Reciprocal mutations of neuropeptide Y receptor Y2 in human and chicken identify amino acids important for antagonist binding

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Abstract The neuropeptide Y (NPY) receptor Y2 antagonist BIIE0246 has sub-nanomolar affinity for the human Y2 (hY2) receptor but binds very poorly to chicken Y2 (chY2) with micromolar affinity. Sequence comparisons identified several amino acids for investigation by mutagenesis. Reciprocal mutagenesis between hY2 and chY2 revealed that three of these, individually and in combination, are important for BIIE0246 binding, namely positions Gln^{135} in transmembrane (TM) 3, Leu^{227} in TM5, and Leu^{284} in TM6. Mutagenesis of hY2 to the corresponding amino in chY2 (generating hY2[Q135H,L227Q, L284F]) made the affinity of BIIE0246 as low as for chY2. Introduction into chY2 of the three human residues resulted in antagonist affinity almost as high as for hY2. To distinguish between direct and indirect effects, each of the three residues in hY2 was replaced with alanine. BIIE0246 bound with 28-fold lower affinity to hY2[L227A], suggesting the Leu^{227} interacts directly with the antagonist. The other two alanine mutants bound with unaltered affinity, suggesting that the corresponding chY2 residues abolish binding through steric hindrance or charge repulsion. Thus, three amino acid residues can in an additive manner completely account for the difference in antagonist binding between the hY2 and chY2 receptors. These results will be useful for construction of three-dimensional models of the widely divergent NPY receptor subtypes.

Key words: Site-directed mutagenesis; Neuropeptide Y; Peptide YY; G-protein-coupled receptor; Modeling

1. Introduction

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the mammalian brain. It forms together with the hormones peptide YY (PYY) and pancreatic polypeptide (PP) a family of structurally related 36 amino acid peptides [1]. NPY, PYY, and PP act on a family of G-protein-coupled receptors: Y1, Y2, Y4, Y5, and y6 [2,3] influencing many important physiological and mental conditions such as mood, feeding, body temperature, blood pressure and reproduction (see [4,5] for reviews). The Y2 receptor [6–8,25,26] subtype has been shown to mainly be located presynaptically, regulating the release of neurotransmitter [9]. It has also been suggested to be involved in NPY's effect on circadian rhythms [10].

Interestingly, the NPY receptor subtypes Y1, Y2 and Y5 differ greatly from each other and display only 27–32% overall amino acid identity although all three do respond to the same peptide ligands NPY and PYY [3,11]. In fact, these receptors may be the most divergent G-protein-coupled receptors that bind the same peptide ligand. Y2 receptors in mammals can be distinguished pharmacologically from Y1, Y4, and Y5 receptors by a Y2-specific antagonist, BIIE0246 [12]. In addition, amino-terminally truncated peptides such as NPY18–36 are full agonists at the Y2 receptor, while the affinities for the Y1 and Y5 receptors decrease with progressive amino-terminal truncation. When amino acids Ile^{31} and Gln^{34} in the NPY molecule are replaced by the corresponding amino acids in PP, leucine and proline, the resulting ligand [Leu^{31},Pro^{34}]NPY does not bind to mammalian Y2 receptors while it is a full agonist at Y1, Y4, Y5 and y6 receptors.

When the Y2 receptor was cloned from the chicken (ch) [13] it was found to differ in ability to bind several of the subtype-specific ligands defined in mammals. The Y2-selective antagonist BIIE0246 did not bind to the chY2 receptor while the PP-mimicking peptide p[Leu^{31},Pro^{34}]NPY did. The chY2 sequence is 77% identical to the human (h) Y2 receptor. When only comparing the transmembrane (TM) regions the identity is 85%. By aligning the hY2 and chY2 receptors (Fig. 1) it was possible to identify several amino acid residues that might help account for the species difference in antagonist binding, particularly one residue in TM5 (Leu^{227} of hY2 which is a glutamine in chicken) and one in TM6 (Leu^{284} in hY2/Phe^{288} in chY2). Leu^{227} and Leu^{284} are conserved in all mammalian Y2 receptors but differ in chY2. When a three-dimensional model was generated, it was striking how close the Gln^{135} in TM3 of the hY2 receptor was to the amino acids in TM5 and TM6. This amino acid is a histidine in chY2 and the mutation hY2[Q135H] was subsequently added to the list of mutants.

Several studies on mutagenesis of the Y1 receptor have been published [14–21]. It has been proposed that the Y1-selective antagonist BIBP3226 binds to a pocket between TM4, 5, and 6 of the hY1 receptor and that the binding sites for NPY and BIBP3226 overlap, but are non-identical [18,19]. The large sequence differences between NPY receptor subtypes mentioned above have hampered the use of this information for modeling of the Y2 and Y5 receptor subtypes. Furthermore,

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Abbreviations: NPY, neuropeptide Y; PYY, peptide YY; PP, pancreatic polypeptide; h, human; ch, chicken; TM, transmembrane; PCR, polymerase chain reaction; WT, wild-type

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TM3
Human Y2    122 LCHLVPYAQGLAAQVSTITLTVIALDRH 149
Chicken Y2  126 .........A..VH...V..N....... 153
Bov. Rhod.  109 G.N.EGFFAT.GGEIALWS.V.L.IE.Y 136

TM5
Human Y2    217 TVYSLSSLLILYVLPLGIISFSYTRI 242
Chicken Y2  221 .I..V.M...Q.....A...YA.... 246
Bov. Rhod.  201 ESFVIYMFVVHFII..IV.F.C.GQL 226

TM6
Human Y2    262 RRQKTTKMLVCVVVVFAVSWLPLHAFQLAVD 292
Chicken Y2  266 ..................N...F.....VS. 296
Bov. Rhod.  226 AEKEV.R.VIIM.IA.LIC...YAGVAFYIF 276
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Fig. 1. α-Helices 3, 5, and 6 of the hY2 and chY2 receptors compared to bovine rhodopsin. The helices are supposed to pierce the membrane and were selected based on [22]. Amino acids that are conserved in all mammalian Y2 receptors (that bind BIIE0246) but differ in the chY2 receptor are shown in bold. Amino acids that differ among mammalian Y2 are underlined. Arrows point at the three positions found to affect BIIE0246 binding.

the new three-dimensional model of bovine rhodopsin [22] has triggered reinterpretation of the published Y1 model. We present here the first mutagenesis study of the Y2 receptor and use the high-resolution structure of bovine rhodopsin [22] to propose a model for the hY2 receptor.

2. Materials and methods

2.1. Generation of mutants of the hY2 and chY2 receptors

The coding sequences of hY2 and chY2 were transferred to a modified pCEP4 vector (Invitrogen, Groningen, The Netherlands) using polymerase chain reaction (PCR). In order to detect mutated receptors that do not bind any radioligand, the nine amino acid long FLAG epitope was inserted at the very carboxy-terminal end of the receptors. Point mutations were introduced using a two-step PCR procedure. All constructs were fully sequenced on both strands by automatic fluorescent dye sequencing using an ABI310 (Perkin Elmer).

2.2. Transfection and binding

Transient transfections of HEK293-EBNA cells and binding to mutant and wild-type (WT) receptors were performed as previously described [13].

2.3. Modeling

Computer modeling of the hY2 and chY2 receptors was performed using Sybyl 6.4 (Tripos) running on a SiliconGraphics O2 computer. The recently presented model of bovine rhodopsin [22] was used as a template.

3. Results

3.1. Generation of mutants of the hY2 and chY2 receptors

The hY2 and chY2 receptor genes were introduced into the eukaryotic expression vector pCEP4. The following hY2 mutant receptors were generated using a PCR-based method and inserted into pCEP4: $Gln^{135} \rightarrow His$ (hY2[Q135H]) in TM3, $Leu^{227} \rightarrow Gln$ (hY2[L227Q]) in TM5, and $Leu^{284} \rightarrow Phe$ (hY2[L284F]) in TM6. The corresponding (reciprocal) mutations were introduced into the chY2 receptor: chY2[H139Q], chY2[Q231L], and chY2[F288L]. Furthermore, the double mutants hY2[L227Q,L284F] and chY2[Q231L,F288L] as well as the triple mutants with all three single mutations, hY2[Q135H,L227Q,L284F] and chY2[H139Q,Q231L,F288L], were also generated.

3.2. Transfection

Each of the constructs was transiently transfected into HEK293-EBNA cells. Stable cell lines expressing the hY2 receptor and the triple mutant of the chY2 receptor, chY2[H139Q,Q231L,F288L], were also established as described previously [13].

3.3. Binding

The radioligand ^{125}I-pPYY bound to all of the mutants with similar affinities and similar B_{max} as to the WT Y2 receptors, as shown by saturation experiments. For hY2, hY2[Q135H,L227Q,L284F], chY2, and chY2[H139Q,Q231L, F288L], the affinities (K_d) were 57 ± 9, 42 ± 1, 36 ± 6, and 91 ± 9 pM ($n = 3$) and the B_{max} values were 270 ± 30, 160 ± 30, 190 ± 40, and 190 ± 20 fmol/mg protein, respectively.

Table 1
Binding of NPY, BIIE0246, and [Leu^{31},Pro^{34}]NPY to WT and mutant hY2 and chY2

Receptor/mutant	NPY			BIIE0246			[Leu^{31},Pro^{34}]NPY		
	pK_i ± S.E.M.	vs. hY2	vs. chY2	pK_i ± S.E.M.	vs. hY2	vs. chY2	pK_i ± S.E.M.	vs. hY2	vs. chY2
hY2	9.12 ± 0.03	1	1.3 ↓	9.15 ± 0.08	1	1300↑	5.95 ± 0.10	1	76 ↓
hY2[Q135H]	9.44 ± 0.10	2.1↑	1.7↑	7.48 ± 0.08	47 ↓	29↑	> 5.5	> 3 ↓	> 100 ↓
hY2[Q135A]	9.08 ± 0.02	1.1 ↓	1.4↑	9.10 ± 0.11	1.1 ↓	1180↑	> 5.5	> 3 ↓	> 100 ↓
hY2[L227Q]	9.22 ± 0.14	1.3↑	1.0	7.33 ± 0.09	64 ↓	21↑	6.33 ± 0.09	2.4↑	32 ↓
hY2[L227A]	9.48 ± 0.05	2.3↑	1.8↑	7.70 ± 0.11	28 ↓	48↑	6.48 ± 0.09	3.3↑	23 ↓
hY2[L284F]	9.24 ± 0.13	1.4↑	1.1↑	8.34 ± 0.10	6.4 ↓	210↑	6.49 ± 0.07	3.4↑	22 ↓
hY2[L284A]	9.29 ± 0.12	1.5↑	1.2↑	8.81 ± 0.13	2.1 ↓	620↑	5.98 ± 0.09	1.1↑	72 ↓
hY2[L227Q,L284F]	9.63 ± 0.03	3.3↑	2.6↑	6.32 ± 0.04	680 ↓	2↑	7.13 ± 0.11	15↑	5 ↓
hY2[Q135H,L227Q,L284F]	9.51 ± 0.07	2.5↑	2.0↑	5.96 ± 0.10	1530 ↓	1.1 ↓	6.11 ± 0.05	1.4↑	54 ↓
chY2	9.24 ± 0.04	1.3↑	1	6.02 ± 0.10	1330 ↓	1	7.83 ± 0.06	76↑	1
chY2[H139Q]	9.47 ± 0.08	2.3↑	1.8↑	7.31 ± 0.07	69 ↓	19↑	7.24 ± 0.08	19↑	3.9 ↓
chY2[Q231L]	9.35 ± 0.06	1.7↑	1.4↑	6.47 ± 0.07	470 ↓	2.8↑	7.71 ± 0.05	57↑	1.3 ↓
chY2[F288L]	9.32 ± 0.08	1.6↑	1.3↑	6.38 ± 0.04	590 ↓	2.2↑	6.61 ± 0.06	4.6↑	16 ↓
chY2[Q231L,F288L]	8.81 ± 0.07	2.1 ↓	2.6 ↓	6.47 ± 0.07	480 ↓	2.8↑	6.45 ± 0.05	3.1↑	24 ↓
chY2[H139Q,Q231L,F288L]	9.20 ± 0.10	1.2↑	1.0	8.74 ± 0.07	2.6 ↓	520↑	6.66 ± 0.06	5.1↑	15 ↓

Inhibition of ^{125}I-pPYY by pNPY, BIIE0246, and [Leu^{31},Pro^{34}]NPY. Data are presented as pK_i ± S.E.M. for three to nine experiments and compared to the human and chicken WT receptors. Numbers in the second and third column for each ligand indicate *X*-fold change in affinity for the mutated receptor and arrows indicate increase (↑) or decrease (↓) in affinity compared to the WT hY2 (second column) and chY2 (third column) receptors, respectively.

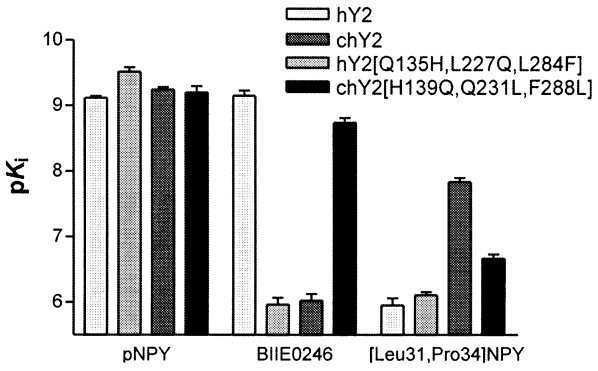

Fig. 2. Binding profiles of WT receptors and triple mutants. Comparison of pNPY, BIIE0246, and [Leu^{31},Pro^{34}]NPY in competition with ^{125}I-pPYY binding to WT and triple mutant hY2 and chY2. Data are presented as pK_i ± S.E.M.

Furthermore, pNPY displaced ^{125}I-pPYY with similar affinities at all receptor mutants tested (no change was more than 2.5-fold, see Table 1). In contrast, the antagonist BIIE0246 showed decreased affinity for the hY2 receptor mutants Q135H, L227Q, and L284F by 47-fold, 64-fold, and 6-fold, respectively. Replacement of all three residues in the same receptor construct generating hY2[Q135H,L227Q,L284F], reduced BIIE0246 affinity to the same level (K_i = 1 μM) as it has for the WT chY2 receptor (Table 1, Fig. 2). Reciprocal mutations of the corresponding amino acids in the chY2 receptor generating chY2[H139Q,Q231L,F288L] almost fully restored BIIE0246 binding (K_i = 2 nM).

When Gln^{135}, Leu^{227}, and Leu^{284} of the hY2 receptor were each mutated to alanine, the mutation in TM5, hY2[L227A] was the only mutation that significantly affected binding of BIIE0246 (28-fold decrease). To further explore the importance of TM5, the mutation hY2[S223M] (located one turn above Leu^{227}) was made. No significant effect on BIIE0246 or NPY binding was found for this mutation (data not shown).

[Leu^{31},Pro^{34}]NPY did not bind to hY2[Q135H] in the concentration range tested (Table 1). At hY2[L227Q] and hY2[L284F] there were only a 2- and a 3-fold increase in affinity respectively. When the TM5 and TM6 mutations were combined in hY2[L227Q,L284F] there was a 15-fold increase in affinity. In the chY2 receptor, [Leu^{31},Pro^{34}]NPY bound to the mutant chY2[H139Q] with 3-fold lower affinity than WT and for chY2[F288L] the effect was 15-fold while chY2[Q231L] did not affect binding.

4. Discussion

Comparison of receptors across species is a powerful tool to identify amino acid residues involved in ligand binding, particularly for synthetic (non-natural) ligands. However, receptor orthologs from different species of mammals often display very similar or indistinguishable pharmacological properties as their sequences are usually highly conserved. In this study we take advantage of differences between the chY2 receptor as compared to Y2 in mammals to identify positions important for antagonist binding.

When the chY2 receptor was cloned, the pharmacological profile was found to be surprisingly different from its ortholog in mammals [13]. The affinity of the Y2 receptor-selective antagonist BIIE0246 [12] was more than 1000-fold lower for the chY2 receptor [13] than for the human, rat and guinea pig Y2 receptors [12,23]. BIIE0246 was designed in a similar way as the first hY1 receptor antagonist, BIBP3226 [24], by modifications of the two most carboxy-terminal amino acids of NPY and PYY, Arg^{35} and Tyr^{36}. BIBP3226 was found to interact mainly with amino acids in TM5 and TM6 but also in TM4 of the hY1 receptor [18]. Assuming that BIIE0246 would interact with the Y2 receptor in a similar manner, several amino acids of potential interest were identified in these regions (Fig. 1). Leu^{227} in TM5 as well as Leu^{284} in TM6 are conserved in all cloned mammalian Y2 receptors but differ in the chY2 receptor where these positions are occupied by glutamine and phenylalanine, respectively. Interestingly, the position corresponding to Leu^{227} is also a glutamine in all known mammalian Y1 and Y5 receptors. In contrast, Leu^{284} is conserved in that position of TM6 in all published and functionally expressed NPY receptors (i.e. both BIIE0246 binding and non-binding) except in chY2. Modeling of the hY2 receptor based on the recently published structure of bovine rhodopsin suggested that Gln^{135} in TM3 is located in close proximity to Leu^{227} and Leu^{284} (11 Å), see Fig. 3. The amino acid that corresponds to Gln^{135} in the chY2 receptor is a histidine. This position is more variable between receptor subtypes than Leu^{227} and Leu^{284}, as it is a threonine in Y1 and Y4, leucine in Y5 and serine in y6. Based on this information the following mutants were made in the hY2 receptor: hY2[Q135H], hY2[L227Q], and hY2[L284F]. As indicated in

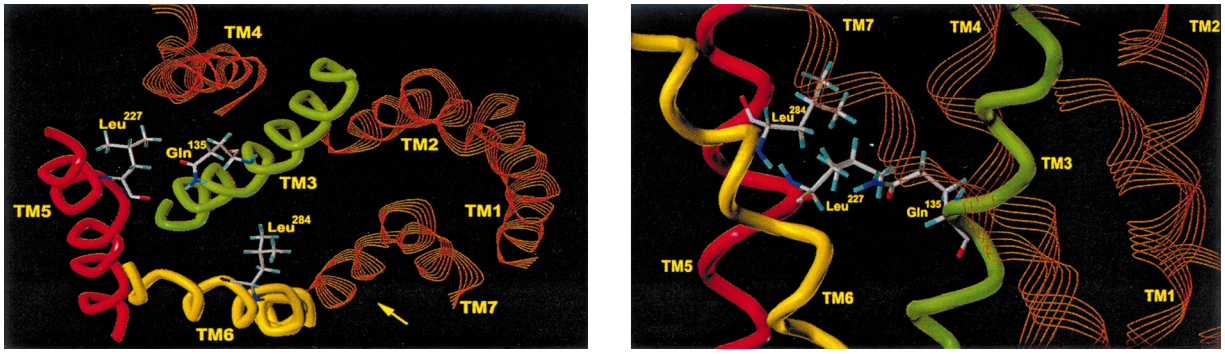

Fig. 3. Model of the hY2 receptor. The hY2 model is based on the X-ray crystallography model of rhodopsin [22]. Left: View from the outside of the cell (TM1–7). Right: View from the side, inside the membrane (TM3–6). The arrow in the left part indicates the viewing angle in the right part.

Table 1, all three mutations reduced antagonist affinity to the hY2 receptor without changing the affinities of the radioligand and NPY. When the mutations in TM5 and TM6 were combined in the mutant hY2[L227Q,L284F] the affinity of BIIE0246 dropped by 680-fold and when all three mutations were combined the affinity of BIIE0246 was as low as for the chY2 receptor indicating that these three amino acid replacements are sufficient to block antagonist binding (Fig. 2).

To further explore the function of Gln^{135}, Leu^{227}, and Leu^{284} in the hY2 receptor, each of these amino acids was also replaced with alanine. As alanine only bears a minimal side chain (a methyl group), it is often the amino acid of choice when studying 'loss of function' of a particular amino acid without affecting the backbone of the protein as glycine often does. In this case alanine mutations can help determine whether the amino acids at these positions in hY2 are involved in direct interaction with the antagonist. BIIE0246 bound with higher affinity to all three alanine single mutants than to the corresponding chicken-resembling mutants. Only for the TM5 mutation hY2[L227A] was there a significant reduction of BIIE0246 affinity compared to the WT receptor (28-fold). Thus, it is likely that Leu^{227} in TM5 of the hY2 receptor interacts directly with BIIE0246, possibly with hydrophobic parts of the antagonist. The importance of Leu^{227} is emphasized by the presence of this amino acid in all NPY receptors that bind BIIE0246 and its absence in all others. In contrast, it appears that the side chains of His^{139} in TM3 and Phe^{288} in TM6 merely block the docking of the antagonist to the chY2 receptor. For His^{139} there may also be a positive charge that repels the antagonist. The corresponding amino acid to Leu^{227} in hY1 (Gln^{219}) has been found to be important for binding of both NPY and the non-peptide antagonist BIBP3226 as both lost affinity for hY1 when Gln^{219} was mutated to alanine [19]. As the structures of BIIE0246 and BIBP3226 resemble each other, it is reasonable that these antagonists can interact with amino acids that are in the corresponding positions in the mammalian Y1 and Y2 receptors.

To address the question if binding to the chY2 receptor could be restored by introduction of the human residues at the three positions discussed above, we made the mutants chY2[H139Q], chY2[Q231L], and chY2[F288L] and the double mutant chY2[Q231L,F288L] as well as the triple mutant chY2[H139Q,Q231L,F288L]. The mutations in TM5 and TM6, either alone or combined, improved only marginally the affinity of BIIE0246 for the chY2 receptor. In contrast, the mutation in TM3, H139Q, improved the binding of BIIE0246 considerably but the affinity was still 69-fold lower than for the hY2 receptor. However, when all three mutations were combined in the triple mutant chY2[H139Q, Q231L,F288L] the affinity increased dramatically (only 2.6-fold lower than for the hY2 receptor, Table 1 and Fig. 2). This agrees with the findings from the human receptor that the histidine in TM3 mainly blocks or repels the antagonist and that the leucine in TM5 is essential for high-affinity binding of BIIE0246. Thus, reciprocal mutations of the amino acids in these three positions can almost completely swap the properties of the hY2 and chY2 receptors with regard to BIIE0246 binding. The minor remaining difference in affinity between the human WT receptor and the triple mutant of the chicken receptor, however, suggests that more subtle and probably indirect interactions may also be involved.

All three mutations are located about two to three full turns down in the TM helices from the outer surface of the cell membrane (Figs. 1 and 3). As none of the mutations affected NPY or ^{125}I-PYY binding it seems likely that the endogenous peptide agonists do not reach very far down into the pocket between the TM regions, but instead have their major sites of interaction with the outer and external parts of the Y2 receptor. The single mutations had very little effect on the affinity of the Y2-discriminating (in mammals) peptide [Leu^{31},Pro^{34}]NPY while a slight increase in affinity was observed for the double mutant (15-fold). In line with this, in chY2 the F288L mutant in TM6 caused [Leu^{31},Pro^{34}]NPY to lose affinity by about 16-fold. Although this position harbors a leucine in all other functionally expressed NPY receptors, even those that allowed binding of [Leu^{31},Pro^{34}]NPY, it appears that a phenylalanine in TM6 is necessary for [Leu^{31},Pro^{34}]NPY binding to the chY2 receptor. Thus, this position in TM6 is important both for BIIE0246 binding to the hY2 receptor and also for the discrimination against [Leu^{31},Pro^{34}]NPY, although being located three turns down in the binding pocket. However, in contrast to the antagonist, additional amino acid residues, possibly in the loops, are likely to contribute to [Leu^{31},Pro^{34}]NPY binding to the chY2 receptor and/or in discrimination against binding of this ligand to the hY2 receptor since the hY2 triple mutant did not allow the affinity displayed for the WT chY2 receptor.

In conclusion, we describe here the first mutagenesis study of the Y2 receptor and have identified three amino acid residues that are important for binding of the Y2-selective antagonist BIIE0246 to the hY2 receptor. We present a model based on the high-resolution bovine rhodopsin structure that places these three amino acids in close proximity to one another. Of these three amino acids, Leu^{227} in TM5 appears to be the most important in the direct interaction with BIIE0246. These results will be helpful to generate three-dimensional models of the widely divergent NPY receptor subtypes Y2 and Y5 as well as improve the Y1 model.

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References

[1] Larhammar, D. (1996) Regul. Pept. 62, 1–11.
[2] Michel, M.C. et al. (1998) Pharmacol. Rev. 50, 143–150.
[3] Larhammar, D., Wraith, A., Berglund, M.M., Holmberg, S.K. and Lundell, I. (2001) Peptides 22, 295–307.
[4] Blomqvist, A.G. and Herzog, H. (1997) Trends Neurosci. 20, 294–298.
[5] Gehlert, D.R. (1998) Proc. Soc. Exp. Biol. Med. 218, 7–22.
[6] Rose, P.M., Fernandes, P., Lynch, J.S., Frazier, S.T., Fisher, S.M., Kodukula, K., Kienzle, B. and Seethala, R. (1995) J. Biol. Chem. 270, 22661–22664.
[7] Gerald, C., Walker, M.W., Vaysse, P.J., He, C., Branchek, T.A. and Weinshank, R.L. (1995) J. Biol. Chem. 270, 26758–26761.
[8] Gehlert, D.R.A., Beavers, L.S., Johnson, D., Gackenheimer, S.L., Schober, D.A. and Gadski, R.A. (1996) Mol. Pharmacol. 49, 224–228.
[9] Wahlestedt, C., Yanaihara, N. and Hakanson, R. (1986) Regul. Pept. 13, 307–318.
[10] Huhman, K.L., Gillespie, C.F., Marvel, C.L. and Albers, H.E. (1996) Neuroreport 7, 1249–1252.
[11] Larhammar, D. (1996) Regul. Pept. 65, 165–174.
[12] Doods, H. et al. (1999) Eur. J. Pharmacol. 384, R3–5.

[13] Salaneck, E., Holmberg, S.K., Berglund, M.M., Boswell, T. and Larhammar, D. (2000) FEBS Lett. 484, 229–234.
[14] Walker, P., Munoz, M., Martinez, R. and Peitsch, M.C. (1994) J. Biol. Chem. 269, 2863–2869.
[15] Munoz, M., Sautel, M., Martinez, R., Sheikh, S.P. and Walker, P. (1995) Mol. Cell. Endocrinol. 107, 77–86.
[16] Munch, G., Walker, P., Shine, J. and Herzog, H. (1995) Recept. Channels 3, 291–297.
[17] Sautel, M., Martinez, R., Munoz, M., Peitsch, M.C., Beck-Sickinger, A.G. and Walker, P. (1995) Mol. Cell. Endocrinol. 112, 215–222.
[18] Sautel, M. et al. (1996) Mol. Pharmacol. 50, 285–292.
[19] Du, P. et al. (1997) Protein Eng. 10, 109–117.
[20] Robin-Jagerschmidt, C., Sylte, I., Bihoreau, C., Hendricksen, L., Calvet, A., Dahl, S.G. and Benicourt, C. (1998) Mol. Cell. Endocrinol. 139, 187–198.
[21] Kanno, T. et al. (2001) Peptides 22, 405–413.
[22] Palczewski, K. et al. (2000) Science 289, 739–745.
[23] Berglund, M.M., Lundell, I., Cabrele, C., Serradeil-Le Gal, C., Beck-Sickinger, A.G. and Larhammar, D. (2000) Biochem. Pharmacol. 60, 1815–1822.
[24] Rudolf, K. et al. (1994) Eur. J. Pharmacol. 271, R11–13.
[25] Ammar, D.A., Eadie, D.M., Wong, D.J., Ma, Y.Y., Kolakowski Jr., L.F., Yang-Feng, T.L. and Thompson, D.A. (1996) Genomics 38, 392–398.
[26] Rimland, J.M., Seward, E.P., Humbert, Y., Ratti, E., Trist, D.G. and North, R.A. (1996) Mol. Pharmacol. 49, 387–390.