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Synthesis of new substance P analogues releasing histamine from rat peritoneal mast cells

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Summary — New analogues of the *N*-terminal fragment of substance P [Arg-Pro-Lys-Pro, SP(1–4)] were synthesized and their activities on histamine release from rat peritoneal mast cells were compared. The potency of these compounds decreases in the following order (in abbreviation): SP(1–4)-C_{12} > C'_{12}-SP(1–4)-OCH_3 > C'_{12}-SP(1–4)-OH, H-Lys-Pro-C_{12} and SP. Benzalkonium chloride, a competitive antagonist of peptide-induced histamine release from rat mast cells, inhibits the effect of SP and SP analogues with IC_{50} in the range of micromolar concentrations. SP(1–4)-C_{12} and C'_{12}-SP(1–4)-OCH_3 have been found to be 10-fold more active than SP on the GTPase activity of purified G proteins (G_o/G_i). The lack of clear structural relationships for agonist activities supports a receptor-less mechanism for histamine release by SP and its analogs. This effect could be elicited by a direct stimulation of G proteins.

mast cells / substance P / histamine release / G proteins / benzalkonium chloride

Introduction

Various compounds, polycations, venoms and peptides such as compound 48/80, polymixin B, mastoparan, mast cell degranulating peptide, lectins, anaphylatoxins, somatostatin, bradykinin and substance P are known to cause mediator release from mast cells and basophils (for review, see [1]). Among these compounds, substance P (SP) is a neuropeptide distributed throughout the peripheral and central nervous system [2, 3]. SP-containing neurons form a close association with mast cells [4] and SP is reported to be involved in inflammatory responses including neurogenic inflammation [5, 6], immediate and delayed hypersensitivity [7] and arthritis [1, 8]. Extensive structure–activity studies of SP-related peptides and of other peptides on rat peritoneal mast cells suggest that basic amino acid residues lysine and (or) arginine are essential for histamine releasing activity [9]. The *N*-terminus of SP (Arg-Pro-Lys-Pro) was found to be responsible for histamine-release. A progressive shortening of the C-terminal sequence of SP produces a decrease in its activities [10]. Substitutions of the *N*-terminal fragment of SP, especially the coupling with dodecylamine, dramatically raise the histamine releasing property [11, 12]. These data suggest that the insertion of a hydrophobic moiety into lipidic membranes facilitates a positively charged peptide fragment to bind receptor sites [13, 14]. Some peptides, natural toxins and polyamines can directly activate G proteins and the hypothesis of SP binding to a membrane receptor on mast cells has been questioned [15–22]. Interestingly, Higashijima *et al* [17] recently showed that benzalkonium chlorides (BAC) can inhibit or stimulate purified G proteins according to the subtype of G protein considered, G_i and G_o, respectively. BAC has been proposed previously as a selective inhibitor of histamine release from mast cells induced by polyamines such as compound 48/80 or by peptides [23]. The mechanism of inhibition involves a competition with the trigger [24].

To further investigate the structure–activity relationships of compounds releasing histamine from rat peritoneal mast cells, we synthesized some analogs of the most active agonist, SP(1–4)-C_{12} [25] and a molecule resembling benzalkonium chloride designed to find the structural factors responsible for the inhibitory activity.

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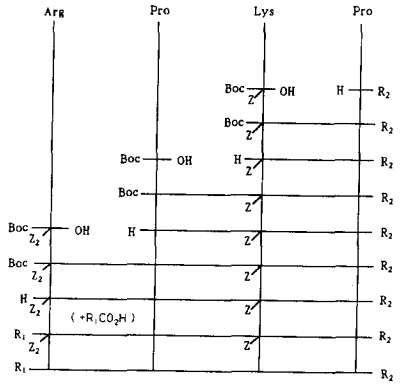
Abbreviations: AcOH, acetic acid; C_{12}, -$NH(CH_2)_{11}$-CH_3; C'_{12}, CH_3-$(CH_2)_{10}$-CO; DIPEA, *N*-ethyldiisopropylamine; DMF, dimethylformamide; AcOEt, ethyl acetate; HCl, hydrochloric acid; IBCF, isobutylchloroformate; MeOH, methanol; NMM, *N*-methylmorpholine; SP, substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2); TFA, trifluoroacetic acid. Other abbreviations used are those recommended by the IUPAC-IUB Commission (*Eur J Biochem* (1984) 138, 9–37).

Chemistry

All peptides were synthesized by conventional solution methods (see scheme 1 and text). Boc peptides were deprotected with TFA and the chain elongation was performed using IBCF/NMM as coupling reagents. Various protocols were used to obtain free peptides. Purifications were accomplished by chromatography or by crystallization. Purities of the final products were checked by TLC, amino acid analysis and fast atom bombardment (FAB) mass spectra.



Scheme 1. Synthesis of $SP(1–4)C_{12}$ and C'_{12}-$SP(1–4)$-OCH_3. R_1 = H, CH_3-$(CH_2)_{10}$-CO-[=C'_{12}]; R_2 = -OCH_3, -NH-$(CH_2)_{11}$-CH_3[= C_{12}].

Materials and methods

All amino acids were of the L-configuration. Peptides and intermediate compounds were obtained by conventional methods [26] or purchased from Fluka or Bachem. Melting points were taken on a Leitz apparatus. Optical rotations were determined with a Perkin–Elmer 121 polarimeter (10 cm cell). FAB mass spectra were obtained on a MS 80 RF Kratos spectrometer.

The amino acid composition was determined with an Applied Biosystem amino acid analyzer, after acid hydrolysis in 6 N HCl at 110 °C for 24 h. Purity of final peptide derivatives was checked by analytical HPLC (Waters 900 analytical system, 220 nm). HPLC purifications of protected peptides were performed on a Waters Delta Prep 3000 preparative system coupled to a Waters Lambda-Max Model 480 UV detector and to a Waters 600 E system controller-computer with a solvent mixture of MeOH–CH_2Cl_2 (5:95, v:v) on a Preppak Cartridge Porasil column (125 Å, 15–20 µm). For the purification of protected intermediates, liquid chromatography was performed on silica gel 60 (230–400 mesh, Merck) columns. Purifications of final compounds were carried out on SP-Sephadex C-25 or Sephadex G-10 columns. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates from Merck, using the following solvent systems (v:v): A) $CHCl_3$–MeOH (95:5); B) propanol–NH_3 (1:1); C) phenol–H_2O (75:25); D) butanol–AcOH–pyridine–H_2O (30:6:20:24). Reagents ninhydrin and *O*-toluidine after chlorination were sprayed on the TLC plates [27]. Samples were considered pure when they showed single spots in more than one solvent system .

Coupling procedure

Method A

To a stirred solution of the Boc-protected amino acid or Boc-protected peptide in CH_2Cl_2 or in a mixture of CH_2Cl_2 and DMF, 1 equiv of NMM was added. The mixture was cooled to less than –10°C, treated with IBCF (1 equiv), and allowed to react for 5 min. A precooled solution (–10°C) of the amino component trifluoroacetate (1.1 mmol) in CH_2Cl_2 or DMF was added, then NMM (1.1 equiv) was added to the mixture. The reaction mixture was stirred for 1 h at –10°C, and kept for 2–3 h at room temperature. The reaction was followed by TLC, then diluted with EtOAc (100 ml). The solution or suspension was washed consecutively with water, 5% citric acid, 5% NaHCO, and saturated brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was crystallized from appropriate solvents or purified by column chromatography.

Deprotection procedures

Method B

Boc-protecting groups were removed by treating the peptide with 50% TFA in CH_2Cl_2 for 20–40 min at 0°C. The mixture, after evaporation of the solvents, was triturated with ether and evaporated *in vacuo*. The last procedure was repeated twice.

Method C

The protected peptide was treated with anhydrous hydrogen fluoride (10 ml) containing anisole (1 ml) for 30 min at 0°C in a Kel-F apparatus. After removal of hydrogen fluoride under nitrogen, the residue was dried *in vacuo* before being dissolved in cold water (10 ml). The aqueous solution was immediately applied to a SP-Sephadex C-25 column (1.5 x 40 cm, H^+ form) washed with cold water (1.5 l). The peptide was eluted with 0.4 N hydrochloric acid, and the acidic eluate was evaporated. The residue was chromatographed on SP-Sephadex C-25 column (61 x 1.5 cm) using a gradient from water to 0.6 N HCl as solvent, or on Sephadex G-10 column (0.5 x 40 cm) using 0.1 N HCl as solvent. It was finally lyophilized to give the peptide chloride.

Method D

The peptide methyl ester was dissolved in a mixture of water-dioxane (2:1) 20 ml, and treated with 1 N NaOH (1.2 eq) for 3 h at room temperature. The solution was neutralized with 1 N HCl at 0°C, chromatographed on SP-Sephadex C-25 column (61 x 1.5 cm) using a gradient from water to 0.6 N HCl as eluent and evaporated to dryness *in vacuo*.

Method E

Catalytic transfer hydrogenations were carried out in a mixture of absolute ethanol (or methanol) and AcOH (2:1, v:v) at room temperature and a gentle stream of nitrogen was passed through the reaction mixture. An equal weight of 10% palladium-carbon (per protecting group) was added following by the addition of 1,4-cychlohexadiene (10 eq). The reaction

Table I. Physicochemical properties of peptides and compounds. All peptides showed correct FAB mass spectra and amino acid analyses. Optical rotation: d = 10 cm in methanol, T = 22°C, Na/cont (589 nm). TLC solvent system: A) $CHCl_3$–MeOH (95:5); B) propanol–NH_3 (1:1); C) phenol–H_2O (75:25); D) butanol–AcOH–pyridine–H_2O (30:6:20:24) (v:v).

Structure	*mp (°C)*	*Crystal solvents*	α_D	*TLC R_f*	*Yield (%)*
Boc-Pro-C_{12}	53	MeOH	–31.9	0.60(A)	92
Boc-Lys(Z)-Pro-C_{12}	Oil	–	–36.9	0.56(A)	75
Boc-Pro-Lys(Z)-Pro-C_{12}	Oil	–	–54.7	0.38(A)	86
Boc-Arg(Z,Z)-Pro-Lys(Z)-Pro-C_{12}	40	MeOH	–44.5	0.43(A)	52
SP(1–4)-C_{12}	Decomp	–	–55.9[a]	0.69(B)	30
Boc-Lys(Boc)-Pro-C_{12}	Oil	–	–40.3	0.50(A)	72
H-Lys-Pro-C_{12}	200	MeOH/Et_2O	–23.3	0.65(B)	95
Boc-Lys(Z)-Pro-OCH_3	Oil	–	–36.5	0.43(A)	86
Boc-Pro-Lys(Z)-Pro-OCH_3	Oil	–	–56.3	0.28(A)	96
Boc-Arg(Z,Z)-Pro-Lys(Z)-Pro-OCH_3	64	MeOH	–45.5	0.31(A)	50
SP(1–4)-OCH_3	163	MeOH	–51.1	0.23(C)	59
C'$_{12}$-SP(1–4)-OCH_3	Oil	–	–66.0	0.67(B)	90
C'$_{12}$-SP(1–4)-OH	Oil	–	–51.0	0.54(B)	95
CH_3-CO-C_{12}	55	AcOEt	–	0.35(A)	80
Boc-NH-$(CH_2)_5$-CO-Pro-C_{12}	73	AcOEt	–24.1	0.48(A)	93
$(CH_3)_3N^+$-$(CH_2)_5$-CO-Pro-C_{12}·Cl^-	177	AcOEt	–21.4	0.42(D)	84

[a]In MeOH–0.1 N HCl aq (50:50) (v:v).

mixture was filtered through Celite, and evaporated *in vacuo* to dryness [28].

Chemical data of final and intermediate compounds are reported in tables I and II.

Synthesis of CH_3-CO-C_{12}

CH_3-$(CH_2)_{11}NH_2$ in CH_2Cl_2 was reacted with Ac_2O (1 eq) in the presence of DIPEA (1 eq) at 0°C for 1 h, then at room temperature for 1 h. The reaction was followed by TLC. The mixture was then diluted with EtOAc. The solution was washed consecutively with water, 5% citric acid, 5% $NaHCO_3$, and saturated brine. The organic phase was dried over $MgSO_4$, stirred with vegetal carbon, filtered through a Celite bed, and evaporated to dryness. The residue was recrystallized from AcOEt at –10°C.

Synthesis of H-Lys-Pro-C_{12}

Boc-Lys(Boc)OH was coupled to H-Pro-C_{12} according to *Method A*. The adducts were treated with a solution of dry hydrochloric acid in anhydrous ether. The peptide chloride was precipitated and recrystallized twice from MeOH/Et_2O.

Synthesis of Boc-NH-$(CH_2)_5$-Pro-C_{12}

According to *Method A*, Boc-NH-$(CH_2)_5$-COOH, obtained by reaction of di-*tert* butyl dicarbonate [26] with 6-aminocaproic acid, was reacted with H-Pro-C_{12} in CH_2Cl_2. The product was purified using a column of silica gel 60 (20–400 mesh, Merck) and eluted by 1.5 MeOH in CH_2Cl_2 (v:v), then recrystallized twice from AcOEt.

Synthesis of $(CH_3)_3N^+$-$(CH_2)_5$-CO-Pro-C_{12}·Cl^-

According to *Method B*, the Boc protecting group of Boc-NH-$(CH_2)_5$-CO-Pro-C_{12} was removed by TFA. The resulting

Table II. HPLC[a,b] and TLC[c] data.

No	*Molecule*	*Retention time (min)*	R_f
1	SP(1–4)-OH	37.0[b]	0.14
2	SP(1–4)-OCH_3	35.0[b]	0.27
3	SP(1–4)-C_{12}	20.8[a]	0.46
4	H-Lys-Pro-C_{12}	4.2[a]	0.47
5	C_{12}-SP(1–4)-OH	20.2[a]	0.45
6	C'$_{12}$-SP(1–4)-OCH_3	17.5[a]	0.58
9	$(CH_3)_3N^+$-$(CH_2)_3$-CO-Pro-C_{12}-Cl^-	22.0[b]	0.42
10	Boc-NH-$(CH_2)_5$-CO-Pro-C_{12}	29.5[a]	0.78

[a]HPLC Waters 900, UV = 220 nm, Column Novapak-C18 (4 μ, 3.9 mm x 150 mm). Purity of peptides was found better than 95% by integration at 220 nm. Solvent system: A) CH_3CN–H_2O (30:70, v:v) and Pic® B-7 (7.5 ml/500 ml); B) CH_3CN–H_2O (50:10, v:v) and Pic® B-7 (7.5 ml/600 ml). Pic B-7 Reagent is an aqueous solution of heptane sulfonic acid (pH 3.5) from Millipore. Gradient program: 0–5 min, 100% A; 5–25 min, 100% A to 100% B; 25–35 min, 100% B. [b]Solvent system: A) H_2O and Pic® B-7 (7.5 ml/500 ml); B) CH_3CN–H_2O (50:10 v:v) and Pic® B-7 (7.5 ml/600 ml). Gradient program: 0–10 min, 100% A; 10–30 min, 100% A to 100% B; 30–40 min, 100% B. [c]Butanol–AcOH–pyridine–H_2O (0:6:20:24, v:v), TLC on silica gel plates 60 F254 from Merck.

product in EtOH was reacted with a large excess of CH_3I in the presence of anhydrous CaO powder (excess) for 2 h. The mixture was filtered, evaporated and crystallized in AcOEt, giving a yellow compound. The compound was redissolved in absolute EtOH, and stirred with an excess of freshly precipitated AgCl [23]. The product was centrifuged, filtered, evaporated and recrystallized twice from AcOEt.

Deprotection of peptides

BocArg(*Z*,*Z*)-Pro-Lys(*Z*)-Pro-C_{12} was deprotected according to *Method B* followed by *Method E*. The free peptide was chromatographed on SP-Sephadex C-25 column (61 x 1.5 cm) using as eluent a gradient from water to 1 N HCl containing 15% methanol. The aqueous solution of the final peptide was purified through a column of AG® 11A8 (Bio-Rad Lab) (30 x 1 cm) and lyophilized to give a white powder.

SP(1–4)-OCH_3 was obtained after deprotection following *Method E* and other protected peptide analogues of SP(1–4) were deprotected according to *Method C*. C'_{12}SP(1–4)-OH was obtained from C'_{12}SP(1–4)-OCH_3 after hydrolysis, following *Method D*.

Biology

Biological experiments were performed as described previously [15]. Briefly, peritoneal mast cells were harvested from male Wistar rats (Iffa-Credo, l'Arbresle, France) weighing 300–350 g. Mast cells were preincubated with 0.3 mM calcium at 37°C for 5 min. Compounds were added with different concentrations and the reaction was quenched by the addition of ice-cold buffer solution after 5 min. These results are reported in table III. The EC_{25} and EC_{50} correspond to the peptide concentration that elicits 25 or 50% of histamine release, respectively.

GTPase assay

Purification of the mixed preparation of G proteins (G_o and G_i) from calf brain membranes was obtained by successive elution from DEAE–Sephacel (Pharmacia), AcA_{34} (LKB) and heptylamine–Sepharose columns as described by Sternweis and Robishaw (29). G proteins were reconstituted into phospholipid vesicles and the GTPase activity was determined as described previously [16] using the [γ-^{32}P]GTP (New England Nuclear-DuPont, Boston, MA).

Results and discussion

Table III shows that the potency of compounds to release histamine from rat peritoneal mast cells decreases in the following order: SP(1–4)-C_{12} > C'_{12}-SP(1–4)-OCH_3 > H-Lys-Pro-C_{12}, C'_{12}-SP(1–4)-OH and SP. Among these compounds, SP(1–4)-C_{12} and C'_{12}-SP(1–4)-OCH_3 are more potent (40 times and 16-fold, respectively) than SP itself. H-Lys-Pro-C_{12} and C'_{12}-SP(1–4)-OH have a potency similar to that of SP while CH_3-$(CH_2)_{10}$-COOH, CH_3-CO-C_{12}, NH_2-$(CH_2)_{11}$-CH_3, H-Lys-Lys-Lys-OH, H-Lys-Lys-OH and SP(1–4) are devoid of activity or have a weak activity. Figure 1 shows the potency of SP(1–4)-C_{12} and C'_{12}-S P (1–4)-OCH_3 compared to SP.

SP(1–4)-C_{12} was found previously to be nearly equipotent as Lys'-SP(1–4)-C_{12} [11, 25, 30]. All active derivatives are composed of two essential components:

Table III. Histamine release from rat peritoneal mast cells. Mast cells were pre-incubated with 0.3 mM calcium at 7°C for 5 min. Values are means ± SEM of four separate experiments. Total amount of histamine (100%) = 25 µg/ 10^6 cells.

No	*Compound*	*EC_{25} (µM)*	*EC_{50} (µM)*
	CH_3-$(CH_2)_{11}$-NH_2[a,b]	No effect	
	CH_3-$(CH_2)_{10}$-COOH[b]	No effect	
	CH_3-CO-C_{12}	> 100	
Substance P		3.6 ± 1.2	8.2 ± 1.8
1	SP(1–4)-OH[b]	> 100	
2	SP(1–4)-OCH_3	> 100	
3	SP(1–4)-C_{12}	0.09 ± 0.02	0.2 ± 0.05
4	H-Lys-Pro-C_{12}	2.8 ± 0.1	8.5 ± 0.3
5	C_{12}-SP(1–4)-OH	3.4 ± 0.3	7.8 ± 1.2
6	C_{12}-SP(1–4)-OCH_3	0.22 ± 0.03	0.57 ± 0.08
7	H-Lys-Lys-OH[b]	>100	
8	H-Lys-Lys-Lys-OH[b]	3 7.4 ± 5.3	75.2 ± 12.5
9	$(CH_3)_3N^+$-$(CH_2)_5$-CO-Pro-C_{12}·Cl^-	8.5 ± 2.3	97.8 ± 14.3
10	Boc-NH-$(CH_2)_5$-CO-Pro-C_{12}	94.5 ± 10.4	

[a]A lytic effect was observed at concentrations over 10 mM; [b]chemicals purchased from Bachem.

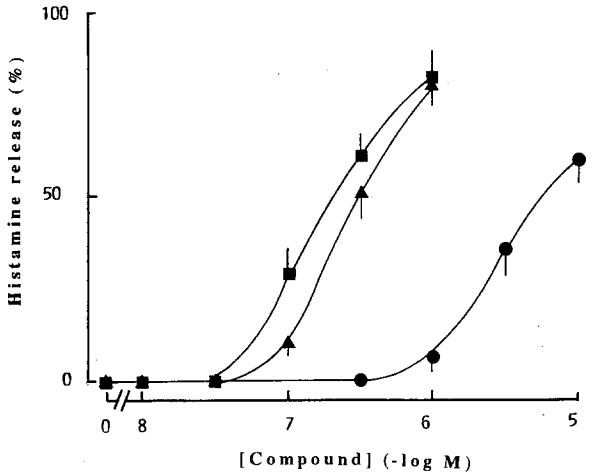


Fig 1. Effect of substance P and substance P analogues on mast cell histamine secretion. Mast cells were purified as described in *Materials and methods* and were preincubated at 37°C for 5 min. Mast cells were then challenged by various concentrations of substance P (●), SP(1–4)-C_{12} (■) and C'_{12}-SP(1–4)-OCH_3 (▲) for 5 min and the reaction was stopped by adding ice-cold buffer. Values are means ± SEM of four separate experiments.

a hydrophobic chain linked to a dipeptide (Lys-Pro) or to a tetrapeptide (Arg-Pro-Lys-Pro). The potency of these peptide derivatives seems to increase rapidly with the number of dipeptide fragments (Lys-Pro or Arg-Pro). The receptor site of Arg-Pro may thus accommodate the fragment Lys-Pro and might contain negative charges. The presence of negative charges in this receptor site can also account for the difference in activity of C'_{12}-SP(1–4)-OCH_3 and C'_{12}-SP(1–4)-OH.

Comparing SP(1–4)-C_{12} with C'_{12}-SP(1–4)-OCH_3 or C'_{12}-SP(1–4)-OH, one finds that the substitutions of the tetrapeptide (Arg-Pro-Lys-Pro) at the N- or C-terminus with a long aliphatic radical afford almost equipotent products. The observed decrease must be due to the global loss of positive charges in the molecule rather than to a specific positioning of the ligand into its target.

Interestingly, some neurokinin analogues release histamine from mast cells. For instance, [Arg^6-Sar^9-$Met(O_2)$]-SP(6–11) is a weak agonist. It becomes inactive after the N-acetylation of the arginine residue [9]. However, N-alkylation with a long aliphatic chain of arginine in C'_{12}-SP(1–4)-OCH_3 and C'_{12}-SP(1–4)-OH does not abolish the activity. These results may be explained by an interaction of the aliphatic chain with the lipidic membrane and/or by an assisted membrane insertion towards the molecular target. In addition, the order of potency on rat peritoneal mast cells SP-OMe > SP [11] and C'_{12}-SP(1–4)-OCH_3 > C'_{12}-SP(1–4)-OH correlates also with the increased hydrophobicity of the molecule at the peptide C-terminus.

The inhibitory activity of a series of BAC analogs increases with the length of the aliphatic substituted chain [23]. In figure 2, we showed that the most active analogue, BAC-C_{14} inhibited the histamine release induced by substance P, SP(1–4)-C_{12} and C'_{12}-SP(1–4)-OCH_3 in a dose-dependent manner between 1–15 μM with the following IC_{50}: 2.46 +0.11 μM, 4.41 + 0.29 μM, 6.14 + 0.39 μM, respectively. Taking these data and the structure of BAC into account, we designed a new molecule 9 (scheme 2) based on the structure of H-Lys-Pro-C_{12}, an agonist equipotent to SP (table III). Another molecule **10**, containing a Boc amino group instead of the quaternary ammonium group of **9**, was proposed to evaluate the possible activity of the backbone.

Results in table III show that compound **9** is an agonist less potent than H-Lys-Pro-C_{12} to release histamine and that compound **10** is devoid of activity although these three compounds have a similar backbone. These results confirm the correlation between the agonist activity and the number of positive charges in the molecule.

The lack of inhibitory activity of **9** and **10** on SP-induced histamine release (data not shown) may result

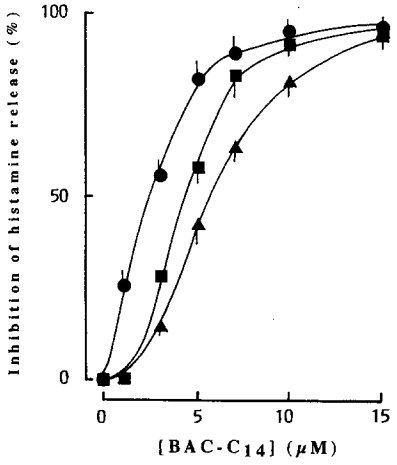


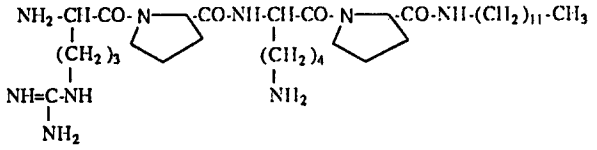
Fig 2. Inhibitory effect of BAC-C_{14} on histamine release induced by substance P and substance P analogues. Purified mast cells were preincubated with various concentrations of BAC-C_{14} for 5 min. Mast cells were challenged by 10 μM of substance P (●), 0.5 μM of SP(1–4)-C_{12} (■) and 1 μM of C'_{12}-SP(1–4)-OCH_3 (▲) were added for 5 min and histamine secretion was stopped as described in figure 1. Results are expressed as a percentage of inhibition and the histamine release of controls in the absence of BAC-C_{14} were 41 ± 2.2%; 66 ± 4.3 %; 61% ± 6.9 %, respectively. Values are means ± SEM of four separate experiments.

from the absence of a free aromatic group existing in BAC. Antagonist properties are sometimes associated with free aromatic residues, as observed in smooth muscle preparations for a series of antagonists of SP: [D-Trp^{7,9,10}]SP, [D-Pro^2, D-Phe^7]SP(l–7) and [D-Pro^2, D-Trp^7]SP(1–7) [32]. Conversely, [D-Trp^{7,9,10}]SP [9] which contains the fragment SP(1–4) and compound 48/80 [24], composed of a buried aromatic polymeric backbone bristled with methylethylamino spikes trigger histamine release.

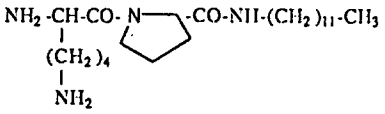
In addition, the structure of compound **9** is more rigid than BAC due to the introduction of a proline in the middle of the backbone. The bend induced by proline and the presence of peptide bonds may be favorable for histamine release but not for inhibitory activity. The antagonist activity of BAC seems to result from the association of three structural requirements: a quaternary ammonium group linked to an aromatic group and to a long aliphatic chain.

Lysine releases histamine at high concentrations. Trilysine is more potent than dilysine, and polylysines are very active [1, 23]. The activity of lysine, much higher in the polymer than in the monomer, has been attributed to the frequency of collision between lysine residues and the membrane matrix. These collisions would synergistically increase in the polymer, after binding one of the residues to the membrane [31]. The potencies of these compounds depend on their charges and their degrees of polymerization. The same explanation could also account for polycationic compounds such as polyarginins and polyamines. In contrast, SP(1–4)-C_{12} and SP(1–4) derivatives, as well as other peptides, which do not contain a large number of cationic residues, might follow a more complex mechanism involving the interaction with G proteins [15–22]. In addition, the hydrophobic moiety (*eg*, C'_{12} or C_{12}) might cooperate to reach G proteins located on the internal face of the plasma membrane.

The effects of SP(1–4)-C_{12}, C'_{12}-SP(1–4)-OCH_3 and SP on the GTPase activity of purified G proteins are shown in figure 3. The potencies of SP(1–4)-C_{12}, C'_{12}-SP(1–4)-OCH are nearly similar whereas SP is very significally less active. These results correlate to those of histamine release on mast cells. The difference of the concentrations range between the effects of these compounds on histamine release from mast cells and on the GTPase activity of purified G proteins might explain the importance of plasma membrane negative charges in the peptide–plasma membrane interaction.

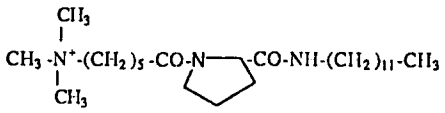


Arg-Pro-Lys-Pro-C12

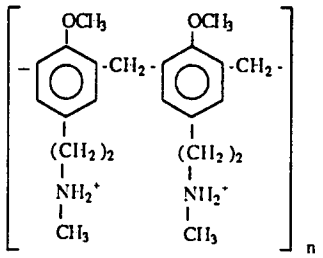


Lys-Pro-C12

9

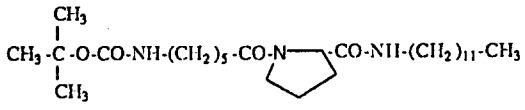


Hybrid compound

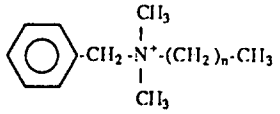


Compound 48/80

10



Boc-NH-$(CH_2)_5$-CO-Pro-C12



Benzalkonium chloride (BAC)
n=1...11...16

Scheme 2. Structure of agonists and antagonists.

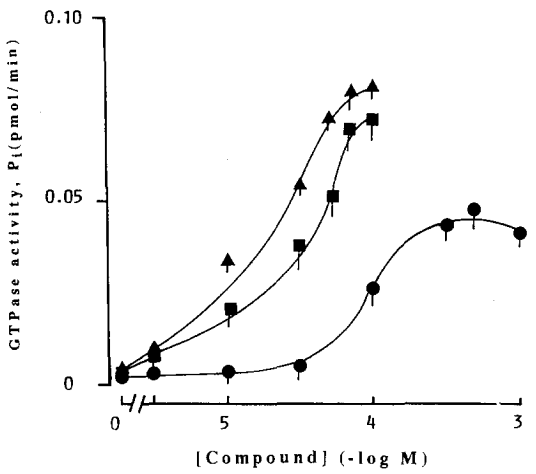


Fig 3. Effect of substance P and substance P analogues on the GTPase activity of purified G proteins (G_o/G_i). The GTPase activity was measured on reconstituted G proteins (1 pmol) after 5 min of incubation with various concentrations of substance P (●), SP(1–4)-C_{12} (■) and C'_{12}-SP(1–4)-OCH_3 (▲) in the presence of 1 μM of [γ-^{32}P]GTP and 0.1 mM of $MgSO_4$. Values are means ± SEM of four separate experiments.

In conclusion, an important modification in the structure of SP(1–4) analogues: SP(1–4)-C_{12} or C'_{12}-SP(1–4), does not lead to dramatic changes in histamine release from mast cells. This is quite different from the usual observations for peptide receptors. These compounds may trigger histamine release *via* other pathways. Furthermore, agonist and antagonist targets may be different. The observed activity could be mediated by activation of pertussis toxin-sensitive G proteins as suggested for substance P [16], various peptides [16, 33, 34] and polyamines [5]. Our results concerning compound **9** and BAC suggest that the inhibitory effect is due to the aromatic quaternary ammonium group.

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