Amino Acids (1993) 5: 233–238

Amino
Acids

Spantide III, a superior tachykinin antagonist with high potency and negligible neurotoxicity

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Accepted February 2, 1993

Summary. Five new antagonists of Substance P were designed and synthesized toward increasing potency and safety. One of them was more effective than Spantide II, which was the basis for the design. It was named Spantide III and has the structure: D-NicLys,Pro,Pal,Pro,D-Cl_2Phe,Asn,D-Trp,Phe,D-Pal,Leu, NleNH_2.

Keywords: Amino acids – Substance P antagonists – Spantide III – Solid phase peptide synthesis

Introduction

During the last decade, a number of increasingly potent tachykinin receptor antagonists have become available. Tachykinin antagonists of the peptide type may be either full length Substance P (SP) analogs (Folkers et al., 1984; Ljungqvist et al., 1989); or truncated C-terminal analogs of six to eight residues (Mizrahi et al., 1982; Regoli et al., 1984, 1985). Recently, non-peptidic tachykinin antagonists have been described (Watling, 1992).

In 1984, the full length SP analog, named Spantide, D-Arg,Pro,Lys,Pro, Gln,Gln,D-Trp,Phe,D-Trp,Leu,LeuNH_2, was introduced by Folkers et al. (1984). It had a pA_2 value (negative logarithm of antagonist concentration producing a 2-fold shift of the antagonist concentration-activity curve) of 7.1 and was used by many investigators as a model antagonist in biological systems.

Spantide was also examined for effects on C-fiber neurotransmission, but its usefulness was restricted by a relatively low potency and by its histamine releasing properties.

SP and many SP analogs release histamine from mast cells, which is apparently due to the presence of the strongly basic amino acids, arginine and lysine, in their N-terminal (Fewtrell et al., 1982).

If antagonists of SP are to be clinically useful, it is essential that they have little or no histamine-releasing activity, and do not cause neurotoxic effects (Post and Panlsson, 1986).

Folkers et al. (1990) used the same approach to eliminate histamine-mobilizing activity of SP analogs, which proved to be successful for LHRH antagonists. The concept was to reduce the basicity of the Lys residue by acylating it with nicotonic acid (Ljungqvist et al., 1985) and to use the resulting N^E-nicotinoyl lysine (NicLys) instead of Arg in position 1. On the basis that both D-Arg^1 and Lys^3 of Spantide may contribute to histamine release, Lys^3 was substituted by the much less basic Pal^3 in the new design.

These changes, with other substitutions, were developed gradually and resulted in Spantide II which is: D-NicLys,Pro,Pal,Pro,D-Cl_2Phe,Asn,D-Trp,Phe, D-Trp,Leu,NleNH_2. Spantide II had a higher potency and a lower histamine-releasing activity than Spantide, and seemed to be without nerotoxicity (Hakanson et al., 1990, 1997; Maggi et al., 1997; Weisenfeld-Hallin et al., 1990).

The new analog, Spantide III, is superior to Spantide II.

Experimental

Materials

The natural amino acid intermediates were purchased from Advanced Chem Tech, Louisville, Kentucky. D-NicLys,Pal and D-Cl_2Phe were synthesized in our laboratory.

Synthesis

The peptides were synthesized by the solid-phase method on a Beckman model 990 peptide synthesizer. The BHA resin was used. After the attachment of the first amino acid was completed, the resin was acetylated by a 25% (vol/vol) acetic anhydride solution in CH_2Cl_2. The peptides were cleaved from the resin with concomitant deprotection by treatment for 45 min at 0°C, with doubly distilled HF containing anisole and thioanisole, as described (Yamaguchi et al., 1978).

Purification and characterization

The crude peptides were first purified by chromatogrphy on silica gel with the solvent system 1-butanol/acetic acid/water, 4:1:2 (vol/vol), or on Sephadex LH-20 (Pharmacia) with the solvent system n-butanol/acetic acid/water/methanol, 10:10:90:15 (vol/vol). Then, they were further purified on a column of Sephadex G-25 (2.5 × 100 cm) with 6–10% acetic acid as the eluant. The peptides were examined for purity on silica gel TLC plates (Merck). At least four different solvent systems were used with the chlorine/o-toluidine test, and the R_f values of the peptides are listed in Table 1.

The purity was further checked by analytical HPLC using a Waters instrument with 660 solvent programmer and a Vydac C_{18} peptide column, 25 × 3.6 mm i.d. Solvent A was 0.01 M KH_2PO_4 adjusted to pH3 with phosphoric acid and solvent B was 80% acetonitrile and 20%A. A linear gradient of 30–80% B in 20 min was used to elute the peptides. The flow rate was 1.5 mL/min. and the absorbance was measured at 210 mm. The purity of peptides was estimated at 97–99%. The HPLC data are in Table 1.

Amino acid analyses

Analyses were performed on a Beckman 118CL automatic amino acid analyzer equipped with a Hewlett-Packard 3390A integrator. The peptides (0.5 mg) were hydrolyzed with

Table 1. Analytical data on Spantide II analogs

Analog	R_f Values				% purity	Retention time (min.)
	A	B	C	D		
I	0.67	0.34	0.67	0.97	98	10.3
II	0.58	0.44	0.62	0.88	98	10.1
III	0.31	0.57	0.58	0.81	99	9.3
IV	0.67	0.34	0.67	0.97	98	10.0
V	0.62	0.34	0.67	0.97	98	9.9

Solvent systems:
A nBuOH-HOAc-H_2O = 4:1:2
B nBuOH-Py-HOAc-H_2O = 4:1:1:2
C nBuOH-Py-HOAc-H_2O = 30:10:3:12
D AcOEt-Py-HOAc-H_2O = 5:5:1:3

Table 2. Amino acid analyses of Spantide II analogs

Analog	Lys	Pro	Pal	Cl_2Phe	Asp	Trp	Phe	Leu	Nle	Ala	Nal
I	0.90	2.15	1.19	+	0.54	+	0.83	2.31			+
II	1.01	2.05	2.02	+	0.60	+	0.87	1.01	1.02		
III	1.03	2.03	1.97	+	0.50	+	0.85	1.04		1.29	
IV	1.01	2.11	1.05		0.56	+	0.87	2.07			+
V	1.04	2.10	1.03		0.57	+	0.90	1.04	1.03		+

When Asn^6 is followed by $D\text{-}Trp^7$, the Asp value is always low, 50–60% of theory. The reason for this is unclear, but may involve acid catalyzed interaction between the $CONH_2$ group of Asn and the indole moiety of D-Trp (Folkers et al., 1986)

constant boiling HCl in an evacuated tubes for 24 hours at 110°C. Tryptophan and the unnatural amino acids were not determined quantitatively. The amino acid analytical data are in Table 2.

Bioassay

The data on the specificity and potency of the analogs to inhibit tachykinin-evoked contraction of the guinea pig *Taenia coli*, and to suppress the contractile response of the rabbit *Iris sphincter* evoked by electrical stimulation of sensory nerve fibers are summarized in Table 4.

Results and discussion

Five new SP antagonists have been designed and synthesized to achieve more potent and non-toxic tachykinin antagonist.

As shown in Table 3, Spantide has changes in four positions as compared to SP. Substituting $D\text{-}Arg^1$ by $D\text{-}NicLys^1$ and Lys^3 by Pal^3 effectively reduced the undesired histamine release due to the very basic N-terminal. Then we designed and synthesized Spantide II, which had seven residues changed compared to SP. Spantide II served as the model for our present structure-activity advances.

Table 3. Amino acid sequences of SP and its analogs

Analog	1	2	3	4	5	6	7	8	9	10	11
SP	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	$MetNH_2$
Spantide	D-Arg	〃	〃	〃	〃	〃	DTrp	〃	DTrp	〃	$LeuNH_2$
Spantide II	DNicLys	〃	Pal	〃	DCl_2Phe	Asn	〃	〃	〃	〃	$NleNH_2$
I	〃	〃	〃	〃	〃	〃	〃	〃	〃	〃	$DLeuNH_2$
II (Spantide III)	〃	〃	〃	〃	〃	〃	〃	〃	DPal	〃	$NleNH_2$
III	〃	〃	〃	〃	〃	〃	〃	〃	〃	〃	$DAlaNH_2$
IV	〃	〃	〃	〃	DNal	〃	〃	〃	DTrp	〃	$DLeuNH_2$
V	〃	〃	〃	〃	〃	〃	〃	〃	〃	〃	$NleNH_2$

NicLys N^E-nicotinoyllysine
Pal 3-(3-pyridyl)alanine
Cl_2Phe 3-(3,4-dichlorophenyl)alanine
Nal 2-naphthylalanine

Peptides I, II and V are analogs of Spantide II with only one substitution, and peptides III and IV have two-residue changes. It is believed that the biological activity of SP may be in its C-terminal fragment. The changes we designed were in positions 5, 9, and 11. D-Leu (analogs I and IV) and D-Ala (analog III) were both tried in position 11 in order to produce antagonists with stronger resistance toward enzymatic degradation, but these three analogs were less potent than Spantide II. The pIC_{50} values are in Table 4.

Analog II, D-NicLys, Pro, Pal, Pro, D-Cl_2Phe, Asn, D-Trp, Phe, D-Pal, Leu,$NleNH_2$ is a [D-Pal^9]Spantide II. This analog is about 0.5 log unit more potent than Spantide II and was named Spantide III. Spantide III was tested for its activity to inhibit the SP-induced contractions in the *Taenia coli* (Wang et al., 1992). The pA_2 value is in Table 4. Analog V has D-Nal^5 instead of D-Cl_2Phe and was the weakest antagonist of this series.

Table 4. Inhibitory effects of Spantide II and its analogs on tachykinin-mediated responses

Analog	pIC_{50}	pA_2
Spantide II	6.1 +/− 0.1	7.6 +/− 0.2
I	5.2 +/− 0.1	ND
II (Spantide III)	6.6 +/− 0.2	6.7 +/− 0.2
III	4.9 +/− 0.1	ND
IV	5.1 +/− 0.1	ND
V	——	ND

——: A weak antagonist, a pIC_{50} value was not calculated
ND Not determined

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Received December 2, 1992