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REVIEW

Neuropeptide Y Y2 receptor in health and disease

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We briefly survey the current knowledge and concepts regarding structure and function of the neuropeptide Y Y2 receptor and its agonists, especially as related to pharmacology of the receptor and its roles in pathological processes. Specific structural features are considered that could be responsible for the known compartmentalization and participation of the receptor in cell and tissue organization. This is further discussed in relation to changes of levels of the Y2 receptor in pathological conditions (especially in epilepsy and drug abuse), to endocytosis and recycling, and to participation in wound healing, retinopathy and angiogenesis. Properties of the receptor and of Y2 agonists are considered and reviewed in connection to the negative regulation of transmitter release, feeding, mood and social behavior. The possible involvement of the Y2 receptor in diabetes, carcinogenesis and bone formation is also reviewed.
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Abbreviations: bFGF, basic fibroblast growth factor; BIIE0246, (S)-N(2)-[[1-[2-[4-[(R,S)-5,11-dihydro-6(6 h)-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]-argininamide; CCK, cholecystokinin; DMPS, 2,3-dimercaptopropane sulphonate; DPPIV, dipeptidyl peptidase IV (EC 3.4.14.5); ECM, the extracellular matrix; fMLF, peptide formylMet-Leu-Phe; NPY, neuropeptide Y; PAO, phenylarsine oxide; PP, pancreatic polypeptide; PYY, peptide YY; RANTES, 'regulated on activation, normal T cell expressed and secreted' chemokine

Introduction

The neuropeptide Y (NPY) Y2 receptor is widely distributed and well expressed in the mammal, with a multitude of physiological and pathological connections (for recent reviews, see Balasubramaniam, 2002; Larhammar and Salaneck, 2004; Harro, 2006). This is a G-protein coupling receptor with seven transmembrane helices, a member of the largest group of plasma membrane receptors. In a differentiation extreme for receptor subtypes that respond to similar agonists, the Y2 receptor supports a virtual antagonism to another well-expressed and important NPY receptor, the Y1 receptor. This functional dualism is connected to a differentiation of agonists within the NPY family of neuropeptides, and obviously is fundamental to the large range of activity of these peptides.

Phylogeny of Y peptides

In addition to NPY itself, the NPY family peptides include peptide YY (PYY), pancreatic polypeptide (PP) and peptide Y (found only in the fish). Both NPY and PYY have five tyrosines, while PP has 4 or 5, and due to that all are frequently referred to as Y peptides. These peptides may have evolved from RFamide ancestors (peptides possessing the C-terminal phenylalanine amide preceded by arginine) similar to neuropeptide F of modern mollusks and flatworms (Dougan *et al.*, 2002). However, NPY and related peptides have not been identified in modern invertebrates. All Y peptides have 36 residues, with arginines in positions 33 and 35, and C-terminal amidated tyrosine (Table 1). The putative ancestral C-terminal phenylalanine is found in some amphibian and reptilian PPs. NPY could be the oldest extant member of a chordate RFamide-related peptide line, and PYY should have evolved along with NPY, while PP appears to be a more recent addition (Larhammar and Salaneck, 2004).

Structure of Y peptides

The C-terminal amidation is critically important for the binding of agonists to all Y receptors. The 'free acid', C-terminal carboxyl NPY has affinities orders of magnitude lower than the parent peptide at either the Y2 or the Y1 receptor, and similar applies to Tyr^{36}-OH PP at the Y4 receptor. Residues 1–3 and 33–36 are critically important in

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the binding of agonists to the Y1 site, and also necessary in the Y4 binding (Keire *et al*., 2002). However, while the C-terminal residues are critical in the Y2 binding (Beck-Sickinger *et al*., 1994), the N-terminal residues 1–2 are not used in attachment to that site, while the presence of N-terminal residue 3 maintains a high binding affinity and agonist activity (Keire *et al*., 2002; Table 2 in this review). Residues 7–16, located mainly in the less organized, nonhelical parts of Y peptide molecules (Lerch *et al*., 2004; Nygaard *et al*., 2006) are marginally involved in the binding of agonists to either the Y1 or the Y2 site (Beck-Sickinger *et al*., 1994), but could be important for the known avid interactions of NPY with membrane lipids (McLean *et al*., 1990) and with environment of the Y1 site (Parker *et al*., 2007a).

Table 1 Sequences of the principal human Y peptides

hNPY	**YPSKPDNP**G**EDA**P**AED**M**ARYYS**A**LRHY**I**N**L**ITRQRY**
hPYY	**YPIKPEA**P**GEDASPEELNRYYASLRHY**L**N**LV**TRQRY**
hPP	A**PLEPVYP**G**DN**A**TPEQ**M**AQY**AA**DLRRY**I**N**M**LTRPRY**

Abbreviations: NPY, neuropeptide Y; h, human.

Table 2 The affinity and activity vs the chain length of agonists, examined with the human Y2 receptor expressed in CHO cells

Peptide	*No. of assays*	K_d *(nM)*	ED_{50} *(nM)*
Human NPY(1–36)	3	0.16±0.025	0.11±0.02
Human PYY(1–36)	3	0.16±0.026	0.13±0.02
Human NPY(3–36)	5	0.23±0.035	0.12±0.027
Human PYY(3–36)	15	0.13±0.045	0.06±0.012
Human NPY(13–36)	5	3.1±0.66	3.4±0.16
NPY(18–36)	5	5.5±0.91	5.7±1.8
NPY(22–36)	5	16±3.9	16±1.3
NPY(26–36)	3	115±17	550±79

Abbreviations: NPY, neuropeptide Y; PYY, peptide YY.
All parameters were obtained with particulates from the expression of the human Y2 receptor in CHO cells (possessing the Y2 receptor density of about 300 fmol mg^{-1} protein) between 28th and 35th passage at 400 μg ml^{-1} of geneticin. The particulates were stored at −75°C for not more than 15 days. The competition and stimulation assays were done as described by Parker *et al*. (2007b), with particulate protein input of 50 μg ml^{-1}. The K_d values in competition of 50 pM [^{125}I]PYY(3–36) by the unlabelled agonists are from least-squares Scatchard fits (8–10 agonist concentrations between 3 pM and 1 μM), and generally correspond with data in Balasubramaniam *et al*. (2000); Keire *et al*. (2002) and Nygaard *et al*. (2006). The ED_{50} values (from logistic or exponential fits) are for the stimulation of the binding of 200 pM [^{35}S]GTP-γ-S by eight agonist concentrations between 10 pM and 10 μM.

Affinity and activity of Y2 agonists

The Y2 receptor, beside presynaptic localization in neurones, is strongly expressed in epithelia of the visceral tissues, including colon and kidney. The receptor shows a high affinity for either NPY or PYY, and a very low affinity for PP (see Keire *et al*., 2002; Nygaard *et al*., 2006). Unlike the Y1 receptor, the Y2 receptor accepts as agonists N-terminally truncated NPY and PYY peptides as short as 11 C-terminal residues (Grundemar and Hakanson, 1990). The Y2 affinity of NPY(3–36) and PYY(3–36) is similar to that of the parent 36-peptides (Grandt *et al*., 1996; Table 2). The relation of the chain length of C-terminal fragments to the binding affinity and activity at the Y2 site is illustrated in Table 2.

Sequence of the Y2 receptor and a comparison with other G-protein coupling receptors

The high scores in comparison of the human Y2R with other human G-protein coupling receptors (Table 3) include the NPY Y1 and PP1 Y4 receptors, all tachykinin receptors, both orexin receptors, two galanin receptors and three somatostatin receptors. The Smith–Waterman index over 600 and sequence identity with the Y2 receptor above 35% are found

Table 3 Twenty human rhodopsin family receptors with the highest sequence similarity to the human Y2 receptor

Receptor	*S-W index*	*% identity*	*Number of residues*	*% of total in the overlap*
Prolactin-releasing peptide (GPR10)	690	35.6	370	89
Glucocorticoid-induced (GPR83)	615	35.6	423	72
Neuropeptide FF-2	596	35.0	522	65
Neuropeptide FF-1	588	32.4	430	72
Pancreatic polypeptide Y4	587	32.6	375	99
Substance P	581	30.1	407	84
Neuropeptide Y Y1	580	30.3	384	89
QRFP (GPR103)	579	31.3	431	74
Neurokinin-4	546	31.0	440	79
Neurokinin-3	523	31.1	465	63
Galanin-1	524	30.7	349	92
Bombesin-3	520	30.2	399	78
Substance K	509	29.9	398	86
Orexin-2	504	30.7	444	82
Orexin-1	504	28.8	425	88
Neuromedin-B	486	28.8	390	82
Somatostatin-4	482	31.6	388	78
Galanin-2	480	30.9	387	75
Somatostatin-2	477	28.9	369	80
Somatostatin-5	474	33.3	364	82

'S-W' denotes the Smith–Waterman index (see Pearson, 2000). The percent identity for a given receptor refers to the largest stretch of its sequence that overlaps with the human Y2 receptor. The parameters were derived from Swiss-Protein database peptide sequences, using SSEARCH3 program by William Pearson.

for the prolactin-releasing peptide receptor, GPR10, and for the glucocorticoid-induced receptor, GPR83, which responds to Y2 agonists (Sah *et al*., 2007). As seen in Table 3, the human Y2 receptor has only about 30% identity with the human Y1 receptor. Similarity to the tachykinin receptors could indicate derivation from a common ancestor.

Cloning of the human Y2 receptor was first reported in 1986 (Rose *et al*., 1995), and the rat receptor was cloned in 1998 (Goumain *et al*., 1998; St-Pierre *et al*., 1998). Sequence of the Y2 receptor derives from a single exon, which is preceded by a large untranslated 5′-region (see Ma *et al*., 2005). Y2 receptors are also found in poikilotherm vertebrates. The zebrafish receptor shows 63% identity with the human (Fredriksson *et al*., 2006). Sequence similarity of Y2 receptors in the homeotherm species is considerable between birds and mammals (the chicken receptor is 80% identical with the human receptor), and very strong across mammalian species (>92%, in comparison with only 68% for Y4 receptors, but >93% for Y1 receptors). The high similarity of sequences of Y2 receptors across mammals should reflect involvement of this Y receptor subtype in critical developmental and metabolic events.

The N-terminal extracellular segment of all Y2 receptors has up to 30% acidic/anionic amino-acid residues, and could be involved in the known support of angiogenesis by the receptor (Zukowska-Grojec *et al*., 1998), as well as in anchoring, embedding and oligomerization of the receptor. This segment could be largely responsible for the low rate of internalization of the Y2 receptor compared to other Y receptors (Parker *et al*., 2001; Gicquiaux *et al*., 2002; Berglund *et al*., 2003a). Also, in comparison with Y1 and Y4 receptors, interaction of the Y2 receptor with β-arrestin is slow (Berglund *et al*., 2003b), possibly due to a much larger anchoring of the Y2 receptor.

Both clonal and native expressions of the Y2 receptor display a large surface compartmentalization (Parker *et al*., 2001, 2002, 2007b), not found with Y1 and Y4 receptors. This is illustrated in Figure 1. This could also be dependent on the N-terminal segment of the Y2 receptor. Unmasking by alkylators or by cholesterol removal, as well as the critical dependence on functional pertussis toxin-sensitive Gi α-subunits (Parker *et al*., 2007b) indicate that this population can also represent a receptor reserve.

Signal transduction involving the Y2 receptor

Interactions of Y receptors with signal transducers and effectors are insufficiently characterized, and more work is obviously necessary. The Y2 receptor is known to interact with Gi and Gq, but probably not with Go and Gs α-subunits (Freitag *et al*., 1995). There is activation of Gq α-subunit by the Y2 receptor transfected into smooth muscle cells (Misra *et al*., 2004). This also was shown in Y2-expressing CHO cells, utilizing a chimaeric Gqi5 α-subunit (Ziemek *et al*., 2006). In CHO cells expressing the human or guinea-pig Y2 receptors, both Gi-related inhibition of cAMP production and the activation of the binding of GTP-γ-S by Y2 agonists are abolished by pertussis toxin, in parallel with inactivation of Gi α-subunits (Parker *et al*., 2007b). Signal transduction involving the Y2 receptor could be influenced by receptor's propensity for anchoring and compartmentalization, but this was not examined thus far.

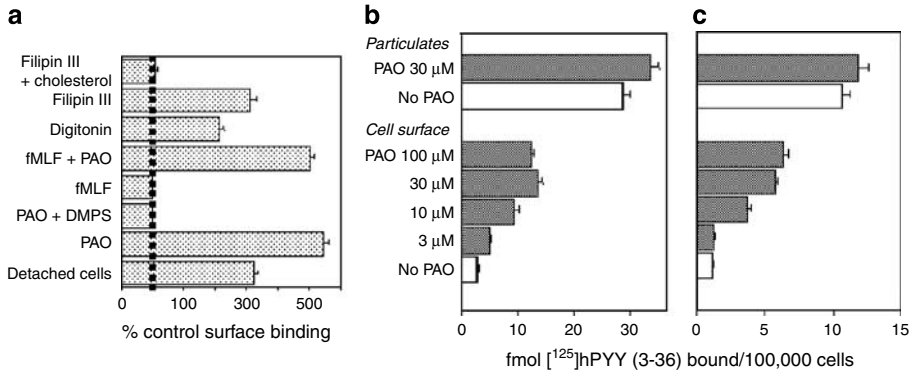

Figure 1 Compartmentalization of surface Y2 receptors in CHO cells and rat forebrain. The masked receptors are exposed, without cell detachment, by alkylators or by cholesterol removal, but not by agents that dissociate receptor complexes with focal adhesion proteins. Mechanical detachment without cell lysis also exposes many masked sites. These results expand the similar findings reported by Parker *et al*. (2002, 2007b). In graph (**a**), the control monolayer level of CHO cell surface sites is indicated by the dashed line. The masked surface sites outnumber the agonist-accessible sites by a factor of 4–5, are rapidly lost in response to pertussis toxin, and can also be activated by agonists (Parker *et al*., 2007b). Monolayers of CHO cells expressing the human Y2 receptor were either detached by silicone rubber, or treated *in situ* with the indicated agents (phenylarsine oxide (PAO) at 30 μM, formylMet-Leu-Phe (fMLF) peptide at 10 μM, 2,3-dimercaptopropane sulphonate (DMPS) at 30 μM, digitonin at 6 μM, filipin III at 3 μM and cholesteryl hemisuccinate at 10 μM). After treatments, the cells were labelled with the Y2 agonist [^{125}I]PYY(3–36), and the surface-attached agonist was extracted by cold acid saline (Parker *et al*., 2007b). PAO crosslinks vicinal cysteines and exposes several types of masked receptors (see Kaplan *et al*., 1985), fMLF detaches the focal adhesion proteins (Bennett *et al*., 2000), DMPS neutralizes PAO, digitonin is a permeabilizing detergent and filipin is a macrolide that extracts membrane cholesterol, and is neutralized with excess cholesterol. Graphs (**b**) and (**c**) show results similar to those by Parker *et al*. (2002). In (**b**), monolayers of CHO cells expressing the guinea-pig Y2 receptor were exposed to PAO at the indicated concentrations to unmask compartmentalized surface receptors before labelling by the Y2 receptor agonist; particulates derived from these cells were also assayed for agonist binding with or without 30 μM PAO, showing that homogenization exposed most of the Y2 binding sites. A very similar unmasking is obtained with collagenase-dispersed cells from rat forebrain (**c**). Note that the Y1 and Y4 receptors expressed in CHO cells present few masked surface sites (⩽30% of the monolayer sites accessible to peptide agonists).

Selectivity of the Y2 agonists starts with truncation to residue 3 without loss of affinity, and possibly with an increase in activity (Grandt *et al*., 1996; Table 2) and is maintained with N-terminal truncation up to residue 26, albeit with a much lower affinity and activity (Table 2). PYY(3–36) has affinity in the intermediate nanomolar range at the Y5 receptor, but not at the Y4 receptor (for example, Nygaard *et al*., 2006). However, PYY(22–36) was modified into feeding-suppressing agonists with very high selectivities for the Y2 receptor (Balasubramaniam *et al*., 2000, 2007).

Release of neurotransmitters and the Y2 receptor

The NPY-linked inhibition of neurotransmitter release could be largely achieved through the Y2 receptor (Smith-White *et al*., 2001). Importantly, the release of glutamate, the principal neuroexcitatory transmitter, is inhibited via the Y2 receptor (Silva *et al*., 2006). Inhibition of glutamate release from the presynaptic bipolar (rod-containing) cells in the retina could be connected to interaction of the Y2 receptor with Ca^{2+} channels (D'Angelo and Brecha, 2004). The Y2 agonists also can inhibit NPY release from PC-12 cells (Chen *et al*., 1997) and from hypothalamic slices (King *et al*., 2000). The release of GABA can be reduced by presynaptic Y2 receptors (Chen and van den Pol, 1996). The Y2 receptor-linked inhibition of noradrenaline release from hypothalamic synaptosomes apparently is not due to a reduced Ca^{2+} influx (Martire *et al*., 1995).

The Y2 receptor in behavior and disease

Appetite and feeding

Central signalling by certain types of gut peptides seems to be important in both initiation and termination of feeding (Moran, 2006). The gut peptides that terminate feeding are believed to work via the vagal inhibitory loop. Short-term feeding inhibitors, such as cholecystokinin, may mainly quench the appetite. However, this can also be an important component of the activity of Y2 agonists in both rodents (Halatchev and Cone, 2005) and humans (Degen *et al*., 2005). Inhibition of feeding by an Y2 agonist, PYY(3–36), was first reported by Stephen Bloom and associates (Batterham *et al*., 2002, 2003). In the rodent, this high-affinity Y2 agonist readily traverses the blood–brain barrier, as different from the parent peptide, PYY(1–36) (Nonaka *et al*., 2003). Moreover, long Y2 agonists have both very high binding site affinity and activity at the Gi α nucleotide site (Table 2), and this should combine to allow intrahypothalamic anorectic influence of PYY(3–36) with the plasma levels observed at satiation (for example, Grandt *et al*., 1994). It also should be noted that PYY(1–36) has a significant affinity preference for the Y2 over the Y1 receptor (Statnick *et al*., 1997), and could also act to inhibit feeding via the abundant Y2 receptors in the arcuate nucleus, or via the medullar Y2 receptors, both not tightly screened from the systemic circulation. The arcuate NPYergic connection to the dorsal vagal complex is documented (Gray *et al*., 1986). Effects of the systemically administered Y2 agonists might also be exerted directly at the dorsal vagal complex in the medulla, since the area postrema and the nucleus of the solitary tract are accessible to the systemic PYY (Hernandez *et al*., 1994). It should be noted that PYY is significantly produced only in the gut.

Variations in sequence of the human Y2 receptor can be linked to obesity. The early-onset obesity does not show a clear association with common single nucleotide polymorphisms of the receptor (Hung *et al*., 2004). However, Ma *et al*. (2005) found an important association of severe obesity in male Pima Indians with the $Ala^{172}Thr$ mutation in the coding region of the Y2 receptor. Protectiveness against human obesity by the common profile of sequence polymorphism of the Y2 receptor, as contrasted by polymorphisms associated with diabetic obesity, is indicated in a recent study (Lavebratt *et al*., 2006).

Similar to the Y2 receptor, the cholecystokinin-A receptor has a quite acidic N-terminus. This points to possible anchoring interactions that could account for the observed slow internalization of this receptor (Tarasova *et al*., 1997). Also, anchoring interactions could be connected to the protracted and similar activities of the cholecystokinin-A receptor and the Y2 receptor via the vagal system. The afferent nodose (vagal) ganglion neurons contain both receptors (Ghilardi *et al*., 1994).

Effectiveness of agonists of the Y2 receptor in the reduction of food intake is a subject of controversy, since some studies show that these peptides may not produce a sustained reduction of feeding, in the rodent (Tschop *et al*., 2004) or in the primate (Koegler *et al*., 2005). However, a lack of protracted reduction of feeding could in part relate to conditions of the treatment (Pittner *et al*., 2004; Chelikani *et al*., 2005). Effectiveness of the Y2 agonists can be influenced by the rate of cycling of the Y2 receptor and the level of saturation of the binding sites. The Y2 receptors are known to internalize slowly (Parker *et al*., 2001, 2002; Gicquiaux *et al*., 2002; Berglund *et al*., 2003b), and also to experience a lower degree of the agonist-induced receptor blockade than either the Y1 or the Y4 receptors (Parker *et al*., 2007a). Activation of both the Y2 and the Y4 receptors, as well as of the cholecystokinin/gastrin and the melanocortin 3 and 4 receptors, should contribute to a composite physiological system controlling satiety, in which messages from several receptors are pooled to adjust food intake, the central signals being sent largely via the vagal loop. An additive anorectic activity is obtained by pairing the highly selective Y2 agonist BT-48 (compound 42 of Balasubramaniam *et al*. (2000) and the potent Y4 agonist BVD-74D (compound 7 of Balasubramaniam *et al*., 2006, 2007).

Studies combining anorexic gut peptides, for example PYY(3–36) and glucagon-like peptide (Talsania *et al*., 2005), show that such co-treatments could also be synergistic. Nonpeptide anorectic agents might also synergize with the Y2 agonists. Important behavioural considerations include the handling and adaptation of animals (Pittner *et al*., 2004). Also, the route of administration appears to be important. It already was shown that the intravenous administration of PYY(3–36) results in a larger reduction in body weight than the intraperitoneal route, especially if administration of the Y2 agonist mimics the wavelike release of PYY (and the associated generation of PYY(3–36)) over a feeding session (Chelikani *et al*., 2006). It should be noted that PYY(3–36)

binds to the Y5 receptor with K_{diss} below 10 nM (for example, Parker *et al*., 2003), and a bolus administration of the peptide could thus even elicit some orexigenic activity. Crossreactivity at the Y5 receptor could be avoided by using agonists obtained by modification of PYY(22–36), such as BT-48 (compound 42 in Balasubramaniam *et al*. (2000)), which due to the large N-terminal truncation should have no affinity for the Y5 receptor (see Hu *et al*., 1996).

Heritable anorexia in anx/anx mice is connected to a deterioration of the hypothalamic arcuate nucleus and a decrease of mRNAs for the Y1 and Y2 receptors (Broberger *et al*., 1999), and possibly also a decrease in functional receptor sites. Such a decrease might help generate a chronic local surplus of NPY that in turn could exacerbate the loss of receptors. Also, in anorectic girls, the levels of circulating PYY are increased fourfold, and there is loss of bone density (Misra *et al*., 2006), making a case for downregulation of the Y2 receptor. It should be noted that the much faster cycling of the Y1 receptor compared to the Y2 receptor (for example Parker *et al*. (2001) and Gicquiaux *et al*. (2002)) could result in a disproportionate decrease of the Y1 receptor in anorectic conditions associated with increased levels of NPY or PYY. The receptor levels, however, were not measured in the above anorectic paradigms. Even at reduced numbers, the Y2 receptor could be expected to contribute to a chronic anorectic tone.

Water and sodium absorption

PYY (Bilchik *et al*., 1993; Liu *et al*., 1996), PYY(3–36) (Eto *et al*., 1997; Litvak *et al*., 1999) and a highly Y2-selective derivative of PYY(22–36), BT-48 (compound 42 of Balasubramaniam *et al*., 2000) have been shown to act as proabsorptive (or anti-secretory) hormones, increasing upon intravenous injection the absorption of both water and sodium in various parts of the bowel. As could be expected from the uneven abundance of PYY in the intestine (Adrian *et al*., 1985), the increase is larger in ileum compared to jejunum (Bilchik *et al*., 1993), and is also quite large in the colon (Liu *et al*., 1996). The proabsorptive effect is obviously carried via Y2 receptors, and is of medical interest in malabsorptive conditions (short bowel syndrome), and in diseases (enterocolitis, cholera, Krohn's disease). Also, deletion of either the Y2 or the Y4 receptor in the mouse was shown to increase the dark-phase water intake (Wultsch *et al*., 2006), indicating a decrease in water absorption due to the receptor knockouts. It should be noted that the antisecretory effects of PYY could involve the Y4 and Y1 receptors in addition to the Y2 receptor (Souli *et al*., 1997).

Seizures and epilepsy

Several studies reported activity of both Y2 and Y5 receptors in neuroprotection against agonists of the ionotropic glutamate receptors, such as kainate (Marsh *et al*., 1999; Silva *et al*., 2003; Woldbye *et al*., 2005). The protection appears to be additive and could be largely due to an increased output of NPY (Woldbye *et al*., 2005). However, levels of the Y2 receptor in the hippocampal formations that are important in the generation of seizures (dentate gyrus, dorsal hippocampus) increase both acutely (Jinde *et al*., 2002) and chronically (Schwarzer *et al*., 1998; Vezzani and Sperk, 2004) following treatments that induce seizures. The Y2 agonists appear to be uniformly neuroprotective, probably via reduction of glutamate release, in paradigms relating to the ionotropic glutamate receptors and/or glutamate release, or following treatment with agonists of the ionotropic glutamate receptor, such as kainate. At high dosages, this activity of Y2 agonists can also reflect their crossreactivity at the Y5 site (El Bahh *et al*., 2005). A clarification of the contributions to neuroprotection by different Y receptor species could benefit from application of more selective Y2 agonists, such as BT-48 and N-α-Ac[Trp^{27}]-PYY(22–36) (compounds 42 and 13, respectively, of Balasubramaniam *et al*. 2000).

The chronic elevation of the Y2 receptor in hippocampal areas following seizures may also point to receptor's participation in neuroprotection via a stabilization of, or an increase in, somatic interactions with the extracellular matrix (ECM), including perineuronal nets (known to be persistently enhanced after convulsions induced by kainite; Okamoto *et al*., 2003). Both the neuroprotection by the Y2 receptor, and the accumulation of the receptor in spasmodic conditions and paradigms could be significantly connected to interactions of the receptor with components of the extracellular and/or plasma membrane matrices. The recently described prokineticin receptors share a strongly anionic N-terminal composition with the Y2 receptor, and could be significantly involved in the maintenance of the streams of precursor neurones in the developing rodent brain (Matsumoto *et al*., 2006). The ubiquitous orexin receptors in the olfactory bulb and the piriform cortex (Caillol *et al*., 2003) may also function as organizers, based on their structural features (see Table 2 in Parker *et al*. (2005)).

Angiogenesis

Angiogenesis is promoted by a number of cytokines, and especially the vascular endothelial growth factor and the basic fibroblast growth factor, via the specific receptors. However, many rhodopsin-like receptors, in particular chemokine receptors, are also known to support angiogenesis. A role for the Y2 receptor in angiogenesis was shown in several systems (Zukowska-Grojec *et al*., 1998; Ekstrand *et al*., 2003; Lee *et al*., 2003a; Movafagh *et al*., 2006). With receptors of the rhodopsin family, an angiogenic activity may mechanistically amount to signalling that would indirectly promote angiogenic interactions, for example, through phospholipase Cβ1 (which is activated via the Gq α-subunit), and of the related metabolic cascades. However, N-terminal domains rich in anionic residues of the rhodopsin-like receptors that support angiogenesis, including the Y2 receptor, could directly link to ECM or to neighbouring cells. In receptors responding to large peptide agonists, especially the cytokine and chemokine receptors, these N-terminal domains may also interact with the ECM or with other cells via the receptor-attached agonists (see reviews by Bauvois (2004) and Parker *et al*. (2005)). It should be noted that the Y2 and Y5 receptors could cooperate in angiogenesis

via similar interactions, since both have N-terminal segments rich in anionic residues (see Parker *et al*. (2005)). A cooperation of Y2 and Y5 receptors in angiogenesis is already documented (Lee *et al*., 2003b; Zukowska, 2005). However, the Y2 receptor and NPY or PYY, rather than having a principal role, appear more as helpers in angiogenesis. The main drivers should be vascular endothelial growth factor and basic fibroblast growth factor (see Ekstrand *et al*. (2003) and Cruze *et al*. (2007) for comparisons of these growth factors with NPY and PYY in the support of angiogenesis in the cornea). A support by the Y2 receptor for a principal activity of chemokine receptors can also be envisaged in motility of leukocytes, which express the Y2 receptor (Nave *et al*., 2004).

Several enzymes are known to influence angiogenesis by modifying agonist peptides (see the review by Bauvois (2004)). With the Y peptides, the chief modifier should be dipeptidyl peptidase IV (DPPIV), which truncates NPY and PYY at Pro2. No involvement of the Y2 receptor is found in the artery balloon-induced angiopathy in the mouse (Li *et al*., 2003), although this procedure increases DPPIV. However, the apparent lack of involvement of the Y2 receptor in this case may relate to a low access of the Y2 antagonist employed, BIIE0246 ((S)-N(2)-[[1-[2-[4-[(R,S)-5,11-dihydro-6(6 h)-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]-argininamide), which poorly enters even CHO cells in monolayers (Ziemek *et al*., 2006).

A study in press at the time of this writing indicates a possible pro-angiogenic role for fat tissue Y2 receptors in the Y2-agonist-elicited acquisition of body weight in the mouse (Kuo *et al*., 2007a). This activity of the Y2 receptor could be connected to antilipolytic effects of NPY, which however, might vary with the mammalian species, and even with strain (Castan *et al*., 1994).

Tubulogenesis, a set of processes involved in the creation and elongation of nonvascular ducts, and in remodelling of the tubuli (Humes *et al*., 1996), could also be helped by Y2 receptors. In the proximal tubuli of the rabbit kidney, high-affinity Y2 receptors (first described by Sheikh *et al*. (1989)) are expressed at up to 1 pmol mg^{-1} membrane protein (that is, above 1% of the total membrane protein), and could participate in arraying of the tubular epithelial cells.

Neurones in the skin show NPY immunoreactivity (Morris *et al*., 2001), but no Y2 receptors have been identified in epidermis or cutis as yet. There could be a role for the Y2 receptor in linking and remodelling of the cutaneous epithelial cells. Also, it is conceivable that interactions with the ECM or with other cells might induce a transition of the receptor to the status of a structural constituent, preventing detection by the conventional techniques.

Tumorigenesis and carcinogenesis

Y2 receptors are not considered as generally supporting tumorigenesis and carcinogenesis (see for example, the review by Korner and Reubi, 2007), and sometimes are viewed as markers of normal breast or lung tissue (Reubi *et al*., 2001). However, there is a generally strong expression of Y2 receptors in neuroblastomas and paragangliomas (Korner *et al*., 2004), and a significant expression in renal carcinomas (Korner *et al*., 2005).

DPPIV can reduce progression of some tumours (Kikkawa *et al*., 2005). The enzyme will increase the availability of the Y2 agonist PYY(3–36), potentially stimulating an angiogenic activity of the Y2 receptor in epithelia and endothelia. However, DPPIV also cleaves several chemokines, for example, RANTES ('regulated on activation, normal T-cell expressed and secreted' chemokine), and may cleave cytokine anchors in the ECM (see Bauvois, 2004). DPPIV also cleaves one of the most potent promoters of angiogenesis, basic fibroblast growth factor, reducing its tumorigenic activity (Wesley *et al*., 2005). The Y2 agonists were reported to reduce the growth of human pancreatic cancer cells in mice (Liu *et al*., 1995). The Y2 receptor might act both to help tumour vascularization, and to reduce tumour growth via a negative metabotropicity.

Ischaemia

Ischaemic conditions result in increased release of NPY, activation of Y2 receptors and of DPPIV (Kuo and Zukowska, 2007b), and could initially engage mainly the negative metabotropicity of the Y2 receptor. The vagal inhibition of NPY-induced bradycardia is achieved exclusively via the presynaptic Y2 receptor (Abrahamsson, 2000). The consistent exacerbation of ischaemic symptoms by NPY via the Y1 receptor, in the rat brain *in vivo* as well as in cell line models (Chen *et al*., 2002; Chen and Cheung, 2004) is not acutely affected by the Y2 receptor antagonist BIIE0246. However, a study with ischaemic Y2(−)/(−) mice suggests a long-term involvement of the Y2 and the Y5 receptors in Y2(+)/(+) mice, leading to angiogenesis in ischaemic conditions (Lee *et al*., 2003b).

Peripheral arterial disease

Involvement of the Y2 receptor in processes and events connected to the vascular system could be dual, including metabolic antagonism to the Y1 receptor, and stabilizing/anchoring interactions, indicated with blood vessels in many systems. Both Y1 and Y2 receptors are expressed in human cardiovascular tissues (Uddman *et al*., 2002) and in mesenteric artery (Gradin *et al*., 2006). Constriction of the human skin arteries by a prostaglandin analogue is counteracted by NPY, and that could represent a negative regulation through the Y2 receptor (Nilsson *et al*., 2000). There could be a role for NPY in the perivascular innervation of arterioles in the eye choroid (Lutjen-Drecoll, 2006). By analogy with other arteries, these vessels should express the Y2 receptor.

Chronic infusion of either PYY or PYY(3–36) was shown recently to result in up to 60% increase of the collateral blood flow capacity in a rat model of peripheral arterial insufficiency (Cruze *et al*., 2007). Maximum effects were indistinguishable for the two Y peptides and the standard angiogenic agent, vascular endothelial growth factor. This could be of interest especially in devising therapies for the common age-related peripheral arterial disease, involving limb ischaemia and the associated leg pain. Selective Y2

agonists, via contributing to angiogenesis and arteriogenesis, could also counteract the stress-related vasoconstriction and ischaemia (Kuo and Zukowska, 2007b).

Wound healing

Activity of the Y2 receptor in wound healing could be distinctly linked to interactive capabilities of the receptor. As shown by Ekstrand *et al*. (2003), wound healing is slow in Y2(−)/(−) mice. In normal mammals, DPPIV after wounding should generally be upregulated, or at least increasingly secreted and embedded on the cell surface. This can also relate to an increased expression of the Y2 receptor. DPPIV is important in wound healing (Ghersi *et al*., 2001), and that could be connected to activity at the Y2 receptor. The actin/integrin assemblies of invadopodia contain DPPIV in complex with prolyl peptidase, important in wound healing (Ghersi *et al*., 2002). This association conceivably could provide the selective Y2 agonist PYY(3–36) for the Y2 receptors that via their N-terminal domains interact with collagen or fibronectin in the ECM. The severity of an eye injury could be reflected in the levels of DPPIV (Cejkova *et al*., 2004). Since internalization of the Y2 receptor at least in epithelial CHO cells is not readily accelerated by agonists (for example, Parker *et al*., 2007a), an increased local activity of DPPIV could generally help saturation of the Y2 receptor with PYY(3–36).

Eye disease

There is NPYergic innervation of the choroid vascular layer in the primate eye (Lutjen-Drecoll, 2006). NPY inhibits adenylyl cyclase in the retina via the Y2 receptor (Bruun *et al*., 1994), which in the choroid could be expressed substantially more than the Y1 receptor (Ammar *et al*., 1998). The Y2 receptor in bipolar rod cells inhibits voltage-regulated Ca^{2+} channels (D'Angelo and Brecha, 2004) and might directly associate with these channels. The N-terminus of the Y2 receptor could be used for such an interaction, since it does not seem to be involved in agonist attachment to the receptor (which is quite insensitive to polyanions), and should be available for association with extracellular oligobasic motifs of channels and transporters. The Y2 receptor is involved in development of the diabetic retinopathy (Koulu *et al*., 2004). In the retinal endothelia, the Y2 receptor can respond to NPY analogous to the response of the Tie2 receptor to angiopoietins (Otani *et al*., 2001). While the retinal blood flow is regulated by NPY via the Y1 receptor (Prieto *et al*., 1995), the Y2 receptor could be more important than the Y1 receptor in the maintenance of the retina (Yoon *et al*., 2002), what again might point to an organizing role for the Y2 receptor. It is of interest that an increased activity of DPPIV is found in both the tear fluid and the ECM after experimental eye injury (Cejkova *et al*., 2004). The corneal microvasculature is one of the favourite models in comparisons of angiogenic effects of the cytokines vascular endothelial growth factor and basic fibroblast growth factor and the agonists of Y receptors, especially the Y2 receptor (Ekstrand *et al*., 2003; Cruze *et al*., 2007), and future studies on the activity of Y receptors in eye diseases are likely to make extensive use of this model.

Diabetes

NPY and the Y2 receptor both participate in the diabetic retinopathy and retinal neovascularization (Koulu *et al*., 2004). The peripheral administration of PYY(3–36) reduces food intake, body weight gain and glycaemic indices in diverse rodent models of metabolic disease in both sexes (Pittner *et al*., 2004). Deletion of the Y2 receptor in ob/ob mice strongly reduces the type 2 diabetic syndrome, including its component of obesity (Sainsbury *et al*., 2002). There is a significant male sex-linked association of Y2 mutations with extreme obesity (Ma *et al*., 2005). Obesity, including that associated with diabetes, could be linked to variants in the 5′-section of the coding region of the Y2 receptor (Torekov *et al*., 2006).

Bone

Bone formation and remodelling are usually considered as locally regulated by growth factors at the level of osteoblasts. However, recent evidence points to a central Y2 receptor-supported mechanism in bone formation (Baldock *et al*., 2002). Also, the recently characterized rhodopsin family receptor GPR103, which has a significant homology to the Y2 receptor (see Table 3), was found to regulate bone formation (Baribault *et al*., 2006). A large increase of circulating PYY could be connected via a downregulation of the Y2 receptor to the loss of bone density observed in anorexia (Misra *et al*., 2006).

Leptin should induce a central negative regulation of bone formation (Karsenty, 2000), possibly via hypothalamic receptors (Takeda and Karsenty, 2001). This finding may support a similar negative regulation by the Y2 receptor. Systemic actions of leptin may outweigh the central effects (Cornish *et al*., 2002), which anyway are due to peripherally supplied hormone. Similar could apply to PYY(3–36). It should again be noted that most PYY(3–36) in the mammal is generated from peripherally made PYY, and of course the systemic levels of PYY(3–36) are larger than its brain levels. In this regard, however, one should also have in mind that PYY(1–36) is in the general blood circulation available at larger levels than PYY(3–36), and that it has a similar affinity and activity at the Y2 receptor (see Table 2). The arcuate hypothalamic Y2 receptors, and the Y2 receptors in the dorsal vagal complex of the medulla (which outnumber the Y1 sites in both areas) should in addition to PYY(3–36) be accessible to, and influenced by, the systemic PYY(1–36), which prefers the Y2 over the Y1 receptor (Statnick *et al*., 1997).

Muscle

The short-term regulation by Y2 receptor in developed and working muscle could be mainly negatively metabotropic, thus through inhibition of adenylate cyclase(s) via the inhibitory Gi α subunits. A negative regulation by Y2 agonists of the contractility of cultured cardiomyocytes (Allen *et al*., 2006) is opposed to the positive regulation via the Y1 receptor. However, Y2 receptors appear to induce contraction of the intestinal circular muscle (Misra *et al*., 2005).

Anxiety, pain, stress, fear and aggression

Participation of the Y2 receptor in the generation of anxiety was originally detected in mice using NPY and selective Y1 and Y2 agonists (Nakajima *et al*., 1998; for a recent review, see Harro, 2006). The functional antagonism of Y1 and Y2 receptors as related to stress, anxiety and depression is evident in responses to antagonists of these receptors (Heilig, 2004). Deletion of the Y2 receptor in the mouse produces an antidepressant phenotype (Tschenett *et al*., 2003; Carvajal *et al*., 2006), with reduced attention and increased reactivity (Greco and Carli, 2006). N-terminally truncated Y peptides are considered as anxiogenic. Inactivation of DPPIV, an enzyme producing Y2 agonists from NPY and PYY, is reported to have anxiolytic effects in the mouse (El Yacoubi *et al*., 2006). A reduced availability of PYY(3–36), the principal brain-accessible (Nonaka *et al*., 2003) systemically produced Y2 agonist, should attenuate anxiogenic activity via the Y2 receptor. The selective Y2 receptor blocker BIIE0246 is anxiolytic in the rat *in vivo* (Bacchi *et al*., 2006).

Y2 receptors in the skin receive nociceptive projections from the dorsal horn, and could be involved in the mechanisms of pain induction (Brumovsky *et al*., 2005). The receptors also accumulate proximally upon spinal nerve ligation, probably in consequence of a post-ligative loss of NPY (Marchand *et al*., 1999), and this could be connected to pain stimuli. Here, it should be noted that ablation of the NPY gene in the mouse results in a massive accumulation of the Y2 (as well as of the Y1) receptor in the brain (Gehlert and Shaw, 2007)). Although a decrease in the release of glutamate release via agonism at the Y2 receptor could contribute to the Y1 receptor-linked reduction of pain (Smith *et al*., 2007), Y1 agonists appear to be more antinociceptive (Mahinda and Taylor, 2004).

The Y2 agonists augment, and the Y1 agonists reduce social stress in the rat (Klemfuss *et al*., 1998). Fear and anxiety in the rat can be reduced by Y1-selective, but not by Y2-selective agonists (Broqua *et al*., 1995). Mice lacking the Y2 receptor show an anxiolytic phenotype, with increased reactivity (Greco and Carli, 2006), and this is particularly pronounced in aged Y2-knockout mice (Carvajal *et al*., 2006). Stress is reduced in the Fischer-344 rat lacking DPPIV (and hence the production of Y2 agonists), and this reduction is associated with an increased sociality, and a decrease in anxiety (Karl *et al*., 2003). Maternal aggression in mice, on the other hand, is reported to be linked to decreases in the Y2 receptor and other metabotropic receptors (Gammie *et al*., 2006).

Substances of abuse and the Y2 receptor

The Y2-selective antagonists to be tested or used in the therapy of human drug abuse need to achieve sufficient levels in the brain. The currently most used Y2-selective antagonist BIIE0246 does not sufficiently saturate the neural matrix, necessitating central administration (Abbott *et al*., 2005).

Alcohol. A recent review by Thorsell (2007) does not assign to the Y2 receptor a principal role among Y receptors in the regulation of alcohol intake, although there is evidence in that direction. Thus, there is reduction of alcohol consumption in mice by the Y2 antagonist BIIE0246 (Rimondini *et al*., 2005), and deletion of the Y2 receptor in the mouse lowers the use of ethanol (Thiele *et al*., 2004).

Nicotine. Chronic administration of nicotine, long known to upregulate the nicotinic acetylcholine receptors, may increase Y2 receptors, while reducing Y1 receptors in rat brain (Li *et al*., 2000b). These changes could be connected to increased NPY levels in conditions of chronic treatment by nicotine at levels typically found in tobacco smokers (Li *et al*., 2000a). Interestingly, a chronic treatment by nicotine also activates the prolactin-releasing peptide receptor (Sun *et al*., 2005), which has a significant sequence homology with the Y2 receptor (see Table 3).

Cocaine. This alkaloid, at least with chronic overdosing, strongly upregulates peptidergic κ opioid receptors (Staley *et al*., 1997; Mash and Staley, 1999), which possess highly interactive (and anionic residue-rich) extracellular domains 1 and 3, and, similar to the Y2 receptor, show a slow internalization (especially in response to high levels of agonists). The Y2 receptor numbers could be significantly increased in hypothalami of rats receiving chronic cocaine (W Sun, personal communication).

Conclusion

As with many other rhodopsin-like receptors, regulatory activity of the Y2 receptor is not strongly specialized, but rather broad and permissive. This lack of specialization can also apply to organizing interactions of the receptor. Physiologically, activities of the Y2 receptor appears to balance those of the Y1 receptor. Negative regulation of food intake in humans and in animal models by Y2 agonists has received the most attention, and appears to have the largest potential for dietary and medical applications, especially with development of agonists (and antagonists) that can sufficiently saturate brain areas principally involved in the regulation of feeding. Another broad area with a significant pharmacological and medical interest is the regulation of anxiety and related conditions of stress, fear, aggression, pain and of social intercourse, by selective Y2 antagonists. Study and/or treatment of these conditions also require development of Y2-affecting agents that efficiently traverse the ependymal barriers. Features especially of the N-terminal sequence should be involved in the anchoring and the compartmentalization of the Y2 receptor, observed in both cell lines and brain tissue. This presents a large potential for metabotropic and organizing activities, and could be importantly involved in the participation of Y2 receptors in angiogenesis, tubulogenesis and other interactions of endothelial and epithelial cells. The application of Y2 agonists could be of medical interest in the management of wound healing and tissue growth, which may not be safely implemented by the use of powerful, but potentially tumorigenic cytokines.

Conflict of interest

Nastech Pharmaceutical Company Inc. (Bothell, Washington, USA) has licensed Y2 agonists invented by one of us (AB) for developing them into anti-obesity drugs.

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