Design and Synthesis of Antagonists of Substance P

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Synthesis and bioassay of about 65 analogs of substance P (SP) over five years yielded the antagonist [D-Arg[1],D-Trp[7,9],Leu[11]]-SP, which was named Spantide, and which was used by many investigators as a "tool". Spantide served as a reference antagonist for the design of 47 new peptides toward the goal of more potent inhibitors. Designs emphasized analogs with D-Trp[7], D-Trp[9], D-Trp[10], D-pClPhe[10], Nle[11], Leu[11], Ile[11] and Met[11], *etc.* Twenty-one/47 antagonists were superior in potency to that of Spantide, the best was [D-Arg[1],D-Nal[5], D-Trp[7,9],Nle[11]]-SP which required a 255-fold increase in SP concentration to give 50 % of the maximum response at a concentration of 10^{-5}M of the antagonist; this potency is *ca.* 5 times that of Spantide. For certain, but not all pairs of undecapeptides and truncated analogs, the undecapeptides may be significantly more potent than the truncated counterparts.

There are many publications on analogs of the tachykinins including substance P from before 1975, and there have been at least 37 publications on agonists and antagonists of substance P since about 1975. Pernow published a comprehensive review on substance P in 1983[1], and a mini-review of structural activity studies of the development of antagonists was published in 1984[2].

Since only six or seven of the *C*-terminal amino acids of the undecapeptide, substance P, are needed for full activity, the design of antagonists of substance P has been based both on truncated peptides as well as undecapeptides. Greater emphasis has possibly been given to undecapeptides than to truncated peptides, and whether an undecapeptide is superior or not to the companion truncated peptide seems dependent upon a specific pair of peptides for a specific activity in a given system.

Of eight analogs, [D-Phe[7]]-SP, found in 1979[3], had 1/50th the agonist activity of SP and weak antagonist activity. Based on seven more analogs[4], [Ile[8]]-SP was found to be about twice as agonistically effective as SP, but it did reveal perplexing antagonistic activity. Of fourteen more analogs[5], six were found to have antagonistic activity, so the productivity of antagonistic analogs was increasing. Of these fourteen analogs, [D-Leu[8],D-Phe[9]]-SP was an antagonist with <0.03 % of the agonistic activity of SP. On the basis that an effective antagonist should have no agonistic activity, this concept was at last being supported by data. [D-Leu[8],D-Phe[9]]-SP was considered at that stage of progress to be a good lead analog. In 1981, [D-Pro[2],D-Phe[7],D-Trp[9]]-SP was found to be a specific competitive SP[6] antagonist, and the focus of design of antagonists at that time was clearly oriented upon the presence of multiple D-amino acids. In 1982, [D-Pro[2] DTrp[9]]-SP of 14 more analogs was found to be the most potent antagonist up to that time.[7] In 1983, [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-SP of ten more analogs was achieved[8] and found to be the most advanced potent antagonist. By 1984, of 11 more analogs, one without a D-amino acid in position 2, [D-Arg[1],D-Trp[7,9],Leu[11]]-SP, was found to be newly superior.[9] Its pA_2 value was 7.1–7.2. This antagonist was named Spantide, and made available for diverse

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studies by different investigators. Spantide was considered a useful step, but not the final step toward the goal of effective and potent inhibitors of substance P.

Here, we describe our relevant background of design and results on new groups of analogs, with emphasis on certain positions, and for both undecapeptides and truncated companion analogs.

Experimental

General. The amino acid derivatives were purchased from Peninsula Laboratories, San Carlos, CA. The α-amino functions were protected by the Boc group. The side chain functions were protected as benzyl esters. Boc-*p*-fluorophenylalanine, D and L-Boc-*p*-chlorophenylalanine, D-Boc-3-pyridylalanine, D-Boc-3-(2-naphthyl)-althreonine. Glutamic acid and aspartic acid were protected as benzyl esters. Boc-*p*-Fluorophenylalanine, D and L-Boc-*p*-Chlorophenylalanine, D-Boc-3-Pyridylalanine, D-Boc-3-(2-Naphthyl)-alanine and D-Boc-3,4-dichlorophenylalanine were kindly provided by Dr. Narashimha Rao of the Southwest Foundation for Research and Education, San Antonio, TX. Benzhydrylamine (BHA) resin hydrochloride was obtained from Beckman Bioproducts, Palo Alto, CA. Dicyclohexylcarbodiimide, triethylamine and other solvents were distilled prior to use. All other chemicals were reagent grade.

Peptide Synthesis. The peptides were synthesized by the solid phase method on a Beckman model 990 Peptide Synthesizer as described[10]. The resin, after the first amino acid was coupled, was acetylated by a 25 % acetic anhydride solution in dichloromethane and pyridine. The completed peptide was cleaved from the resin and the protecting groups on the amino acids were removed by treatment with twice-distilled HF containing 10 % anisole and 10 % thioanisole for 45 min at 0 °C, as described[11].

Purification. The peptides were purified chromatographically. They were first eluted through a column of Sephadex G-25 (2.5×100 cm) with 12 % acetic acid. Then they were further purified over a column of silica gel (1×50 cm) by either of the following solvent systems: (1) nBuOH: HOAc:H_2O=4:1:2; (2) BuOH: HOAc:H_2O= 4:1:5 (upper phase). If the desired peptides were not sufficiently pure, they were repurified over silica gel with the same solvent. The peptides were examined for purity on silica gel TLC (Merck) plates with the following solvent systems:

1. EtOAc:pyridine:HOAc:H_2O=5:5:1:3
2. BuOH:pyridine:HOAc:H_2O=5:5:1:4
3. BuOH:HOAc:H_2O=4:1:2

Single spots were observed in each case and the R_f values for each peptide are listed in Table 1. The purity of the peptide was determined by high pressure liquid chromatography on a column of μ-Bondapak C_{18} (3.9×30 cm). Equipment for HPLC from Waters Associates, Milford, MA, with a gradient programmer was used. The solvent system employed was: Buffer A, 0.1 M potassium phosphate monobasic, pH 3.0; buffer B, 30 % buffer A in acetonitrile, a linear gradient of various percentages of buffer B in 20 or 25 min at a flow rate of 2 ml/min. The purity and retention time of the peptides are shown in Table 1. The amino acid 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 constant boiling HCl in an evacuated tube for 24 h at 110 °C. Tryptophan and unnatural amino acids were qualitatively determined.

Bioassay

The biological activity of the SP analogs was tested using the terminal portion of the guinea pig ileum as described[11].

Results

Spantide, [D-Arg^1,D-Trp^{7,9},Leu^{11}]-undecapeptide, served as a reference antagonist for new designs. Table 2 summarizes [D-Trp^{7,9},Leu^{11}]-analogs. The activity of Spantide was expressed as 625– and 51-fold increases in SP concentration to give 50 % of the maximum response at concentrations of 10^{-4} and 10^{-5}M, respectively. Converting -Gln^5, Gln^6- to -D-Gln^5,D-Gln^6- decreased activity to about 1/10 that of Spantide. Introducing N-Ac-Arg^1 decreased activity to about 1/20. Changing L-Gln^5 to D-Phe^5 of Spantide increased activity from 625– to 717-fold. Changing Phe^8 to pClPhe^8 increased potency from 625- to 980-fold. Changing Leu^{10} to Gly^{10} reduced activity to 1/10. Changing -Gln^5,Phe^8- to -D-Phe^5,pClPhe^8- retained ac-

le 1. Analytical data of the SP analogs*.

Structure											TLC R_f in solvent system+ I	II	III	HPLC Retention time/ min++	Purity/%
D-Arg,	Pro,	Lys,	Pro,	Gln,	Gln,	D-Trp,	Phe,	D-Trp,	Leu,	Leu, NH_2	0.59	0.58	0.25	14.5[a]	98
"	"	"	"	D-Gln	D-Gln	"	"	"	"	" "	0.59	0.64	0.31	14[a]	98
N-Ac-Arg	"	"	"	Gln	Gln	"	"	"	"	" "	0.63	0.71	0.38	15.5[a]	98
D-Arg	"	"	"	D-Phe	"	"	"	"	"	" "	0.77	0.68	0.38	16.5[a]	96
"	"	"	"	Gln	"	"	pClPhe	"	"	" "	0.80	0.70	0.36	16[a]	98
"	"	"	"	"	"	"	Phe	"	Gly	" "	0.68	0.68	0.35	13.5[a]	96
"	"	"	"	D-Phe	"	"	pClPhe	"	Leu	" "	0.98	0.78	0.44	15.8[b]	98
"	"	"	"	DpClPhe	"	"	"	"	"	" "	0.98	0.78	0.41	18.0[c]	96
"	D-Pro	"	D-Pro	Gln	"	D-Phe	Leu	Trp	"	" "	0.86	0.55	0.29	12.5[a]	95
"	"	"	Pro	D-Phe	"	D-Trp	Phe	D-Trp	"	" "	0.91	0.61	0.25	16[c]	97
Arg	pFPhe	"	"	Gln	"	D-Phe	"	"	"	Met "	0.90	0.66	0.28	13.7[a]	93
"	Pro	"	"	"	"	D-Trp	"	pClPhe	"	Leu "	0.65	0.42	0.23	13.5[a]	96
"	"	"	"	"	"	"	Leu	"	"	" "	0.62	0.42	0.22	13.4[a]	98
"	"	"	"	"	"	"	Tyr	"	"	" "	0.84	0.70	0.31	14[a]	96
"	"	"	"	"	"	"	Ile	"	"	" "	0.77	0.68	0.35	15[a]	97
				Glp	Gln	D-Trp	Phe	D-Trp	"	" "	0.93	0.87	0.76	–	–
				"	"	"	"	"	"	Thr "	0.91	0.53	0.07	–	–
				D-Phe	"	"	"	"	"	Leu "	0.56	0.86	0.81	17.5[a]	98
D-Arg	Pro	Lys	Pro	"	"	"	"	"	"	Nle "	0.98	0.78	0.39	14.0[d]	98
				"	"	"	"	"	"	" "	1.0	0.90	0.83	18.0[c]	98
D-Arg	Pro	Lys	Pro	Gln	"	"	"	"	D-Trp	Nle "	0.89	0.64	0.51	15[a]	98
Arg	D-Pro	"	"	"	"	D-Phe	"	"	Leu	Met "	0.97	0.55	0.23	12.5[a]	97
"	"	"	"	"	"	"	"	"	"	– "	0.97	0.38	0.2	10.5[a]	97
D-Arg	"	"	"	"	"	"	"	"	"	– "	0.97	0.32	0.2	11.9[a]	94
"	Pro	"	"	"	"	D-Trp	"	"	"	Nle "	0.93	0.73	0.35	14.3[d]	98
"	"	"	"	D-pClPhe	"	"	"	"	"	" "	0.98	0.78	0.33	17[c]	98
"	"	"	"	"	"	"	pClPhe	"	"	" "	0.94	0.79	0.33	17.5[c]	98
"	"	"	"	D-Leu	"	"	Phe	"	"	" "	0.94	0.79	0.32	16.0[c]	98
"	"	Arg	"	D-Phe	"	"	"	"	"	" "	0.97	0.81	0.34	17.2[c]	98
"	"	Lys	"	DCl_2Phe	"	"	"	"	"	" "	0.96	0.80	0.34	17.6[c]	98
"	"	"	"	D-3-Pal	"	"	"	"	"	" "	0.90	0.63	0.35	16.1[b]	97
"	"	"	"	D-2-Nal	"	"	"	"	"	" "	0.96	0.67	0.40	18.6[c]	97
"	"	"	"	DCl_2Phe	Glu	"	"	"	"	" "	0.97	0.80	0.35	19.0[c]	96
"	"	"	"	"	Asp	"	"	"	"	" "	0.97	0.80	0.33	19.1[c]	96
"	"	Arg	"	"	Gln	"	"	"	"	" "	0.98	0.87	0.29	18.0[c]	96
"	"	Lys	"	Gln	"	"	"	"	D-Trp	" "	0.96	0.72	0.34	13.0[d]	98
"	"	"	"	"	"	"	"	"	DpClPhe	" "	0.98	0.73	0.34	14.5[d]	98
"	"	"	"	D-Phe	"	"	pClPhe	"	D-Trp	" "	0.98	0.79	0.33	16.5[c]	95
"	"	"	"	DpClPhe	"	"	"	"	"	" "	0.98	0.79	0.33	17.5[c]	97
"	"	Arg	"	D-Phe	"	"	Phe	"	Trp	" "	0.97	0.80	0.30	17.4[c]	96
"	"	Lys	Pro	Gln	"	"	"	"	Leu	Ile "	0.67	0.63	0.28	14[a]	97
"	D-Pro	D-Lys	D-Pro	"	"	D-Phe	"	"	"	Met "	0.91	0.55	0.23	14[a]	97
"	"	"	"	D-Gln	D-Gln	D-Trp	"	"	"	" "	0.93	0.55	0.23	18.5[a]	95
"	Pro	Lys	Pro	Gln	Gln	"	"	"	"	" "	0.80	0.55	0.21	17.5[a]	96
Arg	D-Phe	"	"	"	"	"	"	"	"	" "	0.92	0.63	0.21	16.2[a]	92
"	DpClPhe	"	"	"	"	"	"	"	"	" "	0.93	0.65	0.24	16.5[a]	86
"	D-Pro	"	"	"	"	"	"	DpClPhe	"	" "	0.89	0.54	0.18	16.7[a]	90
"	"	"	D-Pro	"	"	"	D-Leu	D-Trp	"	" "	0.89	0.53	0.18	16.2[a]	92

amino acid analyses were in reasonable agreement with theory. +I: EtOAc:Pyr:HOAc:H_2O=5:5:1:3; II: OH:Pyr:HOAc:H_2O=5:5:1:4; III: nBuOH:HOAc:H_2O=4:1:2. ++Buffer A: 0.1 M KH_2PO_4 pH 3; Buffer B: 30 % A in CH_3CN. hear gradient of 20 % to 100 % B in 25 min. [b]Linear gradient of 0 % to 100 % B in 25 min. [c]Linear gradient of 0 % to 100 % B in min. [d]Linear gradient of 20 % to 80 % B in 20 min. In II cases the flow rate was 2 ml min^{-1} and the detection was by UV at 210

-Trp pClPhe D-Trp Leu, Leu, NH2

Table 2. Analogs of SP with D-Trp[7,9], and Leu[11].

	Analog												Activity[a]
1.	[+]D-Arg	Pro,	Lys,	Pro,	Gln,	Gln	D-Trp,	Phe,	D-Trp,	Leu,	Leu,	NH_2	625 (10^{-4}M)
2.	"	D-Pro	"	D-Pro	"	"	D-Phe	Leu	Trp	"	"	"	7 (10^{-4}M)
3.	"	"	"	Pro	D-Phe	"	D-Trp	Phe	D-Trp	"	"	"	64 (10^{-4}M)
4.	"	"	"	"	D-Gln	D-Gln	"	"	"	"	"	"	72 (10^{-4}M)
5.	N-AcArg	"	"	"	Gln	Gln	"	"	"	"	"	"	30 (10^{-4}M)
6.	D-Arg	"	"	"	D-Phe	"	"	"	"	"	"	"	717 (10^{-4}M)
7.	"	"	"	"	Gln	"	"	pClPhe	"	"	"	"	980 (10^{-4}M)
8.	"	"	"	"	"	"	"	Phe	"	Gly	"	"	5 ($10-^{5}$M)
9.	"	"	"	"	D-Phe	"	"	pClPhe	"	Leu	"	"	62 ($10-^{5}$M)
10.	"	"	"	"	D-pClPhe	"	"	"	"	"	"	"	119 ($10-^{5}$M)

[+]Spantide.

[a]– Fold increase in SP concentration to give 50 % of maximum response at a concentration of the analog.

Table 3. Analogs with emphasis on D-Trp[7] Leu[11] and with D-Trp[9] Leu[11].

	Analog												Activity[a]
Spantide: D-Arg,Pro,Lys,Pro,Gln,D-Trp,Phe,D-Trp,Leu,Leu,NH_2													625 (10^{-4}M)
1.	Arg,	pFPhe,	Lys,	Pro,	Gln,	Gln,	D-Phe,	Phe.	D-Trp,	Leu,	Met,	NH_2	44 (10^{-4}M)
2.	"	Pro	"	"	"	"	D-Trp	"	D-pClPhe	"	Leu	"	106 (10^{-4}M)
3.	"	"	"	"	"	"	"	Leu	"	"	"	"	51 (10^{-4}M)
4.	"	"	"	"	"	"	"	Tyr	"	"	"	"	6 (10^{-4}M)
5.	"	"	"	"	"	"	"	Ile	"	"	"	"	15 (10^{-4}M)

[a]– Fold increase in SP concentration to give 50 % of maximum response at a concentration of the analog.

Table 4. Comparison of truncated versus undecapeptides as antagonists.

	Analog	Activity[a]
1.	Spantide: D-Arg, Pro, Lys, Pro, Gln, Gln, D-Trp, Phe, D-Trp, Leu, Leu, NH_2	625 (10^{-4}M) 51 (10^{-5}M)
2.	Glp " " " " " " "	17 (10^{-4}M)
3.	Glp Gln D-Trp Phe D-Trp Leu Thr NH_2	2 (10^{-4}M)
4.	D-Arg, Pro, Lys, Pro, D-Phe, Gln, D-Trp, Phe, D-Trp, Leu, Leu, NH_2	717 (10^{-4}M)
5.	D-Phe " " " " " " "	200 (10^{-4}M)
6.	D-Arg, Pro, Lys, Pro, D-Phe, Gln, D-Trp, Phe, D-Trp, Leu, Nle, NH_2	169 (10^{-5}M)
7.	D-Phe " " " " " " "	27 (10^{-5}M)
8.	D-Arg, Pro, Lys, D-Pro, Gln, Gln, D-Trp, Phe, D-Trp, D-Trp, Nle, NH_2	62 (10^{-5}M)
9.	D-Pro " " " " " " Met "[b]	180 (10^{-5}M)
10.	Arg, D-Pro, Lys, Pro, Gln, Gln, D-Phe, Phe, D-Trp, Leu, Met, NH_2	6 (10^{-4}M)
11.	" " " " " " " " " " NH_2	3 (10^{-4}M)
12.	D-Arg " " " " " D-Trp " " " "	7 (10^{-4}M)

[a]– Fold increase in SP concentration to give 50 % of maximum response at a concentration of the analog.

[b]Kindly provided by Dr. Emanuel Escher.

tivity with a little increase, and the analog with -D-pClPhe[5],pClPhe[8]- had a little more than twice the activity of Spantide.

Table 3 emphasizes D-Trp[7], Leu[11]- and D-Trp[9], Leu[11]-, and the five analogs focus upon positions 7 and 9. But instead of having D-Trp in both positions 7 and 9, diverse combinations of D-Phe and D-pClPhe and D-Trp are in positions 7 and 9. Of these five analogs, number 2 with D-Trp[7] and D-pClPhe[9] was the most potent analog, but it had only about ⅛ of the activity of Spantide. Of these combinations with D-Phe and D-pClPhe, D-Trp in positions 7 and 9 were superior substitutions.

It is well known that the first four or five *N*-terminal amino acids of SP are not essential for full agonist activity, and it is attractive to synthesize truncated analogs of six or seven amino acids. The synthesis of a heptapeptide rather than an undecapeptide saves only about 18 h in a round-the-clock automated synthesis. If a truncated peptide is biologically more effective than the corresponding undecapeptide, then truncation is superior.

Table 4 compares truncated versus undecapeptide antagonists. The Glp-heptapeptide analog of Spantide was only about 3 % as active as Spantide, and truncation was highly deleterious. Changing Leu[11] to Thr[11] in the truncated peptide further reduced activity. The D-Phe truncated heptapeptide represented by analog 5 was only about ¼ as active as the undecapeptide, analog 4. The D-Phe heptapeptide, analog 7, was only about 15 % as active as the corresponding undecapeptide, analog 6. In contrast, analog 9 with -D-Pro[4], Met[11]-, was about three times as active as the undecapeptide with -D-Pro[4] and Leu[11]-, but

Table 5. Analogs of SP with D-Trp[7], D--trp[9], and Nle[11].
Spantide: D-Arg,Pro,Lys,Pro,Gln,Gln,D-Trp,Phe,D-Trp,Leu,Leu,NH_2 625 (10^{-4}M)

	Analog											Activity
1.	D-Arg,	Pro,	Lys,	Pro,	Gln,	Gln,	D-Trp,	Phe,	D-Trp,	Leu,	Nle,	NH_2	146 (10^{-5}M)
2.	"	"	"	"	D-Phe	"	"	"	"	"	"	"	169 (10^{-5}M)
3.	"	"	"	"	D-pClPhe	"	"	"	"	"	"	"	409 (10^{-5}M)
4.	"	"	"	"	"	"	"	pClPhe	"	"	"	"	40 (10^{-5}M)
5.	"	"	"	"	D-Leu	"	"	"	"	"	"	"	50 (10^{-5}M)
6.	"	"	Arg	"	D-Phe	"	"	"	"	"	"	"	139 (10^{-5}M)
7.	"	"	Lys	"	D-Cl_2Phe	"	"	"	"	"	"	"	226 (10^{-5}M)
8.	"	"	"	"	D--3-Pal	"	"	"	"	"	"	"	69 (10^{-5}M)
9.	"	"	"	"	D-Nal	"	"	"	"	"	"	"	255 (10^{-5}M)
10.	"	"	"	"	D-Cl_2Phe	Glu	"	"	"	"	"	"	97 (10^{-5}M)
11.	"	"	"	"	D-Cl_2Phe	Asp	"	"	"	"	"	"	52 (10^{-5}M)
12.	"	"	Arg	"	D-Cl_2Phe	Gln	"		"	"	"	"	135 (10^{-5}M)

[a]– Fold increase in SP concentration to give 50 % of maximum response at a concentration of the analog.

Table 6. Analogs of SP with D-Trp[7,9], D-Trp[10] or D-pClPhe[10], and Nle[11].
Spantide: D-Arg,Pro,Lys,Pro,Gln,Gln,D-Trp,Phe,D-Trp,Leu,Leu,NH_2 625 (10^{-4}M)

	Analog											Activity[a]
1.	D-Arg,	Pro,	Lys,	Pro,	Gln,	Gln,	D-Trp,	Phe,	D-Trp,	D-Trp,	Nle,	NH_2	48 (10^{-5}M)
2.	"	"	"	"	"	"	"	"	"	D-pClPhe	"	"	85 (10^{-5}M)
3.	"	"	"	D-Pro	"	"	"	"	"	D-Trp	"	"	62 (10^{-5}M)
4.	"	"	"	Pro	D-Phe	"	"	pClPhe	"	"	"	"	74 (10^{-5}M)
5.	"	"	"	"	D-pClPhe	"	"	"	"	"	"	"	134 (10^{-5}M)
6.	"	"	Arg	"	D-Phe	"	"	Phe	"	Trp	"	"	146 (10^{-5}M)

[a]– Fold increase in SP concentration to give 50 % of maximum response at a concentration of the analog.

these two peptides differ in position 11 in addition to length. The deletion of Met[11] in analog 11 reduced activity to one half, which is a comparison of a decapeptide with an undecapeptide.

In summary, Table 4 shows three pairs of truncated heptapeptides with the corresponding undecapeptides which greatly favor the undecapeptides over the truncated versions for potency. Table 5 summarizes analogs of SP with -D-Trp[7], D-Trp[9] and Nle[11]-. Sometimes, a very minor structural change, even an isomeric one, such as Leu versus Nle increased or decreased potency. When Leu[11] of Spantide was changed to Nle[11], analog 1, potency was increased about threefold. For the eleven other analogs of Table 5, all with Nle[11], changing Gln[5] to D-Phe[5], analog 2, and changing -Lys[3],Gln[5]- to -Arg[3],D-Phe[5]- and changing Gln[5] to D-Cl_2Phe[5] and changing Gln[5] to D-Nal[5] and changing -Lys[3],Gln[5]- to -Arg[3],D-Cl_2Phe[5]- resulted in analogs which were, in general, 3–5 times as active as Spantide.

Table 6 summarizes six analogs with -D-Trp[7],D-Trp[9] and Nle[11]-. All six analogs contain D-Trp or D-pClPhe in position 10 instead of the Leu[10] of Spantide. The focus of the design of the six analogs was upon position 10. All six analogs were from about 100% to 300% as active as Spantide. Escher *et al.*[12] had reported truncated analogs of SP which had three insertions of D-Trp in positions 7, 9 and 10. In the six analogs, five had D-amino acids in position 10 and the sixth had Trp[10], but the most potent of the six had -Arg[3],D-Phe[5], Trp[10], Nle[11]- in place of -Lys[3],Gln[5],Leu[10],Leu[11]- of Spantide. Koller *et al.*[13] reported the truncated [D-Pro[4],D-Trp[7,9],Nle[11]]-SP (4–11) and found it to be six times more active than the corresponding Met[11]-analog, but apparently, it was a weak inhibitor.

Table 7 shows a comparison of activities of analogs having Leu[11], Nle[11] or Ile[11]. For analogs 1 and 2, the Nle[11] analog of Spantide is about three times as potent. In another pair of analogs, numbers 4 and 5, the Nle[11] analog is possibly more potent than the Leu[11] analog although the different

Table 7. Comparison of activities of analogs having Leu[11], Nle[11] and Ile[11].

	Analog												Activity[a]
1.	[+]D-Arg	Pro,	Lys,	Pro,	Gln,	Gln,	D-Trp,	Phe,	D-Trp,	Leu,	Leu,	NH_2	51 (10^{-5}M)
2.	"	"	"	"	"	"	"	"	"	"	Nle	"	146 (10^{-5}M)
3.	"	"	"	"	"	"	"	"	"	"	Ile	"	202 (10^{-4}M)
4.	D-Arg	Pro	Lys	Pro	D-Phe	Gln	D-Trp	Phe	D-Trp	Leu	Leu	NH_2	717 (10^{-4}M)
5.	"	"	"	"	"	"	"	"	"	"	Nle	"	169 (10^{-5}M)
6.	D-Arg	Pro	Lys	Pro	D-pClPhe	Gln	D-Trp	pClPhe	D-Trp	Leu	Leu	NH_2	119 (10^{-5}M)
7.	"	"	"	"	"	"	"	"	"	"	Nle	"	40 (10^{-5}M)

[+]Spantide

[a]– Fold increase in SP concentration to give 50% of maximum response at a concentration of the analog.

Table 8. Analogs having Met[11].

	Analog												Activity[a]
1.	D-Arg,	D-Pro,	D-Lys,	D-Pro,	Gln,	Gln,	D-Phe,	Phe,	D-Trp,	Leu,	Met,	NH_2	2 (10^{-4}M)
2.	"	"	"	"	D-Gln	D-Gln	D-Trp	"	"	"	"	"	43 (10^{-4}M)
3.	"	Pro	Lys	Pro	Gln	Gln	D-Trp	Phe	D-Trp	"	"	"	178 (10^{-4}M)
4.	Arg	D-Phe	"	"	"	"	"	"	"	"	"	"	18 (10^{-4}M)
5.	"	D-pClPhe	"	"	"	"	"	"	"	"	"	"	8 (10^{-4}M)
6.	"	D-Pro	"	"	"	"	"	"	D-pClPhe	"	"	"	109 (10^{-5}M)
7.	"	"	"	D-Pro	"	"	"	D-Leu	D-Trp	"	"	"	6 (10^{-4}M)

[a]– Fold increase in SP concentration to give 50% of maximum response at a concentration of the analog.

Table 9. Analogs superior in potency to that of spantide.

	Analog												Activity[a]
Spantide: D-Arg,Pro,Lys,Pro,Gln,Gln,D-Trp,Phe,D-Trp,Leu,Leu,NH_2													625 (10^{-4}M)
													51 (10^{-5}M)
1.	D-Arg,	Pro,	Lys,	Pro,	D-Phe,	Gln,	D-Trp,	Phe,	D-Trp,	Leu,	Leu,	NH_2	717(10^{-4}M)
2.	"	"	"	"	Gln	"	"	pClPhe	"	"	"	"	980(10^{-4}M)
3.	"	"	"	"	D-Phe,	"	"	"	"	"	"	"	62 (10^{-5}M)
4.	"	"	"	"	D-pClPhe	"	"	"	"	"	"	"	119(10^{-4}M)
5.	D-Arg	Pro	Lys	Pro	D-Phe,	Gln	D-Trp	Phe	D-Trp	Leu,	Nle	NH_2	169 (10^{-5}M)
6.	D-Arg	Pro	Lys	D-Pro	Gln	Gln	D-Trp	Phe	D-Trp	D-Trp	Nle	"	62 (10^{-5}M)
7.	+			D-Pro	"	"	"	"	"	D-Trp	Met	"	180 (10^{-5}M)
8.	Arg	D-Pro	"	Pro	"	"	"	"	D-pClPhe	Leu	Met	"	109 (10^{-5}M)
9.	D-Arg	Pro	Lys	Pro	Gln	Gln	D-Trp	Phe	D-Trp	Leu	Nle	"	146 (10^{-5}M)
10.	"	"	"	"	D-Phe,	"	"	"	"	"	"	"	169 (10^{-5}M)
11.	"	"	Arg	"	"	"	"	"	"	"	"	"	139 (10^{-5}M)
12.	"	"	Lys	"	D-Cl_2Phe	"	"	"	"	"	"	"	226 (10^{-5}M)
13.	"	"	"	"	D-3-Pal	"	"	"	"	"	"	"	69 (10^{-5}M)
14.	"	"	"	"	D-Nal	"	"	"	"	"	"	"	255 (10^{-5}M)
15.	"	"	"	"	D-Cl_2Phe	Glu	"	"	"	"	"	"	97 (10^{-5}M)
16.	"	"	Arg	"	"	Gln	"	"	"	"	"	"	135 (10^{-5}M)
17.	D-Arg	Pro	Lys	Pro	Gln	Gln	D-Trp	Phe	D-Trp	D-pClPhe	Nle	NH_2	85 (10^{-5}M)
18.	"	"	"	D-Pro	"	"	"	"	"	D-Trp	"	"	62 (10^{-5}M)
19.	"	"	"	"	D-Phe,	"	"	pClPhe	"	"	"	"	74 (10^{-5}M)
20.	"	"	"	"	D-pClPhe	"	"	"	"	"	"	"	134 (10^{-5}M)
21.	"	"	Arg	"	D-Phe,	"	"	Phe	"	Trp	"	"	146 (10^{-5}M)

[a]– Fold increase in SP concentration to give 50 % of maximum response at a concentration of the analog.
+Kindly provided by Dr. Emanuel Escher.

concentrations make comparison uncertain. Another pair of analogs, numbers 6 and 7, show that the Nle[11]-analog is about ⅓ as active as the Leu[11]-analog. It may be unpredictable as to whether Nle is or is not superior to Leu[11].

From time to time, an analog for evaluation as an antagonist was synthesized which had Met[11] as does substance P. Seven such analogs are shown in Table 8. Analog 6 had an activity of 109 at 10^{-5}M. Met[11] can be useful.

Discussion

Table 9 consists of 21 analogs which are superior in potency to that of Spantide. This lists is exclusive of a few which are equal in potency. Of these 21 analogs, the best two are numbers 12 and 14. Interestingly, in design, both are close analogs of Spantide, with Nle[11] in place of Leu[11] and, particularly, with an unnatural D-amino acid in position 5, namely D-Cl_2Phe and D-Nal (D-3,4-dichlorophenylalanine and D-3-(2-naphthyl)-alanine).

The determination of the sequence of the luteinizing hormone-releasing hormone (LHRH) took place in 1971, and the first lead to an antagonist, *in vitro,* of LHRH was [Gly[2]]LHRH and [desHis[2]]-LHRH by Vale *et al.* in 1972[14]. The [D-Phe[7]]-SP of 1979[1], may be considered the equivalent of [Gly[2]]- and [His[2]]-LHRH in which at least one functional moiety for activity is replaced, deleted, or changed to a D-amino acid.

Internationally, since 1972, approximately 2000 or more analogs of LHRH have been internationally synthesized toward the remarkably potent antagonists of LHRH which are known today. Over the past 13 years of research on antagonists of LHRH, potency has increased erratically but steadily. Not nearly the same international effort has been devoted to antagonists of SP as to antagonists of LHRH. Although LHRH is a decapeptide and SP is an undecapeptide, essentially, the full decapeptide of

LHRH is needed for full activity, but only six or seven of the amino acids of the undecapeptide, SP, is needed for activity. A structural relationship betwen the antagonists of LHRH and SP is the presence of multiple D-amino acids, particularly of D-tryptophan and of the L and D forms of synthetic unnatural amino acids. We recognize that the potency of Spantide could be increased desirably, although Spantide would be broadly useful for research. Table 9 shows our acquisition of 21 more peptides, all of which are more potent than Spantide. Antagonists of potency greater than those known today will likely be achieved stepwise. As potency increases, it is more common to lose activity than to gain activity, but occasionally new structural features which increase potency will doubtless continue to appear.

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