The cloned rat pancreatic polypeptide receptor exhibits profound differences to the orthologous human receptor

(peptide YY/heptahelix/7TM/G-protein-coupled)

INGRID LUNDELL*, MICHAEL A. STATNICK†, DWAYNE JOHNSON†, DOUGLAS A. SCHOBER†, PAULA STARBÄCK*, DONALD R. GEHLERT†, AND DAN LARHAMMAR*‡

*Department of Medical Pharmacology, Uppsala University, Box 593, S-751 24 Uppsala, Sweden; and †Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46204

ABSTRACT   Pancreatic polypeptide (PP) is produced in the islets of Langerhans and released in response to meals. It belongs to a family of peptides that also includes neuropeptide Y and peptide YY. In the present communication, we describe a rat receptor with high affinity for PP, therefore named PP1. Clones for the PP1 receptor were obtained by PCR using sequence information for the neuropeptide Y receptor Y1 from several species. The PP1 receptor has 46% overall amino acid sequence identity to the rat Y1 receptor and 56% identity in the transmembrane regions. The PP1 receptor displays a pharmacological profile that is distinct from previously described neuropeptide Y-family receptors. In competition with iodinated bovine PP, it binds rat PP with an affinity (K_i) of 0.017 nM, while the affinities for peptide YY and neuropeptide Y are substantially lower with K_i values of 162 and 192 nM, respectively. In stably transfected CHO cells, the PP1 receptor inhibits forskolin-stimulated cAMP synthesis. Northern blot hybridizations to a panel of mRNAs detected transcripts in testis and lung. A faint band was seen in colon and total brain. In contrast, the human receptor is expressed primarily in colon and small intestine. Whereas rat and human PP1 bind PP with the same affinity, the rat receptor has much lower affinity than its human ortholog for peptide YY and neuropeptide Y. Interestingly, the amino acid sequence identity between rat and human PP1 is only 75%. Thus, the sequence, the tissue distribution, and the binding profile of the PP1 receptor differ considerably between rat and human.

The pancreatic polypeptide (PP)-fold family of neuroendocrine peptides consists of PP, neuropeptide Y (NPY), and peptide YY (PYY). All three peptides have 36 aa and share prominent sequence and three-dimensional structure similarity (1). PP is secreted from pancreatic islets in response to meals, and it inhibits gall bladder contraction, gut motility, and pancreatic secretion (for review, see ref. 2). PP has also been isolated from porcine intestine (3). PYY is found in endocrine cells of the lower intestine (4) as well as in pancreatic islets (5) and a few neuronal populations (6, 7). Its release and actions on the gastrointestinal tract are similar to those of PP (see ref. 2). NPY, in contrast, is found in the central and peripheral nervous systems.

All three peptides have been found in animal experiments to increase food intake. NPY is considered to be one of the most potent orexigenic substances known (8), and intracerebroventricular injection of PP stimulates feeding in rats, mice, and dogs (9–12). Anorectic patients have high levels of circulating PP (13, 14), whereas obese persons have decreased PP concentrations (15) and have lower PP release in response to meals than lean persons (16). Taken together, these studies provide strong support for prominent roles of NPY-family peptides in the regulation of feeding and suggest that PP is involved both in intestinal function and appetite regulation.

Several receptor subtypes have been defined by their ability to bind NPY, PYY, and PP and various peptide fragments and analogs. Both NPY and PYY bind to the Y1 and Y2 receptors, whereas the Y3 receptor binds only NPY. PP does not bind to either of these subtypes. The hypothalamic feeding receptor also appears to be distinct from all of these (for review, see ref. 17). Seemingly distinct PP-preferring receptors have been described based upon binding studies of isolated tissue preparations, namely dog intestinal mucosa (18, 19), rat phaeochromocytoma PC12 cells (20), rat brain area postrema (21), rat adrenal cortex and medulla (22), and rat liver (23). A PP receptor was observed in rat vas deferens in a functional assay (24). Finally, there is a "PP-fold-recognizing" receptor located in the distal colon in rabbit that binds all three peptides (25).

The Y1 receptor has been cloned in rat, mouse, human, and *Xenopus laevis* (26–30). It belongs to the heptahelix [seven transmembrane (7TM) region] family of receptors that couple to G proteins. The cloning of additional receptor subtypes would help determine their binding preferences and physiological roles and would also facilitate development of antagonists. We describe here a rat clone encoding a receptor that binds PP with high affinity, which is hence named PP1. Interestingly, this receptor binds PYY and NPY with substantially lower affinity than its human ortholog (31). Furthermore, the PP1 amino acid sequence differs extensively between the two species.

MATERIALS AND METHODS

Generation of a Rat PP1 (rPP1) Clone by PCR. Degenerate primers based on Y1 sequences were used in different pairwise combinations for PCR on rat genomic DNA using the following conditions: 5 min at 99°C for one cycle, then 1 min at 94°C, 2 min at 42°C, and 3 min at 72°C for 25 cycles with *Taq* polymerase. The product of one primer combination was subcloned. The 5' primer was a 29-mer with the sequence CGG GAT CCT AC(T)A CIC(T) TG(ATC)A TGG AC(T)C AC(T)T GG corresponding to a *Bam*HI cloning site and positions 362–382 (TM2) in the rat Y1 sequence (GenBank accession no. Z11504). The 3' primer had the sequence CGG GAT CCC CA(G)T AA(G)A AG(AT)A TIG GG(A)T TG(ATC)A CA(G)C A corresponding to a *Bam*HI site and positions 1004–1026 (TM7). The PCR product of 670 bp was isolated, reamplified, and cloned into pT7Blue (Novagen).

One clone called R4–7 containing an insert of the expected size was obtained and sequenced.

Screening of Rat Genomic Library. Approximately 600,000 clones from a library in the vector EMBL3 SP6/T7 (Clontech) were screened with a PCR-generated rPP1 fragment. Hybridization was done at 65°C in 25% formamide/6× standard saline citrate (SSC)/10% dextran sulfate/5× Denhardt's solution/0.1% SDS. Filters were washed twice at room temperature in 2X SSC/0.1% SDS and twice for 30 min at 65°C in 0.2× SSC/0.1% SDS. Five individual clones were selected and were found to be nonidentical, but they contained the same hybridizing region. Hybridizing restriction fragments were subcloned in plasmids and sequenced. The clone gR4–24 with a 2.7-kb *Bam*HI fragment contained the entire coding region.

DNA Sequencing. Sequence determinations were performed with dideoxy-nucleotide chain termination in an automated fluorescent dye DNA sequencer (Applied Biosystems) or manually using $dATP[\alpha\text{-}^{35}S]$ followed by autoradiography.

Southern Blot Hybridization. Genomic DNA was purified from rat liver or human leukocytes and digested with restriction enzymes. The probes were PCR-generated fragments corresponding to the entire coding region of the clone gR4–24 or the human PP1 (hPP1) receptor. Hybridization and washes were done at high stringency.

Northern Blot Hybridization. A Northern blot membrane containing 2 μg of poly(A)$^+$ RNA from each of several different rat organs was purchased from Clontech. In addition, a Northern blot membrane was prepared with 2 μg of poly(A)$^+$ RNA from stomach, small intestine, and colon. A single-stranded ^{32}P-labeled probe corresponding to nucleotides 1087–1307 was used. Hybridization was done at 68°C in ExpressHyb (Clontech) with final washes in 0.1× SSC/0.1% SDS at 50°C. The blots were visualized using a Phosphor-Imager (Molecular Dynamics). Blots were reprobed with a human β-actin probe.

Cloning into Expression Vector. A fragment containing the entire coding region of gR4–24 was generated with PCR using Vent DNA polymerase (Biolabs, Northbrook, IL). The 5' primer contained a *Hind*III cloning site and had the sequence CCG GGA AGC TTC CCT TTA GTC TTG AAG TTC CTG GTC T, and the 3' primer had an *Eco*RI cloning site and the sequence CGG AAT TCC TGA AAG GGT GTG TCG AAA GAA AA. The cycle was as follows: 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, for 25 cycles. The reaction mixture was phenol extracted and cut with *Hind*III–*Eco*RI, and the 1.25-kb fragment was purified on an agarose gel using the QIAquick gel extraction kit (Qiagen, Chatsworth, CA); the fragment was then ligated into the expression vector pTEJ-8 (32) to give the clone Ruben-pTEJ. Because this clone was generated with PCR, its insert was completely sequenced and found to be identical to the genomic sequence.

Transient Transfection Protocol. COS1 African green monkey kidney cells were seeded at a density of 1×10^6 cells per 150-mm dish and incubated for 48 h at 37°C. Each dish was transfected with 25 μg of Ruben-pTEJ in 10 ml of OptiMEM containing 300 μl of Lipofectace (GIBCO/BRL) according to kit protocol. Cells were harvested in phosphate-buffered saline (PBS) 48 h after transfection and pelleted by centrifugation.

Binding Assays. The homogenate binding studies were conducted as described (33). Cell pellets were resuspended using a glass homogenizer in 25 mM Hepes (pH 7.4) buffer containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 g of Bacitracin per liter. Saturation experiments were performed in a final volume of 200 μl containing various concentrations of [^{125}I][Tyr^{27}]bPP [SA 2200 Ci/mmol (1 Ci = 37 GBq), Eli Lilly] and 1–3 μg of protein for 2 h at room temperature. Nonspecific binding was defined as the amount of radioactivity remaining bound to the cell homogenate after incubation in the presence of 1 μM unlabeled rat PP (rPP). In competition studies, various

concentrations of the peptides {rPP, rat PYY (rPYY), rat NPY (rNPY), rat [Leu^{31}, Pro^{34}]NPY (r[Leu^{31}, Pro^{34}]NPY), pNPY2–36, pNPY13–36; Peninsula Laboratories and Bachem} were included in the incubation mixture along with 0.025 nM [^{125}I][Tyr^{27}]bPP. Incubations were terminated by rapid filtration through GF/C filters, which had been presoaked in 0.3% polyethyleneimine, using a Tomtec (Orange, CT) cell harvester. The filters were washed with 5 ml of 50 mM Tris (pH 7.4) at 4°C and rapidly dried at 60°C. The dried filters were treated with MeltiLex A (Wallac, Gaithersburg, MD) and melt-on scintillator sheets, and the radioactivity retained on the filters counted using the Wallac model 1205 Betaplate counter. The results were analyzed using the PRISM software package (Graphpad, San Diego). Protein concentrations were measured using Coomassie protein assay reagent (Pierce) with BSA for standards.

cAMP Assay. cAMP was assayed in whole cells treated for 20 min at 37°C with 100 μM isobutylmethylxanthine. Transfected CHO cells were incubated with 15 μM forskolin and various concentrations of rPP, rPYY, rNPY, or r[Leu^{31}, Pro^{34}]NPY for 15 min at 37°C. Reactions were terminated by addition of EDTA to 0.4 μM and heating in a boiling water bath for 4 min. Sample buffer containing cAMP was removed and lyophilized. cAMP was quantitated using radioimmunoassay (Amersham). Protein content of each well was measured using the Coomassie protein assay reagent (Pierce) with BSA as the standard.

RESULTS

Cloning of a Y1-Related Rat PCR Product. Sequence information for the Y1 receptor from three mammals and a frog was used to design degenerate primers. Several primers were used in different combinations for PCR on rat genomic DNA. Two of the primers corresponding to TM2 and TM7 generated a product of expected size. The fragment was cloned, and the clone R4–7 was sequenced and found to have higher sequence identity to the Y1 receptor than to all other receptor sequences.

Characterization of a Full-Length rPP1 Clone. The PCR fragment in clone R4–7 was used to screen a rat genomic library. A *Bam*HI subclone of 2.7 kb called gR4–24 was found by sequencing to contain the entire coding region of a receptor with identical sequence to R4–7 in the overlapping portion. This clone encodes a heptahelix (TM7) receptor of 375 aa (Fig. 1) that we have named PP1 because it has highest affinity to PP. The PP1 receptor has greater amino acid sequence identity to the Y1 receptors than to any other receptors with 56% identity to rat Y1 in the transmembrane regions and 46% overall identity (Fig. 1). The overall nucleotide sequence identity to the rat Y1 sequence is 56%. There are no introns in the coding region.

The deduced PP1 amino acid sequence displays many of the characteristic features of heptahelix receptors (Fig. 1). PP1 shares with Y1 three potential glycosylation sites in the amino-terminal part and a fourth in the second extracellular loop. Four extracellular cysteines, one in the amino-terminal region and one in each of the three extracellular loops, presumably form two disulfide bridges (again like the Y1 receptor). A cysteine in the cytoplasmic tail probably serves as an attachment site for palmitate inserted into the cell membrane. All of these features are present also in the hPP1 sequence (Fig. 1). Sequence similarity between rPP1 and hPP1 is also found throughout the 5'-untranslated and 3'-untranslated segments that are available for comparison—i.e., $\approx$150 nt on either side of the coding region (data not shown).

Southern Blot Hybridization. A single band corresponding to the isolated PP1 gene was observed after hybridization at high stringency (data not shown), indicating that the rat genome contains a single PP1 receptor gene. Hybridization

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                    |       |        |      |       |       |      |     TM2    |        |        |      |       • TM3      |
                                        • TM1
RAT PP1   MMTSHLMASLSPAFLQGKNGTNPLDSLVNLSDGCQDSADLLAFIITTVSVETVLGVLGHLCLIFVTTRQKEKSNVTHLLIANLAFSDFLMCLICOPLTVTYTIMDYWIFGEVLCKMLTFIOCMSVTVSIL   130
HUM PP1   .......L.L.L.KSP..E..ASK..GTP...F..EH....V.UMV..V.S..I...V.........MC..V.....A....................L.....AV.............T....SA............   130
RAT Y1    ..,-..T.FSRVENVSVHY-..VSENSPF.AFEN.D.HLPLAVIFTLALA.GAVIIL..S...A..IIILK...MR....I..V..S...L.VAVM.L.F.FV..L..H.V...TM..LMP.V..V.I....F   128

              TM4                                                            •                 TM5
RAT PP1   SLVLVALERHQLIINPTGUKPSISQAVLGIVVIWFISCFLSLPFLANSILNDLFHVMHSKUVEFLEDKVUCFUSUSSDHHALIVTTFLLLFQVCVPLAFILUCVMRIYQRLQRQRRAFHTHTCSS-RVGQ   259
HUM PP1   ...........................L..V.A.V............ENV..K,....AL...A.....TE..PLA..I.............L..G.......A...R.....G.V..KG.V.L-.A.H   259
RAT Y1    .....I.V.........R..R.NNRH...I..T...VLAVAS....VIVQ..T.EPFQ.V.LAR-.K-..V...DKFP..S...S...L..VL..FG..C..FI..FK..I..K.RNNMMDKIRD.KV.SSE   256

          TM6                              •   TM7
RAT PP1   MKRINGMLMAMVTAFAVLULPLHVFNTLEDUVQEAIPACHGNLIFLMCHLFAMASTCUNPFIVGFLNINFKKDIKALULTCRCRPPQGEPEPLPLSTUHTDLSKGSMRMGSKSNVM       375
HUM PP1   ..QU.UU.UV..V............S....HH....I....,...V..L................T...E........QQSA.LE.S.H........EV....L.LSGR..PI        375
RAT Y1    T..,.V..LSI.V....C....TI...VF..NHQI.AT.NH..L..L...T..I.......IF.....K..QR.LQFFFNF.DF.SRDDDV.TIAM..M...U..T.LKQA.PUAFKKISMHDNEKI     382
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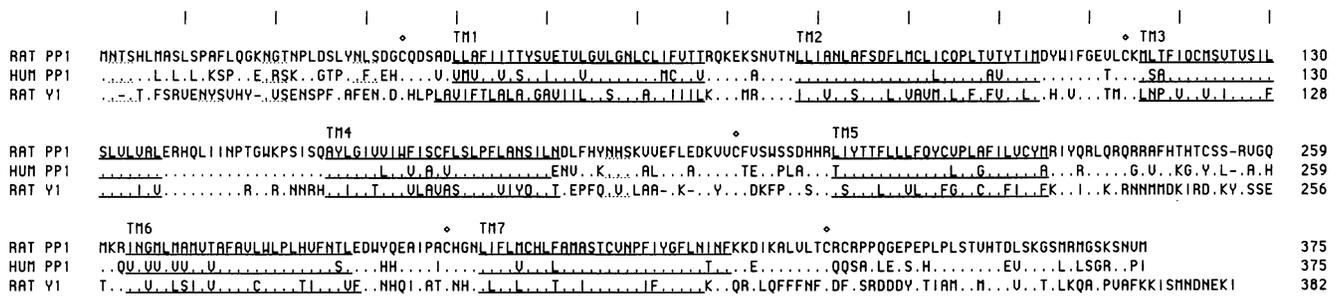
FIG. 1.   Amino acid sequence alignment. The rPP1 receptor serves as master sequence in alignment with the hPP1 receptor (31) and the rat Y1 receptor. In the two latter sequences, only positions that differ from the rPP1 sequence are shown; dots indicate identities. Dashes represent gaps introduced to optimize alignment. The hydrophobic segments assumed to be embedded in the cell membrane are underlined. Four tripeptides in extracellular parts underlined with dotted lines conform to the consensus sequence for N-linked glycosylation. Diamonds show four extracellular cysteines and one intracellular cysteine.

with a hPP1 probe detected the same band supporting an orthologous relationship between the hPP1 and rPP1 genes.

Northern Blot Hybridization. A single mRNA species of ≈ 4.2 kb was observed in testis and lung (Fig. 2). A faint band of this size could also be detected in colon (data not shown) in agreement with hPP1 (31); in addition, an equally faint band at ≈ 1.8 kb could be detected. Brain contained an extremely faint signal visible after long exposure. All other organs in Fig. 2 as well as small intestine and stomach (data not shown) were negative.

Binding Properties. The coding portion of the clone gR 4–24 was cloned into the expression vector pTEJ-8 to generate the clone Ruben-pTEJ and transfected into COS1 cells. Membranes prepared from these cells exhibited concentration-dependent binding of $[^{125}I][Tyr^{27}]bPP$ (Fig. 3), while nontransfected cells exhibited no specific binding (data not shown). This radioligand identified a single class of high-affinity binding sites with an affinity constant (K_d) of 0.038 ± 0.001 nM ($n = 3 \pm$SEM) for $[^{125}I][Tyr^{27}]bPP$ and Bmax of 1560 ± 135 fmol per mg of protein ($n = 2$; $\pm$SEM). The receptor showed no specific binding of $[^{125}I]pPYY$ or $[^{125}I]pNPY$ (data not shown). Competition experiments were performed using PP, PYY, NPY, and various peptide analogues (Fig. 4). rPP was a very strong inhibitor of binding of radiolabeled bPP with an inhibition constant (K_i) of 0.017 ± 0.002 nM, while rPYY and rNPY exhibited much weaker inhibition with K_i values of 162 ± 9 and 192 ± 20 nM, respectively. Interestingly, $r[Leu^{31}, Pro^{34}]NPY$ had >2 orders of magnitude higher affinity for the receptor with a K_i of 0.74 ± 0.06 nM.

cAMP Assay. A CHO cell line stably transfected with the Ruben-pTEJ expression construct was assayed for cAMP after stimulation of adenylyl cyclase with forskolin in the presence of rPP, $r[Leu^{31}, Pro^{34}]NPY$, rNPY, or rPYY. All four peptides caused a dose-dependent inhibition of adenylyl cyclase activity (Fig. 5) with IC_{50} of 0.40, 3.9, 250, and 1500 nM, respectively.

DISCUSSION

Several distinct subtypes of receptors for NPY, PYY, and PP have been demonstrated by physiological and pharmacological studies *in vivo* as well as in tissue preparations and cell lines. We have taken a molecular approach to elucidate the properties and relationships of these receptors by functional expression of their cloned genes. We used sequence information for the cloned Y1 receptor from human, rat, mouse, and *X. laevis* (26) to design several degenerate primers for PCR on rat genomic DNA. One primer pair from TM2 and TM7 generated a product of the expected size that was confirmed by sequencing to encode a receptor with similarity to Y1. A full-length clone was isolated from a rat genomic library. Binding studies of the novel receptor revealed that it binds PP

with high affinity, hence, we have designated it the PP1 receptor.

The rPP1 receptor has 56% sequence identity to the Y1 receptor in the transmembrane regions (Fig. 1), which is typical within other receptor subfamilies. The overall identity to Y1 is 46% (68% similarity). The novel receptor has 375 aa and has many features in common with the Y1 receptor such as three N-terminal glycosylation sites and four extracellular cysteines. Furthermore, the PP1 receptor inhibits forskolin-stimulated cAMP synthesis (Fig. 5) like the Y1 receptor (30). The PP1 gene in both rat and human lacks the short intron after after TM5 in the Y1 gene. rPP1 mRNA is highest in testis and lung (Fig. 2), whereas only a faint band was observed in RNA extracted from colon or brain.

To evaluate the pharmacology of the rPP1 receptor, an expression construct called Ruben-pTEJ was transiently expressed in COS1 cells. The transfected cells exhibited a single high-affinity binding site for $[^{125}I][Tyr^{27}]bPP$ (Fig. 3) but did not bind $[^{125}I]pPYY$ or $[^{125}I]pNPY$ within the concentration ranges tested. rPP exhibited the highest affinity for the receptor with a K_i of 0.017 nM (which corresponds to the circulating levels of the PP hormone), whereas NPY and PYY were $\approx 10,000$-fold less active (Fig. 4). The Y1-selective peptide $[Leu^{31}, Pro^{34}]NPY$ had considerably improved potency ($K_i = 0.74$ nM) when compared with the native NPY molecule. This result points out the potential importance of the Leu^{31} and Pro^{34} residues in the binding to the rPP1 receptor and makes sense as these amino acids were introduced into NPY because they are found at the corresponding positions in PP (34) and because $[Leu^{31}, Pro^{34}]NPY$ has previously been shown to bind to a PP receptor on PC12 cells (35). The Y2-selective peptide, NPY13–36, has a similar low affinity for the rPP1 receptor as observed for NPY. In addition, NPY2–36 had a relatively low affinity (Fig. 4).

The peptide binding results with rPP1 exhibit some important differences when compared with the hPP1 receptor. Whereas both receptors bind PP with high affinity, the hPP1 receptor had substantially higher affinity than rPP1 for both PYY ($K_i = 1.4$ nM) and NPY ($K_i = 9.9$ nM) (31). $[Leu^{31}, Pro^{34}]NPY$ had similar affinity for the hPP1 receptor when compared to NPY. On the other hand, $[Leu^{31}, Pro^{34}]NPY$ had more than a 100-fold improved affinity for rPP1 when compared with NPY. Also, the cAMP response of rPP1 to PYY is much lower than that of the human receptor. In addition to the pharmacological differences between rPP1 and hPP1, there are differences in the distribution of mRNA encoding these orthologs. Expression of mRNA for hPP1 is highest in colon, prostate, and small intestine, whereas rPP1 mRNA is most abundant in testis and lung (Fig. 2) with only a faint band in colon. Both the hPP1 and rPP1 mRNA are found at very low levels in brain, which agrees with reports that PP receptors are only found in small and discrete brain regions (21, 36). *In situ*
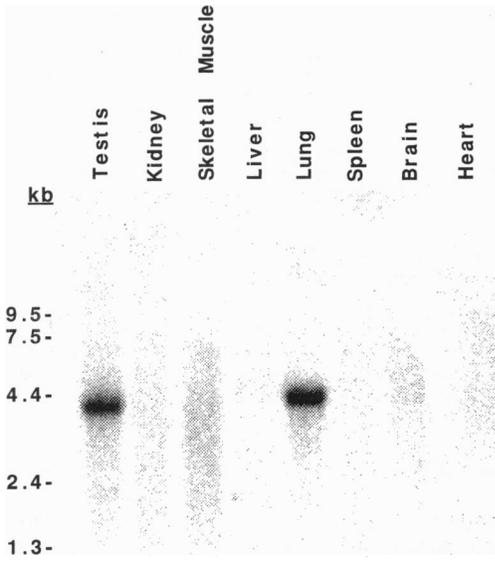
FIG. 2. Northern blot hybridization. Northern blot of rat organ panel. Each lane contains 2 μg of poly(A)$^+$ RNA.

hybridizations will be required to localize the cells that express PP1 mRNA in the brain.

The hPP1 receptor (31) has only 75% overall amino acid sequence identity (85% similarity) to rPP1. This degree of identity raises the question whether they are indeed orthologs, but similar identities also exist between different orders of mammals for the 5HT2B receptor (79%) and the prostaglandin PGI receptor (79%). (Other cases with low identity between different orders of mammals, but with unclear orthology–parology relationships, are interleukin-8, where the two human receptors show 79% and 69% identity to the rabbit F3R receptor, and adenosine A3, with 72% identity between human and rat, but 85% between human and sheep.) Southern blot hybridization with an hPP1 probe to rat genomic DNA detects only the PP1 gene described here. Furthermore, the nucleotide sequence identity between human and rat for PP1 is 80%, which is close to that of Y1 (86%), although the latter has 93% amino acid identity. Thus, PP1 has much lower selection than Y1 against amino acid replacements. In fact, the PP1 receptor seems to be the most variable receptor known between rat and human. This is in agreement with the high variability in the receptor's ligand, PP, which has eight re-
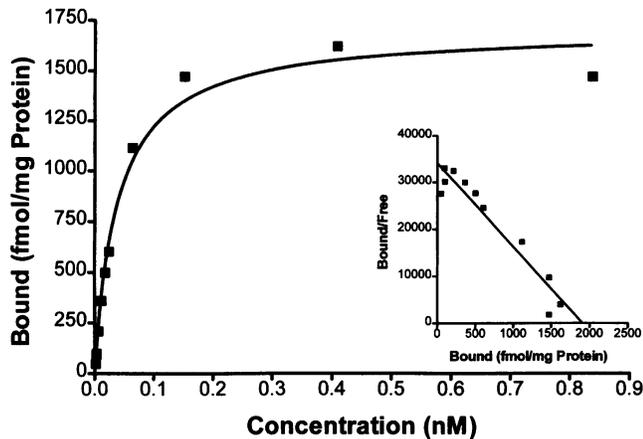

FIG. 3. Saturation and Scatchard (*Inset*) analyses of [^{125}I]bPP binding to membranes prepared from COS1 cells transfected with the PP1 expression plasmid Ruben-pTEJ. Data shown are from a representative experiment performed in quadruplicate. Nonspecific binding was defined by 1 μM rPP.
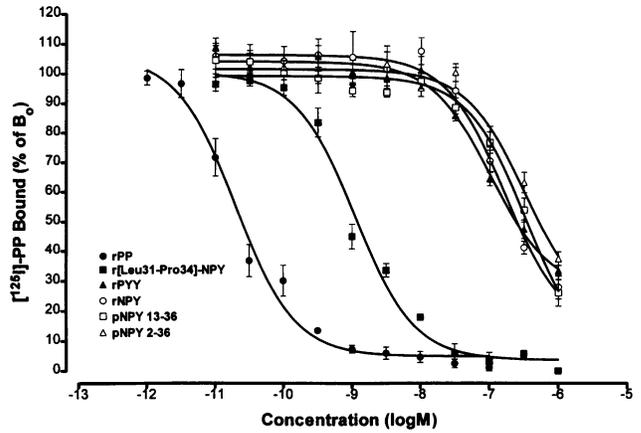

FIG. 4. Inhibition of [^{125}I]bPP binding to membranes from COS1 cells transfected with the PP1 expression plasmid Ruben-pTEJ. Competition data are expressed as percentage binding in the absence of competitor peptide. Data represent the mean ± SEM for eight experiments performed in duplicate. Nonspecific binding was defined as binding in the presence of 1 μM rPP.

placements out of 36 positions between rat and human. PP is one of the most variable neuroendocrine peptides known with only ≈50% identity between mammals, birds, and frogs. On the other hand, NPY has more than 94% identity (see ref. 1). Because PP probably arose through duplication of the PYY gene (the two are located in tandem on the chromosome; see ref. 37), one may speculate that the PP1 gene arose as a copy of a PYY-receptor gene and gradually evolved its strong preference for PP over PYY and NPY. This topic is currently under investigation.

Several different receptors with selective PP binding have previously been found in tissue preparations from various animals or in cell lines. However, they either are incompletely characterized or have features that seem to make them distinct from our PP1 receptor. The PP receptor in the basolateral membranes of dog intestine (18, 19) had much lower affinity
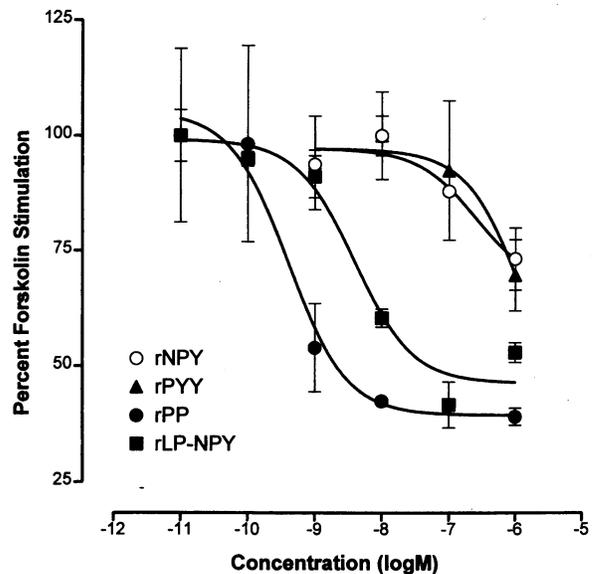

FIG. 5. Inhibition of forskolin-stimulated adenylyl cyclase activity by rPP, r[Leu^{31}, Pro^{34}]NPY, rNPY, and rPYY in CHO cells transfected with the rPP1 receptor clone Ruben-pTEJ. rPP (IC$_{50}$ = 0.40 nM), r[Leu^{31}, Pro^{34}]NPY (IC$_{50}$ = 3.9 nM), rNPY (IC$_{50}$ = 250 nM), and rPYY (IC$_{50}$ = 1600 nM) produced a dose-dependent inhibition of cAMP accumulation. The values are averages of two independent experiments with each sample run in triplicate for each peptide.

for PYY and NPY than rPP1. The rat vas deferens twitch response was mediated by NPY as well as PP, but probably by separate receptors (24). The receptor detected in rat PC12 pheochromocytoma cells (20) also had lower affinity for NPY than the cloned PP1 receptor. The existence of many sublines of PC12 cells makes comparison with this report difficult. Furthermore, the reported PC12 receptor had a very low level of expression (only 750 receptors per cell), which may be too low for detection of its mRNA in a Northern blot. Remarkably, synthetic rat PP bound much less strongly than purified bovine PP to the PC12 receptor. The purified rat liver microsome receptor (23) binds PP with high affinity but lacks NPY and PYY binding and we found no PP1 mRNA in rat liver. Thus, the possibility of additional PP-preferring receptors exists.

An interesting observation with regard to ligand interactions is that the rPP1 receptor lacks one of the four negatively charged residues proposed to be involved in NPY binding as deduced from mutagenesis of the human Y1 receptor (38). Position D-200 in human Y1, which corresponds to Val-203 in rPP1, was hypothesized to form a salt bridge with Arg-35 in NPY. Although this position is an Arg also in all PP sequences known, the rPP1 receptor obviously binds PP with high affinity without this particular salt bridge. The possibility that Val-203 may account for rPP1's selectivity for PP versus PYY and NPY is presently being explored by site-directed mutagenesis.

The PP receptor presented here shows dramatic differences between rat and human both in protein sequence, binding to PYY and NPY, and mRNA distribution. The rapid divergence of PP1 and its ligand may reflect different functions between animal groups, thus making extrapolation from rat to human (and vice versa) difficult.

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