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Characterization of neuropeptide-induced histamine release from human dispersed skin mast cells

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1 Human skin mast cells, unlike other human mast cells so far studied, released histamine in a concentration-related manner in response to substance P, vasoactive intestinal peptide (VIP) and somatostatin (1 μM to 30 μM). In contrast, eledoisin, physalaemin, neurokinin A, neurokinin B, calcitonin gene-related peptide (CGRP), neurotensin, bradykinin and Lys-bradykinin induced negligible histamine release.

2 The low histamine releasing activity of physalaemin, eledoisin, neurokinin A and neurokinin B relative to substance P suggests that the human skin mast cell activation site is distinct from the tachykinin NK-1, NK-2 or NK-3 receptors described in smooth muscle.

3 The relative potencies of substance P and its fragments SP_{2-11}, SP_{3-11}, SP_{4-11} and SP_{1-4} in releasing histamine from human skin mast cells suggests that both the basic N-terminal amino acids and the lipophilic C-terminal portion of substance P are essential for activity.

4 Peptide-induced histamine release, like that induced by compound 48/80, morphine and poly-L-lysine, is rapid, reaching completion in 10–20 s, is largely independent of extracellular calcium but requires intact glycolysis and oxidative phosphorylation.

5 The substance P analogue, [D-Pro^4,D-Trp^{7,9,10}] SP_{4-11} (SPA), not only reduced substance P-induced histamine release in a concentration-related manner but also inhibited that induced by VIP, somatostatin, compound 48/80, poly-L-lysine and morphine but not anti-IgE.

6 The similar characteristics of histamine release induced by substance P, VIP, somatostatin, compound 48/80, poly-L-lysine and morphine suggest that they share a common pathway of activation-secretion coupling distinct from that of IgE-dependent activation. Furthermore, the ability of human skin mast cells to respond to basic non-immunological stimuli including neuropeptides may reflect a specialised function for these cells.

Introduction

The mast cells of human skin are closely associated with both dermal nerve endings and blood vessels (Eady *et al.*, 1979). Increasing evidence suggests that the secretory function of these cells may be regulated by the nervous system (Goetzl *et al.*, 1985; Foreman, 1987). Mammalian skin contains a variety of neuropeptides including substance P, vasoactive intestinal peptide (VIP), somatostatin, neurotensin and calcitonin gene-related peptide (CGRP) (Hartschuh *et al.*, 1983; O'Shaughnessy *et al.*, 1983; Brain *et al.*, 1986) all of which are potential mast cell activators. Each of these peptides is capable of inducing a weal and flare reaction when injected intradermally into human skin (Foreman *et al.*, 1982; Anand *et al.*, 1983; Piotrowski & Foreman, 1986) and it has been suggested that the vasodilatation and, to a lesser extent, plasma extravasation, are secondary to the release of mast cell mediators (Hägermark *et al.*, 1978; Foreman *et al.*, 1982). This hypothesis presupposes that neuropeptides can directly or indirectly activate human skin mast cells.

There is some evidence that the ability of neuropeptides to induce weal and flare reactions in human skin is closely correlated to histamine releasing activity from rat peritoneal mast cells (Foreman *et al.*, 1983). These cells release histamine in response to both IgE-dependent and non-immunological stimuli (Lagunoff *et al.*, 1983). Non-immunological stimuli include compound 48/80, morphine and the basic peptides substance P, VIP, somatostatin, neurotensin and CGRP (Grosman, 1981; Shanahan *et al.*, 1985; Piotrowski & Foreman, 1986).

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In our studies of mast cells dispersed from human skin, lung, tonsils, colon and adenoids, we established that skin mast cells are unique amongst these in releasing histamine in response to compound 48/80, morphine and substance P (Benyon *et al.*, 1987; Lowman *et al.*, 1988). In this paper we extend our observations on the ability of neuropeptides to induce histamine release from human skin mast cells and suggest that they activate histamine release by binding via basic moieties to the same, or a closely related, binding site distinct from the IgE-receptor.

Methods

Mast cell dispersion

This was performed using methods similar to those previously described by Benyon *et al.* (1987). Samples of foreskin from young children (1–12 years) were placed immediately in MEM following circumcision, stored at 4°C, and used within 24 h. Fragments of 0.5 to 2.0 mm, obtained by chopping with scissors, were washed twice with MEM containing 2.0% FCS (MEM/FCS). In experiments to investigate histamine release induced by anti-IgE, skin slices were passively sensitized by incubation for 2 h at 37°C in MEM containing 5% FCS and 10% human atopic serum. To disperse mast cells, tissue was incubated for 60 min at 37°C in MEM (1 g tissue per 10 ml buffer) containing collagenase 1.5 mg ml^{-1}, hyaluronidase 0.75 mg ml^{-1} and BSA 35 mg ml^{-1}. Dispersed cells were separated from undissociated tissue by filtration through 150 μm mesh nylon gauze and were washed twice with MEM/FCS by centrifugation at 500 *g* for 5 min at 20°C. Undigested tissue was subjected to a second identical digestion and the cells from both were pooled, washed once with MEM/FCS, and mast cell numbers were determined by light microscopy following metachromatic staining with Kimura stain (Kimura *et al.*, 1973).

Mast cell activation

Before activation for histamine release, cells were washed once in HEPES-BSS by centrifugation at 500 *g* for 5 min at 20°C and were then resuspended in this buffer to a density of approximately 5×10^4 mast cells ml^{-1}. After warming to 37°C for 10 min, samples of 225 μl were added to polypropylene tubes containing 25 μl of secretagogue, and histamine release reactions allowed to continue for 15 min before termination by addition of 750 μl of ice-cold HEPES-BSS and centrifugation at 500 *g* for 5 min at 4°C. All histamine release reactions were performed in duplicate. Supernatant and cell pellet histamine content was determined by automated spectrofluorimetry (Evans *et al.*, 1973). Net histamine release was calculated as a percentage of total cell-associated histamine following correction for spontaneous release, assessed in duplicate cell samples in the absence of secretagogue. At the secretagogue concentrations used, only somatostatin (10–30 μM) interfered with this assay. Histamine release by somatostatin was corrected for intrinsic fluorescence of the peptide using a series of fluorescence controls.

In experiments to analyse the time course of histamine release, the above procedure was modified as follows. To 180 μl samples of cell suspension prewarmed to 37°C for 10 min were added 20 μl of secretagogue. Reactions were stopped at the stated times by addition of 800 μl of ice-cold, calcium-free HEPES-BSS containing 2 mM EDTA and centrifugation at 1000 *g* for 2 min at 4°C. Supernatant and cell associated histamine was measured as described above. As spontaneous histamine release was constant over the time periods used in the time course experiments, a mean value was used to calculate net histamine release.

Calcium deprivation and energy dependence studies

In studies of the effects of calcium deprivation on histamine release, cell preparations were incubated for 5 min at 37°C in calcium-free HEPES-BSS containing 2 mM EDTA before the 15 min incubation with secretagogue. To assess the energy dependence of histamine release, cells were incubated for 20 min at 37°C in glucose-free HEPES-BSS containing 10 mM 2-deoxy-D-glucose (to inhibit glycolysis) and 1 μM antimycin A (to inhibit oxidative phosphorylation) before the 15 min incubation with secretagogue. Dimethylsulphoxide, used as a solvent for antimycin A to a final concentration of 0.25%, had no significant effect on histamine release.

Substance P antagonist studies

In studies of the effects of [D-Pro^4,D-$Trp^{7,9,10}$] SP_{4-11} on secretagogue-induced histamine release, 200 μl samples of cell suspension were incubated with 25 μl of antagonist for 5 min at 37°C before addition of 25 μl of secretagogue. Histamine release reactions were allowed to proceed for 15 min at 37°C before termination as described above.

Materials

The following chemicals and enzymes were used: collagenase (type I), hyaluronidase (type I), deoxy-

Table 1 Histamine release from human dispersed skin mast cells induced by peptides

Peptide	No. of experiments	Net histamine release (%) 1 μM	3 μM	10 μM	30 μM
Substance P	20	4.3 ± 0.7*	8.7 ± 1.0*	11.9 ± 1.2*	14.8 ± 1.6*
SP_{2-11}	4	0.6 ± 0.2	3.9 ± 1.0*	11.3 ± 3.6*	16.5 ± 4.8*
SP_{3-11}	5	0.6 ± 0.2*	0.4 ± 0.2	4.3 ± 1.4*	11.0 ± 3.3*
SP_{4-11}	7	0.4 ± 0.2	0.3 ± 0.1*	1.0 ± 0.3*	3.3 ± 0.7*
SP_{1-4}	4	0.4 ± 0.2	0.2 ± 0.2	0.4 ± 0.2	0.5 ± 0.4
Eledoisin	5	0.0 ± 0.0	0.4 ± 0.2	0.5 ± 0.4	0.7 ± 0.4
Physalaemin	5	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2*	1.4 ± 0.5*
Neurokinin A	3	0.6 ± 0.2	0.8 ± 0.1*	1.3 ± 0.1*	3.9 ± 0.8*
Neurokinin B	3	0.4 ± 0.1*	1.1 ± 0.1*	0.7 ± 0.4	0.4 ± 0.3
VIP	7	3.2 ± 1.0*	8.2 ± 2.4*	13.5 ± 3.2*	19.3 ± 4.0*
Somatostatin	5	1.8 ± 1.0*	6.7 ± 1.8*	12.3 ± 2.5*	15.6 ± 3.6*
CGRP	3	0.4 ± 0.4	0.8 ± 0.4	1.3 ± 0.7	4.8 ± 0.3*
Neurotensin	4	0.6 ± 0.4	0.2 ± 0.2	0.4 ± 0.2	0.9 ± 0.3*
Bradykinin	4	0.2 ± 0.1	0.5 ± 0.3	0.7 ± 0.3	1.6 ± 0.3
Lys-bradykinin	4	0.8 ± 0.5	0.6 ± 0.2	1.8 ± 0.6	2.8 ± 0.7*

Each result is the mean ± s.e.mean for the stated number of experiments. Histamine release has been corrected for spontaneous release, which was in the range 3.5% to 11.5%, and * indicates release significantly ($P < 0.05$) greater than spontaneous.
VIP, vasoactive intestinal peptide; CGRP, calcitonin gene-related peptide.

ribonuclease (bovine pancreas), bovine serum albumin (Fraction V) (BSA), compound 48/80, poly-L-lysine (average mol wt 45,000 to 55,000 daltons), substance P (SP) (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2), substance P fragment 2-11 (SP_{2-11}), substance P fragment 4-11 (SP_{4-11}), substance P fragment 1-4 (SP_{1-4}), eledoisin, physalaemin, neurokinin A, neurokinin B, vasoactive intestinal peptide (VIP), somatostatin, neurotensin, bradykinin, Lys-bradykinin, [D-Pro^2,D-$Trp^{7,9}$] substance P, dimethylsulphoxide (DMSO), antimycin A, 2-deoxy-D-glucose, *N*-2-hydroxyethyl-piperazine- N'-2-ethanesulphonic acid (HEPES) (Sigma Chemical Co., Poole, Dorset); foetal calf serum (FCS), Eagle's minimum essential medium (MEM) containing 25 mM HEPES and L-glutamine (Gibco Europe Ltd., Paisley, Scotland); ethylenediaminetetraacetic acid (EDTA) (BDH Chemicals, Poole, Dorset); substance P fragment 3-11 (SP_{3-11}), calcitonin gene-related peptide (CGRP), [D-Pro^4,D-$Trp^{7,9,10}$] SP_{4-11} (SPA) (Peninsula Laboratories Europe Ltd., St. Helens, Merseyside); morphine sulphate (Evans Medical Ltd., Greenford, Middlesex). Atopic serum used for mast cell passive sensitization was obtained from an individual allergic to Timothy grass pollen. Goat anti-human IgE serum was heat-inactivated by incubation at 60°C for 1 h. HEPES-buffered salts solution (HEPES-BSS), pH 7.2, contained (mM) NaCl 137, HEPES 10, D-glucose 5, KCl 2.7, NaH_2PO_4 0.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8 and FCS 1.0%, these reagents being of analytical grade.

Statistical analyses

Results are expressed as mean ± 1 standard error of mean (s.e.mean) for the stated number of separate experiments. Differences between means were tested for significance using Student's *t* test for paired data, differences being considered significant when the probability (*P*) was <0.05.

Results

Histamine releasing activity of peptides

Table 1 summarizes histamine release from human skin mast cells activated with various peptides.

In 20 experiments substance P induced a concentration-related release of histamine (Figure 1a). Release from foreskin mast cells with substance P, 30 μM, was 14.8 ± 1.6%, approximately half that released from mast cells obtained from adult breast skin (Lowman *et al.*, 1988). Histamine release

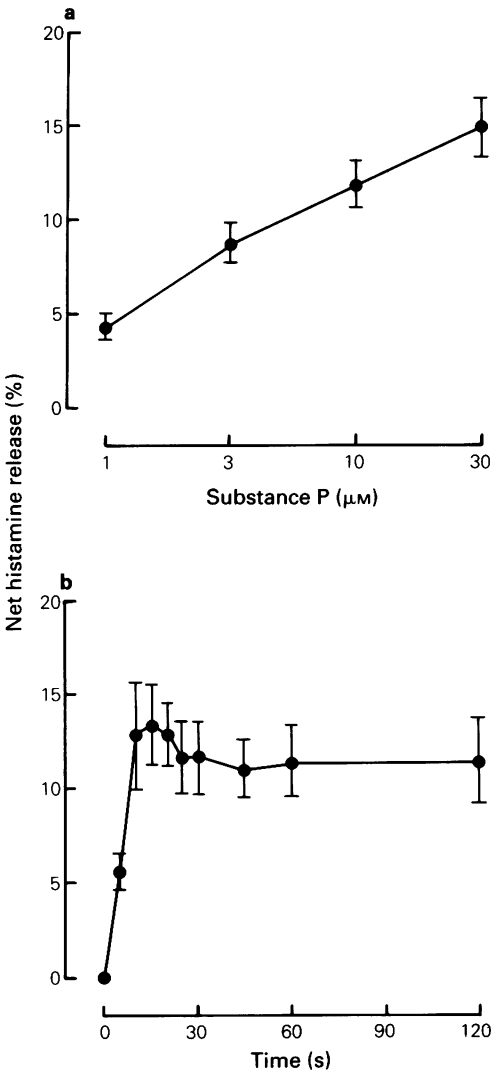

Figure 1 Substance P-induced histamine release from human dispersed skin mast cells. (a) Concentration-dependence of release. Results are mean of 20 experiments in which spontaneous histamine release was 5.6 ± 0.3%. (b) Time course of histamine release induced by substance P, 10 μM. Results are mean of 5 experiments in which spontaneous histamine release was 4.4 ± 0.5%. In (a) and (b), s.e.mean shown by vertical bars.

induced by substance P, 10 μM was rapid, reaching a maximum of 13.4 ± 2.1% at 15 s after addition in five experiments (Figure 1b). This time-course contrasts with that of IgE-dependent histamine release which reaches a maximum 5–7 min after challenge (Benyon *et al.*, 1987).

The ability of substance P fragments SP_{2-11}, SP_{3-11} and SP_{4-11} to release histamine from human skin mast cells was compared with that of substance P in 4–7 experiments (Table 1). In these experiments progressive removal of N-terminal amino acids reduced histamine releasing activity. Relative potencies of substance P and its fragments calculated from the parallel portions of the concentration-response lines were: substance P = 1, SP_{2-11} = 0.46 and SP_{3-11} = 0.16. No potency ratio was calculated for SP_{4-11} as it only released 3.5 ± 0.5% histamine at 30 μM, the maximum concentration used. The basic N-terminal tetrapeptide, SP_{1-4}, induced no significant histamine release, suggesting that the C-terminal portion is essential for expression of activity.

Tachykinin receptors on smooth muscle have been divided into 3 sub-classes, NK-1, NK-2 and NK-3, on the basis of relative potencies of eledoisin, physalaemin, neurokinin A and neurokinin B to cause contraction (Lee *et al.*, 1986). These tachykinins were used to investigate whether the site at which substance P activates skin mast cells falls into such a classification. The results (Table 1) showed that only physalaemin and neurokinin A caused significant concentration-related histamine release and that all were significantly ($P < 0.001$) less potent than substance P.

Vasoactive intestinal peptide (VIP) induced a concentration-related release of histamine from human skin mast cells (Figure 2a), reaching 19.3 ± 4.0% at 30 μM. At no concentration was release induced by VIP significantly different from that induced by substance P in the same experiments. The time course of release, like that of substance P, was rapid, reaching completion within 20 s of VIP addition (Figure 2b). Similar results were obtained with somatostatin (Figure 3), release being 15.6 ± 3.6% at 30 μM and release being complete within 20 s.

Calcitonin gene-related peptide (CGRP), neurotensin, bradykinin and Lys-bradykinin were weak histamine releasing agents in human skin mast cells, significant release being observed only at 30 μM (Table 1).

The effects of calcium deprivation and metabolic inhibitors on histamine release

Challenge of human skin mast cells in calcium-free HEPES-BSS containing 2 mM EDTA reduced histamine release induced by 1% anti-IgE to levels not significantly greater than spontaneous in three experiments (Table 2). Similar results were obtained with nominally calcium-free medium lacking EDTA, suggesting IgE-dependent histamine release to be

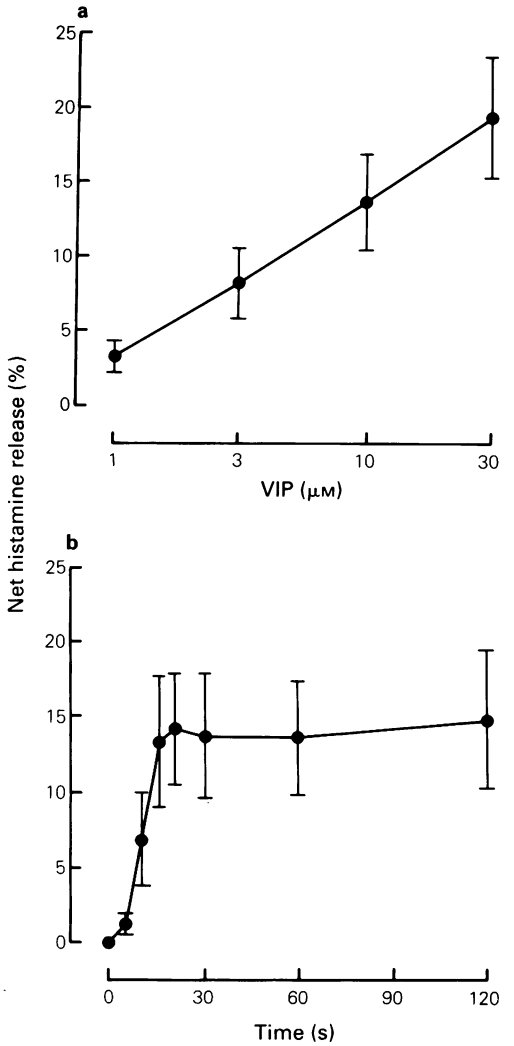

Figure 2 Histamine release from human dispersed skin mast cells induced by vasoactive intestinal peptide (VIP). (a) Concentration-dependence of release. Results are mean of 7 experiments in which spontaneous histamine release was 7.1 ± 0.5%. (b) Time course of histamine release induced by VIP, 10 μM. Results are mean of 3 experiments in which spontaneous histamine release was 13.5 ± 1.8%. In (a) and (b), s.e.mean shown by vertical bars.

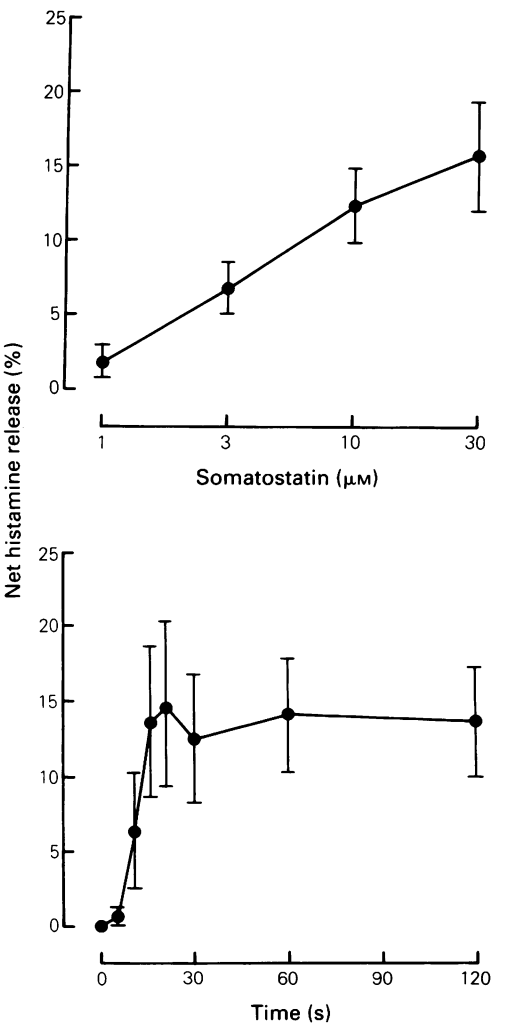

Figure 3 Somatostatin-induced histamine release from human dispersed skin mast cells. (a) Concentration-dependence of release. Results are mean of 5 experiments in which spontaneous histamine release was 7.9 ± 0.4%. (b) Time course of histamine release induced by somatostatin, 10 μM. Results are mean of 3 experiments in which spontaneous histamine release was 13.0 ± 2.0%. In (a) and (b), s.e.mean shown by vertical bars.

dependent on the presence of extracellular calcium. In contrast, histamine release induced by VIP and somatostatin was independent of extracellular calcium in three experiments, there being no significant difference between histamine release induced by VIP, 3 μM, and somatostatin, 3 μM and 10 μM, in the presence and absence of extracellular calcium, Histamine release induced by substance P, 10 μM and 100 μM, was only partially dependent on extracellular calcium. Again, similar results were obtained with nominally calcium-free medium lacking EDTA.

To determine whether histamine release from human skin mast cells induced by substance P, VIP and somatostatin was an energy-dependent process

Table 2 The effect of calcium deprivation and inhibition of cellular ATP generation on histamine release from human dispersed skin mast cells

Secretagogue		*No. of experiments*	*Net histamine release* Control	Test	% *inhibition*
Calcium-free HEPES-BSS containing 2 mM EDTA					
Substance P	10 μM	7	8.5 ± 1.4	5.0 ± 1.2	42.6 ± 12.5*
	100 μM	5	16.9 ± 3.6	9.6 ± 2.6	46.2 ± 7.8*
VIP	3 μM	3	10.7 ± 0.4	12.0 ± 0.8	−12.5 ± 11.1
Somatostatin	3 μM	3	10.9 ± 0.5	11.7 ± 0.3	−7.4 ± 4.7
	10 μM	3	18.2 ± 0.7	14.7 ± 1.0	18.3 ± 8.7
Anti-IgE	1%	6	9.2 ± 1.8	0.3 ± 0.1	96.8 ± 1.6*
10 mM 2-deoxy-D-glucose and 1 μM antimycin A					
Substance P	3 μM	4	9.8 ± 2.6	0.3 ± 0.1	96.1 ± 1.4*
	30 μM	4	16.0 ± 3.5	1.5 ± 0.6	89.5 ± 3.5*
VIP	3 μM	4	8.6 ± 1.8	0.8 ± 0.5	85.1 ± 8.7*
Somatostatin	3 μM	4	7.9 ± 2.4	0.5 ± 0.1	91.2 ± 3.7*
	10 μM	4	15.8 ± 2.9	0.7 ± 0.5	94.6 ± 3.0*
Anti-IgE	1%	4	8.2 ± 1.3	0.2 ± 0.1	96.8 ± 0.7*

Each result is the mean ± s.e.mean of 3–7 experiments in which cells were incubated in either calcium-free HEPES-BSS containing 2 mM EDTA for 5 min or glucose-free HEPES-BSS containing 10 mM 2-deoxy-D-glucose and 1 μM antimycin A for 20 min before activation for histamine release. Histamine release has been corrected for spontaneous release, which was in the range 2.4% to 9.4%, and * denotes results significantly ($P < 0.05$) different from control.

VIP, vasoactive intestinal peptide.

and, presumably, non-cytotoxic, four experiments were undertaken in which cellular glycolysis and oxidative phosphorylation were inhibited by 2-deoxy-D-glucose and antimycin A (Table 2). Metabolic inhibition blocked histamine release induced by anti-IgE, 1%, and almost completely blocked histamine release induced by substance P, 3 μM and 30 μM, VIP, 3 μM, and somatostatin, 3 μM and 10 μM.

The effects of substance P antagonists on histamine release

A number of undecapeptide substance P analogues which antagonize tachykinin-induced smooth muscle contraction and which exhibit N-terminal homology with substance P have been shown to release histamine from rat mast cells (Foreman & Piotrowski, 1985). Substitution of D-Phe or D-Trp residues in the C-terminal sequence of substance P is known to increase the histamine releasing activity of the peptide. The effect of one such analogue, [D-Pro^2,D-Trp^{7,9}] substance P was assessed on human skin mast cells in the concentration range 0.1 μM to 30 μM. In four experiments, histamine release was significantly greater than spontaneous at 0.1 μM, being 1.9 ± 0.6%, and increased in a concentration-related manner from 4.0 ± 0.9% and 6.2 ± 1.2% at 0.3 μM and 1 μM to a maximum of 9.7 ± 1.4% at 30 μM. In the same experiments histamine release induced by substance P was significantly greater than spontaneous at 1 μM, being 1.7 ± 0.4%, and increased in a concentration-related manner to a maximum of 10.4 ± 1.1% at 30 μM. At a response level corresponding to 5% net histamine release [D-Pro^2,D-Trp^{7,9}] SP was 7.2 times more potent than substance P. [D-Pro^2,D-Trp^{7,9}] SP was, therefore, a more potent secretagogue than substance P but had the same maximal effect. Another analogue, [D-Pro^4, D-Trp^{7,9,10}] SP_{4-11} (SPA), although substituted with D-Trp residues in the C-terminal sequence, lacks the N-terminal homology with substance P required for histamine releasing activity. In four experiments, SPA did not induce significant histamine release from human skin mast cells in the concentration range 0.3 μM to 30 μM.

As SPA has been reported to antagonize both substance P-induced histamine release from rat mast cells and substance P-induced flare in human skin (Foreman & Piotrowski, 1985), we tested the ability of SPA to inhibit substance P-induced histamine release from human skin mast cells (Figure 4). In four experiments, increasing concentrations of SPA in the range 0.3 μM to 30 μM produced a graded shift to the right of the substance P concentration-response curve for histamine release. Histamine release induced by substance P, 30 μM, was inhibited by 18.2 ± 3.0% ($P < 0.05$), 50.0 ± 9.0% ($P < 0.05$) and 74.3 ± 6.0% ($P < 0.01$) in the presence of SPA, 3 μM, 10 μM and 30 μM respectively. In contrast to SPA, substance P fragment 4-11, 30 μM, had no sig-

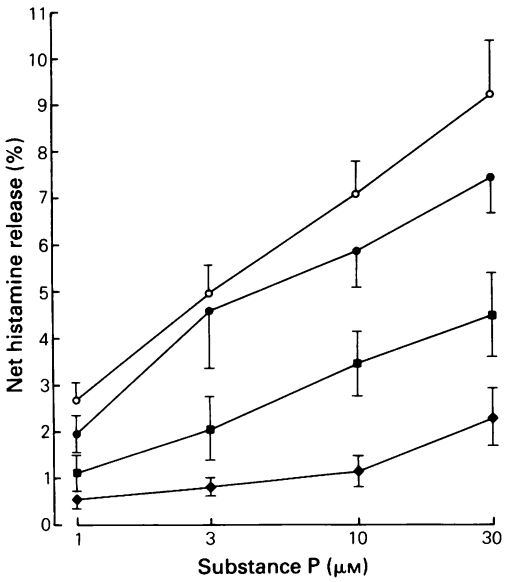

Figure 4 Antagonism of substance P-induced histamine release from human dispersed skin mast cells by [D-Pro^4,D-Trp^{7,9,10}] SP_{4-11}, 0 μM (○), 3 μM (●), 10 μM (■) and 30 μM (◆). Results are mean of 4 experiments in which spontaneous histamine release was 4.7 ± 0.6%; s.e.mean shown by vertical bars.

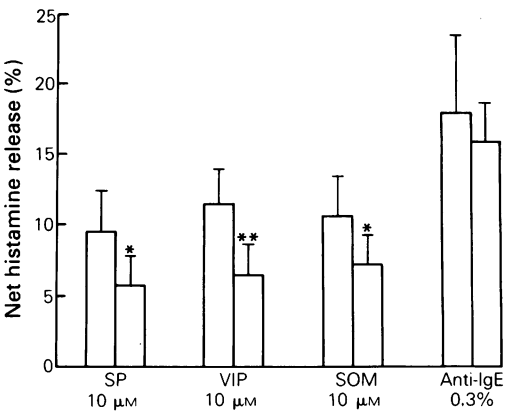

Figure 5 The effect of [D-Pro^4,D-Trp^{7,9,10}] SP_{4-11}, 30 μM, on histamine release from human dispersed skin mast cells induced by anti-IgE, substance P (SP), vasoactive intestinal peptide (VIP) and somatostatin (SOM). Results are mean of 3–5 experiments in which spontaneous histamine release was 8.6 ± 1.5%, s.e.mean shown by vertical lines. Asterisks denote significance of inhibition as follows: * $P < 0.05$; ** $P < 0.01$.

nificant effect on histamine release induced by substance P, 1 μM to 30 μM, in three experiments. A further three experiments showed that neurotensin, which antagonizes substance P-induced histamine release from rat mast cells and flare in human skin (Foreman *et al.*, 1982), had no significant effect on histamine release from human skin mast cells induced by substance P, 1 μM to 30 μM, when present at 30 μM.

In addition to inhibiting substance P-induced histamine release, SPA, 30 μM, also inhibited histamine release induced by VIP, somatostatin and several other non-immunological secretogogues (Figure 5) which we have previously found to induce calcium-independent histamine release from human mast cells (Benyon *et al.*, 1987). In three experiments, histamine release induced by VIP, 10 μM, was reduced by SPA from 11.4 ± 2.6% to 6.3 ± 2.3%, a reduction of 48.8 ± 10.4% ($P < 0.01$). In five experiments, histamine release induced by somatostatin, 10 μM, was reduced by SPA from 10.4 ± 2.8% to 7.1 ± 2.0%, a reduction of 32.5 ± 2.8% ($P < 0.05$). In these experiments, as a positive control, histamine release induced by substance P, 10 μM, was reduced from 9.4 ± 2.9% to 5.6 ± 2.1% in the presence of SPA, a reduction of 46.0 ± 13.4% ($P < 0.05$). Inhibition by SPA was specific for non-immunological secretagogues as in five experiments histamine release induced by anti-IgE, 0.1%, 0.3% and 1%, was not significantly affected by the presence of SPA, 30 μM. Histamine release in the absence and presence of SPA was 13.0 ± 5.8% and 13.3 ± 2.8%, 17.9 ± 5.6% and 15.8 ± 2.8%, and 19.6 ± 6.1% and 17.3 ± 3.3% respectively. Histamine release induced by compound 48/80, 10 μg ml^{-1}, poly-L-lysine, 10 μM, and morphine, 30 μM, was also inhibited in the presence of SPA, 30 μM, by 82.4 ± 6.7% ($P < 0.01$), 80.1 ± 8.6% ($P < 0.02$) and 79.5 ± 11.5% ($P < 0.05$) respectively. In this series of experiments, histamine release induced by substance P, 10 μM, used as a positive control, was inhibited by 85.7 ± 5.1% ($P < 0.01$) in the presence of 30 μM SPA.

Discussion

In this study we have demonstrated that substance P, VIP and somatostatin release histamine from human dispersed skin mast cells with characteristics similar to those previously described for rat mast cells (Shanahan *et al.*, 1985). Histamine release from human skin mast cells induced by these peptides is concentration-related over the range 1 μM to 30 μM. Although cytotoxicity may be suspected at these high concentrations of secretagogue, blockade of his-

tamine release by inhibition of glycolysis and oxidative phosphorylation strongly suggests a non-cytotoxic, energy-requiring process. This conclusion is further supported by the observation that histamine secretion is inhibited by SPA. The time course of neuropeptide-induced histamine release from skin mast cells is rapid, being complete within 30s. This is much faster than substance P-induced histamine release demonstrable *in vitro* from human skin slices where physical barriers to diffusion of both peptide and histamine may give a false time course of mast cell activation (Ebertz *et al.*, 1987). Removal of extracellular calcium reduces substance P-induced histamine release by $< 50\%$ and has little effect on histamine release induced by VIP or somatostatin, indicating that these secretagogues may activate secretion either by mobilization of intracellular calcium or by a calcium-independent mechanism. These findings are similar to those in rat peritoneal mast cells where absence of extracellular calcium does not inhibit histamine release induced by substance P, VIP or somatostatin. Depletion of intracellular calcium by prolonged incubation of rat peritoneal mast cells with EDTA does, however, lead to a 70 to 80% reduction in histamine release (Shanahan *et al.*, 1985).

The low histamine releasing activity of physalaemin, eledoisin, neurokinin A and neurokinin B relative to substance P suggests that the human skin mast cell activation site is a receptor distinct from the tachykinin NK-1, NK-2 or NK-3 types previously described (Lee *et al.*, 1986). Our finding that step-wise removal of N-terminal amino acids of substance P progressively reduces histamine releasing activity supports the hypothesis that the integrity of this portion of the molecule is important for releasing activity. Nonetheless, the inactivity of SP_{1-4} alone, and the increased potency of [D-Pro^2,D-Trp^{7,9}] SP, suggests that the C-terminal sequence does have some role in the full expression of activity, possibly increasing the lipophilicity of the peptides (Fewtrell *et al.*, 1982).

In terms of time course and calcium dependency, histamine release from human skin mast cells induced by substance P, VIP and somatostatin is similar to that previously described for compound 48/80, poly-L-lysine and morphine (Benyon *et al.*, 1987). Furthermore, relatively high concentrations of each secretagogue are required to induce 15–20% histamine release. Our results show [D-Pro^4,D-Trp^{7,9,10}] SP_{4-11} (SPA) to be an antagonist of substance P-induced histamine release from human skin mast cells and to have similar potency in inhibiting histamine release induced by VIP, somatostatin compound 48/80, poly-L-lysine and morphine. This suggests that these stimuli share a common pathway of activation-secretion coupling, possibly acting via a common cell-surface activation site of low affinity and poor specificity. A similar activation site may exist on rat peritoneal mast cells and our findings are consistent with studies showing histamine release from these cells in response to substance P, VIP and somatostatin to be inhibited by SPA (Foreman & Piotrowski, 1985). The activation mechanism employed by substance P, VIP and somatostatin in human skin mast cells appears to be distinct from that for IgE-dependent stimuli. Histamine release in response to anti-IgE is relatively slow, reaching a maximum after 7–10 min (Benyon *et al.*, 1987), is totally dependent on extracellular calcium, and is not significantly inhibited by SPA. There are also differences in the spectrum of mediators released by IgE-dependent and non-immunological stimuli, with anti-IgE being far more effective than substance P as a generator of prostaglandin D_2 and leukotriene C_4 (Benyon, Robinson & Church, unpublished observations).

The ability of human skin mast cells to release histamine in response to substance P, VIP and somatostatin is consistent with *in vivo* findings in human skin where intradermal injection of each of these neuropeptides induces weal and flare reactions (Anand *et al.*, 1983). The flare, or vasodilatory, component of such reactions is considered to be mediated by histamine, being inhibited by H_1-histamine antagonists (Hägermark *et al.*, 1978), is neurogenic, being inhibited by nerve section, prior application of local anaesthetics and depletion of peripheral nerve neuropeptide content by capsaicin (Bayliss, 1901; Foreman *et al.*, 1983; Anand *et al.*, 1983), and therefore is thought to involve an interaction between skin mast cells and neuropeptides (Hägermark *et al.*, 1978; Foreman *et al.*, 1983). Calcitonin gene-related peptide and neurotensin, which we have shown to be poor histamine releasers from human skin mast cells, are much less potent than substance P in inducing both weal and flare reactions in human skin and histamine release from rat peritoneal mast cells (Foreman *et al.*, 1982; Piotrowski & Foreman, 1986). Although at low doses CGRP does produce local vasodilatation, and pronounced long-lasting erythema, these are not associated with a characteristic weal and flare reaction and are not thought to result from a direct action of CGRP on skin mast cells (Brain *et al.*, 1986; Piotrowski & Foreman, 1986).

Whether or not neuropeptide-induced activation of human skin mast cells has a physiological function is uncertain but there is increasing evidence that neuropeptides, acting via receptors on immune effector cells, can modulate a range of immunological responses (Goetzl & Payan, 1984; Payan *et al.*, 1984; Goetzl *et al.*, 1985). A role for neuropeptides in both negative and positive feedback mechanisms regulating mediator release from immune cells is suggested

by the presence of somatostatin-like peptides in rat basophilic leukaemia (RBL) cells (Goetzl *et al.*, 1985) and VIP immunoreactivity in rat peritoneal mast cells (Cutz *et al.*, 1978). Somatostatin-like peptides from RBL cells appear to inhibit mediator release from these cells whereas VIP immunoreactivity, which is released from rat peritoneal mast cells in response to compound 48/80, may stimulate further mast cell mediator release.

In conclusion, we have demonstrated that human dispersed skin mast cells release histamine in response to substance P, VIP and somatostatin. The similarity of this response to that for compound 48/80, poly-L-lysine and morphine, and inhibition of the response to each secretagogue by a substance P antagonist, suggest that they share a common pathway of activation-secretion coupling distinct from that of IgE-dependent activation. Human skin mast cells are distinct from other human mast cells so far studied in their ability to respond to basic, non-immunological stimuli (Lowman *et al.*, 1988) and this may well reflect a specialized function for these cells.

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