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Acylation of the α-Amino Group in Neuropeptide Y(12-36) Increases Binding Affinity for the Y_2 Receptor

Sachiko Murase, Noboru Yumoto, Michael G. Petukhov, and Susumu Yoshikawa[1]

Osaka National Research Institute, AIST, 1-8-31 Midorigaoka, Ikeda, Osaka 563

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Competition assays using three series of analogs of neuropeptide Y (NPY) ([Xaa[11]]NPY(11-36), [Xaa[12]]NPY(12-36), and [Xaa[13]]NPY(13-36)) revealed that the binding affinity for the Y_2 receptor was considerably lowered by truncation of residue 11. Upon acetylation or succinylation of the α-amino group, the binding affinity of [Xaa[12]]NPY(12-36) recovered to a level similar to that of [Xaa[11]]NPY(11-36). No significant difference was observed between the increases caused by acetylation and those caused by succinylation, suggesting that the increase in binding affinity cannot be explained by the change in the net charge at the N-terminus as a consequence of the modification. The scattered data points on a plot of the α-helix content *vs.* IC_{50} of all these analogs revealed the absence of any apparent relationship, an indication that prior formation of the α-helix is not necessary for binding to the Y_2 receptor. It has been widely accepted that fewer than 12 residues from the C-terminus are directly involved in binding of NPY to the Y_2 receptor, while the remaining part of NPY only assists in the adoption of a favorable conformation by the C-terminal hexapeptide for recognition by the receptor. However, the present results suggest that the region around residue 12 does not project from the Y_2 receptor.

Key words: acylation, α-helix stability, binding affinity, neuropeptide Y, Y_2 receptor.

Neuropeptide Y (NPY) is an amidated peptide of 36 amino acids that is widely distributed in the central and peripheral nervous systems. It has been proposed that NPY has a compact globular structure, which is known as the PP-fold, on the basis of X-ray crystallographic analysis of the homologous avian pancreatic polypeptide (PP) (*1*). The PP-fold is composed of two antiparallel helices, an N-terminal polyproline helix (residues 1-8) and an amphipathic α-helix (residues 13-32), connected by a type-I β-turn (residues 9-12). The action of NPY is mediated by at least three types of receptor, Y_1, Y_2, and Y_3 (*2, 3*). Among the subtypes of receptor for NPY, the Y_2 receptor is known to mediate enhancement of memory retention in the hippocampus, inhibition of consummatory behavior in the hypothalamus, suppression of transmitter release in sympathetic and parasympathetic fibers, *etc.* (*2-4*). Previous studies showed that the Y_2 receptor can effectively bind long C-terminal fragments of NPY such as NPY(13-36) (*1-3, 5*), which is often used for the classification of receptor subtypes. It has been widely accepted that the C-terminal hexapeptide is the critical region for recognition by the Y_2 receptor, while the remaining part only assists in the adoption of a favorable conformation by the hexapeptide for recognition by the receptor (*1, 3, 5*).

We have been studying stabilizing effects on the α-helix of amino acids at the helical end using three series of analogs of NPY ([Xaa[11]]NPY(11-36), [Xaa[12]]NPY(12-36), and [Xaa[13]]NPY(13-36)) that contain an α-helical part. Substitution of N-terminal residue of NPY(12-36) significantly affected the stability of the α-helix (*6*). By comparing the IC_{50} values of the series of analogs in binding assays, we found, in the present study, that removal of residue 11 caused a significant decrease in the binding affinity for the Y_2 receptor. Acetylation or succinylation of the α-amino group of [Xaa[12]]NPY(12-36) significantly increased the binding affinity without any exceptions. Upon such modification, the binding affinity returned to a level similar to that of [Xaa[11]]NPY(11-36). Although it has been considered that the region corresponding to the β-turn in the PP-fold structure does not directly interact with the Y_2 receptor, the present results suggest that the region is also recognized by the receptor.

MATERIALS AND METHODS

Materials—Porcine NPY was purchased from the Peptide Institute (Osaka). Reagents for peptide synthesis, 9-fluorenylmethoxycarbonyl (Fmoc)-L-amino acids and Tenta Gel TG-RAM resin were purchased from Shimadzu (Kyoto), and ^{125}I-NPY that had been labeled with Bolton-Hunter reagent was from Amersham International plc (Bucks, UK). All other chemicals were of reagent grade.

Preparation of Analogs of NPY—The C-terminal fragments of NPY were synthesized on an automated solid-phase peptide synthesizer (PSSM-8; Shimadzu) using Tenta Gel TG-RAM resin and "Fmoc chemistry" with benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate and *N*-hydroxybenzotriazole as coupling

[1] To whom correspondence should be addressed. Tel: +81-727-51-9521, Fax: +81-727-51-9628

Abbreviations: CD, circular dichroism; DMPC, dimyristoyl phosphatidylcholine; Fmoc, 9-fluorenylmethoxycarbonyl; NPY, neuropeptide Y; PP, pancreatic polypeptide; TES, 7,*N*-[tris(hydroxymethyl)-methyl]-2-aminomethanesulfonate.

reagents. Fragments were cleaved from the resin by treatment with trifluoroacetic acid and purified by reverse-phase HPLC on a C_{18} column (COSMOSIL, 20×250 mm; Nacalai Tesque, Kyoto). Modification of the α-amino groups of the NPY analogs was carried out before cleavage from the resin. Acetylation was performed as follows. The reaction was started by the addition of 0.1 ml of a 10% (v/v) solution of acetic anhydride in dioxane to the suspension of the resin-bound peptide (5 μmol each) in 2 ml of 0.3 M sodium phosphate buffer (pH 7.2) and was terminated by washing with distilled water after a 3-h incubation at 0°C with stirring. For succinylation, the reaction was started by the addition of 50 μmol of crystalline succinic anhydride to the suspension of the resin-bound peptide (5 μmol each) in 2 ml of 0.5 M 2-(cyclohexylamino)ethanesulfonic acid-NaOH buffer (pH 8.5) and was terminated by washing with distilled water after a 1-h incubation at 25°C with stirring. The purity of each peptide was assessed by analytical reverse-phase HPLC on a C_{18} column (COSMOSIL, 4.6×250 mm). The identity of each peptide was confirmed by mass spectrometry on a time-of-flight mass spectrometer (Shimadzu/Kratos Kompact MALDI II; Shimadzu) with matrix-assisted laser desorption ionization. All the molecular weights were found to be within 0.13% of the expected values.

Receptor-Binding Assay—Porcine hippocampal membranes, which are often used as a homogeneous preparation of Y_2 receptors, were prepared by the method of Shigeri *et al.* (7). Each analog and 30 μM ^{125}I-NPY were incubated for 1 h in 50 mM Tris-HCl buffer (pH 7.4) including 10% glycerol, 1 mM EDTA, and 5 mM 2-mercaptoethanol at 37°C. The binding of ^{125}I-NPY to Y_2 receptors on the hippocampal membranes was also quantitated by the method of Shigeri *et al.* (7). IC_{50} value was defined as the concentration which gives 50% inhibition. IC_{50} values were calculated from profiles of competition for binding between each analog and ^{125}I-NPY. Concentrations of peptides were determined by measuring absorbance due to tyrosine (and absorbance due to tryptophan in the case of [Trp^{12}]NPY-(12-36)) of stock solutions.

Circular Dichroism (CD) Measurements and Determination of α-Helix Content—CD measurements were made with a spectropolarimeter, model J-500A (Jasco International, Tokyo), equipped with a data processor DP-501. CD spectra were recorded at 37°C with a thermostatically regulated cell with a 0.2-cm path-length. Each peptide was dissolved in 5 mM 7,*N*-[tris(hydroxymethyl)methyl]-2-aminomethanesulfonate-NaOH (TES-NaOH) buffer (pH 7.5) at a concentration of approximately 10 μM. To examine the effect of membranes on the stability of α-helix of the analogs, 0.15 mM dimyristoyl phosphatidylcholine (DMPC), which was prepared by the sonication of DMPC films with 5 mM TES-NaOH buffer (pH 7.5), was added as membranes in the peptide solution. α-Helix content was calculated from $[\theta]_{222}$ values using the following equation, proposed by Scholtz *et al.* (8):

$$[\theta]_{222} = -40000 f_H (n-2.5)/n$$

where f_H represents the α-helix content, and n represents the number of peptide bonds.

RESULTS

The inhibitory effects of a series of analogs of NPY ([Xaa^{11}]-NPY(11-36), [Xaa^{12}]NPY(12-36), and [Xaa^{13}]NPY(13-36)) on the binding of ^{125}I-NPY to the porcine hippocampal membranes were examined to evaluate the binding affinity of each peptide for the Y_2 receptor. The IC_{50} values calculated from the results of the competition assays are summarized in Table I. Those analogs with positively charged N-terminal residues had slightly higher binding affinities than those with negatively charged residues, with the exception of [Arg^{13}]NPY(13-36), but no other apparent dependence of the binding affinity on the nature of the N-terminal residue was observed. By contrast, classification according to the length of each peptide revealed significant differences in the distribution of binding affinities between the three series of analogs, as shown in Fig. 1. All the IC_{50} values of [Xaa^{11}]NPY(11-36) analogs were lower than 10 nM; 77% of [Xaa^{12}]NPY(12-36) analogs had IC_{50} values greater than 10 nM; and all the IC_{50} values of [Xaa^{13}]NPY(13-36) analogs were greater than 10 nM. Elimination of residue 11 caused a considerable

TABLE I. **IC_{50} values for analogs of NPY in a binding assay with the Y_2 receptors on porcine hippocampal membranes.**

Peptide	IC_{50} (nM)		
	Unmodified	Acetylated	Succinylated
[Xaa^{11}]NPY(11-36)			
[Lys^{11}]NPY(11-36)	2.5±0.45		
[Arg^{11}]NPY(11-36)	2.5±0.23	12.7±3.9	
[Asp^{11}]NPY(11-36)[a]	9.3±3.6	18.2±5.7	
[Glu^{11}]NPY(11-36)	4.0±0.32		
[Asn^{11}]NPY(11-36)	2.1±0.76	30.4±7.9	
[Gln^{11}]NPY(11-36)	0.49±0.09		
[Thr^{11}]NPY(11-36)	5.0±0.75		
[Ser^{11}]NPY(11-36)	6.9±2.1	23.7±4.0	
[Xaa^{12}]NPY(12-36)			
[Ala^{12}]NPY(12-36)[a]	31.6±0.89[b]	1.3±0.30	1.6±0.20
[Lys^{12}]NPY(12-36)	10.0±0.82[b]	4.0±0.31	3.2±0.23
[Arg^{12}]NPY(12-36)	7.9±0.81[b]	4.0±0.18	2.0±0.15
[His^{12}]NPY(12-36)	5.0±0.30[b]		
[Asp^{12}]NPY(12-36)	20.0±2.1[b]	10.0±0.54	4.0±0.31
[Glu^{12}]NPY(12-36)	31.6±4.0[b]	12.6±1.2	6.3±0.14
[Asn^{12}]NPY(12-36)	20.0±2.0[b]	1.0±0.036	4.0±0.059
[Gln^{12}]NPY(12-36)	15.8±2.3[b]	1.0±0.20	0.8±0.31
[Cys^{12}]NPY(12-36)	100.0±12[b]	6.3±0.49	7.9±0.19
[Thr^{12}]NPY(12-36)	31.6±8.0[b]	12.6±0.85	7.9±0.28
[Ser^{12}]NPY(12-36)	12.6±2.3[b]	6.3±0.29	10.0±1.4
[Tyr^{12}]NPY(12-36)	5.0±0.60[b]		
[Gly^{12}]NPY(12-36)	31.6±1.6[b]	1.0±0.14	0.8±0.29
[Val^{12}]NPY(12-36)	10.0±0.60[b]	3.2±0.26	6.3±1.2
[Phe^{12}]NPY(12-36)	63.1±6.0[b]		
[Trp^{12}]NPY(12-36)	50.1±3.9[b]	20.0±0.82	15.8±0.16
[Xaa^{13}]NPY(13-36)			
[Ala^{13}]NPY(13-36)	39.8±4.0		
[Lys^{13}]NPY(13-36)	15.8±3.4	11.9±1.6	
[Arg^{13}]NPY(13-36)	31.6±1.7		
[Asp^{13}]NPY(13-36)	25.1±3.5	27.7±1.5	
[Glu^{13}]NPY(13-36)	25.1±4.0		
[Pro^{13}]NPY(13-36)[a]	50.1±4.1	41.5±9.0	
[Asn^{13}]NPY(13-36)	15.8±4.7		
[Gln^{13}]NPY(13-36)	12.6±5.3		
[Thr^{13}]NPY(13-36)	15.8±2.1	40.9±11.7	
[Ser^{13}]NPY(13-36)	25.1±3.5		

[a]Peptides having native sequences. [b]Similar results were obtained previously in a preliminary form (6).

decrease in binding affinity. When the N-terminus of [Xaa^{12}]NPY(12-36) was modified with acetic anhydride or succinic anhydride, large increases in binding affinity (1.2- to 40-fold) were observed, without any exceptions (Table I). In particular, the acylation very effectively (by approximately 20-fold) increased the binding affinity of [Ala^{12}]-

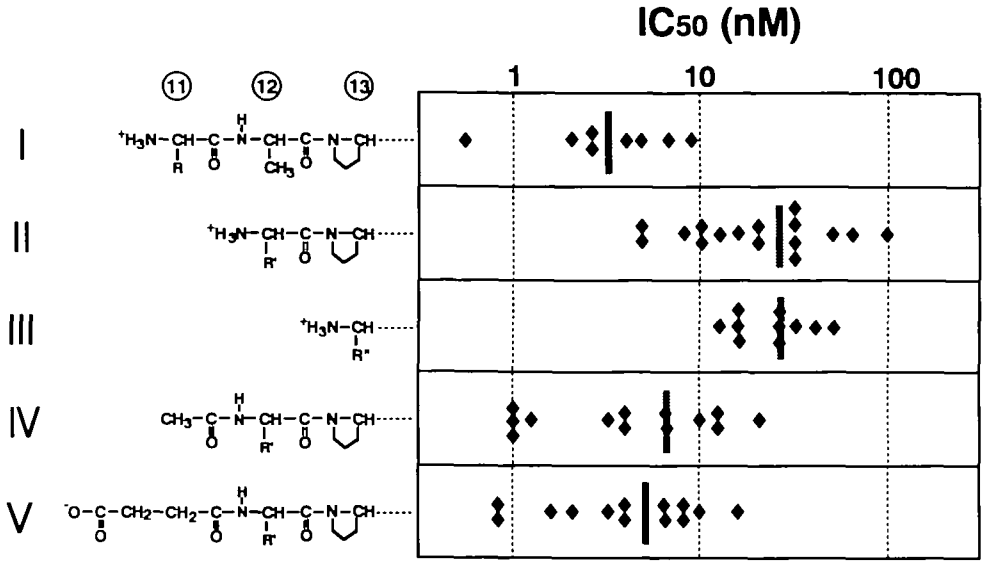

Fig. 1. **Distribution of the IC_{50} values shown in Table I, in terms of the length of the peptide.** Vertical lines represent mean values. I, [Xaa^{11}]-NPY(11-36); II, [Xaa^{12}]-NPY(12-36); III, [Xaa^{13}]-NPY(13-36); IV, acetyl-[Xaa^{12}]-NPY(12-36); V, succinyl-[Xaa^{12}]NPY(12-36).

TABLE II. **The ellipticities for analogs of NPY measured at 222 nm.**

Peptide	$[\theta]_{222}$ (deg·cm^2/dmol)	Peptide	$[\theta]_{222}$ (deg·cm^2/dmol)
[Xaa^{11}]NPY(11-36)		*[Xaa^{13}]NPY(13-36)*	
[Lys^{11}]NPY(11-36)[a]	−2,700[b]	[Ala^{13}]NPY(13-36)	−4,930[b]
[Arg^{11}]NPY(11-36)	−2,590[b]	[Lys^{13}]NPY(13-36)	−4,000[b]
[Asp^{11}]NPY(11-36)	−2,990[b]	[Arg^{13}]NPY(13-36)	−4,070[b]
[Glu^{11}]NPY(11-36)	−2,950[b]	[Asp^{13}]NPY(13-36)	−4,430[b]
[Asn^{11}]NPY(11-36)	−3,090[b]	[Glu^{13}]NPY(13-36)	−5,690[b]
[Gln^{11}]NPY(11-36)	−2,790[b]	[Pro^{13}]NPY(13-36)[a]	−4,430[b]
[Thr^{11}]NPY(11-36)	−2,800[b]	[Asn^{13}]NPY(13-36)	−5,080[b]
[Ser^{11}]NPY(11-36)	−3,350[b]	[Gln^{13}]NPY(13-36)	−4,720[b]
		[Thr^{13}]NPY(13-36)	−6,950[b]
		[Ser^{13}]NPY(13-36)	−6,940[b]
[Xaa^{12}]NPY(12-36)			
[Ala^{12}]NPY(12-36)[a]	−3,090[b]	[Cys^{12}]NPY(12-36)	−6,740[b]
ac[c]-[Ala^{12}]NPY(12-36)	−3,950	ac-[Cys^{12}]NPY(12-36)	−5,070
suc-[Ala^{12}]NPY(12-36)	−6,230	suc-[Cys^{12}]NPY(12-36)	−3,610
[Lys^{12}]NPY(12-36)	−3,540[b]	[Thr^{12}]NPY(12-36)	−9,030[b]
ac-[Lys^{12}]NPY(12-36)	−3,120	[Thr^{12}]NPY(12-36)/lipid[d]	−7,830
suc-[Lys^{12}]NPY(12-36)	−4,620	ac-[Thr^{12}]NPY(12-36)	−5,140
[Arg^{12}]NPY(12-36)	−2,050[b]	suc-[Thr^{12}]NPY(12-36)	−4,900
ac-[Arg^{12}]NPY(12-36)	−4,230	[Ser^{12}]NPY(12-36)	−8,510[b]
suc-[Arg^{12}]NPY(12-36)	−4,000	ac-[Ser^{12}]NPY(12-36)	−4,750
[His^{12}]NPY(12-36)	−2,930[b]	suc-[Ser^{12}]NPY(12-36)	−4,440
[Asp^{12}]NPY(12-36)	−4,430[b]	[Tyr^{12}]NPY(12-36)	−3,220[b]
ac-[Asp^{12}]NPY(12-36)	−4,750	[Gly^{12}]NPY(12-36)	−7,400[b]
suc-[Asp^{12}]NPY(12-36)	−9,830	ac-[Gly^{12}]NPY(12-36)	−4,250
suc-[Asp^{12}]NPY(12-36)/lipid[d]	−6,370	suc-[Gly^{12}]NPY(12-36)	−3,080
[Glu^{12}]NPY(12-36)	−3,840[b]	suc-[Gly^{12}]NPY(12-36)/lipid[d]	−4,380
ac-[Glu^{12}]NPY(12-36)	−5,110	[Val^{12}]NPY(12-36)	−4,320[b]
suc-[Glu^{12}]NPY(12-36)	−6,490	ac-[Val^{12}]NPY(12-36)	−2,740
[Asn^{12}]NPY(12-36)	−6,040[b]	suc-[Val^{12}]NPY(12-36)	−3,850
ac-[Asn^{12}]NPY(12-36)	−4,630	[Phe^{12}]NPY(12-36)	−4,270[b]
suc-[Asn^{12}]NPY(12-36)	−3,050	[Phe^{12}]NPY(12-36)/lipid[d]	−2,600
[Gln^{12}]NPY(12-36)	−6,940[b]	[Trp^{12}]NPY(12-36)	−3,040[b]
ac-[Gln^{12}]NPY(12-36)	−4,220	ac-[Trp^{12}]NPY(12-36)	−2,070
suc-[Gln^{12}]NPY(12-36)	−2,410	suc-[Trp^{12}]NPY(12-36)	−3,660

[a]Peptides having native sequences. [b]Similar results were obtained previously in a preliminary form (*6*). [c]Acetylated and succinylated peptides are represented by ac- and suc-, respectively. [d]Dimyristoyl phosphatidylcholine (0.15 mM) was added to the peptide solution.

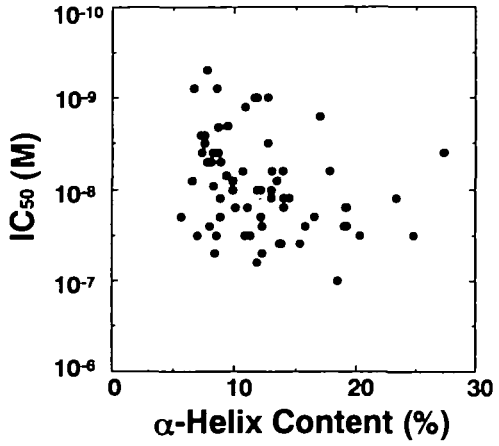

Fig. 2. **Relationship between the α-helix content and the IC_{50} value for analogs of NPY.** The α-helix contents calculated from $[\theta]_{222}$ value were plotted against IC_{50} values for all the analogs of NPY listed in Table I.

NPY(12-36), which has the native sequence, and after succinylation of [Gly^{12}]NPY(12-36) and [Gln^{12}]NPY(12-36), the binding affinity increased to be of the same order as that for the intact NPY molecule (IC_{50} for NPY = 0.20 ± 0.011 nM). In total, the modification of [Xaa^{12}]NPY(12-36) returned the mean value of IC_{50} to a level similar to that of [Xaa^{11}]NPY(11-36), as shown in Fig. 1. By contrast, acetylation of [Xaa^{13}]NPY(13-36) caused no apparent change in the binding affinity, and acetylation of [Xaa^{11}]-NPY(11-36) significantly decreased the binding affinity (Table I).

Next, CD spectra of the analogs were measured to examine whether the formation of the α-helical structure of the analogs affects the binding affinity for the Y_2 receptor. When the α-helix contents calculated from $[\theta]_{222}$ value in Table II were plotted against IC_{50} values for all the analogs of NPY listed in Table I, scattered data points were observed as shown in Fig. 2, which showed the absence of any simple relationship between the α-helix stability and the affinity of binding to the Y_2 receptor. It is necessary to check whether the conformation of peptides in CD measurement is the same as that in receptor-binding assay. Because much higher concentrations of peptides are usually required for CD measurements than for receptor-binding assay, the dependence of concentration of the analogs on their helicity was analyzed. C-terminal fragments of NPY such as NPY(15-36) were reported to have a tendency to associate into oligomers with induction of helical structure at high concentrations (*9*). It was found that the analogs used in this work also had a tendency to associate at high concentrations but exist mainly as monomers at the concentrations used for CD measurements as well as binding assay (data not shown). The effect of interaction with membranes on the stability of α-helix was also examined for the two analogs which showed the highest α-helix contents and those showing rather low α-helix contents. The stability of α-helix decreased by addition of phospholipid vesicles except for the case of succinyl-[Gly^{12}]NPY(12-36). These results also showed no apparent relation between the α-helix stability and the affinity of binding to the Y_2 receptor.

DISCUSSION

In a widely accepted hypothesis (*3-5*), it is proposed that fewer than 12 residues from the C-terminus are directly involved in binding of NPY to the Y_2 receptor, while the remaining part of NPY projects from the receptor. This hypothesis is mainly based on the study using a hybrid peptide, which consists of the C-terminal hexapeptide of NPY and the hairpin loop of homologous PP [PP(1-30)-NPY(31-36)] (*10*). Because the hybrid peptide with a vastly different but structurally homologous hairpin loop retained very high binding affinity for the receptor, the C-terminal hexapeptide was proposed to be the critical region for recognition by the Y_2 receptor, whereas the remaining part of NPY was suggested to make only a structural contribution.

Our present results, however, indicate that the binding affinity for the Y_2 receptor was significantly lowered by truncation of residue 11, and the binding affinity of [Xaa^{12}]-NPY(12-36) recovered to a level similar to that of [Xaa^{11}]-NPY(11-36) upon acetylation or succinylation of the α-amino group. It is notable that the modification of [Xaa^{12}]-NPY(12-36) caused an increase in binding affinity without any exception. The increase in binding affinity cannot be explained by the change in conformation as a consequence of the modification. The plot of the α-helix content *vs.* IC_{50} of all these analogs showed no apparent relationship, which confirmed our preliminary result (*6*). The addition of phospholipid vesicles to the analogs of NPY had no effect on their α-helix contents, although in some regulatory peptides such as cholecystokinin, membrane-induced conformations are thought to meet properly the structural requirements of their receptors (*11*). Therefore, prior formation of a rigid structure does not seem to be necessary for binding to the Y_2 receptor. With regard to the structural contribution to the binding affinity, contradictory results have been reported: analogs of NPY(15-36) modified at the α-amino group revealed good correlation between stability of the α-helix and binding affinity (*9*), whereas the successive removal of the middle region suggests that the α-helix content is not a predominant factor in the control of binding affinity (*12*). However, the set of analogs used in previous studies (*9, 12*) seems to be too small (fewer than 10) to allow elucidation of the structural requirements for the recognition of the Y_2 receptor as compared to that used in our present study (68 analogs).

Although acetylation and succinylation change the net charge at the N-terminus from +1 to 0 and −1, respectively, no significant difference was observed between the increases in the binding affinity for the Y_2 receptor caused by acetylation and those caused by succinylation (results for succinylation/results for acetylation = 1.2 ± 0.6). It is possible that blocking of the α-amino group by acylation caused the increase in the binding affinity. The free α-amino group of [Xaa^{12}]NPY(12-36) may be disadvantageous for the binding to the Y_2 receptor. Alternatively, the additional peptide bond introduced by the modification may be responsible for the recovery of binding affinity. If this is the case, it can be said that a peptide bond between residues 11 and 12 directly interacts with the Y_2 receptor. Such an interaction is seen in peptide-MHC complexes, in which hydrogen bonds between the MHC protein and the main-

chain CO and NH groups of the antigenic peptides are considered to contribute to the tight binding (*13, 14*). In conclusion, the present results suggest that the region around residue 12 does not project from the Y_2 receptor in the peptide-receptor complex.

As shown by the study using the hybrid peptide, the recognition of the side chains other than the C-terminal hexapeptide by the Y_2 receptor seems to be not so strict (*10*). This feature was also supported by a recent study, in which each residue of NPY was systematically replaced by Ala (*15*). Such a tolerant recognition of side chains of a ligand peptide can be found also in peptide-MHC complexes, where only two of nine side chains located near both ends of the peptide are recognized rather strictly (*16*). Our present results are thought to pose no contradiction to the previous results. Although substitution of residues 11, 12, and 13 of NPY with a variety of amino acid residues showed no apparent dependence of the binding affinity on the nature of the N-terminal residue, the following points seem to be interesting: (i) the analogs with positively charged N-terminal residues had slightly higher binding affinities than those with negatively charged residues, (ii) the binding affinity of [Tyr^{12}]NPY(12-36), which was the highest among the analogs of NPY(12-36), was 12-fold higher than that of [Phe^{12}]NPY(12-36), in which the N-terminal residue does not have the OH group.

The role of the N-terminal part of the NPY molecule in the binding to the Y_2 receptor remains to be elucidated. This part is not indispensable for the tight binding, because some analogs including those reported here have quite high affinity without the N-terminal peptide (residues 1-10). However, centrally truncated analogs which lack the β-turn region have been reported to have high binding affinity (*17-19*). Some of the analogs such as cyclo(2/27)-des-AA^{7-24} [Glu^2,Gly^6,D-2,3-diaminopropionic $acid^{27}$] have the affinity equivalent to that of intact NPY (*17*). Therefore, the N-terminal part may act as an alternative anchor in binding to the Y_2 receptor.

These features reported here must be considered in the development of drugs with potential effects at the Y_2 receptor, which is involved in important biological activities such as memory retention or consummatory behavior (*5*).

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REFERENCES

1. Schwartz, T.W., Fuhlendorff, J., Kjems, L.L., Kristensen, M.S., Vervelde, M., O'Hare, M., Krstenansky, J.L., and Bjørnholm, B. (1990) Signal epitopes in the three-dimensional structure of neuropeptide Y. *Ann. N.Y. Acad. Sci.* **611**, 35-47
2. Wahlestedt, C. and Reis, D.J. (1993) Neuropeptide Y-related peptides and their receptors—Are the receptors potential therapeutic drug targets? *Annu. Rev. Pharm. Toxicol.* **32**, 309-352
3. Grundemar, L., Sheikh, S.P., and Wahlestedt, C. (1993) Characterization of receptor types for neuropeptide Y and related peptides in *The Biology of Neuropeptide Y and Related Peptides* (Colmers, W.F. and Wahlestedt, C., eds.) pp. 197-239, Humana Press, Totowa, NJ
4. Heilig, M., Koob, G.F., Ekman, R., and Britton, K.T. (1994) Corticotropin-releasing factor and neuropeptide Y: Role in emotional integration. *Trends Neurosci.* **17**, 80-85
5. Grundemar, L. and Håkanson, R. (1994) Neuropeptide Y effector systems: Perspectives for drug development. *Trends Pharmacol. Sci.* **15**, 153-159
6. Yumoto, N., Murase, S., Hattori, T., Yamamoto, H., Tatsu, Y., and Yoshikawa, S. (1993) Stabilization of α-helix in *C*-terminal fragments of neuropeptide Y. *Biochem. Biophys. Res. Commun.* **196**, 1490-1495
7. Shigeri, Y., Mihara, S., and Fujimoto, M. (1991) Neuropeptide Y receptor in vascular smooth muscle. *J. Neurochem.* **56**, 852-859
8. Scholtz, J.M., Qian, H., York, E.J., Stewart, J.M., and Baldwin, R.L. (1991) Parameters of helix-coil transition theory for alanine-based peptides of varying chain lengths in water. *Biopolymers* **31**, 1463-1470
9. Doughty, M.B. and Hu, L. (1993) The contribution of helical potential to the in vitro receptor binding activity of a neuropeptide Y N-terminal deletion fragment. *Biopolymers* **33**, 1195-1206
10. Fuhlendorff, J., Johansen, N.L., Melberg, S.G., Thøgersen, H., and Schwartz, T.W. (1990) The antiparallel pancreatic polypeptide fold in the binding of neuropeptide Y to Y_1 and Y_2 receptors. *J. Biol. Chem.* **265**, 11706-11712
11. Moroder, L., Romano, R., Guba, W., Mierke, D.F., Kessler, H., Delporte, C., Winand, J., and Christophe, J. (1993) New evidence for a membrane-bound pathway in hormone receptor binding. *Biochemistry* **32**, 13551-13559
12. Fournier, A., Gagnon, D., Quirion, R., Cadieux, A., Dumont, Y., Pheng, L.-H., and St-Pierre, S. (1994) Conformational and biological studies of neuropeptide Y analogs containing structural alterations. *Mol. Pharmacol.* **45**, 93-101
13. Madden, D.R., Garboczi, D.N., and Wiley, D.C. (1993) The antigenic identity of peptide-MHC complexes: A comparison of the conformations of five viral peptide presented by HLA-A2. *Cell* **75**, 693-708
14. Stern, L.J., Brown, J.H., Jardetzky, T.S., Gorga, J.C., Urban, R.G., Strominger, J.L., and Wiley, D.C. (1994) Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* **368**, 215-221
15. Beck-Sickinger, A.G., Wieland, H.A., Wittneben, H., Willim, K.-D., Rudolf, K., and Jung, G. (1994) Complete L-alanine scan of neuropeptide Y reveals ligands binding to Y_1 and Y_2 receptors with distinguished conformations. *Eur. J. Biochem.* **225**, 947-958
16. Falk, K., Rotzschke, O., Stevanovic, S., Jung, G., and Rammensee, H.-G. (1991) Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* **351**, 290-296
17. Kirby, D.A., Koerber, S.C., Craig, A.G., Feinstein, R.D., Delmas, L., Brown, M.R., and Rivier, J.E. (1993) Defining structural requirements for neuropeptide Y receptors using truncated and conformationally restricted analogues. *J. Med. Chem.* **36**, 385-393
18. McLean, L.R., Buck, S.H., and Krstenansky, J.L. (1990) Examination of the role of the amphiphatic α-helix in the interaction of neuropeptide Y and active cyclic analogues with cell membrane receptors and dimyristoylphosphatidylcholine. *Biochemistry* **29**, 2016-2022
19. Beck-Sickinger, A.G., Grouzmann, E., Hoffmann, E., Gaida, W., van Meir, E. G., Waeber, B., and Jung, G. (1992) A novel cyclic analog of neuropeptide Y specific for the Y_2 receptor. *Eur. J. Biochem.* **206**, 957-964