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INTERACTION OF NEUROTENSIN WITH THE SUBSTANCE P RECEPTOR MEDIATING HISTAMINE RELEASE FROM RAT MAST CELLS AND THE FLARE IN HUMAN SKIN

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1 Substance P induced histamine release from rat peritoneal mast cells in a dose-dependent manner over the concentration range 1 to 10 μM.

2 At concentrations in the range 2.5 to 10 μM, neurotensin produced only about 5% release of histamine, which was substantially less than the maximum effect obtained with substance P.

3 Neurotensin, 2.5 to 10 μM produced graded inhibition of histamine release induced by substance P. The inhibitory effect of neurotensin was not seen when histamine release was induced by an antigen-antibody reaction or by the ionophore, A 23187. Some evidence was obtained to suggest that compound 48/80 may interact with the same receptor as substance P and neurotensin.

4 [D-Arg^8]neurotensin, [D-Arg^9]neurotensin, xenopsin and the C-terminal octapeptide of substance P (SP_{4-11}) all inhibited histamine release by substance P, but physalaemin did not.

5 Neurotensin inhibited the wheal and flare reactions induced by substance P in human skin.

6 [D-Trp^{7,9}]substance P released histamine from rat mast cells and was about 12 times more potent than substance P itself. [D-Trp^{7,9}]SP_{1-11} also produced wheal and flare responses in human skin, being 1.8 times more potent than substance P in the production of flare.

Introduction

Intradermal injection of the undecapeptide, substance P (Figure 1) produces a wheal and flare reaction in man (Hägermark, Hökfelt & Pernow, 1978; Foreman & Jordan, 1981) and it is known that this peptide also releases histamine from mast cells (Johnson & Erdös, 1973; Kitada, Ashida, Maki, Fujimo, Hirai, Yasuhara, Nakajima, Takeyama, Koyama & Yajima, 1980; Erjavec, Lembeck, Florjanc-Irman, Skofitsch, Donnerer, Saria & Holzer, 1981; Fewtrell, Foreman, Jordan, Oehme, Renner & Stewart, 1982). Immunohistochemical and neurochemical evidence indicates that substance P is present in the peripheral as well as the central terminals of some primary afferent neurones (Hökfelt, Kellerth, Nilsson & Pernow, 1975; Cuello, Del Fiacco & Paxinos, 1978) and it is conceivable, therefore, that substance P is released from these neurones in the skin and other organs. It has been proposed that substance P, released from sensory nerve endings, plays a part in the triple response of skin to an injury (Hägermark *et al.*, 1978; Lembeck & Donnerer, 1981), and we have already pointed out that the flare, which is believed to be mediated by the so-called 'axon reflex' in the triple response, is induced only by those peptides which also release histamine from rat mast cells *in vitro* (Foreman & Jordan, 1981). The relationship between substance P and mast cells in injury and inflammation is thus of interest, and inhibitors of the peripheral actions of substance P might have a suppressant effect on some inflammatory reactions.

The neuropeptide, neurotensin (Figure 1), has been shown to bind to rat mast cells (Lazarus, Perrin & Brown, 1977; Lazarus, Perrin, Brown & Rivier, 1977) with a dissociation constant for binding of about 0.15 μM. There are several reports that neurotensin releases histamine from rat mast cells *in vitro* (Selbekk, Flaten & Hanssen, 1980; Kurose & Saeki, 1981; Kruger, Aas, Onarheim & Helle, 1982) though the maximum release of histamine that was achieved was much less than that obtained with substance P (Kurose & Saeki, 1981) at concentrations of neurotensin which would be expected to saturate the receptors identified by binding studies.

We show in this paper that neurotensin appears to be a partial agonist at the substance P receptors on rat mast cells and in human skin which mediate histamine release and flare responses respectively. We also describe the actions of another putative substance P antagonist, [D-Trp^{7,9}]SP_{1-11}. A preliminary communication of some of these observations was given to the Physiological Society (Foreman & Jordan, 1982).

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Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Xenopsin	pGlu-Gly-Lys-Arg-Pro-Trp-Ileu-Leu-OH
Neurotensin	Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ileu-Leu-OH
Physalaemin	pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2

Figure 1 Structures of the neuropeptides substance P, neurotensin, xenopsin and physalaemin.

Methods

Histamine release from rat mast cells

A suspension of peritoneal cells containing 2–5% mast cells was obtained by washing the peritoneal cavities of Lister Hooded rats (150 to 250 g) with heparinized (25 u/ml) saline (NaCl, 154 mM). The rats had been sensitized to ovalbumin 15 to 30 days before the experiments by intramuscular injection of a solution of ovalbumin containing *Bordetella pertussis* organisms as previously described (Foreman & Mongar, 1972). The cell suspensions obtained from several rats were pooled and divided by volume into equal aliquots. The aliquots were centrifuged at 50 *g* for 10 min and the supernatants discarded. The cell pellets were resuspended in 900 μl of modified Tyrode solution at pH 7.6 to which calcium chloride had been added from a 1 M stock solution as required. The cell suspensions were brought to 37°C by incubation in a waterbath for 10 min. Additions, usually in a volume of 100 μl, appropriate to the experiment were then made to initiate histamine secretion and the incubation was allowed to proceed for a further 10 min at 37°C. To terminate the reaction, 3 ml of ice-cold Tyrode solution was added to each tube and the cells were separated by centrifugation at 1000 *g* for 5 min. The supernatants were retained for the assay of histamine. Cell pellets were resuspended in Tyrode solution and heated to 100°C for 3 min to release residual cellular histamine.

In experiments where purified, isolated mast cells were used, the mast cells were separated from the other peritoneal cells by density gradient centrifugation on human serum albumin as previously described (Foreman, Hallet & Mongar, 1977). This yielded a population of cells containing more than 80% mast cells with a viability greater than 95% as assessed by trypan blue exclusion.

The Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, NaH_2PO_4 0.4, glucose 5.6, HEPES (4 - (2 - hydroxyethyl) - 1 - piperazine ethane sulphonic acid) 20 mM.

Histamine was assayed fluorometrically by condensation in alkaline medium with *o*-phthalaldehyde and subsequent measurement of the fluorescent product was made at an excitation wavelength of 360 nm and emission at 420 nm. Histamine standards of 50, 100 and 200 ng/ml were routinely used for calibration of the assay. The total histamine content was calculated from the sum of the released and residual histamine levels and histamine release was expressed as a percentage of total. Histamine release occurring in the absence of any stimulus to the cells has been subtracted from evoked histamine release as previously described (Foreman & Mongar, 1972).

Flare and wheal in human skin

The protocol for these experiments received the approval of the Ethics Committee for Experiments at University College London. The experiments were performed in healthy adult volunteers of either sex aged 20 to 35 years. Subjects were not taking drugs at the time of or during the two weeks before the experiments.

Peptides were dissolved in sterile sodium chloride solution (154 mM) and injected at appropriate concentrations, intradermally into the skin on the volar surface of the forearm. A constant volume of injection of 25 μl was used in all experiments and injections were randomly allocated to sites on the forearm. No more than 4 injections were placed in one arm to avoid interaction between injections in adjacent sites. The same person gave all injections to ensure consistency in placing the injections within the skin layers.

The approximately circular wheal and flare reactions were measured in two directions at right angles and a mean diameter was calculated. Assuming the responses to be circular, an area was calculated for the wheal and flare reactions. Preliminary experiments established that the optimum time after injection for measuring the flare response was 3 min and for the wheal response 12 min.

Materials

Substance P, octapeptide (SP_{4-11}), neurotensin and physalaemin were obtained from Beckman, Geneva. Xenopsin was obtained from Peninsula Laboratories. [D-Arg^8]neurotensin and [D-Arg^9]neurotensin were generous gifts from Dr J.E. Rivier. [D-$Trp^{7,9}$]SP_{1-11} was a generous gift from Prof K. Folkers. Compound

48/80 was obtained from Sigma, U.K. A 23187 was obtained from Calbiochem and was prepared and used as previously described (Foreman, Mongar & Gomperts, 1973). Ovalbumin was obtained from B.D.H. All other chemicals were of Analar quality.

All peptides were initially dissolved in 0.1% acetic acid at a concentration of about 1 mM and stored at −25°C. Immediately before use, the stock solutions were thawed, neutralized with sodium hydroxide solution and then diluted in saline for the experiments. The small quantities of sodium acetate which resulted affected neither the skin response to histamine nor histamine release induced by ovalbumin from rat mast cells.

All tubes used for mast cell incubations and all syringes used for peptide injections were polypropylene.

Results

Histamine release by neurotensin

Figure 2 shows histamine release induced by ovalbumin from mast cells obtained from a rat previously sensitized to this antigen, as described in Methods. The histamine release is reduced when extracellular calcium is removed from the incubating medium. In the same cells it can be seen that substance P produced histamine release equivalent to that produced by antigen in the presence of calcium (about 40%) but substance P-induced histamine release does not require calcium, and is, in fact, inhibited by calcium (Fewtrell *et al.*, 1982). Figure 2 further shows that neither in the presence nor in the absence of extracellular calcium does neurotensin at concentrations up to 5 μM release more than about 5% of the total cell histamine in this same population of cells. In a series of 15 experiments, neurotensin, 5 μM produced 4.2 ± 0.8% (mean ± s.e.m.) histamine release in the absence of calcium. Calcium, 1 mM had no significant effect on this release of histamine.

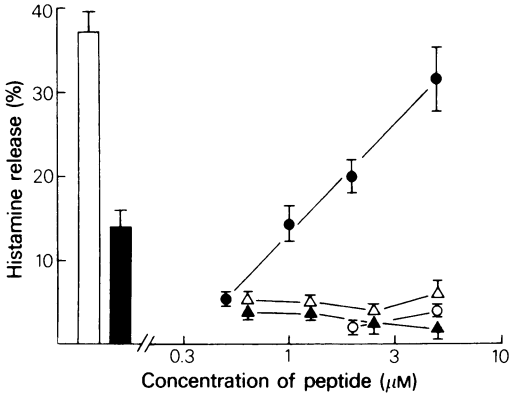

Figure 2 Histamine release from rat peritoneal cells induced by antigen (ovalbumin), substance P and neurotensin. Open column represents histamine release induced by ovalbumin 1 μg/ml in the presence of calcium 1 mM. Filled column represents the release obtained in the presence of the same concentration of ovalbumin but in the absence of added calcium. (●) Histamine release by substance P in the absence of extracellular calcium; (○) histamine release by substance P in the presence of calcium, 1 mM; (▲) histamine release by neurotensin in the absence of calcium; (△) histamine release by neurotensin in the presence of calcium 1 mM. Vertical bars indicate s.e.mean; $n = 4$.

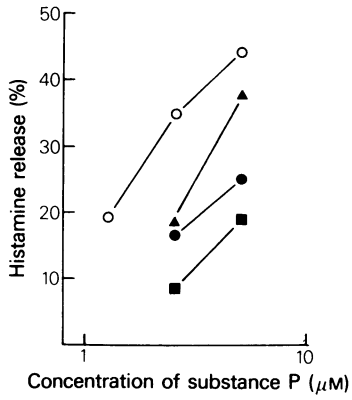

Figure 3 The effect of neurotensin on histamine release induced by substance P. (○) Concentration-response relationship for substance P alone; (▲) substance P in the presence of neurotensin, 2.5 μM; (●) substance P in the presence of neurotensin, 5 μm; (■) substance P in the presence of neurotensin, 10 μM. Neurotensin was added to the cells at 37°C 10 min before the addition of substance P. Points represent the means of duplicate determinations in a single experiment.

Neurotensin-substance P interaction

Neurotensin, 2.5 to 10 μM, produced graded inhibition of the histamine release induced by substance P. Figure 3 shows that increasing concentrations of neurotensin produced a shift of the substance P dose-response curve to the right in a concentration-dependent manner. Dose-ratios for substance P in the presence of various concentrations of neurotensin were calculated from a series of experiments such as the one shown in Figure 3 and these are given in Table 1. It was not possible to construct complete dose-response curves for substance P in the presence of neurotensin because of the very large amounts of peptide which would have been required. An average value for the dose-ratio was calculated over the range of substance P doses giving overlapping histamine release responses in the presence and absence of neurotensin. In some cases, the dose-ratio could be

Table 1 Antagonistic action of a variety of peptides on the histamine release induced by substance P

Peptide	*Concentration of peptide* (μM)		
	2.5	5.0	10.0
Neurotensin (NT_{1-13})	2.1 ± 0.5 (4)	3.2 ± 0.4 (6)	4.6 ± 0.5 (7)
SP_{4-11}	1.5 ± 0.1 (3)	2.4 ± 0.3 (7)	
Physalaemin		1.4 ± 0.2 (8)	
Xenopsin		1.9 ± 1.4 (4)	
[D-Arg^8]NT_{1-13}			4.8 ± 0.2 (3)
[D-Arg^9]NT_{1-13}			5.1 ± 0.9 (3)

The values given are the average dose-ratios produced by the given concentration of the antagonist peptide. Number of experiments is given in parentheses.

measured only at a single response level. A Schild plot of the data for neurotensin in Table 1 gives a straight line with a slope of 0.8 (95% confidence limits ± 0.6) and an intercept on the abscissa of 1.8 μM with 95% confidence limits of 1.4 μM to 6.8 μM.

Neurotensin inhibited histamine release induced by substance P from purified mast cells over the same concentration range as that which produced inhibition of the response to substance P in mixed peritoneal cells.

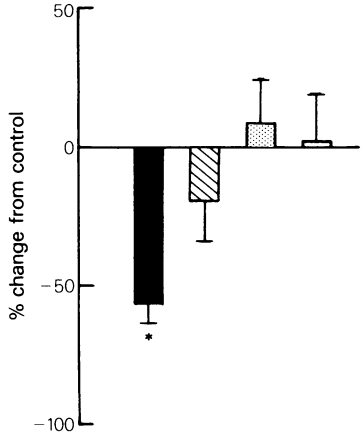

Figure 4 The effect of neurotensin on histamine release induced by substance P, 5 μM (solid column); compound 48/80, 600 ng/ml (hatched column); ovalbumin, 1 μg/ml (stippled column); ionophore A 23187, 600 nM (open column). Inhibition or enhancement of histamine release caused by the addition of neurotensin, 5 μM is expressed in terms of a percentage of the control release obtained with a given releasing agent. Control release for each agent was as follows: substance P 20.0%; compound 48/80 32.4%; ovalbumin, 26.8%; A 23187 56.7%. Experiments with substance P and compound 48/80 were conducted in the absence of extracellular calcium. Calcium, 1 mM was present in the extracellular medium for experiments with ovalbumin and A 23187. The results are mean values obtained from 7 experiments. The value marked * is significantly different from zero ($P < 0.01$). Vertical bars indicate s.e. means.

Selectivity of the action of neurotensin

Neurotensin, 5 μM was tested for its ability to inhibit histamine release induced by substance P, compound 48/80, ovalbumin and the ionophore A23187. It can be seen from Figure 4 that histamine release induced by ovalbumin and A23187 was not inhibited by neurotensin. Neurotensin produced a significant ($P < 0.01$) inhibition of the response to substance P and appeared to inhibit the response to compound 48/80 as well, but this latter effect was not statistically significant ($P > 0.05$).

An attempt to investigate further a possible interaction between compound 48/80-induced histamine release and substance P-induced histamine release is shown in Table 2. Both compound 48/80 and substance P induce histamine release, but it is clear that treatment of mast cells with substance P together with compound 48/80 did not produce additive levels of histamine release. This was true for a range of doses of compound 48/80 producing different levels of histamine release. In Table 2 the histamine release due to substance P alone has also been substracted from that due to substance P plus the particular concentration of compound 48/80, the result being an apparent inhibition by substance P of the histamine releasing action of compound 48/80.

Effects of related peptides

Lazarus *et al.* (1977b), examined the binding to mast cells of a number of peptides structurally related to neurotensin. We have now studied the action of some of these on histamine release. Table 1 shows the dose-ratios for the inhibition of substance P-induced histamine release produced by several peptides related to neurotensin and substance P (for structures see Figure 1). [D-Arg^9] neurotensin, [D-Arg^8] neurotensin and neurotensin itself were equiactive as inhibitors of substance P-induced histamine release. Physalaemin did not inhibit substance P-induced histamine release and the peptides SP_{4-11} and xenopsin had activities less than that of neurotensin. Physalaemin, xenopsin and SP_{4-11} produced no sig-

Table 2 The effect of substance P (SP) on the histamine release induced by compound 48/80 in calcium-free Tyrode solution

Concentration of compound 48/80 (μg/ml)	48/80 alone	*Histamine release (%)* 48/80 + SP 2.5 μM	48/80 + SP 5 μM
0.2	30.2 ± 4.1	26.3 ± 4.7 (10.1)*	29.1 ± 4.2 (4.9)
0.4	40.3 ± 1.1	30.7 ± 3.5 (14.5)	34.8 ± 3.4 (10.6)
0.8	47.6 ± 1.2	36.3 ± 2.4 (20.1)	35.7 ± 3.4 (11.5)
1.6	50.0 ± 3.0	39.1 ± 3.3 (22.9)	45.0 ± 4.0 (20.8)

Substance P, 2.5 or 5 μM was added to the cells and incubation continued for 10 min before the addition of compound 48/80 at the concentration indicated. The reaction was stopped and histamine release measured after a further 10 min incubation at 37°C.

*Figures in parentheses are the histamine releases remaining when the amount of histamine release caused by substance P alone has been subtracted from the total histamine release due to compound 48/80 and substance P. Substance P, 2.5 μM released 16.2 ± 3.2% of total histamine and substance P, 5 μM released 24.2 ± 3.6%. values given are the means and standard errors from three separate experiments.

nificant histamine release by themselves at the concentrations tested. The D-substituted neurotensins at the Arg^8 and Arg^9 positions had minimal histamine releasing action by themselves, which was comparable with that of neurotensin itself.

In view of the findings that neurotensin and its related peptides and also SP_{4-11} are antagonists of the action of substance P, it was of interest to study the action of another putative substance P antagonist. We have previously reported that the putative antagonist of substance P: [D-Pro^2, D-Phe^7, D-Trp^9]SP_{1-11} (Folkers, Hörig, Rosell & Björkroth, 1981; Rosell, Olgart, Gazelius, Panapoulos, Folkers & Hörig, 1981) is more potent than substance P itself as a releaser of histamine from rat mast cells (Fewtrell *et al.*, 1982). The analogue, [D-$Trp^{7,9}$]SP_{1-11} also

Table 3 Action of neurotensin on flare and wheal responses in human skin

Injection	*Experiment*	*Flare area* (mm^2)	*Wheal area* (mm^2)
SP 12.5 pmol		625 ± 109	50 ± 3
SP 50 pmol		931 ± 159	131 ± 29
NT 125 pmol		355 ± 89	47 ± 2
SP 12.5 pmol + NT 125 pmol	A	455 ± 88	62 ± 5
SP 50 pmol + NT 125 pmol	$n = 3$	677 ± 38	79 ± 4
*SP 12.5 pmol + NT 125 pmol		100	15
*SP 50 pmol + NT 125 pmol		322	32
SP 12.5 pmol		71	36
SP 25 pmol		233	57
NT 125 pmol		0	26
SP 12.5 pmol + NT 125 pmol	B	2	39
SP 25 pmol + NT 125 pmol	$n = 2$	100	48
*SP 12.5 pmol + NT 125 pmol		2	13
*SP 25 pmol + NT 125 pmol		100	22

Two separate experiments are shown (A + B). The values given are mean responses with s.e.mean when appropriate. The values in lines marked* represent the responses to the combined substance (SP) and neurotensin (NT) injection with the NT response *subtracted.*

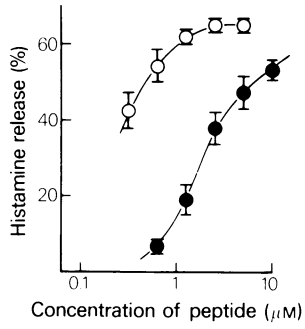

Figure 5 Concentration-response relationships for substance P (●) and [D-Trp^{7,9}]SP$_{1-11}$ (○) as histamine releasing agents in rat peritoneal cells. Vertical bars indicate s.e.means; $n = 4$.

exhibits some antagonist action against substance P in the guinea-pig ileum *in vitro* (W. Piotrowski, unpublished observations), but Figure 5 shows that it, too, was more potent than substance P as a histamine releasing agent by a factor of about twelve fold.

Wheal and flare in human skin

The actions of neurotensin on wheal and flare responses to substance P in human skin are given in Table 3. Neurotensin itself produced both wheal and flare, although it was more than ten times less potent than substance P in producing these effects. Table 3 further shows that the addition of neurotensin to substance P produced responses which were clearly less than additive. If the response to neurotensin itself is subtracted from the response to the combination of substance P and neurotensin, then neurotensin is seen to have produced a marked inhibition of the flare and wheal responses to substance P; the flare response to substance P being inhibited by neurotensin to a greater extent than the wheal response.

Figure 6 shows the wheal and flare responses to intradermal injection of [D-Trp^{7,9}]SP$_{1-11}$. There was little difference between this substituted analogue and substance P itself in terms of ability to produce a wheal response, but the analogue was about 1.8 times more effective than substance P in producing a flare response.

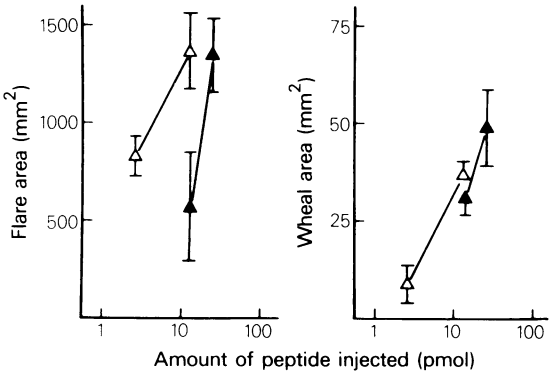

Figure 6 Flare and wheal responses in human skin generated by the intradermal injection of either substance P (▲) or [D-Trp^{7,9}]SP$_{1-11}$ (△) at the doses indicated. The mean responses from ten separate experiments on different subjects are shown. Vertical bars indicate s.e.means.

Discussion

Neurotensin has previously been reported to release histamine from mast cells at several sites (Selbekk *et al.*, 1980; Kurose & Saeki, 1981; Kruger *et al.*, 1982). The results presented in this paper confirm the observation that neurotensin produces some histamine release from rat peritoneal mast cells, but the maximum release which can be achieved with this peptide is a small fraction of that which can be obtained with substance P, compound 48/80 or with an antigen-antibody stimulus. Histamine release induced by neurotensin was found to be insensitive to extracellular calcium, 1 mM.

In contrast to its action in producing trivial levels of histamine release from rat mast cells, neurotensin is capable of inhibiting histamine release induced by substance P. The inhibition by neurotensin of the response to substance P could be reversed by increasing the substance P concentration. Assuming that substance P and neurotensin interact competitively for a common receptor mediating histamine release (neurotensin being a weak partial agonist and substance P a full agonist), Schild analysis of the shifts in the substance P dose-response curves produced by neurotensin yielded a value for the neurotensin-receptor dissociation constant of 1.8 μM. The dissociation constant for neurotensin obtained from binding studies in mixed samples of peritoneal and pleural mast cells (Lazarus *et al.*, 1977a) was found to be 0.15 μM. The discrepancy between the two values for the dissociation constant might be the result of the different origins of the mast cells. The histamine release studies were carried out on peritoneal cells, whereas the binding studies were performed on pleural and peritoneal cells mixed. Neurotensin appears to have greater agonist activity on pleural cells (Kruger *et al.*, 1982).

Inhibition of antigen- and ionophore-induced histamine release by neurotensin was not observed, but neurotensin appeared to produce some inhibition of the histamine release induced by compound 48/80 as

well as that induced by substance P. Histamine release in response to a combination of compound 48/80 and substance P was less than the sum of the histamine releases induced by these two agents acting separately. Doses of substance P which produced relatively low levels of histamine release appeared to inhibit the histamine release induced by compound 48/80. Substance P, neurotensin and compound 48/80 are all basic molecules possessing a positive charge at physiological pH and it is possible that they all interact at a common site on the mast cell, which is involved in the mediation of histamine secretion. In keeping with this possibility, Quirion, Rioux & St. Pierre (1980) found that neurotensin and compound 48/80 appeared to interact in the production of hypotension in the rat. However, Mazurek, Pecht, Teichberg & Blumberg (1981) reported that, with some inhibitors of histamine release, they were able to distinguish between release induced by compound 48/80 and that induced by substance P.

In addition to neurotensin, the peptides xenopsin, SP_{4-11}, [D-Arg^8]NT_{1-13} and [D-Arg^9]NT_{1-13} were all found to inhibit the histamine release produced by substance P. Physalaemin did not produce significant inhibition of the substance P-induced release of histamine. In this study, no difference in activity was detected between [D-Arg^9]NT_{1-13}, [D-Arg^8]NT_{1-13} and NT_{1-13} itself, but SP_{4-11} and xenopsin were less active than neurotensin. It is interesting to compare these findings with those from binding studies. In the binding studies of Lazarus *et al.* (1977b) the relative affinities of these peptides (NT = 100%) for the binding site were: [D-Arg^9]NT_{1-13} 640 : [D-Arg^8]NT_{1-13} 500 : NT_{1-13} 100 : xenopsin 60: physalaemin 0.25. Neurotensin, then, appears to be a partial agonist at a receptor site on rat mast cells which mediates histamine release. The action of the agonist substance P at this receptor is prevented by a number of related peptides whose activities as inhibitors of the action of substance P are similar to their affinities of binding to the receptor. It is interesting that the C-terminal octapeptide of substance P (SP_{4-11}) has lost the agonist activity of the undecapeptide (Fewtrell *et al.*, 1982) and, instead, exhibits antagonist activity towards substance P in this system. In contrast, the peptide [D-$Trp^{7,9}$]SP_{1-11} which is an antagonist of substance P in some smooth muscle preparations (W. Piotrowski, unpublished observations), is a more potent agonist than substance P as an activator of histamine release from mast cells. It has, in fact, already been pointed out that substance P mediates histamine release through an action at a receptor which is different from that which mediates, for example, contraction of the guinea-pig ileum or salivary secretion from the parotid gland (Foreman & Jordan, 1981).

The activities of substance P-related peptides as histamine releasers in rat mast cells is correlated with their activity in producing flare responses in human skin (Foreman & Jordan, 1981) and there is evidence that the flare response to intradermal injection of substance P is mediated by histamine release from skin mast cells (Hägermark *et al.*, 1978; Foreman & Jordan, 1981). We have shown in this study that neurotensin produces flare, and also wheal responses, when injected intradermally into human skin, but it is less potent than substance P in these respects. Furthermore, neurotensin antagonizes the action of substance P in human skin and this, we believe, reflects an antagonistic action of neurotensin at the substance P receptor on skin mast cells, preventing histamine release from skin mast cells and hence the contribution of this histamine to the flare and wheal induced by substance P.

A peptide analogue of substance P, [D-Pro^2, D-$Trp^{7,9}$]SP_{1-11} has been reported to have substance P antagonist activity in the central nervous system and in smooth muscle (Engberg, Svensson, Rosell & Folkers, 1981; Leander, Håkanson, Rosell, Folkers, Sundler & Tornqvist, 1981). The closely related peptide [D-$Trp^{7,9}$]SP_{1-11}, which was found to be about twelve times more potent than substance P as a histamine releasing agent, was about twice as active as substance P in the production of flare. The reason for this difference in potency in the last two systems is not known. In the production of wheal response, SP and [D-$Trp^{7,9}$]SP_{1-11} were found to be equiactive. It has already been pointed out (Foreman & Jordan, 1981) that the wheal response to substance P and some related peptides is largely a result of a direct effect of the peptide on vascular permeability and that histamine release seems to contribute less to the wheal than to the flare. Thus, the equal activities of [D-$Trp^{7,9}$]SP_{1-11} and substance P in producing wheals could reflect equivalence in the ability of these two peptides to activate the receptor mediating increased vascular permeability. However, this seems unlikely in view of the minimal agonist activity of [D-$Trp^{7,9}$]SP_{1-11} on intestinal smooth muscle (W. Piotrowski, unpublished observations). An alternative possibility is that the contribution of histamine release to the wheal response varies according to the particular peptide used.

Neurotensin does not appear to be located in the peripheral endings of the primary afferent neurones where substance P and somatostatin have been identified (Hökfelt, Elde, Johansson, Luft, Nilsson & Arimura, 1976) and there appear to be no reports of the occurrence of neurotensin in human skin. It is noteworthy that neurotensin inhibits gastric acid secretion (Andersson, Rosell, Sjödin, Folkers, 1980) and it may be worth considering whether it does so by preventing histamine release in the stomach in response to the action of an endogenous peptide on

histamine-containing cells. It should be noted however, that Andersson *et al.* (1980) concluded from their experiments that a presynaptic action of neurotensin on the vagus could explain its inhibitory effect on gastric secretion. The significance of the findings described in this paper are, we believe, less related to a possible physiological role for neurotensin in controlling mast cell function, but more to its pharmacological potential as an antagonist of the peripheral actions of substance P. Substance P is believed to mediate neurogenic inflammation (Jancsó, Jancsó-Gábor & Szolcsányi, 1967; Lembeck & Holzer, 1979; Gamse, Holzer & Lembeck, 1980) and so an antagonist of the peripheral actions of substance P would be a useful tool for investigating this phenomenon.

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