Chem. Pharm. Bull. **44**(10) 1880—1884 (1996) Vol. 44, No. 10

Structure–Activity Relationships of Neuromedin U. III. Contribution of Two Phenylalanine Residues in Dog Neuromedin U-8 to the Contractile Activity[1,2]

Katsuro KUROSAWA, Naoki SAKURA,* and Tadashi HASHIMOTO

Faculty of Pharmaceutical Sciences, Hokuriku University, Kanagawa-machi, Kanazawa 920–11, Japan.

Received April 10, 1996; accepted June 7, 1996

Dog neuromedin U-8 (d-NMU-8; pGlu–X^2–Leu–Y^4–Arg–Pro–Arg–Asn–NH_2, X = Y = Phe) has potent biological activity to stimulate an isolated chicken crop smooth muscle preparation with the relative activity (*RA* value) of 5.78 to porcine neuromedin U-8 (p-NMU-8). To elucidate the contribution of the two phenylalanine residues of NMU-8 to the biological activity, fourteen d-NMU-8 analogs modified either at position 2 or 4, [X^2]- or [Y^4]-d-NMU-8, were synthesized, where X and Y were Ala, Tyr, Trp, Thr, Glu, His or cyclohexylalanine (Cha). Most of the analogs retained very low contractile activity, suggesting the importance of both Phe residues in d-NMU-8 for the biological activity. [X^2]-d-NMU-8 analogs had lower biological activity in terms of the *RA* value than the corresponding [Y^4]-d-NMU-8, when X and Y are the same amino acid. Loss of aromaticity of Phe^2 ([Cha^2]-d-NMU-8) resulted in a marked decrease of the contractile activity, while that of Phe^4 ([Cha^4]-d-NMU-8) resulted in retention of considerable activity, with the *RA* value of 2.68. [Tyr^4]-d-NMU-8 was an exceptional analog with higher contractile activity ($p < 0.01$) than the parent compound d-NMU-8, having the *RA* value of 12.6. The results indicated that the aromatic side chain of the Phe residue at position 2 contributes more than that at position 4 to the biological activity.

Key words neuromedin U; structure–activity relationship; smooth muscle contraction; chicken crop; phenylalanine residue

Neuromedin U (NMU) has been isolated from porcine spinal cord as a uterine smooth muscle-stimulating peptide.[3] Subsequently, NMU peptides have been isolated from gastrointestinal tissue of the rat,[4,5] frog,[6] guinea pig,[7] rabbit,[8] dog,[9] and chicken.[10] The primary structures of rat[11] and human[12] NMU precursors have been deduced from cDNA sequence analysis. The structure of the C-terminal heptapeptide amide, Phe–X–Phe–Arg–Pro–Arg–Asn–NH_2 (X = Leu, Val or Phe), of NMU peptides is conserved in all species so far examined (Fig. 1).

NMU receptor from the rat uterus, but not so far from any other organ, has been characterized as G-protein coupled.[13] Immunoreactive NMU is widely observed in gut and brain as well as in genitalia of various animals, and is present at a higher concentration in the gastrointestinal tract than in the central nervous system.[14] NMU peptide has contractile activity not only on the rat uterus, but also on various isolated smooth muscle preparations, such as turtle small intestine,[15] rat stomach circular muscle,[16] and human ileum and urinary bladder.[17] We have demonstrated the motor effects of NMU peptides on various regions of chicken gastrointestinal tract; crop, duodenum, ileum, jejunum, cecum and rectum.[18] It is possible that NMU may be involved in the regulation of the whole gastrointestinal tract in chicken.

Our previous studies on the structure–activity relationships of NMU peptides revealed that the amino acid side chains at positions 2 and 4—8 are important for the contractile activity in the isolated chicken crop assay.[19] The peptide chain length–contractile activity relationships of rat NMU-23 revealed that the hexapeptide portion, Phe–Leu–Phe–Arg–Pro–Arg, is the most important for the contraction of chicken and rat smooth muscle preparations.[20] Elongation of the peptide chain from the N-terminal of NMU-8 increases the biological activity,[21] and the analogs with modification at the N-terminal of NMU-8 to give aminopeptidase resistance showed higher contractile activity than porcine neuromedin U-8 (p-NMU-8).[22] Further studies to develop highly potent

Porcine	F K V D E E F Q G P I V S Q N R R Y F L F R P R N -NH_2
	Y F L F R P R N -NH_2
Dog	F R L D E E F Q G P I A S Q V R R Q F L F R P R N -NH_2
	pE F L F R P R N -NH_2
Rabbit	F P V D E E F Q S P F G S R S R G Y F L F R P R N -NH_2
Rat	Y K V N E * Y Q G P * V A P S G G F F L F R P R N -NH_2
Guinea pig	G Y F L F R P R N -NH_2
Chicken	Y K V D E D L Q G A G G I Q S R G Y F F F R P R N -NH_2
	G Y F F F R P R N -NH_2
Frog	L K P D E E L Q G P G G V L S R G Y F V F R P R N -NH_2
	G Y F V F R P R N -NH_2

*: deletion, pE: pyroglutamyl

Fig. 1. Amino Acid Sequences of NMU Peptides from Various Species

* To whom correspondence should be addressed.

© 1996 Pharmaceutical Society of Japan

NII-Electronic Library Service

analogs modified at the N-terminal of NMU-8 gave succinyl–Phe–Leu–Phe–Arg–Pro–Arg–Asn–NH_2 with about 100-fold greater potency for the contraction of isolated chicken crop.[1] These results demonstrate that the C-terminal heptapeptide amide of NMU peptides is essential for biological activity and the N-terminal of NMU-8 can be extensively modified without decreasing the biological activity.

It is characteristic that the C-terminal half of the NMU-8 molecule is hydrophilic and basic, and the N-terminal half is extremely hydrophobic. The Leu residue at position 3 of p-NMU-8 or dog neuromedin U-8 (d-NMU-8) may not be involved in the active site because Val or Phe are substituted in the corresponding positions in the molecules of the frog and chicken NMU peptides, as shown in Fig. 1. The Phe residues at positions 2 and 4, are completely conserved among various species, suggesting that they play an essential role in the biological activity. Herein, we deal with the contribution of these two Phe residues to the contractile activity.

Results and Discussion

Peptides shown in Fig. 2 were synthesized by standard solid-phase techniques with Boc-amino acid on benzhydrylamine resin 1% divinylbenzene (DVB) polymer using a peptide synthesizer. After HF deprotection and cleavage from the resin, the peptide was purified by reversed-phase (RP)-HPLC, followed by gel filtration. The structures of the synthetic peptides were confirmed by FAB-MS and amino acid analysis of the acid hydrolysate, and homogeneity was evaluated by analytical HPLC and high-performance (HP)-TLC (Tables 1, 2).

The contractile activity of the synthetic peptides was estimated on isolated chicken crop smooth muscle preparation as described previously.[18,22] The activity of p-NMU-8 was taken as the standard and the potency of each peptide is expressed in terms of relative activity (*RA* value: EC_{50} of p-NMU-8/EC_{50} of each peptide) and maximal contraction (% of that of p-NMU-8 at the concentration of 3 μM) (Table 3), which was obtained from the concentration–response curve of each peptide (Fig. 3).

d-NMU-8 has about six times higher contractile activity[22] than p-NMU-8, possibly due to the presence

pGlu–$\overset{2}{X}$–Leu–Phe–Arg–Pro–Arg–Asn–NH_2

pGlu–Phe–Leu–$\overset{4}{Y}$–Arg–Pro–Arg–Asn–NH_2

Peptide No.	X	Peptide No.	Y
1	Ala	8	Ala
2	Tyr	9	Tyr
3	Trp	10	Trp
4	Thr	11	Thr
5	Glu	12	Glu
6	His	13	His
7	Cha	14	Cha

Fig. 2. Synthetic d-NMU-8 Analogs Substituted at Position 2 or 4

Table 1. Characteristics of Synthetic d-NMU-8 Analogs Substituted at Position 2 or 4

Peptide	$[\alpha]_D^{25}$ (c=0.5, 2 M AcOH)	RP-HPLC[a] t_R (min)	HP-TLC[b]	
			Rf^1	Rf^2
1	−67.6	10.3	0.47	0.34
2	−48.3	12.5	0.51	0.36
3	−43.6	16.1	0.52	0.38
4	−63.0	10.3	0.46	0.34
5	−65.5	10.4	0.41	0.33
6	−58.7	7.96	0.39	0.24
7	−61.4	18.2	0.54	0.37
8	−73.1	10.4	0.44	0.32
9	−47.9	12.3	0.50	0.33
10	−46.1	15.8	0.51	0.34
11	−69.5	10.7	0.46	0.30
12	−65.9	10.7	0.39	0.31
13	−62.8	9.11	0.34	0.17
14	−58.8	18.2	0.53	0.35

a) Conditions: column, Puresil 5 μ C_{18} (4.6 × 250 mm); flow rate, 1 ml/min; detection, 210 nm; eluent system, linear gradient from 16% to 40% MeCN (20 min) in 0.1% TFA: t_R, retention time. *b*) Rf^1, *n*-BuOH–pyridine–AcOH–H_2O (30:20:6:24); Rf^2, *n*-BuOH–AcOEt–AcOH–H_2O (1:1:1:1).

Table 2. Amino Acid Analysis and FAB-MS Data for Synthetic d-NMU-8 Analogs Substituted at Position 2 or 4

Peptide	Amino acid analysis[a]								FAB-MS	
	Asp	Glu	Pro	Leu	Phe	Arg	NH_3	Others[b]	Found[c]	Formula
1	0.99 (1)	1.05 (1)	1.02 (1)	0.96 (1)	0.90 (1)	2.06 (2)	2.15 (2)	1.00 (Ala)	983	$C_{44}H_{70}N_{16}O_{10}$
2	1.04 (1)	1.09 (1)	1.07 (1)	0.93 (1)	0.89 (1)	2.11 (2)	2.11 (2)	0.88 (Tyr)	1075	$C_{50}H_{74}N_{16}O_{11}$
3	1.01 (1)	1.06 (1)	0.93 (1)	1.00 (1)	0.97 (1)	2.03 (2)	2.42 (2)	—	1098	$C_{52}H_{75}N_{17}O_{10}$
4	1.01 (1)	1.09 (1)	1.02 (1)	0.96 (1)	0.89 (1)	2.03 (2)	2.18 (2)	1.00 (Thr)	1013	$C_{45}H_{72}N_{16}O_{11}$
5	1.00 (1)	2.06 (2)	0.92 (1)	1.00 (1)	1.01 (1)	2.01 (2)	2.45 (2)	—	1041	$C_{46}H_{72}N_{16}O_{12}$
6	1.00 (1)	1.05 (1)	0.98 (1)	1.01 (1)	0.98 (1)	1.99 (2)	1.88 (2)	0.99 (His)	1049	$C_{47}H_{72}N_{18}O_{10}$
7	1.00 (1)	1.07 (1)	1.02 (1)	0.99 (1)	0.93 (1)	1.99 (2)	2.06 (2)	—	1065	$C_{50}H_{80}N_{16}O_{10}$
8	1.00 (1)	1.05 (1)	0.98 (1)	1.03 (1)	1.00 (1)	1.97 (2)	2.03 (2)	0.96 (Ala)	983	$C_{44}H_{70}N_{16}O_{10}$
9	1.01 (1)	1.07 (1)	1.01 (1)	1.00 (1)	0.96 (1)	1.99 (2)	2.03 (2)	0.96 (Tyr)	1075	$C_{50}H_{74}N_{16}O_{11}$
10	0.98 (1)	1.04 (1)	0.99 (1)	1.03 (1)	0.97 (1)	1.98 (2)	2.46 (2)	—	1098	$C_{52}H_{75}N_{17}O_{10}$
11	0.98 (1)	0.99 (1)	1.00 (1)	1.02 (1)	0.96 (1)	2.00 (2)	1.81 (2)	0.99 (Thr)	1013	$C_{45}H_{72}N_{16}O_{11}$
12	0.98 (1)	2.06 (2)	0.92 (1)	1.02 (1)	1.02 (1)	2.01 (2)	3.19 (2)	—	1041	$C_{46}H_{72}N_{16}O_{12}$
13	0.98 (1)	1.05 (1)	1.02 (1)	0.97 (1)	0.96 (1)	1.94 (2)	1.91 (2)	1.08 (His)	1049	$C_{47}H_{72}N_{18}O_{10}$
14	1.01 (1)	1.06 (1)	0.98 (1)	1.00 (1)	0.96 (1)	1.98 (2)	2.10 (2)	—	1065	$C_{50}H_{80}N_{16}O_{10}$

a) Hydrolysis at 130 °C for 3.0 h in vapor of 6 N hydrochloric acid containing phenol (3%); numbers in parentheses are theoretical values. *b*) Other amino acids analyzed. *c*) For $[M+H]^+$.

NII-Electronic Library Service

of an aminopeptidase-resistant pGlu residue and/or the decrease of basicity at the N-terminal, affording increased affinity for the NMU-receptor. However, when d-NMU-8 is repeatedly subjected to assay, the contractile responses of chicken smooth muscle preparation became more desensitized than in the case of p-NMU-8. Therefore, the contractile effect of p-NMU-8 was taken as the standard biological activity on isolated chicken crop smooth muscle preparation. The d-NMU-8 molecule was selected as a parent compound for structural modification of positions 2 to 8 of NMU-8. To determine the *RA* and the maximum contraction of an analog, the assay was first conducted for p-NMU-8, followed by the analog on the same tissue preparation, as described in our previous study[22] on structure–activity relationships of NMU peptides.

Table 3. Contractile Activity of d-NMU-8 Analogs Substituted at Position 2 or 4 on Isolated Chicken Crop Smooth Muscle Preparations

Peptide	*RA*[a]	Max. contraction[b] (%)	*n*[c]
p-NMU-8	1.00	100	—
d-NMU-8[d]	5.78±0.86	126 ± 4.79	14
1	0.08±0.03	109 ± 3.71	6
2	0.64±0.13	122 ± 7.48	7
3	0.04±0.01	74.6± 5.22	7
4	0.13±0.04	143 ±12.2	7
5	0.04±0.01	104 ± 7.37	8
6	0.08±0.04	110 ± 7.80	6
7	0.08±0.02	146 ±12.1	6
8	0.39±0.06	130 ± 7.31	7
9	12.6 ±1.45	127 ± 8.24	6
10	0.54±0.15	99.6± 6.19	7
11	0.31±0.09	127 ± 3.85	6
12	NA[e]	38.8± 6.94	7
13	1.31±0.69	146 ±17.9	6
14	2.68±1.18	113 ± 4.49	6

a) *RA* was calculated as EC_{50} of p-NMU-8/EC_{50} of analog. *b*) Max. contraction was calculated as [(maximal effect produced by analog/maximal effect produced by p-NMU-8) × 100]. *c*) Number of experiments. *d*) Reported in ref. 22. *e*) Not assessed.

When the Phe at position 2 (Phe^2) was replaced by other L-amino acids, the analogs showed greatly decreased activity to contract chicken crop longitudinal smooth muscle. Substitution of Ala, Trp, Thr, Glu and His for Phe^2 (**1**, **3**, **4**, **5**, **6**) reduced the *RA* to 0.04—0.13 in comparison with p-NMU-8. [Tyr^2]-d-NMU-8 (**2**) had considerable activity with the *RA* value of 0.64, which is only 10% of that of the parent compound, d-NMU-8 (*RA* value of 5.78[22]). [Cha^2]-d-NMU-8 (**7**) had very low activity (*RA* 0.08), indicating the importance of the aromaticity of the phenyl group.

Most of the modifications of d-NMU-8 at position 4, [Y^4]-d-NMU-8, decreased the contractile activity (Table 3), but the degree of the activity reduction was not as marked as that in the case of position 2, [X^2]-d-NMU-8, described above. Introduction of Tyr into position 4 ([Tyr^4]-d-NMU-8 (**9**), *RA* 12.6) increased the activity to twice that of d-NMU-8. This analog (**9**) was exceptional, with significantly higher activity than the parent compound ($p<0.01$), among the analogs examined in this study. When other aromatic amino acids, His and Trp, were substituted for Phe^4, the analogs retained considerable activity (*RA* values of [His^4]- (**13**) and [Trp^4]-d-NMU-8 (**10**): 1.31 and 0.54, respectively). Even the loss of aromatic character of Phe did not change the ability to elicit the contraction, since [Cha^4]-d-NMU-8 (**14**) retained the activity (*RA* 2.68). [Ala^4]- and [Thr^4]-d-NMU-8 (**8**, **11**) had low activity and the drastic structural change

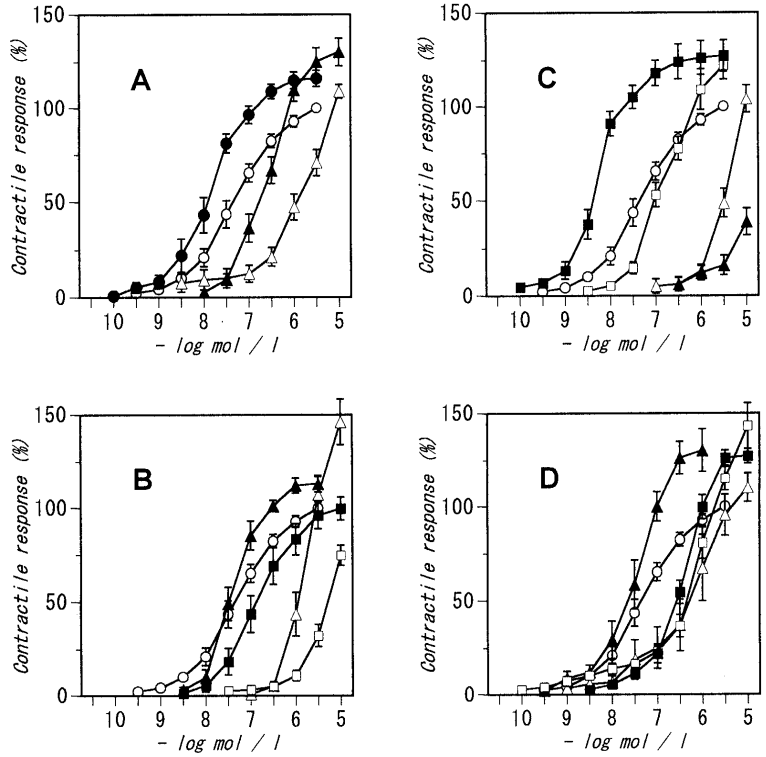

Fig. 3. Concentration–Response Curves of p-NMU-8 and d-NMU-8 Analogs for the Contraction of Isolated Chicken Crop Preparation

○, p-NMU-8; A: ●, d-NMU-8; △, Ala^2-analog; ▲, Ala^4-analog; B: △, Cha^2-analog; ▲, Cha^4-analog; □, Trp^2-analog; ■, Trp^4-analog; C: △, Glu^2-analog; ▲, Glu^4-analog; □, Tyr^2-analog; ■, Tyr^4-analog; D: △, His^2-analog; ▲, His^4-analog; □, Thr^2-analog; ■, Thr^4-analog.

NII-Electronic Library Service

caused by [Glu^4]-d-NMU-8 (**12**) resulted in almost complete loss of the biological activity. Thus, a hydrophobic side chain, preferably a cyclic structure, may be necessary at position 4 of d-NMU-8. Analogs **1**, **3**, **4**, **5**, **6**, **7** and **12** were tested for their antagonistic activity against the contractile effects of p-NMU-8 on chicken crop smooth muscle, but no effects were observed.

Previously we reported that substitution of Gly for Phe at either position 2 or 4 of p-NMU-8 gave almost inactive analogs.[19] A rat NMU-23-related C-terminal hexapeptide amide, H–Leu–Phe–Arg–Pro–Arg–Asn–NH_2, did not induce maximal contraction, but another hexapeptide amide, H–Phe–Leu–Phe–Arg–Pro–Arg–NH_2, did on both chicken crop and rat uterus smooth muscle preparations.[20] The importance of both Phe residues shown in the present study is in accord with the previous results. Furthermore, the roles of the two Phe residues of d-NMU-8 in the biological activity were distinguished by employing analogs with systematic substitution at position 2 or 4. The *RA* values of various [Y^4]-d-NMU-8 analogs are three to thirty times higher than those of the corresponding [X^2]-analogs when X and Y are the same amino acid, as examined in this study. The loss of aromaticity of Phe^2 resulted in a marked decrease of the activity, in contrast to Phe^4, in terms of the *RA* value. The results suggest that the side chain of Phe at position 2 contributes more to the activity than that at position 4 in the d-NMU-8 molecule, and the side chain of Phe^2 of NMU-8 is recognized strictly by a receptor on chicken crop. The side chain of Phe^4 of NMU-8 may be allowed some flexibility in relation to the receptor, and could be modified without decrease of the activity, as in the case of [Tyr^4]-NMU-8 (**9**), which shows higher activity.

Experimental

All reagents and solvents for peptide synthesis were obtained from Watanabe Chem. Ind. Ltd. or Wako Pure Chem. Ind. Ltd., Japan, unless otherwise mentioned, and were used without further purification. Evaporation of organic solvents was carried out *in vacuo* below 40 °C in a rotary evaporator.

Peptide Synthesis Peptides were synthesized by a solid-phase method on benzhydrylamine (BHA) resin with N^{α}-Boc amino protections, employing a model 990C peptide synthesizer (Beckman Instruments Ltd., U.S.A.). N^{α}-Boc-amino acids were purchased from Peptide Institute Inc., Japan, and Boc-L-Cha from Watanabe Chem. Ind. Ltd. Introduction of Boc-Asn onto BHA resin (1% DVB polymer, available amine of the resin: 0.60 mmol/g; Peptide Institute Inc., Japan) was achieved through the use of dicyclohexylcarbodiimide (DCC) in the presence of a 2-fold excess of 1-hydroxybenzotriazole (HOBt). Deprotection of the N^{α}-Boc group was accomplished with 33% trifluoroacetic acid (TFA) in dichloromethane (DCM) for 30 min. The peptide chain was elongated by a coupling reaction for 2 h employing N^{α}-Boc-amino acids (2 eq) *via* benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate[23] (BOP reagent, 2.0 eq) in the presence of *N*-methylmorpholine (3.0 eq) in DMF or *N*-methylpyrrolidone (NMP). DCC-HOBt in DMF was used for the second coupling, and further repeated coupling by BOP was performed, if necessary. Every introduction reaction of an amino acid was repeated until the resin became negative to the Kaiser test.[24] Side-chain protection of N^{α}-Boc-amino acids was as follows: Arg(Tos), Glu(OBzl), His(Bom), Thr(Bzl) and Tyr(Cl_2-Bzl). Z-pGlu was used for the N-terminal. Final deprotection and cleavage from the resin were achieved in HF in the presence of 10% anisole at 0 °C for 45 min. After removal of HF *in vacuo*, the residue was washed with ether and extracted with dilute acetic acid (AcOH). Crude lyophilized peptide was purified by semi-preparative RP-HPLC using a column of YMC-Pack D-ODS-5-A (20 × 250 mm) (YMC Co., Japan) with isocratic elution using a 0.1% TFA–MeCN solvent system at the flow rate of 8 ml/min. The purified peptide was lyophilized from dilute HCl (about 5-fold excess) and finally gel-filtered on a Toyopearl HW-40 superfine column (1.5 × 58 cm) using 25% MeCN in 5 mM HCl as an eluent, and the desired fractions were lyophilized.

Acid hydrolysis of synthetic peptides was carried out with vapor of 6 N HCl containing 3% phenol at 130 °C for 3 h. Amino acid analysis of the acid hydrolysate was performed on a Beckman model 7300 amino acid analyzer system (Table 2). HPLC analysis of the peptides was carried out on a system composed of 590 and 510 pumps, a model U6K injector, a model 680 gradient controller, a model 741 data module (Waters) and a UV-8011 spectrophotometer (Tosoh). Analysis was performed on a column of Puresil C_{18} (4.6 × 250 mm; Waters) with a linear gradient of 16.0—40.0% MeCN over a period of 20 min in 0.1% TFA (Table 1). The FAB-MS of synthetic peptides was measured with a JMS-DX-300 mass spectrometer connected with a JMS-DA 5000 mass data system (JEOL) (Table 2). Optical rotations of peptides were measured with a DIP-370 digital polarimeter (Nippon Bunko Co. Ltd., Japan) employing a 3 × 50 mm cell. Peptides were dissolved in 12% AcOH at a concentration of 0.50% peptide. The *Rf* values in HP-TLC, performed on precoated silica gel plates (Kieselgel 60; Merck), refer to the following solvent systems: Rf^1, *n*-BuOH–pyridine–AcOH–H_2O (30 : 20 : 6 : 24) and Rf^2, *n*-BuOH–AcOEt–AcOH–H_2O (1 : 1 : 1 : 1). Table 1 shows the analytical data.

Smooth Muscle Contraction Assay on Isolated Chicken Crop Preparation The biological activity of synthetic peptides was evaluated by a contraction assay on chicken crop smooth muscle preparation as described previously.[22] Briefly, a chicken crop smooth muscle strip was mounted longitudinally at a resting tension of 0.5 g in an organ bath (10 ml) containing Tyrode's solution at 30 °C. The tissue preparation was equilibrated for 60 min, and then was challenged twice with carbachol (2 μM), and once with p-NMU-8 added cumulatively up to 3 μM. After re-equilibration for 30—40 min, increasing concentrations of p-NMU-8 were added up to 3 μM, followed after a 30 min resting period by those of an analog up to 1 or 10 μM.

The pharmacological parameters, *RA* value (relative activity; EC_{50} of p-NMU-8/EC_{50} of an analog) and maximum contraction (%) [(maximum effect induced by 1—10 μM of an analog/maximum effect induced by 3 μM of p-NMU-8) × 100] were calculated from the cumulative concentration–response curves shown in Fig. 3. An experiment to determine the *RA* value and the maximum contraction of an analog was performed once on a tissue preparation, and the experiment was repeated using different tissue preparations. The values of *RA* and maximum contraction are expressed as mean and S.E. of six to eight experiments. The EC_{50} value of p-NMU-8 was 95.9 ± 6.63 nM, obtained from 92 experiments. Statistical significance of the group differences between the *RA* values of an analog and those of d-NMU-8 was analyzed by using variance and Student's *t* test where applicable. Table 3 shows the assay results. To estimate the antagonist activity of an analog with low contractile activity (**1**, **3**, **4**, **5**, **6**, **7**, **12**), the concentration–response curve of p-NMU-8 was first obtained as described above, followed by that in the presence of the analog (1×10^{-6} M). The latter curves did not significantly shift to the right.

References and Notes

1) Part II: Hashimoto T., Kurosawa K., Sakura N., *Chem. Pharm. Bull.*, **43**, 1154—1157 (1995).
2) The abbreviations for amino acids and peptides are in accordance with the nomenclature of the IUPAC-IUB Joint Commission, *Eur. J. Biochem.*, **138**, 9—37 (1984), and additional abbreviations are as follows: NMU, neuromedin U; Cha, L-cyclohexylalanine; Boc, *tert*-butyloxycarbonyl; DCC, *N*,*N*′-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate; BHA benzhydrylamine; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; MeCN, acetonitrile; AcOH, acetic acid; TEA, triethylamine; TFA, trifluoroacetic acid. Amino acids and their derivatives used in this study are of L-configuration.
3) Minamino N., Kangawa K., Matsuo H., *Biochem. Biophys. Res. Commun.*, **130**, 1078—1085 (1985).
4) Conlon J. M., Domin J., Thim L., DiMarzo V., Morris H. R., Bloom S. R., *J. Neurochem.*, **51**, 988—991 (1988).
5) Minamino N., Kangawa K., Honzawa M., Matsuo H., *Biochem.*

NII-Electronic Library Service

Biophys. Res. Commun., **156**, 355—360 (1988).
6) Domin J., Yiangou Y., Spokes R. A., Aitken A., Parmar K. B., Chrysanthou B. J., Bloom S. R., *J. Biol. Chem.*, **264**, 20881—20885 (1989).
7) Murphy R., Turner C. A., Furness J. B., Parker L., Giraud A., *Peptides*, **11**, 613—617 (1990).
8) Kage R., O'Harte F., Thim L., Conlon J. M., *Regul. Pept.*, **33**, 191—198 (1991).
9) O'Harte F., Bockman C. S., Abel P. W., Conlon J. M., *Peptides*, **12**, 11—15 (1991).
10) *a*) O'Harte F., Bockman C. S., Zeng Y., Abel P. W., Harvey S., Conlon J. M., *Peptides*, **12**, 809—812 (1991); *b*) Domin J., Benito-Orfila M. A., Nandha K. A., Bloom S. R., *Regul. Pept.*, **41**, 1—8 (1992).
11) Lo G., Legon S., Austin C., Wallis S., Wang Z. A., Bloom S. R., *Mol. Endocrinol.*, **6**, 1538—1544 (1992).
12) Austin C., Lo G., Nandha K. A., Meleagros L., Bloom S. R., *J. Mol. Endocrinol.*, **14**, 157—169 (1995).
13) Nandha K. A., Benito-Orfila M. A., Smith D. M., Bloom S. R., *Endocrinol.*, **133**, 482—486 (1993).
14) Nandha K. A., Bloom S. R., *Biomed. Res.*, **14**, Suppl. 3, 71—76 (1993).
15) Bockman C. S., Abel P. W., Hicks J. W., Conlon J. M., *Eur. J. Pharmacol.*, **171**, 225—257 (1989).
16) Benito-Orfila M. A., Domin J., Nandha K. A., Bloom S. R., *Eur. J. Pharmacol.*, **193**, 329—333 (1991).
17) Maggi C. A., Patacchini R., Giuliani S., Turini D., Barbanti G., Rovero P., Meli A., *Br. J. Pharmacol.*, **99**, 186—188 (1990).
18) Sakura N., Kurosawa K., Naminohira S., Sakai T., Hashimoto T., "Peptide Chemistry 1992," ed. by Yanaihara N., ESCOM Science Publishers B. V., Leiden, 1993, pp. 406—408.
19) Hashimoto T., Masui H., Uchida Y., Sakura N., Okimura K., *Chem. Pharm. Bull.*, **39**, 2319—2322 (1991).
20) Sakura N., Ohta S., Uchida Y., Kurosawa K., Okimura K., Hashimoto T., *Chem. Pharm. Bull.*, **39**, 2016—2020 (1991).
21) Okimura K., Sakura N., Ohta S., Kurosawa K., Hashimoto T., *Chem. Pharm. Bull.*, **40**, 1500—1503 (1992).
22) Sakura N., Kurosawa K., Hashimoto T., *Chem. Pharm. Bull.*, **43**, 1148—1153 (1995).
23) Castro B., Dormoy J. R., Evin G., Selve C., *Tetrahedron Lett.*, **1975**, 1219—1222.
24) Kaiser E., Colescott R. L., Bossinger C. D., Cook P. I., *Anal. Biochem.*, **34**, 595—598 (1970).

NII-Electronic Library Service