Acta Dermatovener (Stockholm) 61: 245–250, 1981

STUDIES ON PRURITOGENIC AND HISTAMINE-RELEASING EFFECTS OF SOME PUTATIVE PEPTIDE NEUROTRANSMITTERS

Bo Fjellner and Östen Hägermark

Department of Dermatology, Karolinska Sjukhuset, Stockholm, Sweden

Abstract. Pruritus, whealing and axon-reflex erythema appeared in human skin after intradermal injection of (i) several peptides with a putative transmitter function, i.e. vasoactive intestinal polypeptide (VIP) (10^{-7}–10^{-4} M), [Gln^4]-neurotensin (10^{-7}–10^{-4} M), neurotensin (10^{-5}–10^{-4} M) and secretin (10^{-5}–10^{-4} M), which were compared with substance P (10^{-7}–10^{-5} M) previously shown to be one of the most potent histamine liberators when administered intradermally in humans; (ii) the basic polypeptide protamine (10^{-7}–10^{-4} M); and (iii) histamine (0.3–10 μg/ml) and the histamine liberator compound 48/80 (0.3–10 μg/ml). The reactions were inhibited in a dose-related manner by the antihistamine mepyramine, indicating that the peptide-induced responses were mediated by released histamine. This was further confirmed by the histamine release observed when the peptides were incubated with rat peritoneal mast cells. In human skin, VIP was more potent than the other neuropeptides and had roughly the same potency as substance P. The two adjacent basic amino-acid residues and the amide substitution of the terminal C-group of VIP, in addition to its strong net basic charge, may explain its potency as a histamine releaser.

Key words: Vasoactive intestinal polypeptide; Substance P; [Gln^4]-neurotensin; Neurotensin; Secretin; Protamine; Experimental itch; Rat mast cells

The "receptors" which register pruritic stimuli are situated in the most superficial nerve elements of the skin (30). Pathologic itching is considered to be caused by chemical stimulation of these receptors, but the biochemical events leading to pruritus are still not completely known. Intradermal injection of histamine evokes itching and a "triple response" (redness, wheal and flare). It is probable that histamine acts directly on the nerve endings and is responsible for the itching in urticaria (11). The role of histamine in other pruritic conditions is more doubtful. It has been discussed whether other substances, such as proteolytic enzymes or polypeptides, might stimulate the itch receptors (for ref., see 11). Some of these possible mediators of pruritus have been investigated for itch-eliciting capacity by intradermal injection in humans. However, the pruritogenic effect of most agents, e.g. the proteolytic enzymes trypsin and chymotrypsin and the polypeptides bradykinin and substance P, seems to be due to their release of histamine from cutaneous mast cells (14, 15, 16).

Many biologically active peptides believed to be confined to the central nervous system, have been recovered in the periphery, especially in the intestines (e.g. somatostatin, neurotensin and enkephalins). Conversely some "classical" gut hormones (e.g. gastrin, cholecystokinin and vasoactive intestinal peptide (VIP)) have been demonstrated in neural tissues, both in the CNS and in the peripheral nerves (for ref., see 17, 28). There is accumulating evidence that many of these peptides may have neurotransmitter roles in CNS as well as in the periphery (32).

In the present investigation some of these putative peptide neurotransmitters were injected intradermally in humans to see whether they were pruritogenic and in that case acted without releasing histamine. Histamine-induced responses were inhibited by the H_1-antagonist mepyramine. In addition, the ability of the peptides to release histamine from rat mast cells *in vitro* was investigated.

MATERIALS AND METHODS

Studies on experimental pruritus

Fifty-two healthy volunteers aged 18 to 59 years took part in this investigation. Small volumes, 0.01 ml, of the solutions were injected intradermally on the lateral aspect of the upper arms. The duration of the itch response was recorded. The flare reaction was outlined with a marking pen on the skin 5 min after injection and traced on a transparent plastic film where the area then could be measured planimetrically.

The following agents were studied: VIP and secretin (prepared and donated by Prof. V. Mutt, Department of Biochemistry II, Karolinska Institute, Stockholm, Sweden), neurotensin, [Gln^4]-neurotensin and substance P

Table I. *Amino acid sequences of the peptides*

Vasoactive intestinal polypeptide (VIP)

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2

Secretin

His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

Neurotensin

pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu

[Gln^4]-neurotensin

pGlu-Leu-Tyr-Gln-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu

Substance P

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

Protamine[a]

Pro-Arg-Arg-Arg-Arg-Ser-Ser-Ser-Arg-Pro-Val-Arg-Arg-Arg-Arg-Arg-Pro-Arg-Val-Ser-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Gly-Gly-Arg-Arg-Arg-Arg

[a] The composition of the protamine used in the present study was not obtainable. The structure given in this table is quoted from Jasani et al. (18).

(gifts from Prof. K. Folkers, Austin, Texas, USA), protamine (AB Vitrum, Stockholm), and compound 48/80 (a gift from Prof. B. Högberg, AB Leo, Helsingborg, Sweden). The strongly basic protamine had been prepared from herring sperm. The amino acid sequences of the peptides are shown in Table I. As a histamine H_1-receptor antagonist we used mepyramine (Pharma Rhodia A/S, Copenhagen, Denmark).

The substances were dissolved in physiological saline containing 10% (v/v) Sörensen phosphate buffer ($Na_2HPO_4 + KH_2PO_4$, 67 mM), pH 7.4. The solutions were passed through a Millipore filter (Miltex TM 0.22 μm) before use. They were injected separately, except for mepyramine which was injected in a mixture with the pruritogenic agents.

Studies on histamine release from rat peritoneal mast cells

A peritoneal cell suspension containing about 3–5% mast cells was prepared from male Sprague-Dawley rats as follows. A buffered salt solution (NaCl, 154 mM; KCl, 2.7 mM; $CaCl_2$, 0.9 mM; Na_2HPO_4, 4.0 mM; KH_2PO_4, 2.7 mM), pH 7.0, containing 0.1% serum albumin was injected intraperitoneally. The abdomen was gently massaged and opened after 90 sec. The cell suspension was removed and centrifuged at 140 *g* for 2 min. The cells were resuspended in the buffered salt solution and 0.9 ml portions of the cell suspension were added to centrifuge tubes, each containing 0.1 ml of the agent studied. The samples were incubated in duplicate at 37°C for 10 min, transferred to an ice-water bath and then centrifuged at 350 *g* for 10 min at 0°C. The supernatants were carefully decanted, the sediments resuspended in saline and boiled for 3 min to extract the histamine. Histamine was determined in both supernatants and sediments by the fluorometric method described by Shore et al. (1959) (31), omitting the purification steps (8). Histamine released from the mast cells was expressed as a percen-

Table II*a*. *Itch responses in seconds (mean ± S.E.M.) induced by putative peptide neurotransmitters, protamine, histamine and Compound 48/80*

n denotes number of subjects

Agent	Exp. no.	*n*	Itch duration (sec) for different conc. of the agents			
			10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
VIP	I	6	0	73.0±23.5	128.3±31.0	140.0±32.2
Substance P	II	6	18.2± 8.3	75.0±17.9	110.8±18.1	
[Gln^4]-neurotensin	III	5	13.4± 8.6	18.2±11.3	98.8±30.7	116.0±22.6
Neurotensin	IV	6			0	85.8±22.0
Secretin	I	6			6.8± 6.8	93.5±30.5
Protamine	V	4	0	32.0±12.6	61.3±15.4	99.3±38.4
			0.3 μg/ml	1.0 μg/ml	3.0 μg/ml	10 μg/ml
Histamine	VI	8	1.9± 1.9	42.4±17.2	80.9±26.1	113.4±31.2
Compound 48/80	VI	8	23.0±15.1	46.0±15.2	68.8±25.0	138.4±34.0

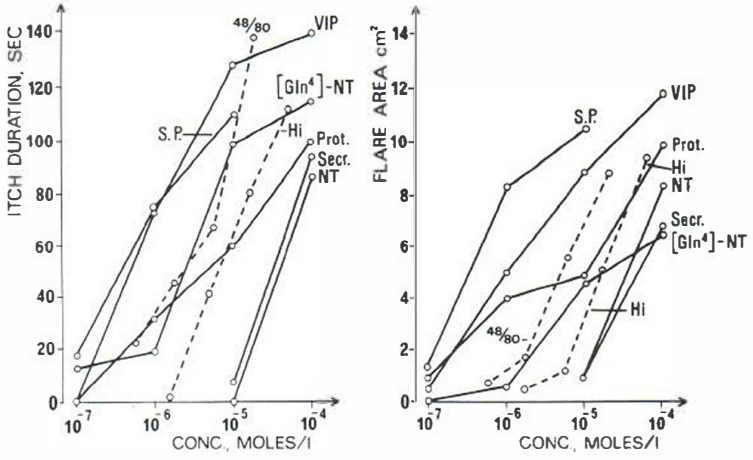

Fig. 1. Dose–response curves for (*a*) itch, and (*b*) flare responses induced by substance P (S. P.), VIP, [Gln⁴]-neurotensin ([Gln⁴]-NT), neurotensin (NT), secretin, protamine, histamine and compound 48/80. Each substance was studied in separate experiments, except VIP used in the same experiment as secretin and histamine used together with compound 48/80. The results are expressed as means based on responses in 6–8 subjects. The curves are based on the data presented in Table II.

tage of the total histamine content of each sample. The spontaneous release, 1–3 %, was deducted.

RESULTS

Cutaneous responses

All peptides induced dose-dependent itch and flare responses at the injection sites (Table II and Fig. 1). The most potent of the peptides were VIP and substance P. Secretin, which is chemically and biologically related to VIP, was less active. Neurotensin had a potency of the same order of magnitude as secretin, whereas [Gln⁴]-neurotensin seemed to be more active. Like [Gln⁴]-neurotensin, the strongly basic peptide protamine was of intermediate potency.

The peptides not only produced itch and flare reactions but also a weal at the site of injection, i.e. the same type of responses which appear when histamine is injected intradermally. To examine the extent to which the responses were caused by histamine, the peptides as well as histamine and the histamine liberator comp. 48/80 were injected in a mixture with the histamine H_1-receptor antagonist mepyramine. The concentrations of the peptides were chosen to give cutaneous responses of approximately the same order of magnitude as histamine, 10 μg/ml. Secretin was not included due to lack of material. With increasing concentrations of mepyramine a dose-dependent inhibition of the itch and flare responses was seen. The inhibition curves (Fig. 2) were approximately parallel for the

Table II *b*. *Flare responses in mm² (mean ± S.E.M.) induced by putative peptide neurotransmitters, protamine, histamine and Compound 48/80*

n denotes number of subjects

Agent	Exp. no.	*n*	Flare area (mm²) for different conc. of the agents			
			10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
VIP	I	6	36.2± 7.5	504.0± 50.7	892.2±103.0	1 192.5±131.4
Substance P	II	6	127.7±65.5	830.8±118.6	1 056.8±138.3	
[Gln⁴]-neurotensin	III	5	0	53.2± 32.8	451.0± 65.5	645.8± 59.3
Neurotensin	IV	6			88.3± 31.9	834.0±143.0
Secretin	I	6			83.0± 38.0	691.0± 48.8
Protamine	V	4	83.3±83.3	393.3±188.8	480.3±169.1	993.8±257.5
			0.3 μg/ml	1.0 μg/ml	3.0 μg/ml	10 μg/ml
Histamine	VI	8	48.9±23.5	121.0± 64.8	504.0± 89.3	950.1± 97.1
Compound 48/80	VI	8	65.6±35.9	163.3± 43.9	563.6±109.3	886.5± 73.3

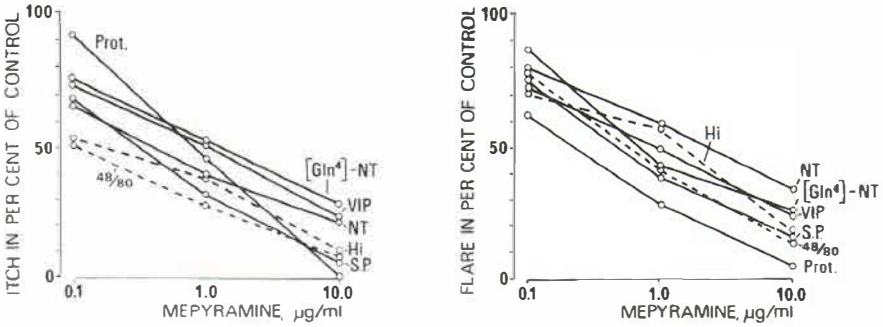

Fig. 2. Inhibitory effects of mepyramine on (*a*) itch, and (*b*) flare responses induced by VIP (10^{-5} M), substance P (3×10^{-6} M), [Gln⁴]-neurotensin (10^{-4} M), neurotensin (10^{-4} M), protamine (10^{-4} M), histamine (10 µg/ml) and compound 48/80 (3.3 µg/ml). Each substance was administered to 6–7 subjects. The values are expressed as means based on percentage of the individuals responses induced by the agents without mepyramine.

agents studied, except for the itch-responses induced by protamine.

Release of histamine from rat mast cells

When incubated with rat mast cells in suspension, the peptides induced histamine liberation in a dose-dependent manner (Fig. 3). Protamine was the most active agent, releasing histamine at a concentration as low as 10^{-7} M. VIP and substance P were less potent. In contrast to the effect in human skin, VIP seemed to be somewhat more potent than substance P in this system. Secretin, neurotensin and [Gln⁴]-neurotensin were the least active agents, releasing significant amounts of histamine only at the concentration 10^{-4} M.

DISCUSSION

The peptides used in the present investigation induced itch when injected intradermally. Wheal and flare always appeared together with the itching. Since this is the typical reaction seen when histamine is injected intradermally, it seemed probable that the pruritic response was due to histamine released from the dermal mast cells. This was confirmed in the studies with the histamine H_1-receptor antagonist mepyramine, which inhibited the peptide-induced reactions in a dose-related manner similar to its inhibiting effects on histamine. Moreover, when incubated with rat mast cells *in vitro*, the peptides released histamine. Thus, there was no indication that any of the peptides had a pruritic effect *per se*.

Histamine-releasing capacity of many basic peptides is a well-recognized phenomenon, studied mostly in rat peritoneal mast cells such as the following peptides: cationic protein from leukocytes (29), the mast cell degranulating peptide of bee venom (MCDP) (2, 8) and wasp venom (Mastoparan' and Mastoparan-X') (12, 13), anaphylatoxins C3a and C5a (20), and corticotropin-derived polypeptides (27). Histamine release has been demonstrated in human skin after injection intradermally of, e.g., anaphylatoxin C3a (21, 33), bradykinin (14) and substance P (16). The anaphylatoxins C3a and C5a also release histamine from human leukocytes (9, 10, 25).

A connection between the histamine-releasing activity and the number of basic groups in peptides was demonstrated by Johnson & Erdös, 1973 (19) who studied the histamine release induced by various vasoactive peptides on rat mast cells. Basicity *per se* is, however, not sufficient for histamine-

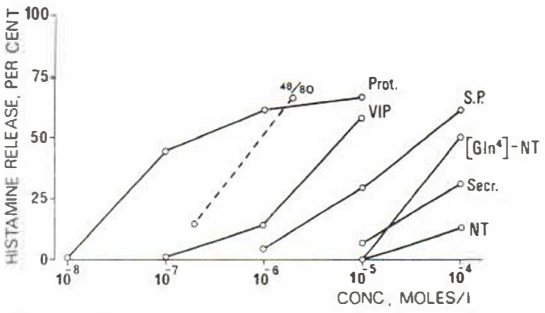

Fig. 3. Histamine release from rat peritoneal mast cells induced by VIP, substance P, [Gln⁴]-neurotensin, neurotensin, secretin, protamine and compound 48/80. The results are means based on 1–5 separate determinations. The data of substance P obtained with permission from Hägermark, Hökfelt & Pernow 1978 (16).

releasing activity, as stated by Rothschild, 1966 (26).

A more detailed quantitative analysis of the structure–activity relationships of polypeptides capable of releasing histamine from rat mast cells has been published by Jasani et al. 1979 (18). They found that a cluster of four basic residues (lysine or arginine) was optimal for histamine release induced by corticotropin and melittin polypeptides, provided that the C-terminal group was substituted. However, two adjacent basic residues were enough to produce histamine release as in dilysine. The release process was enhanced by neighbouring hydrophobic residues. Moreover, appropriately situated aromatic amino acids seemed to play a critical role.

The results of the present investigation are compatible with some of the structure relationships described above. When the structurally related substances VIP and secretin are compared, it is seen that the responses both *in vivo* and *in vitro* were related to the net basic charges of the agents. Thus, VIP, the more basic of the substances was more potent than secretin. In addition, VIP has two adjacent basic residues. Both VIP and secretin have their C-terminal amino acids in amide form (24). Similarly, the very potent substance P has an amide group at its C-terminal. Substance P has relatively few basic residues but, as has been postulated for the interaction with smooth muscle receptor (6), the hydrophobic nature of the side chain and the aromatic character of a pair of amino acids, i.e. phenylalanine in positions 7 and 8, may play a critical role also in optimal stimulation of the histamine release process. Neurotensin like VIP has also two adjacent basic residues (3) which seem crucial for neurotensin–receptor interaction (1). The rather weak effects of neurotensin observed in our systems might in part be due to its unsubstituted C-terminal. On the other hand, the amide analogues of neurotensin and [Gln^4]-neurotensin are practically inactive in other biological systems (7). Our finding that [Gln^4]-neurotensin was more active than neurotensin in human skin might be due to the substitution of the acidic glutamic acid with its non-ionized amide glutamine.

Previously, substance P has been stated to be one of the most potent histamine-releasing agents in man (16). The results presented indicate that VIP has a similar potency. It should also be pointed out that the relative potency between the peptides differed in the in vivo and the in vitro systems. VIP and substance P were the most potent liberators in human skin, whereas protamine was the most active agent on rat mast cell in vitro. Variations between species seem to exist and, for example, Chahl, 1979 (4) recently reported neurotensin to be one of the most potent histamine-releasing peptides in rat skin, releasing histamine when such a low dose as 0.05 ml of a 10^{-7} M solution was injected into rat skin. This should be compared with the rather weak effects of neurotensin found by us in both human skin and rat mast cells, where 10^{-4} M was required for effects.

Both substance P and VIP are present in sensory nerves in which retrograde axonal transportation has been demonstrated (17, 22). Small amounts of substance P have thus been localized in human skin by the immunohistochemical technique (Hökfelt & Hägermark, unpublished). It has previously been speculated that substance P released from sensory neurons may evoke itching via release of histamine from dermal mast cells (16). A similar mechanism could also operate for VIP. It is interesting that VIP seems to occur in certain peripheral cholinergic neurons innervating exocrine glands and blood vessels in muscular tissues (23). Whether such VIP-acetylcholine containing fibres also are present in human skin and thus could be involved in the pruritogenic reactions, is not known. The question also remains unanswered whether there are endogenous mediators other than histamine that can stimulate the dermal itch receptors.

ACKNOWLEDGEMENTS

We are indebted to Prof. Viktor Mutt and Prof. Tomas Hökfelt for a critical reading of this work and to Mrs Halla Bornstein for skilful technical assistance. The work was supported by grants from the Karolinska Institute, the Edvard Welander Foundation, the Finsen Foundation and the Swedish Medical Association.

REFERENCES

1. Bissette, G., Manberg, P., Memeroff, C. B. & Prange, A. J.: Minireview. Neurotensin, a biologically active peptide. Life Sci *23:* 2173, 1978.
2. Breithaupt, H. & Habermann, E.: Mastzelldegranulierendes Peptid (MCD-Peptid) aus Bienengift: Isolierung, biochemische und pharmakologische Eigenschaften. Naunyn Schmiedebergs Arch Pharmacol *261:* 252, 1968.
3. Carraway, R. & Leeman, S. E.: The amino sequence of a hypothalamic peptide, neurotensin. J Biol Chem *250:* 1907, 1975.
4. Chahl, L. A.: The effect of putative peptide neuro-

transmitters on cutaneous vascular permeability in the rat. Naunyn Schmiedebergs Arch Pharmacol *309:* 159, 1979.
5. Chang, M. M., Leeman, S. E. & Niall, H. D.: Amino-acid sequence of substance P. Nature [New Biol] *232:* 86, 1971.
6. Chipkin, R. E., Stewart, J. M., Sweeney, V. E., Harris, K. & Williams, R.: In vitro activities of some synthetic substance P analogues. Arch Int Pharmacodyn Ther *240:* 193, 1979.
7. Folkers, K., Chang, D., Humphries, J., Carraway, R., Leeman, S. E. & Bowers, C. Y.: Synthesis and activities of neurotensin, and its acid and amide analogs: Possible natural occurrence of [Gln⁴]-neurotensin. Proc Natl Acad Sci USA *73:* 3833, 1976.
8. Fredholm, B. & Hägermark, Ö.: Histamine release from rat mast cells induced by a mast cell degranulating fraction in bee venom. Acta Physiol Scand *69:* 304, 1967.
9. Glovsky, M. M., Hugli, T. E., Ishizaka, T., Lichtenstein, L. M. & Erickson, B. W.: Anaphylatoxin-induced histamine release with human leukocytes. J Clin Invest *64:* 804, 1979.
10. Grant, J. A., Dupree, E., Goldman, A. S., Schultz, D. R. & Jackson, A. L.: Complement-mediated release of histamine from human leukocytes. J Immunol *114:* 1101, 1975.
11. Hernon, Jr., J. H.: Pruritus. *In* Clinical Dermatology (ed. D. Joseph Demis, Richard L. Dobson & Joseph McGuire), Harper & Row, Hagerstown, Maryland, 1977.
12. Hirai, Y., Yasuhara, T., Yoshida, H., Nakajima, T., Fujino, M. & Kitada, C.: A new mast cell degranulating peptide "Mastoparan" in the venom of *Vespula lewisii*. Chem Pharm Bull *27:* 1942, 1979.
13. Hirai, Y., Kuwada, M., Yasuhara, T., Yoshida, H. & Nakajima, T.: A new mast cell degranulating peptide homologous to Mastoparan in the venom of Japanese hornet (*Vespa xanthoptera*). Chem Pharm Bull (Tokyo) *27:* 1945, 1979.
14. Hägermark, Ö.: Studies on experimental itch induced by kallikrein and bradykinin. Acta Dermatovener (Stockholm) *54:* 397, 1974.
15. Hägermark, Ö., Rajka, G. & Bergqvist, U.: Experimental itch in human skin elicited by rat mast cell chymase. Acta Dermatovener (Stockholm) *52:* 125, 1972.
16. Hägermark, Ö., Hökfelt, T. & Pernow, B.: Flare and itch induced by substance P in human skin. J Invest Dermatol *71:* 233, 1978.
17. Hökfelt, T., Johansson, O., Ljungdahl, Å., Lundberg, J. M. & Schultzberg, M.: Peptidergic neurones. Nature *284:* 515, 1980.
18. Jasani, B., Kreil, G., Mackler, B. F. & Stanworth, D. R.: Further studies on the structural requirements for polypeptide-mediated histamine release from rat mast cells. Biochem J *181:* 623, 1979.
19. Johnson, A. R. & Erdös, E. G.: Release of histamine from mast cells by vasoactive peptides. Proc Soc Exp Biol Med *142:* 1252, 1973.
20. Johnson, A. R., Hugli, T. E. & Müller-Eberhard, H. J.: Release of histamine from rat mast cells by the complement peptides C3a and C5a. Immunology *28:* 1067, 1975.
21. Lepow, I. H., Willms-Kretschmer, K., Patrick, R. A. & Rosen, F. S.: Gross and ultrastructural observations on lesions produced by intradermal injection of human C3a in man. Am J Pathol *61:* 13, 1970.
22. Lundberg, J. M., Hökfelt, T., Kewenter, J., Petterssson, G., Ahlman, H., Edin, R., Dahlström, A., Nilsson, G., Terenius, L., Uvnäs-Wallensten, K. & Said, S.: Substance P-, VIP-, and enkephalin-like immunoreactivity in the human vagus nerve. Gastroenterology *77:* 468, 1979.
23. Lundberg, J. M., Hökfelt, T., Schulzberg, M., Uvnäs-Wallensten, K., Köhler, C. & Said, S. I.: Occurrence of vasoactive intestinal polypeptide (VIP)-like immunoreactivity in certain cholinergic neurons of the cat: Evidence from combined immunohistochemistry and acetylcholinesterase staining. Neuroscience *4:* 1539, 1979.
24. Mutt, V. & Said, S. I.: Structure of the porcine vasoactive intestinal octacosapeptide. The amino-acid sequence. Use of kallikrein in its determination. Eur J Biochem *42:* 581, 1974.
25. Petersson, B.-Å., Nilsson, A. & Stålenheim, G.: Induction of histamine release and desensitization in human leukocytes. J Immunol *114:* 1581, 1975.
26. Rothschild, A. M.: Histamine release by basic compounds. *In* Handbook of Experimental Pharmacology (ed. O. Eichler & A. Farah), vol. 18/1, pp. 386–430. Springer-Verlag, Berlin, Heidelberg and New York, 1966.
27. Rüegg, M.: Histamine-releasing effect of a corticotrophin derivative. II. Mechanism of action of histamine release by C 44 680-Ba, compared with that of Cpd 48/80, dextran and triton. Agents Actions *9:* 148, 1979.
28. Said, S. I.: Peptides common to the nervous system and the gastrointestinal tract. *In* Frontiers in Neuroendocrinology (ed. L. Martini & W. F. Ganong), vol. 6, pp. 293–331. Raven Press, New York, 1980.
29. Seegers, W. & Janoff, A.: Mediators of inflammation in leukocyte lysosomes. VI. Partial purification and characterization of a mast cell-rupturing component. J Exp Med *124:* 833, 1966.
30. Shelley, W. B. & Arthur, R. P.: The neurohistology and neurophysiology of the itch sensation in man. Arch Dermatol *76:* 296, 1957.
31. Shore, P. A., Burkhalter, A. & Cohn, V. H., Jr.: A method for the fluorometric assay of histamine in tissues. J Pharmacol *127:* 182, 1959.
32. Snyder, S. H. & Innis, R. B.: Peptide neurotransmitters. Ann Rev Biochem *48:* 755, 1979.
33. Wuepper, K. D., Bokisch, V. A., Müller-Eberhard, H. J. & Stoughton, R. B.: Cutaneous responses to human C3 anaphylatoxin in man. Clin Exp Immunol *11:* 13, 1972.

Received April 18, 1980

B. Fjellner, M. D.
Department of Dermatology
Karolinska sjukhuset
S-104 01 Stockholm 60 Sweden