Eur. J. Med. Chem. **23** (1988) 243—247

Original paper

Activity of the C-terminal part of substance P on guinea pig ileum and trachea preparations I. *N*-Acylated pentapeptides SP(7—11)*

Robert MICHELOT, Michel MAYER, Sylviane MAGNENEY,
Paul PHAM VAN CHUONG, Pascal SCHMITT and Pierre POTIER

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France

(Received July 8, 1987, accepted January 15, 1988)

Summary — Acylated pentapeptide X—Phe—Phe—Gly—Leu—$MetNH_2$ analogs of the substance P (7—11) sequence were synthesized by solution method and their spasmogenic activities were evaluated on guinea pig ileum (GPI) and trachea (GPT). Pentapeptide SP(7—11) had the lowest potency and its *N*-acylation increased its activity in both tests, with some derivatives being more active than SP itself. Results obtained on GPI suggest a close dependence of activity upon structural factors in the vicinity of the Phe^7 N-terminus, whereas the activity on GPT seems more dependent upon the hydrophobicity of the analogs.

Résumé — **Activité de la partie C-terminal de la substance P sur les préparations de trachée et d'iléon de cobaye I. Pentapeptides *N*-acylés SP(7—11).** *Des pentapeptides X—Phe—Phe—Gly—Leu—$MetNH_2$, analogues de la séquence substance P (7—11), ont été synthétisés en solution et leur activité spasmogénique a été évaluée sur l'iléon (GPI) et sur la trachée de cobaye (GPT). Le pentapeptide SP(7—11) est le fragment présentant la plus faible activité dans les deux tests et sa N-acylation conduit à des dérivés plus actifs que la SP elle-même. Les résultats suggèrent pour GPI une dépendance étroite entre l'activité et des facteurs structuraux dans la région N-terminale de Phe^7 tandis que l'activité dépendrait plus, pour GPT, de la lipophilie des dérivés pentapeptides.*

substance P (7—11) / SP fragments / smooth muscle preparations / guinea pig ileum / guinea pig trachea

Introduction

Substance P (SP) belongs to the tachykinin family and shares with these peptides the common C-terminal sequence: —Phe—Y—Gly—Leu—$MetNH_2$ (Y = Phe for SP and Val, Tyr or Ile for other tachykinins) [1]. This fragment is able to promote full spasmogenic activity on smooth muscle preparations but is, by itself, a weak agonist. The major potency is usually observed with larger fragments, such as the hepta or octa C-terminal sequences [2—4].

Substance P: Arg^1—Pro^2—Lys^3—Pro^4—Gln^5—Gln^6—Phe^7—Phe^8—Gly^9—Leu^{10}—$Met^{11}NH_2$

Many recent reports have established that modifications starting from the C-terminal hexapeptide sequence may lead to active agonists [5—8] and these data seem to rule out the possibility that shorter fragments afford valuable agonists or antagonists. In fact, Niedrich *et al.* reported [9], more than ten years ago, that *N*-acylation of pentapeptide terminal sequences of tachykinins could greatly modify their activities and, more recently, that the *N*-acylated pentapeptide SP(7—11) could be more active than SP itself on smooth muscle [10].

In the present communication, we examine the activity of *N*-acylated pentapeptides SP(7—11) on guinea pig ileum (GPI) and guinea pig trachea (GPT) in order to evaluate to what extent slight modifications at the N-terminus of this fragment could enhance the activity. GPI remains the most reliable pharmacological test for tachykinins despite the heterogeneity of receptors [11, 12] and GPT has been reported to be more sensitive to shorter SP fragments [13].

Chemistry

The SP pentapeptide analogs have been prepared by the classical stepwise solution method and by fragment cou-

*This work was presented in part at the *Symposium on Substance P and Neurokinins*, Montreal, July 1986.

pling. The N-terminal tripeptide Boc—Phe—Phe—Gly—OH was built up by stepwise N-terminal elongation and subsequently condensed to the C-terminal dipeptide H—Leu—Met—NH_2. The same methodology was used to prepare the C-terminal hexapeptide. Acylation of the deprotected pentapeptide was obtained by reaction with pentachlorophenyl esters, 2,4,5-trichlorophenyl esters or with anhydrides. Acylated peptides were purified by recrystallization and/or by chromatography.

Results and Discussion

The biological activities were determined on guinea pig ileum and on guinea pig trachea and are expressed in comparison with the activity of SP on the same preparations. Activities of *N*-acylated peptides are reported in Table I.

Boc-peptide **9b** is poorly soluble in aqueous medium but is as active as the more water-soluble **9a**. On the contrary, when the lipophilic character is very pronounced, activity is greatly reduced (**10**). 5) Comparison of the relative activities of **8a**, **8b**, **11**, **12**, **13**, **14**, **15** and **17** reveals a sharp dependence upon structural factors in the vicinity of the Phe[7] terminus part. Nevertheless, additional studies, in progress in our laboratory, are still needed in order to definitively determine the exact nature of the high activity of **13**.

On GPT, structural requirements are different: 1) The presence of a free amino group at the N-terminus of the pentapeptide SP(7—11) is unfavorable (**5a**, **5b**, **8a** and **8b**). The apparent exception to this rule (**7a** and **7b**) might be attributed to the possible metabolism by the tissue of Gln[5] into pyroglutamyl (**16**) which is more active. Discrepancies have already been reported for the activities on smooth muscle of fragments containing glutamine [4]. 2) Hexapeptide **7a** and heptapeptide **7c** were reported

Table I. Relative activities *(RA)*[a] of peptides X—Phe—Phe—Gly—Leu—Met—NH_2=X-5-SP.

X	RA_{GPI}	RA_{GPT}	No.
Arg—Pro—Lys—Pro—Gln—Gln—	100	100	SP
H—	0.1	0.7	**5a**
Boc = *t*-Butyloxycarbonyl—	1.3	32	**5b**
Gln—	38	512	**7a**
Boc—Gln—	33	508	**7b**
Gln—Gln—	89[b]	224[c]	**7c**
Tyr = 4-OH—C_6H_4—CH_2—$CH(NH_2)$—CO—	47	6	**8a**
Boc—Tyr—	56	61	**8b**
Aminocaproyl = NH_2—$(CH_2)_5$—CO—	31	152	**9a**
Boc-aminocaproyl—	30	774	**9b**
Pentadecanoyl = CH_3—$(CH_2)_{13}$—CO—	0.7	7	**10**
C_6H_5—CH_2—CO—	74	292	**11**
2-OH—C_6H_4—CH_2—CO—	53	567	**12**
4-OH—C_6H_4—CH_2—CO—	357	742	**13**
4-OH—C_6H_4—CH_2—CH_2—CO—	31	111	**14**
C_6H_5—CH=CH—CO—	3	84	**15**
Pyroglutamyl—	46	774	**16**
4-F—C_6H_4—CH_2—CO—	28	624	**17**

[a] RA as compared to SP = 100 × $(EC_{50})_{SP}/(EC_{50})_{X\text{-}5\text{-}SP}$; for SP: $(EC_{50})_{GPI} = 1.76 \times 10^{-9}$ M; $(EC_{50})_{GPT} = 1.78 \times 10^{-7}$ M ($p < 0.05$; $n = 6$). GPI: atropine 5.2×10^{-6} M; GPT: indomethacin 4.1×10^{-6} M.
[b] Calculated from [15].
[c] Calculated from [12].

Activities on GPI may be analyzed as follows: 1) As reported originally by Bienert *et al.* [10], compound **13** is the best agonist. 2) Gln[6] does not, by itself play, an important role and its replacement by Tyr (**8a**, **8b**) or pyroglutamyl (**16**) is almost equivalent. This residue has been previously thought to form intramolecular hydrogen bonds with the C-terminal carboxamide group in SP [14] and this hypothesis could also provide an explanation of the highest activities observed with the C-terminal sequences in some tests. Our data do not confirm this hypothesis. 3) N-protection with a Boc group does not result in great changes in activity (**7a**, **7b**, **8a**, **8b**, **9a** and **9b**). 4) A slight increase in lipophilicity does not enhance activity: the

to be more potent than SP on GPT [13]. Peptides **7b**, **9b**, **13**, **16** and **17** are also much better agonists than SP and this could be tentatively explained both by a difference in lipophilicity by Boc-protection and/or by the presence of either a cyclic substituent (**13**, **16**, **17**) or again by a chain which fits into the binding site. Spatial requirements could explain the differences in activities between compounds **11**, **12**, **13**, **14** and **15**. 3) The low activity of compound **10** may be due to diffusion through membranes, since the duration from the onset to the final response was much greater than that usually observed with other peptides (result not shown).

In both GPI and GPT assays, pentapeptide **5a** represents

the minimal active sequence. Protection of the amino group by Boc (**5b**, **7b**, **9b**) does not reduce the activity in GPI and even enhances it in GPT. Slight modifications at the N-terminus may induce large changes in activity.

It has appeared, from structure—activity studies on smooth muscle preparations, that C-terminal hepta- and octapeptides are the most potent sequences [15, 16]. Based on these sequences, many attempts have been made to increase activity by modification of side chains [17—22] or backbone [23—27] or by increasing the solubility through coupling to a sugar moiety [10, 28] or replacing methionine by sulfonium or sulfoxide groups [10]. However, significant improvements were generally not observed. To account for the conformational requirements for activity in SP, models for SP have been proposed involving interactions between Gln^5, Gln^6 and the terminal carboxamide group [14]. Such a hypothesis seemed to be supported by NMR and circular dichroism (CD) studies of SP in various solvents, such as methanol or water [29], until studies on the prediction of preferred conformation, orientation and accumulation of SP on lipid membranes indicated an α-helical conformation for the C-terminal part of SP [30].

Recently, even shorter fragments, *e.g.* C-terminal hexapeptide analogs, have been shown to be powerful and selective agonists for SP-P [8] and SP-N [31] receptor subtypes. Peptides shorter than the C-terminal heptapeptide fragment could then be responsible for both activity and selectivity. Our results support this idea and confirm that pentapeptide **13** is far more active than SP on GPI. This increase in activity is probably due to a specific interaction of the *para*-hydroxyphenyl acetic group. Moreover, results obtained on GPT indicate a strong role of lipophilicity and are in agreement with reports proposing as a first step of interaction, an accumulation of the peptide in the plasma membrane. This accumulation was attributed for SP and other positively charged analogs to ionic interactions [30, 32] but could also depend upon hydrophobicity.

GPI in the presence of atropine has been considered as a typical SP-P receptor model [8, 33], whereas GPT seems to be more neurokinin A (or neurokinin B) specific [12, 13]. This preliminary study will be developed on more appropriate preparations [12] that should give better insights into the specificity of modified pentapeptides on SP receptor subclasses.

Experimental protocols

Biological activities

Assays were performed as described before [34]: ileum in the presence of atropine and trachea with indomethacin, according to the procedures described by Regoli *et al.* [11]. Experimental data were processed for statistical evaluation following an Eadie—Hofstee technique [35].

Chemistry

Materials and methods

Reagents and solvents used for the reactions were of analytical grade. Amino acids, all of the L-configuration, were from Fluka and Boc-protected derivatives were prepared according to Keller *et al.* [36]. Substance P was purchased from Peninsula, San Diego, CA.

Melting points were determined on a Leitz apparatus and are reported corrected. Optical rotations were measured on a Perkin—Elmer 121 precision polarimeter (±0.002°). Fast atom bombardment (FAB) (MH^+) mass spectra were obtained from the Service de Spectrométrie de Masse at the Institut de Chimie des Substances Naturelles (ICSN) on an MS 80 RF Kratos spectrometer. Elemental microchemical analyses were performed by the Service de Microanalyses of the ICSN. Amino acid analyses were performed on an LKB Biochrom 4400 coupled to an Enica 10 computerized integrator. Column chromatography was performed on Merck silica gel 60. Thin—layer chromatography (TLC) was performed on analytical Merck silica gel 60 F_{254} plates in the following solvent systems (v/v): A: chloroform/methanol (9/1); B: chloroform/methanol/acetic acid (95/5/3). Spots were detected with ninhydrin or by a spray of *o*-tolidine after chlorination [37]. High pressure liquid chromatography (HPLC) was performed on a Millipore—Waters Nova-pack C-18 column (3.9 mm i.d. × 15 cm) eluted with methanol/sodium phosphate buffer (pH 3.0, 0.1 M, 70/30 v/v, 1 ml/min, UV detection 254 nm). Purity of peptides subjected to biological assays was estimated to be better than 98%.

Peptide coupling procedure

To a stirred solution of Boc—amino acid or Boc—peptide (1 eq.) in dimethylformamide (DMF) an equivalent of *N*-methylmorpholine (NMM) was added. The mixture was cooled at −20°C and isobutylchloroformate (IBCF) (1 eq.) was added to the mixture and allowed to react 5 min. A cooled solution (−20°C) of the amino component (trifluoroacetate or hydrochloride) (1.1 eq.) in DMF and NMM (1.1 eq.) was poured rapidly and reacted for 1—3 h at −15°C. The end of the reaction was monitored by TLC (ninhydrin detection). After completion, DMF was evaporated under reduced pressure (5 mm Hg) and the crude oil was dissolved in ethyl acetate and washed with 5% $NaHCO_3$ aqueous solution, NaCl saturated solution, 5% citric acid aqueous solution and NaCl solution. The organic layer was dried over Na_2SO_4 and processed for purification by crystallization or chromatography on a silica column (CH_2Cl_2/MeOH).

Deprotection

Boc-protected peptides were treated with 2 N HCl/glacial acetic acid (10 eq., 1 h) [38] at room temperature or by trifluoroacetic acid (TFA) (10 eq., 40% in CH_2Cl_2 and 2% anisole, 1 h) at 0°C. After evaporation of the solvents, the residue was triturated in anhydrous diethyl ether and dried under vacuum, over KOH pellets. Purity of deprotected peptides was checked by TLC and amino acid analysis.

Preparation of activated esters

Pentachlorophenyl esters and 2,4,5-trichlorophenyl esters were synthesized according to Kovacs *et al.* [39], Bodanszki and Bodanszki [40] and Handford *et al.* [41]; see Table II for physical data.

Table II. Physical constants of X-activated esters.

Compd.	Formula (MW)	Purification or crystallization method	mp (°C)
8b	$C_{20}H_{18}NO_5Cl_5$ (529.6)	AcOEt/*n*-hexane	166—167
9b	$C_{17}H_{20}NO_4Cl_5$ (479.6)	AcOEt	92—96
11	$C_{14}H_7O_2Cl_5$ (384.5)	AcOEt	105
12	$C_{14}H_9O_3Cl_3$ (331.6)	Merck silica column cyclohexane/AcOEt (97/3)	40—45
13	$C_{14}H_9O_3Cl_3$ (331.6)	AcOEt/*n*-hexane	112—116
14	$C_{15}H_9O_3Cl_5$ (414.5)	AcOEt/*n*-hexane	120—121
15	$C_{15}H_9O_2Cl_3$ (327.5)	AcOEt/petroleum ether	93—95
16	$C_{11}H_6NO_3Cl_5$ (377.4)	EtOH	192—195
17	$C_{14}H_8O_2FCl_3$ (333.6)	AcOEt/*n*-hexane	80—81

Coupling of activated esters
This was done according to Bienert *et al.* [42]; see Table III for physical constants of peptides.

Boc—Phe—Gly—OMe ***1***
Boc—Phe—OH (21.25 g, 80 mmol) dissolved in DMF (200 ml) and NMM (8.8 ml, 80 mmol) was reated at —15°C with IBCF (10.45 ml, 80 mmol) 5 min and with HCl, Gly—OMe (11.04 g, 88 mmol) in DMF (150 ml) and NMM (9.7 ml, 88 mmol). The mixture was processed according to the general procedure. Yield: 29.2 g (85.2%); mp: 91—92°C, lit. 90—91°C [43]. $[\alpha]_D^{20} = -6.4°$ (*c* 1.0, MeOH). TLC R_f(A) = 0.87. TLC R_f(B) = 0.72. Anal. $C_{17}H_{24}N_2O_5$: C, H, N. MH^+ 337.

Boc—Phe—Phe—Gly—OMe ***2***
Boc—Phe—OH (17.5 g, 66 mmol) was coupled to *N*-deprotected **1** (16.37 g, 60 mmol). Yield: 20.30 g (70%). mp: 162—164°C. $[\alpha]_D^{21} = -27°$ (*c* 1.0, MeOH). TLC R_f(A) = 0.88; R_f(B) = 0.62. Anal. $C_{26}H_{33}N_3O_6$: C, H, N. MH^+ 484.

Boc—Phe—Phe—Gly—OH ***3***
2 (15.45 g, 30 mmol) was dissolved in MeOH/dioxane (3/1, v/v) and stirred at 0°C for 5 h at room temperature in the presence of 1 N NaOH (1.5 eq.), completion of the reaction was monitored by TLC. After evaporation of the solvents, the products were extracted in ethyl acetate as in the general procedure. Yield: 11.99 g (85%). mp: 149—150°C. TLC R_f(A) = 0.16; R_f(B) = 0.36. Anal. $C_{26}H_{31}N_3O_6$: *C*, H, N. MH^+ 470.

Boc—Leu—Met—NH₂ ***4***
Boc—Leu, H_2O (12.46 g, 50 mmol) was reacted with L-methionine amide, HCl (10.17 g, 55 mmol), NMM (12.8 ml, 105 mmol), IBCF (6.55 ml, 50 mmol). Yield: 16.9 g (93.6%). mp: 156—158°C (recryst. ethyl acetate/*n*-hexane) lit. [8] mp: 156—158°C. $[\alpha]_D^{24} = -33.8°$ (*c* 1.0 ,DMF). TLC R_f(A) = 0.67; R_f(B) = 0.54. Anal. $C_{16}H_{31}N_3O_4S$: C, H, N. MH^+ 362.

Boc—Phe—Phe—Gly—Leu—Met—NH₂ ***Boc SP(7—11) 5b***
3 (9.38 g, 20 mmol) was reacted with HCl, Leu—Met—NH_2 (6.55 g, 22 mmol), IBCF (2.6 ml, 20 mmol), NMM (4.26 ml, 42 mmol) in DMF. Purification was performed by multiple precipitations from its solution in DMF by diethylether. Yield: 7.78 g (54.6%). mp: 225—227°C (lit. [43] 223—227°C). $[\alpha]_D^{20} = -32.4°$ (*c* 1.0, DMF), lit. [42] —33°. TLC R_f(A) = 0.65; R_f(B) = 0.47. HPLC k' = 6.14. Amino acid analysis: Phe 2.0, Gly 1.02, Leu 1.05, Met 1.05. MH^+ 714.

Boc—Gln—Phe—Phe—Gly—OMe ***6***
As described in [43].

Boc—Gln—Phe—Phe—Gly—Leu—Met—NH₂ ***Boc SP(6—11) 7b***
The same method as that used for **5b**: deprotected **6** (568 mg, 0.95 mmol), HCl, Leu—$MetNH_2$ (367 mg, 1.05 mmol), IBCF (0.124 ml, 0.95 mmol), NMM (0.200 ml, 2.0 mmol). Yield: 724 mg (90%). mp: 235—238°C. $[\alpha]_D^{24} = -43.2°$. Anal. $C_{41}H_{60}N_8O_9S$: C, H, N. MH^+ 842. Amino acid analysis: Glx 1.04, Phe 2.08, Gly 1.00, Leu 0.92, Met 0.97. TLC R_f(A) = 0.28; R_f(B) = 0.1. HPLC k' = 3.0.

Pentadecanoyl—Phe—Phe—Gly—Leu—Met—NH₂ ***10***
10 was prepared by the symmetrical anhydrides method [44]: pentadecanoic acid (170 mg, 0.7 mmol) in CH_2Cl_2 and DMF was added at 0°C to a filtered solution of dicyclohexylcarbodiimide (75 mg, 0.35 mmol) and **5a** (250 mg, 0.4 mmol) in DMF (10 ml), previously reacted at 0°C for 3 min. The reaction mixture was stirred at ambient temperature for 24 h. The precipitate of dicyclohexylurea was filtered and washed with 5 ml of DMF. The filtrate was processed as described in the general coupling method above. Purification on a silica gel column Merck 60 (CH_2Cl_2/MeOH, 95/5). Yield 94 mg (32%). mp: 232—237°C. $[\alpha]_D^{24} = -40.9°$ (*c* 1.0, DMF). TLC R_f(A) = 5.3; R_f(B) = 5.2. HPLC k' = 96.5 (MeOH/sodium phosphate buffer, 0.1 M, pH 3.0; 85/15 v/v, Nova-pak C-18, 1 ml/min (and not eluted under the standard conditions). Amino acid analysis: Phe 2.0, Gly 1.04, Leu 0.99, Met 0.93. Anal. $C_{46}H_{72}O_6N_2S$: C, H, N. MH^+ 837.

Table III. Physical constants of peptides X—Phe—Phe—Gly—Leu—Met—NH_2.

Compd.	Coupling method[a]	Yield (%)	Formula (MW)	MH^+	mp (°C)	TLC		$[\alpha]_D^{24}$ (*c* 1, DMF)	k' (HPLC)
						R_f(A)	R_f(B)		
5b	[b]	54	$C_{36}H_{52}N_6O_7S$ (712.9)	713	225—227	0.65	0.47	—33°	6.14
7b	[b]	90	$C_{41}H_{60}N_8O_9S$ (841.0)	841	235—238	0.28	0.10	—43°	3
8b	P	65	$C_{45}H_{61}O_9N_7S$ (876.1)	876	201—202	0.42	0.33	—34°	5.35
9b	P	67	$C_{42}H_{72}N_7O_8S$ (826.1)	826	232—235	0.53	0.42	—39°	6.64
10	[b]	32	$C_{46}H_{72}O_6N_6S$ (837.2)	837	232—237	0.53	0.52	—41°	[b]
11	P	90	$C_{39}H_{50}N_6O_6S$ (730.9)	731	261—265	0.51	0.44	—33°	13
12	T	83	$C_{39}H_{50}N_6O_7S$ (746.9)	747	229—231	0.42	0.38	—40°	3.14
13	T	72	$C_{39}H_{50}N_6O_7S$ (746.9)	747	220	0.51	0.19	—39°	1.35
14	P	56	$C_{40}H_{52}N_6O_7S$ (760.9)	761	240—243	0.49	0.22	—40°	1.71
15	T	88	$C_{40}H_{50}N_6O_6S$ (742.9)	743	263—264	0.49	0.48	—66°	6.28
16	P	55	$C_{36}H_{49}N_7O_7S$ (723.9)	724	227—232	0.34	0.15	—33°	0.85
17	T	66	$C_{39}H_{49}N_6O_6FS$ (748.9)	749	250—253	0.52	0.32	—45°	4.28

[a]T = trichlorophenylester, P = pentachlorophenylester.
[b]See Experimental protocols.

Acknowledgments

Support for this research by the Institut National de la Santé et de la Recherche Médicale (Contract no. 847007) and Centre National de la Recherche Scientifique (PIRMED ASP Neurodrogues) is gratefully acknowledged.

References

1 Erspamer V. (1981) *Trends Neurosci.* 4, 267
2 Bergmann J., Bienert M., Niedrich H., Mehlis B. & Oehme P. (1974) *Experientia* 15, 401
3 Bury R. W. & Mashford M. L. (1976) *J. Med. Chem.* 19, 854
4 Regoli D., Mizrahi J., D'Orléans-Juste P. & Escher E. (1984) *Eur. J. Pharmacol.* 97, 171
5 Poulos C., Brown J. R. & Jordan C. C. (1986) *J. Med. Chem.* 29, 1281
6 Lipkowski A. W., Drabarek S., Majewski T., Konecka A. M. & Sadowski B. (1981) *Experientia* 37, 499
7 Baizman E. R., Gordon T. D., Hansen P. E., Kiefer D., Lopresti D. M., McKay P. C., Morgan B. A. & Perrone M. H. (1983) *in: Peptides: Structure and Functions, Proceedings of the 8th American Peptide Symposium* (Hruby V. J. & Rich D. H., eds.), Pierce Chem. Co., Rockford, IL, p. 437—440
8 Laufer R., Gilon C., Chorev M. & Selinger Z. (1986) *J. Med. Chem.* 29, 1284
9 Niedrich H., Bergmann J., Bienert M., Albrecht E. & Oehme P. (1975) *in: Peptides, Proceedings of the 4th American Peptide Symposium* (Walter R. & Meienhofer J., eds.), Ann Arbor Sci., Ann Arbor, MI, p. 565—570
10 Bienert M., Forner K., Mehlis B. & Niedrich H. (1983) *in: Peptides 1982* Walter de Gruyter, New York pp. 517—520
11 Regoli D., D'Orléans-Juste P., Escher E. & Mizrahi J. (1984) *Eur. J. Pharmacol.* 97, 161
12 Regoli D., Drapeau G., Dion S. & D'Orléans-Juste P. (1987) *Life Sci.* 40, 109
13 Mizrahi J., Couture R. & Regoli D. (1982) *Pharmacology* 25, 39
14 Sandberg B. E. B. & Iversen L. L. (1982) *J. Med. Chem.* 25, 1009
15 Yanaihara N., Yanaihara C., Hirohashi M., Sato H., Iisuka Y., Hashimoto T. & Sakagami M. (1977) *in: Substance P* (Von Euler V. S. & Pernow B., eds.), Raven Press, New York, p. 27
16 Blumberg S. & Teichberg V. (1980) *in: Neurotransmitters and their Receptors* (Littauer U. Z., ed.), John Wiley & Sons, New York, p. 461
17 Couture R., Fournier A., Magnan J., Saint-Pierre S. & Regoli D. (1979) *Can. J. Physiol. Pharmacol.* 57, 1427
18 Kitagawa K., Ujita K., Kiso Y., Akita T., Nakata Y., Nakamoto N., Segawa T. & Yajima H. (1979) *Chem. Pharm. Bull.* 27, 48
19 Poulos C. P., Pinas N. & Theodoropoulos D. (1980) *Experientia* 36, 1104
20 Blumberg S. & Teichberg V. I. (1981) *Biochem. Biophys. Res. Commun.* 99, 752
21 Theodoropoulos D., Pinas N., Poulos C., Couture R., Mizrahi J., Escher E. & Regoli D. (1982) *Eur. J. Med. Chem.* 17, 527
22 Torigoe K., Katayama T., Sofuku S. & Muramatsu I. (1982) *Chem. Lett.* 4, 563
23 Chassaing G., Lavielle S., Ploux O., Julien S., Convert O., Marquet A., Beaujouan J. C., Torrens Y. & Glowinski J. (1984) *Peptides 1984, Proc. 18th Eur. Pept. Symp., Djurönäset, Sweden,* (Ragnarsson V., ed.), Almqvist and Wiksell, Stockholm, pp. 345—348
24 Theodoropoulos D., Poulos C., Gatos D., Cordopatis P., Escher E., Mirzahi J., Regoli D., Dalietos D., Furst A. & Lee T. D. (1985) *J. Med. Chem.* 28, 1536
25 Neubert K., Hartrodt B., Mehlis B., Rüger M., Bergmann J., Lindau J., Jakubke H. D. & Barth A. (1985) *Pharmazie* 40, 532
26 Cascieri M. A., Chicchi G. G., Freidinger R. M., Colton C. D., Perlow D. S., Williams B., Curtis N. R., McKnight N. T., Maguire J. J., Veber D. F. & Liang T. (1986) *Mol. Pharmacol.* 29, 34
27 Ewenson A., Laufer R., Chorev M., Selinger Z. & Gilon C. (1986) *J. Med. Chem.* 29, 295
28 Poujade C., Lavielle S., Torrens Y. & Marquet A. (1983) *Int. J. Pept. Protein Res.* 21, 254
29 Chassaing G., Convert O. & Lavielle S. (1986) *Eur. J. Biochem.* 154, 77
30 Schwyzer R., Erne D. & Rolka K. (1986) *Helv. Chim. Acta* 69, 1789
31 Wormser U., Laufer R., Hart Y., Chorev M., Gilon C. & Selinger Z. (1986) *EMBO J.* 5, 2805
32 Schwyzer R., Gremlich H.-U., Gysin B. & Fringeli U. P. (1983) *in: Peptides 1982, Proceedings of the 17th European Peptide Symposium* (Blaha K. & Malon P., eds.), Walter de Gruyter, Berlin, p. 55
33 Watson S. P., Sandberg B. E. B., Hanley M. R. & Iversen L. L. (1983) *Eur. J. Pharmacol.* 87, 77
34 Calas B., Michelot R., Le Caer J.-P., Cavé A., Parello J. & Potier P. (1987) *Int. J. Pept. Protein Res.* 29, 170
35 Zivin A. J. & Waud D. R. (1982) *Life Sci.* 30, 1407
36 Keller O., Keller W. E., Van Look G. & Wersin G. (1985) *Org. Chem.* 63, 160
37 Pataki G. (1966) *in: Techniques of Thin Layer Chromatography* Walter de Gruyter, Berlin, pp. 250
38 Bodanszki M. & Bodanszki A. (1984) *in: The Practice of Peptide Synthesis* Springer Verlag, Berlin, p. 169
39 Kovacs J., Kisfaludy L., Ceprini M. Q. & Johnson R. H. (1969) *Tetrahedron* 25, 2555
40 Bodanszki M. & Bodanszki A. (1984) *in: The Practice of Peptide Synthesis* Springer Verlag, Berlin, p. 120
41 Handford B. O., Hylton T. A., Preston J. & Weinstein B. (1967) *J. Org. Chem.* 32, 1243
42 Bienert M., Köller G., Wohlfeil R., Mehlis B., Bergmann J. & Niedrich H. (1979) *J. Prakt. Chem.* 321, 721
43 Sasaki N. A., Michelot R., Morgat J.-L., Genet R. & Potier P. (1986) *Int. J. Pept. Protein Res.* 27, 366
44 Sheehan J. C. & Hess G. P. (1955) *J. Am. Chem. Soc.* 77, 1067