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Mast cell activation – a receptor-independent mode of substance P action?

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Substance P is a representative of a group of amphiphilic neuropeptides which act as mast cell secretagogues. Our experiments with some new substance P derivatives suggest that these effects are dependent on two structural elements: (i) a hydrophobic chain which is not essentially a peptide, and (ii) a hydrophilic part with two positively charged amino acids. The mast cell triggering effect is unlikely to be mediated by a selective substance P receptor, but has strong similarities to the mode of action of polycations.

Mast cell; Substance P; Histamine release

1. INTRODUCTION

A number of different amphiphilic neuropeptides receptor agonists were found to induce mediator release from rat peritoneal mast cells via non-cytolytic mechanisms. These peptides are substance P, neurotensin [1,2], bradykinin [1], somatostatin, vasoactive intestinal polypeptide [2], and dynorphin [3]. However, substance P antagonists [1,2], LHRH antagonists [4] and IgE fragments [5] are amphiphilic peptides which also act as mast cell secretagogues.

Except for neurotensin, the effective doses are in the micromolar range and clear-cut evidence for the presence of the corresponding specific membrane receptor at the mast cell membrane is lacking. Consequently, a receptor-independent mode of mast cell triggering may depend on structural properties common to all of these peptides. Based on previous work on structure-activity relationships (e.g. [1,6,7]), we put forward these hypotheses by examining the histamine-releasing effect of a number of accordingly designed substance P (SP) fragments and analogues, among them five representatives of a new group of SP derivatives composed of an SP-related N-terminal tetrapeptide and non-peptide chains.

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Abbreviations: SP, substance P; Ahx, 6-aminohexanoic acid; Dhp, $\Delta^{3,4}$-dehydroproline; Cpa, *p*-chlorophenylalanine; -$C_{12}H_{25}$, dodecyl; -$C_{17}H_{35}$, stearyl

2. MATERIALS AND METHODS

2.1. *Histamine release*

Peritoneal mast cells were obtained from male Wistar rats (400–500 g) and male Golden hamsters (200–250 g) as described [7] and used without further purification if not stated otherwise. The histamine liberation assay was performed as in [7] using a tyrode of the following composition (mM): 130 NaCl, 2.7 KCl, 5.6 glucose, 0.1 $CaCl_2$, 1 $MgSO_4$, 10 Hepes, pH 7.4.

Briefly, 11000 mast cells per sample were incubated in polypropylene tubes with the corresponding releasing agent for 10 min at 37°C, subsequent to temperature equilibration (5 min). The peptide-induced histamine release was completed within less than 1 min. Each test was performed in quadruplicate. From dose-response

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curves, each consisting of 6–9 points, the concentration of the histamine-releasing agent to induce 50% of the maximal response (ED_{50}) was calculated and expressed as $pD_2 = -\log ED_{50}$. Spontaneous histamine release was less than 10% and was subtracted from the induced release.

2.2. *Peptides and histamine liberators*

The peptides used here were predominantly synthesized by conventional peptide synthesis in solution. This was previously described in detail for the following compounds: SP, SP(5–11) peptide amide, [Met(O)^{11}]-SP, [Met(O_2)^{11}]-SP [8]; [Tyr^8]-SP [9]; [Nle^{11}]-SP, [Cpa^{7,8},Nle^{11}]-SP [10]; and N^{α},N^{ϵ}-bis(gluconyl)-SP(1–11). The synthesis of N-terminal tetrapeptides and [D-Phe^9]-SP will be published elsewhere.

SP(1–4)-NH-$(CH_2)_2$-NH-$C_{17}H_{35}$ and SP(1–4)-(ϵAhx)$_4$ amide were synthesized by stepwise elongation in a two-phase system using Fmoc-amino acid chlorides [12]. The purity of the peptides used in this study was 95–98% according to HPLC analysis.

The retention times of SP and its derivatives were determined using a LiChrosorb RP 18 column which was operated by a Shimadzu LC-6A HPLC equipment and expressed relative to SP (SP=1).

Histamine liberators were obtained from the following sources: Poly(L-lysin) (Acris), mellitin (Serva), protamine sulphate (Hoffmann-La Roche), A23187 (Calbiochem), palytoxin (gift from Professor Habermann, Giessen, FRG), compound 48/80 (Sigma).

3. RESULTS

The amino acid sequence of SP is Arg-Pro-Lys-Pro-Gln-Gln-Phe-Gly-Leu-Met-NH_2, this being the amidated, naturally occurring form. In a first series of experiments, the pD_2 values ($pD_2 = -\log ED_{50}$) of the histamine-releasing effect of a number of SP derivatives were determined using both rat and hamster peritoneal mast cells. The latter cells were found to be less responsive. Substitutions which increase the hydrophobicity of the C-terminal heptapeptide ([Nle^{11}]-SP, [Cpa^{7,8}, Nle^{11}]-SP, [D-Phe^9]-SP) have little effect on the pD_2 value, whereas analogues with decreased hydrophobicity in the same region ([Tyr^8]-SP, [Met(O)^{11}]-SP, [Met(O_2)^{11}]-SP) show a diminution of the histamine-releasing potency of the peptide (fig.1). The blockade of two positive charges in the N-terminal segment [N^{α},N^{ϵ}-bis(gluconyl)-SP] renders SP inactive up to a concentration of 10^{-4} M.

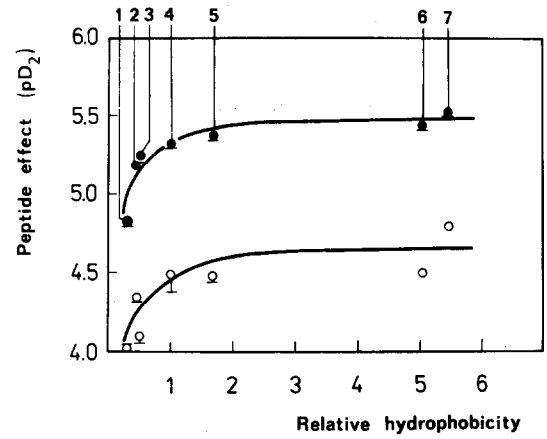

Fig.1. The histamine-releasing activity of SP derivatives correlates with the relative hydrophobicity of the whole molecule, which was determined by HPLC as described in section 2. Modifications were restricted to the hydrophobic, C-terminal part of SP: 1, [Met(O)^{11}]-SP, 2, [Met(O_2)^{11}]-SP; 3, [Tyr^8]-SP; 4, SP; 5, [Nle^{11}]-SP; 6, [Cpa^{7,8},Nle^{11}]-SP; 7, [D-Phe^8]-SP. The maximal histamine release was between 60 and 75% of the total. Closed points: rat peritoneal mast cells; Spearman-Rang correlation coefficient $r_s = 1.0$; open points: hamster peritoneal mast cells, $r_s = 0.955$, $p < 0.01$ (means of 2–4 experiments ± SE).

Neither the N-terminal tetrapeptide SP(1–4) and its derivatives (SP(1–4) amide, [Lys^1]-SP(1–4), [Ac-Arg^1,Lys(Ac)^3]-SP(1–4)) nor the C-terminal heptapeptide SP(5–11) induced histamine release from both rat and hamster mast cells in the concentration range 5×10^{-7}–10^{-4} M.

The substitution of the C-terminal pentapeptide SP(5–11) by different non-peptide chains led to a group of hybrid compounds which act as secretagogues via a non-cytolytic, energy-dependent mechanism in the concentration range examined. This was shown by the absence of any effect after pretreatment of the mast cells with antimycin A (2 μM) in the absence of glucose. The three dodecylamine derivatives are up to 50-fold more potent histamine releasers than SP itself and

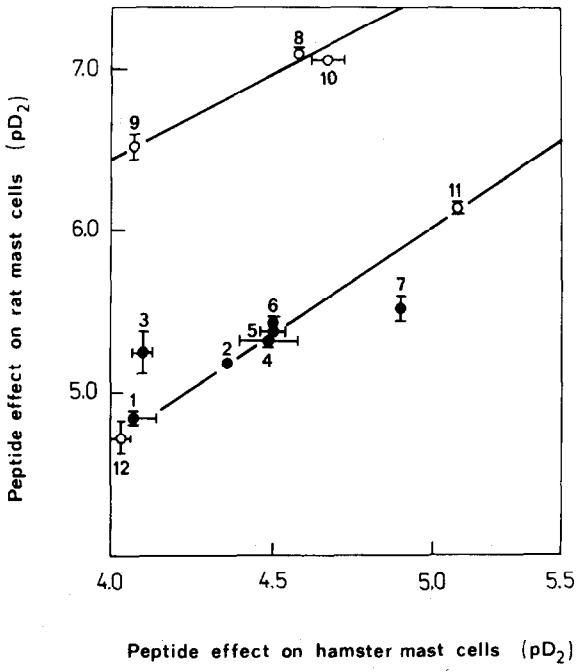

Fig.2. Correlation between the pD_2 values for the effect of SP derivatives on rat and hamster peritoneal mast cells. For compounds 1–7 see fig.1. The numbers 8–12 represent the following new hybrid compounds: 8, SP(1–4)-NH-$C_{12}H_{25}$; 9, [Dhp^4]-SP(1–4)-NH-$C_{12}H_{25}$; 10, [Lys^1]-SP(1–4)-NH-$C_{12}H_{25}$; 11, SP(1–4)-NH-$(CH_2)_2$-NH-$C_{17}H_{35}$; 12, SP(1–4)-NH-$(\epsilon Ahx)_4$. The maximal histamine release of 8–12 was between 70 and 80% of the total. The linear regression coefficient for the bottom regression line was $r = 0.908$, $p < 0.001$. Closed points, peptide derivatives; open points, hybrid compounds (means of 2–5 experiments ± SE).

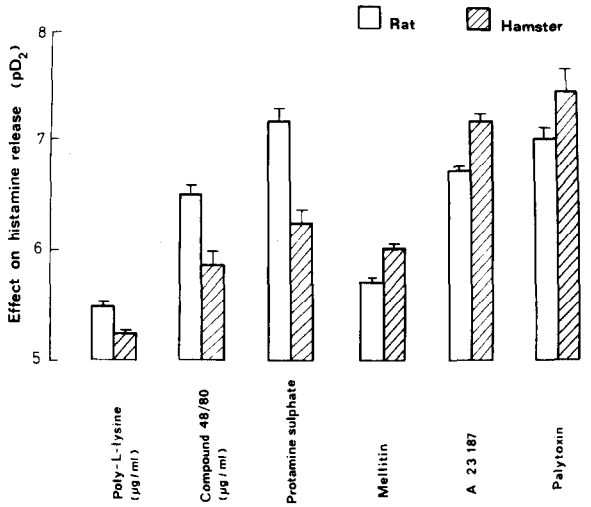

Fig.3. Rat peritoneal mast cells respond more effectively to polycation histamine liberators than hamster mast cells do. This is not the case with the other secretagogues used (means of 2–4 experiments ± SE). The maximal histamine release was between 60 and 80% except for the lytic agent mellitin (95% of total).

do not fit into the correlation of the pD_2 values of the other SP derivatives as shown in fig.2. The results were nearly identical if purified rat peritoneal mast cells (90–95% purity) were used (Repke et al., in preparation).

Dodecylamine acts as a lytic agent by an antimycin A-insensitive mechanism on both hamster ($ED_{50} = 8.7 \pm 2.3\ \mu M$) and rat peritoneal mast cells ($ED_{50} = 8.0 \pm 0.1\ \mu M$).

The substitution of SP(5–11) by -NH-$(CH_2)_2$-NH-$C_{17}H_{35}$ and -NH-$(\epsilon Ahx)_4$ led to a medium and a weak histamine releaser respectively, which fit into the linear correlation of the pD_2 values of the peptide derivatives of SP (fig.2).

As shown in fig.3, hamster mast cells respond weakly to polycationic secretagogues when compared with the same cell type of the rat. With other histamine releasers, however, an inverse potency ratio was found.

4. DISCUSSION

The most comprehensive collection of data on the structure-activity relationship of a neuropeptide-induced mediator release from mast cells has been accumulated for SP [1,7]. The stimulation of mast cells by presynaptically released SP plays a physiological role in the frame of neurogenic inflammation [13]. Therefore, this peptide was chosen as a model compound in our studies.

The following conclusions can be drawn from our results.

(i) Neither the hydrophilic nor the hydrophobic part of the SP molecule or its derivatives can trigger non-cytolytic histamine release. This confirms previous data from others [1,7].

(ii) Amino acid substitutions resulting in increased hydrophobicity of the C-terminal part of SP slightly increase the mast cell triggering potency whereas the incorporation of hydrophilic residues has the inverse effect. This fits into the previously

described strong agonistic effects of the hydrophobic SP receptor antagonists on rat mast cells [1,2].

(iii) The blockade of two of the three positive charges converts SP into an inactive compound although the N-terminal part is still hydrophilic. A minimal number of two positively charged amino acids appears to be essential for the mast cell triggering effect [1,6,7,15]. However, the nature of these amino acids does not seem to be crucial since compounds 8–10 of fig.2 are nearly equipotent.

(iv) Although of essential importance for the interaction of SP and its derivatives with the presently known SP receptor subtypes [14], the amino acid composition of the hydrophobic pentapeptide SP(5–11) is irrelevant for its mast cell triggering action. This is demonstrated by the new hybrid compounds, three of which are among the most potent histamine releasers described so far. In accord with (ii), the derivative with the more hydrophilic chain [SP(1–4)-(ϵAhx)$_4$ amide] is a very weak histamine releaser.

(v) As detailed in [15], the histamine release induced by the model compound SP(1–4)-NH-$C_{12}H_{25}$ is completed within less than 30 s, requires temperatures above 16°C, is independent of external calcium, and can be inhibited by amphiphilic compounds with one or two positive charges. So far, this model compound was found to behave similarly to SP in every respect.

(vi) The dodecylamine derivatives are much more potent on rat mast cells compared to hamster mast cells. This difference might be due to the triggering of calcium influx rather than to protein kinase C activation (Repke, H. et al., in preparation).

(vii) Hamster peritoneal mast cells are much less responsive towards polycations but not to other histamine liberators when compared to the corresponding cell type from the rat. Since the same difference was found with SP and all of its derivatives, we consider this as a further clue that the mode of action of these compounds is similar to that of polycations [15].

The hydrophobic amino acids of SP and other amphiphilic neuropeptides are likely to form an α-helix after insertion into a lipid membrane [16,17]. An association with the hydrophobic phase of the membrane can also be expected for the dodecyl and stearyl chains used in four of our hybrid compounds. It might be speculated that the three positive charges within the hydrophilic part of these compounds and SP itself become associated with negatively charged components of the membrane surface, thus resulting in their decreased mobility which might be the initial event of mast cell activation similar to the action of polycations. Previously, peptides of the same structural feature as discussed above were found to decrease the mobility of band 3 proteins or erythrocytes. The extent of this effect was directly correlated with their histamine-releasing potency [5].

This receptor-independent mechanism of mast cell activation by SP may be physiologically relevant at high local peptide concentrations in the vicinity of presynaptic terminals [13] and may also apply for the action of other neuropeptides on mast cells [1–6].

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