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HUMAN MAST CELL TRYPTASE ATTENUATES THE VASODILATOR ACTIVITY OF CALCITONIN GENE-RELATED PEPTIDE

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Abstract—Calcitonin gene-related peptide (CGRP) is localized in and released from sensory nerves. It is a potent and long acting vasodilator which has been suggested to play a role in the control of blood flow. Using HPLC and trichloroacetic acid precipitation techniques, we have examined the ability of human mast cell lysates and a purified preparation of mast cell tryptase to degrade CGRP. We found that CGRP is effectively cleaved by tryptase ($K_m = 6.8 \times 10^{-6}$ mol/L at 37°). Enzymatic activity was inhibited by antipain, leupeptin, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, benzamidine or aprotinin, but not by soybean trypsin inhibitor or *N*-tosyl-L-phenylalanine chloromethyl ketone. The degradation of CGRP by lysates of purified skin mast cells showed a similar pattern of inhibition suggesting that tryptase may be the major enzyme involved. The activity of tryptase was not affected by the presence of heparin. Incubation of CGRP with tryptase resulted in a loss of its vasodilator activity as observed by intravital microscopy of the hamster cheek pouch microvasculature. CGRP preincubated with tryptase failed to relax arterioles when added topically. It is suggested that the catalysis of CGRP by tryptase could represent an important means by which the activity of this neuropeptide is regulated *in vivo*.

Calcitonin gene-related peptide (CGRP‖) is a 37 amino acid neuropeptide which is widely distributed in the central and peripheral nervous system [1]. CGRP is commonly found in nerves that terminate at sites close to blood vessels suggesting a role in the control of blood flow. The vasodilator activity of CGRP was discovered when it was realised that CGRP has extremely potent and long lasting effects in the cutaneous vasculature of species that include man; and that it acts via relaxation of the resistance arterioles as shown in the hamster cheek pouch using intravital microscopy [2]. It is now well established that CGRP relaxes major blood vessels from organs that include the lung [3]. It has also been reported that CGRP contracts airway smooth muscle *in vitro*, but the importance of CGRP as a bronchoconstrictor has been the subject of some debate [3, 4].

Substance P is often co-stored with CGRP in sensory nerves in skin and other tissues. The injection of substance P with CGRP into human and rat skin has been found to lead to a loss of the sustained vasodilator activity of CGRP [6, 7]. Proteases released from mast cells activated by substance P have been implicated in the breakdown of CGRP *in vivo*. Similar results were obtained with compound 48/80 (which also activates mast cells), and CGRP was shown to be cleaved to fragments by activated mast cells *in vitro* [6].

Purified preparations of mast cell proteases have been demonstrated to be effective in degrading several important neuropeptides. Dog mast cell tryptase and chymase have been found to cleave VIP and substance P, respectively [8], and to abolish VIP-induced relaxation of smooth muscle in the lung [9]. More recently, human lung tryptase has been reported to hydrolyse CGRP, VIP and PHM [10].

The association of mast cells with blood vessels is well documented, and morphometric studies have demonstrated that these cells are frequently in close apposition to peptidergic nerves [11, 12]. Thus, proteases released following degranulation should have ready access to released neuropeptides, and their ability to modulate the potent effects of CGRP on the vasculature deserves further investigation.

Three distinct proteolytic enzymes have been isolated to date from the human mast cell granule, tryptase, chymase and carboxypeptidase, and there is some evidence for an enzyme similar to neutrophil cathepsin G (reviewed in Ref. 13). Of these, tryptase is the most abundant granule component [14], and its selective presence has been demonstrated in mast cells at all anatomical sites so far examined [15, 16], though its biological functions remain poorly defined. The aim of this study has been to investigate the enzymatic actions of mast cell proteases, and particularly of purified human mast cell tryptase on CGRP, and to seek to evaluate their possible role in regulating the activity of this vasodilator peptide.

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‖ Abbreviations: CGRP, calcitonin gene-related peptide; PHM, peptide histidine methionine; SBTI, soybean trypsin inhibitor; TCA, trichloroacetic acid; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; VIP, vasoactive intestinal peptide.

MATERIALS AND METHODS

Purification of tryptase. Tryptase was purified from homogenized human lung tissue obtained at post-mortem using high salt extraction, octyl Sepharose and heparin–agarose affinity chromatography as described previously [17]. On electrophoresis on 10% SDS–polyacrylamide gel, there was a major band with a M_r of 32.5 kDa. This corresponds to the M_r found for the dissociated subunits of tetrameric tryptase [17–19]. The purified protein cleaved the synthetic tryptic substrate *N*-α-benzoyl-DL-arginine *p*-nitroanilide (Sigma Chemical Co., Poole, U.K.), and in ELISA bound monoclonal antibody AA5 specific for tryptase [17].

Mast cell lysates. Foreskin tissue obtained at infant circumcision was chopped finely with scissors, and cells were dispersed enzymatically by incubating with collagenase (1.5 mg/mL) and hyaluronidase (0.5 mg/mL; both Sigma) in Hank's balanced salt solution (pH 7.4) at 37° [20]. Mast cells were purified by centrifugation (500 *g*, 10 min, 22°) with 70% isotonic Percoll (Pharmacia, Uppsala, Sweden). The purity of mast cells was estimated to be between 70 and 80% when cells were stained according to the method of Kimura *et al.* [21], and enumerated in an Improved Neubauer haemocytometer. Preparations of 10^6 mast cells per mL were lysed by two freeze–thaw cycles.

Assays for chemical breakdown of CGRP. Tryptase (1 ng to 80 μg in 100 μL) was incubated at 37° for 1 or 6 hr with ^{125}I-αCGRP (1 pmol in 50 μL; Amersham International, Amersham, U.K.) and buffer or inhibitor (50 μL). All reagents were dissolved in Tris-buffered saline (pH 7.6) containing 0.3% bovine serum albumin (protease-free; Sigma). Unless otherwise indicated, tryptase was added in the presence of heparin (1:10, w/w; lithium salt; Sigma).

Experiments were performed in the same way with 100 μL of the mast cell lysate preparation diluted 1/25, in place of tryptase. Protease inhibitors were preincubated with tryptase or mast cell lysates for 60 min at 4° prior to incubation with CGRP. Inhibitors tested included leupeptin, benzamidine, antipain, aprotinin (bovine lung), SBTI and TPCK (all Sigma).

For analysis of reaction products by HPLC, the reaction was stopped by the addition of 5 μL 10% TFA. The contents of tubes were mixed, centrifuged (8000 *g* for 3 min) and the supernatants frozen (−20°) until analysis. Samples were thawed, and 70 μL was diluted with 430 μL of 0.08% TFA. The sample (300 μL) was subjected to C18 reversed-phase HPLC on a Waters radial compression module with a Deltapak 300 Å pore size column (8 × 100 mm) using a linear gradient of 24–60% acetonitrile over 20 min at a flow rate of 2.4 mL/min. Fractions (30 sec) were collected and radioactivity measured in an automatic gamma counter [6].

A second method was also used for measuring CGRP breakdown. In this assay the intact peptide was precipitated with TCA as follows. Catalysis was stopped by the addition of 5 μL 10% TFA as before, followed by 500 μL 0.3% bovine serum albumin in phosphate-buffered saline and 750 μL 20% TCA. Samples were mixed and centrifuged (8000 *g* for 2 min). The supernatant was removed and the radioactivity counted in the supernatant and pellet samples [22].

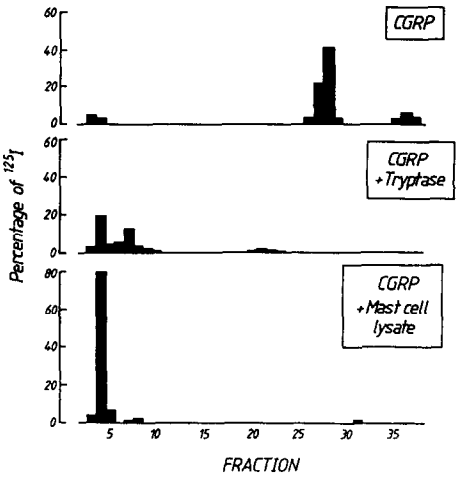

Fig. 1. Breakdown of CGRP following incubation with tryptase or a mast cell lysate. Radioactivity associated with intact ^{125}I-CGRP was eluted in HPLC fractions 26–29, while fragments were eluted in earlier fractions. Results are expressed as the percentage of ^{125}I counts injected. The data shown is representative of five separate experiments.

Assay for vasodilator activity. CGRP (0.1–10 pmol/10 μL; a gift from Dr U. Ney, Celltech, Slough, U.K.) was incubated with an equal volume of tryptase (150 ng) in Tyrode's solution (pH 7.5) for 1 hr at 37°. Samples were centrifuged (8000 *g* for 2 min) and supernatants stored at −20° prior to assay.

Male Syrian hamsters (60–100 g) were anaesthetized with pentobarbitone (60 mg/kg; i.p. injection) and maintenance doses given as required. The cheek pouch was everted and the upper vascular layer and connective tissue removed, leaving the lower vascular layer intact [23].

The preparation was placed on a Letiz Dialux microscope with a specially designed stage; and the vasculature was superperfused throughout the experiment with Tyrode's solution (pH 7.5; 37° 6 mL/min) containing, when necessary, 10^{-7} M phenylephrine to maintain tone. The microvasculature was observed using a video camera and measurements of arteriole diameter made using a Kompira computerized analysis system. Agents under test were added topically in 10 μL volumes, and measurements taken 1 min later.

RESULTS

Demonstration of CGRP catalysis

Intact CGRP was degraded in the presence of tryptase or mast cell lysates. Representative results are shown in Fig. 1 for ^{125}I-CGRP (1 pmol) after incubation with tryptase (80 μg) or a mast cell lysate for 6 hr at 37°, and separation using HPLC. In

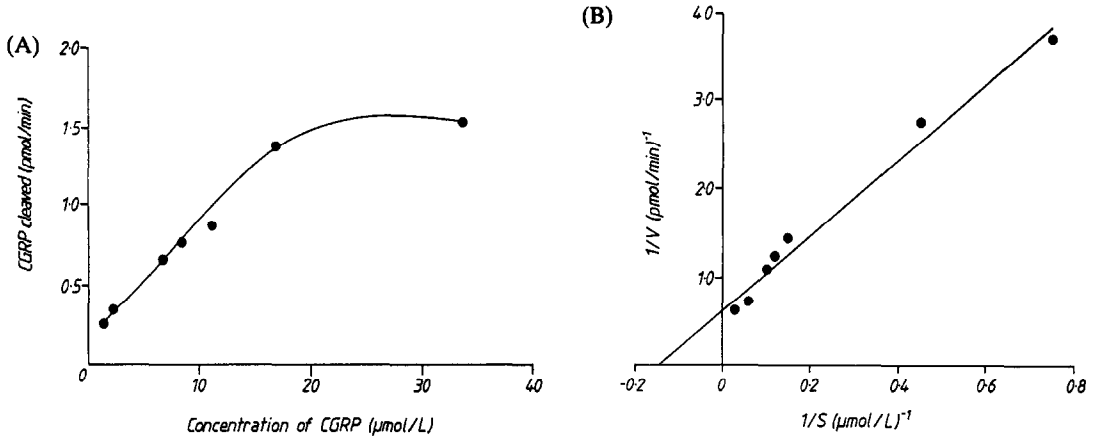

Fig. 2. (A) Substrate titration curve for the catalysis of CGRP by tryptase. The quantity of intact CGRP cleaved was estimated by the TCA precipitation procedure. (B) Double reciprocal plot of the same data.

Table 1. Inhibitor profiles for tryptase and mast cell lysates

Inhibitor	% Inhibition of CGRP cleavage Tryptase	Mast cell lysate
Leupeptin	97 (0.7)	81 (7.7)
TLCK	97 (1.9)	98 (4.3)
Benzamidine	113 (0.7)	75 (7.0)
Antipain	97 (1.9)	83 (7.2)
Aprotinin	74 (22)	96 (4.1)
SBTI	2.8 (2.8)	8.4 (8.4)
TPCK	1.8 (1.8)	1.3 (1.3)

Mean values (and standard errors of the mean) are shown for three separate experiments. All compounds tested were at a final concentration of 10 mmol/L except for aprotinin and SBTI which were at 0.5 mg/mL.

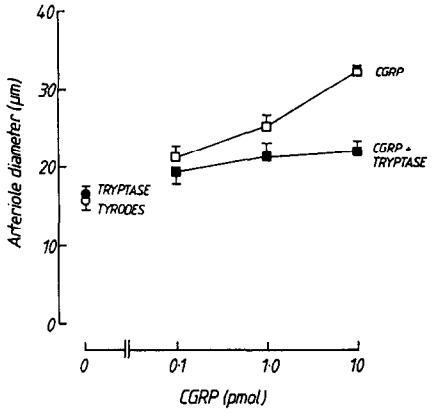

Fig. 3. Vasodilator activity of CGRP and CGRP fragments measured after topical application to the vasculature of the hamster cheek pouch. Mean arteriole diameters (±SEM) are shown for four separate experiments.

further experiments with multiple samples, the fragments of CGRP were separated from the intact peptide by precipitation with TCA. In a comparative experiment, ^{125}I-CGRP incubated alone was assessed as 67.7 ± 4.5% intact by HPLC, and 77.0 ± 3.5% by the TCA precipitation method (mean ± SEM; N = 4).

Kinetic analysis

Substrate titration curves indicated hyperbolic kinetics for the catalysis of CGRP by tryptase. Data is shown for ^{125}I-CGRP incubated with 1 ng tryptase at 37° for 1 hr (Fig. 2A). Conditions were chosen such that maximum cleavage of CGRP was less than 8.0%. Lineweaver–Burk analysis of this experiment indicated values of K_m of 6.8×10^{-6} mol/L and V_{max} of 1.55×10^{-9} mol/min (Fig. 2B).

Inhibition profile

There was effective inhibition of tryptase-induced catalysis of CGRP by leupeptin, TLCK, benzamidine and antipain, but not by SBTI or TPCK at the concentrations tested (Table 1). In all assays, conditions were such that less than 15% of intact CGRP was cleaved in the absence of inhibitor. The inhibitor profile for the mast cell lysate was similar to that for tryptase.

In the absence of heparin there was no significant reduction in the activity of tryptase. The activity of tryptase without heparin represented 100 ± 9.9% (mean ± SEM; N = 3) of that when heparin was added.

Loss of CGRP vasodilator activity

Topical administration of CGRP in 10-μL aliquots to the vasculature of the hamster cheek pouch provoked a dose-dependent increase in arteriole diameter over a range of 0.1 to 10 pmol (Fig. 3). No effect on venules was noted, as reported previously [2]. A significant decrease in vasodilator activity was achieved by preincubation of CGRP with tryptase

($P < 0.05$, Bonferroni's modified t-test). Tryptase alone did not affect arteriole diameter at the concentration tested. Over the period of the experiment, tissues did not become tachyphylactic with tryptase or CGRP.

DISCUSSION

We have demonstrated that human mast cell tryptase is highly effective at cleaving CGRP, and that the products of catalysis do not have the vasodilator activity of the intact peptide. These findings and the known close association between mast cells and sensory nerves suggest that the release of tryptase from mast cells may be an important mechanism by which the potent and long lasting effects of CGRP on the vasculature are regulated.

Lysates of mast cells purified from human skin were found to degrade CGRP, and with an inhibition profile very similar to that found in the present study for purified tryptase. The pattern of inhibition observed is also similar to that found for catalysis of synthetic substrates [19]. Cleavage of CGRP by either tryptase or mast cell lysates was almost completely inhibited by leupeptin, TLCK, benzamidine, antipain and aprotinin, but there was little or no inhibition by SBTI or TPCK at the concentrations tested. Thus, tryptase may have been the major enzyme present in the mast cell lysates which was responsible for the degradation of CGRP.

Skin mast cells are a rich source of the chymotryptic enzyme chymase [24–26], and it is interesting that no inhibition was detected in these studies with the reported chymase inhibitor SBTI or TPCK [24, 27]. There is a potential site for chymotryptic cleavage in CGRP, and the degradation of this peptide by chymotrypsin has been demonstrated [6, 28].

The relative importance of mast cell enzymes in cleaving CGRP may be different in other species. There is a marked variation between species in the composition of mast cell granule proteases [13]. In man, tryptase is the most abundant mast cell enzyme, whereas in rats and mice, tryptase appears to be a relatively minor component and chymases predominate. Such differences may explain why SBTI, which did not inhibit catalysis of CGRP in the present study, has nevertheless been found to inhibit the degradation of CGRP by products of activated rat mast cells [6].

Within the mast cell granule, tryptase will be packed in close association with proteoglycans, and the maintenance of this interaction following degranulation has been postulated as an important means by which the enzymatic activity of tryptase is regulated. It is known that the presence of heparin and other proteoglycans can stabilize the activity of tryptase against synthetic tryptic substrates of small molecular weight [29, 30]. In contrast, it has been reported that the presence of heparin may inhibit the generation of C3a from complement [31]. The addition of heparin to our assays did not appear to affect the catalysis of CGRP by tryptase.

Structure–activity studies of CGRP digested with pancreatic trypsin have suggested that the intact peptide may be required for full expression of biological activity [28]. Different periods of incubation with trypsin were found to result in a variety of fragments with primary hydrolysis occurring at Arg^{11}, Arg^{18}, Lys^{24} and Lys^{35}. Of the fragments produced, only $CGRP_{1-35}$ caused some increase in blood flow in rabbit skin. Under the conditions reported by Tam and Caughey [10], it has been suggested that tryptase may favour cleavage at Arg^{18} and Lys^{24}. The present studies demonstrate that catalysis of CGRP by tryptase is sufficient to cause the loss of vasodilator activity.

There is evidence that the C-terminal fragment $CGRP_{8-37}$ may provide antagonism of the vasodilator [32, 33] and cardiovascular actions [34] of CGRP; while $CGRP_{12-37}$ has been reported to be an antagonist of the inotropic effects of the intact molecule in heart tissue [35]. The products of CGRP catalysis by tryptase could include such C-terminal peptides, and the possibility that mast cell enzymes may actually generate antagonists of intact CGRP deserves further investigation.

Degradation of CGRP by tryptase to either inactive fragments or peptide antagonists, could represent a control mechanism to limit the actions of this peptide in inflammatory conditions where mast cell activation occurs. This has been demonstrated in human skin where the release of proteases from mast cells activated by substance P leads to an attenuation of the vasodilator activity of CGRP [6].

The release of CGRP, subtance P and neurokinins in the lung has been proposed as a component of bronchial asthma, where an axon reflex may be triggered by mediators such as bradykinin acting on exposed unmyelinated nerve endings [36]. It is interesting in this respect that allergen challenge of rhinitic subjects has been found to result in an increase in CGRP concentrations in nasal washings [37].

Concentrations of immunoreactive tryptase have been reported to be elevated following local challenge in skin blister fluid [38] and in lavage fluid from the nose [39] and lung [40] of allergic subjects; while some dramatic increases in blood tryptase levels have been found in systemic anaphylaxis [41]. The concentrations of tryptase in lung lining fluids may be raised in a range of lung diseases including sarcoidosis, extrinsic allergic alveolitis and carcinoma [42]. The relative extents of neuropeptide release and mast cell activation are likely to be important in the development and maintenance of inflammatory changes.

In this study we have demonstrated that tryptase could act to modulate the activity of CGRP. Other biological functions reported for human mast cell tryptase include the activation of C3 to generate C3a anaphylatoxin [31], inactivation of fibrinogen [43], the activation of procollagenase [44] and the degradation of VIP and PHM [10]. The degradation of CGRP by tryptase should serve to reduce the vasodilator or bronchoconstrictor effects of this peptide, and provides an example whereby this mast cell enzyme may also have a role in limiting the extent of inflammation.

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REFERENCES

1. Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vala WW and Evans RM, Production of a novel neuropeptide encoded by the calcitonin gene via tissue specific RNA processing. *Nature* **304**: 129–135, 1983.
2. Brain SD, Williams TJ, Tippins JR, Morris HR and MacIntyre J, Calcitonin gene related peptide is a potent vasodilator. *Nature* **313**: 54–56, 1985.
3. Martling CR, Saria A, Fischer JA, Hokfelt T and Lundberg JM, Calcitonin gene related peptide and the lung: neuronal co-existence with Substance P, release by capsaicin and vasodilatory effects. *Regul Peptides* **20**: 125–139, 1988.
4. Palmer JBD, Cuss FMC, Mulderry PK, Ghatei MA, Springall DR, Cadieux A, Bloom SR, Polak JM and Barnes PJ, Calcitonin gene related peptide is localised to human airway nerves and potently constricts human airway smooth muscle. *Br J Pharmacol* **91**: 95–101, 1987.
5. Gibbins IL, Furness JB, Costa M, MacIntyre I, Hillyard CJ and Girgis S, Co-localisation of calcitonin gene related peptide like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pig. *Neurosci Lett* **57**: 125–130, 1985.
6. Brain SD and Williams TJ, Substance P regulates the vasodilator activity of calcitonin gene-related peptide. *Nature* **335**: 73–75, 1988.
7. Brain SD and Williams TJ, Interactions between the tachykinins and calcitonin gene related peptide lead to the modulation of oedema formation and blood flow in rat skin. *Br J Pharmacol* **97**: 77–82, 1989.
8. Caughey GH, Leidig F, Viro NF and Nadel JA, Substance P and vasoactive intestinal peptide degradation by mast cell tryptase and chymase. *J Pharmacol Exp Ther* **244**: 133–137, 1988.
9. Franconi GM, Graf PD, Lazarus SC, Nadel JA and Caughey GH, Mast cell tryptase and chymase reverse airway smooth muscle relaxation induced by vasoactive intestinal peptide in the ferret. *J Pharmacol Exp Ther* **248**: 947–951, 1989.
10. Tam EK and Caughey GH, Degradation of airway neuropeptides by human lung tryptase. *Am J Respir Cell Mol Biol* **3**: 27–32, 1990.
11. Stead RH, Tomioka M, Quinonez G, Simon G, Felton WY and Bienenstock J, Intestinal mucosa mast cells in normal and nematode infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* **84**: 2975–2979, 1987.
12. Weihe E, Muller S, Fink T and Zentel HJ, Tachykinins calcitonin gene related peptide and neuropeptides in nerves of the mammalian thymus: interactions with mast cells in autonomic and sensory neurimmunomodulation. *Neurosci Lett* **100**: 77–82, 1989.
13. Schwartz LB (Ed.), Neutral proteases of mast cells. *Monographs in Allergy*, Vol. 27. Karger, Basel, 1990.
14. Schwartz LB, Irani AA, Roller K, Castells MC and Schechter NM, Quantitation of histamine, tryptase and chymase in dispersed human T and TC mast cells. *J Immunol* **138**: 2611–2615, 1987.
15. Craig SS, Deblois G and Schwartz LB, Mast cells in human keloid small intestine and lung by an immunoperoxidase technique using monoclonal antibody against tryptase. *Am J Pathol* **124**: 427–435, 1986.
16. Walls AF, Jones DB, Williams JH, Church MK and Holgate ST, Immunohistochemical identification of mast cells in formaldehyde fixed tissue using monoclonal antibodies specific for tryptase. *J Pathol* **162**: 119–126, 1990.
17. Walls AF, Bennett AR, McBride HM, Glennie MJ, Holgate ST and Church MK, Production and characterisation of monoclonal antibodies specific for human mast cell tryptase. *Clin Exp Allergy* **20**: 581–589, 1990.
18. Schwartz LB, Monoclonal antibodies against human mast cell tryptase demonstrate shared antigenic sites on subunits of tryptase and selective localization of the enzyme to mast cells. *J Immunol* **134**: 526–531, 1985.
19. Smith TJ, Hougland MW and Johnson DA, Human lung tryptase, purification and characterization. *J Biol Chem* **259**: 11046–11051, 1984.
20. Benyon RC, Lowman MA and Church MK, Human skin mast cells: their dispersion, purification and secretory characterization. *J Immunol* **138**: 861–867, 1987.
21. Kimura I, Moritani Y and Tanizaki Y, Basophils in bronchial asthma with reference to reagin-type allergy. *Clin Allergy* **3**: 195–202, 1973.
22. Paul S, Volle DJ, Beach CM, Johnson DR, Powell MJ and Massey RJ, Catalytic hydrolysis of vasoactive intestinal peptide by human antibody. *Science* **244**: 1158–1162, 1989.
23. Brain SD, The direct observation of arteriolar constriction induced by endothelin *in vivo*. *Eur J Pharmacol* **160**: 401–403, 1989.
24. Schechter NM, Fraki JE, Geesin JC and Lazarus GS, Human skin chymotryptic proteinase: isolation and relation to cathepsin G and rat mast cell proteinase. *J Biol Chem* **258**: 2973–2978, 1983.
25. Schechter NM, Choi JK, Slavin DA, Deresienski DT, Sayama S, Dong G, Lavaker RM, Proud D and Lazarus GS, Identification of a chymotrypsin-like proteinase from human mast cells. *J Immunol* **137**: 962–970, 1986.
26. Irani AA, Schechter NM, Craig S, Deblois G and Schwartz LB, Two human mast cell subsets with different neutral protease compositions. *Proc Natl Acad Sci USA* **83**: 4464–4468, 1987.
27. Power JC, Tanaka T, Harper JW, Minematsu Y, Barker L, Lincoln D and Crumley KV, Mammalian chymotrypsin-like enzymes. Comparative reactivities of rat mast cell proteases, human and dog skin chymase and human cathepsin G with peptide 4-nitroanilide substrates and with peptide chloromethyl ketone and sulfonyl fluoride inhibitors. *Biochemistry* **24**: 2048–2067, 1985.
28. Zaidi M, Brain SD, Tippins JR, Dimarzo V, Moonga BS, Chambers TJ, Morris HR and MacIntyre I, Structure–activity relationship of human calcitonin gene-related peptide. *Biochem J* **269**: 775–780, 1990.
29. Schwartz LB and Bradford TM, Regulation of tryptase from human lung mast cells by heparin: stabilization of the active tetramer. *J Biol Chem* **261**: 7372–7379, 1986.
30. Alter SC, Metcalfe DD, Bradford TR and Schwartz LB, Stabilisation of mast cell tryptase: effects of enzyme concentration, ionic strength and the structure and negative charge density of polysaccharides. *Biochem J* **248**: 821–827, 1987.
31. Schwartz LB, Kawahara MS, Hugli TE, Vik D, Fearon DT and Austen KF, Generation of C3a anaphylatoxin from human C3 by human mast cell tryptase. *J Immunol* **130**: 1891–1895, 1983.
32. Han SP, Naes L and Westfall TC, Inhibition of periarterial nerve stimulation-induced vasodilation of the mesenteric arterial bed by CGRP(8-37) and CGRP receptor densitisation. *Biochem Biophys Res Commun* **168**: 786–791, 1990.
33. Hughes SR and Brain SD, Effect of calcitonin gene-related peptide (CGRP) antagonist (CGRP 8-37) on blood flow and potentiation of inflammatory oedema induced by CGRP and capsaicin in skin. *Br J Pharmacol* **104**: 738–742, 1991.

34. Gardiner SM, Compton AM, Kemp PA, Bennett T, Bose G, Foulkes R and Hughes B, Antagonistic effect of human α-CGRP (8-17) on the *in vivo* regional haemodynamic actions of α-CGRP. *Biochem Biophys Res Commun* **171**: 938–943, 1990.
35. Dennis T, Fournier A, St. Pierre S and Quirion R, Structure activity profile of calcitonin gene related peptide in peripheral and brain tissues. Evidence for receptor multiplicity. *J Pharmacol Exp Ther* **251**: 718–725, 1989.
36. Barnes PJ, Asthma as an axon reflex. *Lancet* **ii**: 242–245, 1986.
37. Walker KB, Serwonska MH, Valone FH, Harkonen WS, Frick OL, Scriven KH, Ratnoff WD, Browning JG, Payon DG and Goetzl EJ, Distinctive patterns of release of neuroendocrine peptides after nasal challenge of allergic subjects with ryegrass antigen. *J Clin Immunol* **8**: 108–113, 1988.
38. Schwartz LB, Atkins PC, Bradford TR, Fleekop P, Shalit M and Zweiman B, Release of tryptase together with histamine during immediate cutaneous response to allergen. *J Allergy Clin Immunol* **80**: 850–855, 1987.
39. Castells M and Schwartz LB, Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. *J Allergy Clin Immunol* **82**: 348–355, 1988.
40. Wenzel SE, Fowler AA and Schwartz LB, Activation of pulmonary mast cells by bronchoalveolar allergen challenge. *In vivo* release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis* **137**: 1002–1008, 1988.
41. Schwartz LB, Yunginger JW, Miller J, Bokhari and Dull D, Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest* **83**: 1551–1555, 1989.
42. Walls AF, Bennett AR, Godfrey RC, Holgate ST and Church MK, Mast cell tryptase and histamine concentrations in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Clin Sci* **81**: 183–188, 1991.
43. Schwartz LB, Bradford TR, Littman BH and Wintroub BU, The fibrinogenolytic activity of purified tryptase from human lung mast cells. *J Immunol* **135**: 2762–2767, 1985.
44. Gruber BL, Marchese MJ, Suzuki K, Schwartz LB, Okada Y, Nagese H and Ramamurthy NS, Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J Clin Invest* **84**: 1657–1662, 1989.