A Role for Tryptase in the Activation of Human Mast Cells: Modulation of Histamine Release by Tryptase and Inhibitors of Tryptase

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ABSTRACT

Tryptase is a tetrameric serine proteinase that constitutes approximately 20% of the total protein within human mast cells and is responsible for at least 95% of the trypsin-like activity in lysates of mast cells derived from lung and skin tissue (Schwartz, 1990). It is stored in the secretory granules in a catalytically active form (Glenner and Cohen, 1960) and is secreted along with histamine, heparin and other mast cell granule products on mast cell degranulation (Schwartz et al., 1981). Because it is stored almost exclusively in mast cells (Walls et al., 1990a), this proteinase has attracted particular attention as a marker for mast cells and for mast cell activation. Relatively high concentrations of tryptase have been detected in the serum from cases of systemic anaphylaxis (Schwartz et al., 1987a), in bronchoalveolar lavage fluid from patients with bronchial asthma (Broide et al., 1991) or interstitial lung disease (Walls et al., 1991), in nasal lavage fluid of patients with allergic rhinitis (Jarjour et al., 1991), in skin blister fluid from subjects with allergic contact dermatitis (Brockow et al., 1996) and in synovial fluid from patients with arthritis (Buckley et al., 1997). Evidence is emerging that this major secretory product of the human mast cell may be a key mediator of allergic inflammation and a promising target for therapeutic intervention (Walls, 1995).

In seeking to determine the contribution of tryptase in acute inflammatory responses it is particularly important to consider the early cellular events. Studies of human tryptase function in animal models have suggested that certain of the effects noted may depend on the ability of tryptase to activate mast cells. The increase in microvascular permeability induced by tryptase in guinea pig skin, but not that elicited by bradykinin or tissue kallikrein, can be abrogated by pretreatment of guinea pigs with histamine H₁ and H₂ receptor antagonists (He and Walls, 1997). Moreover, addition of tryptase to dispersed guinea pig lung and skin tissue can provoke the release of histamine by a noncytotoxic mechanism in vitro. Although not investigated directly, the finding that both skin microvascular leakage (Molinari et al., 1995) and bronchoconstriction (Molinari et al., 1996) stimulated by tryptase in sheep also could be blocked by a histamine an-

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ABBREVIATIONS: BAPNA N-benzoyl-DL-arginine-p-nitroanilide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NA, nitroanilide; MES, 2-(N-morpholino)ethanesulfonic acid; MEM, minimal essential medium; BSA, bovine serum albumin; HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; Ig, immunoglobulin; FCS, fetal calf serum; HBSS, Hanks’ balanced salt solution.
together provides evidence that this human protease may induce the activation of mast cells in this species also.

Several proteases have been characterized as secretagogues for mast cells. Thus the degranulation of rodent mast cells may be stimulated by bovine pancreatic trypsin (Lagunoff et al., 1975), thrombin (Razin and Marx, 1984), α-chymotrypsin or rat mast cell chymase (Schick et al., 1984). In addition, certain broad spectrum protease inhibitors can reduce IgE-dependent histamine release from chopped guinea pig (Austen and Brocklehurst, 1961) or human lung tissue (Kaliner and Austen, 1973), and inhibitors of chymase reduce IgE-dependent histamine release from human tonsil mast cells (Dietze et al., 1990). However, the actions of human tryptase on human mast cells have not been investigated, and the potential of inhibitors and substrates of tryptase to act as mast cell stabilizers is not known.

In the present study, we investigated the ability of human tryptase to stimulate histamine release from dispersed human skin and tonsil cells and to modulate histamine release by other stimuli. Our findings suggest that tryptase released from degranulated mast cells has the capacity to activate certain mast cell populations. This could provide the basis of an amplification system in allergic disease, which could be susceptible to control by tryptase inhibitors.

**Materials and Methods**

**Reagents.** The following compounds were purchased from Sigma (Poole, Dorset, UK): leupeptin, benzamidine, NA substrates (BAPNA, N-succinyl-L-Ala-L-Ala-L-Phe-p-NA, N-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-p-NA), porcine heparin glycosaminoglycan, histamine dihydrochloride, collagenase (type I), hyaluronidase (type I), BSA (fraction V), penicillin and streptomycin, MEM containing 25 mM HEPES, heparin, ascorbic acid, calcium ionophore A23187, dimethyl sulfoxide, tris-base, MES, antimycin A, 2-deoxy-D-glucose. Goat anti-human IgE (inactive) was purchased from Serotec (Kidlington, Oxford, UK); HEPES and all other chemicals were of analytical grade from BDH (Poole, Dorset, UK); CNBr-activated Sepharose 4B from Pharmacia (Milton Keynes, UK); FCS from Gibco (Paisley, Renfrewshire, UK); o-phthaldialdehyde from Fluka (Gillingham, Woodham, UK); CNBr-activated Sepharose 4B from Pharmacia (Milton Keynes, UK); FCS from Gibco (Paisley, Renfrewshire, UK); o-phthaldialdehyde from Fluka (Gillingham, Woodham, UK); CNBr-activated Sepharose 4B from Pharmacia (Milton Keynes, UK); FCS from Gibco (Paisley, Renfrewshire, UK); o-phthaldialdehyde from Fluka (Gillingham, Woodham, UK);

**Purification and characterization of tryptase.** Tryptase was purified from human lung tissue by high salt extraction, heparinagarose chromatography and an immunoaffinity chromatography procedure with tryptase-specific monoclonal antibody AA5 coupled to agarose as described previously (He et al., 1997). Fractions of purified tryptase were concentrated in C-30 Centron centrifugal concentrators (Amicon, Stonehouse, Gloucestershire, UK) and stored at −80°C until use. Enzymatic activity was determined by measuring spectrophotometrically at 410 nm the hydrolysis of 20 mM BAPNA in 0.1 M Tris-HCl, 1 M glycerol, pH 8.0, containing 1 mg/ml BSA, at 25°C. Protein concentrations were determined by the Coomassie blue dye binding procedure with a BSA standard (according to the manufacturer's protocol). The specific activity of tryptase was 1.8 U/mg, where one unit of enzyme was taken as the amount that catalyzed the cleavage of 1 μmol of BAPNA per minute at 25°C.

Analysis by SDS-PAGE with silver staining revealed a single diffuse band with an apparent molecular weight of approximately 32 kDa and the identity as tryptase was confirmed by Western blotting with monoclonal antibody AA5 (Walls et al., 1990b). Levels of contaminating endotoxin in the final preparations of tryptase were very low. Using the Toxicolor System according to the instructions provided by the manufacturer, endotoxin was found to be present at less than 22 pg/ml in a stock solution, which indicates contamination at the level of less than 40 pg/mg tryptase. No contamination with chymase was detected by use of the substrate 0.7 mM N-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-p-NA in 1.5 M NaCl, 0.3 M Tris, pH 8.0, and 5% ethanol, or by use of elastase with 1.4 mM N-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-p-NA in the same buffer as used with BAPNA.

**Preparation of compounds.** Because tryptase is enzymatically unstable in physiological solutions, considerable care was taken in its preparation and storage. Purified tryptase was maintained in a high salt buffer (10 mM MES, 1 M NaCl, pH 6.8) in the presence or absence of heparin. Immediately before addition to cells, tryptase was diluted first with sterile distilled water, adjusting the NaCl concentration to 0.15 M, and then with normal saline to obtain the required tryptase concentration. Where added, protease inhibitors or the buffer alone were incubated with tryptase for 30 min on ice before adding to the cells.

**Mast cell dispersion and challenge.** Human tonsil and skin tissues were freshly obtained at tonsillectomy or circumcision operations in children. Cells in the macroscopically normal tissue were dispersed enzymatically by a procedure similar to that used previously with human tonsil (Okayama et al., 1994) or skin tissues (Benyon et al., 1987). Tissue was chopped finely with scissors into fragments of 0.5 to 2.0 mm², washed twice with MEM containing 2% FCS (500 × g, 8 min, 25°C), and incubated at 37°C with 1.5 mg/ml collagenase and 0.75 mg/ml hyaluronidase in the same buffer, but containing 200 U/ml penicillin, 200 μg/ml streptomycin (1 g penicillin/10 ml buffer; 1 g streptomycin/15 ml buffer) for 60 min with tonsil tissue and 75 min with skin tissue. Dispersed cells were separated from undigested tissue by filtration through nylon gauze (pore size, 100 μm diameter) and washed twice with MEM containing 2% FCS. Mast cell numbers were determined by light microscopy after staining with the Kimura staining solution. Dispersed cells were maintained in MEM (containing 10% FCS, 200 U/ml penicillin, 200 μg/ml streptomycin) on a roller overnight at room temperature.

After washing twice with HBSS, pH 7.4, without added calcium or magnesium (500 × g, 10 min, 25°C), the cells were resuspended in HBSS with 1.8 mM CaCl₂ and 0.5 mM MgCl₂ (complete HBSS), and warmed at 37°C for 5 min. Aliquots of 100 μl containing 4 to 6 × 10⁵ mast cells were added to a 50-μl aliquot of purified tryptase, control secretagogue or inhibitor in complete HBSS and incubated for 15 min at 37°C. The reaction was terminated by the addition of 150 μl ice-cold HBSS and the tubes centrifuged immediately (500 × g, 10 min, 4°C). All experiments were performed in duplicate. For the measurement of total histamine concentration in some tubes, the suspension was boiled for 6 min. Supernatants were stored at −20°C until histamine concentrations were determined.

**Histamine measurement.** A glass fiber-based, fluorometric assay for histamine was used as described previously (Nolte et al., 1987). Sample, control buffer or histamine standard (50 ng/ml) was pipetted into the well of a 96-well microtiter plate coated with a glass-fiber matrix (Lundbeck Diagnostics, Copenhagen, Denmark) that selectively binds histamine, 50 μl of sample volume per well. After 1 h incubation at 37°C, the plate was rinsed with distilled water, and 150 μl of 0.4% SDS solution was added to each well. After 30 min incubation at 37°C, the plate was rinsed again with distilled water, and 75 μl of coupling reagent [containing 0.5 mg/ml o-phthaldialdehyde, 5% (v/v) methanol and 2 mg/ml NaOH] was added to each well. The plate was then kept in the dark for 10 min at room temperature, and finally 75 μl of 58 mM HClO₄ was added to stop the histamine-o-phthaldialdehyde reaction. The histamine concentration was measured on a spectrophotofluorometer (Perkin-Elmer LS 2, Denmark). Histamine release was expressed as a percentage of total cellular histamine levels and corrected for the spontaneous release measured in tubes in which cells had been incubated with the HBSS diluent alone (i.e., percentage net histamine release = [hista-
mine release with stimulus = spontaneous histamine release/total histamine content × 100. The lower limit of detection of the assay was 1 ng/ml, and interassay variability was less than 3%.

Statistics. All statistical analyses were performed with StatView software (Version 4.02, Abacus Concepts, Berkeley, CA). Data are shown as the mean ± S.E. for the number of experiments indicated. Where analysis of variance indicated significant differences between groups, for the preplanned comparisons of interest, the paired Student’s t test was applied. For all analyses, P < .05 was taken as significant.

Results

Tryptase-induced histamine release. Incubation of mast cells dispersed from tonsil tissue with purified tryptase throughout the concentration range 0.3 to 100 mU/ml stimulated a dose-dependent release of histamine (fig. 1A). Under similar conditions, in preliminary experiments carried out with a range of concentrations of anti-IgE or calcium ionophore A23187, maximal noncytotoxic histamine release for both tonsil and skin tissues was observed with 1% anti-IgE or with 1 μM calcium ionophore (data not shown), and both 1% anti-IgE and 1 μM calcium ionophore were included as positive controls in all experiments (fig. 1B).

The amount of histamine release induced from tonsil cells by tryptase represented two thirds of that which could be elicited with anti-IgE. However, in similar experiments performed with dispersed skin mast cells, tryptase throughout the concentration range 0 to 30 mU/ml failed to stimulate histamine release regardless of whether or not heparin was present (fig. 1A), although the mean net release with anti-IgE was only 9.4 ± 1.7% in these experiments (fig. 1B). Basal levels of tryptic activity (assayed by BAPNA cleavage) in culture media of dispersed tonsil or skin cells were 0.2 ± 0.15 and 1.0 ± 1.0 mU/ml, respectively. There was no significant correlation between the extent of histamine release induced by tryptase (30 mU/ml) and by either anti-IgE (1%) or calcium ionophore (1 μM), when data were analyzed separately for tonsil or skin cells, or with all data considered together. The time course of histamine release stimulated by tryptase was relatively slow and appeared to be biphasic, with maximal release not being achieved until approximately 6 min after addition of the enzyme (fig. 2). In comparison, maximum histamine release in response to 1% anti-IgE was observed by 3 min and in response to 1 μM calcium ionophore by 5 min (data not shown).

Because tryptase is enzymatically unstable, but may be stabilized by heparin in vitro (Schwartz and Bradford, 1986), heparin was added to tryptase (1 μg heparin per mU tryptase) before incubating with cells in certain experiments. However, heparin appeared to reduce histamine release induced by lower concentrations (<100 mU/ml) of tryptase from tonsil mast cells (fig. 1A). It was confirmed that addition of heparin significantly reduced the spontaneous loss of tryptase activity in cell supernatants which occurred after incubation with tonsil or skin cells, as well as reducing the loss of activity which occurred in the absence of cells (table 1). To further investigate the effects of heparin on the activation of tonsil mast cells, the cells were preincubated with 3 or 30 μg/ml of heparin for 0, 5 and 30 min before challenge with anti-IgE or calcium ionophore. At all time points, heparin was without effect on the extent of anti-IgE or calcium iono-
Inhibition of tryptase-induced histamine release.

Heating tryptase at 56°C for 60 min abolished its ability to stimulate histamine release from tonsil cells (fig. 1A), as well as its ability to cleave the chromogenic substrate, BAPNA. The proteinase inhibitor leupeptin and the tryptase inhibitor drug APC366 were investigated for their ability to inhibit histamine release from tonsil cells stimulated by tryptase. Tryptase was preincubated for 30 min on ice with these inhibitors at concentrations which were sufficient to inhibit the cleavage of BAPNA by tryptase but which did not themselves stimulate histamine release. Both leupeptin and APC366 proved effective at reducing tryptase-induced histamine release (table 2). Addition of BAPNA to tryptase before incubation with cells also inhibited histamine release.

When cells were incubated with the metabolic inhibitors 2-deoxy-d-glucose (10 mM) and antimycin A (1 µM) for 40 min at 37°C before challenge with tryptase, no significant release of histamine was induced (table 3). Consistent with the idea that this is a noncytotoxic reaction was the observation that the presence of calcium and magnesium in the medium may be necessary for histamine release, at least with tryptase concentrations up to 10 mU/ml (fig. 3).

Interaction of tryptase with other stimuli.

Dispersed tonsil mast cells were preincubated with concentrations of tryptase ranging from 0.1 to 10 mU/ml in the presence or absence of heparin for 0, 5 or 30 min at 37°C before challenge with the standard doses of anti-IgE (1%) or calcium ionophore (1 µM). Concentrations of tryptase smaller than those that could induce histamine release themselves, neither primed the cells nor inhibited subsequent activation by the other stimuli (data not shown). However, preincubating cells with tryptase at concentrations capable of stimulating histamine release did significantly reduce the degree of histamine release elicited after challenge with either anti-IgE or calcium ionophore (fig. 4). Similar findings were observed when more limited experiments were performed with preparations of skin cells (n = 2 or 3; data not shown).

Inhibition of histamine release by tryptase inhibitors or substrate.

Preincubation of dispersed tonsil or skin cells with various doses of APC366 for periods of 0, 5 or 30 min before challenge with either anti-IgE or calcium ionophore resulted in a dose-dependent inhibition of histamine release (fig. 5). The inhibitory actions of APC366 were particularly potent with skin mast cells, and significant inhibition of histamine release was achieved with a concentration as low as 1 µM after 30 min incubation. With shorter incubation periods, however, higher doses of APC366 were required to achieve significant inhibition of histamine release. Ninety percent inhibition of both anti-IgE and calcium ionophore-induced histamine release from skin cells was

### Table 1

<table>
<thead>
<tr>
<th>Cell Preparation</th>
<th>% Loss in Tryptase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Heparin</td>
</tr>
<tr>
<td>Tonsil</td>
<td>78 ± 8.5†</td>
</tr>
<tr>
<td>Skin</td>
<td>85 ± 5.4†</td>
</tr>
<tr>
<td>Medium alone</td>
<td>92 ± 2.6</td>
</tr>
</tbody>
</table>

* P < .05 in comparison with the loss in activity which occurred without heparin. † P < .05 in comparison with the tryptase activity added.

### Table 2

<table>
<thead>
<tr>
<th>Inhibitor or Substrate</th>
<th>BAPNA cleavage</th>
<th>Net histamine release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>94 ± 4.5*</td>
<td>64 ± 17*</td>
</tr>
<tr>
<td>APC366</td>
<td>102 ± 3.8*</td>
<td>79 ± 17*</td>
</tr>
<tr>
<td>BAPNA</td>
<td>ND*</td>
<td>60 ± 16*</td>
</tr>
</tbody>
</table>

* P < .05 compared with response with the uninhibited controls.

* ND, not done.

### Table 3

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Net Histamine Release</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>With MI</td>
</tr>
<tr>
<td>Tryptase</td>
<td>10 mU/ml + heparin</td>
</tr>
<tr>
<td>100 mU/ml + heparin</td>
<td>1.0 ± 1.1*</td>
</tr>
<tr>
<td>100 mU/ml (no heparin)</td>
<td>2.1 ± 1.9*</td>
</tr>
<tr>
<td>Anti-IgE, 1%</td>
<td>-0.2 ± 0.5*</td>
</tr>
<tr>
<td>Calcium ionophore, 1 µM</td>
<td>0.4 ± 0.4*</td>
</tr>
</tbody>
</table>

* P < .05 compared with response with the uninhibited controls.
achieved with 100 μM APC 366 after 30 min preincubation; IC₅₀ values of about 15 μM were found with both stimuli. For purposes of comparison, more limited studies were performed with either 10 μg/ml or 100 μg/ml leupeptin or benzamidine hydrochloride, concentrations which effectively inhibited the catalytic activity of tryptase (as determined by measuring the cleavage of BAPNA) but which did not themselves cause apparent cell toxicity or liberation of histamine from tonsil mast cells. Both leupeptin and benzamidine at a concentration of 100 μg/ml inhibited IgE-induced histamine release with the tonsil cells (fig. 6). Leupeptin also inhibited IgE-dependent histamine release from skin cells, although benzamidine had little effect at these concentrations with this source of tissue (data not shown). Neither leupeptin nor benzamidine-inhibited histamine release from calcium ionophore challenged tonsil or skin cells in parallel experiments (data not shown).

Addition of the substrate BAPNA to tonsil cells before challenge with anti-IgE elicited a dose-dependent inhibition of histamine release (fig. 7A). The degree of inhibition was less with calcium ionophore as the stimulus, but there was nevertheless significant inhibition of mast cell activation at the highest dose (fig. 7B).
A concentration of either leupeptin or benzamidine for 0 (lease from dispersed human tonsil cells. The cells were preincubated with experiments. * P compared with the responses with the uninhibited controls.

**Fig. 6.** Inhibitory actions of the proteinase inhibitors leupeptin and benzamidine (both at 10 and 100 µg/ml) on anti-IgE induced histamine release from dispersed human tonsil cells. The cells were preincubated with a concentration of either leupeptin or benzamidine for 0, 5, and 30 min at 37°C before challenge. Mean ± S.E. are shown for five separate experiments. * P < .05 compared with the uninhibited controls.

### Discussion

With two sources of human mast cells we have established that inhibitors and substrates of tryptase may have potent mast cell-stabilizing properties. Our observation that tryptase is a stimulus for histamine release, at least from tonsil tissue, suggests that this major secretory product of human mast cells may itself have a key role in processes of mast cell activation. Moreover, the release of tryptase from activated mast cells may stimulate secretion from neighboring mast cells and thus provide an amplification signal in allergic disease.

The proportion of the total cellular histamine released from dispersed tonsil cells by tryptase at concentrations of 30 or 100 mM/ml was of an order similar to the maximum histamine release elicited from these cells by antibody against IgE. Human tonsillar mast cells therefore would seem to resemble mast cells of the guinea pig lung and skin in being responsive to human tryptase (He and Walls, 1997). In contrast, we found that dispersed human foreskin mast cells released negligible quantities of histamine after incubation with tryptase. The possibility that the skin mast cells may have already been activated maximally in response to the addition of these different inhibitors or substrates could not be excluded, although basal levels of tryptase activity measured in the culture supernatants were low. The differences in responsiveness to tryptase are more likely to reflect functional heterogeneity between mast cell populations at different anatomical sites in humans, and between mast cells at comparable sites in different mammalian species. This concept has been established with other secretagogues including neuropeptides and basic compounds (Church et al., 1997). As found in previous studies with human tonsil and skin tissues (Lowman et al., 1988a), the proportion of histamine released from skin cells with anti-IgE and calcium ionophore was substantially less than that from tonsil cells. However, no direct relationship was found in our studies between the degree of releasability to tryptase and that to anti-IgE or to calcium ionophore. Histamine release induced by tryptase and that by anti-IgE are thus likely to be mediated by different processes.

Although detailed information is now available on the mechanism of IgE-dependent mast cell activation (Kennerly and Duffy, 1983), much less is known of the other means by which human mast cells may be activated. One of the best studied non-IgE-dependent processes in human mast cells is that stimulated by the neuropeptide substance P which is able to elicit histamine release from human skin mast cells, but not from tonsil, gut or lung mast cells (Lowman et al., 1988a; Church et al., 1991). Although mast cells which have degranulated in response to substance P appear similar at the ultrastructural level to those which have been activated by anti-IgE (Caulfield et al., 1990), they seem to involve different receptors (Lowman et al., 1988b) and exhibit quite different kinetics of histamine release. Whereas substance P-induced histamine release is complete within 15 to 20 s (Benyon et al., 1987; Lowman et al., 1988b), IgE-dependent histamine release requires some 3 to 6 min to reach completion. Moreover, histamine release stimulated by anti-IgE depends on the presence of calcium in the cell culture medium, whereas that induced by the nonimmunological stimulus is not (Benyon et al., 1987; Lowman et al., 1988b). The activation of human mast cells by other basic compounds, and by complement peptides C3a and C5a seems to share these characteristics (El Lati et al., 1994).

The mechanism of tryptase-induced histamine release must be quite different from that of substance P and related secretagogues. Not only were tonsil mast cells responsive to tryptase, and skin mast cells unresponsive, but the time required to activate maximum histamine release was at 6 min more similar to that found for stimulation with anti-IgE than that with substance P. In addition, we found that the presence of exogenous calcium and magnesium ions may be required for mast cell activation induced by tryptase.

The activation of human mast cells by tryptase seems to involve a novel mechanism quite different from that characterized for other secretagogues. The observation that heat inactivation abrogated its secretagogue properties suggests that an intact catalytic site is required. Moreover, inhibiting tryptase activity with the tryptase inhibitor APC366 markedly reduced its potential to stimulate histamine release, as did the broad spectrum inhibitor leupeptin and the tryptic substrate BAPNA. The possibility cannot be excluded that the addition of these different inhibitors or substrates could have effects on mast cell responsiveness other than those attributable to the inhibition of exogenous tryptase. However, taken together our findings strongly suggest that a proteolytic process is involved in tryptase-induced activation of mast cells.

The ability of heparin to stabilize tryptase activity (Schwartz and Bradford, 1986) was confirmed, even in the presence of the cell preparations. Although the ability of heparin to enhance tryptase-induced cleavage of defined substrates in vitro has been demonstrated (Alter et al., 1987),
this effect has not been seen consistently in investigations of the actions of tryptase on cells (Cairns and Walls, 1996) or tissues (He and Walls, 1997; He et al., 1997), possibly because cell surface or tissue proteoglycans themselves can bind and stabilize tryptase. The finding of that the addition of heparin to tryptase actually inhibited tryptase-induced histamine release was surprising, but may be related to a quite separate effect of heparin on mast cells. Although in the present study the addition of heparin did not alter the extent of either IgE-dependent or calcium ionophore-induced histamine release, Ahmed and colleagues (1993) previously reported that heparin can inhibit IgE-dependent histamine release from human uterine and rat peritoneal mast cells in vitro, as well as reduce allergen-induced airway and cutaneous responses in sheep. The modulating effects of heparin on mast cells seem to differ between mast cell populations and between stimuli of activation.

Although the actions of tryptase on human mast cells have not been investigated previously, studies with several other proteases have highlighted the potential involvement of proteolytic processes in mast cell activation. Most investigations reported to date have used rodent peritoneal cell models, preventing direct comparison with our findings with human cells, but observations that proteases as diverse as pancreatic trypsin (Lagunoff et al., 1975), α-chymotrypsin (Schick et al., 1984), thrombin (Razin and Marx, 1984), rat mast cell chymase (Schick et al., 1984) and a variety of other proteases (Machado et al., 1996) can all stimulate histamine release, suggest that there could be various proteolytic mechanisms whereby these cells may become activated. The cleavage by tryptase of a substrate whether in the membrane or in the extracellular fluid could provide a signal for mast cell activation either directly or by generating an activating peptide. These possibilities have been raised for chymase-induced mast cell activation by Schick (1990) who found that rat chymase appeared to cleave a 90 kDa membrane component of rat mast cells, and by Cochrane and colleagues (Cochrane et al., 1993) who reported that this protease could cleave albumin to release a histamine-releasing peptide. Recently a series of receptors for tryptic proteases have been characterized, termed protease-activated receptor 1 (thrombin receptor) (Vu et al., 1991), 2 (Nystedt et al., 1994) and 3 (Ishihara et al., 1997), and in certain experimental systems tryptase is capable of activating the first two of these (Molino et al., 1997). The extent to which these receptors may be expressed on mast cells remains to be determined, but such a mechanism deserves consideration as a means whereby tryptase can activate human mast cells.

When cells were preincubated for up to 30 min with tryptase at concentrations which were suboptimal for histamine release, before challenging with other stimuli, the response induced by anti-IgE or calcium ionophore was barely affected. Only with concentrations of tryptase that were themselves able to stimulate histamine release was there an apparent reduction in histamine release in response to these other stimuli. Reports that preincubation of rat peritoneal mast cells with trypsin can prevent the subsequent release of histamine in response to chymotrypsin or chymase (Lagunoff et al., 1975; Schick et al., 1984) provide a parallel with our study. It is possible that tryptase may cleave IgE or the FcεR1 receptor. Alternatively, the reduced responsiveness may simply be a consequence of mast cell activation. Repeated antigen challenge at 30-min intervals has been found to inhibit the responsiveness of cultured rat mast cells to antigen (Shalit and Levi-Schaffer, 1995).

Unlike many of the proteases demonstrated to have the capacity to stimulate mast cell activation, tryptase is a physiologically relevant stimulus. It has been estimated that there may be up to 35 pg tryptase per cell stored within secretory granules (Schwartz et al., 1987b), which suggests that high concentrations of this protease will be achieved in the immediate vicinity of degranulating mast cells. The concentrations of tryptase that elicited histamine release from tonsil mast cells are likely to be achieved in vivo (He and Walls, 1997). Moreover, the noncytotoxic nature of tryptase-induced cell activation was indicated by the ability of the metabolic inhibitors antimycin A and 2-deoxy-D-glucose to abrogate the response.

By acting as a stimulus of mast cell degranulation, tryptase could play a key role in allergic disease by amplifying the responses of mast cells to allergen and other stimuli. Consistent with this idea is the observation that the tryptase inhibitor drug APC366 in a dose-dependent manner inhibited both IgE-dependent and calcium ionophore-induced histamine release. The degree of inhibition achieved with APC366 for both tonsil and skin mast cells is high when...
compared with that reported for other drugs with mast cell stabilizing properties. With tonsillar cells, APC366 could be considered to have potency similar to the beta-2 adrenoceptor agonist salbutamol, which at a concentration of 1 μM has been found to inhibit IgE-dependent histamine release by about 25% (Okayama and Church, 1992). In the same study, the antiallergic drug sodium cromoglycate reduced IgE-dependent histamine release from tonsil cells by only about 12% at a concentration of 1000 μM. For skin mast cells, the potency of APC366 was quite remarkable, and at a concentration of 100 μM inhibited histamine release by about 90%. By comparison, salbutamol has been reported to inhibit IgE-dependent histamine release from skin cells by 20%, whereas sodium cromoglycate is without any inhibitory effects on histamine release from this source of mast cells (Okayama and Church, 1992).

Although the addition of exogenous tryptase to skin cells did not elicit histamine release at the concentrations tested, the ability of APC366 to inhibit histamine release from these cells in response to anti-IgE and calcium ionophore does suggest that endogenous tryptase could be involved in cell activation, nevertheless. Tryptase, which is stored in mast cell granules in an active form (Glenner and Cohen, 1960), may interact with substrates or receptors exposed during the degranulation process. When skin or tonsil cells were preincubated with APC366 for 30 min, the inhibitory actions tended to be greater than when this tryptase inhibitor was added at the same time as the stimulus. This may indicate that APC366 can interact with cells, possibly binding irreversibly to tryptase itself in the time-dependent manner described for this inhibitor (McEuen et al., 1996). Leupeptin and benzamidine which are relatively broad spectrum inhibitors of tryptic proteinases, as well as the substrate BAPNA, also inhibited histamine release from tonsil and skin cells. This supports the idea that the actions of APC366 were related to its properties as a proteinase inhibitor. Both leupeptin and benzamidine were less potent than APC366, and in contrast to APC366 and BAPNA, did not inhibit histamine release induced by calcium ionophore. It is possible that the quantities of these inhibitors added was not sufficient for inhibition, but we were constrained by their cytotoxicity at higher concentrations.

The ability of APC366 to inhibit the activation of human mast cells in vitro is in keeping with its actions found in vivo in sheep models. Clark and colleagues (1995) reported that prophylactic administration of this compound can inhibit antigen-induced early as well as late increases in specific lung resistance, airway hyperresponsiveness to inhaled carbachol, microvascular leakage and lung tissue eosinophilia. Moreover, APC366 can inhibit immediate cutaneous responses to Ascaris suum in naturally sensitized sheep (Molinari et al., 1995). Although developed as an inhibitor of tryptase, APC366 is not selective for this proteinase, but it also can inhibit trypsin and thrombin to a certain degree (Molinari et al., 1995). It is possible therefore that the mast cell stabilizing actions of this drug may depend in part on the inhibition of another protease which is yet to be identified in addition to tryptase. The development of selective and potent inhibitors of mast cell tryptase should assist in elucidating some of the mechanisms of action of tryptase, as well as providing a powerful new class of antiallergic drugs which could be effective as mast cell stabilizers.

References


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