it is acting indirectly through contaminating cells. α-Chymotrypsin pretreated with TLCK was still able to produce a pronounced secretion of histamine from rat peritoneal mast cells. The inhibitor TLCK inactivates any trypsin impurities that may be present in the preparation without affecting the chymotryptic activity. This shows that the release was due to α-chymotrypsin and not any tryptic contaminant present in the enzyme.

The failure of the inactive precursor chymotrypsinogen to release histamine from mast cells suggests that the action of α-chymotrypsin depends on its enzymic activity. Similarly, DFP-pretreated α-chymotrypsin was devoid of histamine-releasing activity.

Histamine secretion induced by α-chymotrypsin was strongly dependent on the pH of the incubation medium, being optimal at physiological values (7–7.5). The response was non-cytotoxic and was inhibited by extremes of temperature and by metabolic inhibitors.

Maximal histamine release induced by α-chymotrypsin was dependent on the presence of extracellular Ca^{2+} in the incubation medium but a significant component persisted in the absence of the cation. Release under these conditions is usually attributed to the mobilization of intracellular Ca^{2+} stores, an effect which may be facilitated by brief pretreatment with EDTA (Pearce, 1982). Accordingly, secretion in a Ca^{2+}-free medium was enhanced by the latter treatment.

α-Chymotrypsin (10–300 μg/ml), in addition to stimulating the secretion of preformed mediators, also induced the metabolism of arachidonic acid, resulting in the release of prostaglandin D_2 in a dose-related manner from purified rat peritoneal mast cells. Interestingly, while maximal concentrations of α-chymotrypsin and compound 48/80 induced comparable releases of histamine, the esterase evoked the production of significantly more of the prostaglandin. This suggests that α-chymotrypsin is capable of activating cellular processes which lead to a more effective metabolism of arachidonic acid than compound 48/80.

α-Chymotrypsin significantly potentiated anti-IgE induced histamine release from rat peritoneal mast cells. As α-chymotrypsin is believed to substitute for an endogenous protease, whose activation is a necessary condition for the anaphylactic release of histamine in rat mast cells (Nials et al., 1983), the presence of excess added enzyme may increase the efficiency of IgE-mediated signal transduction.

Histamine release induced by α-chymotrypsin from rat peritoneal mast cells was inhibited by the anti-allergic chromone DSCG. The drug exhibited a rapid tachyphylaxis, very similar to that seen when immunologic stimuli are used to activate the cells. Inhibition of histamine release from mast cells by nedocromil sodium is thought to involve a similar mode of action to DSCG. However, this newer drug was less active than DSCG against histamine release induced by α-chymotrypsin, perhaps indicating some differences in their mechanism of action.

The β-agonist isoprenaline failed to elicit any appreciable inhibition of histamine release induced by α-chymotrypsin, despite the existence of a large number of high affinity β_2-adrenoceptor sites reportedly present on the mast cell membrane (Marquardt and Wasserman, 1982). Similar results were obtained when the more selective β_2-agonist salbutamol was used.

Whether the lack of β-agonist activity in the rat mast cell reflects a lack of coupling between the β-receptor and downstream signal transduction processes is unclear.

Other anti-allergic compounds, including phosphodiesterase inhibitors and cAMP analogues, all inhibited both α-chymotrypsin and anti-IgE induced histamine release to varying degrees. This again may indicate a link between the two secretory stimuli.

In summary, histamine releases induced by anti-IgE and α-chymotrypsin resembled each other in their kinetics, Ca^{2+} and pH dependencies, and the effect of anti-allergic inhibitors. The secretion induced by α-chymotrypsin was, however, not potentiated by phosphatidylserine and the maximum release ($\leq 80\%$) was greater than that generally achievable following immunologic activation.

TLCK, an active-site directed inhibitor of trypsin-like enzymes, exerted a very weak inhibitory action towards α-chymotrypsin, anti-IgE and calcium ionophore A23187-induced histamine release from rat peritoneal mast cells. In contrast, the analogous chymotrypsin inhibitor TPCK inhibited histamine release induced by the calcium ionophore and, rather more effectively, that evoked by anti-IgE. The inhibitory effects of these closely related compounds strongly indicate that the putative mast cell serine esterase is a chymotryptic-like enzyme.

PMSF, which is a general inhibitor of active seryl enzymes, was much less active than TPCK at inhibiting histamine release induced by anti-IgE and calcium ionophore A23187. The suicide inactivator isatoic anhydride, the chymotryptic substrates ATEE, NCDC, and the transition state analogue phenylboronic acid, all significantly inhibited histamine release induced by anti-IgE, and exerted a very weak inhibitory action towards the ionophore A23187. A selection of these inhibitors was also ineffective at inhibiting compound 48/80-induced histamine release. Together, these results suggest that activation of the putative serine esterase is rather more closely related to IgE-directed mast cell degranulation than to cellular stimulation induced by calcium ionophores or compound 48/80.

Having defined the basic characteristics of the effect of α-chymotrypsin on rat peritoneal mast cells, studies were extended to include mast cells isolated from other species and locations. These studies clearly demonstrated the existence of marked functional variations between isolated mast cells from different species. α-Chymotrypsin was most active as a histamine releaser from rat peritoneal mast cells, while mouse peritoneal mast cells failed to respond to the enzyme. In addition, enzymatically dispersed mast cells from the rat lung and mesentery, guinea-pig lung and mesentery, and human lung were only weakly responsive to α-chymotrypsin.

Isolated mast cells from the rat skin were, in general, much less responsive than their connective tissue counterparts from the peritoneum, mesentery and lung to the histamine-releasing action of a variety of immunological and non-immunological secretagogues. These results therefore emphasise the danger in the simple classification of rat mast cells into mucosal type mast cells and connective tissue type mast cells. Clearly, mast cell heterogeneity in the rat is much more complex than this basic division (Pearce, 1988).

The concept of human mast cell heterogeneity is much less clearly defined than in the rat (Barrett and Pearce, 1991). Despite their apparent histochemical differences, isolated human mast cells, in general, are functionally similar in their responses to a variety of immunological and non-immunological stimuli. Thus, human basophils and cell preparations from intestine and lung release histamine upon challenge with IgE-directed ligands and calcium ionophores, but are essentially unresponsive to polybasic compounds (Tainsh et al., 1992). At non-cytotoxic concentrations, α-chymotrypsin was a very weak releaser of histamine from tissue mast cells from human lung, intestine, skin and isolated basophils. To achieve a comparable release to that obtained from rat peritoneal mast cells, cytotoxic concentrations of α-chymotrypsin were required in the human histaminocytes. The fact that skin mast cells, which were obtained mainly from infant foreskin samples, appeared to be less functionally mature than mast cells obtained from other locations in the adult, as indicated by their comparatively weak response to immunological stimuli and calcium ionophores, renders their unique releasability on challenge with polybasic compounds even more striking.

It was experimentally observed that low concentrations of α-chymotrypsin potentiated anti-IgE mediated histamine release from human lung mast cells. Thus, α-chymotrypsin in the human system may produce effects which amplify the immunologic signal transduction mechanism.

Taken as a whole, the results of the present study show that α-chymotrypsin exhibited a marked tissue and species selectivity in its action. These data expand the concept of functional mast cell heterogeneity. The precise mechanisms that account for this phenomenon, however, have still to be clarified.

In conclusion, the current work provides further evidence that activation of a chymotrypsin-like enzyme is involved in immunologic histamine release from rodent and possibly human mast cells. The main questions that still remain to be answered are how the serine esterase is activated by receptor bridging and what kind of biologically active protease is produced. Identification of this enzyme may then provide a new therapeutic target for the modulation of the mast cell function.

Acknowledgements

It is our pleasure to thank the Wellcome Trust for financial support.

References

Ali, H. and F.L. Pearce, 1985, Isolation and properties of cardiac and other mast cells from the rat and guinea pig, Agents Actions 16, 138.

Atkinson, G., M. Ennis and F.L. Pearce, 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. Pharmacol. 65, 395.

Barrett, K.E. and F.L. Pearce, 1991, Heterogeneity of mast cells, in: Handbook of Experimental Pharmacology, ed. B. Uvnas (Springer-Verlag, Heidelberg) p. 93.

Cockroft, S. and B.D. Gomperts, 1979, Evidence for a role of phosphatidylinositol turnover in stimulus secretion coupling, Biochem. J. 178, 681.

Dvorak, A.M., E.S. Hammel, S.P. Peters, D.W. MacGlashan, R.P. Schleimer, H.H. Newball, K. Pynek, H.F. Dvorak, L.M. Lichtenstein and S.J. Galli, 1984, Differences in the behaviour of cytoplasmic and lipid bodies during human lung mast cell degranulation, J. Cell. Biol. 99, 1678.

Ishizaka, T. and K. Ishizaka, 1984, Activation of mast cells for mediator release through IgE receptors, Prog. Allergy 34, 188.

Kaliner, M. and K.F. Austen, 1974, A sequence of biochemical events in the antigen-induced release of chemical mediators from sensitized human lung tissue, J. Exp. Med. 138, 1077.

Kido, H., N. Fukusen and N. Katunuma, 1985, Antibody and inhibitor of chymase inhibit histamine release in immunglobulin E-activated mast cells, Biochem. Int. 10, 863.

Lagunoff, D., E.Y. Chi and H. Wan, 1975, Effect of chymotrypsin and trypsin on rat peritoneal mast cells, Biochem. Pharmacol. 24, 1573.

Mackay, G.A. and F.L. Pearce, 1992, A comparison of rat peritoneal mast cells purified using Percoll and Path-o-cyte 4, Agent Actions (special conference issue), C315.

Marquardt, D.L. and S.I. Wasserman, 1982, Characterization of the rat mast cell beta-adrenergic receptor in resting and stimulated cells by radioligand binding, J. Immunol. 129, 2122.

Nials, A.T., C.J. Vardey and I.F. Skidmore, 1983, The release of histamine from rat mast cells by chymotrypsin, Int. Arch. Allergy Appl. Immunol. 70, 83.

Pearce, F.L., 1982, Calcium and histamine secretion from the mast cell, Prog. Med. Chem. 19, 59.

Pearce, F.L., 1988, Mast cell heterogeneity; the problem of nomenclature, Agents Actions 23, 125.

Schick, B. and K.F. Austen, 1989, Modulation of chymase-mediated rat serosal mast cell degranulation by trypsin and diisopropyl fluorophosphate, Immunology 66, 434.

Tainsh, K.R., W.L. Liu, H.Y. Lau, J. Cohen and F.L. Pearce, 1992, Mast cell heterogeneity in man: unique functional properties of skin mast cells in response to a range of polycationic stimuli, Immunopharmacology 24, 171.

White, J.R. and F.L. Pearce, 1982, Characteristics of histamine secretion from rat peritoneal mast cells sensitized to the nematode *Nippostrongylus brasiliensis*, Immunology 46, 353.