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Modulation of Tryptase and Histamine Release from Human Lung Mast Cells by Protease Inhibitors

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SUMMARY Inhibition of IgE dependent histamine release from human mast cells by protease inhibitors has been observed in skin, tonsil and synovial tissues. However, little is known about the actions of protease inhibitors on tryptase release from human lung mast cells. We therefore examined the ability of protease inhibitors to modulate tryptase and histamine release from human lung mast cells. IgE dependent tryptase release from dispersed lung mast cells was inhibited to a maximum of approximately 53.8% and 44.5% by N-α-tosyl-L-lysine chloromethyl ketone (TLCK) and N-p-Tosyl-L-phenylalanine chloromethyl ketone (TPCK), respectively. A similar degree of inhibition of calcium ionophore A23187 (CI) induced tryptase release was also observed with these two inhibitors. Preincubation of TLCK or TPCK with the mast cells at 37°C for 20 minutes before addition of anti-IgE or CI did not improve their ability to inhibit anti-IgE and CI induced tryptase release. At a concentration of 10 μg/ml, protamine inhibited anti-IgE or CI induced tryptase release; but at 100 μg/ml, it increased anti-IgE and CI induced release of tryptase from lung mast cells. A concentration dependent inhibition of anti-IgE and CI induced release of histamine from lung mast cells was also observed with TLCK, TPCK and protamine. The maximum inhibition of anti-IgE induced histamine release was approximately 40.7%, 40.2% and 33.4% with TLCK, TPCK and protamine, respectively. At the concentrations tested, TLCK and TPCK by themselves did not stimulate tryptase and histamine release from lung mast cells. A specific inhibitor of aminopeptidase, amastatin, had no effect on anti-IgE induced tryptase and histamine release and was used as control. In conclusion, it was demonstrated that protease inhibitors are able to inhibit IgE dependent tryptase and histamine release from human lung mast cells, which suggested that they could be developed to a novel class of anti-inflammatory drugs to treat allergic conditions in man.

It was reported that increased numbers of mast cells and mast cell degranulation are closely associated with allergic airway diseases including asthma[1,2] and rhinitis.[3-5] Through release of their pro-inflammatory mediators including histamine, tryptase, chymase, heparin and some cytokines,[6] mast cells actively participate in the pathogenesis of these airway diseases.

Tryptase is a tetrameric serine proteinase that constitutes some 20% of the total protein within human mast cells and is stored almost exclusively in the secretory granules of mast cells[7] in a catalytically active form.[8] Relatively high concentrations of tryptase have been detected in bronchoalveolar lavage fluid from patients with bronchial asthma[9,10] and in the serum of patients with allergic rhinitis,[11] implicating that this mediator is involved in the pathogenesis of allergic airway diseases. Evidence is emerging that tryptase may be a key mediator of allergic inflammation and a promising target for therapeutic intervention, as it has been found to be able to induce microvascular leakage in the skin of guinea pigs,[12] bronchial hyperresponsiveness in isolated guinea pig bronchi,[13] bronchoconstriction in allergic sheep airways,[14] inflammatory cell accumulation in the peritoneum of mice[15] and release of IL-8 from human epithelial cells.[16] Moreover, tryptase has been proven

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to be a unique marker of mast cell degranulation *in vitro* as it is more selective than histamine to mast cells.[17]

Increased levels of histamine have been observed in allergic rhinitis,[18] and asthma,[19,20] indicating the likelihood of this mediator being involved in the pathogenesis of these diseases. In recent years, inhibitors of tryptase[21,22] and chymase[23] were discovered to possess the ability to inhibit histamine release from human skin, tonsil, synovial[24] and colon mast cells,[25] suggesting they could likely be developed to a novel class of mast cell stabilizers. However, little is known of the actions of tryptase and chymase inhibitors on tryptase release from human lung mast cells. We therefore investigated the effects of these two types of inhibitors on IgE dependent or independent tryptase release from human lung mast cells in the current study. For comparison, the actions of these inhibitors on histamine release were also investigated in the same experimental system.

MATERIALS AND METHODS

Materials

The following compounds were purchased from Sigma (St. Louis, Mo., USA): TLCK, TPCK, protamine, amastatin, calcium ionophore (CI), histamine dihydrochloride, collagenase (type I), hyaluronidase (type I), bovine serum albumin (BSA, fraction V), penicillin, streptomycin, extravidin peroxidase, and *o*-phenylene diamine (OPD). Minimum Essential Medium (MEM) containing 25 mmol/l *N*-2-hydroxylethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES) and fetal calf serum (FCS) were obtained from Gibco (Paisley, Renfrewshire, UK). Anti-IgE (inactivated) was from bought from Serotec (Kidlington, Oxford, UK). A polyclonal antibody and a monoclonal antibody against tryptase were donated by Dr. Andrew F. Walls (University of Southampton, UK). Histamine plates were purchased from RefLab (Copenhagen, Denmark). HEPES and all other chemicals were of analytical grade.

Dispersion of mast cells

Human lung tissue was obtained by lobectomy. Only macroscopically normal tissue was used for the study. The mast cell dispersion procedure employed was the same as described previously in human tonsils.[21] Briefly, finely chopped tissue was incubated with 1.5 mg/ml collagenase and 0.75 mg/ml hyaluronidase in MEM containing 2% FCS (1 g tonsil/10 ml buffer) for 60 minutes at 37°C. Dispersed cells were separated from undigested tissue by filtration through nylon gauze (pore size 100 μm diameter), washed and maintained in MEM (containing 10% FCS, 200 U/ml penicillin and 200 μg/ml streptomycin) on a roller overnight at room temperature. Mast cell population, as determined by light microscopy after staining with alcine blue, ranged from 3.2 to 5.3%.

Mast cell challenge

Dispersed cells were resuspended in a HEPES buffered salt solution (HBSS, pH 7.4) with $CaCl_2$ and $MgCl_2$ (complete HBSS). One hundred microliter aliquots containing 4-6 × 10^3 mast cells were added to 50 μl of anti-IgE, CI, or an inhibitor in complete HBSS and incubated for 15 minutes at 37°C. The reaction was terminated by the addition of 150 μl ice cold incomplete HBSS and the tubes were centrifuged immediately (500 x *g*, 10 minutes, 4°C). All experiments were performed in duplicate. For the measurement of total histamine concentration, the suspension in certain tubes was boiled for 6 minutes. The supernatants were stored at -20°C until histamine and tryptase concentrations were determined (in duplicate for each tube).

Inhibition of tryptase and histamine release

For some experiments, a protease inhibitor was preincubated with the mast cells for 20 minutes before anti-IgE or CI were added. For other experiments, the protease inhibitor and anti-IgE or CI were added to the cells at the same time (no preincubation period). Data were expressed as the percentage inhibition of tryptase and histamine release, taking into account the mediator release in the presence and absence of the inhibitor. As from our previous experiments, the optimal tryptase and histamine releases from colon mast cells were induced by 10 μg/ml anti-IgE or 1 μg/ml CI,[26] and therefore they were chosen as standard concentrations throughout the study.

Histamine measurement

Histamine concentrations were determined using a glass fiber-based, fluorometric assay.[21] The procedure involves the binding of histamine to a glass-fiber matrix (RafLab, Copenhagen, Denmark) and its spectrophotometrical detection with a Perkin-Elmer LS 2 Detector (Denmark), following the addition of OPD. The histamine release was expressed as a percentage

of the total cellular histamine levels, and corrected for the spontaneous release measured in tubes in which cells had been incubated with the HBSS diluent alone.

Tryptase measurement

Tryptase concentrations were measured with a sandwich ELISA procedure with a specific polyclonal antibody against human tryptase as the capture antibody and AA5, a monoclonal antibody specific for human tryptase, as the detecting antibody.[27]

Statistical analyses

Statistical analyses were performed using SPSS software. Data were expressed as mean ± SEM. Where analysis of variance indicated significant differences between groups with ANOVA, for the preplanned comparisons of interest, Student's *t* test was applied. For all analyses, $p < 0.05$ was taken as significant.

Table 1 Effect of protease inhibitors, anti-IgE and calcium ionophore (CI) on tryptase release from lung mast cells

Compound	Concentration (μg/ml)	Tryptase release (ng/ml)	
		15 minutes	35 minutes
Buffer		7.0 ± 0.6	8.2 ± 1.2
TLCK	0.1	7.2 ± 0.8	ND
	1.0	7.1 ± 0.6	8.4 ± 1.2
	10	7.0 ± 0.7	8.7 ± 1.1
	100	5.3 ± 0.8	7.9 ± 0.8
TPCK	0.08	6.7 ± 0.6	ND
	0.8	7.5 ± 0.7	7.6 ± 1.1
	8.0	7.3 ± 0.7	7.6 ± 1.0
	80	7.0 ± 0.7	8.8 ± 1.2
Protamine	0.1	6.9 ± 0.6	ND
	1.0	7.1 ± 0.5	8.1 ± 1.1
	10	7.8 ± 0.7	8.6 ± 1.3
	100	26.3 ± 4.7*	ND
Amastatin	0.05	7.7 ± 0.7	8.3 ± 1.2
	0.5	7.8 ± 0.6	7.6 ± 1.3
	5.0	7.8 ± 0.8	7.8 ± 1.1
Anti-IgE	10	25.3 ± 8.0*	14.8 ± 1.6*
CI	1.0	21.6 ± 7.3*	15.2 ± 1.6*

The values are mean ± SEM for five separate experiments. The mast cells were incubated with TLCK, TPCK, protamine, amastatin, anti-IgE or CI for 15 minutes or 35 minutes, respectively at 37°C. ND = not done. *$p < 0.05$ compared with buffer alone control.

RESULTS

Effect of protease inhibitors, anti-IgE and CI on tryptase and histamine release from mast cells

At 15 minutes following incubation, anti-IgE at 10 μg/ml and CI at 1 μg/ml were able to induce 25.3 ± 8.0 ng/ml and 21.6 ± 7.3 ng/ml tryptase release from lung mast cells, respectively, whereas at the same time point, spontaneous tryptase release (buffer alone) was 7.0 ± 0.6 ng/ml. The same concentrations of anti-IgE and CI were also able to provoke significant tryptase release from lung mast cells after 35 minutes of incubation (Table 1). TLCK, TPCK and amastatin at the concentrations tested did not stimulate tryptase release from lung mast cells after 15 or 35 minutes of incubation. However, protamine by itself at a concentration of 100 μg/ml was able to stimulate a significant release of tryptase from lung mast cells, but at concentrations of 0.1 and 1 μg/ml, it did not alter the tryptase release ability of mast cells (Table 1). While TLCK, TPCK and amastatin (but not protamine) at the concentrations tested had no effect on histamine release from lung mast cells, in the same experiments anti-IgE and CI were able to induce up to 8.3% and 24.7% net histamine release, respectively, following a 15-minute incubation period (Table 2).

Inhibition of tryptase release from mast cells by protease inhibitors

Both TLCK and TPCK were able to inhibit IgE dependent tryptase release from lung mast cells in a concentration dependent manner. Up to approximately 53.8% or 44.5% inhibition of anti-IgE induced tryptase release were achieved with 100 μg/ml TLCK or 80 μg/ ml TPCK, respectively, when they were added to the mast cells at the same time with anti-IgE (Fig. 1). Similarly, TLCK and TPCK were also capable of inhibiting CI induced tryptase release from lung mast cells in a concentration dependent pattern with up to 39.7% or

37.9% inhibition being achieved with 100 μg/ml TLCK or 80 μg/ml TPCK, respectively (Fig. 2). Preincubation of lower concentrations (1 and 10 μg/ml) of TLCK or TPCK with mast cells for 20 minutes at 37°C slightly enhanced their ability to inhibit CI induced tryptase release, though this increased inhibition was not statistically significant (Fig. 2). Preincubation had little effect on the ability of TLCK and TPCK to inhibit anti-IgE induced tryptase release (Fig. 1). A specific inhibitor of aminopeptidase, amastatin, had no effect on anti-IgE induced tryptase release (Fig. 1).

Protamine revealed dual action on anti-IgE and CI induced tryptase release. At a concentration of 10 μg/ml, protamine inhibited anti-IgE or CI induced tryptase release; but at 100 μg/ml, it increased anti-IgE and CI induced release of tryptase from lung mast cells (Table 3). Preincubation of protamine with cells for 20 minutes before the addition of anti-IgE or CI had little effect on its action on tryptase release from lung mast cells (Table 3).

Table 2 Effect of protease inhibitors, anti-IgE and calcium ionophore (CI) on histamine release from lung mast cells

Compound	Concentration (μg/ml)	Net histamine release (%)	
		15 minutes	35 minutes
TLCK	0.1	-0.8 ± 0.5	ND
	1.0	-1.4 ± 0.6	0.1 ± 0.5
	10	-0.8 ± 0.8	3.0 ± 3.0
	100	-2.3 ± 1.0	3.0 ± 1.9
TPCK	0.08	0.5 ± 0.8	ND
	0.8	-1.1 ± 1.0	0.6 ± 0.5
	8.0	0.1 ± 0.8	3.5 ± 3.5
	80	-1.5 ± 0.6	0 ± 0.5
Protamine	0.1	-1.1 ± 0.5	ND
	1.0	-0.4 ± 0.9	2.9 ± 1.5
	10	3.6 ± 0.6	1.9 ± 1.6
	100	5.5 ± 0.9*	ND
Amastatin	0.05	0.1 ± 1.0	0 ± 0.9
	0.5	0.4 ± 0.8	1.3 ± 0.7
	5.0	-0.5 ± 0.7	0.6 ± 0.7
Anti-IgE	10	8.3 ± 1.4*	7.0 ± 1.5*
CI	1.0	24.7 ± 7.9*	18 ± 5.1*

The values are mean ± SEM for five separate experiments. The mast cells were incubated with TLCK, TPCK, protamine, amastatin, anti-IgE or CI for 15 minutes or 35 minutes, respectively at 37°C. ND = not done. *$p < 0.05$ compared with buffer alone control.

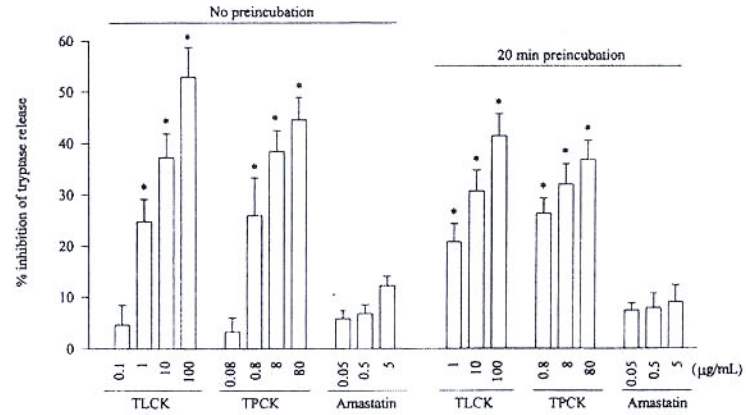

Fig. 1 The effect of the protease inhibitors TLCK, TPCK and amastatin on anti-IgE (10 μg/ml) induced tryptase release from dispersed lung mast cells. The various concentrations of the inhibitors were added to the mast cells at the same time with anti-IgE (no preincubation) or preincubated with the mast cells at 37°C for 20 minutes before the addition of anti-IgE. Data are presented as mean ± SEM for five separate experiments performed in duplicate. *$p < 0.05$ compared with the responses to uninhibited controls.

Inhibition of histamine release from mast cells by protease inhibitors

A concentration dependent inhibition of anti-IgE induced release of histamine from lung mast cells was observed when TLCK, TPCK or protamine were added to the mast cells at the same time. The maximum inhibition of histamine release induced by anti-IgE was approximately 40.7%, 40.2% and 33.4% with 100 μg/ml TLCK, 80 μg/ml TPCK and 10 μg/ml protamine, respectively. Preincubation of various concentrations of TLCK, TPCK and protamine with the mast

Table 3 The effect of protamine on anti-IgE or calcium ionophore (CI) induced tryptase release from human lung mast cells

Protamine concentration (μg/ml)	Tryptase release (ng/ml)			
	No preincubatin		20 minute preincubation	
	Anti-IgE	CI	Anti-IgE	CI
0	25.3 ± 6.5	21.6 ± 7.3	14.8 ± 1.6	15.2 ± 1.6
0.1	21.9 ± 7.4	21.2 ± 7.5	ND	ND
1.0	20.3 ± 8.3	17.4 ± 6.5	13.9 ± 2.0	12.6 ± 1.2
10	18.3 ± 6.9*	15.0 ± 5.0*	11.2 ± 1.6*	11.7 ± 1.2*
100	38.3 ± 14.7†	32.7 ± 8.9†	ND	ND

The values are mean ± SEM for five separate experiments. Protamine was either added to the mast cells at the same time with anti-IgE or CI, or preincubated with the cells for 20 minutes before challenging with anti-IgE or CI. * $P < 0.05$ compared with the uninhibited control (Student's t test). † $P < 0.05$ compared with the corresponding stimulus alone control (Student's t test). ND = not done.

cells at 37°C for 20 minutes before challenging with anti-IgE did not significantly alter the response of the cells to the stimulus (Fig. 3). TLCK, TPCK or protamine were also able to inhibit CI induced histamine release from lung mast cells up to approximately 36%, 33.9% and 37.8%, with 100 μg/ml TLCK, 80 μg/ml TPCK and 100 μg/ml protamine, respectively. Preincubation of TLCK, TPCK or protamine with cells for 20 minutes at 37°C did not improve their ability to inhibit CI induced histamine release (Fig. 4). A specific inhibitor of aminopeptidase, amastatin had no effect on anti-IgE induced histamine release (Fig. 3).

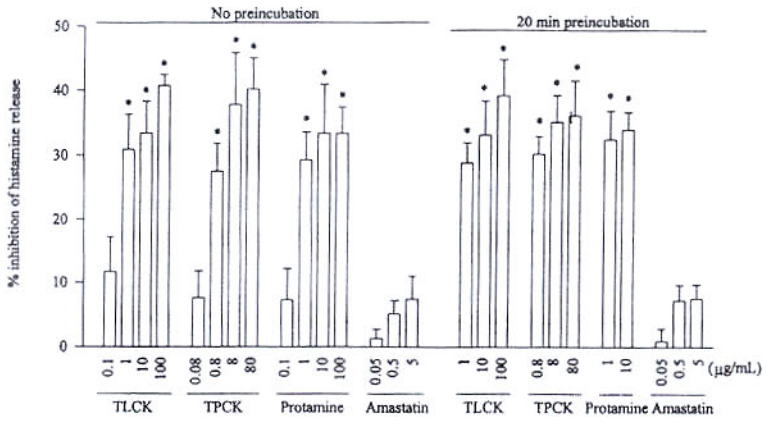

Fig. 2 Inhibition of calcium ionophore (CI, 1 μg/ml) induced tryptase release from dispersed lung mast cells by the protease inhibitors TLCK and TPCK. The various concentrations of the inhibitors were added to the mast cells at the same time with CI (no preincubation) or preincubated with the cells at 37°C for 20 minutes before the addition of CI. Data are presented as mean ± SEM for five separate experiments performed in duplicate. *$p < 0.05$ compared with the responses to uninhibited controls.

DISCUSSION

We found that inhibitors of tryptase and chymase were able to inhibit anti-IgE and CI induced tryptase release from dispersed human lung mast cells. Taking this together with our previous findings that inhibitors of tryptase[21] and chymase[23] could potently inhibit histamine release from human tonsil and skin mast cells, indicates clearly that the inhibitors of tryptase and chymase are mast cell stabilizers with great potential to be developed as a drug that can be used to treat allergic conditions or other mast cell related diseases.

Up to approximately 44.5% inhibition of IgE dependent tryptase release from lung mast cells was observed with a chymase inhibitor, TPCK, indicating that chymase was involved in the process of IgE dependent human mast cell degranulation. This was consistent with our previous finding that chymase inhibitors were able to inhibit IgE

dependent histamine release from tonsil and skin mast cells,[23] which indicated that tryptase and histamine are likely to share a similar degranulation process. Similar to TPCK, a tryptase inhibitor, TLCK, inhibited anti-IgE induced tryptase release from lung mast cells up to 53.8%, which implicated that tryptase is also likely to be involved in the process of mast cell degranulation. This result was consistent with our previous finding that tryptase inhibitors were able to inhibit IgE dependent histamine release from skin and tonsil mast cells.[21] Since the inhibitors at the concentrations used in the current study are able to inhibit more than 95% of tryptase or chymase activity in enzyme assays,[28] the incomplete inhibition of tryptase release from mast cells may suggest that there are other pathways than tryptase and chymase involved in the anti-IgE induced degranulation of lung mast cells. In parallel experiments, TLCK and TPCK were also able to inhibit IgE dependent histamine release from lung mast cells to a maximum of approximately 40.7% and 40.2%. The degree of inhibition was quite similar to that for tryptase release, but much lower than that reported previously, where TPCK was able to completely inhibit FcεRI- mediated histamine release from cultured human mast cells.[29] TPCK was also previously reported to inhibit CI induced histamine release from human lung mast cells.[30]

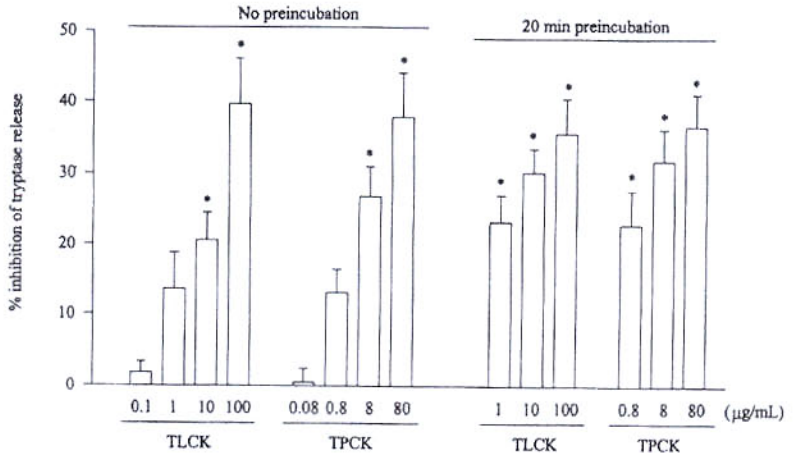

Fig. 3 The effect of the protease inhibitors TLCK, TPCK, protamine and amastatin on anti-IgE (10 μg/ml) induced histamine release from dispersed lung mast cells. The various concentrations of the inhibitors were added to the mast cells at the same time with anti-IgE (no preincubation) or preincubated with the cells at 37°C for 20 minutes before the addition of anti-IgE. Data are presented as mean ± SEM for five separate experiments performed in duplicate. *$p < 0.05$ compared with the responses to uninhibited controls.

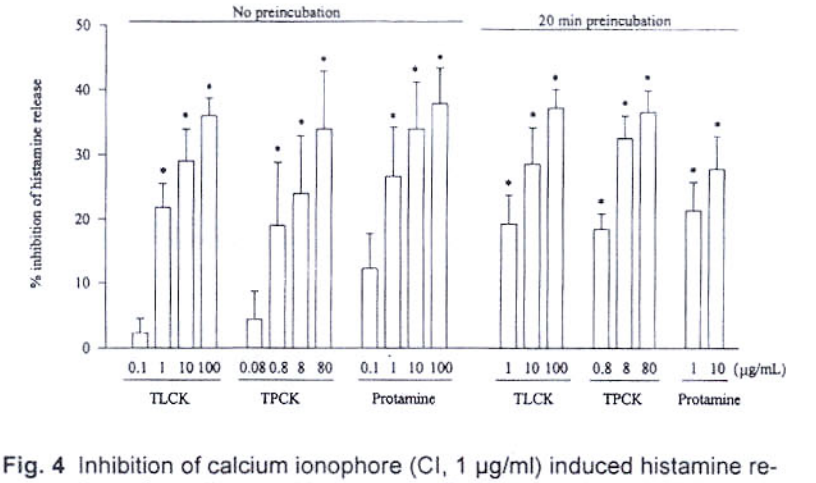

Fig. 4 Inhibition of calcium ionophore (CI, 1 μg/ml) induced histamine release from dispersed lung mast cells by the protease inhibitors TLCK, TPCK and protamine. The various concentrations of the inhibitors were added to the mast cells at the same time with CI (no preincubation) or preincubated with the cells at 37°C for 20 minutes before the addition of CI. Data are presented as mean ± SEM for five separate experiments performed in duplicate. *$p < 0.05$ compared with the responses to uninhibited controls.

CI is a calcium carrier that can elevate the calcium concentration in the cytoplasms of mast cells,[31] and therefore it participates in the late stage of mast cell degranulation. The inhibition of CI induced tryptase release by the inhibitors of tryptase and chymase in the current study may suggest the involvement of tryptase and chymase in the mast cell degranulation process at the late stage, most likely after the influx of

calcium ions into the mast cells. The fact that tryptase and chymase are present in the granules of mast cells in their fully active form[8] supported further the theory that these two mast cell serine proteases are involved in the activation of lung mast cells.

The results that tryptase levels were elevated when 100 μg/ml protamine was added to the mast cells alone or with anti-IgE or CI were unexpected. This was most likely due to the tetrameric structure of tryptase being dissociated by protamine,[32] thus more tryptase monomer existed in supernatants, being recognized by AA5 as an intact tryptase molecule. However, it is difficult to exclude the possibility that protamine stimulated tryptase release from human lung mast cells, as it was reported previously that protamine could stimulate histamine release from guinea pig[33] and rat mast cells.[34] In these cases, protamine may act as an antigen and bind to the IgE antibodies on the surface of mast cells.[35]

A specific inhibitor of aminopeptidase, amastatin, which does not inhibit chymotrypsin and trypsin,[36] was used as an irrelevant protease inhibitor control. It had no significant effects on anti-IgE induced tryptase and histamine release from lung mast cells, which proved the specificity of the actions of tryptase and chymase inhibitors on tryptase and histamine release from lung mast cells.

The observation that preincubation of the inhibitors with the mast cells for 20 minutes before challenging with anti-IgE had little impact on the inhibition of IgE dependent histamine release was unexpected, nevertheless it may suggest that the actions of these inhibitors are rather rapid and that the involvement of tryptase and chymase in anti-IgE induced tryptase and histamine release is likely at the late stage of the degranulation process.

Over the years, many compounds including sodium cromoglycate, lodoxamide, salbutamol, ketotifen, terfenadine and cetirizine have been recognized to possess mast cell stabilizing activities, and have been used as anti-allergic drugs in day-to-day clinical practice to treat asthma or allergic rhinitis. However, only less than 40% inhibition of IgE dependent mast cell degranulation can be achieved with these compounds, which is much less than what was achieved with inhibitors of tryptase[21] and chymase[23] in a similar experimental system. Moreover, some of the latest reports on tryptase inhibitors highlighted the potential of these anti-inflammatory drugs. Inhaled APC366 was able to attenuate allergen-induced late-phase airway obstruction in asthma,[37] and APC2059 could improve the symptomatic scores of patients with mild to moderately active ulcerative colitis in an open-label pilot study.[38] Our findings in the current study may at least partially explain the mechanism by which tryptase inhibitors could treat these diseases. In conclusion, the inhibitors of both tryptase and chymase were able to inhibit IgE dependent and CI induced tryptase release from human lung mast cells, indicating that they could likely be developed to a novel class of anti-inflammatory drugs to treat allergic or other mast cell associated inflammatory conditions in man.

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