Design and discovery of neuropeptide Y_1 receptor antagonists

Jon Wright

Neuropeptide Y (NPY) has been the focus of much attention since its discovery as the most abundant peptide in mammalian brain. It has been implicated in many roles in both the periphery and the CNS. The first cloned receptor subtype, Y_1, has been identified as an important mediator for many of these effects. Potent, selective Y_1 receptor antagonists have been developed and have certainly helped clarify the peripheral roles of NPY and the receptors involved. However, there is still a need for Y_1 antagonists that can be administered peripherally, even orally, and penetrate the CNS. Such compounds would help define the roles of NPY and Y_1 receptors in the CNS, an area rich in potential for novel drug therapy.

Neuropeptide Y (NPY) is a 36-amino-acid polypeptide that was isolated in 1982 from porcine brain[1]. It is a member of the pancreatic polypeptide (PP) family of peptides and shares high structural similarity and sequence homology with PP and peptide YY (PYY)[2]. The amino acid sequence of porcine NPY is shown in Figure 1.

There is evidence that NPY can exist as a dimer in solution[3]. The secondary structure is characterized by a polyproline helix comprising residues 1–10, a tight hairpin bend comprising residues 11–14, and an α-helix comprising residues 15–26 (Ref. 4). The C-terminus was originally thought to be undefined in solution; now evidence shows that the helical nature extends all the way to residue 36 (Ref. 5). It was also believed that both the C- and N-termini of NPY were necessary for Y_1 receptor binding[6] and that the helices and bend served to orientate these termini. These observations show a good correlation with the model of avian PP suggested by Allen[7].

The physiological effects of NPY

Neuropeptide Y is present in a highly conserved manner across species[8,9] and is abundant in mammalian brain and peripheral nervous system. Accordingly, NPY is involved in a variety of peripheral and central processes. It is a potent vasoconstrictor at coronary and cerebral arteries and can potentiate the effects of other vasoconstrictors such as noradrenaline. It is also involved in many central effects, including analgesia, anxiolysis and feeding regulation[10] (for reviews see Refs 11,12).

NPY receptor subtypes

Differential effects of NPY analogs suggested the existence of Y_1, Y_2 and Y_3 receptor subtypes (see Table 1)[13–15]. The Y_1 receptor was cloned in 1992 (Ref. 16) and the Y_2 receptor[17,18] and the Y_4/PP_1 receptor in 1995 (Ref. 19). Very recently, two Y_5 receptors have been reported. The first of these, reported by Synaptic[20], seems to be associated with the feeding effects of NPY and may be the long-awaited 'feeding receptor'. The second, reported by Merck[21], appears to be distinct from the first, thus these receptors may have to be given separate designations. Bayer have cloned a mouse receptor that is highly homologous to the

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Neuropeptide Y	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2
PYX-1	Tyr(2,6-dichlorobenzyl)-Ile-Asn-Leu-Ile-DThr-Arg-Gln-Arg-Tyr-NH_2
PYX-2	Tyr(2,6-dichlorobenzyl)-Ile-Asn-Leu-Ile-DThr-Arg-Gln-Arg-Tyr-(2,6-dichlorobenzyl)-NH_2

Figure 1. *Amino acid sequence of neuropeptide Y (NPY), PYX-1 and PYX-2.*

Y_4/PP_1 receptor, and is currently labelled $Y_{R\text{-}D}$ (Ref. 22). The activity of NPY analogs at some of these cloned receptors agrees with the historical assignments (see Table 2). Use of these selective analogs suggested that the Y_1 receptor was the mediator of several of the important effects of NPY, especially cardiovascular effects. The Y_1 receptor has received the most attention during the past years, although the availability of other cloned NPY receptors should change this. This review outlines the progress towards potent, selective Y_1 receptor antagonists as pharmacological tools and ultimately drug development candidates. The potential roles of Y_1 receptors are also discussed.

Early non-receptor-mediated antagonists

PP-56

Several functional antagonists of NPY-induced effects have been reported. These compounds do not interact with known NPY receptors and are believed to exert their actions elsewhere. PP-56 (D-*myo*-inositol 1,2,6-trisphosphate; α-trinositol) blocks NPY-induced contraction of the guinea pig basilar artery[23,24]. In addition, PP-56 is a selective antagonist of NPY-induced pressor responses in the pithed rat[25]. However, PP-56 does not affect [^{125}I]NPY binding to Y_1 receptors[26], or to Y_2 receptors[27], ruling out possible antagonist effects at known Y_1 or Y_2 receptors.

Table 1. Historical (pre-1995) rank order of potency of NPY family analogs on various NPY effects and the receptor subtype assignment

Receptor	Rank order of potency
Y_1	[Pro34]NPY = NPY = PYY >> NPY_{13-36} >> PP
Y_2	PYY ≥ NPY > NPY_{3-36} >> [Pro34]NPY, PP
Y_3	NPY ≥ [Pro34]NPY ≥ NPY_{3-36} >> PYY, PP

NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY.

PYX-1 and PYX-2

Tatemoto and coworkers introduced two novel NPY antagonists obtained by screening mixtures of NPY analogs[28]. These compounds, designated PYX-1 and PYX-2 (Figure 1), displaced [^{3}H]NPY from rat brain membranes. PYX-2 was later shown to block both normal and NPY-induced feeding in rats after injection into the paraventricular nucleus[29]. However, in a subsequent study, PYX-2 failed to decrease overeating in obese Zucker rats[30]. Although protocol differences may explain these apparently conflicting results, the later report assigns the lack of effect to the weak affinity of PYX-2 for Y_1 receptors. PYX-2 does not inhibit Y_2 receptors in the isolated rat prostatic vas deferens[31]. While PYX-1 and PYX-2 appear to exert their effects at NPY receptors, the exact subtype(s) involved has not been reported, although Wieland and coworkers also concluded that PYX-2 has weak affinity for Y_1 receptors[32].

The first nonpeptide Y_1 antagonists

Benextramine and CC2137

Benextramine (Figure 2), an irreversible α_1-adrenoceptor ligand, was one of the first nonpeptide compounds found to possess weak NPY receptor antagonist activity[33]. Benextramine also binds irreversibly to NPY receptors, suggesting that covalent bonds are formed between the ligand and the receptor. While later studies suggested that benextramine bound selectively to Y_1 receptors[34], a close analog was a selective, competitive antagonist at Y_2 receptors in rat femoral artery[35]. Benextramine has been overshadowed by much more potent Y_1 antagonists, but it has served to raise the question of Y_1 receptor heterogeneity. The Y_1-selective agonist [Leu31,Pro34]NPY contracts rabbit saphenous vein and inhibits electrically stimulated twitching in rabbit vas deferens[36]. Benextramine blocks the first effect but not the second. The Y_2-selective agonist NPY_{8-36} has similar effects in both tests

Table 2. Potency (EC_{50}) of PP family and analogs on forskolin-stimulated cAMP accumulation in LMTK$^-$ cells transfected with rat Y_1, Y_2 or Y_4 receptors, or 293 cells transfected with rat Y_5 receptors[20]

	EC_{50} (nM)			
	Y_1	Y_2	Y_4/PP_1	Y_5
Human NPY	0.14	1.2	>1000	0.96
Porcine [Leu31,Pro34]NPY	0.15	>1000	7.1	1.2
Human PYY	0.70	0.58	>1000	1.0
Human PYY_{3-36}	>1000	0.64	>1000	4.2
Human [Pro34]PYY	0.37	>1000	6.0	1.3
Human PP	150	>1000	0.037	1.4
Porcine NPY_{13-36}	300	2.2	>1000	20

NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY.

but benextramine does not block either effect. These results suggest that the two preparations contain Y_2 receptors and different Y_1 receptor subtypes and that benextramine is discriminating between them. However, recent binding data reveal that benextramine has a K_i of 2 μM at cloned human Y_1 and Y_4 receptors while being a little weaker at Y_2 receptors (K_i = 7.5 μM)[37]. Benextramine also has submicromolar affinity for α-adrenoceptors and dopamine receptors, which limits its use as an NPY ligand *in vivo*. An analog of benextramine, CC2137 (Figure 2), has an approximately tenfold greater affinity for NPY receptors *in vitro*, but again this compound has significant affinity for α-adrenoceptors and dopamine and serotonin receptors. CC2137 binds reversibly to NPY receptors, suggesting that the disulfide link of benextramine is responsible for its irreversible binding.

Arpromidine

A second early series of nonpeptide antagonists was derived from the histamine H_2 agonist arpromidine (Figure 2)[38]. This compound binds weakly to Y_1 receptors. Studies revealed that it was possible to separate Y_1 receptor activity from histamine activity, although the most potent, selective compounds from this series still had micromolar affinity for Y_1 receptors and comparable H_1 antagonist activity. The weak, non-selective activity displayed by these early compounds has undoubtedly fuelled the search for more potent and selective nonpeptide Y_1 antagonists.

The rational design of NPY antagonists from NPY

Early studies identified [Leu31,Pro34]NPY as a selective Y_1 agonist and NPY_{3-36} as a selective Y_2 agonist. Minor modifications of NPY have also produced NPY receptor antagonists. For example, replacement of the Thr in NPY position 32 with D-Trp ([DTrp32]NPY) gave an NPY antagonist. [DTrp32]NPY inhibited [^{125}I]NPY binding to rat hypothalamic membranes and shifted the dose–response curve of NPY on isoproterenol-stimulated rat hypothalamic membrane adenylate cyclase activity to the right[39]. [DTrp32]NPY can competitively antagonize NPY-induced feeding in rats but did not bind to Y_1 receptors. These large peptides are likely to have short half-lives and do not penetrate the blood–brain barrier, limiting their use as tools and making them unlikely drug candidates. Many large endogenous peptides and enzyme substrates have been reduced to small active 'fragments'. These may be as little as a 3- or 4-amino-acid portion of the original substrate. However, it has not been as easy to find a small, active fragment of NPY. Indeed, the smallest non-substituted fragment of NPY found to have potent receptor activity is NPY_{8-36}, a selective Y_2 receptor antagonist[40]. The extensive NPY fragmentation and substitution studies have been reviewed[41], and it was concluded that the C-terminus of NPY is necessary for NPY receptor binding. For example, individual

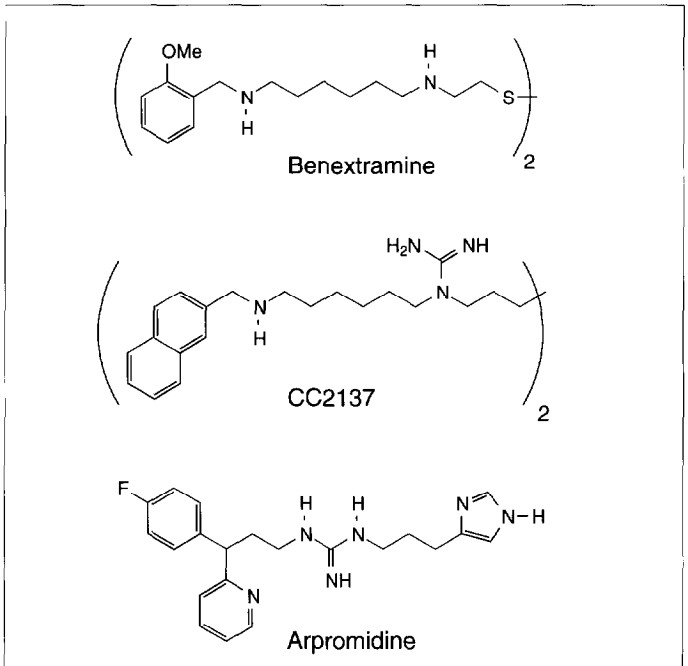

Figure 2. *The first nonpeptide NPY_1 antagonists.*

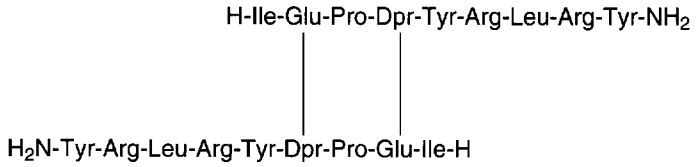

Figure 3. *Chemical structure of 1229U91. Dpr, 2,3-diaminopropionic acid.*

D-residue substitutions in the 29–35 positions each produced a peptide with K_i > 300 nM for the Y_1 receptor[42]. The N-terminus also seemed important, and small agonists were prepared by linking NPY_{1-4} via a spacer to NPY_{25-36} (Ref. 43). This result confirms that the central, α-helical portion of NPY serves mainly to orientate the two termini regions. Other 'centrally truncated' analogs of [DTrp32]NPY were found to be Y_1 receptor antagonists with weak to moderate activity[44]. The breakthrough came with the preparation of dimeric C-terminal NPY analogs. Bis(31/31′)-[Cys31,Trp32,Nva34]NPY_{1-36} (Nva = norvaline) is a moderately potent, selective Y_1 receptor antagonist[45]. The Trp32 and Nva34 modifications appear vital for selective Y_1 receptor antagonist activity. This compound did not affect feeding in rats after intrahypothalamic administration.

Glaxo-Wellcome 1229U91/GW1229

NPY_{3-36} has high affinity for rat brain Y_2 receptors, whereas NPY_{2-36} is essentially inactive (IC_{50} = 15 nM versus >100,000 nM, respectively). In an attempt to discover a Y_2-selective pentapeptide based on the C-terminus of NPY, His-Arg-Leu-Arg-Tyr-NH_2 was found to be a moderately potent Y_2 antagonist (IC_{50} = 0.5 μM)[46]. Unfortunately, this compound also had activity at Y_1 receptors. Extending the peptide further into the α-helix of NPY led to Tyr-Ile-Asn-Leu-Ile-Tyr-Arg-Leu-Arg-Tyr-NH_2 (NPY_{7-36}), a potent Y_2 agonist (ED_{50} = 8.7 nM). In an attempt to stabilize a backbone turn, Leu30 was replaced by Pro ([Pro30]NPY_{7-36}). This compound was slightly weaker at Y_2 receptors (40 nM), but was now an antagonist. Removing Tyr27 gave an analog, [Pro30]NPY_{28-36}, with potent binding to Y_1 receptors (IC_{50} = 5 nM). In order to improve potency further, an attempt was made to further lock the proline-induced turn by linking positions 29 and 31. Thus Asn29 was replaced by Glu and Ile31 by 2,3-diaminopropionic acid (Dpr). Attempts to form the bridge between these two residues gave low yields of the desired bridged species and mainly dimeric product. The bridged species had disappointing Y_1 receptor affinities but the dimeric by-products were extremely potent Y_1 ligands. One dimer, code name 1229U91 (Figure 3), has subnanomolar affinity for Y_1 receptors and micromolar affinity for Y_2 receptors[47]. As a demonstration of Y_1 receptor antagonist ability, 1229U91 inhibits NPY-induced increase in cytosolic calcium in human erythroleukemia (HEL) cells with an IC_{50} of 0.3 nM and blocks NPY-induced constriction of the isolated rat kidney. *In vivo*, 1229U91 produced dose-dependent inhibition of the pressor effect of NPY in rats but had no effect on the NPY inhibition of the electrically evoked twitch response in rat vas deferens, confirming the Y_1 versus Y_2 selectivity[48,49]. Disappointingly, 1229U91 had no effect on resting blood pressure in rats. Centrally, 1229U91 was reported to completely block NPY-induced feeding after intracerebroventricular administration in rats[50]. [^{125}I]PYY does not detect Y_1 receptors in rat hypothalamus; however, [^{125}I]1229U91 reveals high-affinity binding sites[51]. This suggests that 1229U91 is binding to sites other than Y_1 receptors in rat hypothalamus.

The move towards high potency, nonpeptide Y_1 antagonists

BIBP3226

The Karl Thomae company has reported a modified peptide as a Y_1 antagonist[52]. This compound, code name BIBP3226 (Figure 4), was derived from the C-terminal region of NPY. BIBP3226 contains D-Arg, believed to overlap with Arg33 of NPY. Indeed, comparison of NPY and BIBP3226 binding to various mutated Y_1 receptors indicates that NPY and BIBP3226 share an overlapping binding site[53]. BIBP3226 displaced [^{125}I]NPY with a K_i of 7.2 nM from human neuroblastoma (SK-N-MC) cells, which intrinsically express the Y_1 receptor (for a review of the pharmacology of BIBP3226, see Doods and coworkers[54]). The structural requirements for activity were very stringent. For example, the (*S*)-enantiomer, BIBP3435, was essentially inactive at Y_1 receptors (K_i > 10,000 nM)[55]. Replacement of diphenylacetic acid with phenylacetic acid reduced activity over 40-fold. BIBP3226 did not have any effect on blood pressure in pithed rats but blocked the NPY-induced rise in blood pressure. BIBP3226 also blocked NPY-induced vasoconstriction in human cerebral arteries[56,57]. That [Leu31,Pro34]NPY (Y_1-selective agonist) had much greater effects than the Y_2-selective agonist, NPY_{3-36}, in this paradigm suggests that the effect is Y_1-mediated and that BIBP3226 can behave as a Y_1 receptor antagonist *in vivo*.

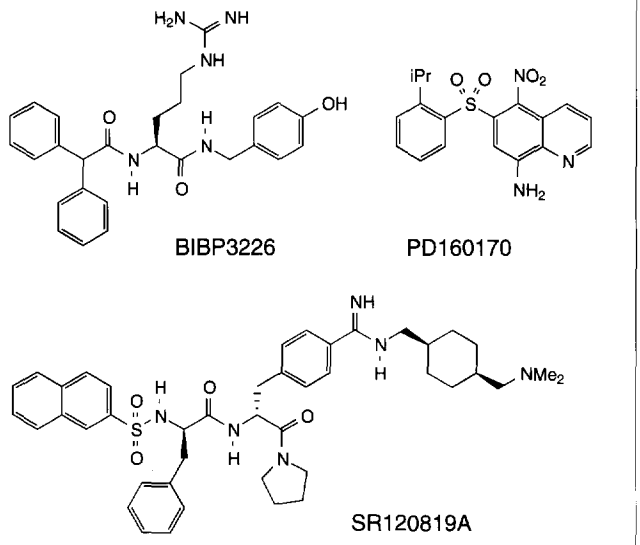

Figure 4. *High-potency, nonpeptide NPY_1 antagonists.*

BIBP3226 did not reduce blood pressure in normal or spontaneously hypertensive rats, but inhibited the blood pressure increase produced by subjecting rats to stress. While BIBP3226 is a very important milestone in Y_1 receptor antagonist discovery, it has a short half-life *in vivo*. Thus it may still be too peptide-like for uses other than as a pharmacological tool.

Sanofi SR120819A/SR120107A

One of the first orally active Y_1 antagonists to be reported is Sanofi's SR120819A (Figure 4)[58]. This compound has (*R,R*) stereochemistry; the (*S,R*)-isomer (SR120107A) is slightly less potent[59]. SR120819A was supposedly designed as a mimic of the C-terminus of NPY. SR120819A is not quite as potent as the peptide ligands (for Y_1, K_i = 15 nM), but it is highly selective versus Y_2 and Y_3 receptor affinity. *In vivo*, SR120819A displays typical Y_1 receptor antagonist activity. After oral administration, it blocks the pressor response of [Leu31,Pro34]NPY in guinea pigs. To date, no bioavailability data have been reported. Malmström's group have reported that the isomer, SR120107A, has a variety of Y_1 receptor-mediated effects in pigs[60,61]. For example, it potently inhibits PYY-induced vasoconstriction in the kidney. These workers noted that SR120107A and BIBP3226 have largely similar effects, but that SR120107A has a longer duration of action.

Parke-Davis PD160170

A series of moderately potent, selective Y_1 antagonists was introduced recently by Parke-Davis[62]. These compounds are unique in structure as they were discovered via random screening and are not obviously related to the structure of any portion of NPY. A representative compound, PD160170 (Figure 4), bound to Y_1 receptors with a K_i of 48 nM and had no affinity for Y_2 receptors up to 10,000 nM. These compounds blocked the inhibition by NPY of adenylate cyclase activity in SK-N-MC cells, demonstrating antagonist activity. Unfortunately, the compounds are highly crystalline; the resulting low solubility is likely to limit their use as NPY receptor tools or drugs.

Recent patent applications

A number of recent patent applications have described novel NPY antagonists. However, without supporting publications, it is often not clear what the most potent and selective compounds are. Eli Lilly[63,64] and Pfizer[65] have filed patents claiming novel nonpeptide Y_1 antagonists; representative compounds (IC_{50} approx. 10 μM and 39 nM, respectively) are shown in Figure 5.

So, what are Y_1 antagonists good for?

There are several reports that Y_1 receptor antagonists block NPY-induced hypertension but have no effect on blood pressure in normal or hypertensive rats. This may limit one of the more promising uses of Y_1 antagonists, as antihypertensive agents – an area already well charted by effective drugs. There have been conflicting reports in the literature about whether the Y_1 receptor mediates the feeding response seen with NPY. No-one has yet reported blockade of feeding effects after peripheral administration of Y_1 antagonists; in all the positive reports so far, compounds have been administered centrally. Thus it is difficult to determine whether the blockade of NPY-induced feeding is a genuine Y_1 effect or is a more general disruption of CNS processes. In addition, the evidence is mounting that the Y_5 receptor is responsible for the feeding effect. More seriously, a recent report

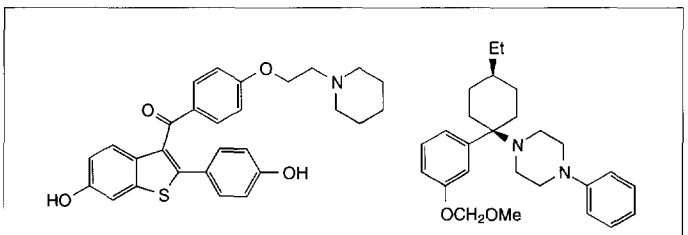

Figure 5. *Representative compounds from Eli Lilly (raloxifene, left) and Pfizer patent applications.*

describes NPY-knockout mice as having normal feeding behavior[66]. Thus NPY may not be *necessary* for normal feeding behavior, and it is possible that other systems may compensate for the lack of NPY. Several systems (e.g. galanin, leptin, NPY) may need to be modulated simultaneously to have robust, long-lasting anti-feeding, and ultimately anti-obesity, effects. In support of studies suggesting that NPY regulates neuronal state[67], the NPY-deficient mice showed a tendency towards spontaneous seizures and an increased susceptibility towards GABA-antagonist-induced seizures. Y_2 receptors are believed to be responsible for this effect.

Conclusions

NPY has important, but not yet fully understood, roles in the CNS. Effects at central NPY receptors are likely to afford new opportunities for drug therapy. The past several years have seen the advent of several potent Y_1-selective receptor antagonists, which are helping to clarify the roles of NPY receptors in the various effects of NPY. The most potent of these new compounds are peptide or peptide-like. Unfortunately, there is still a need for compounds that can be administered peripherally and show central activity. The conversion of these compounds to nonpeptides – compounds with usually more favorable physicochemical characteristics – is a difficult, but not impossible, task. The search for non-peptide antagonists is just starting to uncover potent, selective compounds. The advent of new NPY receptors will undoubtedly expand interest beyond the Y_1 receptor.

REFERENCES

1 Tatemoto, K. (1982) *Proc. Natl. Acad. Sci. U. S. A.* 79, 5485–5489
2 Tatemoto, K., Carlquist, M. and Mutt, V. (1982) *Nature* 296, 659–661
3 Cowley, D.J. *et al.* (1992) *Eur. J. Biochem.* 205, 1099–1106
4 Darbon, H. *et al.* (1992) *Eur. J. Biochem.* 209, 765–771
5 Saudek, V. and Pelton, J.T. (1990) *Biochemistry* 29, 4509–4515
6 Schwartz, T.W. *et al.* (1990) *Ann. New York Acad. Sci.* 611, 35–47
7 Allen, J.M. (1990) *Ann. New York Acad. Sci.* 611, 86–98
8 Larhammar, D., Blomqvist, A.G. and Soderberg, C. (1993) *Comp. Biochem. Physiol.* 106C, 743–752
9 Larhammar, D. (1996) *Regul. Pept.* 62, 1–11
10 Grundemar, L. and Håkanson, R. (1994) *Trends Pharmacol. Sci.* 15, 153–159
11 Dhanoa, D.S. (1995) *Exp. Opin. Ther. Patents* 5, 391–396
12 Wettstein, J.G., Earley, B. and Junien, J.L. (1995) *Pharmacol. Ther.* 65, 397–414
13 Beck-Sickinger, A.G. and Jung, G. (1995) *Biopolymers* 37, 123–142
14 Michel, M.C. (1991) *Trends Pharmacol. Sci.* 12, 389–394
15 Wahlestedt, C. and Reis, D.J. (1993) *Annu. Rev. Pharmacol. Toxicol.* 33, 309–352
16 Herzog, H. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 5794–5798
17 Gerald, C. *et al.* (1995) *J. Biol. Chem.* 270, 26758–26761
18 Gehlert, D.R. *et al.* (1996) *Mol. Pharmacol.* 49, 224–228
19 Bard, J.A. *et al.* (1995) *J. Biol. Chem.* 270, 26762–26765
20 Gerald, C. *et al.* (1996) *Nature* 382, 168–171
21 Weinberg, D.H. *et al.* (1996) *J. Biol. Chem.* 271, 16435–16438
22 Gregor, P. *et al.* (1996) *FEBS Lett.* 381, 58–62
23 Edvinsson, L. *et al.* (1990) *Neuropeptides* 17, 99–105
24 Sun, X. *et al.* (1996) *J. Pharm. Pharmacol.* 48, 77–84
25 Sun, X. *et al.* (1991) *Eur. J. Pharmacol.* 204, 281–286
26 Feth, F. *et al.* (1993) *Life Sci.* 52, 1835–1844
27 Heilig, M. *et al.* (1991) *Eur. J. Pharmacol.* 204, 27–32
28 Tatemoto, K. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 1174–1178
29 Leibowitz, S.F. *et al.* (1992) *NeuroReport* 3, 1023–1026
30 Beck, B. *et al.* (1994) *Neurosci. Lett.* 181, 126–128
31 Palea, S. *et al.* (1995) *Br. J. Pharmacol.* 116, 2401–2406
32 Wieland, H.A. *et al.* (1995) *J. Pharmacol. Exp. Ther.* 275, 143–149
33 Doughty, M.B. *et al.* (1990) *Eur. J. Pharmacol.* 185, 113–114
34 Tessel, R.E. *et al.* (1993) *J. Pharmacol. Exp. Ther.* 265, 172–177
35 Chaurasia, C. *et al.* (1994) *J. Med. Chem.* 37, 2242–2248
36 Palea, S. *et al.* (1995) *Br. J. Pharmacol.* 115, 3–10
37 Dhanoa, D.S. *et al.* (1995) Data presented at ACS National Meeting, 20–24 August, Chicago
38 Knieps, S. *et al.* (1995) *Bioorg. Med. Chem. Lett.* 5, 2065–2070
39 Balasubramaniam, A. *et al.* (1994) *J. Med. Chem.* 37(6), 811–815
40 Balasubramaniam, A. *et al.* (1990) *J. Biol. Chem.* 265, 14724–14727
41 Beck-Sickinger, A.G. and Jung, G. (1995) *Biopolymers* 37, 123–142
42 Kirby, D.A. *et al.* (1993) *J. Med. Chem.* 36, 3802–3808
43 Beck, A. *et al.* (1989) *FEBS Lett.* 1, 119–122
44 Balasubramaniam, A. *et al.* (1996) *J. Med. Chem.* 39, 1142–1147
45 Balasubramaniam, A. *et al.* (1996) *J. Med. Chem.* 39, 811–813
46 Heyer, D. (1996) Data presented at ACS National Meeting, March, New Orleans
47 Daniels, A.J. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 9067–9071
48 Hegde, S.S. *et al.* (1995) *J. Pharmacol. Exp. Ther.* 275, 1261–1266
49 Lew, M.J. *et al.* (1996) *Br. J. Pharmacol.* 117, 1768–1772
50 Ihara, M. *et al.* (1995) *Physiologist* 38, A251
51 Kanatani, A. *et al.* (1996) *Endocrinology* 137, 3177–3182
52 Rudolf, K. *et al.* (1994) *Eur. J. Pharmacol.* 271, R11–R13
53 Sautel, M. *et al.* (1996) *Mol. Pharmacol.* 50, 285–292
54 Doods, H.N. *et al.* (1996) *Regul. Pept.* 65, 71–77
55 Wieland, H.A. *et al.* (1995) *J. Pharmacol. Exp. Ther.* 275, 143–149
56 Abounader, R. *et al.* (1995) *Br. J. Pharmacol.* 116, 2245–2250
57 Nilsson, T. *et al.* (1996) *Neurosci. Lett.* 204, 145–148
58 Serradeil-Le Gal, C. *et al.* (1995) *FEBS Lett.* 362, 192–196
59 Serradeil-Le Gal, C. *et al.* (1994) *Soc. Neurosci. Abstr.* 376.14
60 Malmström, R.E., Modin, A. and Lundberg, J.M. (1996) *Eur. J. Pharmacol.* 305, 145–154
61 Malmström, R.E. and Lundberg, J.M. (1995) *Acta Physiol. Scand.* 155, 329–330
62 Wright, J.L. *et al.* (1996) *Bioorg. Med. Chem. Lett.* 6, 1809–1814
63 Bruns, R. *et al.* (1996) WO 96/12489, 96/12490
64 Bruns, R. *et al.* (1996) US Patent 5,504,094
65 Peterson, J.M. *et al.* (1996) WO 96/14307
66 Erickson, J.C. *et al.* (1996) *Nature* 381, 415–418
67 Colmers, W.F. and Bleakman, D. (1994) *Trends Neurosci.* 17, 373–379