Acta Chemica Scandinavica B 37 (1983) 623–627

Antagonists of Substance P from Emphasis on Position 11

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Ten analogs of substance P (SP) were designed and synthesized. The agonist and antagonist activities against SP were assayed on the isolated guinea pig ileum. The prime designs were changes of the important Met^{11} to include Leu^{11}, Thr^{11}, D-Leu^{11} and D-Ala^{11}. Step-wise designs of changing D-Arg^1 and D-Pro^2 to the corresponding L-configurations resulted in decreasing antagonist activity. Changing Leu^{11} to D-Leu^{11} and D-Ala^{11} reduced antagonist activity. [D-Arg_1,D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP is the most potent antagonist of this group of analogs, and required a 100-fold increase in the concentration of SP to give 50 % of the maximal response caused by SP.

The design and synthesis of effective antagonists of substrance P (SP), H-Arg,Pro,Lys,Pro,Gln,-Gln,Phe,Phe,Gly,Leu,Met,NH_2, evolved,[1–3] and ultimately two analogs were achieved which were found to be useful in physiological systems to study the actions of SP. These two analogs were [D-Pro^2,D-Phe^7,D-Trp^9]-SP and [D-Pro^2,D-$Trp^{7,9}$]-SP.[4] [D-Pro^2,D-$Trp^{7,9}$]-SP was the most potent of these two analogs, which at a concentration of 10^{-4} required a 22-fold increase in SP to allow the same twitch response of the guinea pig ileum as was obtained in the absence of the analog. Mizrahi *et al.*[5] investigated [D-Pro^4,D-$Trp^{7,9}$]-SP-(4–11) and [D-Pro^2,D$Trp^{7,9,10}$]-SP-(4–11) and reported that these analogs inhibited a hypotensive effect of SP in rats and were specific for SP in all pharmacological systems which they studied.

Exemplifying the extensive biological studies which have been made upon [D-Pro^2,D-Phe^7,D-Trp^9]-SP and [D-Pro^2,D-$Trp^{7,9}$]-SP are the following citations. Rosell *et al.*[6] studied the inhibition of antidromic and substance P-induced vasodilatation. Caranikas *et al.*[7] pharmacologically studied SP-antagonists. Bjorkroth *et al.*[8] pharmacologically characterized four SP-antagonists. Rosell and Folkers [9] critiqued SP-antagonists as a new type of pharmacological tool.

Holmdahl *et al.*[10] reported that [D-Pro^2,D-$Trp^{7,9}$]-SP inhibited inflammatory responses in the rabbit eye. Hokfelt *et al.*[11] described immunohistochemical evidence for a neurotoxic action of [D-Pro^2,D-$Trp^{7,9}$]-SP. Piercey *et al.*[12] described sensory and motor functions of spinal cord substance P and intraspinal antagonism of spinal cord substance P receptors which cause sensory and motor deficits.

Although some but not all of our early SP-antagonists [3,4] such as [D-Pro^2,D-$Trp^{7,9}$]-SP showed weak and transient agonist activity,the overriding antagonist activity proved to be useful in the early physiological studies on the actions of SP. For example, [D-Pro^2,D-$Trp^{7,9}$]-SP showed an agonist activity of 0.0007 in comparison with the relative potency of 100 for SP.[4] Even such very low agonist activity was considered to impair perhaps the use of such an antagonist in the pharmacological analysis of SP effects in the spinal cord and sympathetic ganglia, *etc.* Consequently, the design and synthesis of new analogs of SP toward peptides having zero agonist activity and a potency of antagonism considerably greater than that of the early antagonists was sought. We describe herein improved antagonists of substance P, from continuing designs, syntheses and bioassays, which have yielded [D-Arg^1,D-Pro^2,D-$Trp^{7,9}$,Leu^{11}]-SP. The first three reports of biological studies on [D-Arg^1,D-Pro^2,D-$Trp^{7,9}$,Leu^{11}]-

0302-4369/83 $02.50

SP are as follows. Rosell *et al.*[13] described the pharmacological profile of this analog as a new and specific antagonist of substance P, and evidence for the existence of subpopulations of SP-receptors. Lundberg *et al.*[14] found that this new antagonist inhibits vagally induced inflammation and bronchial smooth muscle contraction in the guinea pig. Yanagisawa *et al.*[15] showed that [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-SP blocks slow reflex responses in the spinal cord.

METHODS

The acquisition of protected amino acids, the synthesis of the peptides, the cleavage of the peptides from the resin, were conducted as described for the previous analogs and antagonists of substance P.[3]

The modified purification of the crude peptides, as carried out for the peptides described herein, was conducted as follows. Samples of about 200 mg of the crude peptides were applied to a column of Sephadex G-25 (100×2.5 cm) which had been equilibrated with 12 % acetic acid, and then the chromatography was carried out with the same solvent. Fractions of 10 ml were collected. The peptides were detected by spotting samples of the individual fractions on silica gel plates and conducting the chromatography with n-BuOH–HOAc–H_2O=4:1:2. The fractions containing the desired peptides were pooled and lyophilized, and generally yielded 75–100 mg of material. This lyophilized material was purified over a column of silica gel (1×60 cm) which had been equilibrated with the solvent mixture, n-BuOH–HOAc–H_2O=4:1:2. The chromatography was carried out with this same solvent system, and fractions of 4 ml were collected. The desired peptides, in general, were found in fractions 30–40. Those fractions which contained the pure or nearly pure peptide were collected and lyophilized. If the desired peptide were not sufficiently pure, it was again purified over silica gel using the same solvent system. The yields of the peptides were 20–50 %. Purity was more important than the yield, and ranged from 90 to 99 %, which was acceptable for the first sample for the assay and its error. The time for achievement of purity was balanced with the time of synthesis and assay and the achievement of higher potency of antagonism which was the important goal. The peptidic impurities were not a deterrent toward the goal. High performance liquid chromatography was conducted on a Water Liquid Chromatograph equipped with a Waters 660 solvent programmer. The samples were chromatographed on a μ-Bondapak C_{18} column (10μ), 3.9×300 mm. For elution of the peptides, a linear gradient from 20–100 % of a solvent system was used during 25 min. The solvent system consisted of 70 % of CH_3CN and 30 % 0.1M K H_2PO_4 buffer, pH 3. The flow rate was 2.0 ml/min and 10 μl of a 0.1 % solution of the peptide was injected. The eluted peptide was detected by its UV absorbance at 206 nm.

The amino acid analyses and the optical rotations were conducted as described.[4] The amino acid analytical data are as follows.

III. Glu 1.91(2); Pro 2.03(2); Leu 0.97(1); Phe 0.95(1); Met 0.95(1); Lys 0.95(1); Arg 0.95(1); NH_3 3.27(3); Trp +.

IV. Glu 2.00(2); Pro 2.04(2); Leu 2.07(2); Phe 0.98(1); Lys 0.96(1); Arg 0.96(1); Trp +; NH_3 +.

V. Glu 1.03(2); Pro 2.04(2); Leu 1.01(1); Phe 0.98(1); Thr 0.94(1); Lys 1.13(1); Arg 0.97(1); Trp +; NH_3 +.

VI. Glu 2.03(2); Pro 2.07(2); Leu 1.95(2); Phe 0.98(1); Lys 0.99(1); Arg 0.99(1); Trp +; NH_3 +.

VII. Glu 2.00(2); Pro 2.01(2); Leu 0.95(1); Phe 0.99(1); Ala 1.06(1); Lys 0.99(1); Arg 1.00(1); Trp +; NH_3 +.

VIII. Glu 1.96(2); Pro 2.12(2); Leu 2.01(2); Phe 0.95(1); Lys 1.00(1); Arg 0.96(1); Trp +; NH_3 +.

IX. Glu 1.98(2); Pro 2.35(2); Leu 1.97(2); Phe 0.96(1); Lys 0.97(1); Arg 0.93(1); Trp +; NH_3 +.

X. Glu 1.99(2); Pro 2.03(2); Leu 0.99(1); Val 0.96(1); Phe 1.02(1); Lys 1.01(1); Arg 1.01(1); Trp +; NH_3 +.

XI. Glu 2.04(2); Pro 3.03(2); Leu 2.00(2); Phe 0.87(1); Lys 1.01(1); Arg 1.06(1); Trp +; NH_3 +.

XII. Glu 2.05(2); Pro 1.98(2); Leu 0.94(1); Phe 0.93(1); Ala 1.11(1); Arg 0.98(1); Trp +; NH_3 +.

Table 1. Chemical data of the analogs of substance P Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2.

	Analog	R_F in solvent system [a] I	II	III	IV	V	Retention time in HPLC (min)	Purity (HPLC) *ca.* (%)	$[\alpha]_D$
III.	[D-Arg^1,D-Pro^2,D-Trp^{7,9}]-SP	0.89	0.07	0.60	0.45	0.18	15.6	97	−24 [c]
IV.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP	0.82	0	0.54	0.28	0.20	15.0	96	−50.4 [b]
V.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},Thr^{11}]-SP	0.93	0.01	0.52	0.28	0.22	14.5	96	−38.6 [b]
VI.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},D-Leu^{11}]-SP	0.92	0	0.57	0.23	0.16	13.2	94	−20.8 [b]
VII.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},D-Ala^{11}]-SP	0.85	0.04	0.57	0.25	0.23	12.0	98	−20.4 [b]
VIII.	[D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP	0.82	0	0.57	0.35	0.18	16.5	95	−38.7 [b]
IX.	[D-Trp^{7,9},Leu^{11}]-SP	0.89	0	0.56	0.29	0.27	15.0	97	−36.4 [b]
X.	[D-Trp^{7,9},Val^{11}]-SP	0.89	0	0.59	0.27	0.27	14.5	95	−64.8 [b]
XI.	[D-Trp^{7,9},D-Leu^{11}]-SP	0.97	0	0.58	0.24	0.13	12.8	90	—
XII.	[D-Trp^{7,9},D-Ala^{11}]-SP	0.84	0.02	0.52	0.25	0.27	12.0	98	−10.4 [b]

[a] I: EtOAc:Py:HOAc:H_2O(5:5:1:3); II: n-BuOH:EtOAc:HOAc:H_2O(2:2:1:1); III: n-BuOH:Py:HOAc:H_2O-(30:30:6:24); IV: n-BuOH:Py:HOAc:H_2O(50:33:1:40); V; n-BuOH:HOAc:H_2O(4:1:2). [b] C 0.5 %, 12 % HOAc. [c] C 1 %, MeOH.

Table 2. Assay data from guinea pig ileum system.

	Analog	Agonist activity relative to SP at 100	Antagonist activity.[a] Analog conc. 10^{-4}
III.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9]-SP	0.002	16
IV.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP	0.0001	100
V.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,Thr^{11}]-SP	0.0002	5
VI.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,D-Leu^{11}]-SP	0.0001	9
VII.	[D-Arg^1,D-Pro^2, D-Trp^7,D-Trp^9,D-Ala^{11}]-SP	0.0001	6
VIII.	[D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP	0.0001	70
IX.	[D-Trp^7,D-Trp^9,Leu^{11}]-SP	0.0001	34
X.	[D-Trp^7,D-Trp^9,Val^{11}]-SP	0.0001	31
XI.	[D-Trp^7,D-Trp^9,D-Leu^{11}]-SP	0.0001	14
XII.	[D-Trp^7,D-Trp^9,D-Ala^{11}]-SP	0.0001	2

[a] Fold-increase in SP conc. to give 50 % of max. response.

RESULTS AND DISCUSSION

Structure I represents substance P (SP). The antagonist activity of an analog of SP is expressed as the fold-increase in the concentration of SP in the presence of the antagonist to give 50 % of the maximal response caused by SP alone. The chemical data are in Table 1. The assay data are in Table 2. The biological activity of the SP-analogs was tested using the terminal portion of the guinea pig ileum in a 5-ml organ bath. The tests were carried out as described by Yamaguchi *et al.*[16]

[D-Pro^2,D-Trp^7,D-Trp^9]-SP (II) was found to require a 22-fold increase in SP at a concentration of 10^{-4} to give 50 % of maximal response by SP alone (Table 3).[4]

Arg^1 of II was changed to D-Arg^1 to give III, which reqquired a 16-fold increase, and showed for the pair, II and III, a reduction in activity on changing configuration from L to D for position 1.

It has been known that [desMet^{11}]-SP is substantially less active than SP. For example, on the basis of a relative activity of 1 for SP, [desMet^{11}]-SP had a relative activity of 0.0003, but when

Table 3. Fold increases.

					Fold increase in conc.
I.	Arg^1	$Pro^2Lys^3Pro^4Gln^5Gln^6$	Phe^7Phe^8	$Gly^9Leu^{10}Met^{11}$	
II.	[	Pro^2	D-Trp^7	D-Trp^9]-SP	22
III.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9]-SP	16
IV.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 Leu^{11}]-SP	100
V.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 Thr^{11}]-SP	5
VI.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 D-Leu^{11}]-SP	9
VII.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 D-Ala^{11}]-SP	6

Met^{11} was changed to Leu^{11}, the relative activity was 0.3 according to Yanaihara *et al.*[17] Weak agonist activity was improved 100-fold by the presence of the "non-functional" Leu^{11} in place of deletion of Met^{11}. In other words, the presence of a steric or carbon structure in position 11 improved agonist activity over that of no substituent in position 11.

The exchange of Met^{11} in III for Leu^{11} to give IV increased antagonist activity 6 times, since a 100-fold increase in the concentration of SP was required for a 50 % response in the presence of [D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP.

The change of Leu^{11} in IV to Thr^{11} in V reduced antagonist activity from 100- to 5-fold, showing some specificity in the requirement of the nature of the amino acid in position 11.

Changing Leu^{11} in analog IV to D-Leu^{11} for analog VI reduced activity from 100- to 9-fold, and showed the apparent essentiality of an L-configuration in position 11 in contrast to the corresponding D-configuration.

Substituting D-Ala^{11} as in analog VII for D-Leu^{11} as in analog VI resulted in a negligible difference between these two D-amino acids in position 11 (Table 4)

When D-Arg^1 of the analog IV was changed to Arg^1 of the natural configuration in analog VIII, the increase in concentration was decreased from 100 to 70.

When D-Pro^2 of analog VIII was changed to Pro^2 of the natural configuration to give analog IV, the concentration was decreased from 70 to 34.

When Leu^{11} of analog IX was changed to the closely related Val^{11} to give analog X, there was negligible change in the concentration, *i.e.*, 34 to 31.

When Leu^{11} of analog IV was changed to give the two analogs, D-Leu^{11} and D-Ala^{11} for analogs XI and XII, there were significant decreases in the concentration which were from 34 to 14 and to 2, respectively.

The ten analogs were tested for agonist activity in comparison with SP on the guinea pig ileum. The data are in Table 2 and show that these analogs had little or no agonist activity. It is understood that a most effective antagonist will likely have zero agonist activity.

Table 4. Fold increases.

					Fold increase in conc.
IV.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 Leu^{11}]-SP	100
VIII.	[	D-Pro^2	D-Trp^7	D-Trp^9 Leu^{11}]-SP	70
IX.	[		D-Trp^7	D-Trp^9 Leu^{11}]-SP	34
X.	[		D-Trp^7	D-Trp^9 Val^{11}]-SP	31
XI.	[		D-Trp^7	D-Trp^9 D-Leu^{11}]-SP	14
XII.	[		D-Trp^7	D-Trp^9 D-Ala^{11}]-SP	2

Acknowledgement. Appreciation is expressed to the Robert A. Welch Foundation for their partial support of this research.

REFERENCES

1. Rackur, G., Yamaguchi, I., Leban, J. J., Björkroth, U., Rosell, S. and Folkers, K. *Acta Chem. Scand. B 33* (1979) 375.
2. Leban, J. H., Rackur, G., Yamaguchi, I., Folkers, K., Björkroth, U., Rosell, S., Yanaihara, N. and Yanaihara, C. *Acta Chem. Scand. B 33* (1979) 664.
3. Folkers, K., Horig, J., Rosell, S. and Björk roth, U. *Acta Physiol. Scand. 111* (1981) 505.
4. Folkers, K., Horig, J., Rampold, G., Lane, P., Rosell, S. and Björkroth, U. *Acta Chem. Scand. B 36* (1982) 389.
5. Mizrahi, J., Escher, E., Caranikas, S., D'Orleans-Juste, P. and Regoli, D. *Eur. J. Pharmacol. 82* (1982) 101.
6. Rosell, S., Olgart, L., Gazelius, B., Panopoulos, P., Folkers, K. and Horig, J. *Acta Physiol. Scand. 111* (1981) 381.
7. Caranikas, S., Mizrahi, J., D'Orleans-Juste, P. and Regoli, D. *Eur. J. Pharmacol. 77* (1982) 205.
8. Björkroth, U., Rosell, S., Xu, J.-C. and Folkers, K. *Acta Physiol. Scand. In press.*
9. Rosell, S. and Folkers, K. *Trends in Pharmacol. Sciences, TIPS,* May 1982, 211.
10. Holmdahl, G., Håkanson, R., Leander, S., Rosell, S., Folkers, K. and Sundler, F. *Science 214* (1981) 1029.
11. Hokfelt, T., Vincent, S., Hellsten, L., Rosell, S., Folkers, K., Markey, K., Goldstein, M. and Cuello, C. *Acta Physiol. Scand. 113* (1981) 571.
12. Piercey, M. F., Schroeder, L. A., Folkers, K., Xu, J.-C. and Horig, J. *Science 214* (1981) 1361.
13. Rosell, S., Björkroth, J., Xu, J.-C. and Folkers, K. *Acta Physiol. Scand. Submitted.*
14. Lundberg, J. M., Saria, A., Brodin, E., Rosell, S. and Folkers, K. *Unpublished data.*
15. Yanagisawa, M., Otsuka, M., Konishi, S., Akagi, H., Folkers, K. and Rosell, S. *Acta Physiol. Scand. In press.*
16. Yamaguchi, I., Rackur, G., Leban, J., Folkers, K., Björkroth, U. and Rosell, S. *Acta Chem. Scand. B 33* (1979) 63.
17. Yanaihara, N., Yanaihara, C., Hirohashi, M., Sato, H., Iizuka, Y., Hashimoto, T. and Sakagami, M. In von Euler, U. S. and Pernow, B., Eds., *Substance P Nobel Symposium 37,* Raven, New York 1977, p. 27.

Received October 29, 1982.