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

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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, \([\text{Gln}\text{]}\text{-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\ \(10^{-7}-10^{-3}\) M) previously shown to be one of the most potent histamine liberators when administered intradernally in humans; (ii) the basic polypeptide protamine \(10^{-7}-10^{-4}\) M; and (iii) histamine \(0.3-10\ \mu\text{g}/\text{ml}\) and the histamine liberator compound 48/80 \(0.3-10\ \mu\text{g}/\text{ml}\). 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 \(P\); \([\text{Gln}\text{]}\text{-neurotensin}\;\text{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, \(0.01\ \text{ml}\) of the solutions were injected intradernally 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, \([\text{Gln}\text{]}\text{-neurotensin and substance P}
Table I. Amino acid sequences of the peptides

Vasoactive intestinal polypeptide (VIP)

Secretin

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

[Gln⁺]- Neurotensin

Substance P
Arg-Pro-Lys⁺-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂

Protamine

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).

The substances were dissolved in physiological saline containing 10% (v/v) Sörensen's phosphate buffer (Na₂HPO₄-KH₂PO₄, 67 mM), pH 7.4. The solutions were passed through a Millipore filter (Millrex 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; CaCl₂, 0.9 mM; Na₂HPO₄, 4.0 mM; KH₂PO₄, 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 fluorimetric 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 IIa. Itch responses in seconds (mean ± S.E.M.) induced by putative peptide neurotransmitters, protamine, histamine and Compound 48/80

<table>
<thead>
<tr>
<th>Agent</th>
<th>Exp. no.</th>
<th>n</th>
<th>10⁻³ M</th>
<th>10⁻⁴ M</th>
<th>10⁻⁵ M</th>
<th>10⁻⁶ M</th>
<th>10⁻⁷ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>I</td>
<td>6</td>
<td>73.0 ± 23.5</td>
<td>128.3 ± 31.0</td>
<td>140.0 ± 32.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P</td>
<td>II</td>
<td>6</td>
<td>18.2 ± 8.3</td>
<td>110.8 ± 18.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Gln⁺]-Secretin</td>
<td>III</td>
<td>5</td>
<td>13.4 ± 8.6</td>
<td>98.8 ± 30.7</td>
<td>116.0 ± 22.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurotensin</td>
<td>IV</td>
<td>6</td>
<td>18.2 ± 11.3</td>
<td>85.8 ± 22.0</td>
<td>93.5 ± 30.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretin</td>
<td>I</td>
<td>6</td>
<td>32.0 ± 12.6</td>
<td>32.0 ± 15.4</td>
<td>99.3 ± 38.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protamine</td>
<td>V</td>
<td>4</td>
<td>0.3 μg/ml</td>
<td>1.0 μg/ml</td>
<td>3.0 μg/ml</td>
<td>10 μg/ml</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>VI</td>
<td>8</td>
<td>1.9 ± 1.9</td>
<td>42.4 ± 17.2</td>
<td>80.9 ± 26.1</td>
<td>113.4 ± 31.2</td>
<td></td>
</tr>
<tr>
<td>Compound 48/80</td>
<td>VI</td>
<td>8</td>
<td>23.0 ± 15.1</td>
<td>46.0 ± 15.2</td>
<td>68.8 ± 25.0</td>
<td>138.4 ± 34.0</td>
<td></td>
</tr>
</tbody>
</table>

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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 [Gln\(^n\)]-neurotensin seemed to be more active. Like [Gln\(^n\)]-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 compound 48/80 were injected in a mixture with the histamine H\(_2\)-receptor antagonist mepyramine. The concentrations of the peptides were chosen to give cutaneous responses of approximately the same order of magnitude as histamine, 10 \(\mu\)g/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 IIb. Flare responses in mm\(^2\) (mean ± S.E.M.) induced by putative peptide neurotransmitters, protamine, histamine and Compound 48/80

<table>
<thead>
<tr>
<th>Agent</th>
<th>Exp. no.</th>
<th>(n)</th>
<th>Flare area (mm(^2)) for different conc. of the agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>I</td>
<td>6</td>
<td>(10^{-7}) M: 36.2±7.5; (10^{-6}) M: 504.0±50.7; (10^{-5}) M: 892.2±103.0; (10^{-4}) M: 1192.5±131.4</td>
</tr>
<tr>
<td>Substance P</td>
<td>II</td>
<td>5</td>
<td>(10^{-7}) M: 0; (10^{-6}) M: 127.7±65.5; (10^{-5}) M: 830.8±118.6; (10^{-4}) M: 1056±138.3</td>
</tr>
<tr>
<td>[Gln(^n)]-neurotensin</td>
<td>III</td>
<td>5</td>
<td>(10^{-7}) M: 0; (10^{-6}) M: 53.2±32.8; (10^{-5}) M: 451.0±65.5; (10^{-4}) M: 645.8±59.3</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>I</td>
<td>6</td>
<td>(10^{-7}) M: 127.7±65.5; (10^{-6}) M: 830.8±118.6; (10^{-5}) M: 883±31.9; (10^{-4}) M: 834.0±143.0</td>
</tr>
<tr>
<td>Secretin</td>
<td>I</td>
<td>6</td>
<td>(10^{-7}) M: 0; (10^{-6}) M: 83.3±33.3; (10^{-5}) M: 393.3±188.8; (10^{-4}) M: 993.8±257.5</td>
</tr>
<tr>
<td>Protamine</td>
<td>V</td>
<td>4</td>
<td>(10^{-7}) M: 127.7±65.5; (10^{-6}) M: 830.8±118.6; (10^{-5}) M: 53.2±32.8; (10^{-4}) M: 83.0±38.0</td>
</tr>
<tr>
<td>Histamine</td>
<td>VI</td>
<td>8</td>
<td>(10^{-7}) M: 48.9±23.5; (10^{-6}) M: 121.0±64.8; (10^{-5}) M: 504.0±89.3; (10^{-4}) M: 950.1±97.1</td>
</tr>
<tr>
<td>Compound 48/80</td>
<td>VI</td>
<td>8</td>
<td>(10^{-7}) M: 48.9±23.5; (10^{-6}) M: 121.0±64.8; (10^{-5}) M: 504.0±89.3; (10^{-4}) M: 950.1±97.1</td>
</tr>
</tbody>
</table>
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 [Gln⁵]-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 H₁-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 & Erdős, 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-

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**Fig. 2.** Inhibitory effects of mepyramine on (a) itch, and (b) flare responses induced by VIP (10⁻⁶ M), substance P (3×10⁻⁹ M), [Gln⁵]-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.

**Fig. 3.** Histamine release from rat peritoneal mast cells induced by VIP, substance P, [Gln⁵]-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 dilysoine. 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]-neurotensin are practically inactive in other biological systems (7). Our finding that [Gln]-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 transport 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.

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