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Identification of multiple neuropeptide Y receptor subtypes in the human frontal cortex

Michael A. Statnick, Douglas A. Schober, Donald R. Gehlert *

CNS Research, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

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Abstract

Recently, we found abundant mRNA and binding sites for neuropeptide Y Y_1-like receptors in the human cerebral cortex. However, an earlier study using indirect labeling methods failed to detect substantial neuropeptide Y_1-like receptor binding in numerous areas of the human brain, including the cerebral cortex. To resolve the disparity in these findings, we characterized the neuropeptide Y receptor subtypes labeled with [^{125}I]peptide YY in homogenates of human frontal cortex. Competition experiments using 100 pM [^{125}I]peptide YY binding to human frontal cortex homogenates indicated predominantly neuropeptide Y Y_2 receptors are labeled with this concentration of ligand. However, saturation analysis of [^{125}I]peptide YY binding to frontal cortex membranes resulted in isotherms best characterized by a two-site fit. Binding of [^{125}I]peptide YY to the high affinity (K_d = 40 pM) binding site was prevented using a 100 nM concentration of the neuropeptide Y Y_2 receptor agonist peptide YY-(3–36). By masking the higher affinity site, we found a low affinity [^{125}I]peptide YY binding site (K_d = 1.4 nM) exhibiting a pharmacology consistent with a neuropeptide Y Y_1-like receptor. It appears that neuropeptide Y Y_2 receptors are the predominant subtype labeled with low concentrations of [^{125}I]peptide YY and that the neuropeptide Y Y_1 receptor is a low affinity [^{125}I]peptide YY binding site in the human frontal cortex. © 1997 Elsevier Science B.V.

Keywords: Radioligand binding; Neuropeptide Y; Neuropeptide Y_1 receptor; Neuropeptide Y_2 receptor; (Human)

1. Introduction

Neuropeptide Y is a 36-amino-acid peptide that is widely distributed in the peripheral and central nervous systems (Tatemoto et al., 1982; DiMaggio et al., 1985; O'Donohue et al., 1985; Wahlestedt and Reis, 1993; Heilig and Widerlov, 1995). In the brain, neuropeptide Y modulates food intake, anxiety, cardiovascular function and the release of neuroendocrine hormones (Tatemoto et al., 1982; DiMaggio et al., 1985; O'Donohue et al., 1985; Wahlestedt and Reis, 1993; Gehlert, 1994; Heilig and Widerlov, 1995; Wettstein et al., 1995). Peptide YY and pancreatic polypeptide are two closely related peptides appear to function as circulating endocrine peptides (Wahlestedt and Reis, 1993). Collectively, these peptides share considerable amino acid sequence and structural homology across several phylogenetic classes (Larhammar, 1996) and form the pancreatic polypeptide-fold family (Schwartz et al., 1987).

Several different classes of neuropeptide Y receptors have been described by investigators using molecular cloning and/or radioligand binding. The neuropeptide Y Y_1 and neuropeptide Y Y_2 receptors are the best characterized of the at least 6 different molecular sequences of neuropeptide Y receptors. The neuropeptide Y Y_1 receptor recognizes intact neuropeptide Y, peptide YY and Pro^{34}-substituted analogs (Sheikh et al., 1989; Fuhlendorff et al., 1990), while C-terminal fragments of neuropeptide Y and peptide YY are less potent agonists. Conversely, neuropeptide Y Y_2 receptors have low affinity for Pro^{34}-substituted analogs of neuropeptide Y and peptide YY, while neuropeptide Y, peptide YY and the C-terminal fragments of these peptides have high affinity (Wahlestedt and Håkanson, 1986; Wahlestedt et al., 1990). The neuropeptide Y Y_3 receptor (Wahlestedt and Reis, 1993; Gehlert, 1994) has a preferential affinity for neuropeptide Y over peptide YY, while the pancreatic polypeptide receptor has a higher affinity for pancreatic polypeptide and Pro^{34}-substituted analogs of neuropeptide Y and peptide YY (Gehlert et al., 1996b). The neuropeptide Y Y_5 receptor (Gerald et al., 1996) recognizes certain C-terminal fragments as well as Pro^{34}-substituted analogs of neuropeptide Y and peptide YY and the neuropeptide Y Y_6 receptor (Weinberg et al.,

* Corresponding author. Tel.: (1-317) 276-1810; Fax: (1-317) 276-5546; e-mail: gehlert_donald_r@lilly.com

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1996) closely resembles the neuropeptide Y Y_1 receptor in both amino acid sequence and pharmacology.

The distribution of neuropeptide Y binding sites has been examined in the mammalian brain using membrane homogenate and autoradiographic techniques. The rat brain is known to contain a mixture of neuropeptide Y Y_1 and neuropeptide Y Y_2 binding sites. Although several areas of the rat brain contain both receptor subtypes, neuropeptide Y Y_1 binding sites predominate in the cerebral cortex, inferior colliculus and certain thalamic nuclei, while neuropeptide Y Y_2 binding sites predominate in the hippocampus, hypothalamus, amygdala and cerebellum (Gehlert et al., 1992; Dumont et al., 1993, 1996a; Widdowson, 1993). Unlike the rat, the human brain appears to contain abundant neuropeptide Y Y_2 binding sites, with very little neuropeptide Y Y_1-like binding (Widdowson, 1993). Using indirect labeling methods, Widdowson reported that the neuropeptide Y Y_2 receptor agonist neuropeptide Y-(13–36) (300 nM) was effective at reducing 100 pM [^{125}I]peptide YY binding in all regions of the human brain examined, while the neuropeptide Y Y_1 receptor agonist [Leu^{31}, Pro^{34}]neuropeptide Y (100 nM) was relatively ineffective at reducing this binding in the structures examined. The most notable exceptions were the molecular layer of the dentate gyrus, layer IV of the frontal cortex and the stratum radiatum and oriens of the hippocampus where [Leu^{31}, Pro^{34}]neuropeptide Y reduced [^{125}I]peptide YY binding by approximately 20–40% (Widdowson, 1993).

Recently, we reported that the human frontal cortex contains abundant neuropeptide Y Y_1 receptor mRNA and binding sites for the radioligand [^{125}I][Leu^{31}, Pro^{34}]peptide YY (Statnick et al., 1997). Moreover, we found the density of neuropeptide Y Y_1-like and neuropeptide Y Y_2 binding sites in the frontal cortex was comparable with estimated B_{max} values of 313 and 444 fmol/mg protein, respectively. Therefore, it appears the indirect labeling methods used by Widdowson may have underestimated the density of neuropeptide Y Y_1-like receptors in the human brain. To test this hypothesis, we examined the nature of [^{125}I]peptide YY binding in the frontal cortex using membrane homogenate techniques. Herein we report that [^{125}I]peptide YY labels at least two distinct receptor subtypes in the frontal cortex with differing affinities. Moreover, we show that the high affinity binding of [^{125}I]peptide YY is to the neuropeptide Y Y_2 subtype, while the low affinity component is to a receptor population with a neuropeptide Y Y_1-like pharmacology.

2. Materials and Methods

2.1. Tissue homogenates

Frozen samples of human frontal cortex were obtained from the National Neurological Research Specimen Bank (Los Angeles, CA, USA). All patients were males, 49–57 years of age with no evidence of neurological disease. The postmortem delay for the tissue used in this study was 7–12 h. Tissues were thawed and homogenized in 40 volumes (w/v) of Tris–HCl buffer (50 mM, pH 7.4) using a Polytron (Brinkmann, Westbury, NY, USA). The homogenate was centrifuged once for 10 min at $800 \times g$, 4°C. The resulting supernatant was centrifuged again for 30 min at $20\,000 \times g$, 4°C. The pellet was washed in 40 volumes (original tissue weight) of Tris–HCl buffer and centrifuged for 30 min at $20\,000 \times g$, 4°C. The final pellet was suspended in 40 volumes (w/v) of Hepes buffer (25 mM, pH 7.4) containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.2% bacitracin.

2.2. Radioligand binding and competition studies

Saturation experiments were conducted as previously described (Gehlert et al., 1992). Membranes (0.2–0.4 mg protein) were incubated for 2 h at room temperature with 0.01–1.8 nM [^{125}I]peptide YY (2200 Ci/mmol, NEN, Wilmington, DE, USA) in a final volume of 200 μl. Nonspecific binding was defined as the amount of radioactivity remaining after incubation with 1 μM neuropeptide Y. For competition studies, binding of 100 pM [^{125}I]peptide YY was displaced using various concentrations of neuropeptide Y/peptide YY fragments and analogs (Peninsula, Belmont, CA; Bachem, King of Prussia, PA), or the nonpeptide neuropeptide Y_1 receptor antagonist BIBP3226 ((R)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)-methyl]D-arginine amide) (Rudolf et al., 1994; Peninsula, Belmont, CA, USA) using conditions identical to the saturation experiments.

To characterize the receptor subtypes labeled with [^{125}I]peptide YY, masking experiments were conducted on frontal cortex homogenates. The high affinity [^{125}I]peptide YY binding site(s) was characterized by preincubating membranes for 30 min with 100 nM of the neuropeptide Y Y_2 agonist peptide YY-(3–36), and incubating with 0.01–1.8 nM [^{125}I]peptide YY as described above. This concentration of unlabeled peptide YY-(3–36) was chosen as it was the concentration that maximally displaced 100 pM [^{125}I]peptide YY binding in our competition analysis. To characterize the low affinity component of [^{125}I]peptide YY binding, membranes were incubated with 1.2 nM [^{125}I]peptide YY in the presence of a saturating concentration of peptide YY-(3–36) (to mask neuropeptide Y Y_2-like binding sites). This binding was then displaced using various concentrations of neuropeptide Y/peptide YY fragments and analogs, pancreatic polypeptide, or BIBP3226 ((R)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)-methyl]D-arginine amide).

Following incubation with [^{125}I]peptide YY, unbound radioligand was removed by rapid filtration using a TOMTEC 96-well cell harvester (Orange, CT, USA) through GF/C filters (Wallac, Gaithersburg, MD) that

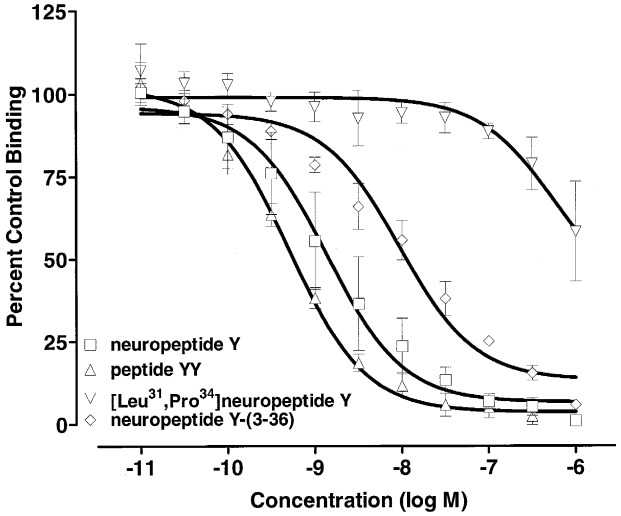

Fig. 1. Competition curves for 100 pM [^{125}I]peptide YY binding to membranes isolated from human frontal cortex. Membranes were labeled in the absence or presence of increasing concentrations of unlabeled neuropeptide Y/peptide YY analogs and fragments. Specific binding was calculated as the difference in binding in the absence and presence of 1 μM neuropeptide Y. IC_{50} values were calculated by nonlinear regression analysis as described in the Section 2. Data represent the mean ± S.D. of two independent determinations in postmortem samples from two patients.

were presoaked in 0.3% polyethyleneimine (Sigma, St. Louis, MO, USA). The filters were washed with 5 ml of Tris–HCl (50 mM, pH 7.4, 4°C) and dried at 60°C. The dried filters were treated with MeltiLex A melt-on scintillator sheets (Wallac) and the radioactivity measured using a Wallac 1205 Betaplate counter. Results were analyzed using Prism (Graphpad, San Diego, CA, USA) or the Cheng-Prushoff equation. Protein concentrations were measured using Commassie Protein Plus Assay Reagent (Pierce, Rockford, IL, USA) and bovine serum albumin standards.

Table 1
Affinity of neuropeptide Y/peptide YY fragments and analogs at high affinity [^{125}I]peptide YY binding sites in the human frontal cortex

Peptide	IC_{50} (nM) ± S.D.	Hill coefficient
hNeuropeptide Y	1.9 ± 1.6	1.4
hPeptide YY	0.61 ± 0.14	1.3
h[Pro^{34}]Neuropeptide Y	> 1000	—
h[Leu^{31}, Pro^{34}]Neuropeptide Y	> 1000	—
h[Leu^{31}, Pro^{34}]Peptide YY	> 1000	—
BIBP3226	> 1000	—
Neuropeptide Y-(3–36)	12.7 ± 4.0	1.8
Neuropeptide Y-(13–36)	16.0 ± 8.7	1.7
Peptide YY-(13–36)	10.8 ± 6.6	1.9
Neuropeptide Y-(2–36)	3.6 ± 1.8	1.6

Approximately 0.2–0.4 mg protein of human frontal cortex homogenate was labeled with 100 pM [^{125}I]peptide YY in the presence of various unlabeled compounds. Specific binding was defined as the difference in binding in the presence and absence of 1 μM neuropeptide Y. IC_{50} values were calculated by nonlinear regression analysis. Each value represents the mean ± S.D. of two independent determinations in postmortem samples from two patients. BIBP3226 ((R)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)-methyl]D-arginine amide).

3. Results

To characterize the neuropeptide Y/peptide YY receptor subtypes expressed in the human frontal cortex, we examined the ability of various unlabeled neuropeptide Y/peptide YY analogs and fragments to inhibit the binding of 100 pM [^{125}I]peptide YY to membrane ho-

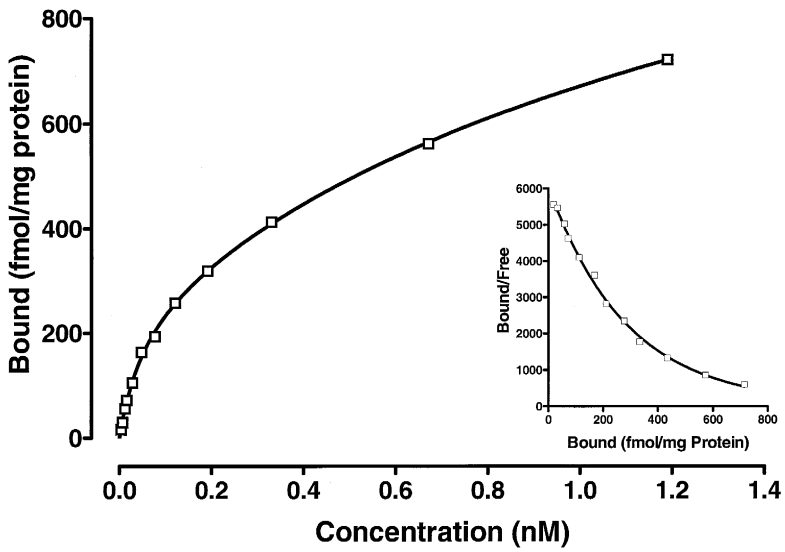

Fig. 2. Representative saturation binding analysis of [^{125}I]peptide YY to human frontal cortex membranes. Specific binding was calculated as the difference in binding in the absence and presence of 1 μM neuropeptide Y. Estimated kinetic constants for [^{125}I]peptide YY binding were calculated by nonlinear regression analysis: $K_d1 = 40$ pM and $K_d2 = 1.2$ nM with $B_{max}1 = 231$ fmol/mg protein and $B_{max}2 = 974$ fmol/mg protein. Data represent the mean ± S.E.M. of three independent determinations in postmortem samples from two patients.

Table 2
Pharmacological characterization of low affinity [^{125}I]peptide YY binding sites in human frontal cortex homogenates

Peptide	K_i (nM) ± S.E.M.	Hill coefficient	Maximal displacement (%)
hNeuropeptide Y	20.9 ± 6.0	0.82	91
hPeptide YY	9.8 ± 2.5	0.91	76
hPancreatic polypeptide	839 ± 198	0.38	36
h[Leu^{31}, Pro^{34}]peptide YY	4.8 ± 1.5	0.71	90
pNeuropeptide Y-(13–36)	692 ± 375	0.57	69
BIBP3226	5.2 ± 2.6	0.87	68

Approximately 0.2–0.4 mg protein of human frontal cortex homogenate was labeled with 1.2 nM [^{125}I]peptide YY following preincubation with 10 nM unlabeled peptide YY-(3–36) in the presence of various compounds. Specific binding was defined as the difference in binding in the presence and absence of 1 μM neuropeptide Y. Percent maximal displacement was calculated from the amount of specific binding remaining at 1 μM of each tested ligand. K_i values were calculated by nonlinear regression analysis. Each value represents the mean ± S.E.M. of three independent determinations in postmortem samples from two patients. BIBP3226 ((R)-*N*^{2}-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)-methyl]D-arginine amide).

mogenates. Neuropeptide Y-(3–36) potently inhibited the binding of [^{125}I]peptide YY to frontal cortex homogenates, having an IC_{50} of 12.7 ± 4 nM (Fig. 1). In comparison, [Leu^{31}, Pro^{34}]neuropeptide Y had substantially lower affinity, having a IC_{50} of greater than 1000 nM. The rank order potencies of peptide YY and neuropeptide Y analogs and fragments to compete with [^{125}I]peptide YY at these sites were: peptide YY > neuropeptide Y > neuropeptide Y-(2–36) > peptide YY-(3–36) = neuropeptide Y-(3–36) = neuropeptide Y-(13–36) ⋙ [Pro^{34}]neuropeptide Y = [Leu^{31}, Pro^{34}]neuropeptide Y = [Leu^{31}, Pro^{34}]peptide YY = BIBP3226 (Table 1).

Further characterization of [^{125}I]peptide YY binding in frontal cortex homogenates was accomplished using saturation analysis. From these experiments, the isotherms for [^{125}I]peptide YY binding to frontal cortex membranes was best fitted to two sites ($r^2 = 0.99$) (Fig. 2). The estimated kinetic constants for this binding were $K_d1 = 40$ pM and $K_d2 = 1.2$ nM with $B_{max}1 = 231$ fmol/mg protein and $B_{max}2 = 974$ fmol/mg protein. Interestingly, the binding of [^{125}I]peptide YY to the high affinity site could be prevented by preincubating the homogenate with 100 nM peptide YY-(3–36) (Fig. 3). The isotherms for this binding were best fit to a single site ($r^2 = 0.91$) with a $K_d = 1.4$ nM and a $B_{max} = 725$ fmol/mg protein.

To characterize the pharmacology of the low affinity [^{125}I]peptide YY site(s), we examined the ability of unlabeled neuropeptide Y/peptide YY fragments and analogs to displace 1.2 nM [^{125}I]peptide YY binding in the presence of 10 nM peptide YY-(3–36) (to mask the high affinity binding of [^{125}I]peptide YY to neuropeptide Y Y_2 receptors). In addition, we also included the nonpeptide neuropeptide Y Y_1 receptor antagonist BIBP3226 in our competition experiments. The binding of [^{125}I]peptide YY to the low affinity site was displaced potently by [Leu^{31}, Pro^{34}]peptide YY and BIBP3226, while peptide YY-(13–36) and pancreatic polypeptide were much less potent at competing for this binding (Table 2). Interestingly, BIBP3226 displaced approximately 70% of the bound [^{125}I]peptide YY.

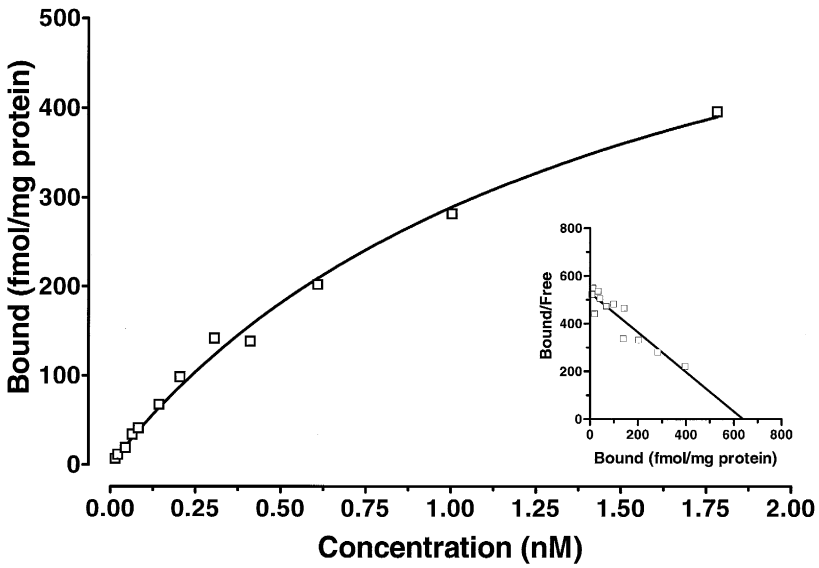

Fig. 3. Representative saturation analysis of [^{125}I]peptide YY binding to neuropeptide Y/peptide YY receptor subtypes in the human frontal cortex. Membranes were incubated with increasing concentrations of [^{125}I]peptide YY in the presence of 100 nM peptide YY-(3–36). Specific binding was calculated as the difference in binding in the absence and presence of 1 μM neuropeptide Y. Estimated kinetic constants for [^{125}I]peptide YY binding were calculated by nonlinear regression analysis: $K_d = 1.4 \pm 49$ nM, $B_{max} = 725 \pm 39$ fmol/mg protein. Data represent the mean ± S.E.M. of three independent determinations in postmortem samples from two patients.

4. Discussion

Recently, we reported that the human brain and in particular the frontal cortex, contains abundant neuropeptide Y Y_1 receptor mRNA and binding sites for the neuropeptide Y Y_1 receptor agonist [^{125}I][Leu^{31}, Pro^{34}]peptide YY (Statnick et al., 1997). These findings contrasted from an earlier autoradiography study that found very little of the [^{125}I]peptide YY binding in human brain was displaceable with 100 nM [Leu^{31}, Pro^{34}]neuropeptide Y (Widdowson, 1993), suggesting that neuropeptide Y Y_2 receptors are the predominant receptor subtype in this tissue. Moreover, Jacques et al. (1996) reported that with the exception of the dentate gyrus of the hippocampal formation, only very low levels of [^{125}I][Leu^{31}, Pro^{34}]peptide YY binding were detected in the human brain. To extend the autoradiography data (Widdowson, 1993; Jacques et al., 1996), we evaluated the affinity of neuropeptide Y and peptide YY fragments and analogs to displace low concentrations (100 pM) of [^{125}I]peptide YY from membranes of frontal cortex. Consistent with earlier reports, the pharmacology of [^{125}I]peptide YY binding sites in the frontal cortex was characteristic of the neuropeptide Y Y_2 subtype. These findings led us to question: why are we able to detect abundant neuropeptide Y Y_1-like receptors in homogenates of frontal cortex by direct labeling with [^{125}I][Leu^{31}, Pro^{34}]peptide YY, while autoradiographic analysis with [^{125}I]peptide YY or [^{125}I][Leu^{31}, Pro^{34}]peptide YY reveals only the neuropeptide Y Y_2 subtype? To answer this question we examined the nature of [^{125}I]peptide YY binding by saturation analysis in membrane preparations. Using this approach, we found that [^{125}I]peptide YY binds to at least two distinct receptor sites in homogenates of frontal cortex with different affinities. Moreover, we found that saturating concentrations of the neuropeptide Y Y_2 receptor agonist peptide YY-(3–36) prevented binding of [^{125}I]peptide YY to the high affinity (K_d = 40 pM) site. By masking the neuropeptide Y Y_2 subtype with peptide YY-(3–36), we were able to characterize the lower affinity (K_d = 1.2 nM) [^{125}I]peptide YY binding site. Interestingly, we found that the neuropeptide Y Y_1-selective ligands [Leu^{31}, Pro^{34}]peptide YY and BIBP3226 potently displaced 1.2 nM [^{125}I]peptide YY binding under these conditions. Collectively, our data support the hypothesis that, in the postmortem human brain, high affinity [^{125}I]peptide YY binding is to neuropeptide Y Y_2 receptors, while low affinity binding is to a neuropeptide Y Y_1-like subtype.

While our data offer explanation for the underestimation of neuropeptide Y Y_1 receptors in human cerebral cortex detected by [^{125}I]peptide YY binding, they do not resolve inconsistencies in the estimation of these sites detected by autoradiography with [^{125}I][Leu^{31}, Pro^{34}]peptide YY (Jacques et al., 1996). However, the literature offers a possible explanation. Recently, the neuropeptide Y Y1 receptor was found to exhibit a profound sensitivity to cations (Parker et al., 1996). These investigators found that binding of [^{125}I]neuropeptide Y or [^{125}I][Leu^{31}, Pro^{34}]peptide YY to neuropeptide Y Y_1 receptors is selectively enabled by Ca^{+2} and strongly attenuated by Na^+ and other alkali cations. Moreover, [^{125}I]peptide YY-(3–36) binding to neuropeptide Y Y_2 receptors showed little sensitivity to these cations (Parker et al., 1996). Interestingly, the Krebs phosphate buffers used in the previous autoradiography studies (Widdowson, 1993; Jacques et al., 1996) contained 120–137 mM NaCl, 4.7–5.4 mM KCl and 1.3–2.2 mM $CaCl_2$, while our laboratory employs a Hepes buffer containing 2.5 mM $CaCl_2$ without the monovalent cations for membrane homogenate studies (this study and Statnick et al., 1997). Therefore, the buffer used in our assay may have enabled us to detect neuropeptide Y Y_1 binding, while the buffer used by previous investigators may have prevented detection of these binding sites.

Several lines of evidence support our hypothesis that in the human brain the neuropeptide Y Y_2 receptor binds [^{125}I]peptide YY with high affinity and that this receptor is the primary subtype labeled with low concentrations of [^{125}I]peptide YY. First, we found that in membranes of frontal cortex C-terminal fragments of neuropeptide Y/peptide YY potently displaced 100 pM [^{125}I]peptide YY binding (a pharmacology consistent with a neuropeptide Y Y_2 subtype). There was no indication that neuropeptide Y Y_1-like receptors were labeled with this concentration of [^{125}I]peptide YY, since the Pro^{34}-substituted analogs of neuropeptide Y/peptide YY and BIBP3226 were ineffective at competing for binding (IC_{50} values were greater than 1000 nM). Interestingly, the slight deflection in the neuropeptide Y-(3–36) competition curve suggests that multiple neuropeptide Y Y_2 receptor subtypes may exist in the human frontal cortex. However, we found no difference in the correlation coefficients for a single site versus two sites (r^2 = 0.96 and 0.99, respectively). Moreover this neuropeptide Y Y_2 binding observed with low concentrations of [^{125}I]peptide YY showed Hill coefficients above unity. Similar Hill coefficients were reported for neuropeptide Y Y_2-selective binding in rat hypothalamus membranes and synaptosomes that was modeled to two receptor populations (Parker et al., 1996). Therefore, the possible existence of multiple neuropeptide Y Y_2 receptor subtypes in the human brain is intriguing and warrants a more detailed investigation.

Additional evidence that the neuropeptide Y Y_2 receptor may preferentially bind low concentrations of [^{125}I]peptide YY is provided from our finding that [^{125}I]peptide YY binds to at least two distinct receptor sites in homogenates of frontal cortex with different affinities (K_d1 = 40 pM, K_d2 = 1.2 nM). Interestingly, the affinity of the cloned human neuropeptide Y Y_2 receptor for [^{125}I]peptide YY (Gehlert et al., 1996a) and that of the high affinity [^{125}I]peptide YY binding site in the frontal cortex are strikingly similar (K_d values of 50 pM and 40 pM, respectively). Consistent with these observations, we

demonstrated that incubating membranes with saturating concentrations of peptide YY-(3–36) prevented high affinity [^{125}I]peptide YY binding. Moreover, several laboratories have reported that neuropeptide Y Y_2 receptors have a higher affinity for [^{125}I]peptide YY than the neuropeptide Y Y_1 subtype (Gehlert et al., 1992; Walker et al., 1988; Wieland et al., 1995). These data provide compelling evidence that the neuropeptide Y Y_2 receptor is the high affinity [^{125}I]peptide YY binding site in the human frontal cortex.

The pharmacological identity of the lower affinity [^{125}I]peptide YY binding site(s) is less clear, although our data suggest it is predominantly a neuropeptide Y Y_1-like subtype. In the present study, we found that [Leu^{31}, Pro^{34}]peptide YY and the BIBP3226 potently displaced 1.2 nM [^{125}I]peptide YY in the presence of saturating concentrations of peptide YY-(3–36). Under the same experimental conditions, pancreatic polypeptide and neuropeptide Y-(13–36) were far less potent (174 times and 143 times, respectively), discounting a significant contribution from pancreatic polypeptide/neuropeptide Y Y_4 and neuropeptide Y Y_2 receptors, respectively. These data provide direct evidence that neuropeptide Y Y_1-like receptors in frontal cortex homogenates have relatively low affinity for [^{125}I]peptide YY. However, our laboratory (Gehlert et al., 1996a) and several others (Tong et al., 1995; Wieland et al., 1995) have shown that peptide YY is a high affinity agonist at the cloned human neuropeptide Y Y_1 receptor. The neuropeptide Y Y_1-like receptor in the frontal cortex that we have identified exhibits 2–12 times lower affinity for [^{125}I]peptide YY than the cloned human neuropeptide Y Y_1 receptor. Therefore, one could argue that the neuropeptide Y Y_1-like receptor in the frontal cortex is not the same neuropeptide Y Y_1 receptor that has been cloned. However, our findings do not support this hypothesis. Recently, we found abundant neuropeptide Y Y_1 receptor mRNA and binding sites for [^{125}I][Leu^{31}, Pro^{34}]peptide YY in the human frontal cortex (Statnick et al., 1997). Moreover, the K_i values for BIBP3226 at the low affinity [^{125}I]peptide YY binding site and at the cloned human neuropeptide Y Y_1 receptor are equivalent (Gehlert et al., 1996a). Considering these data, we suggest the major component of neuropeptide Y Y_1-like binding in the frontal cortex is to the neuropeptide Y Y_1 subtype with a lower than expected affinity for the agonist radioligand, [^{125}I]peptide YY.

The reason for the reduced affinity of neuropeptide Y Y_1-like binding sites in the frontal cortex is presently unknown. A possible explanation may relate to postmortem changes in the agonist affinity state of the neuropeptide Y Y_1 receptor. This is likely since the tissue that was used in the present study was obtained 7–12 h postmortem. Supporting this hypothesis, Hurd et al. (Yasmin Hurd, personal communication) have found that binding of [^{125}I]peptide YY to the neuropeptide Y Y_1 receptor (but not to the neuropeptide Y Y_2 subtype) is reduced by postmortem delay. Whether this or an alternative mechanism(s) is responsible for the low affinity agonist conformation of neuropeptide Y Y_1-like receptors in the human brain awaits future study.

Another exciting finding from the present study is that while [Leu^{31}, Pro^{34}]peptide YY completely displaced specific [^{125}I]peptide YY binding, BIBP3226 displaced only 70% of the bound ligand. We suggest that another neuropeptide Y Y_1-like, BIBP3226-insensitive site may exist in the human frontal cortex. These results are supported by Dumont et al. (1996b) who identified [^{3}H]BIBP3226-insensitive binding sites in the rat brain including the external plexiform layer of the olfactory bulb, septal area, nucleus of the solitary tract and area postrema. These authors suggested that the rat BIBP3226-insensitive binding site may be the neuropeptide Y Y_4/pancreatic polypeptide receptor. In the human frontal cortex we found that pancreatic polypeptide was essentially inactive at displacing [^{125}I]peptide YY binding from the low affinity binding site, discounting a significant contribution from neuropeptide Y Y_4/pancreatic polypeptide receptors for the BIBP3226-insensitive binding that we have observed. While the identity of the BIBP3226-insensitive site is currently unknown, two new genes encoding neuropeptide Y/peptide YY receptor subtypes were recently cloned. It appears that [Leu^{31}, Pro^{34}]neuropeptide Y binds to neuropeptide Y Y_5 and neuropeptide Y Y_6 receptors with high affinity (Gerald et al., 1996; Weinberg et al., 1996). Interestingly, BIBP3226 does not antagonize the effects of neuropeptide Y or peptide YY on forskolin-stimulated adenylyl cyclase activity in cells expressing the neuropeptide Y Y_5 receptor (Gerald et al., 1996). Although the exact distribution of the neuropeptide Y Y_5 receptor in the human brain is unknown, we have detected neuropeptide Y Y_5 receptor mRNA in the frontal cortex by reverse transcriptase-polymerase chain reaction (RT-PCR) and by Northern blot (Statnick et al., unpublished data). Recently, Matsumoto et al. (1996) have cloned the human orthologue of the neuropeptide Y Y_6 receptor cDNA. It appears that the human neuropeptide Y Y_6 gene contains a frameshift mutation (a single T base insertion) within the sixth transmembrane domain which leads to premature termination of the receptor protein. Interestingly, while lower mammals (i.e. rabbits, rats and mice) have retained a functional neuropeptide Y_6 receptor, it appears that primates have universally lost the function of this receptor (Matsumoto et al., 1996). Therefore, the neuropeptide Y Y_5 receptor is the more probable contributor to the BIBP3226-insensitive binding that we observed in the human frontal cortex. Whether the neuropeptide Y Y_5 receptor also represents the [^{3}H]BIBP3226-insensitive binding site previously described in the rat brain (Dumont et al., 1996b) awaits further investigation.

The present study is the first to describe binding of [^{125}I]peptide YY to a heterogeneous population of neuropeptide Y receptor subtypes in the human brain. These

findings provide direct evidence that the neuropeptide Y Y_2 subtype is the primary receptor labeled with low concentrations of [^{125}I]peptide YY. We also report that a low affinity [^{125}I]peptide YY binding site(s) exists in the frontal cortex that has a neuropeptide Y Y_1-like pharmacology. Moreover, we found that approximately 30% of this binding is to a BIBP3226-insensitive site, suggesting the existence of another neuropeptide Y Y_1-like receptor subtype in the human brain. Therefore, it appears that neuropeptide Y Y_1 receptors exist in a low affinity agonist conformation in postmortem samples of frontal cortex. Future studies should carefully consider assay conditions and the concentration of radioligand to be used when identifying various subtypes of neuropeptide Y/peptide YY receptors that are present in postmortem tissues.

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