British Journal of Pharmacology (2000) 129, 1075–1088 

www.nature.com/bjp

# BIIE0246, a potent and highly selective non-peptide neuropeptide Y $Y_2$ receptor antagonist

[1]Yvan Dumont, [2]Alain Cadieux, [3]Henri Doods, [2]Leng Hong Pheng, [4]Roger Abounader, [4]Edith Hamel, [1]Danielle Jacques, [2]Domenico Regoli & *,[1]Rémi Quirion

[1]Douglas Hospital Research Centre, Department of Psychiatry, McGill University, 6875 LaSalle Blvd., Verdun, QC, H4H 1R3, Canada; [2]Department of Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, QC, J1H 5N4, Canada; [3]Preclinical Res. Department, Boehringer-Ingleheim, 88397 Biberach, Germany and [4]Montreal Neurological Institute, Department of Neurology, McGill University, 3801 University St., Montreal, QC, H3A 2B4, Canada

**1** BIIE0246, a newly synthesized non-peptide neuropeptide Y (NPY) $Y_2$ receptor antagonist, was able to compete with high affinity (8 to 15 nM) for specific [$^{125}$I]$PYY_{3-36}$ binding sites in HEK293 cells transfected with the rat $Y_2$ receptor cDNA, and in rat brain and human frontal cortex membrane homogenates.

**2** Interestingly, in rat brain homogenates while NPY, C2-NPY and $PYY_{3-36}$ inhibited all specific [$^{125}$I]$PYY_{3-36}$ labelling, BIIE0246 failed to compete for all specific binding suggesting that [$^{125}$I]$PYY_{3-36}$ recognized, in addition to the $Y_2$ subtype, another population of specific NPY binding sites, most likely the $Y_5$ receptor.

**3** Quantitative receptor autoradiographic data confirmed the presence of [$^{125}$I]$PYY_{3-36}$/BIIE0246-sensitive ($Y_2$) and-insensitive ($Y_5$) binding sites in the rat brain as well as in the marmoset monkey and human hippocampal formation.

**4** In the rat vas deferens and dog saphenous vein (two prototypical $Y_2$ bioassays), BIIE0246 induced parallel shifts to the right of NPY concentration-response curves with $pA_2$ values of 8.1 and 8.6, respectively. In the rat colon (a $Y_2/Y_4$ bioassay), BIIE0246 (1 $\mu$M) completely blocked the contraction induced by $PYY_{3-36}$, but not that of [Leu$^{31}$,Pro$^{34}$]NPY (a $Y_1$, $Y_4$ and $Y_5$ agonist) and hPP (a $Y_4$ and $Y_5$ agonist). Additionally, BIIE0246 failed to alter the contractile effects of NPY in prototypical $Y_1$ *in vitro* bioassays.

**5** Taken together, these results demonstrate that BIIE0246 is a highly potent, high affinity antagonist selective for the $Y_2$ receptor subtype. It should prove most useful to establish further the functional role of the $Y_2$ receptor in the organism.
*British Journal of Pharmacology* (2000) **129,** 1075–1088

**Keywords:** Receptor subtype; NPY; binding assay; bioassay; antagonist

**Abbreviations:** BIBO3304, ((R)-N-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N2-(diphenylacetyl)-argininamide trifluoroacetate); BIBP3226, R-N$^2$-(diphenylacetyl)-N-(4-hydroxyphenyl)-methyl argininamide; BIIE0246, (S)-N2-[[1-[2-[4-[(R,S)-5,11-dihydro-6(6h)-oxodibenz[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl] cyclopentyl] acetyl]-N-[2-[1,2-dihydro-3,5 (4H)-dioxo-1,2-diphenyl-3H-1,2,4-triazol-4-yl]ethyl]-argininamid; SR120819A, (R,R)-(1-[2-[2-(2-naphthylsulphamoyl)-3-phenylpropionamido]-3-[4-[N-[4-(dimethylaminomethyl)-cis-cyclohexylmethyl] amidino] phenyl] propionyl]-pyrrolidine); LY357897, 1-(1-[3-((3s)(3-piperidyl))-propyl]-2-[(4-chlorophenoxyl)-methyl]indol-3-yl]-2-(4-piperidylpiperidyl)ethan-1-one; GR231118, homodimeric Ile-Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-$CONH_2$; h, human; HEK293; human embryonic kidney cells, KRP, Krebs Ringer phosphate buffer; NPY, neuropeptide Y, p, porcine; PP, pancreatic polypeptide; PYY, peptide YY; r, rat.

## Introduction

Several studies have addressed the physiological functions of neuropeptide Y (NPY) and its homologues, peptide YY (PYY) and the pancreatic polypeptides (PP) in the central nervous system (CNS) (for reviews see: Colmers & Bleakman, 1994; Dumont *et al*., 1992; Gehlert, 1998; Heilig & Widerlov, 1995; Inui, 1999; Kalra & Crowley, 1992; Wahlestedt & Reis, 1993; Vezzani *et al*., 1999) and demonstrated a broad range of effects. For example, these peptides stimulate feeding behaviours and water consumption (Jolicoeur *et al*., 1991a,b; Morley & Flood, 1989; Stanley & Leibowitz, 1984), facilitate learning and memory processes (Flood *et al*., 1987), inhibit glutamatergic excitatory synaptic transmission at Schaffer collaterals and mossy fibres (Colmers & Bleakman, 1994; Klapstein & Colmers, 1993), have anticonvulsive properties (Klapstein & Colmers, 1997; Woldbye, 1998; Vezzani *et al*., 1999), modulate neuroendocrine secretions (Kalra & Crowley, 1984; 1992) and are anxiolytics (Heilig *et al*., 1993). Several of these effects appear to be physiologically relevant based on data obtained using NPY antibody, antisense oligonucleotides or knockout mice (for more details see Dumont *et al*., 2000a).

Thus far, five classes of receptors have been cloned and classified as the $Y_1$, $Y_2$, $Y_4$, $Y_5$ and $y_6$ subtypes on the basis of their molecular and pharmacological profiles (Michel *et al*., 1998). All NPY receptor subtypes are expressed in several species including man (Larhammar, 1996), except for the $y_6$ receptor subtype which is not expressed in the rat (Burkhoff *et al*., 1998) while in human and primates, the translated protein is a non-functional receptor due to a truncation from the half of the sixth transmembrane domain (Gregor *et al*., 1996; Matsumoto *et al*., 1996). The respective structure-activity relationships of each of these receptors has been established

*Author for correspondence; E-mail: mcou@musica.mcgill.ca

using several analogues and fragments of NPY, PYY and PPs (Blomqvist & Herzog, 1997; Michel *et al*., 1998). However, most of the currently available agonists such as [Pro$^{34}$]NPY, [Leu$^{31}$,Pro$^{34}$]NPY, [Leu$^{31}$,Pro$^{34}$]PYY and $PYY_{3-36}$, first reported as selective for the $Y_1$ or $Y_2$ receptors (Dumont *et al*., 1994; Fuhlendorff *et al*., 1990; Schwartz *et al*., 1990) are now known to also have significant affinities for the more recently cloned $Y_4$ and $Y_5$ subtypes (Blomqvist & Herzog, 1997; Dumont *et al*., 1998a; Gehlert *et al*., 1996a,b; Gerald *et al*., 1996; Michel *et al*., 1998). Accordingly, a series of agonists must be used to tentatively establish the possible role of a given receptor subtype in mediating an effect induced by NPY and its homologues (Michel *et al*., 1998).

The use of highly selective antagonists is often preferable to precisely classify receptor subtypes. Over the past few years, few peptides and especially non-peptide NPY antagonists have been developed. The first non-peptide $Y_1$ antagonist to be reported was R-N$^2$-(Diphenylacetyl)-N-(4-hydroxyphenyl)-methyl argininamide, known as BIBP3226 (Rudolf *et al*., 1994). This antagonist has been extensively studied and most data have shown that BIBP3226 behaves as a competitive, selective and specific $Y_1$ receptor antagonist in various binding assays as well as in *in vitro* and *in vivo* bioassays (Abounader *et al*., 1995; Bergdahl *et al*., 1996; Doods *et al*., 1995; 1996; Jacques *et al*., 1995; Lundberg & Morin, 1995; Lundberg *et al*., 1996; Malmstrom *et al*., 1997; Nilsson *et al*., 1996a,b; Racchi *et al*., 1996; Rudolf *et al*., 1994; Tough & Cox, 1996; Wieland *et al*., 1995; Zukowska-Grojec *et al*., 1996), without any significant affinity for the $Y_2$ (Doods *et al*., 1996; Dumont *et al*., 1998a; Gehlert *et al*., 1996c; Gerald *et al*., 1996; Jacques *et al*., 1995; Rudolf *et al*., 1994), $Y_4$ (Doods *et al*., 1996; Gehlert *et al*., 1996a; Gerald *et al*., 1996) and $Y_5$ (Doods *et al*., 1996; Dumont *et al*., 1998a; Gerald *et al*., 1996) receptors. More recently, ((R)-N-[[4-(aminocarbonylaminomethyl)-phenyl]-methyl]-N2-(diphenylacetyl)-argininamide trifluoroacetate) or BIBO3304 was reported to be highly selective for the human and rat $Y_1$ receptors having a 10 fold greater affinity for this subtype than BIBP3226 (Dumont *et al*., 2000b; Wieland *et al*., 1998). Other $Y_1$ antagonists including (R,R) -(1-[2-[2-(2-naphthylsulphamoyl) -3- phenylpropionamido]-3-[4-[N-[4-(dimethylaminomethyl)-cis-cyclohexylmethyl] amidino] phenyl] propionyl]-pyrrolidine) known as SR120819A (Serradeil-Le Gal *et al*., 1995) and 1-(1-[3-((3s)(3-piperidyl))-propyl]-2-[(4-chlorophenoxyl) -methyl] indol-3-yl] -2-(4-piperidylpiperidyl)ethan-1-one known as LY357897 (Hipskind *et al*., 1997) have also been reported although not as extensively studied as BIBP3226 and BIBO3304. Additionally, a $Y_1$ peptide antagonist, homodimeric Ile-Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-$CONH_2$, firstly known as 1229U91 (Daniels *et al*., 1995a) or GW1229 (Bitran *et al*., 1997) and now as GR231118 (Michel *et al*., 1998; Parker *et al*., 1998) has been rather extensively investigated. GR231118 was first characterized as a specific $Y_1$ receptor antagonist on the basic of its selective blockade of $Y_1$-like vs $Y_2$-like effects (Bitran *et al*., 1997; Daniels *et al*., 1995a,b; Leban *et al*., 1995). However, more recent data have shown that GR231118 is also a potent agonist on the $Y_4$ (Dumont & Quirion, 2000; Kanatani *et al*., 1998; Parker *et al*., 1998; Schober *et al*., 1998) and $y_6$ (Parker *et al*., 1998) receptors with some weak agonistic properties on the $Y_5$ receptor (Dumont *et al*., 1998a; Kanatani *et al*., 1998; Parker *et al*., 1998). Recently, few non-peptide $Y_5$ antagonists have been characterized including [(4-{[(4-amino-quinazolin-2-yl)amino]-methyl}-cyclohexyl)methyl](naphthyl-suphonyl)amine, known as CGP71683A (Criscione *et al*., 1998a; Dumont *et al*., 2000b) and L-152804 (Kanatani *et al*., 1997). Preliminary studies seem to demonstrate their potent antagonistic properties and selectivity for the $Y_5$ receptor (Criscione *et al*., 1998; Kanatani *et al*., 1997) although further characterization is certainly warranted.

Considering the high amounts of specific [$^{125}$I]$PYY_{3-36}$/$Y_2$-like binding sites detected in the brain of various species, especially in the hippocampal formation (Dumont *et al*., 1996;1998b) and the possible implication of the $Y_2$ receptor subtype in various biological effects induced by NPY (for review see Colmers & Bleakman, 1994; Dumont *et al*., 2000a; Gehlert, 1998; Vezzani *et al*., 1999), it was deemed critical to develop selective $Y_2$ receptor antagonists. One group has proposed $T_4$-[$NPY_{33-36}$]$_4$ as potent $Y_2$ antagonist (Grouzmann *et al*., 1997). However, subsequent studies by this group (Grouzmann *et al*., 1998) and ours (Pheng *et al*., 1999) demonstrated its rather low affinity. Most recently, Doods *et al*. (1999; 5th International NPY Meeting, Cayman Island, April 17–22, 1999) reported on the development of BIIE0246 ((S)-N2-[[1-[2-[4-[(R,S)-5,11-Dihydro-6(6h)-oxodibenz[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl] cyclopentyl] acetyl]-N-[2-[1,2-dihydro-3,5(4H)-dioxo-1,2-diphenyl-3H-1,2,4-triazol-4-yl]ethyl]-argininamid) as the first potent non-peptide $Y_2$ antagonist devoid of apparent agonistic or antagonistic activities for the $Y_1$, $Y_4$ and $Y_5$ receptors.

In the present study, we investigated in details the profile of BIIE0246 for various NPY receptors using cloned transfected receptors in HEK 293 cells, rat and human brain membrane homogenates, quantitative receptor autoradiography in mammalian CNS as well as a variety of *in vitro* bioassays. Our data clearly demonstrate that BIIE0246 is the first potent and highly selective $Y_2$ receptor antagonist to be developed.

## Methods

### *Materials*

Male Sprague Dawley CD rats (200–250 g) and Albino New-Zealand rabbits of either sex (1.5–2.0 Kg) were obtained from Charles River Canada (St-Constant, Québec, Canada). Mongrel dogs of either sex (20–50 Kg) were obtained from

**Table 1** Competition binding parameters of various antagonists of the NPY family against either [$^{125}$I]GR231118, [$^{125}$I]$PYY_{3-36}$, [$^{125}$I]hPP or [$^{125}$I][Leu$^{31}$,Pro$^{34}$]PYY binding in HEK 293 cells transfected with the rat $Y_1$, $Y_2$, $Y_4$ or $Y_5$ receptor cDNA

| | | | $IC_{50}$ (nM) | | |
|---|---|---|---|---|---|
| *HEK 293 cells transfected with* | *BIBP3226* | *BIBO3304* | *GR231118* | *BIIE0246* | *CGP71683A* |
| Rat $Y_1$/[$^{125}$I]GR231118 | 1.2±0.4 | 0.2±0.03 | 0.4±0.06 | >10000 | >10000 |
| Rat $Y_2$/[$^{125}$I]$PYY_{3-36}$ | >10000 | >10000 | 2000±400 | 15±3 | >10000 |
| Rat $Y_4$/[$^{125}$I]hPP | >10000 | >10000 | 0.1±0.03 | >10000 | >10000 |
| Rat $Y_5$/[$^{125}$I][Leu$^{31}$,Pro$^{34}$]PYY | >10000 | >10000 | 350±90 | >10000 | 5±1 |

Data represent the mean+s.e.mean of 3–5 determinations, each performed in triplicates. $IC_{50}$ represents the concentration of competitors needed to inhibit 50% of the specific binding.

the Laboratory of the Animal Protection Branch (Sherbrooke, QC, Canada). All animals were kept on a 12 h light-dark cycle (light on at 07:00) in temperature and humidity controlled rooms. Animals were fed with standard laboratory chow and had access to tap water *ad libitum*. Animal care was according to protocols and guidelines approved by McGill University, University of Sherbrooke and the Canadian Council of Animal Care. Human cerebral arteries reactivity studies were conducted in small ramification of middle cerebral arteries obtained post-morterm from patients with no cerebrovascular pathologies following approval of the research ethic committees from the Douglas Hospital or the Montreal Neurological Institute. Sanofi Recherche (Montpellier, France) generously provided marmoset (*C. jacchus*) monkey brains. Human frontal cortex and brain blocks were provided by the Douglas Hospital Research Centre Brain Bank from tissues obtained according to protocols and methodology described elsewhere (Quirion *et al*., 1987). These brains were obtained from human with post-morterm delays varying between 8–23 h in which the neuropathological examinations revealed no evidence of neurological disorders, significant cell losses, plaques, neurofibrillary tangles or excessive gliosis.

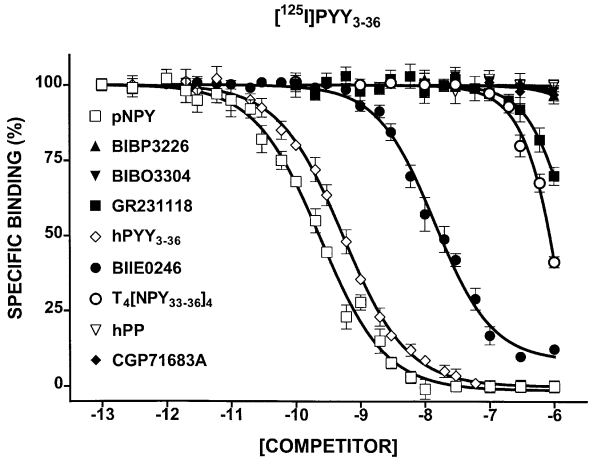


**Figure 1** Competition binding profile of pNPY, C2-NPY, $hPYY_{3-36}$, BIIE0246 and $T_4$-$[NPY_{33-36}]_4$ against specific $[^{125}I]PYY_{3-36}$ binding sites in rat brain membrane preparations. Data represent the mean ± s.e.mean of 4–6 determinations, each performed in triplicate.

Analogues and fragments of human (h) PYY, porcine (p) NPY and hPP were synthesized as previously described (Forest *et al*., 1990). BIBP3226, BIBO3304 and BIIE0246 were generously provided by Boerhinger Ingelheim (Germany) while GR231118 (first known as 1229U91 or GW1229) and CGP71683A were a gift from Glaxo Wellcome (Research Triangle Park NC, U.S.A.) and Servier (Paris, France), respectively. $T_4$-$[NPY_{33-36}]_4$ was purchased from Dr E. Grouzmann (Lausanne, Switzerland) and C2-NPY was obtained from Dr M. Aubert (Geneva, Switzerland). Bovine serum albumin (BSA) and Iodine-125 were obtained from ICN Pharm. Canada Ltd. (Montréal, Québec, Canada) and bacitracin was purchased from Sigma Chemical (St-Louis, MI, U.S.A.). Schleicher and Schuell #32 glass filters were obtained from Xymotech (Montréal, Québec, Canada). [$^3$H]Hyperfilms and $^{125}$I-microscale standards were purchased from Amersham (Mississauga, Ontario, Canada). All tissue culture media, antibiotics and reagents were obtained from Gibco-BRL (Burnington, Ontario, Canada). The rat $Y_1$, $Y_2$, $Y_4$ and $Y_5$ receptor cDNA were generously provided by Dr H. Herzog (Sydney, Australia). The expression vector, pcDNA3, was purchased from Invitrogen (San Diego, CA, U.S.A.). All other chemicals were of analytical grade and obtained from Fisher Scientific (Montreal, QC, Canada) or Sigma Chemical (St-Louis, MI, U.S.A.).

Iodine-125 was incorporated into the tyrosine residue of [Leu$^{31}$,Pro$^{34}$]PYY, $PYY_{3-36}$, hPP and GR231118 using the chloramine T method as previously described (Dumont *et al*., 1995; Dumont & Quirion, 2000) and the specific activity was assumed to be of the theoretical value (2000 Ci mmol$^{-1}$).

### *Membrane preparations*

Membranes were prepared as previously described (Dumont *et al*., 1995). Briefly, rats were killed by decapitation and their brains rapidly removed and homogenized in a Krebs Ringer phosphate (KRP) buffer at pH 7.4 of the following composition (mM: NaCl 120, KCl 4.7, $CaCl_2$ 2.2, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, dextrose 5.5 and $NaHCO_3$ 25 using a

**Table 2** Comparative binding parameters of porcine (p) NPY, human (h) PYY, their C-terminal fragments and BIBP3226 and BIBO3304 (two $Y_1$ non-peptidergic antagonists), $T_4$-$[NPY_{33-36}]_4$ (a purported $Y_2$ peptide antagonist) and BIIE0246 (a $Y_2$ non-peptidergic antagonist) against $[^{125}I][Leu^{31},Pro^{34}]PYY$ and $[^{125}I]PYY_{3-36}$ binding sites in a rat brain and human frontal cortex membrane homogenates

| | *Rat brain* | | *Human frontal cortex* |
|---|---|---|---|
| | $[^{125}I][Leu^{31},Pro^{34}]PYY$ | $[^{125}I]PYY_{3-36}$ | $[^{125}I]PYY_{3-36}$ |
| *Competitors* | $IC_{50}$ (nM) | $IC_{50}$ (nM) | $IC_{50}$ (nM) |
| pNPY | 1.1 ± 0.3 | 0.2 ± 0.05 | 2.4 ± 0.9 |
| $pNPY_{2-36}$ | 40 ± 9 | 0.4 ± 0.1 | 47 ± 16 |
| $pNPY_{13-36}$ | 100 ± 15 | 0.5 ± 0.1 | 290 ± 40 |
| C2-NPY | 150 ± 18 | 1.0 ± 02 | ND |
| hPYY | 1.7 ± 0.2 | 0.08 ± 0.02 | 0.25 ± 0.04 |
| $hPYY_{3-36}$ | 780 ± 80 | 0.5 ± 0.1 | 1.0 ± 0.4 |
| $hPYY_{13-36}$ | 450 ± 120 | 0.4 ± 0.1 | ND |
| hPP | 130 ± 30 | > 1000 | ND |
| BIBP3226 | 2.1 ± 0.6 | > > 1000 | > > 1000 |
| BIBO3304 | 0.3 ± 0.1 | > > 1000 | > > 1000 |
| $T_4[NPY_{33-36}]_4$ | 2000 ± 850 | 750 ± 200 | ND |
| BIIE0246 | > > 1000 | 10 ± 3 | 8 ± 2 |
| CGP71683A | > > 1000 | > > 1000 | ND |

Data represent the mean ± s.e.mean of 3–8 determinations. $IC_{50}$ represents the concentration of competitors needed to inhibit 50% of the specific binding. ND, not determined.

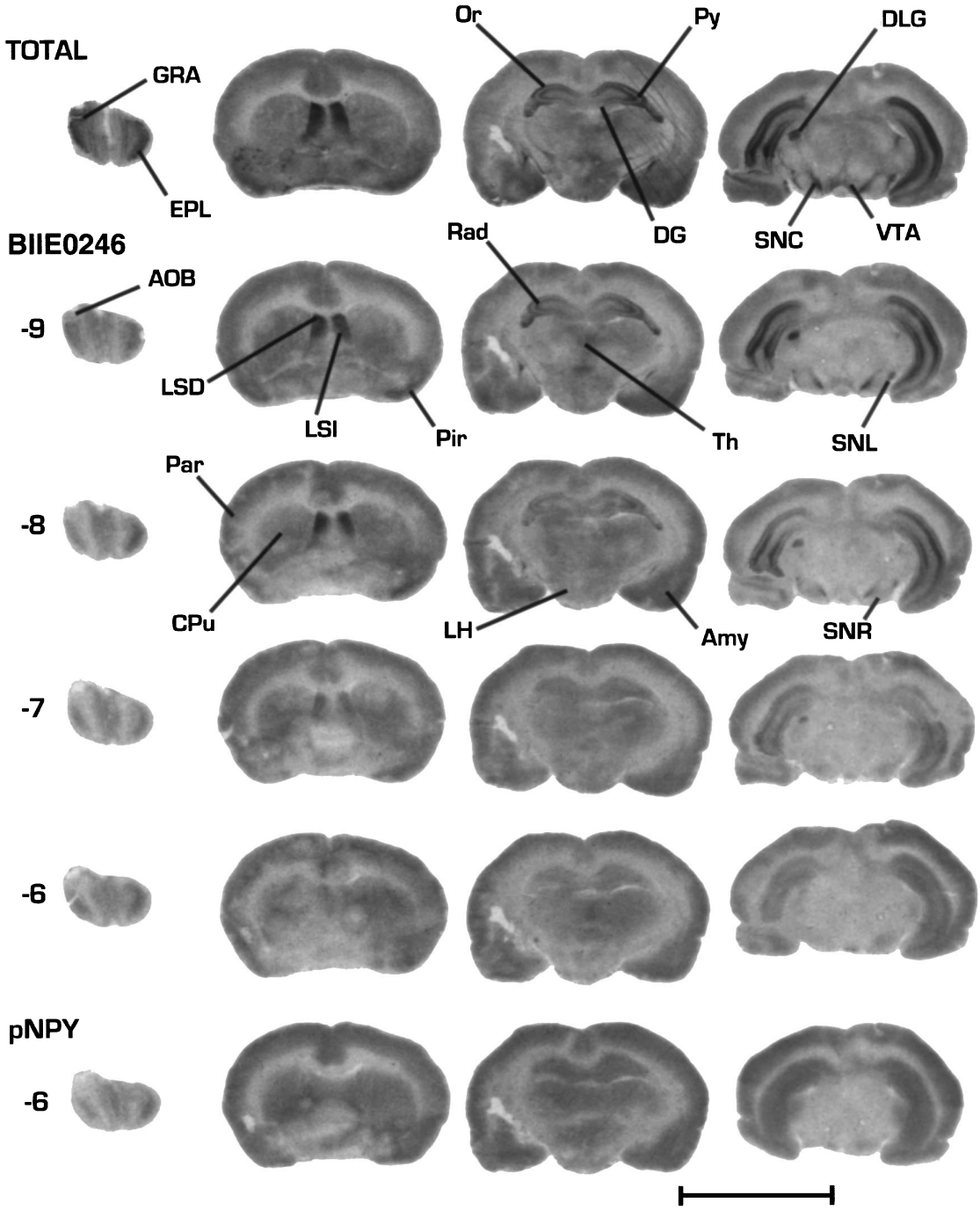


**Figure 2** Photomicrographs of the autoradiographic distribution of [$^{125}$I]$PYY_{3-36}$ binding sites in presence and absence of various concentrations of BIIE0246 in the rat brain. Adjacent coronal rat brain sections were incubated in the presence of 30 pM [$^{125}$I]$PYY_{3-36}$ and in the presence of BIIE0246 from 1–1000 nM. Non-specific binding was determined in the presence of 1 $\mu$M pNPY. See list of abbreviations for anatomical identification. Scale bar represents 10 mm.

Brinkman polytron (at setting 6 for 15–20 s). Human frontal cortex membrane homogenates were prepared as described elsewhere (Jacques *et al*., 1997). Homogenates were centrifuged at 49,000 × *g* for 20 min, supernatants discarded and pellets washed, resuspended, and recentrifuged twice. Protein concentration was determined with BSA as the standard (Bradford, 1976).

### *Transfected cells*

HEK 293 cells were maintained in Dulbecco's modified Eagle medium (D-MEM) supplemented with 10% foetal calf serum and antibiotics (penicillin G sodium, streptomycin sulphate and amphotericin B). Cultured cells were transfected with either of the rat $Y_1$, $Y_2$, $Y_4$ or $Y_5$ receptor cDNA using a calcium phosphate method (Tong *et al*., 1995). Briefly, 125 $\mu$l of 2.5 M calcium phosphate was added to 1.125 ml water containing 50 $\mu$g of either rat $Y_1$, $Y_2$, $Y_4$ or $Y_5$ receptor cDNA which was previously inserted in expressing pcDNA3 vectors and was slowly mixed with 1.25 ml 2× HEPES buffer at pH 7.05 and left at room temperature for 20 min. The mixture was added to a 150 mm dish containing HEK 293 cells at 30% confluent and returned to the incubator. The medium was changed the next morning. Forty-eight hours later, cells were washed with KRP buffer pH 7.4 and scraped. Detached cells were then centrifuged at 400 × *g* for 10 min and the pellet

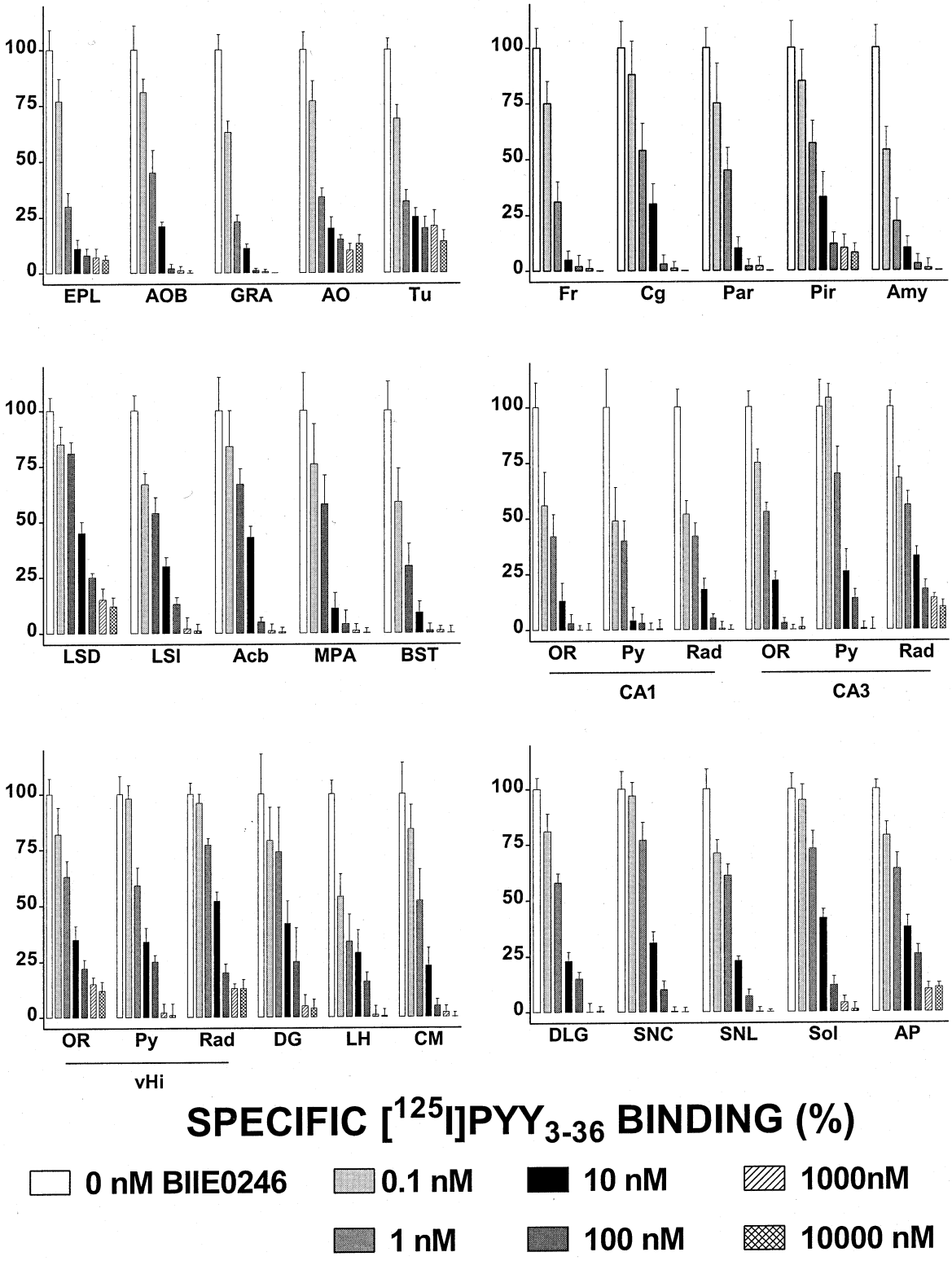


**Figure 3** Quantitative autoradiographic data of [$^{125}$I]PYY$_{3-36}$ in the presence of increasing concentrations of BIIE0246 (0.1–10,000 nM) in various rat brain regions. See list of abbreviations for anatomical identification.

washed with KRP buffer (pH 7.4), recentrifuged twice, and resuspended in 8 ml of KRP buffer pH 7.4 and used for receptor binding assay.

*Binding assays*

All binding assays were initiated by adding 100 $\mu$l of membrane or cell preparations in a final volume of 500 $\mu$l of KRP containing 0.1% (w v$^{-1}$) BSA, 0.05% (w v$^{-1}$) bacitracin radiolabelled probes and unlabelled peptide or competitor as needed. Isotherm saturations were performed in the presence of increasing concentrations of radiolabelled probes while competition binding experiments were performed using 30–35 pM of radiolabelled probes in the presence and absence of various competitors at concentrations ranging from $10^{-12}$–$10^{-6}$ M. In the rat brain homogenates, $Y_1$-like and $Y_2$-like receptors were

**Table 3** Comparative affinities ($K_d$) of various radioligands for the $Y_1$, $Y_2$, $Y_4$ and $Y_5$ receptors expressed in HEK 239 cells transfected with their respective receptor cDNA

| | *HEK 293 transfected cells* | | | |
|---|---|---|---|---|
| | *$Y_1$* | *$Y_2$* | *$Y_4$* | *$Y_5$* |
| *Radioligand* | $K_d$ (nM) | $K_d$ (nM) | $K_d$ (nM) | $K_d$ (nM) |
| [$^{125}$I][Leu$^{31}$,Pro$^{34}$]PYY | 0.85±0.13 | no binding | 0.92±0.3 | 0.21±0.03 |
| [$^{125}$I]PYY$_{3-36}$ | no binding | 0.25±0.05 | no binding | 1.05±0.12 |
| [$^{125}$I]hPP | no binding | no binding | 0.09±0.01 | 2.5±0.4 |
| [$^{125}$I]GR231118 | 0.11±0.01 | no binding | 0.31±0.04 | no binding |

Data represent the mean±s.e.mean of three determinations each performed in triplicate. $K_d$ represents the affinity obtained from isotherm saturation binding experiments performed using at least six different concentrations of each radioligand and analysed using a non-linear regression with the GraphPad Prism software. No binding means no specific binding detected at 1 nM of the radioligand.

studied using [$^{125}$I[Leu$^{31}$,Pro$^{34}$]PYY and [$^{125}$I]PYY$_{3-36}$, respectively and as previously described (Dumont *et al*., 1995). [$^{125}$I]GR231118 and [$^{125}$I][Leu$^{31}$,Pro$^{34}$]PYY were used in HEK 293 cells transfected with rat $Y_1$ and $Y_5$ receptor cDNA, respectively. Binding in HEK 293 cells transfected with rat $Y_2$ and $Y_4$ receptor cDNA was performed using [$^{125}$I]PYY$_{3-36}$ and [$^{125}$I]hPP, respectively. Non-specific binding was determined in the presence of 1 $\mu$M pNPY. Following a 2 h incubation, the binding reaction was terminated by rapid filtration through Schleicher and Schuell #32 glass filters (previously soaked in 1.0% polyethyleneimine) using a cell harvester filtering apparatus (Brandel Instruments, Gaithersburg, MD, U.S.A.). Filters were rinsed three times with 3 ml of cold KRP and the radioactivity remaining on filters was quantified using a gamma counter with 85% efficiency (Packard Instruments).

All binding experiments were repeated three to six times, each in triplicate, and results expressed as percentage of specific binding (competition) or fmol (saturation). $K_d$ values (i.e. the concentration of radioligand needed to occupy 50% of the total receptor population) were calculated from data obtained in saturation isotherm binding experiments using GraphPad Prism software (GraphPad Software Inc. San Diego, CA, U.S.A.) with a fit to a one site hyperbola curve. $IC_{50}$ values (i.e. concentration of unlabelled peptide required to compete for 50% of specific binding of the radioligand) of the various peptides and antagonists were calculated from the competition binding assays data using the GraphPad Prism software with a fit to a sigmoidal dose response curve.

### *Quantitative receptor autoradiography*

Receptor autoradiography was performed as described in detail elsewhere (Dumont *et al*., 1996; 1998a,b; Jacques *et al*., 1997). All sections (20 $\mu$m) were obtained using a cryomicrotome at −17°C, mounted on gelatin-chrome-alum-coated slides, dried overnight in a desiccator at 4°C, and then kept at −80°C until use.

On the days of the experiments, adjacent coronal sections were preincubated for 60 min at room temperature in a KRP buffer at pH 7.4 and then incubated for 120 min in a fresh preparation of KRP buffer containing 0.1% BSA, 0.05% bacitracin, 30 pM [$^{125}$I]PYY$_{3-36}$ and various concentrations of BIIE0246 ($10^{-10}$–$10^{-5}$ M). Following a 2 h incubation, sections were washed four times, 2 min each in ice-cold KRP buffer then dipped in deionized water to remove salts and rapidly dried. Non-specific binding was determined using 1 $\mu$M NPY. Incubated sections were apposed against $^3$H-Hyperfilms for 6 days alongside radioactive standards. Films were developed and quantified as described in detail elsewhere (Dumont *et al*., 1996; 1998a).

### In vitro *bioassays*

The rabbit (Cadieux *et al*., 1993) and dog (Pheng *et al*., 1997) saphenous veins, the rat vas deferens (Martel *et al*., 1990; Dumont *et al*., 1994), the rat colon (Pheng *et al*., 1999) and human cerebral arteries (Abounader *et al*., 1995) were prepared as described in details elsewhere. Concentration-response curves to NPY were generated by the cumulative addition of peptides for the rabbit saphenous vein and human cerebral arteries (NPY-induced contractions), and the rat vas deferens (NPY-inhibition of electrically stimulated twitch response) while a non-cumulative manner was used for the rat colon (NPY-induced contraction). In these tissues, the antagonistic properties of BIIE0246 were investigated by applying various concentrations or a single maximal concentration (1 $\mu$M) of BIIE0246 10 min prior to NPY. In the dog saphenous vein, the ability of BIIE0246 to block the contractile effects of NPY was investigated by the cumulative addition of BIIE0246 on tissues pre-contracted with NPY.

Concentration-response curves were constructed by plotting the molar concentration of NPY versus response expressed as percentage of the maximal response. From these plots, $EC_{50}$ values were calculated by non-linear regression analysis (sigmoidal dose-response curve). $EC_{50}$ values were calculated from each individual curve and the mean±s.e.mean was calculated from these data for the rat vas deferens, the rabbit saphenous vein and the human cerebral arteries. For each concentration of antagonist used, concentration-ratio was calculated by dividing the $EC_{50}$ value for NPY in the presence of the antagonist by the $EC_{50}$ obtained in the absence of the blocker. Schild plots were constructed and linear regression used to determine the x-intercept ($pA_2$ value). For the dog saphenous vein, the $pA_2$ value was determined as the concentration of BIIE0246 required to reduce by 50% the contractile effects of NPY.

## Results

Receptor binding assays in HEK 293 cells transfected with the rat $Y_2$ receptor cDNA demonstrated that BIIE0246 competed with high affinity ($IC_{50}$=15±3 nM) against specific [$^{125}$I]PYY$_{3-36}$ binding sites (Table 1). In contrast, BIIE0246, at concentrations up to 10 $\mu$M, failed to compete for significant amounts of specific [$^{125}$I]GR231118, [$^{125}$I]hPP and [$^{125}$I][Leu$^{31}$,Pro$^{34}$]PYY binding sites in HEK 293 cells transfected with the rat $Y_1$, $Y_4$ or $Y_5$ receptor

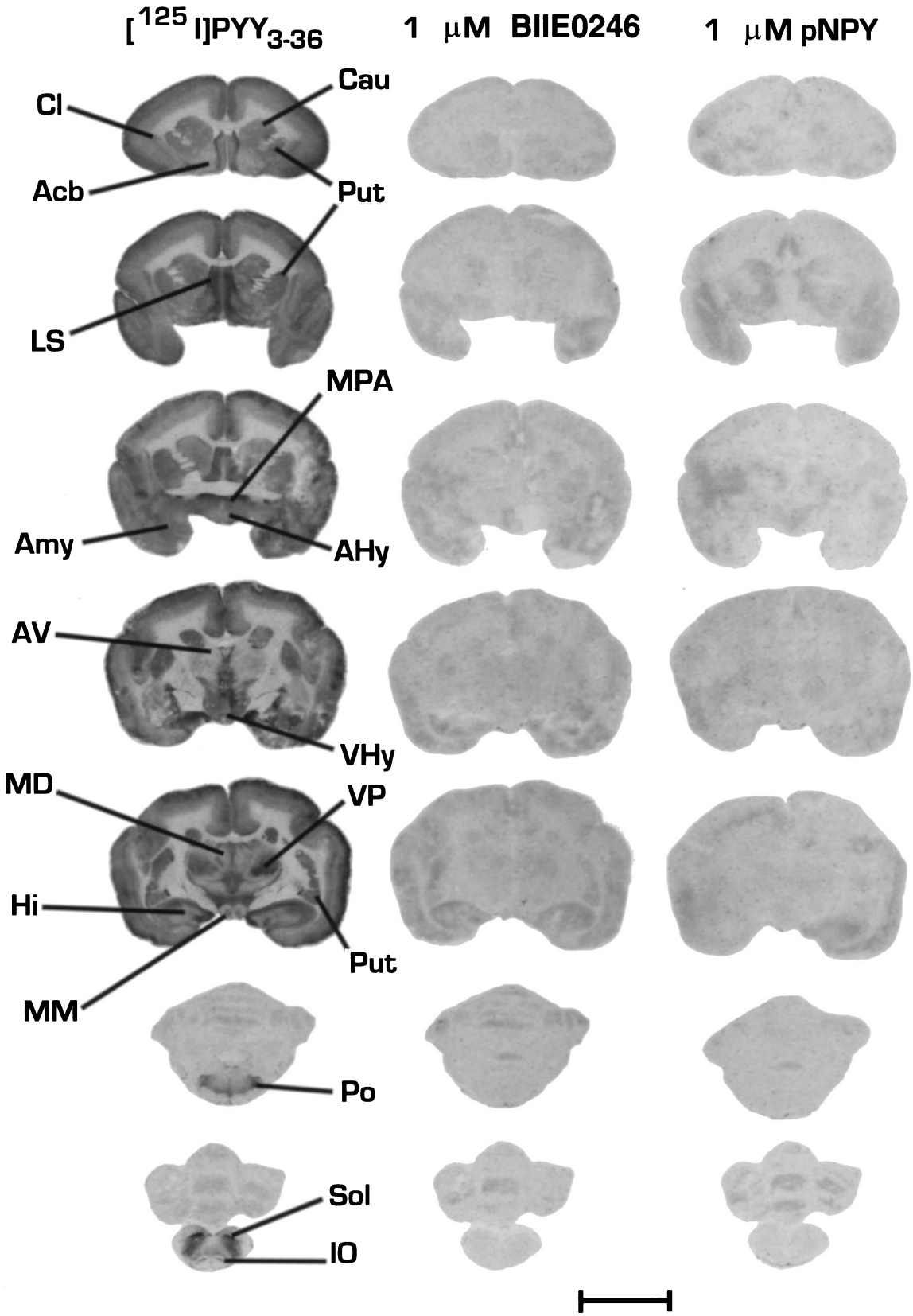


**Figure 4** Photomicrographs of the autoradiographic distribution of [$^{125}$I]$PYY_{3-36}$ binding sites in the marmoset monkey brain. Adjacent coronal brain sections were incubated with 30 pM [$^{125}$I]$PYY_{3-36}$ and in the presence of 1000 nM BIIE0246. Non-specific binding was determined in the presence of 1 $\mu$M pNPY. See list of abbreviations for anatomical identification. Scale bar represents 10 mm.

cDNA, respectively (Table 1). On the other hand, the $Y_1$ antagonists, BIBP3226 and BIBO3304 were potent competitors in HEK 293 cells expressing the $Y_1$ but not $Y_2$, $Y_4$ and $Y_5$ subtypes (Table 1) while the $Y_5$ antagonist, CGP71683A, had high affinity in HEK 293 cells transfected with the rat $Y_5$ receptor cDNA and GR231118 was a potent competitor for the $Y_1$ and $Y_4$ receptor binding sites expressed in HEK 293 cells (Table 1).

In rat brain homogenates, BIIE0246 was able to compete for specific [$^{125}$I] $PYY_{3-36}$ binding sites with an affinity similar

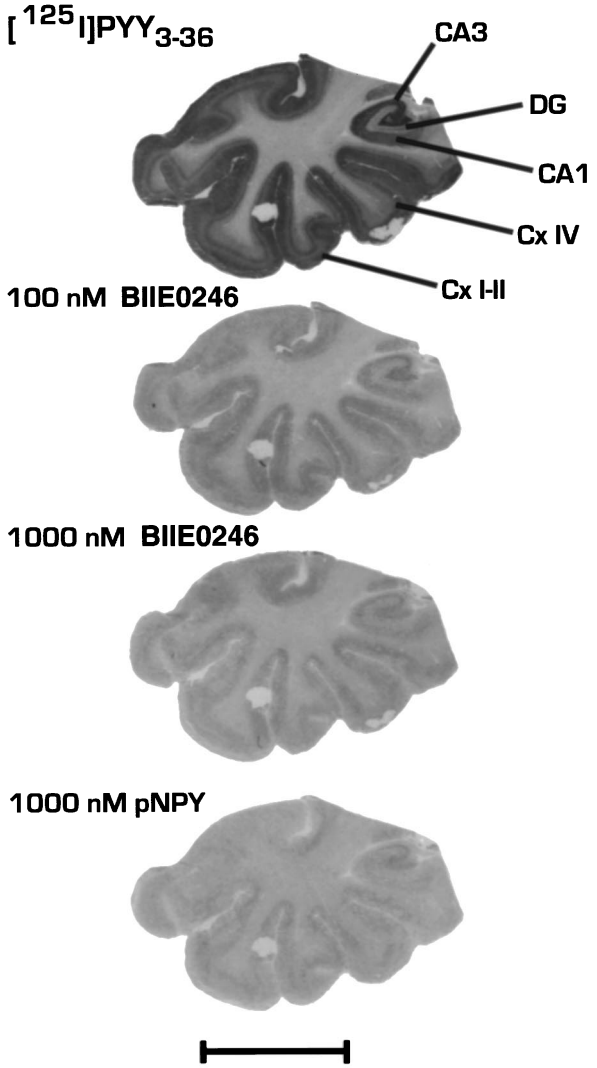


**Figure 5** Photomicrographs of the autoradiographic distribution of $[^{125}I]PYY_{3-36}$ binding sites in the human brain hippocampal area. Adjacent coronal human brain sections were incubated with 30 pM $[^{125}I]PYY_{3-36}$ and in the presence of either 100 or 1000 nM BIIE0246. Non-specific binding was determined in the presence of 1 $\mu$M pNPY. See list of abbreviations for anatomical identification. Scale bar represents 20 mm.

to that observed in HEK 293 cells transfected with the rat $Y_2$ receptor cDNA (Figure 1; Table 2). Specific $[^{125}I][Leu^{31},Pro^{34}]$-PYY binding was not competed by BIIE0246 at concentrations up to 1 $\mu$M (Table 2). BIIE0246 was about 70 times more potent to inhibit $[^{125}I]$ $PYY_{3-36}$ binding and possessed higher selectivity than $T_4$-$[NPY_{33-36}]_4$, a purported $Y_2$ peptide antagonist (Figure 1; Table 1). In contrast to pNPY, C2-NPY and $hPYY_{3-36}$ which all competed for 100% of specifically bound $[^{125}I]PYY_{3-36}$ (as established using 1 $\mu$M pNPY), BIIE0246 was able to inhibit only up to 95% of the labelling ($P<0.05$; Figure 1), suggesting that $[^{125}I]PYY_{3-36}$ could recognize an additional population of binding sites in the rat brain.

The existence and the distribution of specific $[^{125}I]PYY_{3-36}$ binding sites that are highly sensitive or not to BIIE0246 was established next using quantitative receptor autoradiography. Adjacent coronal rat brain sections were incubated with $[^{125}I]PYY_{3-36}$ in the presence of increasing concentration (0.1 nM – 10 $\mu$M) of BIIE0246 and demonstrated that specific $[^{125}I]PYY_{3-36}$ binding was fully competed by BIIE0246 in most

**Table 4** Quantitative autoradiographic distribution of $[^{125}I]PYY_{3-36}$ binding sites in the marmoset monkey and human brains

| *Regions* | *Specific binding nCi/g tissue, wet weight* | *% of specific binding in the presence of BIE0246* 100 nM | 1000 nM |
|---|---|---|---|
| *Marmoset temporal cortex* | | | |
| Superficial layers | 489±43 | ND | 0 |
| Mid layers | 210±24 | ND | 0 |
| Deep layers | 130±15 | ND | 0 |
| *Marmoset hippocampus* | | | |
| CA1 subfield | 310±26 | ND | 24±6 |
| CA3 subfield | 532±49 | ND | 18±5 |
| dentate gyrus | 84±12 | ND | 0 |
| *Human temporal cortex* | | | |
| Superficial layers | 326±41 | 35±8 | 0 |
| Mid layers | 295±36 | 35±7 | 0 |
| Deep layers | 156±24 | 37±6 | 0 |
| *Human hippocampus* | | | |
| CA1 subfield | 288±23 | 28±6 | 27±5 |
| CA3 subfield | 446±24 | 17±4 | 8±2 |
| Dentate gyrus | 125±19 | 9±3 | 0 |

Data represent the mean±s.e.mean of three determinations. Adjacent sections were incubated with 30 pM $[^{125}I]PYY_{3-36}$ in the presence or absence of BIIE0246. Non-specific labelling in the presence of 1 $\mu$M pNPY was digitally subtracted from all readings. ND, not determined.

brain structures (Figure 2). However, the quantitative analysis of $[^{125}I]PYY_{3-36}$ labelling revealed that BIIE0246 failed to compete for all specific $[^{125}I]PYY_{3-36}$ binding sites in few regions such as the external plexiform layer of the olfactory bulb, the anterior olfactory nucleus, the olfactory tubercle, the dorsal part of the lateral septum, the CA3 subfield of the ventral hippocampus and the area postrema (Figure 3). The specific $[^{125}I]PYY_{3-36}$ binding sites resistant to the $Y_2$ receptor antagonist may represent the $Y_5$ subtype. In HEK 293 cells expressing the rat $Y_5$ receptor gene, $[^{125}I][Leu^{31},Pro^{34}]PYY$, $[^{125}I]PYY_{3-36}$ and $[^{125}I]hPP$ were all able to recognize the expressed $Y_5$ receptor protein (Table 3). However, $[^{125}I][Leu^{31},$-$Pro^{34}]PYY$ has higher affinity than $[^{125}I]PYY_{3-36}$ and $[^{125}I]hPP$ for the transfected $Y_5$ receptor subtype (Table 3). In fact, at concentrations used in receptor binding and autoradiographic studies, $[^{125}I][Leu^{31},Pro^{34}]PYY$ specifically labelled three to four times more $Y_5$ sites than $[^{125}I]PYY_{3-36}$ (data not shown). This may explain the lower signal detected using $[^{125}I]PYY_{3-36}$/BIIE0246-insensitive sites (Figure 2) as compared to $Y_5$ receptors characterized as specific $[^{125}I][Leu^{31},Pro^{34}]PYY/Y_1$ antagonists-insensitive sites (Dumont *et al*., 1998a; 2000b).

Further characterization of specific $[^{125}I]PYY_{3-36}$ binding sites sensitive to BIIE0246 was done in other species. In human frontal cortex homogenates, BIIE0246 was able to compete against specific $[^{125}]PYY_{3-36}$ binding with an apparent affinity of 8 nM (Table 1). Additionally, autoradiographic studies demonstrated that specific $[^{125}I]PYY_{3-36}$ binding was fully inhibited by BIIE0246 (1 $\mu$M) in most areas of the marmoset monkey (Figure 4) and human (Figure 5) brains. However, some regions such as the hippocampal formation revealed the existence of $[^{125}I]PYY_{3-36}$/BIIE0246-insensitive sites in both species (Figures 4 and 5; Table 4).

The antagonistic properties of BIIE0246 were investigated next using various *in vitro* bioassays. In the rabbit saphenous vein and human cerebral arteries (two $Y_1$ bioassays; Cadieux *et al*., 1993; Abounader *et al*., 1995), the $Y_2$ antagonist (1 $\mu$M)

**Table 5** Comparative potencies of pNPY in the presence of various concentrations of BIIE0246 in several *in vitro* bioassays

| | *pNPY $EC_{50}$* (nM) | | | | |
|---|---|---|---|---|---|
| *+BIIE0246* | *Rabbit saphenous vein ($Y_1$)** | *Human cerebral arteries ($Y_1$)*** | *Rat vas deferens ($Y_2$)†* | *Dog saphenous vein ($Y_2$)††* | *Rat colon ($Y_2/Y_4$)#* |
| 0 nM | 19±4 | 4.5±2.3 | 23±5 | 60±17 | 46±8 |
| 1 nM | — | — | 20±4 | — | — |
| 10 nM | — | — | 42±6 | — | — |
| 100 nM | — | — | 160±12 | — | 107±20 |
| 1000 nM | 23±4 | 2.1±1.0 | — | — | 65±15 |
| $pA_2$ value | No inhibition | No inhibition | 8.1 | 8.6 | Block $Y_2$ component |

Data represent the mean±s.e.mean of 4–7 determinations. $pA_2$ represents the concentration of antagonist necessary to inhibit 50% of pNPY responses. No inhibition means that no antagonistic effect was observed in the presence of 1 μM of the blocker. *Cadieux *et al.*, 1993; **Abounader *et al.*, 1995; †Wahlestedt *et al.*, 1986; ††Pheng *et al.*, 1997; #Pheng *et al.*, 1999.

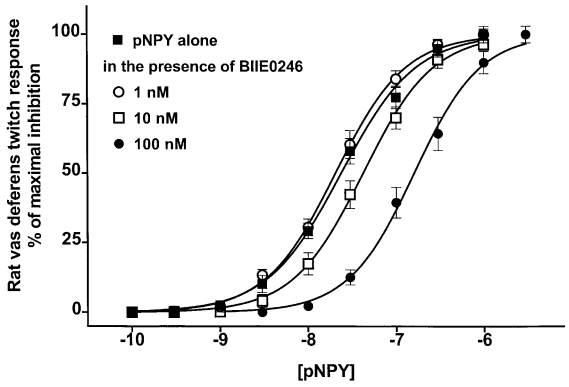


**Figure 6** Concentration-response curves of pNPY in the presence and absence of various concentrations of BIIE0246 in the electrically stimulated rat vas deferens. Data represent the mean±s.e.mean of 4–8 determinations.

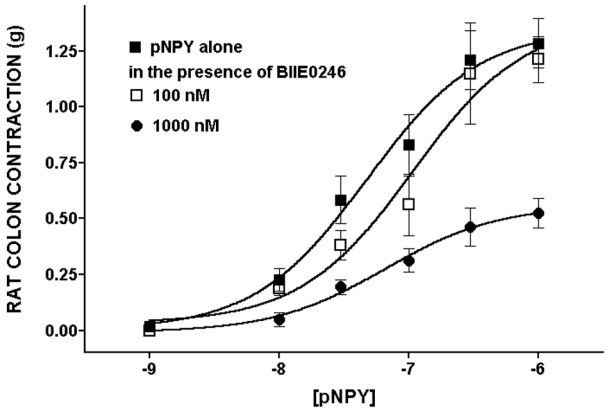


**Figure 7** Concentration-response curves of pNPY in the presence and absence of various concentrations of BIIE0246 in the isolated rat colon. Data represent the mean±s.e.mean of 4–6 determinations.

had no agonistic properties by itself (data not shown) and failed to block NPY responses in those tissues (Table 5). In contrast, in the rat vas deferens (a prototypical pre-junctional $Y_2$ bioassay; (Wahlestedt *et al.*, 1986), increasing concentrations of BIIE0246 resulted in a parallel shift to the right of NPY concentration-response curves (Figure 6; Table 5) with a $pA_2$ value of 8.1 as determined using a Schild plot (Table 5). Similarly, in the dog saphenous vein (a post-junctional $Y_2$ bioassay; Pheng *et al.*, 1997), BIIE0246 blocked the vasoconstriction induced by NPY with an apparent $pA_2$ value of 8.6 (Table 5). In the rat colon (a $Y_2/Y_4$ bioassay; Pheng *et al.*, 1999), 100 nM BIIE0246 induced a slight shift to the right of the NPY-induced contraction response curves (Figure 7, Table 5) while in the presence of 1 μM of the $Y_2$ antagonist, the effect of NPY was markedly reduced, resulting in a decrease in the maximal response (Figure 7). Moreover, BIIE0246 (1 μM) was able to fully block the contractile effects induced by $PYY_{3-36}$ but not those produced by [$Leu^{31}$,$Pro^{34}$]NPY and hPP (Figure 8).

## Discussion

We have demonstrated that BIIE0246 has high affinity for the $Y_2$ receptor subtype while being inactive at the $Y_1$, $Y_4$ and $Y_5$ subtypes in HEK 293 cells transfected with the respective receptor cDNA. In tissues containing heterogeneous population of NPY receptors such as the CNS, BIIE0246 was able to inhibit specific [$^{125}$I]$PYY_{3-36}$ binding sites with an affinity of 8–10 nM while failing to compete against [$^{125}$I][$Leu^{31}$,$Pro^{34}$]-PYY binding sites. Quantitative receptor autoradiographic studies demonstrated further that BIIE0246 competed for all specifically bound [$^{125}$I]$PYY_{3-36}$ labelling in most regions of the rat, marmoset monkey and human brains. However, few areas especially in the hippocampal formation, also revealed the existence of a small but significant proportion of [$^{125}$I]$PYY_{3-36}$/BIIE0246-insensitive sites possibly of the $Y_5$ subtype (Dumont *et al.*, 1998a,b). Additionally, the antagonistic property and selectivity of BIIE0246 for the $Y_2$ receptor was confirmed using various *in vitro* bioassays. No agonistic or antagonistic activities of BIIE0246 were observed in isolated tissues in which NPY-induced effects are mediated by the activation of the $Y_1$ and $Y_4$ receptor subtypes. In contrast, BIIE0246 acted as a potent antagonist in the rat vas deferens and dog saphenous vein, two prototypical $Y_2$ *in vitro* bioassays (Pheng *et al.*, 1997; Wahlestedt *et al.*, 1986). Hence, these functional and radioligand binding data confirm that BIIE0246 is the first potent and selective $Y_2$ receptor antagonist to be characterized and represents a useful tool to investigate the physiological role(s) of the $Y_2$ subtype.

Most recently, Doods and collaborators from Boerhinger-Ingelheim in Germany briefly described the characterization of the first non-peptide $Y_2$ receptor antagonist, BIIE0246 (Doods *et al.*, 1999; 5th International NPY Meeting, Cayman Island, April 17–22, 1999). They reported that BIIE0246 antagonized the effects of NPY in the rat vas deferens with an apparent $pA_2$ value of 7.8 while having no apparent affinity for the human and rat $Y_1$, $Y_4$ and $Y_5$ receptor subtypes. Our results confirm

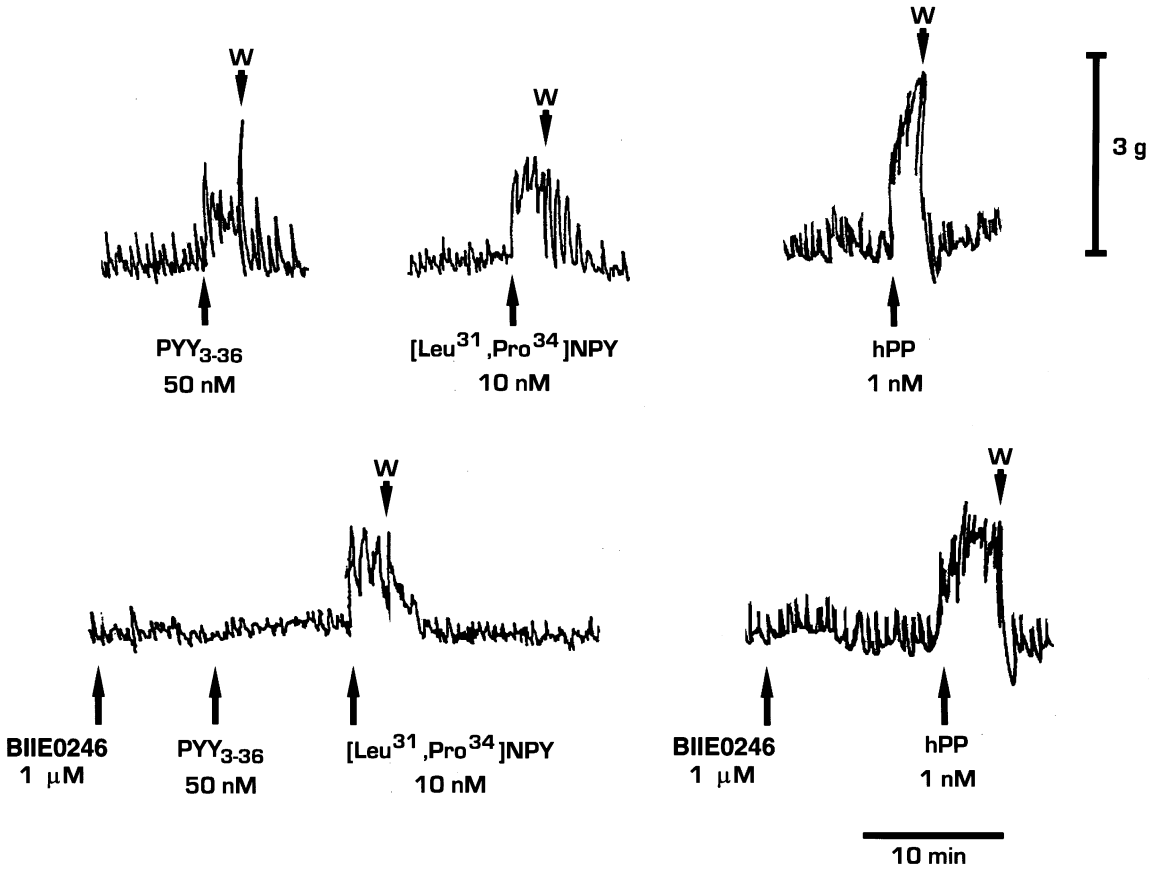


**Figure 8** Prototypical recording showing changes of tension (g) induced by $PYY_{3-36}$, [Leu$^{31}$,Pro$^{34}$]NPY and hPP in the presence and absence of 1 $\mu$M BIIE0246 in the rat colon. W, represents washout.

and extend these preliminary results by demonstrating that BIIE0246 is a potent competitor for NPY receptors expressed in HEK 293 cells transfected with the rat $Y_2$ receptor cDNA while being inactive in HEK 293 cells expressing the rat $Y_1$, $Y_4$ or $Y_5$ receptor gene. Moreover, using BIIE0246 we were able to show that most but not all specific [$^{125}$I]$PYY_{3-36}$ binding sites observed in the rat, marmoset monkey and human brains are of the $Y_2$ subtype. These data reveal the usefulness of BIIE0246 to clearly establish the genuine presence of the $Y_2$ receptor subtype in tissues expressing heterogeneous populations of NPY receptors. Using *in vitro* bioassays, we have also demonstrated that BIIE0246 can block the activation of the $Y_2$ receptor subtype without affecting the action of NPY or its homologues on the $Y_1$ and $Y_4$ receptors. This is especially evident in the rat colon, in which BIIE0246 (1 $\mu$M) abolished the contractile effects of $PYY_{3-36}$ but not that induced by [Leu$^{31}$,Pro$^{34}$]NPY and hPP and only partly blocking that of NPY. These data clearly demonstrate the ability of BIIE0246 to discriminate between the $Y_2$ vs $Y_1$, $Y_4$ and $Y_5$ receptor subtypes.

Earlier on, other molecules were proposed to act as $Y_2$ antagonists. In the rat femoral artery, an analogue of benextramine, N,N′-bis[6-[N-(2- naphthylmethyl)amino]hexyl]-N,N′-(1,6- hexanediyl)diguanidine tetrahydrochloride was reported to block the effect of the preferential $Y_2$ agonist, $NPY_{13-36}$, without altering the vasoconstriction induced by [Leu$^{31}$,Pro$^{34}$]NPY (Chaurasia *et al*., 1994). However, receptor binding assays in the rat brain homogenates failed to demonstrate the selectivity of this compound for $Y_2$-like vs $Y_1$-like receptors (Chaurasia *et al*., 1994) and we were unable to confirm its significant affinity for any NPY receptors (unpublished results). More recently, a peptidergic $Y_2$ receptor antagonist, $T_4[NPY_{33-36}]_4$ has been developed (Grouzmann *et al*., 1997). As shown here, this analogue weakly inhibits specific [$^{125}$I]$PYY_{3-36}$ binding sites in rat brain membrane preparations, confirming subsequent studies performed by Grouzmann (1998). Moreover, *in vitro* bioassays revealed that $T_4[NPY_{33-36}]_4$ is only a weak $Y_2$ antagonist (Pheng *et al*., 1999) while binding assays performed in HEK 293 cells transfected with the rat $Y_1$, $Y_2$, $Y_4$ or $Y_5$ receptor cDNA demonstrated its rather poor selectivity (Dumont *et al*., 1999b). Accordingly, BIIE0246 is by far more potent and selective than $T_4[NPY_{33-36}]_4$ as an antagonist of the $Y_2$ receptor subtype.

The functional role(s) of the $Y_2$ receptor in the organism remains to be clearly established. However, some data have been reported supporting its involvement in a variety of NPY-induced CNS effects based on the pharmacological profile using various agonists, especially C-terminal fragments. For example, electrophysiological data suggest that NPY, by acting on presynaptic $Y_2$ subtypes, can inhibit glutamatergic excitatory synaptic transmission in the hippocampus (Colmers, 1990; Colmers & Bleakman, 1994; McQuiston & Colmers, 1996; Qian *et al*., 1997) and hence suppress epileptiform activity (Klapstein & Colmers, 1997). On the other hand, it is not clear if the anticonvulsive activity of NPY is mediated by a $Y_2$ receptor as suggested by Klapstein & Colmers (1997) or by a $Y_5$-like subtype as proposed by Woldbye *et al*. (1997). The use of BIIE0246 and the newly developed $Y_5$ receptor antagonist (Criscione *et al*., 1998) should be most helpful to establish the implication of the $Y_2$ and/or the $Y_5$ receptor subtypes in that regard.

The $Y_2$ receptor subtype has also been suggested to modulate NPY-induced effects on circadian rhythms (Golom-

bek *et al*., 1996; Huhman *et al*., 1996) and baroreceptor reflexes (Barraco *et al*., 1990; Narvaez *et al*., 1993). However, various NPY receptor subtypes may be involved in the modulation of circadian rhythms (Chen & van den Pol, 1996) and the purported '$Y_3$' receptor subtype has been proposed to be involved in modulating cardiorespiratory responses, (Glaum *et al*., 1997; Grundemar *et al*., 1991). The use of BIIE0246 should help to clarify if these effects of NPY and homologues are mediated solely by the $Y_2$ subtype or by more than one population of NPY receptors as seen in the rat colon (Pheng *et al*., 1999). Furthermore, the existence of a PYY-preferring receptor has been suggested to explain the 3–5 fold differences in potency between PYY and NPY in rat crypt intestinal cells (Laburthe, 1991; Laburthe *et al*., 1986; Servin *et al*., 1989) and dog adipocytes (Castan *et al*., 1992). However, the pharmacological profile reported for the 'PYY-preferring receptor subtype' is most similar to that of the cloned $Y_2$ receptor (Gehlert *et al*., 1996c; Gerald *et al*., 1995; Michel *et al*., 1998). The use of BIIE0246 should establish whether the so called 'PYY-preferring receptor' is in fact the $Y_2$ receptor, or not. Finally, the $Y_2$ receptor antagonist should be most helpful to clearly establish the role of the $Y_2$ receptor subtype in various cardiovascular tissues as demonstrated in the dog saphenous vein (this study) and as suggested by others (Modin, 1994; Nilsson *et al*., 1996b). The use of selective antagonist(s) is more suitable to determine the exact nature of the receptor subtype involved in a given physiological response. This is especially evident for the NPY receptor family, since none of the agonists developed thus far are truly selective for one receptor subtype (Michel *et al*., 1998). Accordingly, BIIE0246 should prove most useful to establish the physiological and/or pathophysiological implication(s) of the $Y_2$ receptor subtype.

In summary, we have demonstrated using several receptor binding assays and *in vitro* bioassays that BIIE0246 is a potent and selective $Y_2$ receptor antagonist devoid of high affinity for the $Y_1$, $Y_4$ and $Y_5$ subtypes. To our knowledge, BIIE0246 represents the first potent and selective tool to precisely establish the potential roles of the $Y_2$ receptor in various tissues and to molecularly dissect features of agonist vs antagonist recognition sites on this receptor. The availability of BIIE0246, in addition to $Y_2$ knockout mice (Naveilhan *et al*., 1999) should also prove critical to demonstrate the involvement of this subtype in a given effect induced by NPY and related peptides.

## List of Abbreviations used in figures

I-VI, cortical layer 1-6; Acb, Accumbens nucleus; A Hy, anterior hypothalamic area; Amy, Amygdaloid complex; AOB, accessory olfactory bulb; AO, anterior olfactory nucleus; AP, area postrema; AV, anteroventral thalamic nucleus; BST, bed nucleus of the stria terminalis; CA1, field CA1 of hippocampus; CA3, field CA3 of hippocampus; Cau, caudate; Ce, cerebellum; Cg, cingulate cortex; Cl, claustrum; CM, central medial thalamic nucleus; CPu caudate putamen (striatum); Cx, cortex; DG, dentate gyrus; DLG, dorsolateral geniculate nucelus; EP1, external plexiform layer of the olfactory bulb; Fr, frontal cortex; GrA, granular cell layer of the accessory olfactory bulb; Hi, hippocampus; IO, inferior olive; LH, lateral hypothalamic area; LS, lateral septal nucleus; LSD, lateral septal nucleus, dorsal part; LSI, lateral nucleus, intermediate part; MD, mediodorsal thalamic nucleus; MM, medial mammillary nucleus, medial part; MPS, medial preoptic area; NS, non-specific binding; Or, oriens layer of the hippocampus; Par, Parietal cortex; Pir, piriform cortex; Po, pontine nucleus; Put, putamen; Py, pyramidal cell layer of the hippocampus; Rad, stratum radiatum of the hippocampus; SNC, substantia nigra, compact part; SNL, substantia nigra, lateral part; SNR, substantia nigra, reticular part; Sol, nucleus of the solitary tract; Th, thalamus; Tu, olfactory tubercle; vHi, ventral part of the hippocampus; VHy, ventral part of the hypothalamus; VP, ventral posterior thalamic nucleus; VTA, ventral tegmental area

This study was supported by the Medical Research Council of Canada. A. Cadieux, E. Hamel and R. Quirion are 'Chercheur-Boursiers' of the 'Fonds de la recherche en Santé du Québec'. L.H. Pheng has a studentship from the Heart and Stroke Foundation of Canada and D. Regoli has salary support from the Medical Research Council of Canada. The authors would like to thank Mrs Danielle Cécyre, Coordinator of the Douglas Hospital Research Centre Brain Bank, for supplying us with human brain tissues.

## References

ABOUNADER, R., VILLEMURE, J.G. & HAMEL, E. (1995). Characterization of neuropeptide Y (NPY) receptors in human cerebral arteries with selective agonists and the new $Y_1$ antagonist BIBP 3226. *Br. J. Pharmacol.,* **116,** 2245–2250.

BARRACO, R.A., ERGENE, E., DUNBAR, J.C. & EL-RIDI, M.R. (1990). Cardiorespiratory response patterns elicited by microinjections of neuropeptide Y in the nucleus tractus solitarius. *Brain Res. Bull.,* **24,** 465–485.

BERGDAHL, A., NILSSON, T., CATERA, L., NILSSON, L., SUN, X.Y., HEDNER, T., ERLINGE, D., VALDEMARSON, S. & EDVINSSON, D. (1996). Neuropeptide Y potentiates noradrenaline-induced contraction through the neuropeptide Y $Y_1$ receptor. *Eur. J. Pharmacol.,* **316,** 59–64.

BITRAN, M., DANIELS, A.J. & BORIC, M.P. (1997). GW1229, a novel neuropeptide Y $Y_1$ receptor antagonist, inhibits the vasoconstrictor effect on neuropeptide Y in the hamster microcirculation. *Eur. J. Pharmacol.,* **319,** 43–47.

BLOMQVIST, A.G. & HERZOG, H. (1997). Y-receptor subtypes–how many more? *Trends Neurosci.,* **20,** 294–298.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72,** 248–254.

BURKHOFF, A., LINEMEYER, D.L. & SALON, J.A. (1998). Distribution of a novel hypothalamic neuropeptide Y receptor gene and it's absence in rat. *Mol. Brain Res.,* **53,** 311–316.

CADIEUX, A., PHENG, L.H., ST-PIERRE, S., FOURNIER, A. & BENCHEKROUN, M.T. (1993). The rabbit saphenous vein: a tissue preparation specifically enriched in NPY-$Y_1$ receptor subtype. *Regul. Pept.,* **46,** 557–564.

CASTAN, I., VALET, P., VOISIN, T., QUIDEAU, N., LABURTHE, M. & LAFONTAN, M. (1992). Identification and functional studies of a specific peptide YY-preferring receptor in dog adipocytes. *Endocrinology,* **131,** 1970–1976.

CHAURASIA, C., MISSE, G., TESSEL, R. & DOUGHTY, M.B. (1994). Nonpeptide peptidomimetic antagonists of the neuropeptide Y receptor: benextramine analogs with selectivity for the peripheral $Y_2$ receptor. *J. Med. Chem.,* **37,** 2242–2248.

CHEN, G. & VAN DEN POL, A.N. (1996). Multiple NPY receptors coexist in pre- and postsynaptic sites: inhibition of GABA release in isolated self-innervating SCN neurons. *J. Neurosci.,* **16,** 7711–7724.

COLMERS, W.F. (1990). Modulation of synaptic transmission in hippocampus by neuropeptide Y: presynaptic actions. *Ann. N. Y. Acad. Sci.,* **611,** 206–218.

COLMERS, W.F. & BLEAKMAN, D. (1994). Effects of neuropeptide Y on the electrical properties of neurons. *Trends Neurosci.,* **17,** 373–379.

CRISCIONE, L., RIGOLLIER, P., BATZL-HARTMANN, C., RUEGER, H., STRICKER-KRONGRAD, A., WYSS, P., BRUNNER, L., WHITEBREAD, S., YAMAGUCHI, Y., GERALD, C., HEURICH, R.O., WALKER, M.W., CHIESI, M., SCHILLING, W., HOFBAUER, K.G. & LEVENS, N. (1998). Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide $Y_5$ receptor. *J. Clin. Invest.,* **102,** 2136–2145.

DANIELS, A.J., MATTHEWS, J.E., SLEPETIS, R.J., JANSEN, M., VIVEROS, O.H., TADEPALLI, A., HARRINGTON, W., HEYER, D., LANDAVAZO, A. & LEBAN, J.J. (1995a). High-affinity neuropeptide Y receptor antagonists. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 9067–9071.

DANIELS, A.J., MATTHEWS, J.E., VIVEROS, O.H., LEBAN, J.J., CORY, M. & HEYER, D. (1995b). Structure-activity relationship of novel pentapeptide neuropeptide Y receptor antagonists is consistent with a noncontinuous epitope for ligand-receptor binding. *Mol. Pharmacol.,* **48,** 425–432.

DOODS, H., GAIDA, W., WIELAND, HA., DOLLINGER, H., SCHNORRENBERG, G., ESSER, F., ENGEL, W., EBERLEIN, W. & RUDOLF, K. (1999). BIIE0246: A selective and high affinity neuropeptide Y $Y_2$ receptor antagonist. *Eur. J. Pharmacol.,* **384,** R3–R5.

DOODS, H.N., WIELAND, H.A., ENGEL, W., EBERLEIN, W., WILLIM, K.D., ENTZEROTH, M., WIENEN, W. & RUDOLF, K. (1996). BIBP 3226, the first selective neuropeptide $Y_1$ receptor antagonist: a review of its pharmacological properties. *Regul. Pept.,* **65,** 71–77.

DOODS, H.N., WIENEN, W., ENTZEROTH, M., RUDOLF, K., EBERLEIN, W., ENGEL, W. & WIELAND, H.A. (1995). Pharmacological characterization of the selective nonpeptide neuropeptide Y $Y_1$ receptor antagonist BIBP 3226. *J. Pharmacol. Exp. Ther.,* **275,** 136–142.

DUMONT, Y., CADIEUX, A., DOODS, H., FOURNIER, A. & QUIRION, R. (2000b). Potent and selective tools to investigate neuropeptide Y receptors in the central and peripheral nervous systems: BIBO3304 ($Y_1$) and CGP71683A ($Y_5$). *Can. J. Physiol. Pharmacol.,* (In Press)

DUMONT, Y., CADIEUX, A., PHENG, L.H., FOURNIER, A., ST PIERRE, S. & QUIRION, R. (1994). Peptide YY derivatives as selective neuropeptide Y/peptide YY $Y_1$ and $Y_2$ agonists devoided of activity for the $Y_3$ receptor sub-type. *Mol. Brain Res.,* **26,** 320–324.

DUMONT, Y., FOURNIER, A. & QUIRION, R. (1998a). Expression and characterization of the neuropeptide Y $Y_5$ receptor subtype in the rat brain. *J. Neurosci.,* **18,** 5565–5574.

DUMONT, Y., FOURNIER, A., ST-PIERRE, S. & QUIRION, R. (1995). Characterization of neuropeptide Y binding sites in rat brain membrane preparations using [$^{125}$I][Leu$^{31}$,Pro$^{34}$]peptide YY and [$^{125}$I]peptide $YY_{3-36}$ 36 as selective $Y_1$ and $Y_2$ radioligands. *J. Pharmacol. Exp. Ther.,* **272,** 673–680.

DUMONT, Y., FOURNIER, A., ST-PIERRE, S. & QUIRION, R. (1996). Autoradiographic distribution of [$^{125}$I][Leu$^{31}$,Pro$^{34}$]PYY, and [$^{125}$I]$PYY_{3-36}$ binding sites in the rat brain evaluated with two newly developed $Y_1$ and $Y_2$ receptor radioligands. *Synapse,* **22,** 139–158.

DUMONT, Y., JACQUES, D., BOUCHARD, P. & QUIRION, R. (1998b). Species differences in the expression and distribution of the neuropeptide Y $Y_1$, $Y_2$, $Y_4$, and $Y_5$ receptors in rodents, guinea pig, and primates brains. *J. Comp. Neurol.,* **402,** 372–384.

DUMONT, Y., JACQUES, D., ST-PIERRE, J.A., TONG, Y., PARKER, R., HERZOG, H. & QUIRION, R. (2000a). Neuropeptide Y, peptide YY and pancreatic polypeptide receptor proteins and mRNAs in mammalian brains. In: Quirion, R., Hokfelt, T. & Borjklund, A. (eds). *Handbook of Chemical Neuroanatomy*. Elsevier Science Publishers: London. In Press.

DUMONT, Y., MARTEL, J.C., FOURNIER, A., ST-PIERRE, S. & QUIRION, R. (1992). Neuropeptide Y and neuropeptide Y receptor subtypes in brain and peripheral tissues. *Prog. Neurobiol.,* **38,** 125–167.

DUMONT, Y. & QUIRION, R. (2000). [$^{125}$I]GR231118: A high affinity radioligand to investigate neuropeptide Y $Y_1$ and $Y_4$ receptors. *Br. J. Pharmacol.,* **129,** 37–46.

FLOOD, J.F., HERNANDEZ, E.N. & MORLEY, J.E. (1987). Modulation of memory processing by neuropeptide Y. *Brain Res.,* **421,** 280–290.

FOREST, M., MARTEL, J.C., ST-PIERRE, S., QUIRION, R. & FOURNIER, A. (1990). Structural study of the N-terminal segment of neuropeptide tyrosine. *J. Med. Chem.,* **33,** 1615–1619.

FUHLENDORFF, J., GETHER, U., AAKERLUND, L., LANGELAND-JOHANSEN, N., THOGERSEN, H., MELBERG, S.G., OLSEN, U.B., THASTRUP, O. & SCHWARTZ, T.W. (1990). [Leu$^{31}$, Pro$^{34}$]neuropeptide Y: a specific $Y_1$ receptor agonist. *Proc. Natl. Acad. Sci. U.S.A.,* **87,** 182–186.

GEHLERT, D.R. (1998). Multiple receptors for the pancreatic polypeptide (PP-fold) family: physiological implications. *Proc. Soc. Exp. Biol. Med.,* **218,** 7–22.

GEHLERT, D.R., BEAVERS, L.S., JOHNSON, D., GACKENHEIMER, S.L., SCHOBER, D.A. & GADSKI, R.A. (1996c). Expression cloning of a human brain neuropeptide Y $Y_2$ receptor. *Mol. Pharmacol.,* **49,** 224–228.

GEHLERT, D.R., GACKENHEIMER, S.L., SCHOBER, D.A., BEAVERS, L., GADSKI, R., BURNETT, J.P., MAYNE, N., LUNDELL, I. & LARHAMMAR, D. (1996b). The neuropeptide Y $Y_1$ receptor selective radioligand, [$^{125}$I][Leu$^{31}$,Pro$^{34}$]peptide YY, is also a high affinity radioligand for human pancreatic polypeptide 1 receptors. *Eur. J. Pharmacol.,* **318,** 485–490.

GEHLERT, D.R., SCHOBER, D.A., BEAVERS, L., GADSKI, R., HOFFMAN, J.A., SMILEY, D.L., CHANCE, R.E., LUNDELL, I. & LARHAMMAR, D. (1996a). Characterization of the peptide binding requirements for the cloned human pancreatic polypeptide-preferring receptor. *Mol. Pharmacol.,* **50,** 112–118.

GERALD, C., WALKER, M.W., CRISCIONE, L., GUSTAFSON, E.L., BATZL-HARTMANN, C., SMITH, K.E., VAYSSE, P., DURKIN, M.M., LAZ, T.M., LINEMEYER, D.L., SCHAFFHAUSER, A.O., WHITEBREAD, S., HOFBAUER, K.G., TABER, R.I., BRANCHEK, T.A. & WEINSHANK, R.L. (1996). A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature,* **382,** 168–171.

GERALD, C., WALKER, M.W., VAYSSE, P.J., HE, C., BRANCHEK, T.A. & WEINSHANK, R.L. (1995). Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY $Y_2$ receptor subtype. *J. Biol. Chem.,* **270,** 26758–26761.

GLAUM, S.R., MILLER, R.J., RHIM, H., MACLEAN, D., GEORGIC, L.M., MACKENZIE, R.G. & GRUNDEMAR, L. (1997). Characterization of $Y_3$ receptor-mediated synaptic inhibition by chimeric neuropeptide Y-peptide YY peptides in the rat brainstem. *Br. J. Pharmacol.,* **120,** 481–487.

GOLOMBEK, D.A., BIELLO, S.M., RENDON, R.A. & HARRINGTON, M.E. (1996). Neuropeptide Y phase shifts the circadian clock in vitro via a $Y_2$ receptor. *Neuroreport,* **7,** 1315–1319.

GREGOR, P., FENG, Y., DECARR, L.B., CORNFIELD, L.J. & MCCALEB, M.L. (1996). Molecular characterization of a second mouse pancreatic polypeptide receptor and its inactivated human homologue. *J. Biol. Chem.,* **271,** 27776–27781.

GROUZMANN, E., BUCLIN, T., MARTIRE, M., CANNIZZARO, C., DORNER, B., RAZANAME, A. & MUTTER, M. (1997). Characterization of a selective antagonist of neuropeptide Y at the $Y_2$ receptor. Synthesis and pharmacological evaluation of a $Y_2$ antagonist. *J. Biol. Chem.,* **272,** 7699–7706.

GROUZMANN, E., BUCLIN, T., MARTIRE, M., CANNIZZARO, C., DORNER, B., RAZANAME, A. & MUTTER, M. (1998). Characterization of a selective antagonist of neuropeptide Y at the $Y_2$ receptor. Synthesis and pharmacological evaluation of a $Y_2$ antagonist [Erratum]. *J. Biol. Chem.,* **273,** 7699.

GRUNDEMAR, L., WAHLESTEDT, C. & REIS, D.J. (1991). Neuropeptide Y acts at an atypical receptor to evoke cardiovascular depression and to inhibit glutamate responsiveness in the brainstem. *J. Pharmacol. Exp. Ther.,* **258,** 633–638.

HEILIG, M., MCLEOD, S., BROT, M., HEINRICHS, S.C., MENZAGHI, F., KOOB, G.F. & BRITTON, K.T. (1993). Anxiolytic-like action of neuropeptide Y: mediation by $Y_1$ receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology,* **8,** 357–363.

HEILIG, M. & WIDERLOV, E. (1995). Neurobiology and clinical aspects of neuropeptide Y. *Crit. Rev. Neurobiol.,* **9,** 115–136.

HIPSKIND, P.A., LOBB, K.L., NIXON, J.A., BRITTON, T.C., BRUNS, R.F., CATLOW, J., DIECKMAN-MCGINTY, D.K., GACKENHEIMER, S.L., GITTER, B.D., IYENGAR, S., SCHOBER, D.A., SIMMONS, R.M., SWANSON, S., ZARRINMAYEH, H., ZIMMERMAN, D.M. & GEHLERT, D.R. (1997). Potent and selective 1,2,3-trisubstituted indole NPY Y-1 antagonists. *J. Med. Chem.,* **40,** 3712–3714.

HUHMAN, K.L., GILLESPIE, C.F., MARVEL, C.L. & ALBERS, H.E. (1996). Neuropeptide Y phase shifts circadian rhythms in vivo via a $Y_2$ receptor. *Neuroreport,* **7,** 1249–1252.

INUI, A. (1999). Neuropeptide Y feeding receptors: are multiple subtypes involved? *Trends Pharmacol. Sci.,* **20,** 43–46.

JACQUES, D., CADIEUX, A., DUMONT, Y. & QUIRION, R. (1995). Apparent affinity and potency of BIBP3226, a non-peptide neuropeptide Y receptor antagonist, on purported neuropeptide Y $Y_1$, $Y_2$ and $Y_3$ receptors. *Eur. J. Pharmacol.,* **278,** R3–R5.

JACQUES, D., DUMONT, Y., FOURNIER, A. & QUIRION, R. (1997). Characterization of neuropeptide Y receptor subtypes in the normal human brain, including the hypothalamus. *Neuroscience,* **79,** 129–148.

JOLICOEUR, F.B., MICHAUD, J.N., MENARD, D. & FOURNIER, A. (1991b). In vivo structure activity study supports the existence of heterogeneous neuropeptide Y receptors. *Brain Res. Bull.,* **26,** 309–311.

JOLICOEUR, F.B., MICHAUD, J.N., RIVEST, R., MENARD, D., GAUDIN, D., FOURNIER, A. & ST-PIERRE, S. (1991a). Neurobehavioral profile of neuropeptide Y. *Brain Res. Bull.,* **26,** 265–268.

KALRA, S.P. & CROWLEY, W.R. (1984). Norepinephrine-like effects of neuropeptide Y on LH release in the rat. *Life Sci.,* **35,** 1173–1176.

KALRA, S.P. & CROWLEY, W.R. (1992). Neuropeptide Y: a novel neuroendocrine peptide in the control of pituitary hormone secretion, and its relation to luteinizing hormone. *Front Neuroendocrinol.,* **13,** 1–46.

KANATANI, A., FUKAMI, T., FUKURODA, T., IWAASA, H., MACNEIL, D.J. & VAN DER PLOEG, L.H. (1997). $Y_5$ receptors are not involved in physiologically relevant feeding in rodents. *Regul. Pept.,* **71,** 212.

KANATANI, A., ITO, J., ISHIHARA, A., IWAASA, H., FUKURODA, T., FUKAMI, T., MACNEIL, D.J., VAN DER PLOEG, L.H. & IHARA, M. (1998). NPY-induced feeding involves the action of a $Y_1$-like receptor in rodents. *Regul. Pept.,* **75–76,** 409–415.

KLAPSTEIN, G.J. & COLMERS, W.F. (1993). On the sites of presynaptic inhibition by neuropeptide Y in rat hippocampus in vitro. *Hippocampus,* **3,** 103–111.

KLAPSTEIN, G.J. & COLMERS, W.F. (1997). Neuropeptide Y suppresses epileptiform activity in rat hippocampus in vitro. *J. Neurophysiol.,* **78,** 1651–1661.

LABURTHE, M. (1990). Peptide YY and neuropeptide Y in the gut: availability, biological actions and receptors. *Trends Endocrinol. Meta.,* **1,** 168–174.

LABURTHE, M., CHENUT, B., ROUYER-FESSARD, C., TATEMOTO, K., COUVINEAU, A., SERVIN, A. & AMIRANOFF, B. (1986). Interaction of peptide YY with rat intestinal epithelial plasma membranes: binding of the radioiodinated peptide. *Endocrinology,* **118,** 1910–1917.

LARHAMMAR, D. (1996). Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. *Regul. Pept.,* **65,** 165–174.

LEBAN, J.J., HEYER, D., LANDAVAZO, A., MATTHEWS, J., AULABAUGH, A. & DANIELS, A.J. (1995). Novel modified carboxy terminal fragments of neuropeptide Y with high affinity for $Y_2$-type receptors and potent functional antagonism at a $Y_1$- type receptor. *J. Med. Chem.,* **38,** 1150–1157.

LUNDBERG, J.M. & MODIN, A. (1995). Inhibition of sympathetic vasoconstriction in pigs in vivo by the neuropeptide Y-$Y_1$ receptor antagonist BIBP 3226. *Br. J. Pharmacol.,* **116,** 2971–2982.

LUNDBERG, J.M., MODIN, A. & MALMSTROM, R.E. (1996). Recent developments with neuropeptide Y receptor antagonists. *Trends Pharmacol. Sci.,* **17,** 301–304.

MALMSTROM, R.E., BALMER, K.C. & LUNDBERG, J.M. (1997). The neuropeptide Y (NPY) $Y_1$ receptor antagonist BIBP 3226: equal effects on vascular responses to exogenous and endogenous NPY in the pig in vivo. *Br. J. Pharmacol.,* **121,** 595–603.

MARTEL, J.C., FOURNIER, A., ST-PIERRE, S., DUMONT, Y., FOREST, M. & QUIRION, R. (1990). Comparative structural requirements of brain neuropeptide Y binding sites and vas deferens neuropeptide Y receptors. *Mol. Pharmacol.,* **38,** 494–502.

MATSUMOTO, M., NOMURA, T., MOMOSE, K., IKEDA, Y., KONDOU, Y., AKIHO, H., TOGAMI, J., KIMURA, Y., OKADA, M. & YAMAGUCHI, T. (1996). Inactivation of a novel neuropeptide Y/peptide YY receptor gene in primate species. *J. Biol. Chem.,* **271,** 27217–27220.

MCQUISTON, A.R. & COLMERS, W.F. (1996). Neuropeptide $Y_2$ receptors inhibit the frequency of spontaneous but not miniature EPSCs in CA3 pyramidal cells of rat hippocampus. *J. Neurophysiol.,* **76,** 3159–3168.

MICHEL, M.C., BECK-SICKINGER, A., COX, H., DOODS, H.N., HERZOG, H., LARHAMMAR, D., QUIRION, R., SCHWARTZ, T. & WESTFALL, T. (1998). XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol. Rev.,* **50,** 143–150.

MODIN, A. (1994). Non-adrenergic, non-cholinergic vascular control with reference to neuropeptide Y, vasoactive intestinal polypeptide and nitric oxide. *Acta Physiol. Scand. Suppl.,* **622,** 1–74.

MORLEY, J.E. & FLOOD, J.F. (1989). The effect of neuropeptide Y on drinking in mice. *Brain Res.,* **494,** 129–137.

NARVAEZ, J.A., AGUIRRE, J.A. & FUXE, K. (1993). Subpicomolar amounts of NPY(13-36) injected into the nucleus tractus solitarius of the rat counteract the cardiovascular responses to L-glutamate. *Neurosci. Lett.,* **151,** 182–186.

NAVEILHAN, P., HASSANI, H., CANALS, J.M., EKSTRAND, A.J., LAREFALK, A., CHHAJLANI, V., ARENAS, E., GEDDA, K., SVENSSON, L., THOREN, P. & ERNFORS, P. (1999). Normal feeding behavior, body weight and leptin response require the neuropeptide Y $Y_2$ receptor. *Nature Med.,* **5,** 1188–1193.

NILSSON, T., CANTERA, L. & EDVINSSON, L. (1996a). Presence of neuropeptide Y $Y_1$ receptor mediating vasoconstriction in human cerebral arteries. *Neurosci. Lett.,* **204,** 145–148.

NILSSON, T., YOU, J., SUN, X., HEDNER, T. & EDVINSSON, L. (1996b). Characterization of neuropeptide Y receptors mediating contraction, potentiation and inhibition of relaxation. *Blood Press.,* **5,** 164–169.

PARKER, E.M., BABIJ, C.K., BALASUBRAMANIAM, A., BURRIER, R.E., GUZZI, M., HAMUD, F., MUKHOPADHYAY, G., RUDINSKI, M.S., TAO, Z., TICE, M., XIA, L., MULLINS, D.E. & SALISBURY, B.G. (1998). GR231118 (1229U91) and other analogues of the C-terminus of neuropeptide Y are potent neuropeptide Y $Y_1$ receptor antagonists and neuropeptide Y $Y_4$ receptor agonists. *Eur. J. Pharmacol.,* **349,** 97–105.

PHENG, L.H., FOURNIER, A., DUMONT, Y., QUIRION, R. & REGOLI, D. (1997). The dog saphenous vein: a sensitive and selective preparation for the $Y_2$ receptor of neuropeptide Y. *Eur. J. Pharmacol.,* **327,** 163–167.

PHENG, L.H., PERRON, A., QUIRION, R., CADIEUX, A., FAUCHERE, J.L., DUMONT, Y. & REGOLI, D. (1999). Neuropeptide Y-induced contraction is mediated by $Y_2$ and $Y_4$ receptors in the rat colon. *Eur. J. Pharmacol.,* **374,** 85–91.

QIAN, J., COLMERS, W.F. & SAGGAU, P. (1997). Inhibition of synaptic transmission by neuropeptide Y in rat hippocampal area CA1: modulation of presynaptic $Ca^{2+}$ entry. *J. Neurosci.,* **17,** 8169–8177.

QUIRION, R., ROBITAILLE, Y., MARTIAL, J., CHABOT, J.G., LEMOINE, P., PILAPIL, C. & DALPE, M. (1988). Human brain receptor autoradiography using whole hemisphere sections: a general method that minimizes tissues artefacts. *Synapse,* **1,** 446–454.

RACCHI, H., SCHLIEM, A.J., DONOSO, M.V., RAHMER, A., ZUSIGA, A., GUZMAN, S., RUDOLF, K. & HUIDOBRO-TORO, J.P. (1996). Neuropeptide Y $Y_1$ receptors are involved in the vasoconstriction caused by human sympathetic nerve stimulation. *Eur. J. Pharmacol.,* **329,** 79–83.

RUDOLF, K., EBERLEIN, W., ENGEL, W., WIELAND, H.A., WILLIM, K.D., ENTZEROTH, M., WIENEN, W., BECK-SICKINGER, A.G. & DOODS, H.N. (1994). The first highly potent and selective non-peptide neuropeptide Y $Y_1$ receptor antagonist: BIBP3226. *Eur. J. Pharmacol.,* **271,** R11–R13.

SCHOBER, D.A., VAN ABBEMA, A.M., SMILEY, D.L., BRUNS, R.F. & GEHLERT, D.R. (1998). The neuropeptide Y $Y_1$ antagonist, 1229U91, a potent agonist for the human pancreatic polypeptide-preferring (NPY $Y_4$) receptor. *Peptides,* **19,** 537–542.

SCHWARTZ, T.W., FUHLENDORFF, J., KJEMS, L.L., KRISTENSEN, M.S., VERVELDE, M., O'HARE, M., KRSTENANSKY, J.L. & BJORNHOLM, B. (1990). Signal epitopes in the three-dimensional structure of neuropeptide Y. Interaction with $Y_1$, $Y_2$, and pancreatic polypeptide receptors. *Ann. N.Y. Acad. Sci.,* **611,** 35–47.

SERRADEIL-LE, GAL C., VALETTE, G., ROUBY, P.E., PELLET, A., OURY-DONAT, F., BROSSARD, G., LESPY, L., MARTY, E., NELIAT, G. & DE COINTET, P. (1995). SR 120819A, an orally-active and selective neuropeptide Y $Y_1$ receptor antagonist. *FEBS Lett.,* **362,** 192–196.

SERVIN, A.L., ROUYER-FESSARD, C., BALASUBRAMANIAM, A., SAINT, P.S. & LABURTHE, M. (1989). Peptide-YY and neuropeptide-Y inhibit vasoactive intestinal peptide- stimulated adenosine 3′,5′-monophosphate production in rat small intestine: structural requirements of peptides for interacting with peptide-YY-preferring receptors. *Endocrinology,* **124,** 692 – 700.

STANLEY, B.G. & LEIBOWITZ, S.F. (1984). Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci.,* **35,** 2635 – 2642.

TONG, Y., DUMONT, Y., SHEN, S.H., HERZOG, H., SHINE, J. & QUIRION, R. (1995). Expression of the neuropeptide Y $Y_1$ receptor in human embryonic kidney 293 cells: ligand binding characteristics, in situ hybridization and receptor autoradiography. *Mol. Brain Res.,* **34,** 303 – 308.

TOUGH, I.R. & COX, H.M. (1996). Selective inhibition of neuropeptide Y $Y_1$ receptors by BIBP3226 in rat and human epithelial preparations. *Eur. J. Pharmacol.,* **310,** 55 – 60.

VEZZANI, A., SPERK, G. & COLMERS, W.F. (1999). Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends Neurosci.,* **22,** 25 – 30.

WAHLESTEDT, C. & REIS, D.J. (1993). Neuropeptide Y-related peptides and their receptors – are the receptors potential therapeutic drug targets? *Annu. Rev. Pharmacol. Toxicol.,* **33,** 309 – 352.

WAHLESTEDT, C., YANAIHARA, N. & HAKANSON, R. (1986). Evidence for different pre-and post-junctional receptors for neuropeptide Y and related peptides. *Regul. Pept.,* **13,** 307 – 318.

WIELAND, H.A., ENGEL, W., EBERLEIN, W., RUDOLF, K. & DOODS, H.N. (1998). Subtype selectivity of the novel nonpeptide neuropeptide Y $Y_1$ receptor antagonist BIBO 3304 and its effect on feeding in rodents. *Br. J, Pharmacol.,* **125,** 549 – 555.

WIELAND, H.A., WILLIM, K. & DOODS, H.N. (1995). Receptor binding profiles of NPY analogues and fragments in different tissues and cell lines. *Peptides,* **16,** 1389 – 1394.

WOLDBYE, D.P. (1998). Antiepileptic effects of NPY on pentylenetetrazole seizures. *Regul. Pept.,* **75 – 76,** 279 – 282.

WOLDBYE, D.P., LARSEN, P.J., MIKKELSEN, J.D., KLEMP, K., MADSEN, T.M. & BOLWIG, T.G. (1997). Powerful inhibition of kainic acid seizures by neuropeptide Y via $Y_5$- like receptors. *Nat. Med.,* **3,** 761 – 764.

ZUKOWSKA-GROJEC, Z., DAYAO, E.K., KARWATOWASKA-PROKOPCZUK, E., HAUSER, G.J. & DOODS, H.N. (1996). Stress-induced mesenteric vasoconstriction in rats is mediated by neuropeptide Y $Y_1$ receptors. *Am,. J. Physiol.,* **270,** H796 – H800.

*(Received September 20, 1999*
*Revised November 25, 1999*
*Accepted December 13, 1999)*