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Characterization of the Peptide Binding Requirements for the Cloned Human Pancreatic Polypeptide-Preferring Receptor

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SUMMARY

Traditionally, neuropeptide Y (NPY) receptors have been divided into Y1 and Y2 subtypes based on peptide pharmacology and synaptic localization. Other receptor subtypes have been proposed based on preferences for NPY, peptide YY (PYY), or pancreatic polypeptide (PP). Recently, we discovered a novel human member of this receptor family exhibiting high affinity for PP and PYY. In the current study, we expressed a DNA clone encoding this human PP-preferring receptor [hPP1 (or Y4)] in Chinese hamster ovary cells and performed a peptide structure-activity study. [^{125}I]pPYY bound to homogenates of hPP1-Chinese hamster ovary cells with a K_d of 0.064 ± 0.006 nM and a B_{max} of 244 ± 12 fmol/mg protein. Human PP inhibited binding with a K_i of 0.023 nM, whereas human PYY (K_i = 0.31 nM) and human NPY (K_i = 12 nM) were significantly less potent. Rat, porcine, and bovine PP inhibited binding with similar affinities to human PP, whereas avian PP was substantially less potent (K_i = 1 nM). Deletion of the first four amino acids reduced the affinity of bovine PP to 1 nM. Carboxyl-terminal fragments of NPY and PYY also had reduced potency compared with the native peptides. In addition, deletion of Tyr36-amide produced a substantial reduction in affinity. Pro34-substituted NPY and PYY had modestly increased affinity compared with the native peptides, although Gln34-bPP had similar affinity compared with bovine PP. The carboxyl-terminally derived Y1 antagonist 1229U91 was a very potent (K_i = 0.042 nM) inhibitor of binding to hPP1. Thus, the carboxyl-terminal region of PP seems to be the most important part of the peptide for high affinity binding to hPP1. A few key residues (amino acids 2 and 3) in the amino-terminal region of PP contribute to the high affinity of the native peptide. Thus, features required for peptide recognition by the hPP1 receptor seem to be distinct from the Y1 and Y2 receptor.

The endocrine peptide PP was first discovered in extracts of bovine (1, 2) and chicken (3) insulin. Interestingly, these two forms of the peptide have <50% sequence homology. Subsequently, the ovine, porcine, canine, and human peptides were isolated and found to differ from the bovine sequence by only a few amino acids (2). Two related peptides were discovered in the 1980s, NPY (4, 5) and PYY (6), and they have been sequenced in several species (7). These peptides share a common structural motif consisting of two antiparallel helices, an amino-terminal polyproline helix (residues 2–8), and a long amphipathic α helix (residues 14–32) connected by a β turn (8, 9). The characteristic helical regions of this peptide family have led to the designation of this structure as the "PP-fold" (10). All members of this peptide family are carboxyl-terminally amidated, which is critical to their biological activity (11, 12). Although NPY is primarily found in neurons, PYY and PP are primarily endocrine peptides that exert their actions on target tissues via the circulation. A number of receptors for this peptide family have been proposed, including a postsynaptic Y1 receptor, a presynaptic Y2 receptor, an NPY-preferring Y3 receptor, and a PP receptor (for reviews, see Refs. 13 and 14). The Y1 and Y2 receptors seem to have similar high affinities for NPY and PYY, whereas the Y3 receptor has higher affinity for NPY. In several tissues, a distinct PP receptor with high affinity only for PP has been described. In addition to these receptors, several other receptors have been proposed, including a receptor that mediates the hypothalamic feeding response to NPY and PYY and a PYY-preferring receptor.

The cloning of these receptors began several years ago when it was recognized that the distribution of mRNA coding for the orphan clone FC5 (15) was similar to that described for the Y1 receptor (16). Subsequently, human clones encoding for the Y1 receptor were isolated that had high sequence identity to FC5 (17, 18). A number of cell lines express the Y2 receptor (19), and several groups have reported successful

ABBREVIATIONS: PP, pancreatic polypeptide; NPY, neuropeptide Y; PYY, peptide YY; hPP1, human PP-preferring receptor; CHO, Chinese hamster ovary; rPP, rat pancreatic polypeptide; bPP, bovine pancreatic polypeptide; aPP, avian pancreatic polypeptide; hPP, human pancreatic polypeptide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

expression cloning of this receptor subtype (20–22). A proposed bovine Y3 receptor clone was described in 1991 (23), although subsequent efforts to repeat this work have not been able to identify any specific [^{125}I]NPY binding to cell lines transfected with this gene (24, 25). Recently, we identified a human clone coding for a receptor that we named hPP1 (26); another group has cloned this receptor and named it Y4 (27). This clone encodes a receptor of the heptahelix family with 43% sequence identity with the Y1 receptor. When expressed transiently in COS7 cells, hPP1 had very high affinity for PP, somewhat lower affinity for PYY, and much lower affinity for NPY. As such, this receptor is a novel member of the PP-fold peptide receptor family.

Peptide structure-activity relationships have made important contributions to the understanding of how the PP-fold peptides interact with their receptors. Extensive studies have been conducted to define the interaction of NPY with the Y1 and Y2 receptor (for a review, see Ref. 28). In general, the Y1 receptor seems to require amino acid residues found in positions 31–36 as well as the first few amino acids of the amino terminus. Substitution of a Pro34 into NPY or PYY results in an analogue with high affinity for Y1 and little affinity for the Y2 receptor. On the other hand, the Y2 receptor does not seem to require amino acids in the amino terminus, with fragments such as NPY3–36 and NPY13–36 having affinity that is similar to that of the intact peptide. PYY has affinity similar to that of NPY for the Y1 and Y2 receptors and fragments, and analogues of PYY show similar selectivity. In contrast to the Y1 and Y2 receptor, less is known about how the PP-fold peptides interact with the PP receptor. To further define the peptide binding domain of the novel hPP1, we expressed this receptor in a stable CHO cell line and evaluated the affinities of a variety of PP-fold peptide fragments and analogues.

Materials and Methods

Stable transfection of CHO cells with hPP1. The vector pTEJ-8 containing the hPP1 insert (26) was linearized with the use of *Pvu*I and transfected into CHO cells with the use of Lipofectin (GIBCO-BRL, Gaithersburg, MD). The cells were maintained under 5% CO_2/95% O_2 in Dulbecco's modified Eagle's medium/Ham's F-12 medium (3:1) containing 10% fetal bovine serum, 2 mM glutamine, 100 IU of penicillin, and 100 μg/ml streptomycin. Stably transfected cells were selected with 500 μg/ml G-418 and tested for their ability to bind [^{125}I]pPYY (see below). Several cell lines were selected that exhibited high levels of binding.

[^{125}I]pPYY binding. Cells were scraped from the culture plates into phosphate-buffered saline and pelleted in tubes. The homogenate binding studies were conducted as described previously (16). The cell pellets were resuspended with the use of a polytron in 25 mM HEPES, pH 7.4, buffer containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 g/liter bacitracin. Incubations were performed in a final volume of 200 μl that contained various concentrations of [^{125}I]pPYY (specific activity, 2200 Ci/mmol; DuPont-NEN, Boston, MA) and 0.2–0.4 mg of protein for 2 hr at room temperature. Nonspecific binding was defined as the amount of radioactivity remaining bound to the tissue after incubation in the presence of 1 μM hPP. In pharmacological studies, various concentrations of peptides were included in the incubation mixture. Incubations were terminated by rapid filtration through GF/C filters (Wallac, Gaithersburg, MD) that had been presoaked in 0.3% polyethyleneimine (Sigma Chemical Co., St. Louis, MO) with the use of a TOMTEC (Orange, CT) cell harvester. The filters were washed with 5 ml of 50 mM Tris, pH 7.4, at 4° and rapidly dried at 60°. The dried filters were treated with MeltiLex A melt-on scintillator sheets (Wallac), and the radioactivity retained on the filters was counted with a Wallac 1205 Betaplate counter. The results were analyzed using the Prism software package (GraphPAD, San Diego, CA). Protein concentrations were measured with Commassie protein assay reagent (Pierce, Rockford, IL) using bovine serum albumin as standards.

Drugs. NPY, PYY, rPP, hPP, aPP, and various analogues were purchased from Bachem California (Torrance, CA), Bachem Biosciences (King of Prussia, PA) or Peninsula (Belmont, CA). pPP, bPP, bPP fragments, [Pro14]rPP, BIBP3226, and 1229U91 were prepared at Eli Lilly and Co., Indianapolis, IN.

Results

Stable expression of hPP1 in CHO cells. To characterize the binding domain of hPP1, we examined the ability of a variety of peptides to inhibit the binding of 100 pM [^{125}I]pPYY to hPP1. After transfection, several cell lines were selected that contained substantial [^{125}I]pPYY binding. One of these cell lines (designated CHO-PP1) was used for complete characterization. Initial saturation experiments were performed to determine the affinity and density of hPP1 binding sites in this cell line. [^{125}I]pPYY bound to a single site with a K_d of 0.064 ± 0.006 nM and a B_{max} of 244 ± 12 fmol/mg protein (Fig. 1). On the concentration-dependent portion of the curve, specific binding exceeded 90% of the total binding. Nontransfected CHO cells exhibited no specific binding of [^{125}I]pPYY (data not shown). Kinetic experiments indicated that specific [^{125}I]pPYY binding approached equilibrium within 2 hr and had very slow dissociation (data not shown). The cells also exhibited functional coupling. In cells in which forskolin was used to stimulate adenylate cyclase, hPP and hPYY produced a dose-dependent inhibition of adenylate cyclase activity (26).

Analysis of PP and PP fragments. Because hPP is the most potent inhibitor of [^{125}I]pPYY (K_i = 0.023 nM), we evaluated the affinity of PP from various species (Fig. 2). Compared with hPP, bPP exhibits similar affinity for hPP1 (K_i = 0.035 nM). bPP differs from the human sequence by two amino acid: a substitution of Glu6 and a conservative substitution of Glu23. Both of these substitutions are in the loop region of the peptide. pPP contains two conservative amino acid differences in the loop region and has similar affinity (K_i = 0.034 nM). The rPP sequence contains eight amino acid differences, principally in the loop region, and also has similar affinity for hPP1 (K_i = 0.052 nM). On the other hand, the aPP or chicken PP sequence contains 21 differences from hPP, and these differences are found in both the loop region and the carboxyl- and amino termini. Particularly notable are the substitutions of His34 and Ser3, whereas most of the other substitutions proximal to these are conservative. In the loop region, a number of conservative and nonconservative substitutions can be found. As might be expected, aPP has substantially lower affinity for hPP1 (K_i = 0.96 nM).

Because bPP retained a similar affinity for hPP1 as that seen with hPP, fragments of this peptide were used to define the binding requirements of PP (Table 1). Deletion of Ala1 did not seem to affect the affinity of bPP for hPP1, whereas sequential deletion of the next two amino acids produced about an additional 6-fold reduction in affinity. bPP 4–36, bPP 5–36, and bPP 13–36 had similar affinities for hPP1. To explore the role of the carboxyl terminus in the binding to hPP1, a series of carboxyl-terminal bPP fragments were eval-

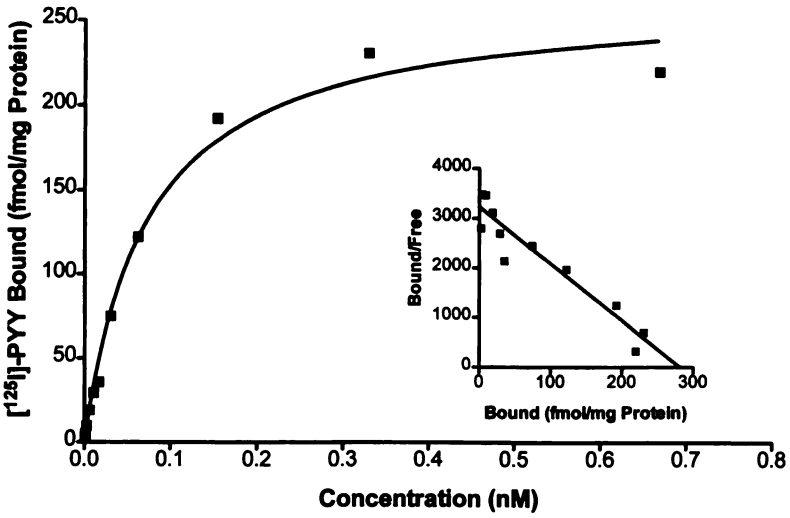


Fig. 1. Saturation studies of [^{125}I]pPYY binding to hPP1. Membranes obtained from CHO cells transfected with hPP1 were incubated with various concentrations of [^{125}I]pPYY. Nonspecific binding was determined by the addition of 1 μM hPP. Data are from a representative experiment. Mean K_d = 0.064 ± 0.006 nM and B_{max} = 244 ± 12 fmol/mg protein from four experiments performed in duplicate.

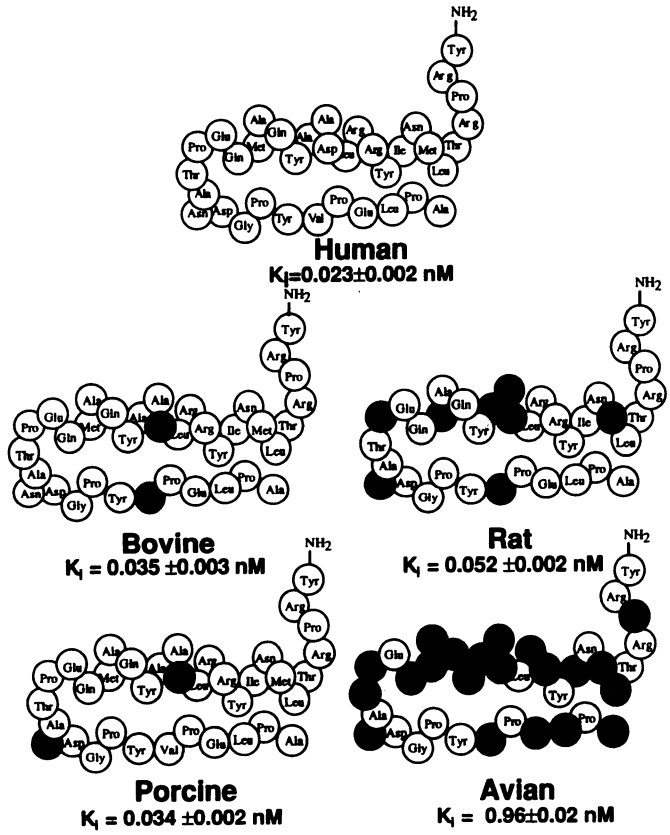
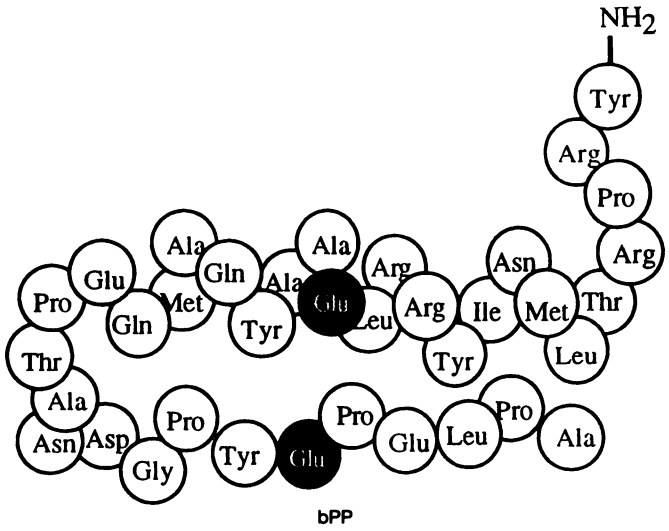


Fig. 2. Affinity of PP from various species for the hPP1 expressed in CHO cells. The schematic peptide structures are modeled after those in Ref. 40. *Black*, differences in the amino acid sequence compared with hPP; *gray*, conservative substitutions. Results are mean ± standard error of four experiments performed in duplicate.

TABLE 1

Affinity of bPP and carboxyl-terminal fragments for the hPP1 receptor expressed in CHO cells

Results are the mean (± standard error) of four experiments performed in duplicate.



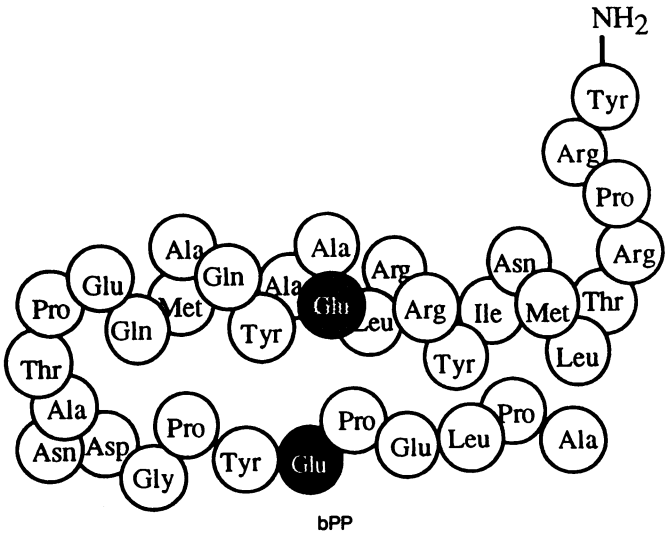
Peptide	K_i
	nM ± standard error
bPP	0.035 ± 0.003
bPP 2-36	0.035 ± 0.003
bPP 3-36	0.11 ± 0.01
bPP 4-36	0.31 ± 0.06
bPP 5-36	1.8 ± 0.7
bPP 13-36	0.46 ± 0.04
bPP 24-36	22 ± 0.1
bPP 31-36	71 ± 7

uated (Table 2). Deletion of the Tyr36-amide produced a substantial reduction in the potency of bPP (K_i = 2.8 nM). bPP 27–35 had very low affinity (K_i = >1000 nM) compared with the native peptide or carboxyl-terminal fragments containing the Tyr36-amide such as bPP 24–36 (K_i = 22 nM) or bPP 31–36 (K_i = 71 nM). Removal of only the carboxyl-terminal amide reduced the affinity of the peptide for hPP1 (K_i = 0.89 nM). Replacement of the carboxyl-terminal amide with a methyl group restored the affinity of the peptide but not completely (K_i = 0.071 nM). Fragments missing this carboxyl-terminal region, such as bPP 1–26, bPP 1–15, and bPP 1–17, were inactive at concentrations of ≤1 μM. Fragments containing only portions of the loop region such as bPP 16–23 or bPP 18–30 also had no appreciable affinity for the receptor. Finally, several analogues of bPP were tested to evaluate the role of specific amino acids. Oxidation of Met30 did not affect the affinity of bPP for hPP1. Substitution of the Gln34 found in NPY and PYY into bPP produced a slight reduction

TABLE 2

Affinity of bPP and various fragments and analogues for the hPP1 receptor expressed in CHO cells

Results are the mean (± standard error) of four experiments performed in duplicate.



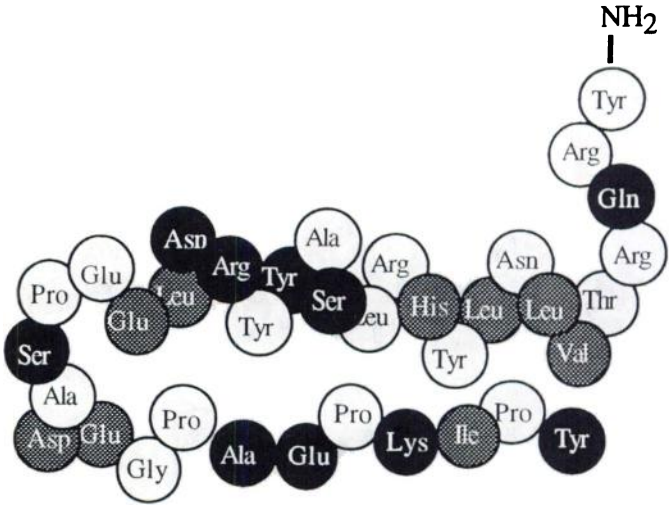
bPP

Peptide	K_i
	nM ± standard error
bPP	0.035 ± 0.003
bPP 1-35	2.8 ± 0.1
bPP (Tyr36-OH)	0.89 ± 0.04
bPP (Tyr36-OCH_3)	0.071 ± 0.006
bPP 1-26	>1000
bPP 1-15	>1000
bPP 1-17	>1000
bPP 16-23	>1000
bPP 27-35	>1000
bPP 18-30	>1000
bPP [Met(O)30]	0.035 ± 0.002
Gln34-bPP	0.042 ± 0.002
Pro14-rPP	0.071 ± 0.002

TABLE 3

Affinity of hPYY and various fragments and analogues for the hPP1 receptor expressed in CHO cells

Results are the mean (± standard error) of four experiments performed in duplicate.



hPYY

Peptide	K_i
	nM ± standard error
hPP	0.023 ± 0.002
hPYY	0.31 ± 0.03
pPYY	0.12 ± 0.01
hPYY3-36	9.9 ± 0.2
hPYY 13-36	8.5 ± 0.2
h[Pro34]PYY	0.077 ± 0.002
h[Leu31,Pro34]PYY	0.14 ± 0.01

in potency, whereas exchanging the Pro14 found in hPP, pPP, and bPP into rPP slightly reduced the affinity.

Analysis of PYY and PYY analogues. To characterize the structural requirements of PYY binding to hPP1, a variety of PYY analogues were tested for their ability to inhibit [^{125}I]PYY binding. The data from these studies are summarized in Table 3. hPYY was >10-fold less active than hPP (K_i = 0.31 nM), whereas pPYY was a little more potent than hPYY (K_i = 0.12 nM). hPYY contains a number of amino acid differences compared with hPP, including Gln34 instead of Pro34. Deletion of the first two amino acids of PYY resulted in a ~30-fold reduction in the affinity compared with the intact peptide. pPYY13–36 had a similar affinity as hPYY3–36. Substitution of the Pro34 found in PP into PYY produced an improvement in the affinity to 0.077 nM. Introducing the Leu31 and Pro34 of PP into PYY also improved the potency but not to the same extent as seen with the Pro34 substitution alone.

Analysis of NPY and NPY analogues. Although hPP1 exhibits highest affinity for hPP and hPYY, hNPY exhibits a lower affinity (K_i = 12 nM) (Table 4). Because fewer analogues of hNPY are available commercially, pNPY analogues were evaluated. pNPY differs from hNPY only by substitution of leucine for Met17 and exhibits similar inhibition of [^{125}I]PYY binding. Similar to what was observed with PYY fragments, deletion of the Tyr1 residue and the Tyr1/Pro2 fragment resulted in peptides with lower affinity for hPP1. Deletion of the first 12 amino acids did not produce any further reduction in affinity. Substitution of both Leu31 and Pro34 produced a ~3-fold increase in the potency of NPY. Finally, we evaluated putative NPY receptor antagonists. The first contains a substitution of dTrp into position 32, which reduced the affinity of NPY for hPP1 by almost 20-fold. The modified carboxyl-terminal fragments of NPY that are putative NPY receptor antagonists, PYX-1 and PYX-2, did not affect binding in the concentration range tested. The nonpeptide Y1 antagonist BIBP3226 also did not inhibit binding at concentrations of ≤10 μM. Interestingly, the peptide Y1 antagonist 1229U91 was a very potent inhibitor of binding (K_i = 0.042 nM).

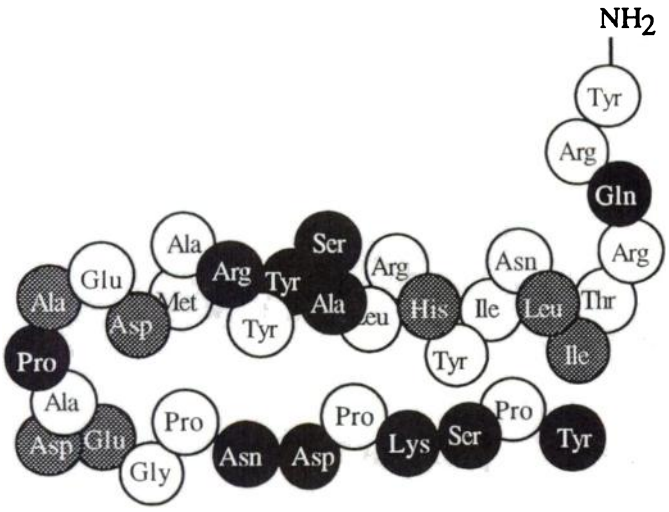
Discussion

hPP1 is the second receptor to be cloned from the family of receptors for the PP-fold peptides. The first member to be cloned was the Y1 receptor, and it has high affinity for both NPY and PYY and lower affinity for PP (17, 18). In contrast, hPP1 has high affinity for PP and PYY and lower affinity for NPY (26). In the current study, we stably expressed hPP1 in a CHO cell line and characterized its binding properties. Much like what occurred in transient expression (26), hPP1 binds hPP with very high affinity, PYY with somewhat lower affinity, and NPY with relatively low affinity. Similar to the Y1 (29) and Y2 (30, 31) receptors, hPP1 expressed in CHO cells can couple to the inhibition of adenylate cyclase (26).

TABLE 4

Affinity of hNPY and various fragments and analogues for the hPP1 receptor expressed in CHO cells

Results are the mean (± standard error) of four experiments performed in duplicate.



hNPY

Peptide	K_i
	nM ± standard error
hPP	0.023 ± 0.002
hNPY	12 ± 1
fNPY	12 ± 1
pNPY 2-36	34 ± 1
pNPY 3-36	146 ± 5
pNPY 13-36	110 ± 10
p[Leu31,Pro34]NPY	3.8 ± 0.7
p[dTrp32]NPY	200 ± 10
PYX-1	>1000
PYX-2	>1000
BIBP3226	>10,000
1229U91	0.042 ± 0.001

The most distinguishing feature of hPP1 is its extremely high affinity for PP. In the current study, we identified key amino acid residues in the PP sequence that are involved in the binding of PP to hPP1 (Fig. 3).

To characterize the structure-activity binding to hPP1, we evaluated fragments and analogues of PP, PYY, and NPY. From these studies, several amino acids were identified that contribute to the binding of these peptides to the receptor. In the amino terminus of bPP, Ala1 can be deleted with little effect on the affinity for the receptor. However, deletion of each of the next two amino acids produces a reduction, whereas deletions to bPP 13–36 produce no further reduction in affinity. Binding is reduced an additional ~10-fold in the 24–36 fragment of bPP compared with bPP 5–36. Therefore, the prolyl-prolyl helical region of the peptide does not directly participate in the binding of bPP to the receptor but rather may serve to properly position amino acids 2 and 3 for optimal binding. In NPY, deletion of Tyr1 produced a small reduction in affinity, whereas NPY3–36 had 10-fold lower affinity compared with the intact peptide. Interestingly, NPY13–36 exhibited no further reduction in affinity. Similarly, PYY3–36 and PYY13–36 had ~30-fold lower affinity compared with PYY. Thus, it is likely that several residues near the amino terminus, excluding Ala1, participate in the binding. Particularly, the Pro2 seems to participate in the binding of all three peptides to the receptor. The prolyl-prolyl helical region between 3 and 13 does not seem to play an important role in the binding of PP, PYY, and NPY. Similar binding affinity is retained in the bPP 31–36 fragment compared with bPP 24–36, indicating that residues in the α helical region also do not directly participate in binding. To further substantiate this finding, bPP 16–23, bPP 27–35, and bPP 18–30 were inactive. bPP, pPP, and rPP all have similar affinity to hPP and contain amino acid substitutions in the loop regions of the peptide. Replacement of the His14 found in rPP with Gln14 found in the other mammalian PP sequences produced a peptide with similar affinity to rPP, indicating that this amino acid does not play an important role in binding. Collectively, these data suggest that like the binding of NPY Y1 and Y2, the loop region of the peptide does not play a strong role in the binding of PP to the PP-preferring receptor. On the other hand, aPP contains both differences in the loop region and, importantly, differences in the amino-terminal (Ser3) and carboxyl-terminal (His34) regions. This peptide has >40-fold lower affinity for the receptor, which, again, is consistent with the hypothesis that residues in the amino- and carboxyl-terminal regions participate in binding.

To explore the participation of the carboxyl-terminal region, we studied a number of bPP fragments. Deletion of Tyr36-amide or just the carboxyl-terminal amide substantially reduced binding. Replacing the amide with a methyl group restored binding to a similar affinity as the native peptide. Peptide fragments such as bPP 1–26 that were missing the carboxyl-terminal region were inactive, further establishing the importance of this region. Although the Tyr36-amide residue seems to be very important for peptide binding, the Pro34 residue seems to contribute to a small increase in affinity. Replacing the Gln34 found in NPY and PYY with a Pro34, as found in PP, modestly increases the affinity of both of these peptides for hPP1. However, Gln34-bPP has a similar affinity to bPP, suggesting that this residue is of only minimal importance in obtaining high affinity in the PP molecule. Thus, the Pro34 substitution of NPY and PYY may serve to improve the positioning of Arg33 and Arg35 to improve binding of these peptides. Also, it is likely that other amino acids in this region of the peptide participate in the binding in that [dTrp32]NPY has a ~8-fold lower affinity than NPY. To further define both the amino- and carboxyl-terminal regions, a systematic substitution of the amino acids with either alanine or d-amino acids is necessary. In addition, deletion of the loop region with bridged analogues will allow further analysis of the importance of this region in binding to the receptor.

Although the carboxyl-terminal region of bPP seems to be very important for binding, interesting results were obtained with NPY fragments and analogues. Like PYY and PP, deletion of the first two amino acids produced a substantial reduction in affinity, whereas additional deletion from NPY3–36 to NPY13–36 did not affect binding. [dTrp32]NPY also exhibited a substantial reduction in affinity, whereas the [dTrp32]-containing, chemically modified fragments PYX-1 and PYX-2 (32, 33) were inactive in the concentration ranges tested. Interestingly, the peptide Y1 antagonist 1229U91 (34) was extremely potent, with similar affinity to PP for hPP1. This high affinity is similar to the affinity of this peptide for the hY1 receptor and for [^{3}H]NPY binding to rat brain. This

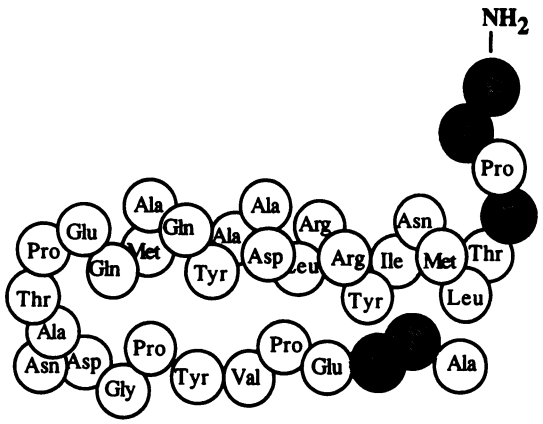


Fig. 3. Important amino acids involved in the binding of PP to the hPP1. *Shading and bold lines*, key amino acids.

peptide analogue is also derived from the carboxyl-terminal region of NPY (residues 28–36) but is an antiparallel dimer, with two Dpr31/Glu29 links. The peptide contains several conserved amino acids from NPY, PYY, and PP, including Ile28, Arg33, Arg35, and Tyr36-amide. Interestingly, the nonpeptide Y1 antagonist BIBP3226 (35, 36) was designed to incorporate the Arg33, Arg35, and Tyr36-amide motif of the carboxyl-terminal region of NPY, and this compound was inactive. This suggests key differences in how this region of the peptide is recognized by hPP1 compared with Y1. In addition, the unexpected high affinity of 1229U91 suggests that the amino-terminal regions of PP, PYY, and NPY are necessary for high affinity binding of the native peptides to hPP1 but not for the design of high affinity peptide and nonpeptide analogues. Also, caution must be exercised when using 1229U91 as a Y1 antagonist because this analogue has very high affinity for hPP1.

In general, very little information is available in the literature on the structure-activity relationship of PP at the PP receptor. In addition, a PP receptor with the unique pharmacology of hPP1 has not been described in the literature. Nevertheless, a comparison of our results with several other studies points out the importance of key amino acid residues in PP. Perhaps the most complete study involved [^{125}I]bPP binding to the basolateral membranes of the canine intestinal mucosa (37). In this study, bPP, pPP, chicken PP, hPP, and ovine PP inhibited binding with similar affinities (ED_{50} = ~1 nM). rPP was significantly less potent, with ~30-fold lower affinity. All fragments of bPP had affinities of >500 nM, including bPP 1–35 and bPP 31–36. From this limited study, it seems that the canine intestinal PP receptor also requires intact amino and carboxyl termini for bPP to bind. Rat-derived PC-12 cells also express a PP receptor that is capable of binding [^{125}I]bPP (10). Interestingly, this receptor binds bPP with 10-fold higher affinity than rPP, and pNPY displayed little inhibition at concentrations of ≤1 μM. Similar to the present study, bPP 24–36 was ~1000-fold less active compared with the native peptide. Surprisingly, [Ile31/Gln34]PP exhibits a >100-fold lower affinity for the PP receptor expressed in PC-12 cells (38). In the current study, we observed little change in the affinity of Gln34-bPP compared with bPP. This may indicate some heterogeneity in the PP receptor family or that there are species differences in how the peptide is recognized. Nevertheless, the PP receptor expressed in PC-12 cells displays some of the structural requirements we observed with hPP1. Finally, a receptor has been identified in chicken cerebellar membranes that binds both [^{125}I]pPYY and [^{125}I]aPP (39). Ligand binding to this receptor exhibits a potency rank order of aPP > pPYY > pPP > pNPY. Carboxyl-terminal fragments of PYY (17–36 and 24–36) had much lower affinity for the binding to this tissue. Again, these data suggest that two amino acids in the amino-terminal region contribute to PP-fold peptide recognition at this receptor.

Our results suggesting that the amino- and carboxyl-terminal regions participate in binding of the native peptides to hPP1 are similar with the known peptide structure-activity relationship for the Y1 receptor but differ significantly from that seen for the Y2 (for a review, see Ref. 28). In general, NPY3–36 and PYY3–36 show substantial reduction in affinity for the Y1 receptor while retaining most of their affinity for the Y2 receptor. NPY13–36 and PYY13–36 have similar affinities at both the Y1 and Y2 receptors compared with the 3–36 fragments. This suggests that amino acids 3–12 in these peptides do not participate significantly in the binding to either receptor. However, Tyr1 is critical to the binding of NPY and PYY to the Y1 receptor. With the hPP1, we observed that bPP requires amino acids in the 3–24 region of the peptide to optimize binding. On the other hand, NPY13–36 seems to retain much of the affinity of the 3–36 fragment at hPP1. Thus, NPY may recognize hPP1 a bit differently from bPP. In the carboxyl-terminal region, [Pro34]NPY and [Pro34]PYY retain most of the affinity of the native peptides at the Y1 receptor while almost completely losing affinity at Y2. At hPP1, [Pro34]NPY and [Pro34]PYY actually have increased affinity over the native peptides, suggesting that this substitution is not only tolerated but also improves receptor affinity. This is in agreement with the presence of proline in the 34 position of the preferred endogenous ligand, PP. The Tyr36-amide is critical for the recognition of both NPY and PYY by the Y1 and Y2 receptors. Substitution of Tyr36 of NPY with alanine results in a >1000-fold loss of affinity at the Y1 and Y2 receptors (28). In the hPP1, deletion of the Tyr36-amide reduces binding by almost 100-fold. Desamido (Tyr36) bPP has a similar affinity, most likely because of the free carboxyl-terminal carboxylate. Interestingly, block of this functional group with a methyl ester peptide restored affinity to almost that of the native carboxyl-terminally amidated bPP.

In conclusion, we achieved functional expression of hPP1 in a stable CHO cell line. This receptor seems to recognize the PP-fold family of peptides in a manner similar to the Y1 receptor in that it recognizes both the amino- and carboxyl-terminal regions of PP, PYY, and NPY. The varying affinities of carboxyl-terminally derived Y1 antagonists for hPP1 suggests there are key differences in how this region of the peptide is recognized by the receptor. The carboxyl-terminal region of PP will undoubtedly be an important area for the design of PP mimetics and antagonists.

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