Synthesis and Biological Activity of Substance P Analogs Containing Pyrohomoglutamic Acid and Homoglutamine

Tadashi HASHIMOTO,* Naoki SAKURA, Kyoko HIROSE, Yoshiki UCHIDA, Miwako NISHIJIMA, and Tadashi MORO
School of Pharmacy, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-11
(Received August 26, 1986)

Synopsis. Eight substance P analogs substituted by L- or D-pyrohomoglutamic acid (6-oxo-2-piperidinecarboxylic acid) and L- or D-homoglutamine at the position 5 and 6 were prepared by the solid phase method. The contractile activities of the analogs were compared with that of SP on isolated guinea-pig organs.

Synthetic study[1] on thyrotropin-releasing hormone [TRH: pGlu-His-Pro-NH_2[2]] revealed that TRH analogs containing pyrohomoglutamic acid (pHgu-OH: 6-oxo-2-piperidinecarboxylic acid) at the N-terminus showed relative selectivity for action in the central nervous system. Therefore, the substitution of pHgu residue for pGlu at N-terminus of a bioactive peptide may be able to provide a new knowledge of the structure-activity relationships.

Up to date, L-pHgu moiety has been incorporated into peptide chain using L-pHgu-OH and the usual peptide bond forming reagent. However, the preparation of L-pHgu-OH involves a troublesome work, that is, generally an enzymatic resolution[3] of N-acetyl-DL-homoglutamic acid (DL-Ac-Hgu-OH) to give L-H-Hgu-OH, followed by cyclization procedure. Our investigation[4] developed a simple method for synthesis of pHgu-peptide that Hgn residue at N-terminus in peptide chain could be cyclized to produce pHgu residue. D-Hgn-peptide is readily converted and yields a new amino acid D-pHgu residue. This conversion reaction was applied to the synthetic study on analogs of substance P (SP) related peptides, pGlu-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (**1**) and pGlu-Phe-Phe-Gly-Leu-Met-NH_2 (**2**), which potencies (Table 1) are higher than the contracting activity of SP itself of guinea-pig ileum.[5]

This paper describes syntheses and biological properties of eight analogs (Fig. 1) of the SP related peptides containing L- or D-pHgu and L- or D-Hgn at position 5 and 6. The contracting activities of the synthetic peptides were compared with that of our synthetic SP in order to examine the biological effects of the substitution of L- or D-pHgu for pGlu moiety and of the replacement of Gln residue by L- or D-Hgn on pGlu-peptides.

The SP analogs were synthesized in the same way with the preparation of SP analogs containing L-Hgn[6] and D-Hgn[7] by the solid phase technique[8] on an automated peptide synthesizer. *t*-Butoxycarbonyl (Boc) protection was used for α-amino function except benzyloxycarbonyl(Z) for pGlu-OH, and the amide nitrogen (N^{ca}) of Hgn. N-Terminal Hgn-peptide was kept at 40 °C for 4 h in aq acetic acid (50%) to produce pHgu moiety. The crude product was purified by preparative reverse-phase high-performance liquid chromatography (HPLC) using aq trifluoroacetic acid (0.1%) in acetonitrile as eluent. Highly purified peptides (Table 2, 3) 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 peaks and single spots were observed for a synthetic 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 results of biological properties of the synthetic analogs are shown in Table 4.

The potency of D-pHgu heptapeptide analog **4** was as high as the contractile activity of **1** and higher than that of L-pHgu-peptide **3** on isolated guinea-pig ileum. The both replacements of pGlu and Gln

Table 1. Relative Contracting Activities of Synthetic Analogs on Guinea-Pig Ileum

Peptide	Amino acid sequence	Relative activity
	1 5 6 11	
SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	1
1 [pGlu^5]-SP (5—11)	pGlu-Gln-Phe-Phe-Gly-Leu-Met-NH_2	1.7
2 [pGlu^6]-SP (6—11)	pGlu-Phe-Phe-Gly-Leu-Met-NH_2	2.0

		5 6 11	
[L-pHgu^5]-SP	(5—11)	L-pHgu—Gln-Phe-Phe-Gly-Leu-Met-NH_2	**3**
[D-pHgu^5]-SP	(5—11)	D-pHgu—Gln-Phe-Phe-Gly-Leu-Met-NH_2	**4**
[pGlu^5, L-Hgn^6]-SP	(5—11)	pGlu-L-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**5**
[pGlu^5, D-Hgn^6]-SP	(5—11)	pGlu-D-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**6**
[L-pHgu^5, L-Hgn^6]-SP	(5—11)	L-pHgu-L-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**7**
[D-pHgu^5, D-Hgn^6]-SP	(5—11)	D-pHgu-D-Hgn-Phe-Phe-Gly-Leu-Met-NH_2	**8**
[L-pHgu^6]-SP	(6—11)	L-pHgu-Phe-Phe-Gly-Leu-Met-NH_2	**9**
[D-pHgu^6]-SP	(6—11)	D-pHgu-Phe-Phe-Gly-Leu-Met-NH_2	**10**

Fig. 1. Synthetic analogs.

Table 2. Physical Properties and Yields of Synthetic Analogs

Analog	$[\alpha]_D$/°	Retention time[c]	R_f^I	R_f^{II}	Yield/%
3	−25.3 (*c* 0.3)[a]	16.0	0.46	0.81	21.0
4	−42.3 (*c* 0.3)[b]	16.1	0.45	0.84	8.3
5	−34.0 (*c* 0.5)[a]	16.3	0.45	0.81	14.0
6	−42.0 (*c* 0.3)[b]	16.9	0.46	0.83	7.6
7	−28.3 (*c* 0.3)[a]	17.4	0.44	0.82	12.3
8	−43.6 (*c* 0.5)[b]	16.8	0.43	0.83	12.1
9	−37.6 (*c* 0.5)[a]	19.7	0.72	0.87	18.5
10	−56.6 (*c* 0.5)[b]	19.3	0.73	0.87	20.8

a) and b) $[\alpha]_D$ values were measured in DMF at 25 °C and 14 °C respectively. c) Condition of analytical HPLC: column, NOVA-PAK C_{18}; flow rate, 1 ml min^{-1}; Detection, 210 nm; eluent system, linear gradient from 21% to 35% CH_3CN (15 min) in 20 mM phosphate buffer (pH 3.0).

Table 3. Amino Acid Analyses of the Synthetic Peptides

Analog	Found (Calcd)						
	Glu	Gly	Hgu[a]	Met	Leu	Phe	NH_3
3	0.99 (1)	0.95 (1)	1.06 (1)	0.95 (1)	0.98 (1)	2.06 (2)	2.49 (2)
4	0.99 (1)	1.01 (1)	1.04 (1)	0.98 (1)	0.99 (1)	1.99 (2)	2.04 (2)
5	1.01 (1)	0.96 (1)	1.09 (1)	0.97 (1)	0.97 (1)	1.96 (2)	2.92 (2)
6	1.03 (1)	0.99 (1)	1.08 (1)	0.93 (1)	0.99 (1)	1.99 (2)	2.08 (2)
7	—	1.00 (1)	2.10 (2)	0.94 (1)	0.94 (1)	2.01 (2)	2.42 (2)
8	—	1.00 (1)	2.16 (2)	0.88 (1)	0.98 (1)	1.98 (2)	2.47 (2)
9	—	0.96 (1)	1.11 (1)	0.96 (1)	1.00 (1)	1.98 (2)	1.07 (1)
10	—	0.97 (1)	1.03)1)	1.03 (1)	1.04 (1)	1.93 (2)	1.33 (1)

a) Homoglutamic acid.

Table 4. Relative Potencies of Synthetic Analogs on Guinea-Pig Organs

Analog	Relative activity accepted SP as 1	
	Ileum	Trachea
3	0.65	1.10
4	1.76	0.78
5	2.08	3.64
6	0.07	<0.01
7	0.88	1.74
8	0.10	<0.01
9	1.50	3.01
10	0.17	0.14

residues with L- or D-pHgu and L- or D-Hgn brought the decreases of the contracting activities on the ileum assay. Analogs **7** and **8** had 90 and 10% of the activity of SP, and 50 and 6% of that of **1** respectively. pGlu-peptide analogs **5** possessed the highest activities among the eight analogs **3**—**10** on the both assays, while D-Hgn analog **6** showed the lowest potency. Hexapeptide analog **9** was found to be highly active on the both assays, and had 150 and 300% of the activity of SP on the ileum and trachea assays respectively and 75% of that of **2** on the ileum assay. Similarly to SP analogs containing L-Hgn[6] or D-Hgn,[7] the replacement of Gln moiety with L-Hgn on pGlu-peptide enhanced the biological activity, while D-Hgn analog **6** possessed reduced activity. The data indicate that L-pHgu-peptide analogs exert potent agonistic effects and the substitution of D-pHgu for pGlu, except for analog **4**, brings the drastic decrease of the activity. The results also suggest that the L-configuration of the amino acid residue at the position 6 may be more important than that of the residue at the position 5 for the contractile activities of SP related peptides.

Experimental

Amino acid derivatives were purchased from Peptide Institute, Inc., Osaka, and benzhydrylamine (BHA) resin (available amine of the resin: 0.6 mmol g^{-1} of support) from Beckman Inc. 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 were carried out on silica-gel plates (Merck). The following solvent systems were used: 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 were 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 990 C Peptide Synthesizer as described previously.[6] Half gram of BHA-resin hydrochloride for each analog served as the solid support. A 2.5 fold excess of amino acid derivative was used for all coupling. The coupling was affected with DCC/HOBt.† The protected peptide resin was treated with anhydrous liquid HF containing 10% anisole. After evaporation of HF in vacuo, the peptide was extracted with 50% AcOH. The extract of N-terminal Hgn-peptide was kept at 40 °C for 4 h to yield pHgu moiety.

Purification of the Peptides. The crude peptide was subjected to HPLC as reported previously.[6] The apparatus was

†DCC=dicyclohexylcarbodiimide, HOBt=1-hydroxybenzotriazole.

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 27—34% CH_3CN contents of the solvent system. The desired fraction was passed through a Sephadex G-10 column (16×930 mm) eluted with 2 M 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.[6] 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.

References

1) R. F. Nutt, F. W. Holly, C. Homnick, R. Hirshmann, D. F. Veber, and B. H. Arison, *J. Med. Chem.*, **24**, 692 (1981).

2) Amino acids, peptides and their derivatives mentioned in this paper are of L-configuration. The abbreviations for amino acids and peptides are in accordance with the rules of the IUPAC-IBU Commission on Biochemical Nomenclature in *Eur. J. Biochem.*, **138**, 9 (1984).

3) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, New York (1961), Vol. 3, p. 2411.

4) K. Hirose, N. Sakura, Y. Uchida, M. Nishijima, and T. Hashimoto, *Chem. Pharm. Bull.*, in press.

5) N. Yanaihara, C. Yanaihara, M. Hirohashi, H. Sato, Y. Iizuka, T. Hashimoto, and M. Sakagami, "Substance P," ed by U. S. von Euler and B. Pernow, Raven Press, New York (1977), p. 27.

6) Y. Uchida, M. Nishijima, T. Moro, N. Sakura, K. Hirose, H. Kontani, and T. Hashimoto, *Bull. Chem. Soc. Jpn.*, **59**, 4003 (1986).

7) T. Hashimoto, Y. Uchida, M. Nishijima, T. Moro, N. Sakura, and K. Hirose, *Bull. Chem. Soc. Jpn.*, **59**, 4009 (1986).

8) R. B. Merrifield, *Adv. Enzymol.*, **32**, 221 (1969).