Synthesis and Biological Activities of D-Homoglutamine Analogs of Substance P

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Synopsis. Six substance P (SP) analogs substituted by D-homoglutamine (Hgn) at position 5 or/and 6 were prepared by the solid phase method. The activity of the peptides was assayed on the smooth muscles of the isolated guinea pig ileum and trachea. Results indicate that the substitution of D-Hgn for Gln residue(s) reduces the activity. [D-Hgn^6]-SP (4—11) **5** acted as an antagonist of SP.

Substance P[1] (SP), an undecapeptide with the sequence [H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2[2]], is a putative neurotransmitter or a neuromodulator. Up to date, numerous SP analogs have been synthesized and their structure-activity relationships have been studied.

Our study[3] on the structure-activity relationships of SP revealed that the elongation of the methylene group of the side-chain in the Gln residue(s), that is, the substitution of L-homoglutamine (Hgn) for Gln moiety at position 5 or/and 6, enhanced the biological activity on the smooth muscle of the isolated guinea pig ileum.

This paper describes the first application of a new amino acid D-Hgn to the synthesis of bioactive peptide analogs. D-Hgn residue as well as L-Hgn can be incorporated into a peptide chain by the usual solid phase technique and solution method using N^{α},N^{ca}-$(Boc)_2$-D-Hgn-OH,[4,5] which is derived from the ester of $(Boc)_2$-D-Lys-OH by ruthenium tetraoxide oxidation. The oxidative transformation[6,7] of Lys made it possible to prepare quite easily the novel Hgn derivative, which is applicable to present synthetic study of the structure-activity relationships. The protecting group Boc on the amide nitrogen (N^{ca}) of N^{α},N^{ca}-$(Boc)_2$-D-Hgn-OH can be eliminated by the same deblocking reagents as those for the protecting group on the α-amino function to produce the Hgn moiety.[5,7]

The aim of our present study is to examine not only the structure-activity relationships between L- and D-Hgn-substituted SP analogs at the same position(s), but also a capability of D-Hgn moiety to change the pharmacological spectrum of SP from that of an agonist to that of an antagonist by the substitution of D-Hgn for the natural amino acid residue in SP, as D-Trp and D-Ala residues have been used to obtain antagonist for SP[8] and its related peptide.[9] It is suggested[10] that the octapeptide SP (4—11) appears to be more favorable for antagonist than the undecapeptide SP itself. Thus three SP (1—11) and three SP (4—11) analogs replaced by D-Hgn at the same position 5 or/and 6 with L-Hgn SP analogs were synthesized by the solid phase method. The biological properties of the synthetic SP analogs were examined on the isolated guinea pig organs.

The SP analogs were synthesized in the same way with the preparation of L-Hgn analogs[3] by the solid phase technique[11] on an automated peptide synthesizer. α-Amino functions were protected by *t*-butoxycarbonyl (Boc) group. The side-chain protecting groups were Z for Lys, tosyl (Tos) for Arg and Boc for the amide of D-Hgn. The synthetic products were purified by preparative reverse-phase high-performance liquid chromatography (HPLC) using 0.1% TFA in acetonitrile (CH_3CN) as eluent. Highly purified peptides (Table 1 and 2) were obtained after gel filtration on Sephadex G-10 column. The yield was sacrificed for the purity. Homogeneity of the peptides was demonstrated by analytical HPLC and thin-layer chromatography (TLC). When single peak and single spot were observed for a peptide in all chromatographic systems, the peptide was considered appropriately pure for bioassay. These chromatographic data were reinforced by the amino acid analytical data.

The agonistic effects of the synthetic peptides were compared with that of our synthetic SP on the isolated guinea pig ileum and trachea. Three analogs were tested for their antagonistic effects against SP on the isolated guinea pig ileum. The results are presented in Table 3.

The replacement of the Gln residue(s) with D-Hgn brought the decrease of the contracting activity on the isolated guinea pig ileum and the trachea in contrast with the SP analogs containing L-Hgn residue(s). The L-configuration of both amino acid residues at positions 5 and 6 may be essential for the contractile activity of SP. Monosubstituted undecapeptide **1** and **2** possessed signifcant activities on the guinea pig ileum,

	1 4 5 6 11	
[D-Hgn^5]-SP	H-Arg-Pro-Lys-Pro-D-Hgn—Gln-Phe-Phe-Gly-Leu-Met-NH_2	**1**
[D-Hgn^6]-SP	H-Arg-Pro-Lys-Pro—Gln-D-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**2**
[D-Hgn^{5,6}]-SP	H-Arg-Pro-Lys-Pro-D-Hgn-D-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**3**
[D-Hgn^5]-SP (4—11)	H-Pro-D-Hgn—Gln-Phe-Phe-Gly-Leu-Met-NH_2	**4**
[D-Hgn^6]-SP (4—11)	H-Pro—Gln-D-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**5**
[D-Hgn^{5,6}]-SP (4—11)	H-Pro-D-Hgn-D-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**6**

Fig. 1. Synthetic SP analogs.

Table 1. Physical Properties and Yields of the Synthetic Peptides

Analog	$[\alpha]_D^{14}$ (c 0.5, 3M AcOH)/°	Retention time min	R_f^I	R_f^{II}	Yield %
1	−40.0	9.1	0.00	0.58	11.0
2	−57.8	11.2	0.00	0.57	13.7
3	−32.6	10.7	0.00	0.57	15.3
4	−29.6	13.5	0.13	0.71	15.6
5	−33.8	14.4	0.15	0.71	23.8
6	−15.6	14.8	0.13	0.71	10.1

Table 2. Amino Acid Analyses of the Synthetic Peptides

Analog	Found (Calcd)									
	Lys	Arg	Glu	Pro	Gly	Hgu[a)]	Met	Leu	Phe	NH_3
1	0.89 (1)	0.85 (1)	1.08 (1)	2.13 (2)	1.02 (1)	0.89 (1)	1.06 (1)	1.02 (1)	2.05 (2)	3.14 (3)
2	0.86 (1)	1.16 (1)	1.01 (1)	1.95 (2)	0.99 (1)	1.09 (1)	1.01 (1)	1.01 (1)	1.90 (2)	3.11 (3)
3	0.91 (1)	1.15 (1)	—	2.11 (2)	0.97 (1)	1.89 (2)	1.01 (1)	1.01 (1)	1.94 (2)	3.58 (3)
4	—	—	1.01 (1)	0.94 (1)	0.97 (1)	1.08 (1)	1.02 (1)	1.00 (1)	1.97 (2)	3.39 (3)
5	—	—	1.04 (1)	0.80 (1)	1.00 (1)	1.09 (1)	1.01 (1)	1.06 (1)	2.00 (2)	3.07 (3)
6	—	—	—	1.10 (1)	1.01 (1)	2.14 (2)	0.88 (1)	0.99 (1)	1.91 (2)	3.10 (3)

a) Homoglutamic acid; α-aminoadipic acid.

Table 3. Biological Activities of the Synthetic SP Analogs on Guinea Pig Organs

Analog	GPI		GPT
	RA	ANT	RA
1	0.48	——	0.04
2	0.42	——	0.10
3	0.02	−	<0.01
4	0.09	——	0.04
5	0.01	+	0.04
6	0.06	−	0.03

GPI, guinea pig ileum; GPT, guinea pig trachea; RA, relative contractile activity to SP=1. ANT, antagonism; antagonist activity concentration, 10^{-6}M: +, positive: −, negative: ——, not tested.

while the disubstituted analog **3** showed the lowest potencies on both assays. The potencies of monosubstituted octapeptide **4** and **5** were lower than the activities of the undecapeptides **1** and **2** on the guinea pig ileum assay. However, disubstituted octapeptide analog **6** was found to show higher potency than that of the undecapeptide **3** on the both assays. Analogs **3**, **5**, and **6** were tested to determine their antagonistic properties against SP on the guinea pig ileum. Analogs **3** and **6**, in which both Gln residues at positions **5** and **6** were replaced by D-Hgn, exerted no antagonistic effects against SP. [D-Hgn^6]-SP (4—11) **5** was found to act as an antagonist of SP. The results indicate that the replacement of the Gln residue with D-Hgn causes the reduction of the agonistic activity of SP and D-Hgn can be used to change the pharmacological spectrum of SP from that of an agonist to that of an antagonist. The data also suggest that octapeptide SP (4—11) analog is favorable for antagonist than undecapeptide SP analog.

Experimental

Amino acid derivatives were purchased from Peptide Institute, Inc., Osaka, Japan and benzhydrylamine (BHA) resin (available amine of the resin: 0.6 mmol g^{-1} of support) from Beckman Inc., Palo Alto, Calif. Optical rotations were measured in a Nipponbunkoh DIP-4 Polarimeter. Amino acid analyses on samples previously hydrolyzed with 6 M HCl (1 M=1 mol dm^{-3}) (110 °C, 24 h) were performed on a Hitachi KLA-5 Amino Acid Analyzer. TLC was carried out on silica-gel plates (Merck). The following solvent systems were used and allowed to ascend for 10 cm: R_f^I, *n*-BuOH : AcOH : H_2O (4 : 1 : 5, upper phase); R_f^{II}, *n*-BuOH : pyridine : AcOH : H_2O (30 : 20 : 6 : 24). Analytical HPLC was effected on the following systems: column, NOVA-PAK C_{18} (3.9×150 mm); flow rate, 1 ml min^{-1}; detection, 210 nm; eluent system, linear gradient for 15 min from 21% to 35% CH_3CN in 20 mM phosphate buffer (pH 3.0).

General Procedure for the Preparation of Analogs. The solid phase synthesis was carried out using a Beckman System 990C Peptide Synthesizer as described previously.[3] Half gram of BHA-resin hydrochloride for each analog served as the solid support. A 2.5 fold excess of the amino acid derivative was used for all coupling. The coupling was affected with dicyclohexylcarbodiimide/1-hydroxybenzotriazole. The protected peptide resin was treated with anhydrous liquid hydrogen fluoride (HF)[12] containing 10% anisole. After evaporation of HF in vacuo, the peptide was extracted with 10% AcOH.

Purification of the Peptides. The crude peptide was subjected to HPLC as reported previously.[3] The apparatus was composed of a model 590 pump and a U6K injector (Waters) connecting with a column (20×300 mm) of Chemcosorb ODS (Chemco). The eluates were monitored with a UV detector S-310A model-II (Soma) at 210 nm wavelength. CH_3CN-0.1% TFA solvent system was used as eluent at flow rate 10 ml min^{-1}. Each peptide was emerged at 40—60 min by isocratical elution with 21—23% CH_3CN contents of the solvent system. The desired fraction was passed through a Sephadex G-10 column (16×930 mm) eluted with 2M AcOH. Homogeneity of the peptides was analyzed by analytical HPLC and TLC.

Bioassay. The agonistic activity of the synthetic analog was measured on ileum and trachea taken from guinea pig, as described before.[3] The contraction was recorded by means of an isotonic transducer (Nippon Kohden, TD-111T) with load of 1 or 2 g (for trachea and ileum respectively) on a Servocorder (Watanabe Instruments, SR6204). Concentration-response curves were obtained using a cumulative dose-assay, and the time between two consecutive dose-response curves was longer than 10 min. In the tests for antagonistic activity on guinea pig ileum, SP analog was added 10 min before SP was added. The cumulative dose-response curve of SP in the presence or the absence of the analog was obtained.

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