Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus


ABSTRACT: The mast cell plays a pivotal role in the early asthmatic response via release of mediators, which directly influence airway smooth muscle tone. Canine mast cell tryptase has been reported to potentiate the contractile response of canine isolated airways to histamine. The aim of this study was to investigate whether human mast cell tryptase potentiated contractile responses in human isolated bronchi.

The effect of tryptase differed according to the sensitization status of the bronchi. In lung tissue from sensitized patients (those whose bronchial tissue contracted in response to the application of any of four common antigens) 90 ng·mL−1 of human purified lung tryptase markedly potentiated the contractile response to histamine. The maximal response as a percentage of maximal contraction to acetylcholine was 80±8% in control tissues and 119±6% in tryptase treated tissues (n=4; p<0.05). Tryptase, at a dose of 200 ng·mL−1, also potentiated responses but to a lesser degree, 100±5% (n=4; p<0.05). In nonsensitized bronchi, neither 90 nor 200 ng·mL−1 tryptase had any significant effect on histamine responses. The increased response in the presence of tryptase in sensitized tissue was inhibited by the calcium voltage-dependent channel antagonist, verapamil (10−4 M).

We have shown, for the first time, that human mast cell tryptase potentiates contraction in sensitized bronchi via a calcium-related mechanism. These findings provide a link between a mast cell derived product and in vitro human airway hyperresponsiveness.

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Mast cells are pivotal to the immediate or early response in asthma, and contain mediators which have profound effects on airway smooth muscle tone. The relationship between mast cell products and a heightened contraction of human airway smooth muscle, which could contribute to airway hyperresponsiveness, has not been established in human airways. Although there is some evidence to support in vitro differences between asthmatic and nonasthmatic muscle [1−5], results are conflicting [2, 3, 6, 7]. In 1989, Sekizawa et al. [8] observed that supernatants derived from canine degranulated mast cells markedly increased the contractile response of canine airways to histamine. They extended these findings to report that the changes were due to tryptase, a proteolytic enzyme contained in mast cells. More recently, Tanaka et al. [9] reported that a selectively selective tryptase inhibitor reduces bronchoconstriction and hyperresponsiveness in a model of allergic asthma in sheep. Thus, in animal tissue, there is a link between the effects of a specific mast cell product, tryptase, and in vitro hyperresponsiveness. The aim of the current study was to investigate whether the same phenomenon occurs in human airways with human mast cell tryptase.

Human lung tissue was obtained from 11 patients, six of whom were undergoing lobectomy or pneumonectomy (usually for suspected carcinoma) and five who were undergoing pulmonary transplantation. Details of the patients are presented in table 1. Approval for all experiments with human lung was provided by the Human Ethics Committee of the University of Sydney and the Central Sydney Area Health Service. Eight bronchial rings, measuring 3–5 mm in internal diameter and 4 mm in length, were dissected from the tissue from each patient and stored overnight at 4°C in Krebs Henseleit solution (composition in mmol·L−1: NaCl 118.4, KCl 4.7, CaCl2·2H2O 2.5, MgSO4·7H2O 1.2, KH2PO4 1.2, NaHCO3 25.0, and d-glucose 11.1) aerated with 5% CO2 in O2 (carbogen). On the day following retrieval, the tissues were suspended in 5 mL organ baths containing Krebs Henseleit solution, bubbled with carbogen and maintained at 37°C. A load of 1–2 g was placed on the tissues as determined by tissue size [10], as we have previously shown that these loads are optimal in tissues of these measurements [11]. Tissues were washed at 15 min intervals until a stable tone was established. Changes

Methods

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Results) and prepared as 9 µg·mL−1 aliquots in a buffer to the organ bath, tryptase activity was assessed (see photometer (Biochrom, Cambridge, UK). For addition recorded using an LKB Novaspec Model 4049 spectro-
tion coefficient=8,800. Two microlitres of tryptase was
tically, in the laboratories in Sydney, by measuring the
134 kDa) its activity was determined spectrophotomet-

case of patients from whom tissue was obtained

<table>
<thead>
<tr>
<th>Pt No</th>
<th>Condition</th>
<th>Sex</th>
<th>Age yrs</th>
<th>Sensitization status*</th>
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<tbody>
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<td>M</td>
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<td>F</td>
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<td>8</td>
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<td>11</td>
<td>Carcinoma</td>
<td>M</td>
<td>72</td>
<td>S</td>
</tr>
</tbody>
</table>

*: as assessed by the presence or absence of a contraction to antigen in vitro. Pt: patient; M: male; F: female; S: sensitized; NS: nonsensitized.

in tension were measured isometrically using Grass FTO3
force transducers and recorded on a Grass 7P polygraph
(Grass Instruments, Quincy, MA, USA).

Sensitization status was assessed, as described pre-
viously [12, 13], in two bronchial rings from each pati-
ent. Ten microlitres of a solution of Dermatophagoides
pteronyssinus, Timothy grass pollen, Alternaria tenuis
and cat pelt allergens to which more than 97% of the
atopic population of Australia exhibits a positive skin-
prick test [14], were added sequentially to the bath. Contraction in response to any of these antigens indi-
cated that the tissue was sensitized [12, 13]. If tissues
from a patient did not contract to any of these antigens
but contracted to the subsequent addition of acetylcholine
(1 mM), then these tissues were classified as nonsensi-
tized [12, 13].

Assay of tryptase activity

Mast cell derived tryptase from a human lung extract
was prepared in the laboratory by one of the research
team (GC) using the method described by HARTMANN
et al. [15], and was transported to Australia on dry ice. As tryptase is a heat labile tetrameric enzyme (E.C. 3.4.21.59;
134 kDa) its activity was determined spectrophotomet-
rically, in the laboratories in Sydney, by measuring the
rate of hydrolysis of the chromogenic substrate, N-p-
Tosyl-Gly-Pro-Lys-p-Nitroanilide with a molar extinc-
tion coefficient=8,800. Two microlitres of tryptase was
added to 1 mL of reaction mixture containing 0.05 M
Tris-HCl (pH 7.6), 0.12 M NaCl, 20 µg·mL−1 heparin
and 0.1 mM N-p-Tosyl-Gly-Pro-Lys-p-Nitroanilide at
37°C. The rate of change of absorbance (at 405 nm) was
recorded using an LKB Novaspec Model 4049 spectro-
photometer (Biochrom, Cambridge, UK). For addition
to the organ bath, tryptase activity was assessed (see Results) and prepared as 9 µg·mL−1 aliquots in a buffer of
10 mM bis-Tris, pH 6.1, with 0.5 M NaCl and 60
µg·mL−1 heparin. These aliquots were stored at -70°C
until use.

Responses to histamine

In the remaining six tissues, an initial response to a
maximal concentration of acetylcholine (1 mM) was eli-
cited and, after the contraction had reached a plateau,
the tissues were washed repeatedly until baseline tension
was re-established. Tissues then received 250 µg heparin,
to stabilize the tryptase [8], followed 15 min later either
by tryptase, at a concentration of 90 ng·mL−1 [8] or 200
ng·mL−1 (the highest concentration that could be achieved
with the tryptase available), or by appropriate volumes
of tryptase buffer (10 mM bis-Tris, pH 6.1, with 0.5 M
NaCl and 60 µg·mL−1 heparin). After an equilibration
period of 15 min, a cumulative concentration-response
curve to histamine was obtained.

The effect of verapamil

In a separate series of experiments, the effect of the
calcium voltage-dependent channel antagonist, verapa-
ml, on responses to histamine in the presence of tryptase
was investigated in tissues from three patients. The exper-
imental procedure followed was as above, except that
in two tissues from each of the three patients, verapamil
was added at a bath concentration of 10−6 M approximate-
ly 15 min before the addition of 90 ng·mL−1 tryptase. In
addition, in another two tissues, in which histamine re-
ponses were conducted in the presence of tryptase buff-
er alone, verapamil (10−6 M) was also added 15 min prior
to the buffer to assess the effect of the calcium channel
antagonist alone on histamine responses. Histamine cu-
mulative concentration-response curves were also per-
formed in the presence of the vehicles used for verapamil
and tryptase, i.e. water and tryptase buffer, respective-
ly.

Drugs and chemicals

Antigen extracts of Dermatophagoides pteronyssinus
standardized mite DP 30,000 bioequivalent allergen units
(BAU·mL−1), Timothy Phleum pratense 1:20 w/v, Alternaria
tenuis 1:10 w/v and cat pelt 10,000 BAU·mL−1 were ob-
tained from Miles Laboratories Inc. (Elkhart, IN, USA)
and stored at 4°C. Stock solutions of histamine acid phos-
phate, acetylcholine perchlorate, verapamil hydrochlo-
ride and bovine lung heparin sodium salt (149 United
States Pharmacopeia (USP) units·mg−1) were prepared
in distilled water and stored at -20°C. Dilute solutions
of histamine were prepared in Krebs-Henseleit solution
on the day of the experiment and kept on ice. All drugs
and chemicals, other than allergens, were purchased from
the Sigma Chemical Co. (St. Louis, MO, USA).

Analysis of results

All responses to histamine were expressed both as a
value in milligram tension and as a percentage of the
contraction to the maximal concentration of acetylcholine
in that tissue. Where duplicate responses were obtained
within the one experiment, a mean value was derived
for that treatment. In each tissue in each experiment,
the concentration of histamine producing half the max-
imal response (EC50) and thence the negative log value

<table>
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of EC50 (pD2) was obtained from the response curve. Data were analysed using the Student's t-test, linear regression or analysis of variance (ANOVA) with Fisher protected least squares difference (PLSD) to compare entire curves and then responses at specific concentrations of histamine. Differences were considered significant at a p-value equal to or less than 0.05.

Results

Tissues from seven patients contracted in response to antigens, and these were considered sensitized, whereas tissue from the other four patients failed to respond to the application of any of the four allergens, and these were designated as nonsensitized. Responses were consistent within tissues from the same patient. The magnitude of the response to antigen was highly variable between patients and ranged 433–1,500 mg, which represented 25–155% of the maximal response to acetylcholine in tissues from that patient.

Figure 1 shows the spectrophotometric assay of human tryptase activity. The concentration of active tryptase was calculated using the absorbance rate of 0.096 absorbence units·min⁻¹ and the specific activity conversion factor derived from SCHWARTZ and BRADFORD [16] (i.e. 1 mg·mL⁻¹ human tryptase gives an absorbance rate of change-min⁻¹ of 352 absorbance units at 405 nm). After taking the dilution factor (i.e. 500) into account, the concentration of active tryptase was calculated to be 136 µg·mL⁻¹. For addition to the organ bath, tryptase was prepared as 9 µg·mL⁻¹ aliquots in a buffer of 10 mM bis-Tris, pH 6.1, with 0.5 M NaCl and 60 µg·mL⁻¹ heparin.

The mean contractions to acetylcholine were 1,610±103 (n=4) and 2,489±564 mg (n=4) in sensitized and nonsensitized tissues, respectively, and were not significantly different (p>0.05). There was no change in baseline tone in response to the addition of either tryptase or tryptase buffer solution. All tissues from all patients contracted in response to histamine over a concentration range of 10 nM to 1 mM. There was no significant difference between the value for mean maximum tension (Tmax) (mg) in response to histamine in tissue from sensitized patients and that from nonsensitized patients (table 2). However the addition of 90 ng·mL⁻¹ tryptase to sensitized tissues significantly potentiated the contractile response to histamine (fig. 2a), while producing

<table>
<thead>
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<th>Tryptase concentration</th>
<th>0</th>
<th>90</th>
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<tr>
<td>NS</td>
<td>2704 (724)</td>
<td>2190 (408)</td>
<td>2645 (442)</td>
</tr>
<tr>
<td>S</td>
<td>1180 (203)</td>
<td>1968 (322)</td>
<td>1692 (442)</td>
</tr>
<tr>
<td>pD2</td>
<td>5.53 (0.20)</td>
<td>5.49 (0.13)</td>
<td>5.73 (0.27)</td>
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<td>5.70 (0.11)</td>
<td>5.51 (0.27)</td>
<td>5.71 (0.16)</td>
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<td>5.73 (0.27)</td>
<td>5.71 (0.16)</td>
<td>5.71 (0.22)</td>
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Values are presented as mean of four experiments, and SEM in parenthesis. Response to histamine is expressed as maximum tension (Tmax); pD2: negative log value of the concentration of histamine producing half the maximal response (EC50).
increased in the presence of 200 ng·mL⁻¹ tryptase, but mne responses, at the highest concentrations, were also water (tryptase buffer plus the diluent for verapamil water (i.e. whose bronchi exhibit a contractile response to antigen, tentiation occurs only in tissues derived from patients histamine in human isolated airways. Moreover, this po-

mast cell tryptase potentiates the contractile response to

elicited in the presence of tryptase buffer alone (fig. 3). Ver-

amil had no significant effect on histamine responses (p<0.05) from 117±2 to 84.5±10% (n=3) (fig. 3). Vera-

pD2 value for histamine in the presence of 200 ng·mL⁻¹ tryptase (fig. 2a). There was no significant change in the

table 2). In nonsensitized patients, neither 90 nor 200 ng·mL⁻¹ produced any significant change in con-

tractile responses to histamine (fig. 2b and table 2).

In the presence of verapamil, the tryptase-induced in-

crease in histamine responses was significantly inhibited (p<0.05) from 117±2 to 84.5±10% (n=3) (fig. 3). Vera-

pamil had no significant effect on histamine responses elicited in the presence of tryptase buffer alone (fig. 3).

Discussion

This study has demonstrated, for the first time, that mast cell tryptase potentiates the contractile response to histamine in human isolated airways. Moreover, this poten-
tiation occurs only in tissues derived from patients whose bronchi exhibit a contractile response to antigen, i.e. which are sensitized. The potentiation was not ob-
served in nonsensitized tissue. The mechanism underly-
ing the tryptase-induced potentiation is related to Ca²⁺ flux through voltage-dependent channels, since it was inhibited by verapamil.

These findings confirm, in human airways, observations made in canine airways more than 7 yrs ago [8], but with several significant differences. Firstly, Sekizawa et al. [8] found that dog tissue, regardless of sensitization sta-
tus, exhibited hyperresponsiveness to histamine, where-
as we found that responses were only increased in sen-
sitized tissue, i.e. tissue which responded to antigen. However, no mention was made of the allergic status of the dogs in the study by Sekizawa et al. [8], and it is possible that all the mongrel dogs they studied were, in fact, sensitized to a common allergen such as Ascaris. Another difference between our study in human bron-
chi and that conducted in dog airways, is that we found that responses to histamine were markedly increased without a significant change in sensitivity, whereas in dog bronchus, both sensitivity and efficacy were increased [8].

It is possible that the difference that we observed between sensitized and nonsensitized tissue resulted from the fact that there was a tendency for histamine responses, expressed as a percentage of the maximal acetylcholine response, to be lower in the former than in the latter group, and that potentiation by tryptase restored res-

ponses in sensitized tissues to that in controls. However, the difference was not significant and, furthermore, in all experiments, the effect of tryptase was assessed by making comparisons between tissues from the same pa-

tient tested in the presence and absence of tryptase, as opposed to between tissues from different patients.

It is often suggested that preoperative medication and indication for surgery may be confounding variables when tissue obtained from patients with a variety of preopera-
tive conditions is acquired. We have recently made an extensive study of reactivity in tissues obtained from pa-

ients with very different pulmonary diseases, for which they were receiving a variety of preoperative medica-
tions. We compared these with tissue from patients free of pulmonary disease and found that histamine responses from patients with cystic fibrosis, emphysema, α1-antitrypsin deficiency, carcinoma and Eisenmenger’s syn-

drome did not differ from those in tissues derived from patients with no pulmonary disease [17]. Thus, it is un-

likely that, in the present study, the observed differences could be attributable to preoperative disease state or medica-

tion.

In the present study, the increased response in sensi-
tized tissue in the presence of tryptase was not dose-
dependent, in that potentiation of histamine responses with 200 ng·mL⁻¹ was not significantly greater than that occurring with 90 ng·mL⁻¹. It is possible that there is a form of downregulation occurring at the higher con-

centrations of tryptase. Sekizawa et al. [8] studied only a single concentration of tryptase (90 ng·mL⁻¹) in full response curves to histamine in canine airways. These authors did find, however, that when they used increas-
ing concentrations of tryptase (3–90 ng·mL⁻¹) there was, in fact, a dose-related increase in the response to histo-

mine. However, these experiments were performed on only single concentrations of histamine (10⁻⁶ M).

The relationship between the concentrations of tryptase studied in the present experiments and those found in situ in human lung can be considered in the following manner. Shanahan et al. [18] reported that mast cell density in airways of a size comparable to those studied here, was 3×10⁶ cells·cm³, although others have reported lower values, depending on the fixative used and area sampled [19–21]. Eleven micrograms of tryptase is stored in 10⁷ cells [22], and subjects with systemic ana-

phylaxis exhibit serum tryptase levels up to 88 ng·mL⁻¹ [23]. Not all tryptase contained in the mast cell is released after antigen challenge, and once released, tryptase dif-

fuses into the circulation and is cleared. Thus, it would seem that both 90 and 200 ng·mL⁻¹ are concentrations
of tryptase that are achievable locally in human bronchus in situ after mast cell degranulation has occurred.

This is not the first time that we have found sensitization status to be an important factor in determining human airway responsiveness. We and others [11, 24, 25] have reported that passive sensitization of human bronchus produces an increase in some contractile responses, as well as a decrease in the relaxant response to some agonists. Moreover, in a series of experiments which investigated the effect of supernatants from stimulated neutrophils on responsiveness of human bronchus to electrical field stimulation, responses were potentiated, but only in sensitized tissue [26]. These results, together with those of the current study, suggest that sensitized tissue, whether this occurs passively or "actively", is primed in some way to produce these heightened contractile responses. The nature of the priming factor(s) merits investigation.

As in our previous studies, in which we examined airway hyperresponsiveness in the light of sensitization status [12, 13], tissues from a single patient exhibited a homogeneous response to antigen, i.e. tissues either contracted or did not. Moreover, based on data collected in large epidemiological studies in the Australian population [14], it is unlikely that the spectrum of antigens used would fail to detect sensitized tissue. The concentrations of antigen used in the present study were based on those used in our previous work, in which we had established dose-response relationships to the antigens and, hence, an optimal, maximal dose to detect sensitization. No information was available to us on the atopic status of the subjects as they did not undergo skin-prick testing prior to surgery. Had these data been available to us, we would still have designated our patients as nonsensitized or sensitized on the basis of their in vitro bronchial response to antigen.

Sekizawa et al. [8] found that the potentiation of histamine contraction by tryptase observed in dogs could be inhibited by a calcium channel antagonist, and suggested that tryptase may cause proteolytic cleavage of a protein within the calcium channel. We also found that verapamil inhibited tryptase-induced potentiation. What was surprising, however, was that verapamil itself did not markedly inhibit histamine-induced contraction, as we had previously observed [27]. This may be a reflection of the sensitization status of the tissue. When we made our original observation that 10^{-6} M verapamil decreased histamine responses [27], we were unaware of the sensitization state of the tissues studied and unaware that it could influence tissue responsiveness. Since then, we have shown that when human bronchus is passively sensitized, verapamil-induced relaxation responses are markedly decreased [13]. If this is also the case in innately sensitized tissue, as used in the present study, then this may explain the decreased efficacy of verapamil in decreasing histamine-induced contraction.

If tryptase released from degranulated mast cells within the airways is involved in the airway hyperresponsiveness associated with asthma, it would be expected that airway tissue taken from asthmatic subjects would be hyperresponsive in vitro. However, in vitro hyperresponsiveness has not been consistently demonstrated in isolated asthmatic airways. The effect of tryptase may not be sufficiently long-lived to demonstrate an effect after tissue removal. Alternatively, multiple factors may contribute to the effects of tryptase, including the constant presence of heparin to stabilize its activity. Thus, in the absence of these stabilizing factors, differences in vitro may not be observed.

In summary, we have demonstrated, for the first time, that human mast cell-derived tryptase potentiates contraction to histamine in sensitized, but not nonsensitized human bronchus. Tryptase is likely to cause these effects, as in the dog, via increased calcium influx through voltage-dependent calcium channels. These data extend the observations made in canine airways to human lung and underline the importance of our previous findings on the association between sensitization status and the demonstration of hyperresponsiveness in vitro.

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