Pharmacological Characterization of $^{125}$I-1229U91 Binding to Y1 and Y4 Neuropeptide Y/Peptide YY Receptors

DOUGLAS A. SCHOBER, SUSAN L. GACKENHEIMER, MARK L. HEIMAN, and DONALD R. GEHLERT

Lilly Neuroscience (D.A.S., S.L.G., D.R.G.) and Lilly Endocrine Research (M.L.H.), Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, Indiana

Accepted for publication December 10, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

1229U91 (GW1229 or GR231118) [lle,Glu,Pro,Dpr,Tyr,Arg,Leu,Arg,Tyr-NH$_2$]$_2$ cyclic (2,4'),(2,4')-diamide has been reported by several research groups to be a potent antagonist at the Y1 neuropeptide Y (NPY) receptor subtype. However, 1229U91 also displaces $^{125}$I-peptide YY (PYY) with high affinity from the Y4 subtype. Previously, we reported that 1229U91 had full agonist properties for the Y4 receptor. To characterize the pharmacological properties of 1229U91 directly, we had it radiiodinated with the chloromine-T method. $^{125}$I-1229U91 bound to cell lines expressing the human Y1 and Y4 receptors with high affinity. The $K_d$ and $B_{max}$ for $^{125}$I-1229U91 binding to Y1 were 14.9 pM and 1458 fmol/mg protein, respectively. The Y4 receptor bound $^{125}$I-1229U91 with a $K_d$ of 12.5 pM and a $B_{max}$ of 1442 fmol/mg protein. When competing $^{125}$I-1229U91 binding from Y1 and Y4 receptors, a similar rank order of potency was observed: 1229U91 > [Leu$^{31}$,Pro$^{34}$]-NPY ≥ [Leu$^{31}$,Pro$^{34}$]-PYY > PYY ≥ NPY > NPY(2–36) > PYY(3–36).

Pancreatic polypeptide (PP) potently displaced $^{125}$I-1229U91 from the Y4 receptor, but displayed little affinity for Y1. In autoradiographic studies with rat brain sections, $^{125}$I-1229U91 bound with a distribution similar to that reported for the Y1 receptor when localized with $^{125}$I-[Leu$^{31}$,Pro$^{34}$]-PYY. Brain regions exhibiting binding sites for $^{125}$I-PP were not detected with this radioligand. Those include the interpeduncular nucleus and the periventricular nucleus of the hypothalamus. Furthermore, $^{125}$I-labeled rat PP was not displaced from these areas with 10 nM 1229U91. Thus, $^{125}$I-1229U91 is a high affinity Y1 and Y4 radioligand and binds with a distribution in the rat brain consistent with the localization of the Y1 receptor.

Since the discovery (Tatemoto, 1982) of neuropeptide Y (NPY), its receptor has been the focus of research for potential antagonist design and drug discovery. A novel peptide antagonist 1229U91 [lle,Glu,Pro,Dpr,Tyr,Arg,Leu,Arg,Tyr-NH$_2$]$_2$ cyclic (2,4'),(2,4')-diamide was synthesized based on the C terminus of NPY (Daniels et al., 1995) that displaced $^{125}$I-NPY (Daniels et al., 1995) and $^{125}$I-peptide YY (PYY) (Kanatani et al., 1996) with high affinity from the human Y1 receptor containing cell line SK-N-MC. Furthermore, the displacement was competitive in nature, and the compound was reported to be a pure antagonist at the Y1 receptor found in human erythroleukemia cells (Daniels et al., 1995). The selectivity of 1229U91, however, remains somewhat controversial. In rat brain homogenates, 1229U91 displaced $^3$H-NPY with 10-fold higher affinity than the Y1 containing SK-N-MC cells (Daniels et al., 1995). From their findings, these investigators concluded that 1229U91 did not appear to be selective for any particular NPY receptor subtype. In contrast, other studies (Hegde et al., 1995; Kanatani et al., 1996) have reported that 1229U91 selectively inhibited $^{125}$I-PYY binding to the Y1 receptor with SK-N-MC cells. To clarify 1229U91 selectivity, we evaluated it with the Y1 containing SK-N-MC and clonal cell lines containing Y2, Y4, and Y5 receptor subtypes (Schober et al., 1998). We found that not only did 1229U91 displace $^{125}$I-PYY with high affinity for the Y1 but also Y4 receptors. Therefore, we concluded that 1229U91 was a nonselective inhibitor of NPY binding. Subsequent work was performed to look at functional activity of 1229U91 at the Y1 and Y4 receptors. With adenylate cyclase assays, we (Schober et al., 1998) and others (Parker et al., 1998) found that 1229U91 was a potent antagonist at the Y4 receptor but an equally potent agonist at the Y1 receptor.

To better understand the pharmacology of 1229U91, a radiolabeled version of the molecule was necessary. In the present study, the binding of $^{125}$I-1229U91 to the cloned Y1 and Y4 receptors was evaluated. In addition, autoradiographic studies were performed to investigate the distribution of $^{125}$I-1229U91 throughout the rostral-caudal extent of the rat brain.

ABBREVIATIONS: NPY, neuropeptide Y; 1229U91, [lle,Glu,Pro,Dpr,Tyr,Arg,Leu,Arg,Tyr-NH$_2$]$_2$ cyclic (2,4'),(2,4')-diamide; PYY, peptide YY; rPP, rat pancreatic polypeptide; pPYY, porcine PYY; CHO, Chinese hamster ovary; bPP, bovine pancreatic polypeptide; hPP, human pancreatic polypeptide; IPN, interpeduncular nucleus.
Materials and Methods

Cell Culture. Cells stably expressing human Y1 (Gehlert et al., 1996b), Y4 (Lundell et al., 1995), and rat Y1 (Eva et al., 1990) receptors were grown in T-150 flasks containing Dulbecco’s minimal essential media with 5% fetal calf serum (Life Technologies, Gaithersburg, MD). The flasks were placed in a humidified incubator at 37°C containing 5% CO₂. The confluent cells were removed manually from the flasks by scraping. Cells were then washed with PBS, pelleted by centrifugation, and stored at −70°C until assayed.

Rat Brain Tissue Preparation. Male, 250- to 350-g Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were anesthetized with halothane and rapidly decapitated. The brains were quickly removed and placed on ice. The tissues were homogenized in 50 mM Tris (pH 7.4) with a Polytron (Brinkmann Instruments, Westbury, NY) with three 10-s bursts. After an initial spin at 800g for 10 min, pelleting the supernatant at 35,000g for an additional 20 min isolated membranes. Membranes were stored at −70°C until assayed.

Homogenate-Binding Studies. Binding assays were conducted as previously described (Gehlert et al., 1992) with isolated crude membrane homogenates. The cell pellets were resuspended with a Polytron homogenizer (Brinkmann) in 25 mM HEPES (pH 7.4) buffer containing 2.5 mM CaCl₂, 1.0 mM MgCl₂, and 2 g/l bacitracin. Incubations were performed for 2 h at room temperature in a final volume of 200 μl containing known amounts of 125I-1229U91 or 125I-porcine PYY (pPYY) (specific activity 2200 Ci/mM; NEN, Boston, MA). In the saturation experiments, 12 different concentrations of 125I-1229U91 were used. A 96-well cell harvester (Tomtec, Orange, CT) was used to terminate the incubations by rapid filtration through GF/C filters (Wallac, Gaithersburg, MD) presoaked in 0.3% polyethyleneimine (Sigma Chemical Co., St. Louis, MO). After a 5-min wash with ice-cold 50 mM Tris (pH 7.4), the filters were dried at 60°C. The dried filters were treated with MeltiLex A melt-on scintillation sheets (Wallac), and the radioactivity retained on the filters was counted with the Wallac 1205 Betaplate β-scintillation counter. The amount of radioactivity remaining on the filter after conducting the incubation in the presence of 1 μM 1229U91 (Eli Lilly and Co., Indianapolis, IN) was defined as nonspecific binding. At a radioligand concentration of 10 nM, ~70% of the 125I-1229U91 binding to the Y1 and Y4 cell lines was specific. In rat brain homogenates, 50% of the 125I-1229U91 binding was specific at 140 pM. Eleven point displacement curves were used in the pharmacology studies to determine specificity of various peptides and peptide analogs (Peninsula, Belmont, CA; Bachem, King of Prussia, PA). The Kᵣ and Kₛ values and Hill coefficients were determined with the Prism software package (GraphPad, San Diego, CA). Protein concentrations were measured with Coomassie protein plus assay reagent (Pierce, Rockford, IL) with BSA for standards.

Autoradiographic Studies. Male, 250- to 350-g Sprague-Dawley rats were anesthetized with halothane and decapitated. The brains were rapidly removed and stored at −70°C. The brains were mounted onto cryostats and sectioned at 12 μm with a cryostat (Hacker, Fairfield, NJ). Coronal sections were thaw mounted onto gelatin-coated slides, placed at −20°C overnight, and stored at −70°C until assayed. Sections were incubated according to a previously described protocol (Gehlert et al., 1992) with slight modifications. Sections were initially preincubated for 30 min in Krebs-Ringer buffer containing 0.4% BSA and 0.5% bacitracin. The sections were then placed into glass jars either containing 100 nM 125I-1229U91 or 25 nM 125I-rat pancreatic polypeptide (rPP) (specific activity 2200 Ci/mM; NEN, Boston, MA). Nonspecific binding was defined by incubating near adjacent sections with the addition of 1 μM 1229U91 or 10 nM rat pancreatic polypeptide (PP). Following a 2-h incubation, the sections were rinsed four times in fresh buffer without BSA for 5 min each and dried rapidly. The labeled sections were exposed for 3 or 7 days to Hyperfilm β-max (Amersham, Arlington Heights, IL) for 125I-1229U91 or 125I-rPP, respectively. The films were developed in D-19 developer (Kodak, Rochester, NY) for 5 min.

Results

Saturation-Binding Analysis of 125I-1229U91 to Membranes Prepared from Rat Brains and Cell Lines Expressing Human Y1 and Y4 Receptors. The affinity of 125I-1229U91 binding to both the Y1 and Y4 receptors, as well as well as forreinbrain membranes was examined. Figure 1 illustrates a saturation isotherm for the human Y1 receptor stably expressed in an AV12 cell line. Nonlinear regression analysis of the specific binding revealed a single (r² = 0.97) saturable high-affinity site with a Kₛ = 14.9 ± 1.5 pM and a Bₘₐₓ = 1458 ± 43 fmol/mg protein. In Fig. 2, 125I-1229U91 binding to the human Y4 receptor stably expressed in a Chinese hamster ovary (CHO) cell line is illustrated. A single (r² = 0.97) saturable high-affinity site also was observed and had a Kₛ = 12.5 ± 1.1 pM and a Bₘₐₓ = 1442 ± 35 fmol/mg protein. To characterize the binding to rat brains, similar saturation studies were performed (Fig. 3). In this case, 125I-1229U91 also bound to a single (r² = 0.99) saturable site with a Kₛ = 136 ± 6 pM and a Bₘₐₓ = 549 ± 8 fmol/mg protein. No specific binding was observed to cell lines expressing the Y2 and Y5 receptor subtypes (data not shown).

Pharmacology of 125I-1229U91 and 125I-pPYY Binding to Rat Brain and Cell Lines Expressing Y1 and Y4 Receptors. Because 125I-1229U91 bound with similar affinity to the human Y1 and Y4 containing cell lines, the pharmacology of 125I-1229U91 binding to their respective cell lines was examined. These data are presented in Table 1. When competing 10 pM 125I-1229U91 binding from human Y1 (Fig. 4) and Y4 (Fig. 5) receptors with various peptides and peptide analogs, a similar rank order of potency was observed: 1229U91 > [Leu31,Pro34]-NPY ≥ [Leu31,Pro34]-PYY > PYY ≥ NPY > NPY(2-36) > PYY(3-36). The displacement curves were best fit to a one-site model. Human PP inhibited with high-affinity 125I-1229U91 binding to the
human Y4 receptor-expressing cell line. Similarly, 1229U91 displaced $^{125}$I-1229U91 with high affinity from the human Y4 subtype. However, inhibition by PP at the human Y1 receptor was significantly lower. An identical pharmacological profile ($^{1229}$U91 > [Leu]$^{31}$,Pro$^{34}$]-NPY > PYY ≥ NPY > NPY(2–36) > PP) was observed between the rat brain (Fig. 6) and the human Y1 (Fig. 4) receptor, although a difference in $K_i$ values was noticed for 1229U91. This difference was examined with cloned cell lines expressing the human and rat Y1 receptors. Cell membranes with these receptors were incubated with 100 pM $^{125}$I-pPYY with various concentrations of 1229U91 (Fig. 7). 1229U91 inhibited $^{125}$I-pPYY binding to the human Y1 receptor with a $K_i$ of 34.5 ± 6.6 pM, whereas the rat Y1 receptor had a $K_i$ of 131.9 ± 22 pM.

**Discussion**

The Y1 NPY receptor subtype was the first member of the PP-fold peptide receptor family to be cloned (Herzog et al., 1992; Larhammar et al., 1992). This subtype displays high affinity for both PYY and NPY with little affinity for PP (Herzog et al., 1992; Larhammar et al., 1992). In contrast, the second receptor cloned from the family of PP-fold peptide receptors, the Y4/PP1, has high affinity for PP and lower affinity for PYY and NPY (Bard et al., 1995; Lundell et al., 1995). With expression-cloning techniques, the Y2 receptor subtype was the next receptor cloned in this receptor family (Gerald et al., 1995; Gehlert et al., 1996a). Unlike the Y1 subtype, the Y2 receptor binds C-terminal fragments of NPY/PYY as well as intact NPY and PYY. A novel receptor from the rat hypothalamus was the fourth NPY receptor subtype cloned and was designated the Y5 (Gerald et al., 1996). This was the first receptor that appeared to have a pharmacological profile that correlates with in vivo feeding studies. The Y5 subtype binds NPY and PYY, C-terminal fragments of NPY and PYY, and Pro$^{34}$-substituted analogs of NPY and PYY as well as PP. One distinguishing feature of the Y5 receptor is its high affinity for N[Trp]$^{32}$]NPY. In another study, a novel NPY receptor was cloned and expressed from a mouse genomic cDNA library (Weinberg et al., 1996). Also designated the Y5, the pharmacology of this novel receptor resembles that of the Y1 subtype and is distinct from that described for the Y2, Y3, and Y4 receptors. Referred to as both Y5 and/or Y2b, and/or PP2, this murine receptor has now been designated y6 by the IUPHAR organization (Burkhoff et al., 1998). The y6 sequence does not encode a functional receptor in either the rat or human (Gregor et al., 1996).

The bridged antiparallel dipeptide compound 1229U91 was first described as a potent NPY receptor antagonist. Subsequently, it was found to be selective for Y1 over Y2 receptors. The high potency and metabolic stability of this peptide indicated that a radioiodinated version would make a suitable radioligand. In the present study, we have used $^{125}$I-1229U91 binding to the Y1 and Y4 NPY/PYY Receptors.

**Autoradiographic Localization of $^{125}$I-1229U91 and $^{125}$I-rPP Binding to Rat Brain.** A series of coronal sections through the rat brain were incubated with 100 pM $^{125}$I-1229U91 (Fig. 8) to examine the distribution of labeling in different brain structures. In general, $^{125}$I-1229U91 binding exhibited a broad distribution, localized to many distinct brain regions. Some of the highest levels of binding with $^{125}$I-1229U91 were observed in the superficial (I–III) laminae of the cerebral cortex, claustrum, medial and lateral dorsal thalamus, medial geniculate, and dorsal area of the hypothalamus. A moderate amount of binding was observed in anterior olfactory nucleus, lateral septum, caudate putamen, and area postrema, and in the striatum orien and radiatum of the hippocampus. In sections incubated with 25 pM $^{125}$I-rPP, the greatest density of binding sites was observed in the interpeduncular nucleus (Fig. 9). This binding was completely inhibited with the addition of 10 nM PP and only slightly by 10 nM [Leu]$^{31}$,Pro$^{34}$]-PYY. No inhibition of $^{125}$I-rPP binding to the interpeduncular nucleus was noted when the sections were incubated in the presence of 10 nM 1229U91 or 10 nM PYY(3–36).

![Fig. 2. Saturation-binding analysis of the human Y4 receptor expressed in CHO cells. Twelve different concentrations of $^{125}$I-1229U91 were incubated with the homogenized membranes for 2 h at room temperature. A saturation isotherm obtained from four experiments performed in duplicate is shown. The data were best fit to a single-site model ($r^2 = 0.97$). A $K_i$ of 12.5 ± 1.1 pM and a $B_{max}$ of 1442 ± 35 fmol/mg protein (mean ± S.E.).](image)

![Fig. 3. Rat forebrain membrane homogenates were incubated for 2 h at ambient temperature with 12 different concentrations of $^{125}$I-1229U91. A saturation isotherm obtained from four experiments performed in duplicate is shown. A $K_i$ of 136 ± 6 pM and a $B_{max}$ of 549 ± 8 fmol/mg protein (mean ± S.E.). The data were best fit to a single-site model ($r^2 = 0.99$).](image)
Furthermore, the distribution of 125I-1229U91 binding is with a pharmacological profile consistent for the Y1 receptor. tani et al., 1996) and forebrain (present study) membranes species differences between the rat and human receptors in the human Y1 or Y4 receptors. This suggests there may be lower affinity compared with the cell lines expressing either.

However, 125I-1229U91 in the rat brain had a significantly evaluated at the Y1 and Y4. As expected, 125I-1229U91 exhibited high affinity for both the human Y1 and Y4 receptors expressed in AV12 and CHO cell lines, respectively. The pharmacology of the binding to these receptors was consistent with that described with other radioligands. Interestingly, 125I-1229U91 was displaced from rat hypothalamic (Kanatani et al., 1996) and forebrain (present study) membranes with a pharmacological profile consistent for the Y1 receptor. Furthermore, the distribution of 125I-1229U91 binding is identical with that observed for Y1 receptors with 125I-[Leu31,Pro34]-PP (Schober et al., 1998), so it was likely that a majority of 125I-1229U91 binding in rat brain tissue is to the Y1 subtype. However, 125I-1229U91 in the rat brain had a significantly lower affinity compared with the cell lines expressing either the human Y1 or Y4 receptors. This suggests there may be species differences between the rat and human receptors in the potency for 1229U91. In this study, the Ki values for 1229U91 in both the rat brain and the cloned rat Y1 receptor were similar to the Ki values obtained for 125I-1229U91 in the rat forebrain. The Ki and Kd values for 1229U91 and 125I-1229U91, respectively, at the human Y1 receptor were also similar, but, interestingly, 1229U91 was a log order more potent at the human receptor than the rat. Species differences in the potency of 1229U91 also were noted in a study by Parker et al. (1998).

There are several lines of evidence that suggest another PP-fold peptide receptor subtype may exist in the rat brain. First, at concentrations up to 1 \mu M, both NPY and PYY will not inhibit 125I-bovine PP (bPP) from the area postrema (Whitcomb et al., 1990). This differs substantially from the pharmacology described for the Y1, Y2, Y4, and Y5 receptor subtypes. Secondly, Schwartz et al. (1987) demonstrated that 1229U91 was also a potent agonist for the Y4 receptor as a radioligand for NPY receptors. Because we had shown that 1229U91 was also a potent agonist for the Y4 receptor (Schober et al., 1998), the binding of 125I-1229U91 was evaluated at the Y1 and Y4. As expected, 125I-1229U91 exhibited high affinity for both the human Y1 and Y4 receptors expressed in AV12 and CHO cell lines, respectively. The pharmacology of the binding to these receptors was consistent with that described with other radioligands. Interestingly, 125I-1229U91 was displaced from rat hypothalamic (Kanatani et al., 1996) and forebrain (present study) membranes with a pharmacological profile consistent for the Y1 receptor. Furthermore, the distribution of 125I-1229U91 binding is identical with that observed for Y1 receptors with 125I-[Leu31,Pro34]-PP (Dumont et al., 1996; Gehlert and Gackenheimer, 1997). Finally, previous studies have shown very low levels of Y4 receptor mRNA expression in rat brain (Lundell et al., 1996), so it was likely that a majority of 125I-1229U91 binding in rat brain tissue is to the Y1 subtype. However, 125I-1229U91 in the rat brain had a significantly lower affinity compared with the cell lines expressing either the human Y1 or Y4 receptors. This suggests there may be species differences between the rat and human receptors in the potency for 1229U91. In this study, the Ki values for 1229U91 in both the rat brain and the cloned rat Y1 receptor were similar to the Ki values obtained for 125I-1229U91 in the rat forebrain. The Ki and Kd values for 1229U91 and 125I-1229U91, respectively, at the human Y1 receptor were also similar, but, interestingly, 1229U91 was a log order more potent at the human receptor than the rat. Species differences in the potency of 1229U91 also were noted in a study by Parker et al. (1998).

There are several lines of evidence that suggest another PP-fold peptide receptor subtype may exist in the rat brain. First, at concentrations up to 1 \mu M, both NPY and PYY will not inhibit 125I-bovine PP (bPP) from the area postrema (Whitcomb et al., 1990). This differs substantially from the pharmacology described for the Y1, Y2, Y4, and Y5 receptor subtypes. Secondly, Schwartz et al. (1987) demonstrated that a rat phaeochromocytoma cell line PC-12 expressed a receptor that bound 125I-bPP with high affinity. Similarly to the Whitcomb study, bPP, but not NPY potently inhibited 125I-bPP binding to this receptor. Trinh et al. (1996), with 125I-human (h/rPP, postulated that the area postrema most likely contained the Y4 and/or Y5 receptor subtypes. These

### Table 1

<table>
<thead>
<tr>
<th>Peptide</th>
<th>NPY Y1 (nM)</th>
<th>Hill</th>
<th>NPY Y4 (nM)</th>
<th>Hill</th>
<th>Rat Brain (nM)</th>
<th>Hill</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>192.2 ± 36.8</td>
<td>0.89</td>
<td>0.024 ± 0.005</td>
<td>0.96</td>
<td>706.1 ± 282.8</td>
<td>1.10</td>
</tr>
<tr>
<td>PYY</td>
<td>1.49 ± 0.21</td>
<td>0.85</td>
<td>2.55 ± 0.57</td>
<td>0.83</td>
<td>2.31 ± 0.43</td>
<td>0.83</td>
</tr>
<tr>
<td>[Leu31,Pro34]-PYY</td>
<td>1.10 ± 0.14</td>
<td>0.86</td>
<td>0.53 ± 0.15</td>
<td>0.82</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PYY (3–36)</td>
<td>204.5 ± 44.4</td>
<td>0.94</td>
<td>16.71 ± 1.75</td>
<td>0.83</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PYY (3–36)</td>
<td>2.93 ± 0.71</td>
<td>0.84</td>
<td>4.83 ± 1.24</td>
<td>0.85</td>
<td>3.08 ± 0.51</td>
<td>0.84</td>
</tr>
<tr>
<td>NPY</td>
<td>0.30 ± 0.04</td>
<td>0.81</td>
<td>0.31 ± 0.05</td>
<td>0.83</td>
<td>0.96 ± 0.26</td>
<td>1.01</td>
</tr>
<tr>
<td>NPY (2–36)</td>
<td>12.32 ± 2.44</td>
<td>0.83</td>
<td>5.59 ± 1.09</td>
<td>0.78</td>
<td>18.29 ± 5.39</td>
<td>0.83</td>
</tr>
<tr>
<td>1229U91</td>
<td>0.004 ± 0.006</td>
<td>0.90</td>
<td>0.027 ± 0.004</td>
<td>0.91</td>
<td>0.10 ± 0.03</td>
<td>1.20</td>
</tr>
</tbody>
</table>

ND, not determined.
incubation was carried out in the presence of 100 pM 125I-pPYY for 2 h. [Leu31,Pro34]-NPY; of 1229U91 was evaluated at the rat (M) and human (M) NPY receptors. In an 11-point displacement curve, the affinity various peptides or peptide analogs (PP; PYY; NPY; [Leu31,Pro34]-NPY; NPY(2–36); 1229U91). The incubation was carried out over a 2-h period in the presence of 140 pM 125I-1229U91. Data are expressed as a percentage of specific binding and were obtained from four experiments performed in duplicate. Nonspecific binding was defined as the binding remaining in the presence of 1 μM 1229U91.

In conclusion, we have examined the pharmacology and binding of 125I-1229U91 to cloned NPY Y1 and Y4 receptors and the rat brain. This radioligand bound with high affinity to both the human Y1 and Y4 receptors. In rat brain, the binding pharmacology and distribution was consistent with the Y4 subtype. However, 125I-1229U91 had significantly lower affinity for rat Y1 receptors compared with the cell lines expressing either the human Y1 or Y4 receptors, suggesting species differences in the potency for 1229U91. Thus, 125I-1229U91 is a useful antagonist ligand for the study of Y1 and Y4 receptors.

References

![Fig. 6](image6.png)

Fig. 6. Inhibition of 125I-1229U91 binding to rat forebrain homogenates. Eleven point displacement curves were used to determine the affinity various peptides or peptide analogs (PP; PYY; NPY; [Leu31,Pro34]-NPY; NPY(2–36); 1229U91). The incubation was carried out over a 2-h period in the presence of 140 pM 125I-1229U91. Data are expressed as a percentage of specific binding and were obtained from four experiments performed in duplicate. Nonspecific binding was defined as the binding remaining in the presence of 1 μM 1229U91.

![Fig. 7](image7.png)

Fig. 7. Inhibition of 125I-pPYY binding to rat and human Y1 containing membrane homogenates. In an 11-point displacement curve, the affinity of 1229U91 was evaluated at the rat and human Y1 receptors. The incubation was carried out in the presence of 100 pM 125I-pPYY for 2 h. The data shown were obtained from four experiments performed in duplicate. Nonspecific binding was defined as the binding remaining in the presence of 1 μM NPY.

![Fig. 8](image8.png)

Fig. 8. Autoradiographic localization of 125I-1229U91-binding sites throughout the rostral-caudal extent of the rat brain (A–H). Rat brain sections were incubated with 100 pM 125I-1229U91 as described in Materials and Methods. In addition, some sections were incubated with the addition of 1 μM 1229U91 to define nonspecific binding. Representative autoradiograms for nonspecific binding are presented in I. Autoradiograms were obtained by exposing the radiolabeled sections to X-ray film for 3 days. Abbreviations are AON, accessory olfactory nucleus; CPu, caudate-putamen; ICj, islands of Calleja; AM, anterior medial thalamus; AV, anterior ventral thalamus; Re, thalamic reuniens nucleus; VP, ventral posterior nucleus; VL, ventral lateral thalamus; LG, lateral dorsal thalamus; MG, medial geniculate; DG, dentate gyrus; SuG, superficial gray layer of the superior colliculus; sp5, spinal trigeminal nucleus; NTS, nucleus of the solitary tract; MD, medial dorsal thalamus; and Cg, cingulate cortex.

Y1 and Y4 receptors (Schober et al., 1998) nor PYY(3–36) that has high affinity for the Y2 and Y5 (Gerald et al., 1996) receptors displaced the IPN-binding sites. The peptide analog (Leu31,Pro34)-PYY slightly inhibited 125I-rPP binding from the IPN. In addition, this receptor uniquely possesses high affinity for 125I-PP because both 125I-1229U91 (present study) and 125I-[Leu31,Pro34]-PYY (Gehlert et al., 1997) did not exhibit detectable binding to the rat IPN. Thus, these data are suggestive that the cloned Y4 receptor subtype is not the same receptor identified by radiolabeled PP in the rat IPN.


Send reprint requests to: Dr. Ronald D. Gehlert, Lilly Neurosciences, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. E-mail: gehlert_ron@dilly.com


Fig. 9. Autoradiographic localization of 125I-rPP binding sites through coronal sections containing the interpeduncular nucleus. Rat brain sections were incubated with 25 pM 125I-rPP as described in Materials and Methods. An additional section was incubated with 10 nM rPP to define nonspecific binding. A representative autoradiogram for nonspecific binding is presented in E. Autoradiograms were obtained by exposing the radiolabeled sections to X-ray film for 7 days.

Total

10nM 1229U91

10nM [Leu[31-Pro[34]]PY

10nM PYY[3-36]

10nM Pancreatic Polypeptide

Downloaded from jpet.aspetjournals.org at ASPET Journals on May 13, 2017