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Design and Synthesis of Effective Antagonists of Substance P

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The agonist/antagonist activities of four background analogs of substance P (SP) facilitated design and synthesis of 12 new analogs to achieve effective antagonists. (D-Pro^2, D-Phe^7, D-Trp^9)-SP, (D-Pro^2, D-Trp^{7,9})-SP and (D-Arg^1, D-Phe^7, D-Trp^9)-SP showed no agonist activity; 9 analogs showed weak agonist activity of SP.

(D-Pro^2, D-Trp^{7,9})-SP was the most potent antagonist which at a concentration of 10^{-5} required a 3-fold increase in SP to allow a 50 % response by SP. (D-Pro^2, Lys^6, D-Phe^7)-SP and (D-Pro^2, D-pClPhe^7, D-Trp^9)-SP were also potent, and the antagonism was competitive.

For specific pairs of peptides, Lys^6 is a promising substituent. D-Trp^{7,9} was as effective as Lys^6, D-Phe^7. D-pClPhe^7 was three times as effective as D-Phe^7. D-Dln^6 was 1.33-fold better than D-Gln^5. D-Pro^2 and D-Pro^4 were equally effective. D-Pro^2 was 1.5 times as effective as D-Lys^3. D-Pro^2 may not be important. D-pClPhe^9 and D-Trp^9 were equally effective.

Von Euler and Gaddum[1] discovered the physiological actions of an entity in fractions from equine intestinal tissue which they names substance P (SP). Diverse biological and chemical investigations followed including progress on purification. In 1970, Chang and Leeman[2] isolated a sialogogic peptide from hypothalamic tissue which was proven to be identical in physiological activities and chemical properties to those described for SP.

Substance P is the undecapeptide, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, elucidated by Chang *et al.*[3] The physiological activities of SP include the contraction of the guinea pig ileum, the sialogogic effect in the rat,[4] depolarization of motor neurons of the spinal cord, and a lowering of blood pressure.[5]

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Jessell and Iversen[6] reported the release of SP from neurons of the brain stem is inhibited by the action of [D-Ala^2]-Met-encephalin, and interpreted this effect as support for the hypothesis that SP is a neurotransmitter in nociception.

Before the elucidation of the structure of SP, the chemistry of eledoisin and physalemin was elucidated as peptides which were later recognized to be closely related in sequence to that of SP.[7,8] The *C*-terminal pentapeptide moiety of these two tachykinins is similar to the *C*-terminal pentapeptide moiety of SP. The amino acid in position 8 is the only difference. Phe^8 is in SP, Ile^8 is in eledoisin, and Tyr^8 is in physalemin. The analogs of these tachykinins which have been described[9,10,11] are also analogs of SP, and provide information about the sequence – activity relationships of these peptides. These and subsequent studies revealed the importance of the substituents Phe^7, Leu^{10} and Met^{11}.

Despite these years of investigations and the innumerable available analogs, no meaningful progress was made on the design and synthesis of effective inhibitors of SP, eledoisin and physalemin.[12,13] As biological progress was made with the newly avaulable synthetic SP, the need for effective inhibitors of SP became emphasized.

Yamaguchi *et al.*[14] synthesized [D-Phe^7]-SP, which was found to have a weak antagonistic activity in the guinea pig ileum system. Leban *et al.*[15] found that [D-Leu^8, D-Phe^9]-SP antagonized SP, but only at high concentrations. [D-Pro^2]-SP exhibited some antagonistic activity.

Described herein are the syntheses and the chemical and biological properties of newly designed analogs which were based upon the structural features and the antagonistic activities of [D-Phe^7]-SP, [D-Leu^8, D-Phe^9]-SP and [D-Pro^2]-SP.

EXPERIMENTAL

The protected amino acids were purchased from Peninsula Laboratories, Inc., San Carlos, California. The benzhydrylamine hydrochloride resin was purchased from Beckman Inc., Palo Alto, California. All solvents (except TFA and isopropranol) were distilled before use. To check homogeneity, the peptides (5 μg in 5 μl of water) were chromatographed on precoated TLC plates (silica gel, 5 × 20 cm, Merck, Darmstadt, W. Germany) in the following solvent systems: I. $CHCl_3$ – conc. NH_4OH – CH_3OH = 60:20:45; II. EtOAc – Pyr – AcOH – H_2O = 5:5:1:3; III. n-BuOH – EtOAc – AcOH – H_2O = 2:2:1:1; IV. n-BuOH – Pyr – AcOH – H_2O = 30:30:6:24; V. i-PrOH – 1 N AcOH = 2:1. The spots on the developed thin layer plates were detected with the chlorine-o-tolidine reagent.

Synthesis of the peptides. The peptides were synthesized by the solid phase method[17] using a Beckman Model 990 Peptide Synthesizer. The benzhydrylaminehydrochloride resin (BHA-resin) was used as a solid support. The program of the synthesizer was divided into subprograms to increase the versatility of the synthesizer, as follows.

Deprotection: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 50 % TFA in CH_2Cl_2 containing 0.1 % indole 1 × wash, 2 min); 3. 50 % TFA in CH_2Cl_2 (deprotection, 30 min); 4. CH_2Cl_2 (2 × wash). Neutralization: 1. CH_2Cl_2 (2 × wash, 2 min); 2. Et_3N (10 % in CH_2Cl_2) (2 × wash, 2 min); 3. Et_3N (10 % in CH_2Cl_2) (Neutralization, 20 min); 4. CH_2Cl_2 (2 × wash, 2 min). DCC Coupling: 1. CH_2Cl_2 (2 × wash, 2 min); 2. Amino acid derivative in CH_2Cl_2 (delivery, transfer, mix, 2 min); 3. DCC (10 % in CH_2Cl_2) (delivery and mix, 180 min); 4. CH_2Cl_2 (2 × wash, 2 min). Active Ester Coupling: 1. CH_2Cl_2 (2 × wash, 2 min); 2. Amino acid derivative and 1-hydroxybenzotriazole in DMF (delivery, transfer, mix, 180 min); 3. CH_2Cl_2 (2 × wash, 2 min). Final Wash: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 2-ProOH (3 × wash, 2 min); 3. DMF (3 × wash, 2 min); 4. CH_2Cl_2 (3 × wash, 2 min). Wash after TFA treatment: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 2-ProOH (3 × wash, 2 min); 3. CH_2Cl_2 (3 × wash, 2 min). Acetylation: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 10 % Ac_2O and 10 % Pyr in CH_2Cl_2 (1 × wash, 2 min); 3. 10 % Ac_2O and 10 % Pyr in CH_2Cl_2 (Acetylation, 20 min); 4. CH_2Cl_2 (2 × wash, 2 min).

The first amino acid was attached to the resin by the program sequence 2-3-5. Before placing the resin into the reaction vessel, it was washed twice in a separate funnel with 25 ml of CH_2Cl_2/g resin to remove the finer particles. In all couplings, usually a 4 – 5-fold excess of the Boc-amino acid over the nitrogen content of the resin (nitrogen content was about 0.5 mequ/g dry resin) was used. This procedure generally resulted in a complete coupling reaction. If a negative ninhydrin color reaction[18] was not obtained, a second coupling using the same excess of the amino acid derivative was performed (program sequence 3 – 5). Then, the resin was acetylated (program sequence 7 – 5).

The next amino acid was attached by the program sequence 1-6-2-3-5. All amino acid derivatives were used as their Boc derivatives except Arg, which was used as Aoc-Arg(Tos). The ε-NH_3-group of Lys was protected by the Cl-Z group. For DCC coupling, all amino acids were dissolved in CH_2Cl_2 (0.5 – 1 mmol/ml). To dissolve Boc-Trp, it was necessary to add 10 % of DMF to the suspension. Gln was coupled to the resin by its Boc-Gln-ONp derivative using the active ester coupling program sequence 1-6-2-4-5. The Boc-Gln-ONp (4 – 5-fold excess over the N-content of the resin) was dissolved in DMF 1 mmol/ml), and 20 mg/ml 1-hydroxybenzotriazole was added as a catalyst.[19]

The volume of the solvents and the reagents used for washing and performing chemical reactions was about 10 ml/g resin. The acetylation mixture was freshly prepared before each use.

Cleavage of the peptides from the resin. After all of the amino acids had been coupled, the resin was dried overnight, *in vacuo*, by an oil pump. It was then treated with double-distilled and dried (over CoF_3) liquid hydrogen fluoride (10 ml/g resin) containing 10 – 25 % of distilled anisole for 1 h at 0 °C. Then, the HF was evaporated under reduced pressure and the residue was dried overnight, *in vacuo*, by an oil pump. The mixture was then extracted with EtOAc (25 ml/g resin), and then twice with 25 ml of 20 % AcOH, and once with 25 ml water. The combined aqueous layers were pooled and lyophilized to yield the crude peptide.

Purification of the crude peptide. Gel filtration. 200 mg of the crude peptide was applied to a column of Sephadex G-25 (100 × 2.5 cm) which was equilibrated with 6 % AcOH, and then chromatographed with the same solvent. Fractions of 10 ml were collected. The peptide was detected by spotting samples of the individual fractions on silica gel plates and chromatographing them in solvent system II. The fractions containing the product in a partially purified state were pooled and lyophilized. The yield was 60 – 130 mg of dry material.

First partition chromatography. The lyophilized material was applied to a column of Sephadex G-25 (3.5 × 53 cm). Before use, the column was first equilibrated with 1 l of lower phase of BuOH – 0.1 % AcOH – Pyr = 7:10:3, and then with 1 l of the upper phase of the same system. The sample was then dissolved in 3 – 5 ml of the upper phase, applied to the column and chromatographed. Fractions of 10 ml were collected (0.5 ml/min). The peptides, in general, were eluted in fractions 18 – 23 except for peptides V and VI, which were eluted with fractions

60 – 80. The fractions which contained the pure or nearly pure peptide were collected and lyophilized.

Second partition chromatography. When the product was not sufficiently pure, after having been chromatographed in the first two systems, it was further purified on a column of Sephadex G-25 (2.4 × 100 cm) prepared in the same manner as the first partition column, but with the upper phase of the solvent system BuOH-AcOH-H_2O = 4:1:5. Fractions of 10 ml were collected. The peptide was eluted in fractions 21 – 36 (flow rate: 0.5 ml/min).

Column chromatography on Sephadex LH 20. If not sufficiently pure, after the second partition chromatography, the peptide was chromatographed on a column of Sephadex LH 20 (2.5 × 100 cm) with the solvent system BuOH-AcOH-H_2O = 6:10:90. The peptide was eluted with fractions 35 – 45 (fraction size, 10 ml). The fractions containing the pure peptide were lyophilized.

High pressure liquid chromatography. The HPLC was performed on a Waters Liquid Chromatograph equipped with a Waters 660 Solvent Programmer. The samples were chromatographed either on a Waters analytical μ-Bondapak column (3.9 × 300 mm) or a Chrompak Lichrosorb 5 RP C_{18}-column (5 μ) (4.6 × 250 mm). For elution of the analogs, a linear gradient from 0 – 100 % of solvent system B in 25 min was used (solvent A: 0.1 M K-phosphate buffer, pH 3.0; solvent B: 50 % of solvent system A, 50 % CH_3CN). The flow rate was 2.0 ml/min. 10 μl of a 0.1 % solution of the peptide was injected. The eluted peptide was detected by its UV-absorbance at 210 nm.

Amino acid analysis. The automated amino acid analysis was performed on a Beckman Model 119 Automated Amino Acid Analyzer. The peptides were hydrolyzed for 24 h in a sealed glass tube at 110 °C in 6 N HCl. The mixture was then dried, *in vacuo*. The residue was dissolved in 1.5 ml of sodium citrate buffer, pH 2.2 (0.2 N), and 0.2 ml of the solution was applied to the analyzer and chromatographed.

Optical rotation. The optical rotation (α_D) was measured at room temperature (25 °C) with a Perkin Elmer 141 Polarimeter. All peptides were dissolved in distilled MeOH (10 mg/ml).

Properties of background analogs of substance P showing antagonistic activity. The chemical properties of four analogs of substance P which showed some antagonistic activity, and which provided some guidance for the design of the initial analogs of the group of fourteen peptide in Table 1 are as follows. The TLC systems were: Rf^1: BuOH – AcOH – EtOAc – H_2O = 1:1:1:1; Rf^2: EtOAc – Pyr – AcOH – H_2O = 5:5:1:3; Rf^3: BuOH – Pyr – AcOH – H_2O = 30:30:6:24; Rf^4: 2-PrOH – 1 N AcOH = 2:1; Rf^5: $CHCl_3$ – MeOH – AcOH = 60:20:45.

[D-Leu^8, D-Phe^9]-SP showed single spots in five systems in TLC: Rf^1, 0.51; Rf^2, 0.78; Rf^3, 0.39; Rf^4, 0.13; Rf^5, 0.31. Amino acid analysis: Glu, 1.96 (2); Pro, 2.01 (2); Met, 1.01 (1); Leu, 1.92 (2); Phe, 1.99 (2); Lys, 1.11 (1); Arg 1.00 (1).

[D-Pro^2,Ile^7]-SP showed single spots in three systems by TLC: Rf^2, 0.81; Rf^3, 0.53; Rf^5, 0.43. Amino acid analysis: Glu, 2.05 (2); Pro, 1.87 (2); Gly, 1.06 (1); Met, 0.97 (1); Ile, 0.90 (1); Leu, 1.06 (1); Phe, 0.91 (1); Lys, 1.04 (1); Arg 1.00 (1).

[D-Pro^2]-SP showed single spots in three systems by TLC: Rf^3, 0.3; Rf^1, 0.1; Rf^2, 0.8. Amino acid analysis: Glu, 2.10 (2); Pro, 2.00 (2); Gly, 1.04 (1); Met, 0.95 (1); Leu, 1.07 (1); Phe, 1.89 (2); Lys, 0.94 (1); Arg, 1.02 (1).

[D-Pro^2,D-Leu^8,D-Phe^9]-SP showed single spots in three systems by TLC: Rf^1, 0.08; Rf^2, 0.6; Rf^3, 0.4. Amino acid analysis: Gly, 1.97 (2); Pro, 1.97 (2); Met^a, 0.81 (1); Leu, 1.94 (2); Phe, 1.91 (2); Lys, 1.11 (1); Arg, 0.97 (1).

RESULTS AND DISCUSSION

Chemical results. The fourteen peptides in Table 1 were synthesized by stepwise coupling of Boc-amino acids to the growing peptide chain on the benzhydrylamine (BHA)-resin. When the reaction cycles were finished, the peptides were deprotected and cleaved from the resin by liquid HF, and the crude peptides were purified by column chromatography. The data of Table 1 include *Rf* values from five solvent systems by TLC, and the retention times in HPLC on a column of lichrosorb RP-5, estimated purities, and the optical rotations.

The crude peptides were first partially purified by gel filtration on Sephadex G-25, and then further purified by partition chromatography on Sephadex G-25. These two steps frequently resulted in peptides which showed single spots in the five TLC systems, and a sharp symmetrical peak by HPLC with only negligible impurities, as exemplified by Fig. 1 for [D-Pro^2,D-Phe^7,D-D-Trp^9]-substance P. Since analog IX was not sufficiently pure by this two-step purification, it was subjected to a second column of partition chromatography. Analog XIV was not pure even after three-step purification, and was further purified by chromatography on Sephadex LH 20. This system was found to be particularly suitable for the purification of these analogs of SP.

The data in Table 2 summarizes the details of the syntheses of the peptides of Table 1 and the yields which were obtained after the chromatographic purifications. The resin had an N-content of 0.47 –

Table 1. Analogs of substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2.

Analog	*Rf* in solvent I	II	III	IV	V	Retention time in HPLC(min)	Purity (HPLC)(%)	$[\alpha]_D$ (°)
I. [D-Pro^2,Ile^7,D-Leu^8,D-Phe^9]-SP	0.43	0.75	0.02	0.64	0.05	19.5	>97	−21.6
II. [D-Arg^1,D-Pro^2,Ile^7,D-Leu^8, D-Phe^9]-SP	0.47	0.73	0.02	0.59	0.03	19.5	>98	−35.7
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP	0.46	0.76	0.03	0.61	0.04	22.3	>98	−38.2
IV. [D-Arg^1,D-Pro^2,D-Phe^7,D-Trp^9]-SP	0.53	0.75	0.02	0.59	0.04	22.3	>98	−46.1
V. [D-Pro^2,Lys^6,D-Phe^7]-SP	0.3	0.54	0	0.54	0	17.6	>94	−26.6
VI. [D-Arg^1,D-Pro^2,Lys^6,D-Phe^7]-SP	0.35	0.54	0	0.52	0	17.6	>96	−35.4
VII. [D-Pro^2,D-Trp^{7,9}]-SP	0.4	0.78	0.02	0.59	0.03	22.3	>99	−40.1
VIII. [D-Arg^1,D-Phe^7,D-Trp^9]-SP	0.33	0.77	0.03	0.6	0.03	22.3	>95	−71.4
IX. [D-Lys^3,D-Phe^7,D-Trp^9]-SP	0.49	0.74	0.02	0.57	0.03	22.3	>95	−37.9
X. [D-Pro^4,D-Phe^7,D-Trp^9]-SP	0.4	0.75	0.02	0.62	0.03	22.3	>96	−47.1
XI. [D-Gln^5,D-Phe^7,D-Trp^9]-SP	0.44	0.81	0.02	0.62	0.03	22.3	>96	−38.7
XII. [D-Gln^6,D-Phe^7,D-Trp^9]-SP	0.43	0.77	0.02	0.58	0.03	22.3	>95	−51.2
XIII. [D-Pro^2,D-pClPhe^7,D-Trp^9]-SP	0.71	0.78	0.01	0.61	0.03	23.1	>90	−42.0
XIV. [D-Pro^2,D-Phe^7,D-pClPhe^9]-SP	0.74	0.78	0.01	0.62	0.03	23.5	>89	−40.3

0.5 meq/g resin. The first amino acid was coupled to the extent of 60 − 100 % as determined by amino acid analysis. The yields of the crude peptides were 0.2 − 0.5 mmol/g resin, which were 40 − 100 % of the theoretical values. These results show that during deprotection of the Boc-protected peptide in 50 % TFA and CH_2Cl_2 only little or no loss of peptide occurred, which is presumed to have been due to the stability of the amide linkage between the peptide and the resin toward hydrolysis. This stability of the BHA-resin is a great advantage over that of the Merrifield-type resin.

The data from the amino acid analysis of all analogs, which are described herein, revealed the expected ratios of the amino acids. These data alone may not be regarded as a proof for the purity of such

Table 2. Data on syntheses of analogs of substance P.

No.	Resin (g)	Peptide-resin (g)	Peptide after HF (mg)	Peptide for 1st column (mg)	Peptide after 1st column (mg)	Peptide after 1st pat. chrom. (mg)	Peptide after 2nd part. chrom. (mg)
I	3.0	3.48	910	100	78	44	[b]
II	3.0	3.7	990	150	[a]	17	[b]
III	1.0	1.57	324	200	[a]	75	[b]
IV	1.0	1.26	299	200	[a]	42	[b]
V	1.0	2.54	740	200	[a]	92	[b]
VI	1.0	2.39	550	200	[a]	78	[b]
VII	0.8	1.68	380	200	103	50	[b]
VIII	0.64	1.16	348	200	64	24	[b]
IX	0.64	1.11	315	200	120	[a]	25
X	0.64	1.16	280	200	121	68	[b]
XI	0.64	1.28	362	200	122	47	[b]
XII	0.64	1.28	326	200	142	80	[b]
XIII	1.0	2.29	627	200	87	76	[b]
XIV	1.0	[a]	460	200	94	42	[c]

[a] Not determined. [b] Not performed. [c] Peptide was chromatographed in addition on an LH-20 column (yield: 6 ng).

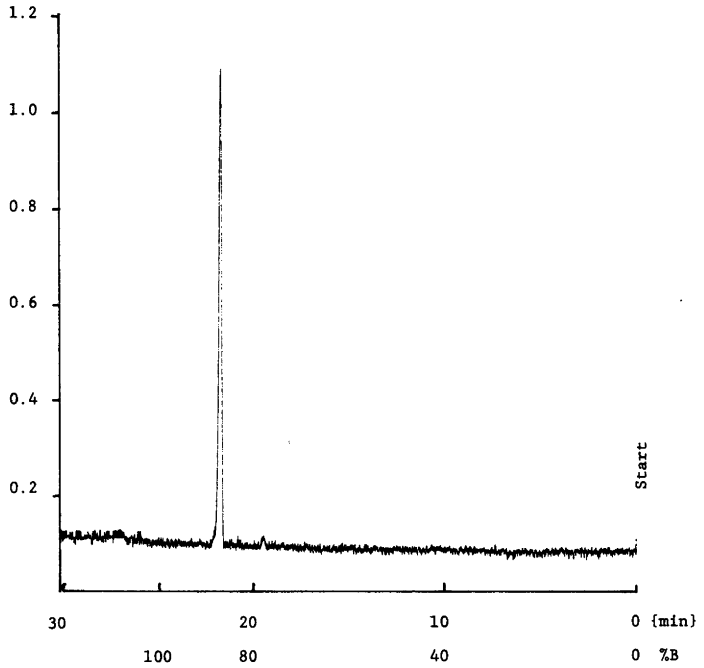

Fig. 1. HPLC of [D-Pro^2,D-Phe^7,D-Trp^9]-substance P. A Lichrosorb 5 RP C_{18} column was used. The flow rate was 2 ml/min. The column was equilibrated with 0.1 m K-phosphate buffer pH 3.0 (solvent A). At "start", the compound was injected and a linear gradient of 0 – 100 % of solvent B (50 % solvent A, 50 % CH_3CN) was applied within 25 min. The diagram shows the absorpance of the effluent at 210 nm. HPLC was performed only on fractions which were selected from column purification for purity rather than yield. Generally, samples of about 10 μg were used for HPLC and which had been found to be adequate to reveal impurities and purity for each peptide.

peptides, since it is common for analyses of crude preparations of peptides after cleavage by HF to reveal acceptable amino acid analytical data. However, these data must be acceptable as a component of the total data of purity.

During the purification by chromatography of the peptides, fractions were selected in such manner that purity was emphasized rather than yield. In the final chromatography step, only fractions which appeared pure by HPLC were taken. This procedure usually gave yields of 12 – 45 % of the pure peptides as calculated on the basis of the weights of the crude deprotected peptides.

Biological results. Twelve peptides (Table 3) were tested for smooth muscle stimulatory or substance P inhibitory actions on the isolated guinea pig ileum, which was suspended in a 5 ml organ bath containing Kreb's solution.

Three of the twelve peptides, (D-Pro^2, D-Phe^7, D-Trp^9)-SP, (D-Pro^2, D-Trp^{7,9})-SP and (D-Arg^1, D-Phe^7, D-Trp^9)-SP did not stimulate smooth muscle even at very high concentrations. All the other analogs very weakly stimulated smooth muscle (Table 3).

At a concentration of 10^{-4}, ten of the peptides showed antagonistic activity requiring concentrations of 3 to 22-fold of SP to allow a 50 % response. Of these ten peptides, (D-Pro^2, D-Trp^{7,9})-SP, (D-Pro^2, Lys^6, D-Phe^7)-SP and (D-Pro^2, D-pClPhe^7, D-Trp^9)-SP were the most potent at a concentration of 10^{-4} by requiring 22, 18 and 17-fold increases, respectively, in the SP concentration to allow a 50 % response.

[D-Pro^2,D-Phe^7,D-pClPhe^9]-SP not only inhibited the smooth muscle contraction induced by SP, but also of histamine, indicating that the inhibitory activity is less specific. None of the other peptides affected the smooth muscle stimulating activity of

Table 3. Assay data from guinea pig ileum system.

Analog	Agonist activity[a] Relative potency SP-100	Analog conc. (M)	Antagonist activity[a] Increase in SP-conc. to give 50 % of max. response
I	0.001	10^{-4}	0
III	0.0007	10^{-4}	6×
		10^{-3}	30×
V	0.034	10^{-4}	18×
VI	0.110	10^{-5}	0
VII	0.0009	10^{-4}	22×
		10^{-3}	430×
VIII	0.0005	10^{-4}	5×
IX	0.003	10^{-4}	4×
X	0.004	10^{-4}	6×
XI	0.001	10^{-4}	3×
XII	0.001	10^{-4}	4×
		10^{-3}	29×
XIII	0.001	10^{-4}	17×
		10^{-3}	[b]
XIV	0.001	10^{-4}	6×

[a] The tests were made on isolated guinea pig ileum suspended in a 5-ml organ bath containing Krebs' solution. [b] Inhibits also histamine.

histamine or acetylcholine, which indicated that these peptides are specific antagonists of substance P.

In the presence of each of the ten peptides (III, V, VII, VIII, IX, X, XI, XII, XIII, XIV) in the bath, the dose-response curve for SP was shifted in parallel to the right with no change in a maximally obtained response, which indicated that the antagonistic activity of these peptides is of a competitive type.

A comparison of the substitution in the sequences of these peptides reveals the following relationships, which may contribute to the design of new analogs or greater potency and perhaps greater specificity of diverse antagonistic effects.

The following style of comparisons of substitutions and antagonism facilitate interpretations of the more effective substitutions.

V. [D-Pro^2,Lys^6,D-Phe^7]-SP; 18-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
Lys^6 is a promising substituent.
VII. [D-Pro^2,D-Trp^{7,9}]-SP; 30-fold/10^{-4}
V. [D-Pro^2,Lys^6,D-Phe^7]-SP; 18-fold/10^{-4}
D-Trp^{7,9} was as effective as Lys^6,D-Phe^7.
XIII. [D-Pro^2,D-pClPhe^7,D-Trp^9]-SP; 17-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-pClPhe^7 was three times as effective as D-Phe^7. D-Arg^1 and L-Arg^1 are equally effective.
XII. [D-Gln^6,D-Phe^7,D-Trp^9]-SP; 4-fold/10^{-4}
XI. [D-Gln^5,D-Phe^7,D-Trp^9]-SP; 3-fold/10^{-4}
D-Gln^6 was 1.33-fold better than D-Gln^5.
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
X. [D-Pro^4,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-Pro^2 and D-Pro^4 were equally effective.
IX. [D-Lys^3,D-Phe^7,D-Trp^9]-SP; 4-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-Pro^2 was 1.5 times as effective as D-Lys^3.
VIII. [D-Arg^1,D-Phe^7,D-Trp^9]-SP; 5-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-Pro^2 may not be important.
XIV. [D-Pro^2,D-Phe^7,D-pClPhe^9]-SP; 6-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-pClPhe^9 and D-Trp^9 were equally effective.

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