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Structure-Antagonistic Activity Relationships of an NK-2 Tachykinin Receptor Antagonist, L-659,877 and Its Analogues

Jong-Myung Ha*, Song Yub Shin[1], Hea Nam Hong[2], Duk Joon Suh[3], Tae Sik Jang[4], Shin Won Kang[4], Sun Jin Kuean[5] and Bae Jin Ha

Department of Chemistry, College of Natural Sciences, Pusan Women's University, Pusan 616-736, [1]Peptide Engineering Research Unit, Korea Research Institute of Bioscience and Biotechnology, KIST, Taejon 305-600, [2]Department of Anatomy, College of Medicine, University of Ulsan, Seoul 138-040, [3]Department of Physiology, College of Medicine, Dong-A University, Pusan 602-103, [4]Department of Chemistry, College of Natural Sciences, Pusan National University, Pusan 609-735, [5]Department of Food Science & Nutrition, College of Home Economics, Pusan National University, Pusan 609-735, Korea

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Abstract: To investigate the structure-antagonistic relationship of the cyclohexapeptide L-659,877, a selective NK-2 tachykinin receptor antagonist, seven analogues were chemically synthesized by a solid phase method. The agonistic and antagonistic activities of the analogues were evaluated by contraction assay using the smooth muscle of guinea pig trachea (GPT) containing the NK-2 receptor. It was shown that the aromatic ring of Phe at position 3 and the sulfur group of Met at position 6 in L-659,877 were essential for binding to the NK-2 receptor. Decrease in antagonistic activity of L-659,877 caused by substituting Leu for Nle at position 5 indicates that the γ methyl group and side chain length of Leu plays an important role in its antagonistic action. Although the activity was slightly lower than L-659,877, cyclo [βAla[8]]NKA(4-10) (analogue 1) showed potential antagonistic activity for the NK-2 receptor. It was confirmed that the expansion of the ring in L-659,877 by substitution of βAla for Gly at position 4 stabilized its conformation monitored by CD spectra. The results suggest that analogue 1 can be used as a new leader compound to design a more powerful, selective, and stable NK-2 receptor antagonist.

Key words: antagonist, cyclic hexapeptide, guinea pig trachea, L-659,877, neurokinin-2 (NK-2).

Tachykinins are a family of neuropeptide which share the common carboxyl terminal sequence, Phe-Xaa-Gly-Leu-Met-NH_2. In mammalians, the three main tachykinin peptides are: substance P(SP) (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) (Euler and Gaddum, 1931; Chang *et al.*, 1971), neurokinin A(NKA) (His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2)(Kimura *et al.*, 1983), and neurokinin B(NKB) (Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2) (Kimura *et al.*, 1983). SP, NKA and NKB are widely distributed throughout the central and peripheral nervous systems, where they are believed to act as neurotransmitters or neuromodulators (Maggio, 1985; Takano *et al.*, 1990; Munekata, 1991).

It has been accepted that their diverse actions of the mammalian tachykinins are mediated by three distinct tachykinin receptors, named as NK-1, NK-2 and NK-3 (Buck *et al.*, 1984; Lee *et al.*, 1986; Regoli *et al.*, 1989). SP displays the highest affinity for the NK-1 receptor, whereas NKA and NKB preferentially bind to NK-2 and NK-3 receptors, respectively. All three receptors have been cloned and turned out to be members of the G-protein-coupled seven-transmembrane spanning receptor superfamily (Masuo *et al.*, 1987; Shigemoto *et al.*, 1990). Complete characterization of the multiple tachykinin receptor and a study of the relative physiological functions of tachykinins have been hampered by the absence of more selective receptor antagonists. There have been several attempts to develop a selective antagonist for tachykinin receptors.

Although there a large number of selective NK-1 receptor antagonists reported (Folkerts *et al.*, 1986; Dutta, 1987; Wollborn *et al.*, 1993), only a few NK-2 receptor antagonists have been discovered (Martling *et al.*, 1987; Rogers *et al.*, 1989). Selective NK-2 receptor antagonists are valuable for therapeutic purposes since NK-2 is responsible for bronchoconstriction (Uchita

*To whom correspondence should be addressed.
Tel: 82-51-309-5467, Fax: 82-51-309-5176.

et al., 1987; Maggi *et al.*, 1993).

Particularly, cyclic peptide antagonists may be very useful because of their enhanced enzymatic stability and selectivity toward distinctive receptors. Recently, Williams *et al.* (1993) reported L-659,877 as a selective NK-2 receptor antagonist. This cyclic hexapeptide is of considerable interest because it is derived from carboxy-terminal six amino acids of SP.

In this study, to investigate structure-antagonistic relationships of L-659,877 and to develop a powerful selective antagonist for the NK-2 receptor, seven analogues of L-659,877 were synthesized by a stepwise solid phase method using Fmoc(9-fluorenylmethoxycarbonyl) strategy. The agonistic and the antagonistic activities of peptides were investigated with contraction assay of the smooth muscle on guinea pig trachea (GPT) containing the NK-2 receptor, preferentially (Shin *et al.*, 1993; Shin *et al.*, 1994).

Materials and Methods

Chemical reagents

Fmoc-amino acid derivatives were purchased from Watanabe Chemical IND., LTD (Hiroshima, Japan). Bop [benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate] was purchased from Kokusan Chemical Works, LTD. (Tokyo, Japan). DCC (dicyclohexylcarbodiimide), DMAP (4-methylaminopyridine), Wang-resin, HOBt (1-hydroxybenzotriazole), NMP (1-methyl-2-pyrridone), DIEA (diisopropylethylamine), TFA (trifluoroaceticacid), HBTU (O-benzotriazole-1-yl-tetra-methylmethyluronium hexafluorophosphate) and piperidine were purchased from Applied Biosystems (Foster city, USA). Phenol, TEA (triethylamine), EDT (1,2-ethanedithiol), DCM (dichoromethane), DMSO (dimethyl sulfoxide), anisole, thioanisole, and benzoic anhydride were supplied from Wako Pure Chemical IND., LTD. (Osaka, Japan).

Synthesis of the linear hexapeptide of L-659,877 and its analogues

Loading of Fmoc-Leu onto the Wang-resin was performed by the DCC/ DMAP method for 18 h at 0℃ (Fmoc-Leu : DMAP : HOBt : DCC : Wang-Resin=2 : 2 : 4 : 2 : 1 equiv.). The substitution degree (0.25 mmol/g, Fmoc-Leu-Wang resin) was determined by amino acid analysis (HITACHI L-8500 Amino Acid Analyzer). Coupling of Fmoc-amino acid at each step was performed using Bop/HOBt/DIEA (1 : 1 : 1) or HBTU/ HOBt/DIEA (1 : 1 : 1) in NMP. Coupling completion was checked by Kaiser s ninhydrin test (Kaiser *et al.*, 1970). Incomplete coupling reaction was either recoupled until a negative Kaiser result was obtained or acetylated by treatment with benzoic anhydride in DMF-DCM (1 : 3, v/v). After completion of chain elongation, the protected-peptide-Wang resin (250 mg) was treated with TFA (10 ml) in the presence of scavengers (Phenol; 0.75 g, EDT; 0.25 ml, thioanisole; 0.5 ml, H_2O; 0.5 ml) at room temperature for 2 h. After removing volatile materials under a vacuum, the crude residues were washed with cold ethylether 3 times to remove trace amount of scavengers. The crude product was extracted in 1 M of acetic acid and the resultant solution was lyophilized. The fluffy product was purified with a preparative RP-HPLC (GL Science, Inertsil ODS-2, 32.0×250 mm).

Cyclization of the linear hexapeptide of L-659,877 and its analogues and purification

Cyclization of linear peptide was completed with BOP/HOBt/DIEA in DMF for overnight at room temperature. A solution of the linear peptide (1.0 equiv.) in DMF was dropped in a solution of BOP (10 equiv.) and HOBt (10 equiv.) in DMF (final concentration of peptide: 10^{-3} M). The product was checked by analytical RP-HPLC during cyclization. The crude product was purified by a preparative RP-HPLC (GL Science, Inertsil ODS-2, 32.0×250 mm), and detected UV at 280 nm. The correct amino acid composition of purified linear hexapeptides was identified by amino acid analysis. The molecular weight of cyclic peptides was checked by fast atom bombardment mass spectrometry (FAB-mass).

Pharmacological assay

We measured the peptide induced contraction of guinea pig trachea (GPT) as described by Shanab *et al.* (1991). Briefly, Hartley guinea pig (280~320 g) was sacrificed by a blow on the head and the trachea was rapidly dissected away and put in cold Krebs-Henseleit Bicarbonate solution (NaCl 119.0 mM, KCl 3.5 mM, KH_2PO_4 1.5 mM, $CaCl_2$ 1.25 mM, $NaHCO_3$ 25.0 mM, Glucose 11.0 mM, pH 7.4). Two rings of trachea were connected to a steel hook vertically jointed in series and incubated in a 5 ml organ bath filled with warm (37℃) oxygenated (95% O_2, 5% CO_2) Krebs solution. The muscle was equilibrated, under a tension of 1.0 g prior to the assay for 60 min, while being washed every 20 min with Krebs-Henseleit solution. The preperated muscles were excised by the addition of 10^{-6} M carbachol. Contractions were recorded isotonically under a resting tension of 1 g *via* FD-pick up (TB-612T, Nihon Koden) connected to an amplifier (AP 601G, Nihon Koden) and recorder (WI-621G, Nihon Koden). Peptides were dissolved in saline and were applied at intervals of 10~15 min, consecutively. Peptide derivatives insoluble in aqueous media were dissolved in saline with the aid of a small amount of

DMSO.

The agonistic and antagonistic L-659,877 and seven cyclic hexapeptides were evaluated by contraction assay of the smooth muscle on GPT. The pD2 value is a negative logarithm of molar concentration of agonist needed to exhibit 50% of the maximal response. In this study, [βAla^8] NKA(4-10) was used as the NK-2 receptor agonist (Rovero *et al.*, 1989; Maggi *et al.*, 1990). [βAla^8] NKA(4-10) is known to be more potent and selective to NK-2 receptor than NKA (Maggi *et al.*, 1990). In all preparations, full concentration-response curves to the [βAla^8]NKA(4-10) were determined in the absence and presence of various concentrations of agonists. The pA_2 value is a negative logarithm of the concentration of antagonist that reduces the effect of a double dose to that of a single dose of selective agonist [βAla^8]NKA(4-10). It was estimated according to Van Rossum (1963).

Circular Dichroism (CD) Measurement

The CD measurements of cyclic hexapeptides were made on a JASCO J20-A Automatic Recording Spectropolarimeter using a quartz cell of 2 mm pathlength. The concentration of cyclic hexapeptides was determined by amino acid composition analysis. CD spectra in 100% trifluoroethanol (TFE) was measured at a peptide concentration of 100 μM. All measurements were carried out at 25℃.

Results and Discussion

Cyclic hexapeptide synthesis

Many synthetic peptides have been used to develop a more potent and selective antagonist for tachykinin receptors. The L-659,877 is the most selective cyclic peptide as an NK-2 receptor antagonist. Interestingly, the sequence of this cyclic hexapeptide is based on the carboxy-terminal six amino acid residues of SP. In order to understand the relationships between the structure and antagonistic activity of L-659,877 for the NK-2 receptor, seven cyclic hexapeptides (Fig. 1) were chemically synthesized by substituting several amino acid residues, and their antagonistic activities were measured. Seven cyclic hexapeptides were prepared by a solid phase method using Fmoc chemistry. The synthetic route of a cyclic hexapeptide is indicated in Fig. 2. Here, analogue 6 and 5 are designed from carboxy-terminal six amino acids of NKA and NKB, respectively.

After protected-peptide-resins were treated with TFA in the presence of scavengers, crude linear hexapeptides were obtained. The crude linear hexapeptides were purified by a preparative RP-HPLC (GL Science, Inertsil ODS-2, 32.0×250 mm). The elution profiles purified

L-659,877	: cyclo (Gln-Trp-Phe-Gly-Leu-Met)
Analogue 1	: cyclo (Gln-Trp-Phe-βAla-Leu-Met)
Analogue 2	: cyclo (Gln-Trp-Phe-Gly-Nle-Met)
Analogue 3	: cyclo (Gln-Trp-Phe-Gly-Leu-Nle)
Analogue 4	: cyclo (Gln-Trp-Tyr-Gly-Leu-Met)
Analogue 5	: cyclo (Phe-Trp-Val-Gly-Leu-Met)
Analogue 6	: cyclo (Ser-Trp-Val-Gly-Leu-Met)
Analogue 7	: cyclo (Gln-Trp-Phe-Pro-Leu-Met)

Fig. 1. Amino acid sequences of L-659,877 and its analogues.

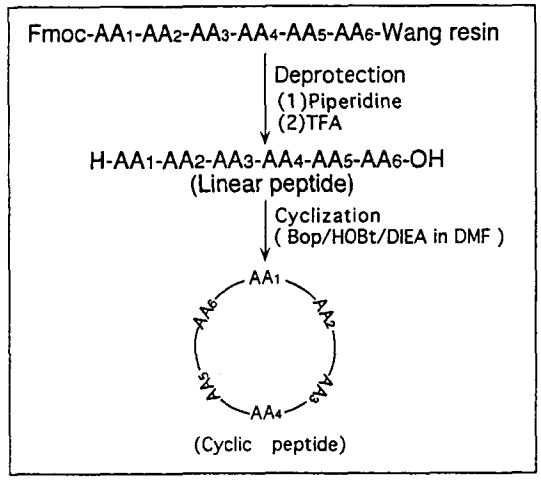

Fig. 2. Synthetic route of L-659,877 and its analogues.

linear hexapeptides before cyclization from analytical RP-HPLC (Beckman ODS, 4.5×250 mm) were shown as a single peak (data not shown). Amino acid compositions of linear hexapeptides were determined by amino acid analysis (Table 1). The purified linear hexapeptides were cyclized by the head-to-tail (amino-terminus to carboxy-terminus) cyclization method with BOP/HOBt/DIEA in DMF. After purification, the synthetic cyclic hexapeptides were eluted as a single symmetrical peak in analytical RP-HPLC as shown in Fig. 3. The molecular weight of the purified cyclic hexapeptides was assessed by a fast atom bombardment mass spectrometer (FAB-mass). The observed values of purified cyclic hexapeptides in the FAB-mass agreed with the molecular weights calculated (Table 2). Thus, all synthetic cyclic hexapeptides were homogeneous.

Biological activities

DMSO had no effect on muscle contraction of the GTP assay. Analogues 5 and 7 showed very weak ago-

Table 1. Amino acid compositions of synthetic linear peptides of L-659,877 and its analogues determined by amino acid analysis

Amino acid	L-659,877	Analogue 1	Analogue 2	Analogue 3
Glu	1.06(1)	1.09(1)	1.02(1)	0.95(1)
Phe	1.03(1)	1.00(1)	1.00(1)	0.93(1)
Gly	1.04(1)		1.00(1)	0.94(1)
Leu	1.00(1)	0.99(1)		1.00(1)
Met	1.00(1)	0.93(1)	0.98(1)	
βAla		0.85(1)		
Nle			0.95(1)	1.02(1)

Amino acid	Analogue 4	Analogue 5	Analogue 6	Analogue 7
Glu	1.06(1)			1.06(1)
Phe		1.01(1)		1.03(1)
Gly	0.90(1)	0.94(1)	1.00(1)	
Leu	1.00(1)	1.00(1)	1.07(1)	1.00(1)
Met	1.00(1)	1.03(1)	0.97(1)	0.96(1)
Tyr	1.01(1)			
Val		0.99(1)	1.07(1)	
Ser			1.10(1)	
Pro				0.98(1)

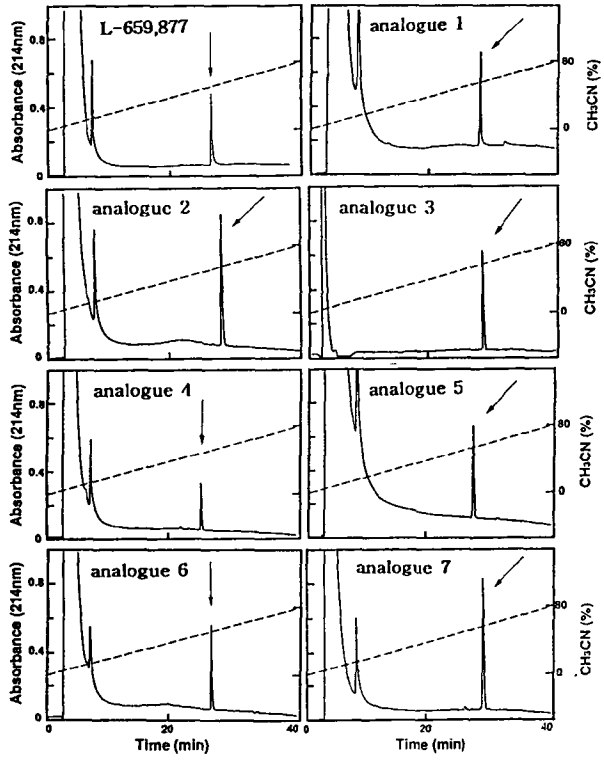

Fig. 3. Analytical RP-HPLC profiles of synthetic L-659,877 and its analogues. Peptides were applied to a Backmann C_{18} column (4.5×250 mm) using a Backmann HPLC system. Conditions, mobile phase; A=0.1% trifluoroacetic acid (TFA) in water, B=80 % acetonitrile in 0.1% TFA; gradient, linear, 0~60% B in 60 min; flow, 1.0 ml/min; detection, UV (210 nm).

Table 2. Molecular weights of L-659,877 and its analogues determined by FAB-mass

Peptides	Theoretical value	Experimental value
L-659,877	763.4	763.4
Analogue 1	777.4	777.2
Analogue 2	763.4	763.2
Analogue 3	745.3	745.4
Analogue 4	779.4	779.1
Analogue 5	734.3	734.3
Analogue 6	674.3	674.3
Analogue 7	803.4	803.2

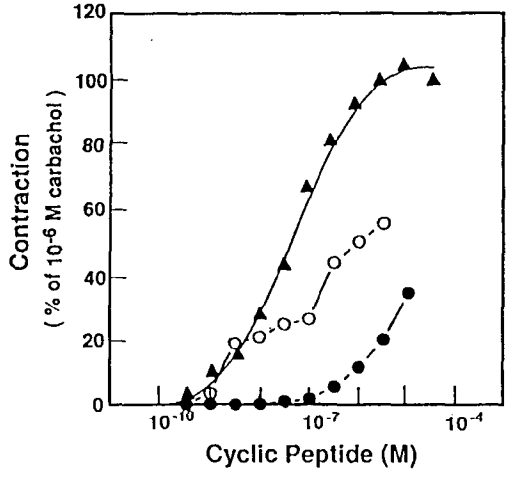

Fig. 4. Concentration-response curves of [βAla^8] NKA(4-10) (▲) analogue 5 (○) and analogue 7 (●) measured in GPT. Abscissa: the molar concentration of cyclic peptide. Ordinate: percent of contraction induced by carbachol (10^{-6} M).

nistic activity in the GPT assay (Fig. 4). The pD_2 values of [βAla^8]NKA(4-10) were 7.5, analogue 5 being less than 5.7 and analogue 7 being less than 4.5, respectively. On the other hand, the other five analogues, analogue 1, 2, 3, 4 and 6, exerted no agonistic activities. Therefore, the antagonistic activity of these five analogues was investigated. The two cyclic analogues, analogue 1 and 2, caused a rightward shift of the concentration-response curve at their 10^{-5} M concentrations as [βAla^8]NKA(4-10) increased (Fig. 5). However, analogues 3, 4 and 6 showed no antagonistic activity. Substitutions Phe with either Tyr or Val at position 3 and Met with Nle at position 6 removed the antagonistic activity of L-659,877. In addition, these analogues showed no agonistic activity. Thus, these results indicate that the aromatic ring of Phe at position 3 and the sulfur group of Met at position 6 in L-659,877 are essential for binding to the NK-2 receptor.

The contractile activity of [βAla^8]NKA(4-10) was antagonized by a 10^{-5}M pretreatment of analogue 1 and

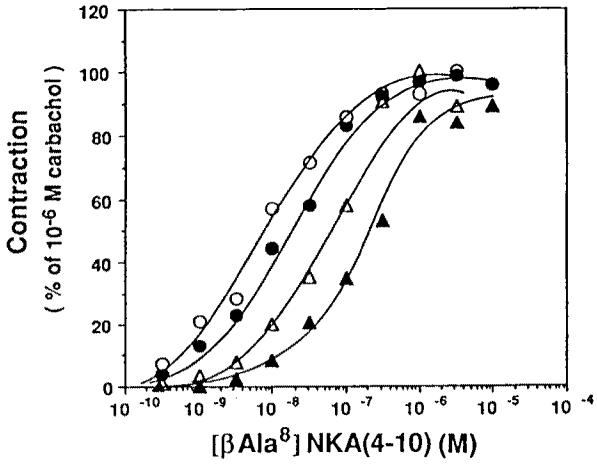

Fig. 5. Concentration-response curves of [βAla^8]NKA(4-10) in GPT in the absence (○) and in the presence of 10^{-5} M L-L-659,877 (▲), 10^{-5} M analogue 1 (△) and 10^{-5} M analcgue 2 (●). Abscissa: the molar concentration of [βAla^8]NKA(4-10). Ordinate: percent of contraction induced by carbachol (10^{-6} M).

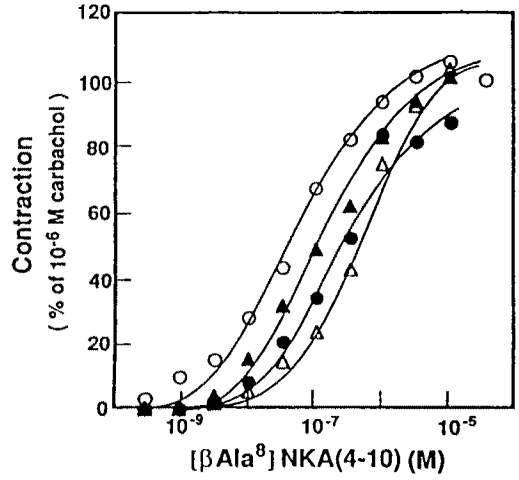

Fig. 6. Concentration-response curves of [βAla^8] NKA(4-10) in GPT in the absence (○) and in the presence of 10^{-5} M (△), 10^{-6} M (●) or 10^{-7} M (▲) of L-659,877. Abscissa: the molar concentration of [βAla^8]NKA(4-10). Ordinate: percent of contraction induced by carbachol (10^{-6} M).

2 [rank order of antagonistic activity: L-659,877> analogue 1> analogue 2]. The replacement of Leu at position 5 in L-659,877 with Nle brings a remarkable decrease in antagonistic activity. This result suggested that the γ methyl and side chain length of Leu at position 5 play an important role in the antagonistic activity of L-659,877.

The antagonistic activity of analogue 1 was investigated in detail by pretreatment (analogue 1) at various concentrations (10^{-7}, 10^{-6} and 10^{-5} M). The contractile response of [βAla^8]NKA(4-10) was antagonized by analogue 1 in a concentration-dependent manner like L-659,877 (Fig. 6, 7). The pA_2 values of L-659,877 and analogue 1 were 7.0 and 6.7, respectively. The pA_2 values of the antagonists were obtained using the constrained Schild plot method (Schild, 1947; Tallarida *et al.*, 1979). Although the activity was slightly lower than L-659,877, analogue 1 showed potent antagonistic activity against [βAla^8] NKA(4-10).

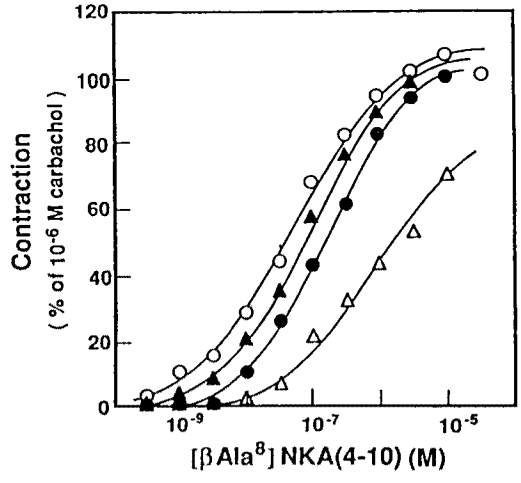

Fig. 7. Concentration-response curves of [βAla^8]NKA(4-10) in GPT in the absence (○) and in the presence of 10^{-5} M (△), 10^{-6} M (●) or 10^{-7} M (▲) of analogue 1. Abscissa: the molar concentration of [βAla^8]NKA(4-10). Ordinate: percent of contraction induced by carbachol (10^{-6} M).

Solution conformation of L-659,877 and cyclo-[βAla^8] NKA(4-10)

It is known that cyclization does not always constrain flexible linear peptides to one stable conformation (Malikayil and Harbeson, 1992; Wollborn *et al.*, 1993). In fact, Gly→βAla substitution can confer conformational freedom to the backbone dihedral angles of a cyclic peptide (Narita *et al.*, 1993). Amodeo *et al.* (1994) reported that the converged structure of L-659,877 was not obtained presumably due to the presence of multiple conformations which are in a fast exchange with each other on a NMR chemical shift scale.

In this study, CD spectra were measured in order to investigate the predicted structure of L-659,877 and analogue 1. The CD spectra of L-659,877 and analogue 1 in 100% TFE are shown in Fig. 8. L-659,877 exhibited a negative band near the 217 nm representative of a β-structure and a negative band near the 200 nm representative of a random coil. On the other hand, analogue 1 showed a negative band near 217 nm and a positive band near 200 nm characteristic of a β-structure. Unexpectedly, CD spectra data indicated that analogue 1 forms a more stable β-structure when compared with L-659,877. This result suggested that the extension in the ring size by addition of an

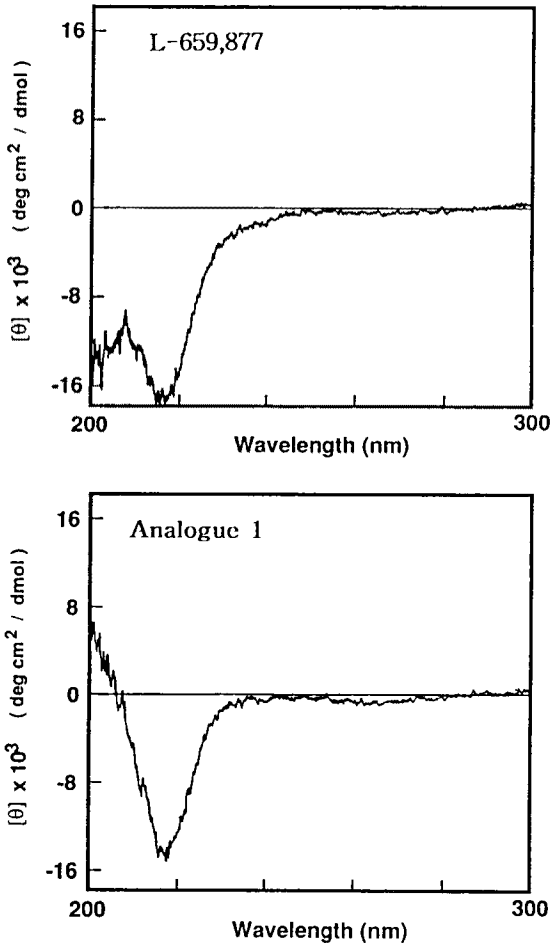

Fig. 8. Circular dichroism (CD) spectra of L-659,877 and analogue 1 in 100% trifluoroethanol (TFE). Peptide concentration is 100 μM.

extra methylene group to Gly residue resulted in increasing conformational stabilization of L-659,877. Kessler (1992) suggested that the inclusion of βAla in cyclic peptides results in a less defined conformation. Such modification may actually help to analogue structure of cyclic peptides when the original cyclic peptide is comprised of a mixture of multiple conformations.

Now, we are planning to compare the defined conformation of analogue 1 and L-659,877 by high resolution proton NMR spectra in DMSO and structure calculations. We expect that analogue 1 serves as a new leader compound for designing a more selective, powerful, and stable NK-2 receptor antagonist in conformation. Moreover, potent as well as selective agonist/antagonist peptides with a defined conformation will be useful for probing structural differences existing in different receptors.

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References

Amodeo, P., Rovero, P., Saviano, G. and Temussi, P. A. (1994) *Int. J. Pept. Protein Res.* **44**, 556.

Buck, S. H., Burcher. E., Schultz. C. W., Lovenberg, W. and O'Donarce, T. L. (1984) *Science* **226**, 987.

Chang, M. M., Leeman. S. E. and Nial, H. D. (1971) *Nature* **232**, 86.

Dutta, A. S. (1987) *Drugs Future* **12**, 781.

Euler, U. S. von and Gaddum, J. H. (1931) *J. Physiol. Lond.* **72**, 74.

Folkerts, K., Rosell, S., Chu, J. -Y., Lu, L. -A., Tang, P.-F. L. and Ljungqvist, A. (1986) *Acta Chem. Scand. Ser.* **B40**, 295.

Kaiser, E., Colescott, R. L., Bossinger C. D. and Cook, C. D. (1970) *Anal. Biochem.* **34**, 595.

Kimura, S., Okada, M., Sugita, Y., Kanazawa, I. and Munekata, E. (1983) *Proc. Jpn. Acad.* **58B**, 101.

Lee, C. M., Campbell. N. J., William. B. J. and Iversen. L. L. (1986) *Eur. J. Pharmacol.* **130**, 209.

Maggi, C. A., Patacchini, R., Giuliani, S., Rovero, P., Dion, S., Regoli, Giachetti, A. and Meli, A. (1990) *Br. J. Pharmacol.* **100**, 588.

Maggi, C. A., Quartara, L., Giuliani, S. and Patacchini, R. (1993) *Drugs Future* **18**, 155.

Maggio, J. E. (1985) *Peptides* **6**, 237.

Malikayil, J. A. and Harbeson, S. L. (1992) *Int. J. Peptide Protein Res.* **39**, 497.

Martling, C.-R., Theodorsson-Norheim, E. and Lundberg, J. M. (1987) *Life Sci.* **40**, 1633.

Masuo, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M. Nakanishi, S. (1987) *Nature* **329**, 836.

Munekata, E. (1991) *Comp. Biochem. Physiol.* **98C**(1), 171.

Narita, M., Doi, M., Kubo, K. and Terauchi, Y. (1986) *Bull. Chem. Soc. Jpn.* **59**, 3553.

Regoli, D., Drapeau, G., Dion, S. and D'Orleans-Juste, P. (1989) *Pharmacology* **38**, 1.

Rogers, D. F., Aursudkij, B. and Barnes, P. J. (1989) *Eur. J. Pharmacol.* **174**, 283.

Rovero, P., Pestellini, R., Patacchiini, R., Giuliani, S., Santicioli, P., Maggi, C. A., Meli, A. and Giachetti, A. (1989) *Peptides.* **10**, 593.

Schild, H. O. (1947) *Br. J. Pharmacol.* **2**, 189.

Shanab, A. A. A., Allen, J. M., Guthrie, D. J. S., Irvine, G. J., Murphy, R. F. and Walker, B. (1991) *Peptides.* **12**, 1069.

Shigemoto, R., Yokota, Y., Tsuchida, K. and Nakanishi, S. (1990) *J. Biol. Chem.* **265**, 623.

Shin, S. Y., Ha, J.-M., Ha, B. J. and Munekata, E. (1993) *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **26**, 720.

Shin, S. Y., Ha, J.-M., Ha, B. J., Jang, T. S., Kang, S. Y.

and Munekata, E. (1994) *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **27**, 436.
Takano, T., Nagashima, A., Hagio, T., Tateishi, K. and Kamiya, H. (1990) *Brain Res.* **528**, 231.
Tallarida, R. J., Cowan, A. and Adler, M. W. (1979) *Life Sci.* **25**, 637.
Uchita, Y., Nomura, A. and Ohtsuka, M. (1987) *Am. Rev. Resp. Dis.* **136**, 718.
Van Rossum, J. M. (1963) *Arch. Int. Pharmacodyn.* **143**, 299.
Williams, B. J. Curtis, N. R., McKnight, A. T., Maquire, J. J., Young, S. C., Veber, D. F. and Baker, R. (1993) *J. Med. Chem.* **36**, 2.
Wollborn, U., Brunne, R. M., Harting, J., Holzmann, G. and Leibfritz, D. (1993) *Int. J. Pept. Protein Res.* **41**, 376.