The PP-Fold Solution Structure of Human Polypeptide YY and Human PYY3-36 As Determined by NMR[†,‡]

Rie Nygaard,[§] Steen Nielbo,[§] Thue W. Schwartz,[∥,⊥] and Flemming M. Poulsen*[,§]

Structural Biology and NMR Laboratory, Institute of Molecular Biology and Physiology, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K, Denmark, Laboratory for Molecular Pharmacology, Department of Pharmacology, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark, and 7TM Pharma A/S Hørsholm, Denmark

Received February 21, 2006; Revised Manuscript Received May 9, 2006

ABSTRACT: PYY3-36 is a biopharmaceutical antiobesity agent under development as well as an endogenous satiety hormone, which is generated by dipeptidyl peptidase-IV digestion of polypeptide YY (PYY), and in contrast to the parent hormone, PYY is highly selective for the Y_2 versus the Y_1 receptor. NMR analysis revealed a highly ordered, back-folded structure for human PYY in aqueous solution similar to the classical PP-fold structure of pancreatic polypeptide. The NMR analysis of PYY3-36 also showed a folded structure resembling a PP-fold, which however was characterized by far fewer long distance NOEs than the PP-fold observed in the full-length peptide. This suggests that either a conformational change has occurred in the N-terminal segment of PYY3-36 or that this segments is characterized by larger dynamics. The study supports the notion that the PP-fold is crucial for establishing simultaneous interactions with two subsites in the receptor for binding of, respectively, the N- and C-terminal ends of PYY. The Y_2 receptor only requires recognition of the C-terminal segment of the molecule as displayed by the Y_2 selective PYY3-36.

Polypeptide YY (PYY)[1] is a gastrointestinal hormone, which is a member of the so-called PP-fold family of regulatory peptides, which includes also the widespread neuropeptide, neuropeptide Y (NPY), and the hormone pancreatic polypeptide (PP) (*1*). These peptides all have 36 amino acid residues and are amidated at the C-terminal end. The PP-fold is characterized by a poly proline-like, N-terminal segment (Pro2-Pro5-Pro8), which is folded back on a long amphipathic α-helix, which is followed by a relatively disordered C-terminal hexapeptide, which is important for receptor recognition. Originally, this structure was identified for avian PP by X-ray analysis reaching a resolution of 0.98 Å (*2*, *3*). Subsequent NMR analysis demonstrated that bovine PP (*4*) and porcine PYY (*5*, *6*) adopt similar structures in dilute aqueous solution. In lipid micelles, the PP-fold of PYY is disrupted, conceivably because the strongly amphipathic α-helix instead partitions at the surface of the micelles and the N-terminal segment consequently appears in an unfolded structure (*6*). In one study, the solution structure of NPY is in a similar PP-fold as that of PP and PYY (*7*), whereas only the helical segment is observed in several other studies, which, however, all appear to have been performed under conditions where dimer formation occurred (*7*−*10*). Importantly, enzymatic stability studies have demonstrated that the well-ordered PP-fold is highly important for protection against endoproteolytic digestion, at least for PP (*1*).

The primary sequences of the PP-fold peptides differ considerably (*11*). However, the conserved residues are either important for the PP-fold—Pro2, Pro5, Pro8, Gly9, Tyr20, and Tyr27—or they are located in the C-terminal segment, which has been shown to be important for binding to the receptors—Thr32, Arg33, Arg35, and Tyr36 (*1*). The peptides mediate their effect via a family of receptors termed the Y-receptors; so far, this family consists of five receptors Y_1, Y_2, Y_4, Y_5, and Y_6 (*12*). This family of receptors belongs to the G-protein-coupled receptors having seven transmembrane segments and to the 7TM super-family of protein architectures.

PYY was originally isolated from colonic extracts in 1980 (*13*) and is synthesized and released from endocrine cells, which also release the hormone GLP-1. PYY3-36 is generated by removal of the N-terminal Tyr−Pro residues by dipeptidyl peptidase IV (DPP-IV) (*14*). The potential role of PYY3-36 as a satiety signal from the GI-tract to the CNS has recently been subject to much debate after the characterization in 2002 of the inhibitory effect on food intake of this peptide by Batterham and co-workers (*15*). This activity

[†] We thank "The John and Birthe Meyer Foundation" for the donation to establish the SBiN-Lab, and "The Biocampus Program of The University of Copenhagen" for a scholarship to Rie Nygaard.

[‡] Coordinates have been deposited in the Protein Data Bank (PDB entries: 2DF0 and 2DEZ).

* Corresponding author: Flemming M. Poulsen, Structural Biology and NMR Laboratory, Institute of Molecular Biology, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K. E-mail, fmp@apk.molbio.ku.dk; phone, +45 3532 2077; fax, +45 3532 2075.

[§] Institute of Molecular Biology and Physiology, University of Copenhagen.

[∥] The Panum Institute, University of Copenhagen.

[⊥] 7TM Pharma A/S.

[1] Abbreviations: DPP-IV, dipeptidyl peptidase IV; DQF−COSY, double quantum filtered correlation spectroscopy; NMR, nuclear magnetic lesonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser spectroscopy; NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, polypeptide YY; PYY3-36, polypeptide YY containing only the residues from 3 to 36; rmsd, root-mean-square deviation; TOCSY, total correlation spectroscopy.

10.1021/bi060359l CCC: $33.50
Published on Web 06/16/2006

of PYY3-36 was eliminated in Y_2-receptor knock-out mice, indicating that it is mediated through the Y_2 receptor (*15*). Initially a number of groups had difficulties in reproducing the effects on food intake in rodents (*16*). However, it became clear that the effect of PYY3-36 especially in rats was particularly susceptible to interference by stress mediated by the sympathetic nervous system (*17*, *18*). Importantly, the suppressive effect of PYY3-36 on appetite and on food intake has been demonstrated in man as well as in obese human subjects by two independent groups (*19*, *20*). Thus, currently, several pharmaceutical companies are in the process of developing PYY3-36 as an antiobesity agent for either subcutaneous or nasal delivery.

Keire et al. (*5*) first determined the solution structure of porcine PYY to have the expected PP-fold. The experiments also showed that PYY was in the monomer form at the concentrations used for the NMR experiments. Four years later, Lerch et al. (*6*) also determined the structure of porcine PYY. In the first structure, the helix was discontinued from residues 23−25, but in the second structure, this interruption was not present (*5*, *6*). In both structures the N-terminal end folds back onto the α-helix. So far, no structures of PYY3-36 have been published, but the secondary structure of PYY3-36 has been compared to PYY (*21*) and shown to have less α-helical content than full-length PYY. Molecular dynamics simulations of PYY3-36 suggest that the N-terminal does not fold back onto the α-helix (*21*). In the present study, we have determined the NMR structure of full-length human PYY and the structure of PYY3-36.

MATERIALS AND METHODS

PYY and PYY3-36 were synthesized by standard solid-phase synthesis.

Experimental Procedure. PYY and PYY3-36 were dissolved in water and 10% D_2O to a concentration of approximately 1 mM, and the pH was adjusted to 4.6 by adding NaOH and HCl.

NMR Studies. NMR experiments were performed on a Varian INOVA 750 MHz spectrometer at 25 °C. Chemical shift assignments for PYY were performed by the standard method (*22*) using DQF−COSY (*23*) and TOCSY (*24*) spectra. For NOE assignment, NOESY spectra (*25*) with a mixing time of 150 ms were recorded. The spectra were all processed using NMRPipe (*26*)

Structure Calculation. Sequential and side-chain assignments were performed using Pronto3D (*27*). NOEs were assigned automatically using the CANDID algorithm in CYANA (*28*, *29*). The assigned NOEs were used as distance restraints in the XPLOR-NIH structure calculations (*30*). Finally, a water refinement was performed using CNS (*31*).

Restraints. The upper restraint limits determined in CYANA were converted to XPLOR-NIH format, and a lower limit of 1.8 Å was used in the XPLOR-NIH structure calculations.

Hydrogen bonds were restrained using an N−O distance of 2.85 Å and upper and lower bound of 3.00 and 2.60 Å, respectively, and using an O−HN distance of 1.90 Å with an upper and lower bound of 2.05 and 1.65 Å, respectively.

RESULTS

Confirmation of Receptor Affinity and Potency for the PYY Peptides. The affinity and potency of the hPYY and hPYY3-36 peptides used for structural analysis was determined in transiently transfected COS-7 cells expressing the human Y receptors (Table 1). This confirmed (*12*) that the two peptides had similar affinity on the Y_2 receptor, 0.23 and 0.36 nM, respectively, whereas hPYY3-36 showed more than 100-fold decreased affinity toward the Y_1 receptor as compared to full-length hPYY, with 74 versus 0.6 nM, respectively. This difference was even more pronounced when signal transduction was studied, as the potency of hPYY was 16 nM in contrast to >1000 nM for hPYY3-36 on the Y_1 receptor, whereas the two peptides were equipotent on the Y_2 receptor, 0.22 and 0.20 nM (Table 1).

Table 1: Affinity and Potency of hPYY and hPYY3-36 to the Receptors[a]

	Affinity (nM)				Potency (nM)			
	hY_1	hY_2	hY_4	hY_5	hY_1	hY_2	hY_4	hY_5
hPYY	0.6	0.23	34	8.5	16	0.22	39	1.2
hPYY3-36	74	0.36	>1000	82	>1000	0.20	343	22

[a] Affinity: COS-7 cells transfected with human Y_1, Y_2, Y_4, and Y_5, respectively. Competition assays performed with 125I-PYY for the Y_2 receptor, 125I-NPY for the Y_1 and Y_4 receptors, and 125I-PP for the Y_4 receptor. Potency: dose−response experiments in COS-7 cells transfected with human Y_1, Y_2, Y_4, and Y_5 as well as promiscuous G protein, Gqi5 that ensures that the Y_2 receptor couples through a Gq pathway leading to an increase in inositol phosphate turnover.

Table 2: Nonredundant NOEs Used for Structure Calculations in XPLOR-NIH

	PYY	PYY3-36
intraresidue NOEs	20	23
sequential NOEs	67	76
medium-range NOEs	80	107
long-range NOES	25	7
total	192	213

Assignment and Structure Calculation. DQF−COSY, TOCSY, and NOESY spectra were used for the assignment of the ^1H of the backbone and side chains. NMR signals from Tyr1 and Tyr36 of hPYY could not be observed, which can be due to the flexibility in these regions. For hPYY3-36, NMR signals from all residues were assigned.

For both peptides, most of the cross-peaks in the NOESY spectra were assigned by normal analysis. However, the program CYANA CANDID was also used to assign the NOEs and subsequently to calculate the two structures. Calculations were continued in XPLOR-NIH using a set of nonredundant NOEs as shown in Table 2. This implies that most of the intraresidue NOEs were not included in the structure calculations.

The distribution of the NOEs for hPYY and hPYY3-36 can be seen in Figure 1. The difference in the pattern of NOEs between the two peptides is the absence in hPYY3-36 of several long range NOEs between the N- and C-terminal part of the sequence seen in hPYY. Whereas 23 long-range NOEs were seen between hydrogen atoms of the helix residues Leu17, Tyr20, Leu24, and Tyr27 and atoms in Pro2, Ile3, Lys4, Pro5, and Ala7 in the N-terminal of hPYY, only seven long-range NOEs were seen in hPYY3-36 between the same residues. Figure 2 shows the presence and absence of NOEs between the phenol HD and HE hydrogen atoms of Tyr20 and Tyr27 and the hydrogen atoms of the N-terminal residues Pro2, Ile3, and Lys4 in hPYY

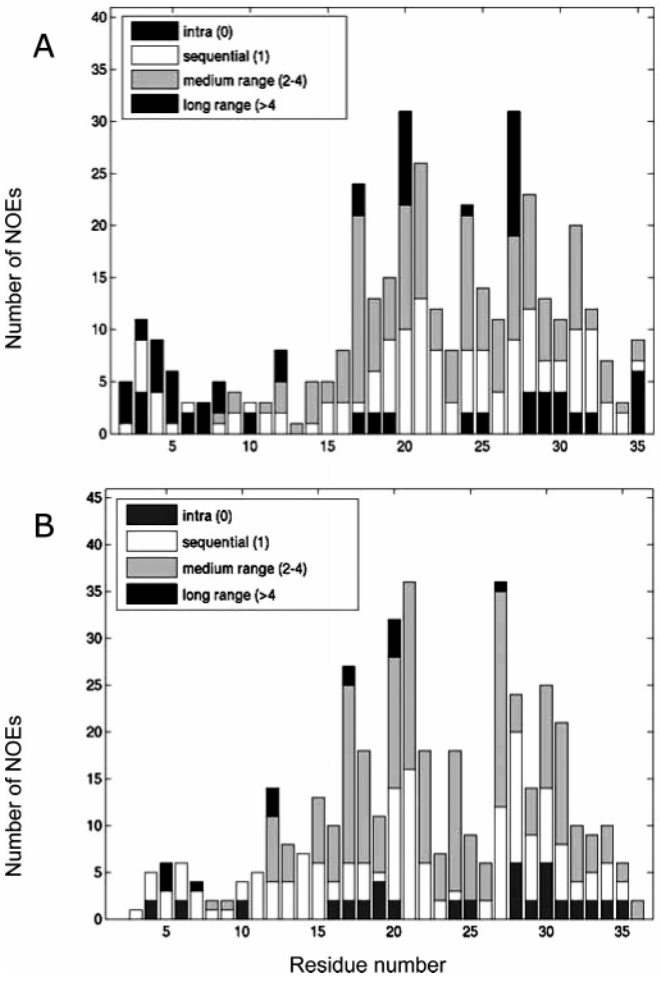

FIGURE 1: (A) Distribution of NOEs for hPYY; (B) distribution of NOEs for hPYY3−36.

and hPYY3-36, respectively. A table of the long-range NOEs in hPYY and hPYY3-36 is provided in the Supporting Information. The reduced number of long-range NOEs may suggest that removal of the two N-terminal residues abolish and weakens a number of hydrophobic interactions involving the side chains mentioned above, which results in a conformational change. However, since obvious line broadening was seen for many signals in the hPYY3-36 NMR spectra, the absence of the two residues may also result in more peptide chain dynamics. The presence of dynamics may also explain the reduced number of long-range NOEs. Hence, it is difficult to distinguish whether the reduced number of long-range NOEs is due to increased dynamics and line broadening or to a conformational change or to both.

In Figure 3, the observed backbone-related sequential and medium-range NOEs of hPYY and hPYY3-36 are shown. In the stretch of residues from 15 to 34 in both peptides, there is a clear pattern of NOEs, suggesting that this part of the peptide chain forms a regular α-helix, as supported by the observation of 15 and 10 $d_{\alpha N}(i,i+3)$ NOEs in hPYY and hPYY3-36, respectively, and similarly 9 and 6 NOEs of the $d_{\alpha N}(i,i+4)$ type. The $d_{\alpha\beta}(i,i+3)$ NOE type is also expected in α-helical stretches, but because of overlap in the region of the NMR spectra of both hPYY and hPYY3-36, where these NOEs occur, not all of them can be resolved. Still, however, 6 and 7 of this type of NOEs were observed in PYY and hPYY3-36, respectively.

A secondary chemical shift analysis comparing the chemical shift difference between the experimentally determined H^{α} chemical shifts and random coil shifts for the individual amino acid residues in peptide chains was performed using the random coil shifts determined by Schwarzinger et al. (*32*), Figure 4. The NOE and chemical shift data are in support of the helix structure present in the C-terminal part of the two peptides. The relatively small differences between the secondary chemical shift in the sequence between residue 15 and 34 of hPYY and hPYY3-36 compared to the differences in the N-terminal part also suggest that the peptide backbone structures in this part of the sequence in the two forms are very similar. The secondary H^{α} chemical shift reported for porcine PYY(*5*) is, residue by residue, qualitatively very similar to those observed here for human PYY, suggesting also the similarity between the two structures. In the best 20 structures from the CANDID protocol, no NOEs were violated more than 0.5 Å, in either of the two peptides.

This initial structure calculation suggested, in agreement with the sequential NOE analysis and the chemical shift analysis, that an α-helix is present starting at residue 15 and ending at residue 34. However, the characteristic hydrogen bonds in an α-helix were not being calculated consistently for the C-terminal hydrogen bonds of the proposed 15−34 helix element. An analysis of the hydrogen bonds found in the two samples of the 20 initially calculated structures showed that in hPYY hydrogen bonds were formed for residues 15−30 in more than 18 of the 20 structures and similarly for hPYY3-36 from residue 17 to 31. Hydrogen bond restraints were subsequently applied for these bonds in the structure calculations of hPYY and hPYY3-36. A second round of structure calculation including the hydrogen bond restraints was performed calculating 200 structures and selecting the 20 structures with the lowest energy. In these 20 structures, no distance restraint was violated by more than 0.5 Å. The structures were further refined using CNS (*31*), a simulated annealing protocol with a layer of water added. The refined structures were validated in the program PROCHECK-NMR (*33*). Ramachandran plot analysis for the two peptides can be seen in the Supporting Information.

The Structure of Human PYY and PYY3-36. In Figure 5, the 20 structures with the lowest energy computed by CNS are shown for the two peptides. The hPYY structures have been aligned by the backbone atoms residues 5−6 and 15−32, and the alignment yields an rmsd of 0.78 Å. PYY has the classical PP-fold. The hPYY3-36 structures have been aligned by the backbone atoms residues 5−6 and 15−34, and the alignment yields an rmsd of 0.92 Å. The two structures are, apart from the missing two residues in hPYY3-36, essentially identical and propose that the PP-fold is present in both PYY and PYY3-36. However, the smaller number of long-range NOEs in the hPYY3-36 spectra clearly results in a less well-defined structure in the N-terminal residues as compared to full-length hPYY. In the final ensemble of both structures, the C-terminal helix was found to start at residue 15 and end at residue 32. This is in good agreement with the direct experimental observation of NOEs and chemical shift data, which suggested an α-helix between residues 15 and 34 in both peptides.

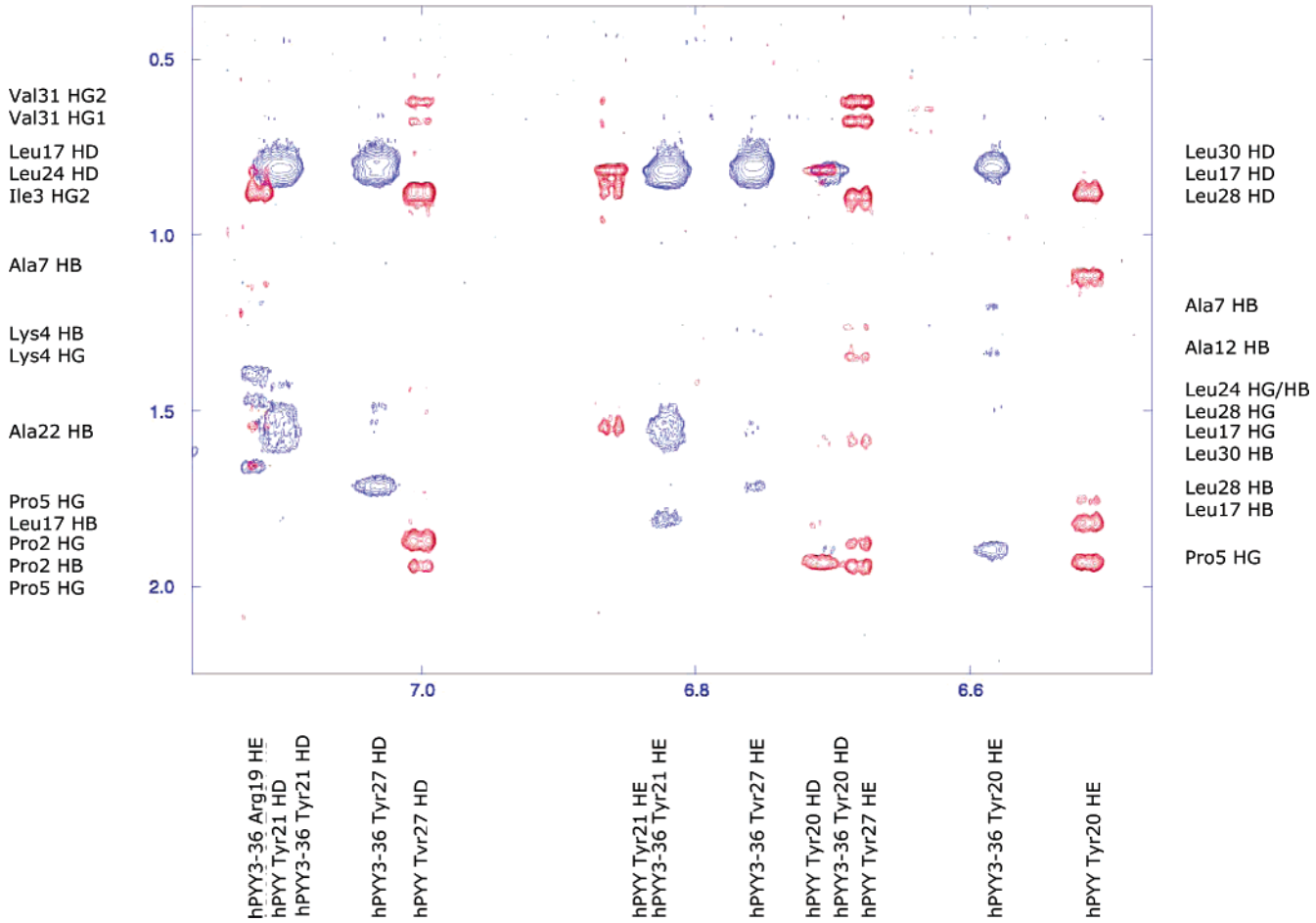

FIGURE 2: Long-range NOEs in hPYY NOESY spectrum compared to hPYY3-36 NOESY spectrum. Both spectra are recorded on peptide samples at pH 4.6 and 25 °C. The red cross-peaks are from the hPYY NOESY spectrum, and the blue cross-peaks are from the hPYY3-36 NOESY spectrum. Residue numbers to the left refer to residues from hPYY, and residue numbers to the right refer to residues from hPYY-36.

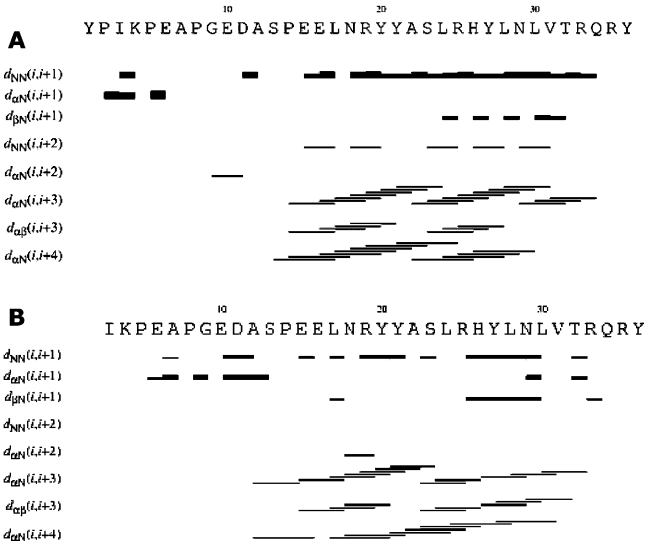

FIGURE 3: (A) Schematic presentation of NOEs for hPYY; (B) schematic presentation of NOEs for hPYY3-36.

DISCUSSION

The present study provides the first 3D NMR structure of human PYY and the first 3D structure of human PYY3-36 demonstrating that both molecules have a PP-fold in aqueous solution. The results suggest that the difference in receptor binding of hPYY and hPYY3-36 may be entirely associated with the absence of the two N-terminal residues of hPYY. The missing affinity of PYY3-36 to Y_1 suggests, therefore, that the PP-fold, which brings the N- and the C-terminal ends in close contact to each other, is the scaffold required to bring the ligand residues in the N- and C-terminal of the peptide together to be recognized jointly by the Y_1 receptor.

Stability of the PP-Fold in Solution. It is somewhat surprising that PYY and the other relatively small 36 amino acid residue peptides of this family are able to maintain a stable, well-defined 3D structure in dilute aqueous solution without, for example, any intramolecular-stabilizing disulfide bridges. The PP-fold can be considered to constitute a miniature of a "real protein". Thus, it has a hydrophobic core, comprising the highly conserved poly-proline sequence Pro2, Pro5, and Pro8, which are interdigitating with the conserved hydrophobic residues Leu17, Tyr20, Leu24, and Tyr27 in the hydrophobic patch along the amphipathic α-helix. The poly-proline helical structure of the N-terminal segment and its similarity to collagen structures has previously been characterized through CD analysis of NPY in solution (*34*). This poly-proline segment and the α-helix are connected by a *β*-turn region, which is induced by a highly conserved Gly9, and which includes a Pro residue situated either at position 13 or 14. The amphipathic, long α-helix of the PP-fold peptides is stabilized by a conserved, strong dipole moment oriented antiparallel to the dipole moment of the α-helix

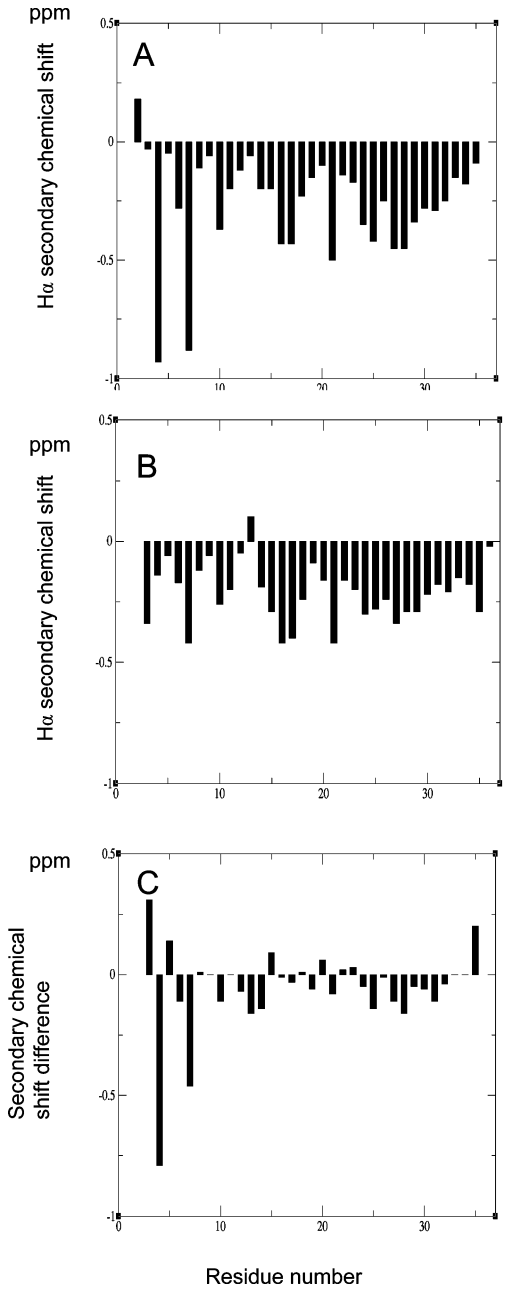

FIGURE 4: (A) hPYY and (B)hPYY3-36 H^{α} chemical shift differences from random coil chemical shift. Random coil chemical shift with the experimentally determined chemical shift subtracted. (C) Secondary chemical shift difference between hPYY and hPYY3-36.

resulting from the alignment of the peptide dipoles parallel to the helix (*35*). The helix-stabilizing, antiparallel dipole moment of the PP-fold is a result of the occurrence of a number of acidic residues at the N-terminal end of the helix, that is, around the turn region, plus a number of basic residues located at the C-terminal end. Interestingly, the nature and the precise location of the individual acidic residues in the loop region are not conserved. However, the occurrence of an overall cluster of acidic residues in this loop region is nevertheless conserved from, for example, Lamprey to man conceivably to create the helix-stabilizing dipole moment (*35*). The PP-fold of the solution structure of hPYY3-36 observed in the present study indicates that the stability of the PP-fold is strong enough to resist the elimination of the Pro2−Tyr27 interaction (Figure 5). The

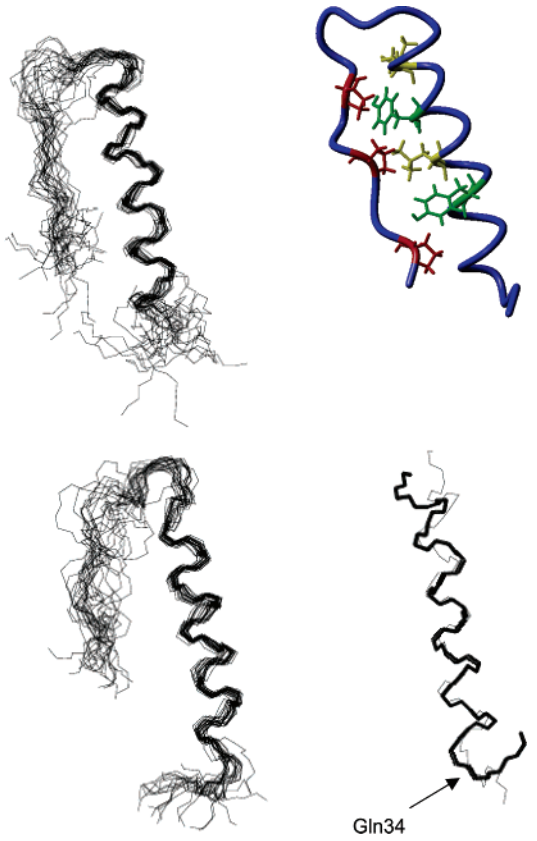

FIGURE 5: Structure of hPYY and hPYY3-36. Top left, hPYY backbone atoms aligned from residue 5−6 and 15−32 with a rmsd of 0.789 Å. Top right, hydrophobic residues in the hydrophobic core of hPYY (proline red, tyrosine green, leucines yellow). Bottom left, hPYY3-36 backbone atoms aligned from residue 5−6 and 15−32 rmsd 0.923 Å. Bottom right, backbone atoms of hPYY and hPYY3-36 aligned from residue 17−34, the bold line represents hPYY, and the thin line represents hPYY3-36.

NMR data, though, suggest that the removal of this introduces both dynamics and conformational changes in the structure.

It has been demonstrated that whereas full-length PP is digested very slowly by, for example, protease Asp-N−directed against Asp10 in the loop region—this rate is highly increased either by deleting the far C-terminal 27−36 segment of the molecule or by introducing a Pro residue in the middle of the α-helix, that is, far away from the actual cleavage site (*1*). These studies clearly indicate that the majority of the peptide molecules in solution are found in a conformation, which protects against attack by the protease, that is, conceivably the PP-fold. The deletion of the C-terminal 27−36 segment removes Tyr27, which is part of the hydrophobic "zipper" in the core of the PP-fold, which most likely results in a destabilization or partly unfolding of the PP-fold in the resulting N-terminal 1−26 peptide. In PYY3-36, another part of the hydrophobic "zipper" is missing, that is, Pro2, which from the N-terminal poly-Pro segment is the main interaction partner for Tyr27. The NMR analysis of the present study suggests that although the PP-fold apparently still is present in PYY3-36 in solution it is not as well-defined, conceivably as a result of increased dynamics and destabilization of the molecule. This is interesting, because such a destabilization of the PP-fold of PYY3-36 could make the molecule more instable in general and, for example, more susceptible to proteolytic degradation.

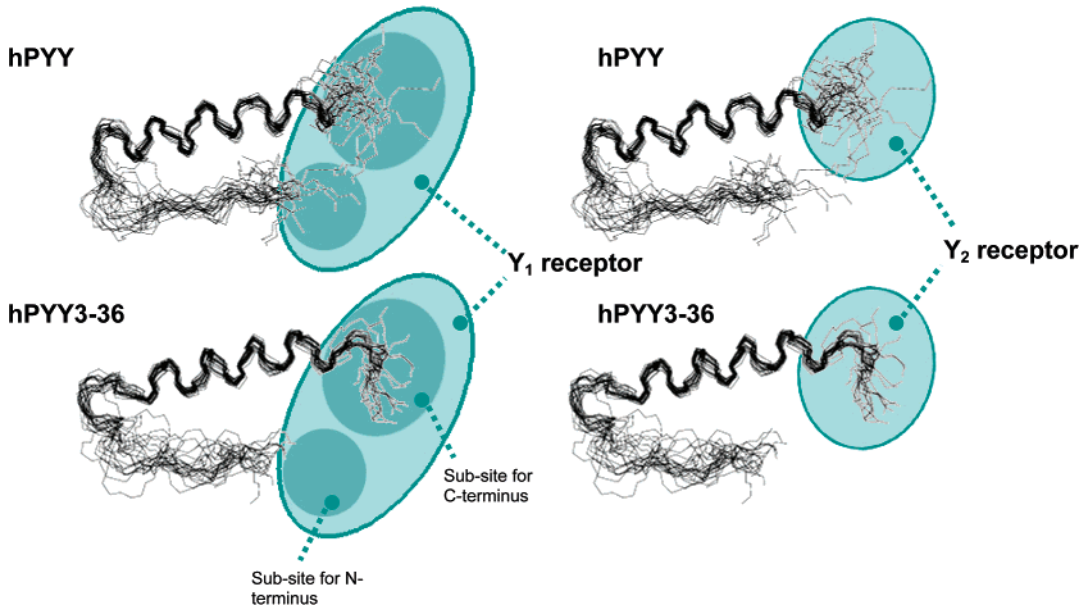

FIGURE 6: Schematic illustration of proposed binding sites for the Y_1 and Y_2 receptors. Top, structures of hPYY; bottom, structure of hPYY3-36.

This could be important because PYY currently is under development as an antiobesity biopharmaceutical agent.

PP-Fold in Solution versus in Micelles. Recently, structures of porcine PYY bound to micelles have been solved (*6*). In these structures, the N-terminal part of the full-length PYY molecule is disordered and only the C-terminal helix structure is maintained. Conceivably, in the presence of the micelles, the hydrophobic patch along the amphipathic α-helix and their hydrophobic counterparts in the N-terminal part favor an interaction with the lipid. It is of interest to note that removal of the two N-terminal residues from PYY reduced the number of long-range NOEs dramatically, presumably due to reduction of hydrophobic interactions and increased dynamics as a consequence of this. This emphasizes that the observation of the PP-fold by NMR spectroscopy very much depends on the observation of a set of long-range NOEs, and that any perturbation of the system, which destabilizes it and increases the dynamics of the peptide, may obscure the observation of these NOEs and the detection of a more dynamic PP-fold

Lerch and co-workers suggested that PYY and NPY are recognized by the Y receptors from the membrane-bound state and that the PP-fold, which is not observed in the micelle-associated structures, therefore, is not important for the receptor recognition (*6*). We would argue that the solution structures, as determined for example in the present study, are highly relevant for the receptor recognition, but that the amphipathic nature of the peptides will lead to an accumulation of the peptides at the surface of the cells in accordance with the classical theory of Kaiser and Kezdy (*36*, *37*). In this fashion, the peptides concentrate near the receptor, which makes the peptide−receptor interaction more likely to occur. We will, however, also argue that to bind at least to the Y_1 receptor, it is nevertheless necessary for the peptides to adopt the PP-fold structure observed in solution in order to present the conjoined N- and C-terminal ends to this receptor (Figure 6). It is likely that PP-fold peptides such as PYY accumulate at the cell membrane, but that they will regain the PP-fold structure observed in solution before binding to the receptor. In the case of the Y_2 receptor, the PP-fold is not required as this receptor only interacts with the C-terminal end of the peptide, Figure 6.

Implications for Receptor Recognition. It has previously been suggested that the main function of the PP-fold is to present the conjoined C- and N-terminal segments of the PP-fold peptides to the receptor, as depicted for PYY and the Y_1 receptor in Figure 6 (*1*, *21*). In 1994, a complete alanine-scan was performed on the NPY peptide by Beck-Sickinger, revealing that the C-terminal hexapeptide is especially important for binding to both the Y_1 and the Y_2 receptors (*38*). The N-terminal part of the molecule proved to be very important for Y_1-receptor binding but not for Y_2-receptor binding (*38*). In a study by Fuhlendorff (*39*), fragments of the NPY molecule missing variable lengths of pieces of the N-terminal part were synthesized. The C-terminal hexapeptide did not show any affinity for the Y_1 receptor; neither did longer fragments of NPY, for example, NPY23-36 and NPY19-39. The C-terminal hexapeptide does not have any appreciable affinity for the Y_2 receptor either. However, longer fragments of the C-terminal part of NPY bind with high affinity to the Y_2 receptor in contrast to the Y_1 receptor (*39*, *40*). This implies that the scaffold of the C-terminal helix, which this work has shown to exist in both hPYY and hPYY3-36, is important for the formation of the binding conformation for both the Y_1 and the Y_2 receptors. It also suggests that the N-terminal part of the molecule is important for binding to the Y_1 receptor, but not for binding to the Y_2 receptor, Figure 6. Therefore, the solution structures of hPYY and hPYY3-36 as determined in the present work provide a clear structural explanation for the specificities of the two molecules to the Y_1 and Y_2 receptors, respectively, Figure 6. The model proposed by Beck-Sickinger (*38*) in which the degree of helicity of the two peptides should explain the different activities does not agree with the data presented here, because the NMR data strongly suggest that the helicity of the two forms are similar.

In summary, three-dimensional structures determined by NMR spectroscopy of hPYY and hPYY3-36 in aqueous

solution have suggested a structural explanation for the difference in the activity of the two molecules toward the Y_1 and Y_2 receptors. The removal by dipeptidyl peptidase IV of the first two N-terminal residues, of which Pro2 probably is structurally most important, switches the peptide hormone from binding to both the Y_1 and the Y_2 receptors to only binding to the Y_2 receptor due to the missing two residues. Interestingly, especially the N-terminal part of the PP-fold of PYY3-36 appears to be less well-defined and conceivably more dynamic than in full-length PYY

ACKNOWLEDGMENT

Christian E. Elling and Susanne Hummelgaard, 7TM Pharma A/S, are thanked for peptide materials and for performing the receptor binding and signaling analysis. Birthe Brandt Kragelund is thanked for help with the use of the programs Pronto3D and XPLOR-NIH and with many other aspects.

SUPPORTING INFORMATION AVAILABLE

Table of long-range NOEs in hPYY and hPYY3-36, Ramachandran plot analysis of the peptides, and HN-Hα fingerprint region of of NOESY spectra from hPYY and hPYY3-36. This material is available free of charge via the Internet at http://pubs.acs.org.

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. Interaction with Y1, Y2, and pancreatic polypeptide receptors, *Ann. N.Y. Acad. Sci. 611*, 35−47.
2. Blundell, T., Pitrs, J., Tickle, S., Wood, S., and Wu, C. (1981) X-ray analysis (1.4-Å resolution) of avian pancreatic polypeptide: Small globular protein hormone, *Proc. Natl. Acad. Sci. U.S.A. 78*, 4175−4179.
3. Glover, I., Haneef, I., Pitts, J., Wood, S., Moss, D., Tickle, I., and Blundell, T. (1983) Conformational flexibility in a small globular hormone. X-ray analysis of avian pancreatic polypeptide at 0.98 Angstroms resolution, *Biopolymers 22*, 293−304
4. Li, X. A., Sutcliffe, M. J., Schwartz, T. W., and Dobson, C. M. (1992) Sequence-specific 1H NMR assignments and solution structure of bovine pancreatic polypeptide, *Biochemistry 31*, 1245−1253.
5. Keire, D. A., Kobayashi, M., Solomon, T. E., and Reeve, J. R., Jr. (2000) Solution structure of monomeric peptide YY supports the functional significance of the PP-fold, *Biochemistry 39*, 9935−9942.
6. Lerch, M., Mayrhofer, M., and Zerbe, O. (2004) Structural similarities of micelle-bound peptide YY (PYY) and neuropeptide Y (NPY) are related to their affinity profiles at the Y receptors, *J. Mol. Biol. 339*, 1153−1168.
7. Darbon, H., Bernassau, J. M., Deleuze, C., Chenu, J., Roussel, A., and Cambillau, C. (1992) Solution conformation of human neuropeptide Y by ^{1}H nuclear magnetic resonance and restrained molecular dynamics, *Eur. J. Biochem. 209*, 765−771.
8. Saudek, V., and Pelton, J. T. (1990) Sequence-specific ^{1}H NMR assignment and secondary structure of neuropeptide Y in aqueous solution, *Biochemistry 29*, 4509−4515.
9. Cowley, D. J., Hoflack, J. M., Pelton, J. T., and Saudek, V. (1992) Structure of neuropeptide Y dimer in solution, *Eur. J. Biochem. 205*, 1099−1106.
10. Monks, S. A., Karagianis, G., Howlett, G. J., and Norton, R. S. (1996) Solution structure of human neuropeptide Y, *J. Biomol. NMR 8*, 379−390.
11. Larhammar, D. (1996) Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide, *Regul. Pept. 62*, 1−11.
12. Michel, M. C., Beck-Sickinger, A., Cox, H., Doods, H. N., Herzog, H., Larhammar, D., Quirion, R., Schwartz, T., and Westfall, T. (1998) XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors, *Pharmacol. Rev. 50*, 143−150.
13. Tatemoto, K., and Mutt, V. (1980) Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides, *Nature 285*, 417−418.
14. Grandt, D., Schimiczek, M., Beglinger, C., Layer, P., Goebell, H., Eysselein, V. E., and Reeve, J. R., Jr. (1994) Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36, *Regul. Pept. 51*, 151−159.
15. Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., Brynes, A. E., Low, M. J., Ghatei, M. A., Cone, R. D., and Bloom, S. R. (2002) Gut hormone PYY(3-36) physiologically inhibits food intake, *Nature 418*, 650−654.
16. Tschop, M., Castaneda, T. R., Joost, H. G., Thone-Reineke, C., Ortmann, S., Klaus, S., Hagan, M. M., Chandler, P. C., Oswald, K. D., Benoit, S. C., Seeley, R. J., Kinzig, K. P., Moran, T. H., Beck-Sickinger, A. G., Koglin, N., Rodgers, R. J., Blundell, J. E., Ishii, Y., Beattie, A. H., Holch, P., Allison, D. B., Raun, K., Madsen, K., Wulff, B. S., Stidsen, C. E., Birringer, M., Kreuzer, O. J., Schindler, M., Arndt, K., Rudolf, K., Mark, M., Deng, X. Y., Whitcomb, D. C., Halem, H., Taylor, J., Dong, J., Datta, R., Culler, M., Craney, S., Flora, D., Smiley, D., and Heiman, M. L. (2004) Physiology: Does gut hormone PYY3-36 decrease food intake in rodents?, *Nature 430*, 1.
17. Halatchev, I. G., Ellacott, K. L., Fan, W., and Cone, R. D. (2004) Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism, *Endocrinology 145*, 2585−2590.
18. Boggiano, M. M., Chandler, P. C., Oswald, K. D., Rodgers, R. J., Blundell, J. E., Ishii, Y., Beattie, A. H., Holch, P., Allison, D. B., Schindler, M., Arndt, K., Rudolf, K., Mark, M., Schoelch, C., Joost, H. G., Klaus, S., Thone-Reineke, C., Benoit, S. C., Seeley, R. J., Beck-Sickinger, A. G., Koglin, N., Raun, K., Madsen, K., Wulff, B. S., Stidsen, C. E., Birringer, M., Kreuzer, O. J., Deng, X. Y., Whitcomb, D. C., Halem, H., Taylor, J., Dong, J., Datta, R., Culler, M., Ortmann, S., Castaneda, T. R., and Tschop, M. (2005) PYY3-36 as an anti-obesity drug target, *Obes. Rev. 6*, 307−322.
19. Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., Ghatei, M. A., and Bloom, S. R. (2003) Inhibition of food intake in obese subjects by peptide YY3-36, *N. Engl. J. Med. 349*, 941−948.
20. Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J., and Beglinger, C. (2005) Effect of peptide YY3-36 on food intake in humans, *Gastroenterology 129*, 1430−1436.
21. Keire, D. A., Mannon, P., Kobayashi, M., Walsh, J. H., Solomon, T. E., and Reeve, J. R., Jr. (2000) Primary structures of PYY, [Pro(34)]PYY, and PYY-(3-36) confer different conformations and receptor selectivity, *Am. J. Physiol.: Gastrointest. Liver Physiol. 279*, G126−G131
22. Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley-Interscience, New York.
23. Rance, M., Sørensen, O. W., Bodenhausen, G., Wagner, G., Ernst, R. R., and Wüthrich, K. (1983) Improved spectral resolution in cosy ^{1}H NMR spectra of proteins via double quantum filtering, *Biochem. Biophys. Res. Commun. 117*, 479−485.
24. Braunschweiler, L. and Ernst, R. R. (1983) Coherence transfer by isotropic mixing: Application to proton correlation spectroscopy, *J. Magn. Reson. 53*, 521−528
25. Jeener, J., Meier, B. H., Bachmann, P., and Ernst, R. (1979) *J. Magn. Reson. 71*, 4546.
26. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe: A multidimensional spectral processing system based on UNIX pipes, *J. Biomol. NMR 6*, 277−293.
27. Kjær, M., Andersen, K. V., and Poulsen, F. M. (1994) Automated and semiautomated analysis of homo- and heteronuclear multidimensional nuclear magnetic resonance spectra of proteins: The program Pronto, *Methods Enzymol. 239*, 288−307.
28. Herrmann, T., Güntert, P., and Wüthrich, K. (2002) Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA, *J. Mol. Biol. 319*, 209−227.

29. Güntert, P., Mumenthaler, C., and Wüthrich, K. (1997) Torsion angle dynamics for NMR structure calculation with the new program DYANA, *J. Mol. Biol. 273*, 283−298.
30. Schwieters, C. D., Kuszewski, J. J., Tjandra, N., and Clore, G. M. (2003) The Xplor-NIH NMR molecular structure determination package, *J. Magn. Reson. 160*, 65−73.
31. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination, *Acta Crystallogr., Sect. D.: Biol. Crystallogr. 54*, 905−921.
32. Schwarzinger, S., Kroon, G. J., Foss, T. R., Chung, J., Wright, P. E., and Dyson, H. J. (2001) Sequence-dependent correction of random coil NMR chemical shifts, *J. Am. Chem. Soc. 123*, 2970−2978.
33. Laskowski, R. A., Rullmannn, J. A., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996) AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR, *J. Biomol. NMR 8*, 477−486.
34. Fuhlendorff, J., Johansen, N. L., Melberg, S. G., Thøgersen, H., and Schwartz, T. W. (1990a) The antiparallel pancreatic polypeptide fold in the binding of neuropeptide Y to Y1 and Y2 receptors, *J. Biol. Chem. 265*, 11706−11712.
35. Bjørnholm, B., Jørgensen, F. S., and Schwartz, T. W. (1993) Conservation of a helix-stabilizing dipole moment in the PP-fold family of regulatory peptides, *Biochemistry 32*, 2954−2959.
36. Kaiser, E. T. and Kezdy, F. J. (1983) Secondary structures of proteins and peptides in amphiphilic environments. (A review), *Proc. Natl. Acad. Sci. U.S.A. 80*, 1137−1143.
37. Kaiser, E. T. and Kezdy, F. J. (1984) Amphiphilic secondary structure: Design of peptide hormones, *Science 223*, 249−255.
38. 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 Y1 and Y2 receptors with distinguished conformations, *Eur. J. Biochem. 225*, 947−958.
39. Fuhlendorff, J., Gether, U., Aakerlund, L., Langeland-Johansen, N., Thøgersen, H., Melberg, S. G., Olsen, U. B., Thastrup, O., and Schwartz, T. W. (1990b) [Leu31, Pro34]neuropeptide Y: A specific Y1 receptor agonist, *Proc. Natl. Acad. Sci. U.S.A. 87*, 182−186.
40. Wahlestedt, C., Yanaihara, N., and Hakanson, R. (1986): Evidence for different pre-and post-junctional receptors for neuropeptide Y and related peptides. *Regul. Pept. 13*, 307−318.

BI060359L