ELSEVIER

European Journal of Pharmacology 414 (2001) 295–303

ejp

www.elsevier.nl/locate/ejphar

Immunologically induced histamine release from rat peritoneal mast cells is enhanced by low levels of substance P

Alaster H.Y. Lau *, Sharron S.M. Chow, Yeung S. Ng

Department of Pharmacology, Faculty of Medicine, Basic Medical Sciences Building, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong S.A.R., People's Republic of China

Received 20 November 2000; received in revised form 15 January 2001; accepted 26 January 2001

Abstract

Although direct activation of mast cells by high concentrations ($>10^{-6}$ M) of substance P is well established, the effect of sub-micromolar concentrations of the neuropeptide on mast cell activation has not been reported. We hence investigated if substance P would modulate immunologic activation of mast cells by studying the effect of the neuropeptide on anti-rat immunoglobulin E antibody (anti-IgE)-induced histamine release from purified rat peritoneal mast cells. We observed that substance P could dose-dependently potentiate anti-IgE-induced histamine release from rat peritoneal mast cells at concentrations (3×10^{-9} M to 3×10^{-7} M) which alone induced insignificant or low level of histamine release. While the potentiating effect of substance P was not suppressed by any of the non-peptide tachykinin receptor antagonists CP99994 ((2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine), SR48968 ((*S*)-*N*-methyl-*N*-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl-benzamide) and SR142801 ((*S*)-(*N*)-(1-[3-{1-benzoyl-3(3,4-dichlorophenyl)piperidine-3-yl)propyl]-4-phenylpiperidin-4-yl)-*N*-methyl-acetamide), it was mimicked by compound 48/80 and suppressed by benzalkonium chloride. Hence, substance P enhanced anti-IgE-induced histamine release through a similar receptor-independent mechanism as the direct mast cell activating action of polybasic compounds. Since high concentrations of substance P required for directly activating mast cells may not be achievable physiologically, the enhancing actions of the neuropeptide on the immunologic activation of mast cells may be more clinically relevant in the pathogenesis of various inflammatory conditions.

Keywords: Substance P; Tachykinin receptor antagonist; Anti-IgE; Mast cell; Histamine release; Benzalkonium chloride

1. Introduction

Injury or chemical stimulation of the sensory nerve endings sends impulses to the spinal cord and at the same time antidromically back towards nerve fibres of the lateral branches of the nerve through axon reflex, leading to the release of neuropeptides from these fibres (Barnes et al., 1990; Pernow, 1983a). The released neuropeptides then cause vasodilatation and increased vascular permeability resulting in a condition known as neurogenic inflammation at and around the site of stimulation (Pernow, 1983b). Among the various neuropeptides, substance P, an 11-amino acid peptide localised in peripheral sensory C-fibres, is believed to be the major mediator of neurogenic inflammation (Lembeck and Holzer, 1979). In addition to the direct effects of substance P on the microvasculature, degranulation of mast cells has also been demonstrated to contribute towards the symptoms of neurogenic inflammation (Kiernan, 1990a). Degranulation of mast cells releases inflammatory mediators such as histamine, prostaglandins and various enzymes including kinin activating tryptase and, hence, have always been associated with the pathogenesis of inflammatory and allergic diseases (Wasserman, 1983). Morphological studies provided visual evidence which confirms the close anatomical association between mast cells and nerve fibres, especially those containing substance P (Bienenstock et al., 1991; Stead et al., 1987). Mast cell degranulation has also been documented in various in vivo models of neurogenic inflammation in the skin (Fantini et al., 1995), airways (Kiernan, 1990b) and gut (Stead et al., 1987).

Although evidence supporting the participation of mast cells in substance P-induced neurogenic inflammation is abundant, the exact mechanisms for mast cell activation by substance P at various neurogenic inflammatory sites still

* Corresponding author. Tel.: +852-2609-6846; fax: +852-2603-5139.
E-mail address: hyalau@cuhk.edu.hk (A.H.Y. Lau).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00805-6

remain unresolved. The original hypothesis is the direct activation of mast cells by substance P. In fact, it has long been known that substance P can induce histamine release from mast cells of the rat peritoneal cavity (Fewtrell et al., 1982) and small intestine mucosa as well as human skin (Tainsh and Pearce, 1992) and bladder (Frenz et al., 1994). The direct activation of mast cells by substance P has been shown to be mediated by a common receptor-independent mechanism proposed for the actions of various polybasic mast cell secretagogues such as compound 48/80 (Mousli et al., 1990a). However, the concentration of substance P required is relatively high compared to the concentrations required for their vascular actions (Amano et al., 1997; Fewtrell et al., 1982). Moreover, mast cells derived from human lung, adenoids, tonsils, intestinal mucosa and intestinal muscle all failed to respond to substance P (Lowman et al., 1988). The different responses of mast cells from different species and anatomical locations have been addressed by the now widely accepted concept of mast cell heterogeneity (Pearce, 1986; Tainsh et al., 1992). Since not all mast cells can be directly activated by substance P, the interaction between them in neurogenic inflammation is more complicated than the original hypothesis.

Instead of directly activating mast cells, substance P may increase the sensitivity of these cells to external stimuli. During inflammation, mast cells may interact with various exogenous and endogenous substances simultaneously. Although substance P at concentrations detected during neurogenic inflammation is insufficient to directly activate mast cells, the sensitivity of mast cells to other stimuli may be enhanced. The most common pathophysiological activation of mast cells is the cross linking of cell surface IgE molecules by antigens. Substance P may increase the sensitivity of tissue mast cells to sub-threshold levels of antigens. The subsequent mediator release then facilitates the symptoms of neurogenic inflammation. The literature so far has concentrated mainly on the mechanisms of direct activation of mast cells by substance P whereas the possible potentiating activity of substance P on mast cells has not been investigated. The present paper hence reports our study on the possibility of a substance P enhanced mast cell sensitivity. In addition, the effects of substance P on mast cells were characterised with non-peptide tachykinin antagonists.

2. Materials and methods

2.1. Preparation and protocols

2.1.1. Sensitization of rats with ovalbumin

Male Sprague–Dawley rats (200–250 g) obtained from the Joint Animal House, Faculty of Medicine, Chinese University of Hong Kong, were sensitised by a single intraperitoneal injection of 0.5 ml of an emulsion containing ovalbumin (60 mg/ml), aluminium hydroxide (240 mg/ml) and pertussis vaccine (1.33 IU/ml) in 0.01 M phosphate buffered saline. The animals were used 3 to 6 weeks after sensitization.

2.1.2. Isolation and purification of rat peritoneal mast cells

Sensitized rats were killed by decapitation and exsanguination. Rat peritoneal mast cells were isolated and purified over Percoll density gradient as previously described (Chan et al., 2000). Briefly, mixed peritoneal cells (containing 4–5% mast cells) were obtained by peritoneal lavage following the intraperitoneal injection of HEPES buffer (137 mM NaCl. 5.6 mM glucose, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid), 2.7 mM KCl, 0.4 mM NaH_2PO_4 and 1 mM $CaCl_2$, pH 7.4) supplemented with 50 IU/ml of heparin. The mixed peritoneal cells were then washed twice by centrifugation ($180 \times g$, 5 min, 4°C) and were resuspended in 1 ml of HEPES buffer supplemented with 1 mg/ml of bovine serum albumin. The cell suspension was then mixed with 4 ml of 90% Percoll and 1 ml of bovine serum albumin supplemented HEPES buffer was then carefully layered over the Percoll–cell mixture. Purification was then performed by centrifugation ($150 \times g$, 25 min, 4°C) which allowed cell separation and gradient formation to perform simultaneously. Harvesting of the mast cells posed no problem since these cells gathered in a layer at the bottom of the tube whereas other cells formed a rather compact layer on top of the gradient and could easily be removed by aspiration. The cell fraction was then washed twice in HEPES buffer by centrifugation and finally resuspended to the desired cell density in HEPES buffer prewarmed at 37°C.

2.1.3. Experimental procedures

Mast cells pooled from three rats were resuspended to a cell density of 10^5 cells/ml and were equilibrated at 37°C for 10 min with HEPES buffer. Two hundred microliters of the equilibrated cells was then further incubated at 37°C for 10 min with 200 μl of the appropriate secretagogue or secretagogue combination (or buffer for spontaneous release). When the effect of substance P preincubation was studied, cells were first incubated with the neuropeptide for 20 min before the addition of an anti-rat immunoglobulin E antibody (anti-IgE). When the effects of pharmacological agents were studied, cells were either activated simultaneously with anti-IgE and/or substance P in the presence of the agent (benzalkonium chloride or a tachykinin receptor antagonist) or after a 10-min preincubation with the agent in the case of tachykinin receptor antagonists. Histamine release from activated cells was then stopped 10 min later with the addition of 1 ml of ice cold HEPES buffer. Cells and supernatants were separated by centrifugation ($180 \times g$, 5 min, 4°C). The cell pellets

were resuspended in 1.4 ml of HEPES buffer and were boiled for 10 min to liberate the residual histamine. Histamine content in both supernatants and cell pellets were determined spectrofluorimetrically without extraction. All tubes were centrifuged ($2000 \times g$, 20 min, 4°C) prior to fluorescence detection.

2.2. Materials

Substance P, compound 48/80, benzalkonium chloride, bovine serum albumin, HEPES, Percoll and ovalbumin were purchased from Sigma (St. Louis, MO, USA); sheep anti-rat IgE from ICN Biomedicals (Costa Mesa, CA, USA); Pertussis toxin from Pasteur Merieux SV (France). CP99994 ((2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine) is a gift from Pfizer Central Research (Groton, USA) whereas SR48968 ((*S*)-*N*-methyl-*N*-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl benzamide) and SR142801 ((*S*)-(*N*)-(1-[3-{1-benzoyl-3(3,4-dichlorophenyl)piperidine-3-yl)propyl]-4-phenylpiperidin-4-yl)-*N*-methyl-acetamide) are free gifts from Sanofi Recherche (Montpellier, France). All other chemicals were of analytical grade.

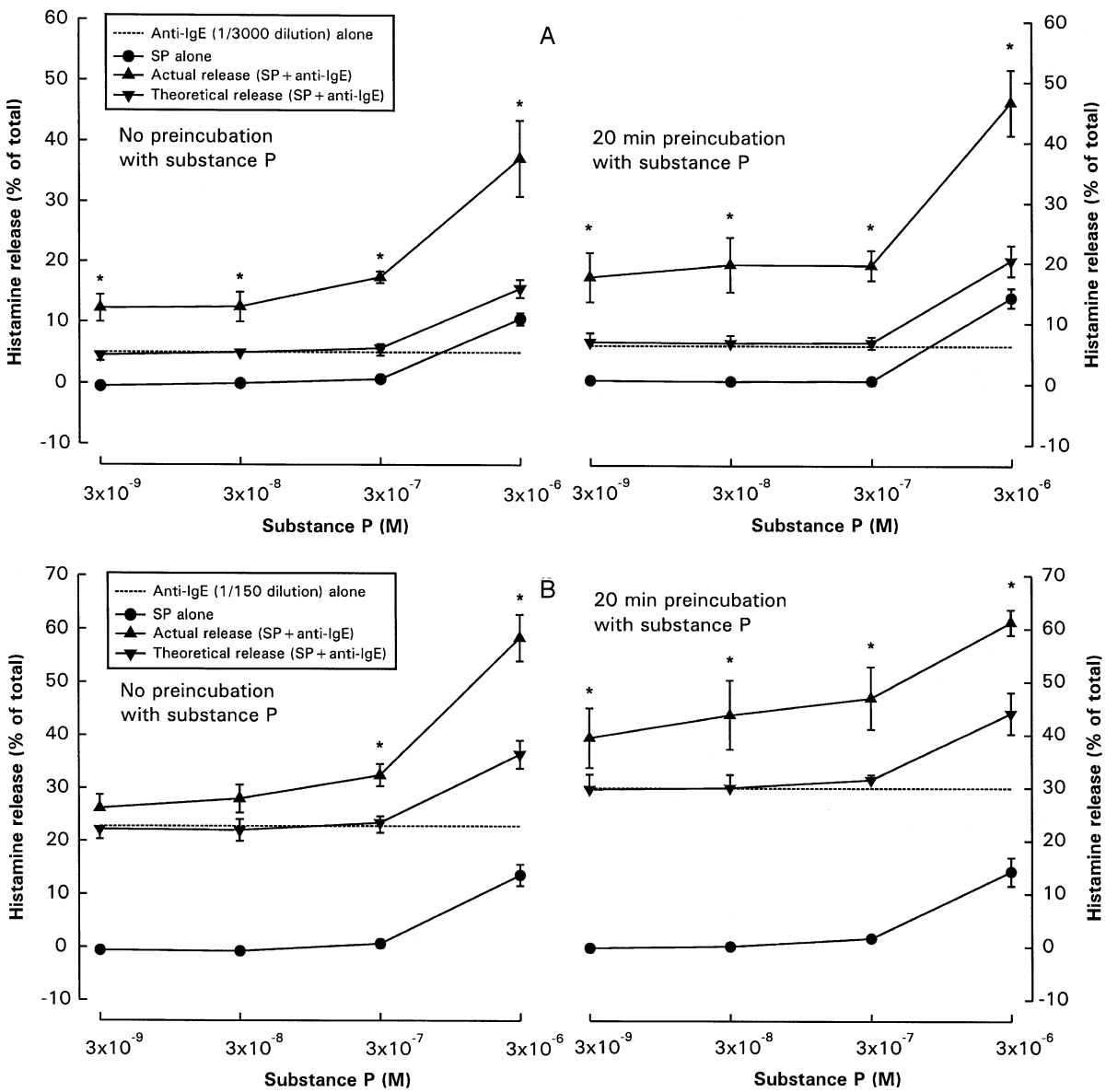

Fig. 1. Effects of substance P (SP) on histamine release from mast cells (A) marginally activated by 1/3000 and (B) fully activated by 1/150 dilution of anti-IgE. All results were corrected for spontaneous histamine release of 8.9 ± 0.5% (no preincubation with SP) and 11.7 ± 0.3% (20 min preincubation with SP) in buffer alone. 5.0 ± 0.5% and 22.8 ± 1.8% of histamine release were, respectively, induced by 1/3000 and 1/150 dilution of anti-IgE in the no-preincubation experiments. The corresponding values for the 20-min preincubation experiments were 6.2 ± 1.3% and 29.9 ± 2.6%. Data are shown as means ± S.E.M. for $n = 5$. * Indicates $P < 0.05$ compared with the theoretical histamine release calculated by adding the histamine release induced independently by anti-IgE and substance P.

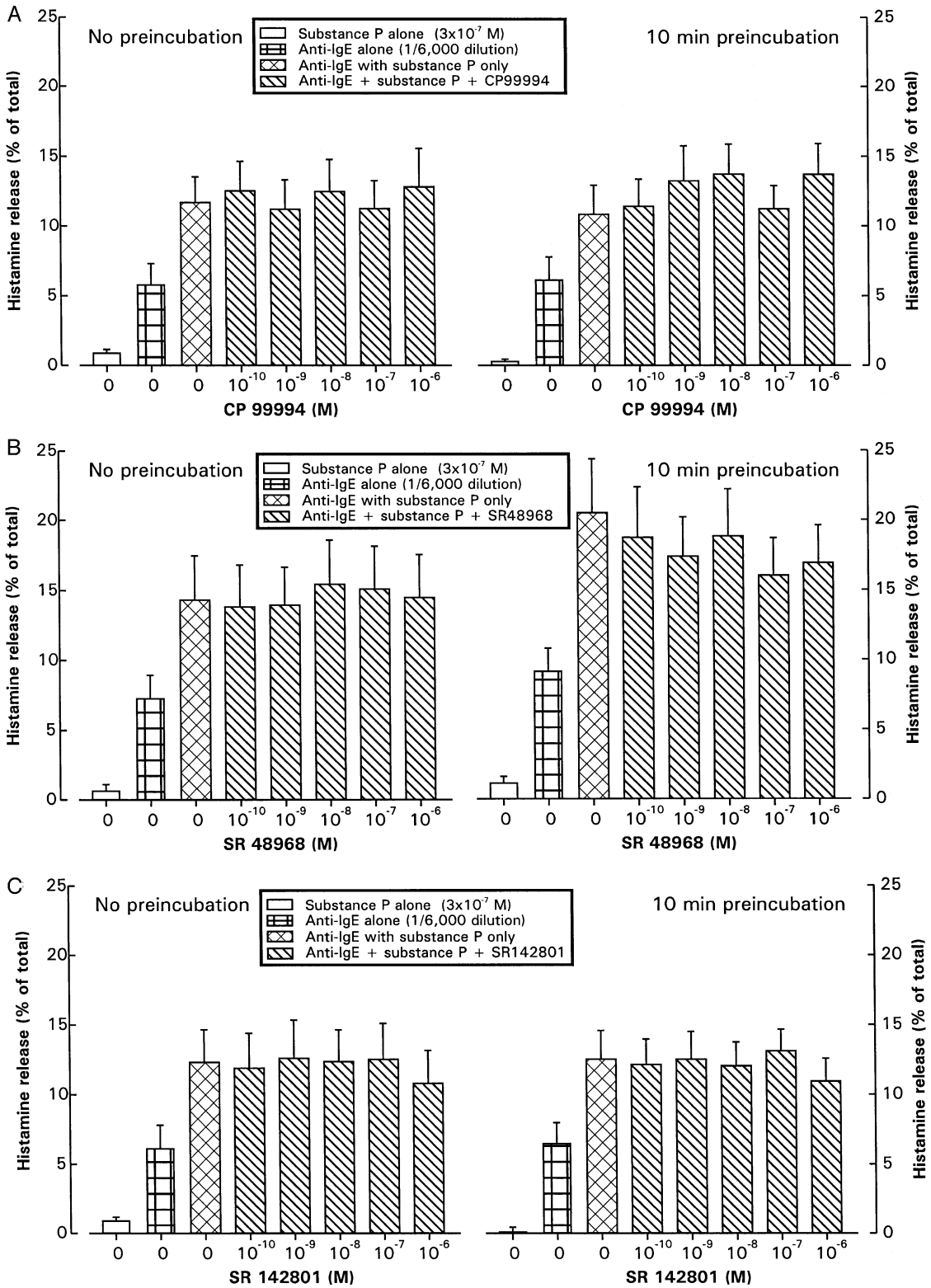

Fig. 2. Effects of the non-peptide neurokinin receptor antagonists (A) CP99994, (B) SR48968 and (C) SR142801 on the potentiating effect of substance P on anti-IgE-induced histamine release. Mast cells were activated with anti-IgE and substance P at the time of antagonist addition (no preincubation) or after 10-min incubation with the tachykinin receptor antagonist. All results were corrected for spontaneous histamine release of 8.2 ± 1.6% (no preincubation with antagonist) and 8.8 ± 1.8% (10-min preincubation with antagonist) in buffer. Data are shown as means ± S.E.M. for $n = 5$.

2.3. Data presentation

The results are expressed as a percentage of the total histamine content of the cells which had been released into the supernatant:

$$\text{Histamine release}\,(\%) = \{S/(S+C)\} \times 100\%$$

where S = amount of histamine released into the supernatant, C = amount of histamine remaining in the cell pellet. All results were adjusted for the spontaneous histamine release in buffer only and were presented as mean $\pm$ standard error of the mean (S.E.M.) for n experiments. Where appropriate, data were compared using paired Student's t-test and the null hypothesis was rejected when $P < 0.05$.

3. Results

3.1. Effects of substance P on anti-IgE-induced histamine release from rat peritoneal mast cells

For investigating the effects of substance P on histamine release induced by other secretagogues, concentrations which produced less than 30% histamine release were used (i.e. 3×10^{-9} M to 3×10^{-6} M). The effect of substance P on marginally activated mast cells was first investigated. Following a 10-min incubation with 1/3000 dilution of anti-IgE, only $5.0 \pm 0.5\%$ of total cellular histamine was released from rat peritoneal mast cells (Fig. 1A). However, when substance P at the concentrations of 3×10^{-8} M, 3×10^{-7} M or 3×10^{-6} M was added together with anti-IgE to the cells, the observed histamine release was greater than the theoretical sum of histamine release calculated by adding the values of histamine release induced independently by anti-IgE and substance P. The enhancement of anti-IgE-induced histamine release by substance P is dose dependent. Although both 3×10^{-8} M and 3×10^{-7} M substance P induced less than 5% release of total cellular histamine, 3×10^{-7} M of substance P produced a more significant potentiation. The effect of substance P on fully activated mast cells was also investigated by stimulating the cells with 1/150 dilution of anti-IgE which induced $22.8 \pm 1.8\%$ of histamine release. Significant potentiation was observed only at 3×10^{-7} M and 3×10^{-6} M (Fig. 1B). However, enhancement of anti-IgE-induced histamine release by 3×10^{-8} and 3×10^{-9} M of substance P was significantly improved by preincubating the cells with substance P for 20 min (Fig. 1) while histamine release induced by substance P alone was not significantly increased following extended incubation with the neuropeptide.

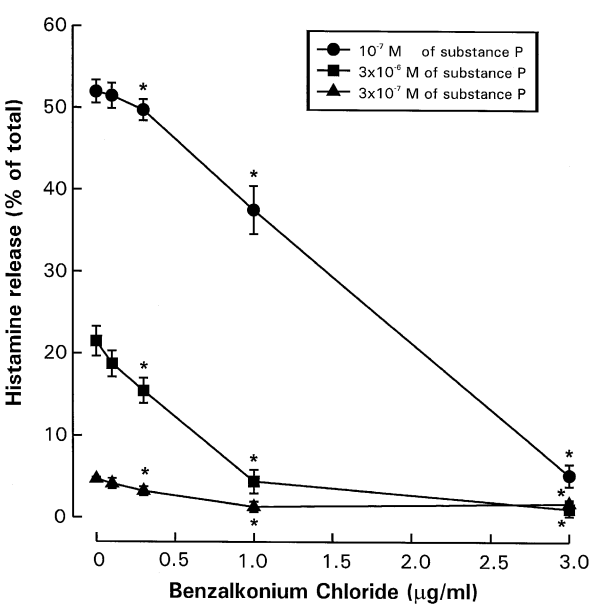

Fig. 3. Effect of benzalkonium chloride on histamine release induced by substance P. Cells were exposed to substance P and benzalkonium chloride simultaneously. All results were corrected for spontaneous histamine release of $9.5 \pm 0.6\%$ in buffer. Data are shown as means $\pm$ S.E.M. for $n = 6$. * Indicates $P < 0.05$ compared with the histamine release induced by substance P in the absence of benzalkonium chloride.

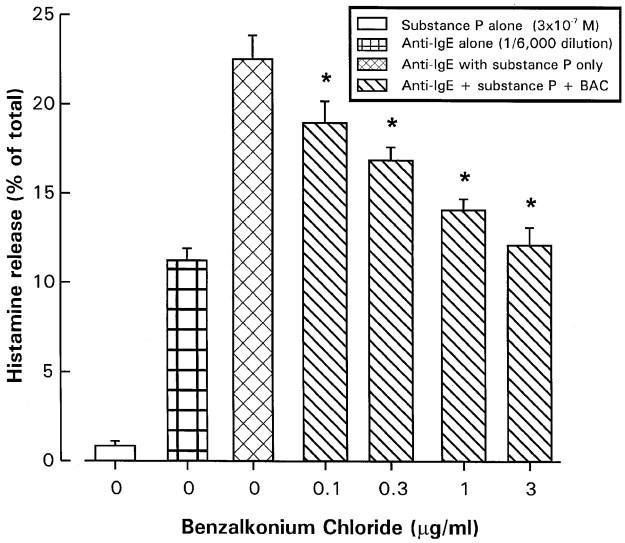

Fig. 4. Effect of benzalkonium chloride (BAC) on the potentiating effect of substance P on anti-IgE induced histamine release from rat peritoneal mast cells. Anti-IgE, substance P and benzalkonium chloride were added simultaneously to mast cells. All results were corrected for spontaneous histamine release of $9.1 \pm 0.6\%$ in buffer alone. Data are shown as means $\pm$ S.E.M. for $n = 6$. * Indicates $P < 0.05$ compared with the substance P enhanced anti-IgE-induced histamine release in the absence of benzalkonium chloride.

3.2. Effects of tachykinin antagonists on the potentiating effect of substance P on anti-IgE-induced histamine release from mast cells

In order to investigate if the enhancing effects of substance P on immunological activation of rat peritoneal

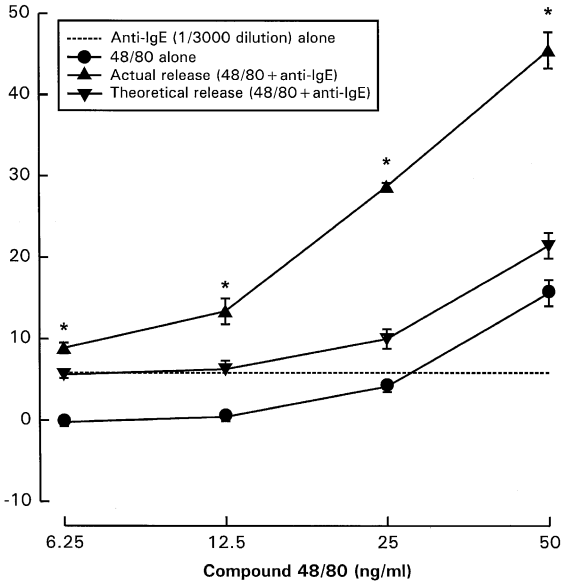

Fig. 5. Effect of compound 48/80 on anti-IgE-induced histamine release from rat peritoneal mast cells. Cells were activated simultaneously with compound 48/80 and anti-IgE. All results were corrected for spontaneous histamine release of 12.9 ± 3.5% in buffer alone. Data are shown as means ± S.E.M. for $n = 6$. * Indicates $P < 0.05$ compared with the theoretical histamine release calculated by adding the histamine release induced independently by anti-IgE and compound 48/80.

mast cells was mediated through the conventional tachykinin receptors, the effects of non-peptide antagonists of the three known tachykinin receptor subtypes were studied. CP99994, SR48968 and SR142801 have selectivity on the tachykinin NK_1, NK_2 and NK_3 receptors, respectively. The reason for choosing non-peptide antagonists is to avoid possible mast cell activating actions of peptide antagonists as previously reported (Håkanson et al., 1983; Wang et al., 1994). It was observed that all three antagonists tested did not cause changes in the level of spontaneous histamine release and had no effects on histamine release induced by anti-IgE or substance P. When mast cells were incubated together with 1/6000 dilution of anti-IgE (releasing around 5% of cellular histamine) and 3×10^{-7} M substance P (releasing around 1% of cellular histamine), the level of histamine release (> 10%) was significantly higher than the theoretical additive response. As illustrated in Fig. 2, the potentiation of anti-IgE-induced histamine release by substance P was not affected by the three tachykinin antagonists (10^{-10} to 10^{-6} M). The results remained the same even after preincubating the cells with the tachykinin receptor antagonists for 10 min.

3.3. Effects of benzalkonium chloride on the potentiating effect of substance P on anti-IgE-induced histamine release from mast cells

Histamine release induced by substance P has been reported to involve the direct activation of a G protein in rat peritoneal mast cells (Mousli et al., 1990b). Benzalkonium chloride has been described in previous studies to be a selective inhibitor to this pathway (Piotrowski and Foreman, 1985; Read et al., 1982; Read and Kiefer, 1979). We hence investigated if the enhancing activity of substance P was mediated through the activation of this pathway. Benzalkonium chloride alone did not affect the spontaneous or anti-IgE-induced histamine release. However, when added

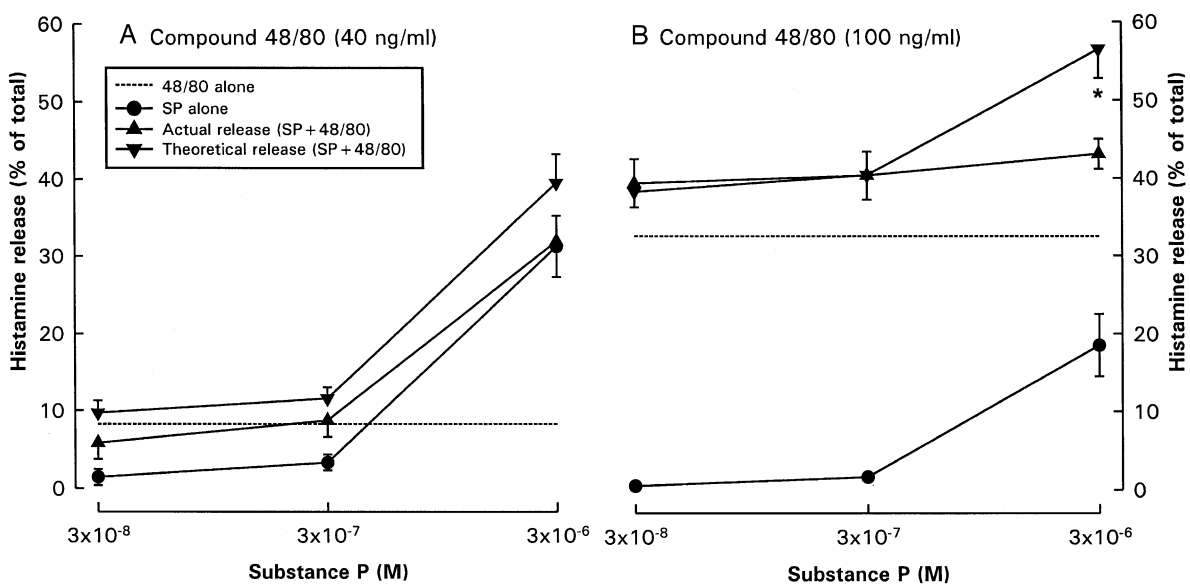

Fig. 6. Effect of substance P (SP) on compound 48/80-induced histamine release from rat peritoneal mast cells. Cells were marginally activated by 40 ng/ml or fully activated by 100 ng/ml of compound 48/80 after preincubation with substance P for 20 min. All results were corrected for spontaneous histamine release of around 10% in buffer alone. 8.3 ± 1.6% and 37.7 ± 2.6% of total histamine were, respectively, released by 40 ng/ml or 100 ng/ml of compound 48/80. Data are shown as means ± S.E.M. for $n = 5$. * Indicates $P < 0.05$ compared with the theoretical histamine release calculated by adding the histamine release induced independently by substance P and compound 48/80.

simultaneously with various concentrations of substance P, dose-dependent inhibition of substance P-induced histamine release was observed between 0.1 and 3 μg/ml of benzalkonium chloride (Fig. 3). Similarly, the potentiating action of non-releasing concentration of substance P (3×10^{-7} M) on anti-IgE-induced histamine release was dose-dependently reduced by benzalkonium chloride (Fig. 4).

3.4. Effects of compound 48/80 on anti-IgE-induced histamine release from rat peritoneal mast cells

Since the peptidergic pathway can also be activated by compound 48/80, we investigated if this compound could enhance immunological activation of rat peritoneal mast cells in a similar fashion as substance P. As illustrated in Fig. 5, compound 48/80 could also significantly potentiate anti-IgE-induced histamine release at concentrations which alone induced none (6.25 and 12.5 ng/ml) or low level (25 and 50 ng/ml) of histamine release. This potentiation was also dose dependent and was apparently more significant than with substance P.

3.5. Effects of substance P on compound 48/80-induced histamine release from rat peritoneal mast cells

In order to evaluate if the potentiating effect of substance P on anti-IgE-induced histamine release is specific, the effect of substance P on histamine release induced by compound 48/80 was investigated (Fig. 6). Mast cells were preincubated with substance P for 20 min before being activated by 40 ng/ml or 100 ng/ml of compound 48/80 which independently induced $8.3 \pm 1.6\%$ and $37.7 \pm 2.6\%$ of histamine release, respectively. As illustrated in Fig. 6, the percentage of total cellular histamine release from mast cells activated with compound 48/80 in the presence of substance P in general was not significantly different from the sum of histamine release from cells separately activated by substance P or compound 48/80. The only exception was when mast cells were activated by 100 ng/ml of compound 48/80 following a 20-min preincubation with 3×10^{-6} M of substance P; the actual release of histamine was significantly lower than the theoretical sum of histamine release separately induced by the two secretagogues.

4. Discussion

The direct histamine releasing activity of substance P at concentrations higher than 10^{-6} M is well known and is confirmed once again in the present study. However, the potentiation of immunologically induced histamine release by substance P at concentrations which do not induce significant release alone has not been documented. Although the exact concentration of substance P at inflammatory sites has not been reported, the requirement of only nM concentration of the neuropeptide to produce its effects on the microvasculature and its rapid breakdown in the body (Fewtrell et al., 1982; Pernow, 1983a) suggests that the high concentrations required for the direct histamine releasing action of substance P may not occur even at inflammatory sites. The immunological potentiating effect of substance P may be a more pathophysiologically related action of substance P. This effect in fact is specific for immunologic activation of mast cells since co-incubation of mast cells with substance P and compound 48/80 produced only additive effect with no synergism.

The failure of the non-peptide tachykinin receptor antagonists to block the direct histamine releasing action of substance P is also in accordance with the general belief that such action of the neuropeptide is independent of the conventional tachykinin receptors. Early experiments by Foreman and Jordan (1983) concluded that while the N-terminus tetrapeptide of substance P binds to anionic sites on the cell membrane to activate mast cells, a lipophilic region of the remaining peptide sequence is required to anchor the peptide molecule by inserting into the cell membrane. The N-terminal fragments of substance P have also been shown to modulate the secretory response of rat parotid gland, the excitation of the rat substantia nigra neurons, the release of γ-aminobutyric acid in the isolated spinal cord of the new born rat and the catecholamine release from the adrenal medulla of various species (Maggi et al., 1993). New substance P receptors have been suggested to mediate these actions of the N terminus of substance P (Devillier et al., 1986; Piercey et al., 1982). However, the identification or characterisation of such receptors remains to be documented.

More recent studies have demonstrated that compound 48/80, substance P and other cationic amphiphilic peptides can activate isolated G_i and G_o proteins (Mousli et al., 1990a,b). In addition, it was reported that the removal of sialic acid residues from mast cell membrane and incubation with pertussis toxin could reduce the histamine-releasing activity of these polycationic substances (Mousli et al., 1990b). It was hence postulated that cationic amphiphilic substances might interact with negatively charged components of the cell membrane and consequently insert into the membrane to activate a pertussis sensitive G proteins of mast cells independent of surface receptors. This G_i-like protein has subsequently been named G_E to distinguish it from the phospholipase C-coupled G_p protein activated by the cross-linking of $Fc_\varepsilon R$ (Barrowman et al., 1986). This hypothesis is currently most accepted for explaining why such a diverse polycationic structures can induce mast cell activation despite the lack of direct evidence such as the identification of the binding sites or the putative G protein. However, it has also been pointed out that this hypothesis is insufficient in explaining why there is heterogeneity in the response of different mast cells and basophils to basic peptides and why the activity of polybasic compounds

should be limited to the G-protein of some mast cells and basophils (Bienenstock et al., 1985; Foreman, 1993).

In the present study, it was also observed that enhanced anti-IgE-induced histamine release by substance P was not affected by the three classes of tachykinin receptor antagonists. A receptor-independent mechanism similar to that for the direct histamine-releasing action of the neuropeptide may also be involved with the immunologic potentiating effect of low concentrations of substance P. This postulation was first supported by the observation that compound 48/80 could enhance anti-IgE-induced histamine release in a similar fashion as substance P. Furthermore, the elimination of the immunologic enhancing activity of substance P by benzalkonium chloride, a specific inhibitor for histamine release induced by polybasic compounds, confirms this speculation. The specific action of benzalkonium chloride was also confirmed in the present study by demonstrating that while suppressing effectively the histamine release from compound 48/80 and substance P-activated mast cells, the compound failed to affect anti-IgE-induced histamine release. The fact that the combination of 3×10^{-6} M of substance P and 100 ng/ml of 48/80 induces a lower level of histamine release than the theoretical sum of histamine release separately induced by the secretagogues may indicate that the two secretagogues were competing for the same binding sites.

Priming of mast cells to subsequent stimulus by picomolar of substance P had been previously suggested by patch-clamp studies (Janiszewski et al., 1994). Non-releasing concentration of substance P as low as 5 pM was demonstrated to trigger an outwardly rectified Cl^- current in mast cells. This electrical response appeared as brief current pulses that was believed to promote Ca^{2+} influx induced by subsequent stimuli. Although such observation could be applied to explain our current finding, it cannot explain why the augmentation applies only to anti-IgE but not to compound 48/80. The synergistic amplification of the effects of non-releasing concentrations of anti-IgE and substance P may be the consequence of the simultaneous sub-optimal activation of the polycation sensitive G protein and various anti-IgE-related signalling in the rat peritoneal mast cells. In fact, amplification of physiological responses due to synergistic cross-talks between different G protein-coupled receptors or between G protein-coupled receptors and other classes of receptors has been reported and comprehensively reviewed by Selbie and Hill (1998). Accordingly, sub-optimal activation of G_E may augment G_p-mediated intracellular events such as enhanced phospholipase C activation result in the enhanced increase of intracellular Ca^{2+}. In fact, it is well known that substance P and other polycationic compounds can mediate histamine release in the absence of extracellular Ca^{2+}. Crosstalk between G_E and the tyrosine kinase activated by the cross-linking of $Fc_\varepsilon R$ may also be responsible for enhancing downstream signal transduction pathways which facilitate mast cell exocytosis. Further studies investigating the effects of substance P on various anti-IgE-activated mast cell signal transduction pathways will be useful for identifying such interactions.

In total, the present study has clearly demonstrated that low, physiological concentrations of substance P can enhance mast cell response to the allergic stimulus, anti-IgE, through a non-neuropeptide receptor related mechanism. The clinical significance of this potentiating effect of substance P remains to be evaluated in future studies using human mast cell models. Human basophils and mast cells from human skin, bladder and broncholavage fluid have been demonstrated to be directly activated by high concentration of substance P in neuropeptide receptor-independent manners. Similar potentiating effect of substance P as currently reported may also occur in these histaminocytes and contributes to the pathogenesis of neurogenic inflammation in various allergic reactions. In fact, antigen-evoked mediator release from human mucosa has been reported to be enhanced by substance P (Baumgarten et al., 1996). Moreover, the potentiating effect of substance P also helps to explain the recently reported elimination of IgE-mediated reactivity in allergic patients following a period of treatment with substance P co-administered with low doses of allergens (Patterson et al., 1999). Substance P may increase the reactivity of mast cells marginally to non-releasing concentrations of an allergen so that non-symptom inducing level of mediators are continuously released from mast cells leading to depletion of stored mediators. Furthermore, chronic sub-optimal stimulation of mast cells may lead to desensitization of essential signal transduction pathways and subsequently reduce the reactivity of mast cells.

Acknowledgements

This work was supported by grants from the University Grant Committee of Hong Kong. The generous gifts of drugs by companies listed in the Materials and methods section are gratefully acknowledged.

References

Amano, H., Kurosawa, M., Miyachi, Y., 1997. Possible mechanisms of the concentration-dependent action of substance P to induce histamine release from rat peritoneal mast cells and the effect of extracellular calcium on mast-cell activation. Allergy, 52, 215–219.

Barnes, P.J., Belvisi, M.G., Rogers, D.F., 1990. Modulation of neurogenic inflammation: novel approaches to inflammatory disease. Trends Pharmacol. Sci., 11, 185–189.

Barrowman, M.M., Cockcroft, S., Gomperts, B.D., 1986. Two roles for guanine nucleotides in the stimulus-secretion sequence of neutrophils. Nature, 319, 504–507.

Baumgarten, C.R., Witzel, A., Kleine-Tebbe, J., Kunkel, G., 1996. Substance P enhances antigen-evoked mediator release from human nasal mucosa. Peptides, 17, 25–30.

Bienenstock, J., Befus, D., Denburg, J., Goto, T., Lee, T., Otsuka, H., Shanahan, F., 1985. Comparative aspects of mast cell heterogeneity in different species and sites. Int. Arch. Allergy Immunol., 77, 126–129.

Bienenstock, J., MacQueen, G., Sestini, P., Marshall, J.S., Stead, R.H., Perdue, M.H., 1991. Mast cell/nerve interactions in vitro and in vivo. Am. Rev. Respir. Dis., 143, S55–S58.

Chan, C.L., Jones, R.L., Lau, H.Y., 2000. Characterization of prostanoid receptors mediating inhibition of histamine release from anti-IgE-activated rat peritoneal mast cells. Br. J. Pharmacol., 129, 589–597.

Devillier, P., Regoli, D., Asseraf, A., Descours, B., Marsac, J., Renoux, M., 1986. Histamine release and local responses of rat and human skin to substance P and other mammalian tachykinins. Pharmacology, 32, 340–347.

Fantini, F., Magnoni, C., Pincelli, C., Giannetti, A., 1995. Neurogenic inflammation and the skin: neural modulation of cutaneous inflammatory reactions. Eur. J. Dermatol., 5, 349–357.

Fewtrell, C.M., Foreman, J.C., Jordan, C.C., Oehme, P., Renner, H., Stewart, J.M., 1982. The effects of substance P on histamine and 5-hydroxytryptamine release in the rat. J. Physiol., 330, 393–411.

Foreman, J.C., 1993. Non-immunological stimuli of mast cells and basophil leucocytes. In: Foreman, J.C. (Ed.), Immunopharmacology of Mast Cells and Basophils. Academic Press, London, pp. 57–69.

Foreman, J., Jordan, C., 1983. Histamine release and vascular changes induced by neuropeptides. Agents Actions, 13, 105–116.

Frenz, A.M., Christmas, T.J., Pearce, F.L., 1994. Does the mast cell have an intrinsic role in the pathogenesis of interstitial cystitis? Agents Actions, 41, C14–C15.

Håkanson, R., Leander, S., Andersson, R.G., Horig, J., 1983. Substance P antagonists release histamine from peritoneal mast cells. Acta Physiol. Scand., 117, 319–320.

Janiszewski, J., Bienenstock, J., Blennerhassett, M.G., 1994. Picomolar doses of substance P trigger electrical responses in mast cells without degranulation. Am. J. Physiol., 267, C138–C145.

Kiernan, J., 1990a. Interactions between mast cells and nerves. Neurogenic inflammation. Trends Pharmacol. Sci., 11, 316.

Kiernan, J.A., 1990b. Degranulation of mast cells in the trachea and bronchi of the rat following stimulation of the vagus nerve. Int. Arch. Allergy Immunol., 91, 398–402.

Lembeck, F., Holzer, P., 1979. Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn-Schmiedeberg's Arch. Pharmacol., 310, 175–183.

Lowman, M.A., Rees, P.H., Benyon, R.C., Church, M.K., 1988. Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. J. Allergy. Clin. Immunol., 81, 590–597.

Maggi, C.A., Patacchini, R., Rovero, P., Giachetti, A., 1993. Tachykinin receptors and tachykinin receptor antagonists. J. Auton. Pharmacol., 13, 23–93.

Mousli, M., Bronner, C., Landry, Y., Bockaert, J., Rouot, B., 1990a. Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80. FEBS Lett., 259, 260–262.

Mousli, M., Bueb, J.L., Bronner, C., Rouot, B., Landry, Y., 1990b. G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. Trends Pharmacol. Sci., 11, 358–362.

Patterson, R., Harris, K.E., Grammer, L.C., Greenberger, P.A., Ditto, A.M., Shaughnessy, M.A., 1999. Potential effect of the administration of substance P and allergen therapy on immunoglobulin E-mediated allergic reactions in human subjects. J. Lab. Clin. Med., 133, 189–199.

Pearce, F.L., 1986. On the heterogeneity of mast cells. Pharmacology, 32, 61–71.

Pernow, B., 1983a. Substance P. Pharmacol. Rev., 35, 85–141.

Pernow, B., 1983b. Substance P—a putative mediator of antidromic vasodilation. Gen. Pharmacol., 14, 13–16.

Piercey, M.F., Dobry, P.J., Einspahr, F.J., Schroeder, L.A., Masiques, N., 1982. Use of substance P fragments to differentiate substance P receptors of different tissues. Regul. Pept., 3, 337–349.

Piotrowski, W., Foreman, J.C., 1985. On the actions of substance P, somatostatin, and vasoactive intestinal polypeptide on rat peritoneal mast cells and in human skin. Naunyn-Schmiedeberg's Arch. Pharmacol., 331, 364–368.

Read, G.W., Kiefer, E.F., 1979. Benzalkonium chloride: selective inhibitor of histamine release induced by compound 48/80 and other polyamines. J. Pharmacol. Exp. Ther., 211, 711–715.

Read, G.W., Hong, S.M., Kiefer, E.F., 1982. Competitive inhibition of 48/80-induced histamine release by benzalkonium chloride and its analogs and the polyamine receptor in mast cells. J. Pharmacol. Exp. Ther., 222, 652–657.

Selbie, L.A., Hill, S.J., 1998. G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signalling pathways. Trends Pharmacol. Sci., 19, 87–93.

Stead, R.H., Tomioka, M., Quinonez, G., Simon, G.T., Felten, S.Y., Bienenstock, J., 1987. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. Proc. Natl. Acad. Sci. U. S. A., 84, 2975–2979.

Tainsh, K.R., Pearce, F.L., 1992. Mast cell heterogeneity: evidence that mast cells isolated from various connective tissue locations in the rat display markedly graded phenotypes. Int. Arch. Allergy Immunol., 98, 26–34.

Tainsh, K.R., Liu, W.L., Lau, H.Y., Cohen, J., Pearce, F.L., 1992. Mast cell heterogeneity in man: unique functional properties of skin mast cells in response to a range of polycationic stimuli. Immunopharmacology, 24, 171–180.

Wang, Z.Y., Feng, D.M., Wang, Y.L., Tung, S.R., Wong, K., Strichartz, G.R., Folkers, K., Hakanson, R., 1994. Pharmacological assessment of spantide II analogues. Eur. J. Pharmacol., 260, 121–128.

Wasserman, S.I., 1983. Mediators of immediate hypersensitivity. J. Allergy. Clin. Immunol., 72, 101–119.