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Review

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Towards improved receptor targeting: anterograde transport, internalization and postendocytic trafficking of neuropeptide Y receptors

Abstract: The neuropeptide Y system is known to be involved in the regulation of many central physiological and pathophysiological processes, such as energy homeostasis, obesity, cancer, mood disorders and epilepsy. Four Y receptor subtypes have been cloned from human tissue (hY_1, hY_2, hY_4 and hY_5) that form a multiligand/multireceptor system together with their three peptidic agonists (NPY, PYY and PP). Addressing this system for medical application requires on the one hand detailed information about the receptor-ligand interaction to design subtype-selective compounds. On the other hand comprehensive knowledge about alternative receptor signaling, as well as desensitization, localization and downregulation is crucial to circumvent the development of undesired side-effects and drug resistance. By bringing such knowledge together, highly potent and long-lasting drugs with minimized side-effects can be engineered. Here, current knowledge about Y receptor export, internalization, recycling, and degradation is summarized, with a focus on the human Y receptor subtypes, and is discussed in terms of its impact on therapeutic application.

Keywords: arrestin; G protein-coupled receptor (GPCR); neuropeptide Y system; oligomerization; short consensus motif.

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Introduction

G protein-coupled receptors (GPCR) represent the largest family of cell surface receptors and are responsible for the majority of signal transduction across cell membranes (Millar and Newton, 2010). Thus, it is not surprising that nearly half of the pharmaceuticals prescribed worldwide act on those receptors (Bridges and Lindsley, 2008). Extensive investigations on GPCR have revealed that the sensitive balance between receptor activation and desensitization is crucial to maintain cellular homeostasis. One component of this balancing act is the agonist-driven desensitization, internalization and recycling process of GPCR. Understanding the mechanism underlying these processes tremendously enlarges the scope of clinical intervention, when GPCR are used as a drug target. One group of GPCR that is being focused on in current investigations are the neuropeptide Y receptors (Y receptors). Besides their very prominent role in food intake and cancer (Korner and Reubi, 2007; Nguyen et al., 2011; Zhang et al., 2011), further involvement of these receptors in ischemic diseases, reproduction, memory retention (Flood et al., 1989), epilepsy (Vezzani and Sperk, 2004; El Bahh et al., 2005), and mood disorders (Heilig, 2004) has been reported during the past few decades. Consequently, these GPCR are extraordinarily interesting in terms of medical applications.

Y receptors belong to the group of rhodopsin-like GPCR and act preferentially via pertussis toxin-sensitive, heterotrimeric $G_{i/o}$ proteins (Michel et al., 1998). Like all GPCR, the Y receptors consist of seven transmembrane-spanning helices, three intra- and extracellular loops (ECLs), and an external amino(N-)- and an internal carboxy(C-)-terminus. Their activation leads to the inhibition of the adenylate cyclase, the modulation of potassium and calcium channels and, in some cell types, to the phosphorylation of p44/42 mitogen-activated protein kinases (MAPKs) and their downstream effectors (Howell et al., 2005; Lu et al., 2010; Thiriet et al., 2011; Shimada et al., 2012). Five Y receptors have been cloned from mammals, namely Y_1, Y_2, Y_4, Y_5 and y_6. The last, y_6, is not expressed in primates

due to a frame-shift mutation resulting in a truncated nonfunctional protein (Rose et al., 1997). Y receptors can be found in various tissues including the central nervous system, blood vessels, intestine and kidneys, where they convey various physiological effects.

The endogenous ligands of Y receptors are the closely-related neuropeptide Y (NPY), peptide YY (PYY) and the pancreatic polypeptide (PP). NPY is the most abundantly expressed neuropeptide in the brain, whereas PYY and PP are gut-derived hormones. All three members of the NPY family consist of a 36-amino-acid(aa)-long peptide chain with an amidated C-terminus. The spatial arrangement of the NPY family peptides was long thought to be a hairpin-like so-called PP-fold, as determined from the crystal structure of avian PP in 1981 (Blundell et al., 1981). This structure comprises an N-terminal polyproline helix (residues 1–8), a consecutive turn and a C-terminal α-helix (residues 14–31) arranged in a U-shaped tertiary structure. Nuclear magnetic resonance (NMR) studies in the presence of membrane-mimicking dodecylphosphocholine confirmed the formation of the C-terminal α-helical structure. However, those studies revealed that the N-termini of all three peptides are more flexible and do not form the hairpin-like fold under physiological conditions (Bader et al., 2001; Lerch et al., 2002, 2004) (Figure 1). Notably, the peptides were found to pre-associate with the membrane via their amphipathic α-helix, and for the PP also with its N-terminus, presumably to get into the correct orientation for specific Y receptor subtype binding (Lerch et al., 2002; Thomas et al., 2005). Y receptors have unique but overlapping ligand binding profiles, thereby constituting a multiligand/multireceptor system. NPY and PYY are both bound by the Y_1, Y_2 and Y_5 receptors with relatively high affinities (Cabrele and Beck-Sickinger, 2000; Lindner et al., 2008a). In contrast, PP, which evolved most recently, binds predominantly to the Y_4, and with lower but still nanomolar affinity to the Y_5 receptor. Moreover, truncation of NPY and PYY by dipeptidyl peptidase IV leads to Y_2/ Y_5 selective agonists, namely NPY_{3-36} and PYY_{3-36} (Mentlein et al., 1993; Borowsky et al., 1998).

Targeting such a multiligand/multireceptor system for clinical application is challenging because one distinct Y receptor subtype has to be addressed specifically to favor the desired outcome while minimizing side effects that might occur from the co-activation of other Y receptor subtypes. In order to unravel the similarities and differences in Y receptor structures, and more precisely in their binding pockets, chimeric receptor approaches, peptide alanine-scans (Pedragosa Badia et al., 2013), recombinant receptor expression (Schmidt et al., 2009) and NMR studies were performed. Several peptide residues were identified as being involved in receptor binding, while determination of the receptor counterpart was more difficult. The first direct interaction sites between Y receptors and their ligands were determined by Merten et al. (2007). In those studies, it was shown that the aspartic acid$^{6.59}$ residue within the ECL3 of Y_1 and Y_4 receptor forms a salt-bridge with the arginine^{35} of NPY and PP. A similar interaction was confirmed for aspartic acid$^{6.59}$ in the Y_2 and Y_5, however, the counterpart within NPY was found to be arginine^{33} instead of arginine^{35}. In addition, Y_2 and Y_5 receptor binding required the presence of arginine^{25} within the peptide ligands, while Y_1 and Y_4 were insensitive to the alanine mutation of arginine^{25}. Shortly after, the counterpart of arginine^{25} was identified to be aspartic acid$^{2.68}$ in the ECL2 of the Y_5 receptor (Lindner et al., 2008b). These studies point towards distinct binding modes for the Y_2/Y_5 and Y_1/Y_4 receptors. Based on this knowledge, improved subtype selective peptides as well as small non-peptidic agonists and antagonists have been developed. These ligands can specifically modify receptor signal transduction in order to favor a physiological response (Figure 2).

Peptide ligands, including the endogenous ligands of Y receptors, and some organic agonists or antagonists are not cell permeable; thus modulation of Y receptor activity with any ligand requires initial receptor availability at the cell surface. This is not only determined by the expression levels of GPCR but also by trafficking processes that comprise:

– the anterograde transport of the receptor to the cell membrane;

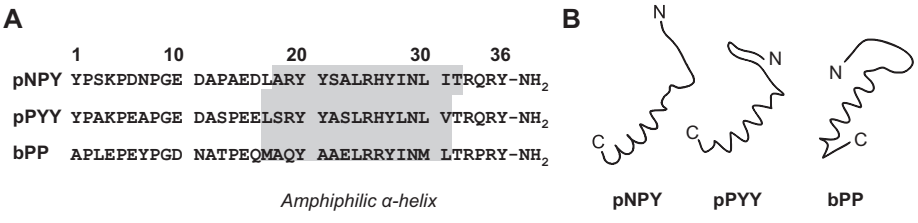

Figure 1 (A) Primary and (B) tertiary structure of NPY, PYY and PP, as determined by nuclear magnetic resonance in the presence of membrane mimicking dodecylphosphocholine-micelles (PDB ID: pNPY: 1F8P, pPYY: 1RUU, bPP: 1LJV).

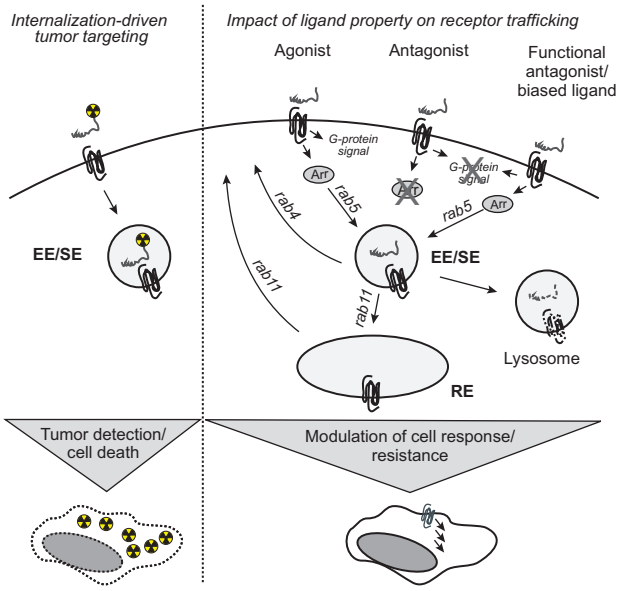

Figure 2 Internalization and postendocytic trafficking of G protein-coupled receptors.
Internalization of radio-ligands enables the cell-specific targeting of tumor cells (left panel). Likewise, the receptor function can be influenced by different ligands, thereby modulating the cellular response (right panel). While classic antagonists do not promote internalization, agonist and functional antagonist lead to receptor endocytosis mediated by arrestin and rab5. Once internalized, the receptor–ligand complex is either degraded in the lysosome or recycled back passing the rab4- (direct recycling) or rab11-dependent (indirect recycling) pathway. Arr, arrestin; EE, early endosome; RE, recycling endosome; SE, sorting endosome.

- the internalization; and
- the postendocytic trafficking that may lead to receptor recycling or degradation.

Usually, these receptor trafficking processes are tightly regulated by a mutual interplay of receptor conformation and consensus sequences on the one site and accessory proteins on the other site. Remarkably, interaction partners involved in receptor internalization were also found to alter GPCR signaling pathways by serving as a scaffold for further enzymes and regulators. This clearly shows an interdependence of receptor localization and signaling following agonist-induced activation and endocytosis. Key players in this phenomenon are G protein-coupled receptor kinases (visual GRK1 and GRK7, non-visual GRK2-6) and arrestins (Arr; visual Arr1 and Arr4, non-visual Arr2 and Arr3) (Gurevich and Gurevich, 2006; Gurevich et al., 2012; Magalhaes et al., 2012). These proteins usually bind to the receptor subsequent to its activation and G protein-dependent signaling and mediate receptor desensitization, endocytosis and alternative G protein-independent signaling pathways. The observation of an Arr-induced alternative signaling pathway led to the identification of a new class of receptor ligands, named biased ligands. Such compounds can trigger a discrete receptor conformation that either corresponds to G protein activation or Arr binding and consecutive internalization. In the latter case, the receptor is removed from the cell surface independent from G protein signaling, which is referred to as functional antagonism (Figure 2).

Besides mediating receptor activation and localization, GPCR trafficking can also be used as a shuttling system when receptors are specifically addressed and overexpressed in cells that are related to cancer. The underlying mechanism is the co-internalization of a ligand with its receptor subsequent to its binding and activation. Thus, radioactive or cytotoxic ligands can be applied that accumulate within the tumor cells subsequent to receptor stimulation and internalization. This enables improved diagnosis and therapy of distinct types of cancer and has been successfully shown for several GPCR, including the bombesin (gastrin-releasing peptide) and the Y_1 receptor (Santos-Cuevas et al., 2008; Khan et al., 2010) (Figure 2).

Contrary to the benefits of using GPCR trafficking as a shuttling system, postponed export or simultaneous internalization and degradation of the receptor may lead to drug resistance when Y receptors are addressed for clinical intervention. Consequently, detailed knowledge about GPCR trafficking is essential for the design of highly effective diagnostic and therapeutic tools that do not only modulate classic Y receptor subtype activation but also control receptor localization and signaling pathways. Therefore, molecular mechanisms that are responsible for membrane targeting, internalization and the recycling of Y receptors were investigated in detail to contribute to a better understanding of how to use these receptors as drug targets. Here, recent findings on Y receptor trafficking are reviewed with a focus on the human proteins and discussed with respect to their relevance for addressing those receptors in therapeutic applications.

Human Y receptors

Y_1 receptor

The human Y_1 (hY_1) receptor consists of 384 aa and shows a sequence identity of more than 92% with its orthologs expressed in pig, guinea pig, mouse, rat and *Bos taurus*. Generally, the sequence identity among the Y receptor subtypes is rather low, but is highest for the Y_1 and the Y_4 receptor. Hence, hY_1 and hY_4 (and y_6 in other mammals) form one Y receptor superfamily, while hY_2 and hY_5 each

form an autonomous group (Larhammar and Salaneck, 2004). Tissues in which the hY_1 receptor can be found are predominantly in the brain (dentate gyrus of the hippocampus) (Dumont et al., 1998) but also in the vascular smooth muscle cells, adipose tissue, kidney and the gastrointestinal tract (Michel et al., 1998). The most prominent role of this Y_1 receptor subtype is the induction of food intake and the regulation of energy homeostasis in synergy with Y_5 (Nguyen et al., 2012). Moreover, the Y_1 receptor modulates vasoconstriction (Hodges et al., 2009) and conveys neuroregenerative effects *in vitro* and *in vivo* (Howell et al., 2005; Thiriet et al., 2011), as well as antidepressant and anxiolytic effects in rodents (Wahlestedt et al., 1993; Verma et al., 2012). The hY_1 is overexpressed in more than 84% of primary human breast (Reubi et al., 2001) and Ewing sarcoma tumors (Korner et al., 2008), representing a promising diagnostic and therapeutic target for these types of cancer.

Y_2 receptor

The hY_2 receptor is a 381-aa-protein that is expressed predominately in neuronal tissue, but also in the spleen, liver, blood vessels, gastrointestinal tract, white and brown fat tissue. This receptor shares a sequence identity of more than 92% with its orthologs in mouse, *Bos taurus*, pig and guinea pig. In the brain, activation of presynaptically-expressed Y_2 receptors inhibits neurotransmitter release (Klapstein and Colmers, 1993), including NPY and glutamate, which makes this receptor an interesting target for antiepileptic drugs (Vezzani and Sperk, 2004). Furthermore, the Y_2 conveys anorexigenic effects in mice, rats and humans (Batterham et al., 2002) and is involved in memory retention, mood disorders (Verma et al., 2012), angiogenesis (Lee et al., 2003), and the reward system following alcohol consumption (Hayes et al., 2012). In addition, the Y_2 receptor is overexpressed in glioblastoma and neuroblastoma, where it induces tumor growth and vascularization (Kitlinska et al., 2005; Korner and Reubi, 2008; Lu et al., 2010). Interestingly, it was recently found that Y_2 receptors expressed in the central nervous system and in non-neuronal tissues might have different assignments. Only Y_2 receptors of the central nervous system regulate bone mass while peripherally-expressed receptors were not found to contribute to bone metabolism at all (Shi et al., 2011).

Y_4 receptor

With a sequence identity of <86%, the hY_4 receptor has the lowest proportion of identical sequence compared to its orthologs in relation to other Y receptor subtypes. The hY_4 receptor consists of 375 aa and is mainly expressed in the gastrointestinal tract and, to a much lesser extent, in the hippocampus, the hypothalamus and the area postrema, a region with an incomplete blood-brain barrier (Dumont et al., 1998; Lindner et al., 2008a; Bellmann-Sickert et al., 2011). There, the Y_4 receptor can receive signals from peripherally-circulating PP and NPY, modulating energy homeostasis and emotional behavior in synergy with the Y_2 receptor (Lin et al., 2009; Tasan et al., 2009). Moreover, the Y_4 receptor inhibits gastric emptying in humans, followed by a delayed postprandial increase in blood glucose levels and subsequent insulin secretion (Schmidt et al., 2005), thereby inducing anorexigenic effects. As the Y_4 receptor also has an inhibitory effect on gastrointestinal secretion, it might be a potent target in diarrheal disorders (Tough et al., 2006). Besides this, the Y_4 receptor was found to be expressed in several human colon adenocarcinoma cell lines (Cox et al., 2001), pointing towards this receptor having a role in the development of colon cancer in humans.

Y_5 receptor

The hY_5 receptor exists in two isoforms with similar pharmacological profiles (Rodriguez et al., 2003). The long isoform of the hY_5 receptor consists of 455 aa, while the short isoform constitutes a splice variant that lacks the first 10 aa. With respect to the other Y receptor subtypes, the Y_5 receptor possesses a relative large third intracellular loop (ICL3) with approximately 140 aa, while the C-terminus is rather short. The Y_5 receptor shares a sequence identity with its orthologs of over 85% and is abundantly expressed in the hypothalamus, thalamus, amygdala and temporal cortex (Durkin et al., 2000). It contributes to energy homeostasis and food intake together with Y_1 (Nguyen et al., 2012), and mediates anticonvulsant effects (Benmaamar et al., 2005). Moreover, recent findings suggest that the Y_5 receptor also contributes to anxiolysis in rats (Morales-Medina et al., 2012). Similar to the Y_1 receptor, the Y_5 receptor is expressed in distinct human breast cancer cell lines, where it promotes cell growth and migration (Sheriff et al., 2010).

The anterograde transport of Y receptors

GPCR are translated in the rough endoplasmic reticulum (ER) and transported to the cell surface passing the

ER–Golgi intermediate complex, the Golgi apparatus and the trans-Golgi network (Wang and Wu, 2012) (Figure 3C). The anterograde transport is a very well regulated process and ER export requires correct folding, optional N-glycosylation and protein assembly of GPCR. Membrane proteins are then packed into COPII vesicles, which require Sar1 GTPase and Sec23/24 proteins to bud off from the ER. This first step in GPCR trafficking is mediated by a variety of distinct receptor sequence-dependent motifs within the proximal C-terminus (dileucine, triple phenylalanine, diacid, and diphenyl motifs) as reviewed by Duvernay et al. (2005). For some receptors, ER export is also controlled by the N-terminus and its glycosylation (Dong and Wu, 2007). Once the receptor is sorted into COPII vesicles, its trafficking from the ER to the membrane is mediated by ras-related in brain (rab) proteins 1, 2, 6, and 8 (Wang and Wu, 2012). These small GTPases associate with their cargo directly or indirectly and take part in vesicle targeting, tethering and fusion.

Y receptors are predominately expressed at the cell surface, however, the anterograde transport mechanisms responsible were not known until recently. Pioneer studies in our laboratory were performed to shed light on the membrane-targeting of human Y receptors. A combination of truncated and point mutants of human Y receptors in HEK293 cells revealed that the molecular mechanisms

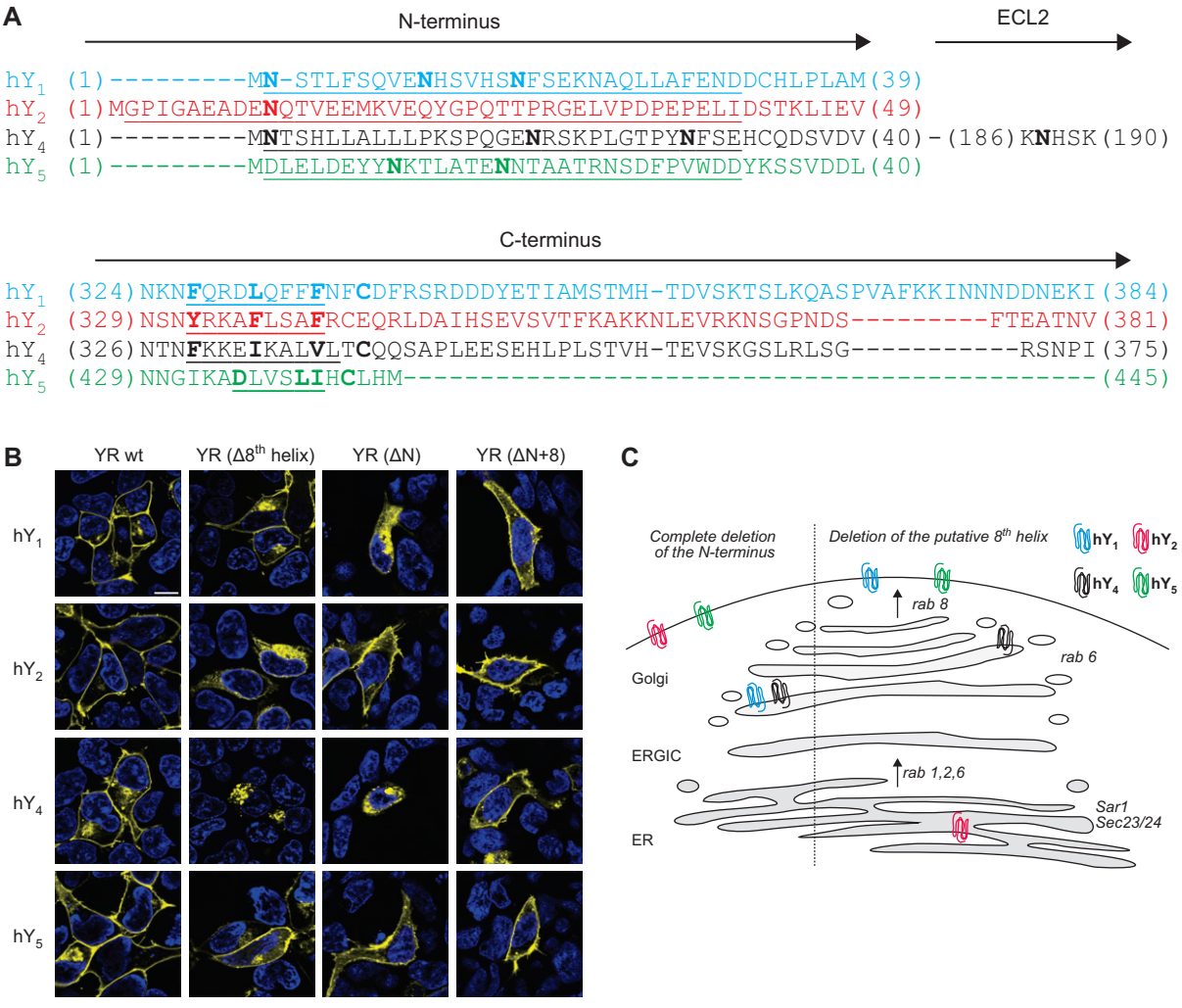

Figure 3 Regulation of the anterograde transport of human Y receptors.
(A) Comparison of N- and C-terminal sequences of human Y receptors. Underlined sequences represent the deleted region in YR(Δ8th helix) and YR(ΔN+8), respectively. In YR(ΔN), the whole N-terminus as presented here was truncated except for the starting methionine. Asparagines that are proposed to be glycosylated as well as residues that might contribute to the formation of the putative eighth helix are written in bold. (B) Representative fluorescence images show the distribution of wild-type and mutant receptors in HEK293 cells (Lindner et al., 2009; Walther et al., 2012). (C) Schematic illustration of the impact of N- and C-terminal deletion on human Y receptor export. Scale bar: 10 µm; ECL2, extra cellular loop 2; ERGIC, ER-Golgi intermediate complex; wt, wild type; YR, human Y receptor.

of all four human Y receptors seem to differ dramatically (Lindner et al., 2009; Walther et al., 2012) (Figure 3).

Role of C-terminal sequences

With a focus on hY_2 receptor, the sequence motif $^{332}Y(x)_3F(x)_3F^{340}$ located at the very proximal C-terminus was identified to be crucial for ER export (Walther et al., 2012). Complete or partial deletion of the motif as well as point mutations from tyrosine/phenylalanine to alanine led to dramatic ER retention of the receptor. Interestingly, transferring the motif to a more distal region of the hY_2 receptor C-terminus did not rescue the anterograde transport and the receptor still accumulated in the ER. As the very proximal C-terminus comprises the putative eighth helix, we concluded that the $^{332}Y(x)_3F(x)_3F^{340}$ motif must be essential for its formation, and thus for the correct receptor conformation. This hypothesis was further confirmed by performing a low-temperature (28°C) experiment to rescue receptor folding. Thereby, only receptors with single mutations that affected the ER export motif (tyrosine/phenylalanine→alanine) or the formation of the eighth helix ($^{338}S\rightarrow P$) were partly rescued when cells were cultured at 28°C. In contrast, complete deletion as well as relocation of the motif to a more distal C-terminal region still prevented ER export despite lower temperature cultivation. These findings are in good agreement with the function and position of the triple phenylalanine motif of the dopamine D_1 receptor, which was found to bind to the ER chaperone DRiP78 (Bermak et al., 2001).

Like the hY_2 receptor, the hY_4 receptor comprises a comparable motif within the proximal C-terminus, namely $^{329}F(x)_3I(x)_3V^{337}$. Interestingly, deletion of this motif did not result in impaired ER export but prevented transport of the receptor from the Golgi apparatus to the cell surface (Walther et al., 2012). Similarly, the motif of the hY_1 receptor $^{327}F(x)_3L(x)_3F^{335}$ resembles the motif of the hY_2 and hY_4 receptors and is also located within the putative eighth helix. Nonetheless, deletion of this motif had no influence on the cell surface targeting of the hY_1 receptor (Walther et al., 2012).

As mentioned above, the hY_5 receptor exhibits a short C-terminus and a large ICL3. Thus, it seems reasonable that export motifs or structural determinants might be displayed by the ICL3 instead of the C-terminus. Indeed, deletion of the putative eighth helix of the hY_5 receptor C-terminus had no influence on cell surface expression of the receptor as it has been observed for the hY_1 receptor (Walther et al., 2012). Replacing the ICL3 of the hY_5 with the ICL3 of the hY_2 receptor, as well as deletion of large parts of Y5 ICL3 sequences (unpublished results), had no effect on membrane targeting either (Bohme et al., 2008). Thus, it can be suggested that the hY_5 receptor displays its export motif in some region other than the C-terminus/ICL3.

With respect to palmitoylation, which is also a common posttranslational modification of GPCR stabilizing the eighth helix, mutation of the Cys within the proximal C-terminus of the Y_1 and Y_2 receptor led to opposite results. While the palmitoylation motif is crucial for the G protein-coupling and desensitization of the Y_1 receptor, mutation of the Cys within the Y_2 receptor sequence had no effect on receptor signaling properties or cell surface targeting (Holliday and Cox, 2003; Walther et al., 2012).

Role of the N-terminus and its glycosylation motifs

All human Y receptors have an N-terminus of about 40–50 aa, with at least one putative N-glycosylation site. An additional glycosylation site is postulated within the ECL2 of the hY_4 receptor. Generally, N-glycosylation was found to be a prerequisite for the association of chaperons like calnexin and calreticulin, which facilitate GPCR folding in the ER (Dong and Wu, 2007). However, not all GPCR require their N-termini and receptor glycosylation for ER export. The Y_1, Y_2 and Y_4 receptors were reported to be glycosylated, however, the impact of this posttranslational modification on membrane targeting was not addressed (Hansen and Sheikh, 1992; Voisin et al., 2000; Holliday and Cox, 2003). To answer the question of whether the N-termini and their potential glycosylation sites of human Y receptors are important for membrane integration, N-terminally truncated mutants were investigated in our laboratory (Lindner et al., 2009). Interestingly, complete deletion of the hY_1 and hY_4 receptor N-termini prevented membrane integration. Receptors accumulated in intracellular compartments that resembled the Golgi apparatus (Figure 3B). However, N-terminal extension with arbitrary sequences of at least eight residues rescued membrane targeting, despite the absence of any glycosylation motifs. Here, the N-terminal extension seems to stabilize the overall receptor conformation of the hY_1 and hY_4 receptors. In contrast, complete deletion of the N-terminus was very well tolerated for the hY_2 and hY_5 receptors and no N-terminal extension was required for membrane integration. Consequently, it can be speculated that glycosylation does not play any role in the membrane targeting of Y receptors. Nonetheless, it cannot be excluded that the hY_4 displays a glycosylation within its ECL2 that might promote cell surface expression.

Receptor oligomerization

It is very likely that the N-terminus or the putative eighth helix contribute to the organization of the correct receptor conformation and thus are essential elements required for cell surface expression. Those structural elements may therefore favor receptor dimerization in the ER and promote transport to the membrane and/or enhance the membrane retention time, as has been shown for other GPCR (Salahpour et al., 2004). However, the role of class A GPCR oligomerization is discussed controversially, since its physiological relevance is still in question (Gurevich and Gurevich, 2008). Oligomerization of GPCR is generally thought to be achieved by three types of interaction:

- the most stable covalent cysteine-bridge like in metabotropic glutamate receptors;
- coiled-coil interactions favored by the putative eighth helix, such as in $GABA_B$ receptors; and
- non-covalent contacts between transmembrane helices that might lead to the domain swapping of the helices (Breitwieser, 2004).

Non-covalent interactions were recently evaluated in more detail: the crystal structures of different GPCR revealed that receptor protomers contact each other via two types of interfaces. While some receptors, such as rhodopsin and the κ-opioid receptor, form less compact homodimers via their transmembrane helices I, II and their intracellular eighth helix, other GPCR such as CXCR4, tightly interact through its transmembrane helix V and VI also involving intracellular regions of helix III and IV (Filizola, 2013; Katrich, 2013). Moreover, some receptors seem to be capable of interacting via both interfaces, as shown for the μ-opioid receptor.

Although the crystal structures of Y receptors and the corresponding oligomerization interfaces have not been available up to now, Y receptor homodimerization at the cell membrane has been shown *in vitro* (Berglund et al., 2003a; Dinger et al., 2003) and *in vivo* (Estes et al., 2008) using ultracentrifugation, bioluminescence and fluorescence resonance energy transfer (BRET/FRET) as well as Western blot techniques. Moreover, Y receptor dimers were found to be pre-associated with heterotrimeric G proteins at the cell surface in a ratio of 2:1 or 2:2 (Estes et al., 2011; Kilpatrick et al., 2012). Pre-association with G protein was also observed for other GPCR, such as the GABA-B_2 receptor, and most likely occurs shortly after biosynthesis in the ER (David et al., 2006). Thus, Y receptor homodimerization and pre-formation of the G-protein-receptor complex might be in fact a prerequisite for anterograde transport and full functionality of the receptor at the cell surface. In addition to homodimers, heterodimerization was observed for the Y_1 and Y_5 receptors (Gehlert et al., 2007), presumably due to the fact that these receptors are often co-expressed in the same tissues, and are located on the same but opposite and overlapping chromosomal region. When stimulating the Y_1/Y_5 heterodimer, Gehlert et al. observed an increase in Y_5-receptor internalization with respect to its mono- and homodimer (Gehlert et al., 2007). These results were contradicted by the findings of later studies by Bohme et al, showing that co-expression of Y_1/Y_5 receptors did not influence Y_5 receptor endocytosis (Bohme et al., 2008). *Vice versa*, the Y_1 receptor was found to be internalized alone after stimulation despite being co-expressed with the Y_5 receptor (Bohme et al., 2008). Moreover, while some groups report on Y receptor dimer dissociation upon agonist stimulation (Berglund et al., 2003a; Estes et al., 2008), others have observed an increase in oligomerization following agonist-induced receptor activation (Gehlert et al., 2007). Clearly, the role of Y receptor dimerization, as for many other class A GPCR, is still a matter of debate. Thus, further studies will be necessary to identify additional sequences and interaction partners that promote Y receptor export and putative oligomerization to gain further insights into the exact trafficking mechanisms.

Internalization and postendocytic trafficking of Y receptors

Extensive investigation revealed that many GPCR, upon agonist binding and activation, undergo receptor endocytosis via clathrin-coated pits, resulting in down-regulation or altered receptor signaling. In brief, the common mechanism of GPCR internalization requires agonist-induced receptor activation followed by G protein-mediated signaling. Subsequently, GRKs recognize and bind to the activated GPCR, thereby displacing the G protein from the receptor binding site (receptor desensitization). Once having bound, GRKs phosphorylate the active receptor at the serine/threonine residues within the C-terminus or ICL3, which enables the binding of Arr. Finally, Arr recruits the endocytosis machinery, such as adapter protein 2 (AP2) or clathrin, and serves as a scaffold for components involved in further signaling cascades. Some internalized GPCR are reported to preferentially bind Arr3, from which they dissociate immediately after endocytosis ('class A' receptor), while other GPCR bind Arr2 and Arr3 in the same manner and with higher affinity ('class B' receptors). Dissociation of Arr in combination with dephosphorylation

of GPCR promotes recycling and resensitization either via direct or indirect pathways. In contrast, prolonged Arr association and ubiquitination favors receptor trafficking to lysosomes and subsequent degradation (Figure 2). In addition to the general internalization process, which is briefly explained above, several more mechanisms and accessory proteins were found to be involved in GPCR signaling, trafficking, de- and resensitization. All of these findings point towards a coupling of receptor activity to the endocytic machinery and *vice versa* and emphasize the importance of comprehensive studies on GPCR trafficking when modulating receptor activity. With respect to Y receptors, detailed knowledge about endocytosis and trafficking is rather limited. The Y_1 receptor subtype has been studied in the greatest detail and similar and complementary results have been found. In contrast, trafficking properties of the remaining three human Y receptor subtypes have been more controversial, especially for the Y_2 receptor. Taking the latest findings into account, it can be concluded that the hY_1, hY_2 and hY_4 receptors are internalized quickly, while endocytosis of the hY_5 receptor is slow. Clearly, different structural components located in the ICL2, the ICL3 and the C-terminus were identified as contributing to the observed subtype-specific desensitization, Arr recruitment and trafficking behavior of the Y receptors.

Y_1 receptor

The hY_1 receptor was found to be rapidly internalized upon agonist stimulation by different groups (Fabry et al., 2000; Gicquiaux et al., 2002; Bohme et al., 2008; Ouedraogo et al., 2008; Lindner et al., 2009; Kilpatrick et al., 2010; Lecat et al., 2011; Lundell et al., 2011), consistent with the results that were obtained for its orthologs in the rat (Holliday and Cox, 2003; Pheng et al., 2003; Holliday et al., 2005; Kilpatrick et al., 2012) or guinea pig (Parker et al., 2001, 2002b). Several studies on the internalization mechanism revealed that the Y_1 receptor undergoes clathrin-dependent endocytosis in an Arr3-dependent fashion and is transported back to the cell membrane, involving direct and indirect recycling mechanisms (Gicquiaux et al., 2002; Berglund et al., Pheng et al., 2003b; Holliday et al., 2005; Ouedraogo et al., 2008; Kilpatrick et al., 2010; Lecat et al., 2011). Beside the interaction with Arr3, the Y_1 receptor was also found to bind efficiently to Arr2, suggesting that it belongs to the group of 'class B' receptors (Ouedraogo et al., 2008; Kilpatrick et al., 2012). As mentioned before, interaction with Arr is usually a prerequisite for activated and phosphorylated receptors. For the identification of phosphorylation motifs, C-terminally truncated mutants of the rat and hY_1 receptor (whose C-terminal sequences only vary in three distal residues) were investigated with respect to G protein coupling, Arr binding and internalization (Holliday et al., 2005; Ouedraogo et al., 2008; Lecat et al., 2011). These studies showed that the truncation of the whole C-terminus is very well tolerated with respect to G protein signaling, supporting the idea that the formation of the correct Y_1 receptor structure does not require the presence of the C-terminus (see also anterograde transport). In contrast, truncation of the C-terminus had a dramatic impact on receptor desensitization, internalization and Arr recruitment. Detailed studies on point mutations clarified that individual serine/threonine residues within the C-terminal (S/T)-(S/T)-Φ-H-(S/T)-(E/D)-V-(S/T)-x-T motif (where x represents any aa and Φ a bulky hydrophobic residue) are phosphorylated by GRK2, and thus are required for receptor desensitization and Arr recruitment (Holliday et al., 2005; Ouedraogo et al., 2008; Kilpatrick et al., 2010, 2012) (Figure 4). Within this cluster, the number of serine/threonine residues rather than their actual position was found to be crucial for efficient Arr binding, suggesting that multiple phosphorylation events occur within this sequence (Kilpatrick et al., 2010).

Another sequence within the C-terminus, namely the Y-x-x-Φ (YETI) motif, lays upstream of the serine/threonine cluster. This tyrosine-based sequence is known to promote direct interaction with the μ2 subunit of AP2, thereby mediating internalization (Pandey, 2009). In fact, the YETI motif was found to induce constitutive clathrin-dependent internalization of the Y_1 receptor once the downstream phosphorylation and Arr binding motif was absent (Holliday et al., 2005; Lecat et al., 2011). Interestingly, mutation of the tyrosine residue (AETI) in combination with the full length Y_1 receptor had no impact on internalization but impaired receptor reappearance at the plasma membrane after agonist-induced endocytosis (Lecat et al., 2011). Thus, the YETI motif within the C-terminus of the wild-type Y_1 receptor is believed to regulate receptor recycling rather than internalization. In addition, a second Y-x-x-Φ (YIRL) motif is present in the ICL3 of the Y_1 receptor and further adapter protein interaction sequences based on dileucine (D/E)-$X_{2\text{-}3}$-L-(L/I) or triple basic motifs (R/K)-(R/K)-(R/K) are predicted at the beginning of ICL2 (ERHQLI) and ICL3 (KRR), respectively (Pandey, 2009; Smith et al., 2012). However, the impact of these motifs on receptor internalization, recycling and Arr binding has not been estimated yet and is therefore speculative.

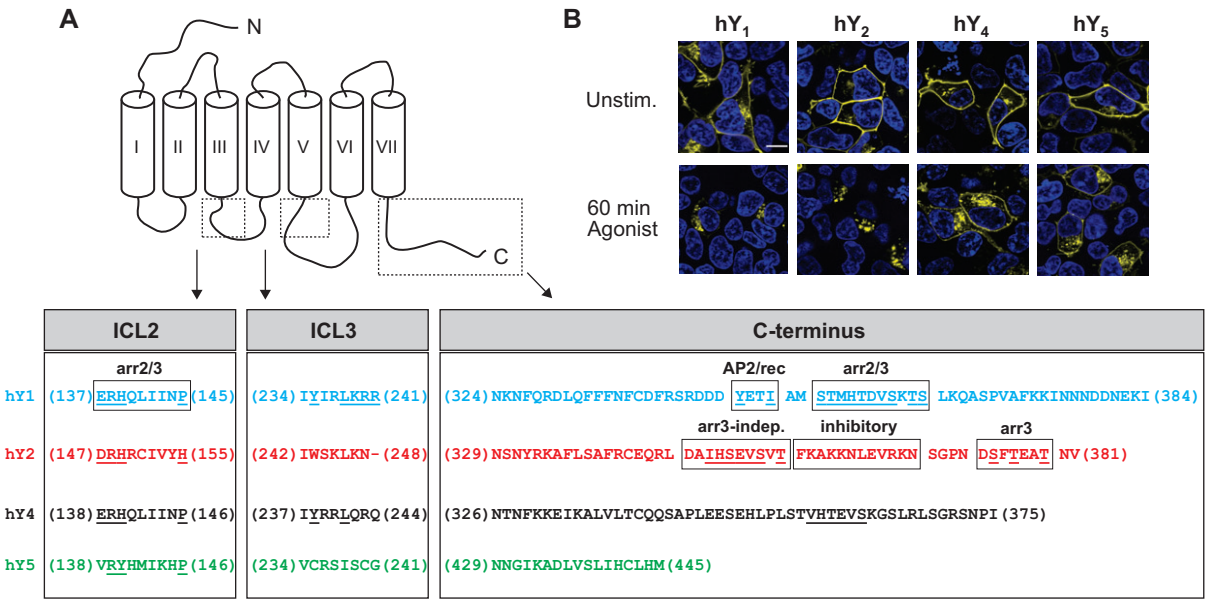

Figure 4 Internalization motifs within human Y receptors.
(A) Position and sequence of identified and putative motifs are displaced. Motifs within boxes were identified as being responsible for either receptor internalization or postendocytic trafficking. Underlined letters represent key residues in postulated or identified consensus sequences. (B) Localization of human Y receptor fused to the enhanced yellow fluorescent protein at the C-termini in living HEK293 cells prior to (unstimulated) or after agonist stimulation. hY_1, hY_2 and hY_5 were stimulated with 1 µM pNPY, while hY_4 was stimulated with 100 nM hPP for 60 min. Scale bar: 10 µm; indep., independent; rec, recycling.

Beside the relevance of C-terminal sequences, a proline residue located in the ICL2 was identified as acting in concert with the DRY motif as an Arr anchor in the $5\text{-}HT_{2c}$ receptor (Marion et al., 2006). This proline residue can also be found in the ICL2 of the Y_1 receptor. However, none of the Y receptors displays a classical DRY motif, and substitution of the proline residue to an alanine only had a minor impact on Y_1 receptor internalization and Arr association (Ouedraogo et al., 2008). In conclusion, the C-terminus seems to be most crucial for the regulation of Y_1 receptor desensitization and trafficking, as summarized in Figure 4. However, it cannot be ruled out that further motifs within the three ICLs contribute synergistically to receptor internalization and trafficking properties, binding either to Arr or to proteins of the endocytic machinery.

Clearly, those protein-receptor interactions depend on the conformation of both binding partners and the corresponding accessibility of consensus motifs exposed by the cytoplasmic receptor site. According to the hypothesis of 'functional selectivity or biased agonism', different ligands can promote discrete receptor conformations and thus favor one downstream effect over another, as it has been observed for several GPCR, including adrenergic receptors and the vasopressin V2 receptor (Rahmeh et al., 2012). Interestingly, Pheng et al. reported that the peptidic ligand GR231118 induced sequestration of Y_1 receptor independent of G protein signaling (Pheng et al., 2003), which points towards a biased agonism of this compound. It is worth noting that GR231118 was initially identified to have agonistic effects only for Y_4, while displaying a classical antagonistic function towards Y_1 (Schober et al., 1998; Dumont and Quirion, 2000). In fact, Y_1 receptor internalization induced by GR231118 could not be confirmed in later experiments, even though the receptor species and cell types were consistent with the initial study (Tough et al., 2006). One possible explanation for this might be that internalization experiments by Pheng et al. were based on the internalization of the radio-labeled GR231118, while later studies focused on receptor redistribution subsequent to ligand stimulation. Thus, further investigations will be necessary to unravel how GR231118 is internalized and whether the Y_1 receptor can undergo biased signaling.

Y_2 receptor

For a long time, the internalization and postendocytic trafficking properties of the Y_2 receptor were controversial.

Early radio-ligand binding studies on the guinea pig Y_2 receptor revealed that this receptor subtype does not undergo sequestration (Parker et al., 2001). Similar studies on the human and rhesus receptor, respectively, confirmed that Y_2 is resistant to endocytosis and shows only a weak interaction with Arr3 (Gicquiaux et al., 2002; Berglund et al., 2003b; Ouedraogo et al., 2008). In contrast, Bohme et al. clearly showed that the hY_2 receptor desensitizes and undergoes fast internalization in response to agonist stimulation comparable to rates observed for the hY_1 and hY_4 receptors (Bohme et al., 2008). Recent studies confirmed those results and revealed that internalization of the hY_2 receptor strongly depends on the agonist concentration used in the experiment (Lundell et al., 2011). In addition, Parker et al. reported that the internalization rate observed for hY_2 is much higher than for its ortholog in the guinea pig (Parker et al., 2008). It is worth noting that the C-terminal sequence of the guinea pig Y_2 receptor displays significant differences with respect to the serine/threonine cluster in comparison to other species, including humans. In a chimeric approach Bohme et al. showed that both the ICL3 and the C-terminus of the hY_2 receptor can individually promote endocytosis of the usually non-internalizing hY_5 receptor, thereby clearly demonstrating that these structural components determine agonist-induced internalization (Bohme et al., 2008). In addition, recent studies on Y_1/Y_2 chimeras revealed an enhanced internalization rate of the Y_1 receptor when its C-terminal sequence is exchanged with the Y_2 receptor C-terminus (Lundell et al., 2011).

Detailed investigation of the molecular mechanism underling Y_2 receptor internalization unraveled three key motifs within the C-terminus responsible for the regulation of hY_2 receptor trafficking (Walther et al., 2010). The first motif within the very distal C-terminus, namely D-S-x-T-E-x-T (DSFTEAT), was found to promote Arr3-dependent internalization subsequent to GRK2 phosphorylation. A second motif, D-x-Φ-H-(S/T)-(E/D)-V-(S/T)-x-T, that resembles the phosphorylation motif of the Y_1 receptor, was found in the more proximal C-terminus, comprising residues DAIHSEVSVT. This sequence also induces agonist-mediated internalization, but surprisingly in an Arr3-independent manner (Walther et al., 2010). The Arr3-independent internalization mechanism was only observed when all further distal C-terminal parts were truncated. Conversely, the presence of the intermediate basic sequence FKAKKNLEVRKN impeded Arr3-independent receptor internalization, presuming that this inhibitory motif masks the proximal internalization motif D-x-Φ-H-(S/T)-(E/D)-V-(S/T)-x-T in the full-length receptor (Walther et al., 2010). Moreover, the proximal motif was also found to promote Y_2 receptor recycling rather than internalization in the wild type receptor, similar to the function assigned to the YETI motif in the Y_1 receptor.

By comparing the Y_1 and Y_2 subtypes, it becomes clear that the regulation of receptor trafficking strongly depends on sequences within the C-terminus. Importantly, consensus sequences and exact mechanisms seem to vary between those two Y receptor subtypes. However, each receptor displays one primary phosphorylation motif within the C-tail that functions as a molecular switch, thereby regulating Arr recruitment and internalization. In addition, a second latent internalization motif is present that controls receptor recycling and, apart from that, becomes involved in receptor endocytosis under the condition that the primary motif is missing.

Interestingly, while all other Y receptors possess a proline residue within the ICL2 for the proposed Arr interaction, the Y_2 receptor displays a histidine at this position. Replacing this histidine with proline resulted in an enhanced Arr binding and internalization of the Y_2 receptor (Marion et al., 2006; Kilpatrick et al., 2010, 2012), suggesting that Arr also recognized motifs within the ICL. Clearly, the role of additional trafficking sequences within the cytoplasmic region of the Y_2 receptor requires further investigation.

Beside C-terminal residues, the N-terminus of the receptor has been found to contribute to receptor internalization properties. Even though hY_2 receptor export is not affected when the entire N-terminus is truncated, ligand binding, receptor activation, and consequently the internalization process were drastically impaired (Lindner et al., 2009). Importantly, elongation of the first transmembrane residues by any eight aa rescued receptor activation and internalization, suggesting a sequence-independent, stabilizing effect of the N-terminus towards the ligand-binding pocket and/or the active receptor conformation (Lindner et al., 2009). Similarly, Parker et al. confirmed that the Y_2 receptor N-terminus is not directly involved in agonist binding (Parker et al., 2008). However, this group discovered an acidic and proline-rich motif within the N-terminus contributing to receptor internalization. By mutating one aspartic acid residue within this motif to alanine, the receptor internalization was found to be accelerated. Since no other receptor characteristic was affected by this alanine substitution, it was suggested that the acidic motif within the N-terminus of the Y_2 receptor binds to components of the extracellular matrix. This, in turn, might affect Y_2 receptor internalization kinetics (Parker et al., 2012).

In conclusion, the hY_2 receptor is internalized in an Arr3-dependent manner, but higher agonist concentration

seems to be required to promote receptor endocytosis (Walther et al., 2010; Lundell et al., 2011). Even though the serum concentration of NPY and PYY might be too low to drive receptor internalization in the periphery, local concentrations in the synaptic gap might be high enough to induce hY_2 endocytosis in the brain. That the Y_2 receptor desensitization might indeed have an important physiological relevance was impressively shown by Ortiz et al. (2007). Here, a stabilized PYY(13–36) analog was observed to reduce food intake in mice. In a long-term study, however, food intake returned to the control level after 3 days of daily drug administration. This might be due to receptor desensitization or down-regulation.

Y_4 receptor

Similar to the Y_2 receptor, studies on Y_4 receptor desensitization and trafficking revealed opposing results. Initially, the hY_4 receptor was reported to be resistant to agonist-promoted desensitization and internalization when being expressed in Chinese hamster ovary cells (Voisin et al., 2000). In contrast, several other groups independently showed that the Y_4 receptor is rapidly internalized and subsequently transported back to the cell surface, most probably via the indirect recycling pathway (Parker et al., 2001, 2002a; Tough et al., 2006; Bohme et al., 2008). The determined internalization and recycling rates were either comparable or slightly slower than those observed for the Y_1 receptor. With respect to the exact internalization mechanism, Y_4 receptor endocytosis was found to be promoted by Arr3, suggesting a clathrin-dependent process (Berglund et al., 2003b). In these studies, however, Arr3 recruitment was less pronounced for the Y_4 than for the Y_1 receptor, supporting the idea that Y_4 receptor internalization and recycling rates might indeed be slightly prolonged.

In addition to these findings, the rate of Y_4 receptor sequestration was found to be highly sensitive to agonist affinity and efficacy, i.e., full agonists induced a higher internalization rate than partial agonists (Parker et al., 2002a; Tough et al., 2006). This is in agreement with the common mechanism of GPCR activation followed by sequestration, as described above, and underlines the fact that the endogenous Y_4 ligands generate two conformations, which are equally important for G protein and GRK/Arr binding. It is worth noting that the Y_4 receptor and its ligand PP emerged last and presumably quite quickly in evolution, leading to greater divergences in peptide and receptor sequences across mammalian species (Yulyaningsih et al., 2011). Thus, a combination of receptors and ligands originating from different species was observed to result in a decreased internalization rate due to reduced peptide efficacy (Tough et al., 2006).

Very little information is available about consensus sequences that drive internalization and recycling processes of the Y_4 receptor. As the Y_4 receptor is most closely related to the Y_1 receptor, it is not surprising that a variant of the serine/threonine cluster, which is known to promote Arr binding and internalization of the Y_1 receptor subtype, also occurs within the distal C-tail of the Y_4 receptor (STVHTEVSKG). Further sequences that might be involved in Y receptor trafficking include the proline-based Arr-binding motif and the tyrosine-based AP-interaction sequence within ICL2 and ICL3, respectively. Even though, all these motifs are present in the Y_4 receptor, their role in internalization and trafficking is still a matter of investigation.

Y_5 receptor

The regulation of Y_5 receptor trafficking is not well understood. Microscopic studies using EYFP-fused receptors and radio-ligand internalization experiments revealed an extremely slow internalization rate for both ligand and receptor (Parker et al., 2003; Bohme et al., 2008). Nonetheless, the receptor was still found to desensitize in inositol phosphate (IP)-accumulation assays (Bohme et al., 2008). The relatively small fraction of internalized receptor was even smaller in the presence of phenylarsine oxide, an inhibitor of clathrin-coated pit formation (Parker et al., 2003), suggesting a clathrin-dependent internalization mechanism. Studies on Arr recruitment are rather limited but we were unable to detect a pronounced Arr recruitment subsequent to Y_5 receptor stimulation in our lab (data unpublished). Contrasting with this, BRET studies showed that the Y_5 receptor can recruit Arr3 after stimulation, even though the signals were much lower than observed for the Y_1 receptor (Berglund et al., 2003b).

Comprehensive studies are necessary to unravel the structural determinants that modulate and inhibit Y_5 receptor internalization. It is reasonable that these structures being distinct to all other Y receptors, namely the long ICL3 and the short C-terminus, have an impact on Y_5 receptor trafficking. The Y_5 receptor chimera bearing either the ICL3 or C-terminus of the Y_2 receptor showed an accelerated internalization rate (Bohme et al., 2008). Still, it cannot be determined whether this effect is due to the deletion of inhibitory segments lying in the Y_5 receptor ICL3 and C-terminus or to the addition of internalization motif present in the Y_2 receptor sequence. Thus, further

studies are required to estimate the mechanisms underlying Y_5 receptor trafficking.

Taking everything into account, it can be concluded that Y receptor subtypes internalize with rates in the following order: $Y_1 > Y_2 \geq Y_4 >> Y_5$. All receptors seem to undergo clathrin-mediated endocytosis, involving Arr in case of the Y_1, Y_2 and Y_4 receptors. The three latter receptors were also found to be recycled subsequent to endocytosis.

Conclusion and future directions

The NPY system has frequently been shown to be a promising target for the treatment of several diseases, with an emphasis on obesity and cancer. Beside the Y_1 receptor-selective agonist for breast cancer diagnosis (Khan et al., 2010), various Y receptor agonists and antagonists have been developed for therapeutic applications. Among those are several Y_5 receptor antagonists designed for antiobesity medication. Two such antagonists, MK-0557 and Velneperit, have already been tested in Phase II trials, but failed to show a clinically-meaningful effect (Sato et al., 2009). In contrast, another antiobesity approach using the dual Y_2/Y_4 receptor peptidic agonist Obinepitide (produced by 7TM Pharma) caused a significant reduction in food intake during Phase I and II trials. Despite this desired clinical outcome of Obinepitide, side-effects were reported such as nausea (Sato et al., 2009).

Thus, the development of specific agonists, antagonists and even biased ligands is still a major issue when using GPCR as drug targets. However, the examples provided above emphasize that engineering specific ligands acting on GPCR can only be the first step in the development of well-tolerated drugs with high potentials.

Here, we report on specific Y receptor-trafficking processes, such as anterograde transport, internalization and recycling, which clearly intervene with receptor response and signaling. These processes might be responsible for the undesired side effects. They can influence the response via the modulation of cell surface availability of receptors. In order to understand and avoid desensitization mechanisms and drug resistance, it will be crucial to unravel the mechanisms of subcellular receptor trafficking. Also for the receptor-mediated uptake of cytostatic compounds for cancer therapy, it will be essential to elucidate internalization or degradation pathways and their regulation. Intervention in alternative signaling pathways related to internalization might open further possibilities of specifically modifying Y receptor signaling. Consequently, intracellular receptor trafficking should be included in a second step in drug development.

With respect to the Y receptor system, it is to be noted that despite the formation of one multiligand/multipeptide system, each Y receptor subtype apparently has a distinct molecular mechanism responsible for the regulation of its export or internalization. This provides the possibility to specifically modulate the localization/signaling of one subtype receptor while leaving others unaffected. Surely, further studies will be helpful in generating an overall picture that, in combination with subtype-selective ligands, will provide a powerful tool in the engineering of highly effective and long-acting pharmaceuticals that address Y receptors.

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References

Bader, R., Bettio, A., Beck-Sickinger, A.G., and Zerbe, O. (2001). Structure and dynamics of micelle-bound neuropeptide Y: comparison with unligated NPY and implications for receptor selection. J. Mol. Biol. *305*, 307–329.

Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A., et al. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. Nature *418*, 650–654.

Bellmann-Sickert, K., Elling, C.E., Madsen, A.N., Little, P.B., Lundgren, K., Gerlach, L.O., Bergmann, R., Holst, B., Schwartz, T.W., and Beck-Sickinger, A.G. (2011). Long-acting lipidated analogue of human pancreatic polypeptide is slowly released into circulation. J. Med. Chem. *54*, 2658–2667.

Benmaamar, R., Richichi, C., Gobbi, M., Daniels, A.J., Beck-Sickinger, A.G., and Vezzani, A. (2005). Neuropeptide Y Y5 receptors inhibit kindling acquisition in rats. Regul. Pept. *125*, 79–83.

Berglund, M.M., Schober, D.A., Esterman, M.A., and Gehlert, D.R. (2003a). Neuropeptide Y Y4 receptor homodimers dissociate upon agonist stimulation. J. Pharmacol. Exp. Ther. *307*, 1120–1126.

Berglund, M.M., Schober, D.A., Statnick, M.A., McDonald, P.H., and Gehlert, D.R. (2003b). The use of bioluminescence resonance

energy transfer 2 to study neuropeptide Y receptor agonist-induced β-arrestin 2 interaction. J. Pharmacol. Exp. Ther. *306*, 147–156.

Bermak, J.C., Li, M., Bullock, C., and Zhou, Q.Y. (2001). Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. Nat. Cell Biol. *3*, 492–498.

Blundell, T.L., Pitts, J.E., Tickle, I.J., Wood, S.P., and Wu, C.W. (1981). X-ray analysis (1. 4-Å resolution) of avian pancreatic polypeptide: small globular protein hormone. Proc. Natl. Acad. Sci. USA *78*, 4175–4179.

Bohme, I., Stichel, J., Walther, C., Morl, K., and Beck-Sickinger, A.G. (2008). Agonist induced receptor internalization of neuropeptide Y receptor subtypes depends on third intracellular loop and C-terminus. Cell. Signal. *20*, 1740–1749.

Borowsky, B., Walker, M.W., Bard, J., Weinshank, R.L., Laz, T.M., Vaysse, P., Branchek, T.A., and Gerald, C. (1998). Molecular biology and pharmacology of multiple NPY Y5 receptor species homologs. Regul. Pept. *75–76*, 45–53.

Breitwieser, G.E. (2004). G protein-coupled receptor oligomerization: implications for G protein activation and cell. signal. Circ. Res. *94*, 17–27.

Bridges, T.M. and Lindsley, C.W. (2008). G-protein-coupled receptors: from classical modes of modulation to allosteric mechanisms. ACS Chem. Biol. *3*, 530–541.

Cabrele, C. and Beck-Sickinger, A.G. (2000). Molecular characterization of the ligand-receptor interaction of the neuropeptide Y family. J. Peptide Sci. *6*, 97–122.

Cox, H.M., Tough, I.R., Zandvliet, D.W., and Holliday, N.D. (2001). Constitutive neuropeptide Y Y_4 receptor expression in human colonic adenocarcinoma cell lines. Br. J. Pharmacol. *132*, 345–353.

David, M., Richer, M., Mamarbachi, A.M., Villeneuve, L.R., Dupre, D.J., and Hebert, T.E. (2006). Interactions between GABA-B1 receptors and Kir 3 inwardly rectifying potassium channels. Cell. Signal. *18*, 2172–2181.

Dinger, M.C., Bader, J.E., Kobor, A.D., Kretzschmar, A.K., and Beck-Sickinger, A.G. (2003). Homodimerization of neuropeptide y receptors investigated by fluorescence resonance energy transfer in living cells. J. Biol. Chem. *278*, 10562–10571.

Dong, C. and Wu, G. (2007). Regulation of anterograde transport of adrenergic and angiotensin II receptors by Rab2 and Rab6 GTPases. Cell. Signal. *19*, 2388–2399.

Dumont, Y. and Quirion, R. (2000). [^{125}I]-GR231118: a high affinity radioligand to investigate neuropeptide Y Y_1 and Y_4 receptors. Br. J. Pharmacol. *129*, 37–46.

Dumont, Y., Jacques, D., Bouchard, P., and Quirion, R. (1998). Species differences in the expression and distribution of the neuropeptide Y Y_1, Y_2, Y_4, and Y_5 receptors in rodents, guinea pig, and primates brains. J. Comp. Neurol. *402*, 372–384.

Durkin, M.M., Walker, M.W., Smith, K.E., Gustafson, E.L., Gerald, C., and Branchek, T.A. (2000). Expression of a novel neuropeptide Y receptor subtype involved in food intake: an in situ hybridization study of Y_5 mRNA distribution in rat brain. Exp. Neurol. *165*, 90–100.

Duvernay, M.T., Filipeanu, C.M., and Wu, G. (2005). The regulatory mechanisms of export trafficking of G protein-coupled receptors. Cell. Signal. *17*, 1457–1465.

El Bahh, B., Balosso, S., Hamilton, T., Herzog, H., Beck-Sickinger, A.G., Sperk, G., Gehlert, D.R., Vezzani, A., and Colmers, W.F. (2005). The anti-epileptic actions of neuropeptide Y in the hippocampus are mediated by Y and not Y receptors. Eur. J. Neurosci. *22*, 1417–1430.

Estes, A.M., Wong, Y.Y., Parker, M.S., Sallee, F.R., Balasubramaniam, A., and Parker, S.L. (2008). Neuropeptide Y (NPY) Y_2 receptors of rabbit kidney cortex are largely dimeric. Regul. Pept. *150*, 88–94.

Estes, A.M., McAllen, K., Parker, M.S., Sah, R., Sweatman, T., Park, E.A., Balasubramaniam, A., Sallee, F.R., Walker, M.W., and Parker, S.L. (2011). Maintenance of Y receptor dimers in epithelial cells depends on interaction with G-protein heterotrimers. Amino Acids *40*, 371–380.

Fabry, M., Langer, M., Rothen-Rutishauser, B., Wunderli-Allenspach, H., Hocker, H., and Beck-Sickinger, A.G. (2000). Monitoring of the internalization of neuropeptide Y on neuroblastoma cell line SK-N-MC. Eur. J. Biochem. *267*, 5631–5637.

Filizola, M. and Devi, L.A. (2013). Grand opening of structure-guided design for novel opioids. Trends Pharmacol. Sci. *34,* 6–12.

Flood, J.F., Baker, M.L., Hernandez, E.N., and Morley, J.E. (1989). Modulation of memory processing by neuropeptide Y varies with brain injection site. Brain Res. *503*, 73–82.

Gehlert, D.R., Schober, D.A., Morin, M., and Berglund, M.M. (2007). Co-expression of neuropeptide Y Y_1 and Y_5 receptors results in heterodimerization and altered functional properties. Biochem. Pharmacol. *74*, 1652–1664.

Gicquiaux, H., Lecat, S., Gaire, M., Dieterlen, A., Mely, Y., Takeda, K., Bucher, B., and Galzi, J.L. (2002). Rapid internalization and recycling of the human neuropeptide Y Y_1 receptor. J. Biol. Chem. *277*, 6645–6655.

Gurevich, E.V. and Gurevich, V.V. (2006). Arrestins: ubiquitous regulators of cellular signaling pathways. Genome Biol. *7*, 236.

Gurevich, V.V. and Gurevich, E.V. (2008). GPCR monomers and oligomers: it takes all kinds. Trends Neurosci. *31*, 74–81.

Gurevich, E.V., Tesmer, J.J., Mushegian, A., and Gurevich, V.V. (2012). G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. Pharmacol. Ther. *133*, 40–69.

Hansen, A.P. and Sheikh, S.P. (1992). Y2 receptor proteins for peptide YY and neuropeptide Y. Characterization as N-linked complex glycoproteins. FEBS Lett. *306*, 147–150.

Hayes, D.M., Fee, J.R., McCown, T.J., Knapp, D.J., Breese, G.R., Cubero, I., Carvajal, F., Lerma-Cabrera, J.M., Navarro, M., and Thiele, T.E. (2012). Neuropeptide Y signaling modulates the expression of ethanol-induced behavioral sensitization in mice. Addict. Biol. *17*, 338–350.

Heilig, M. (2004). The NPY system in stress, anxiety and depression. Neuropeptides *38*, 213–224.

Hodges, G.J., Jackson, D.N., Mattar, L., Johnson, J.M., and Shoemaker, J.K. (2009). Neuropeptide Y and neurovascular control in skeletal muscle and skin. Am. J. Physiol. Regul. Integr. Comp. Physiol. *297*, 546–555.

Holliday, N.D. and Cox, H.M. (2003). Control of signalling efficacy by palmitoylation of the rat Y1 receptor. Br. J. Pharmacol. *139*, 501–512.

Holliday, N.D., Lam, C.W., Tough, I.R., and Cox, H.M. (2005). Role of the C-terminus in neuropeptide Y Y_1 receptor desensitization and internalization. Mol. Pharmacol. *67*, 655–664.

Howell, O.W., Doyle, K., Goodman, J.H., Scharfman, H.E., Herzog, H., Pringle, A., Beck-Sickinger, A.G., and Gray, W.P. (2005). Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. J. Neurochem. *93*, 560–570.

Katritch, V., Cherezov, V., and Stevens, R.C. (2013). Structure-function of the G protein-coupled receptor superfamily. Annu. Rev. Pharmacol. Toxicol. *53*, 531–556.

Khan, I.U., Zwanziger, D., Bohme, I., Javed, M., Naseer, H., Hyder, S.W., and Beck-Sickinger, A.G. (2010). Breast-cancer diagnosis by neuropeptide Y analogues: from synthesis to clinical application. Angew. Chem. Int. Ed. Engl. *49*, 1155–1158.

Kilpatrick, L.E., Briddon, S.J., Hill, S.J., and Holliday, N.D. (2010). Quantitative analysis of neuropeptide Y receptor association with β-arrestin2 measured by bimolecular fluorescence complementation. Br. J. Pharmacol. *160*, 892–906.

Kilpatrick, L.E., Briddon, S.J., and Holliday, N.D. (2012). Fluorescence correlation spectroscopy, combined with bimolecular fluorescence complementation, reveals the effects of β-arrestin complexes and endocytic targeting on the membrane mobility of neuropeptide Y receptors. BBA-Mol. Cell Res. *1823*, 1068–1081.

Kitlinska, J., Abe, K., Kuo, L., Pons, J., Yu, M., Li, L., Tilan, J., Everhart, L., Lee, E.W., Zukowska, Z., et al. (2005). Differential effects of neuropeptide Y on the growth and vascularization of neural crest-derived tumors. Cancer Res. *65*, 1719–1728.

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

Korner, M. and Reubi, J.C. (2007). NPY receptors in human cancer: a review of current knowledge. Peptides *28*, 419–425.

Korner, M. and Reubi, J.C. (2008). Neuropeptide Y receptors in primary human brain tumors: overexpression in high-grade tumors. J. Neuropathol. Exp. Neurol. *67*, 741–749.

Korner, M., Waser, B., and Reubi, J.C. (2008). High expression of neuropeptide Y1 receptors in ewing sarcoma tumors. Clin. Cancer. Res. *14*, 5043–5049.

Larhammar, D. and Salaneck, E. (2004). Molecular evolution of NPY receptor subtypes. Neuropeptides *38*, 141–151.

Lecat, S., Ouedraogo, M., Cherrier, T., Noulet, F., Ronde, P., Glasser, N., Galzi, J.L., Mely, Y., Takeda, K., and Bucher, B. (2011). Contribution of a tyrosine-based motif to cellular trafficking of wild-type and truncated NPY Y_1 receptors. Cell. Signal. *23*, 228–238.

Lee, E.W., Michalkiewicz, M., Kitlinska, J., Kalezic, I., Switalska, H., Yoo, P., Sangkharat, A., Ji, H., Li, L., Michalkiewicz, T., et al. (2003). Neuropeptide Y induces ischemic angiogenesis and restores function of ischemic skeletal muscles. J. Clin. Invest. *111*, 1853–1862.

Lerch, M., Gafner, V., Bader, R., Christen, B., Folkers, G., and Zerbe, O. (2002). Bovine pancreatic polypeptide (bPP) undergoes significant changes in conformation and dynamics upon binding to DPC micelles. J. Mol. Biol. *322*, 1117–1133.

Lerch, M., Mayrhofer, M., and Zerbe, O. (2004). Structural similarities of micelle-bound peptide YY (PYY) and neuropeptide Y (NPY) are related to their affinity profiles at the Y receptors. J. Mol. Biol. *339*, 1153–1168.

Lin, S., Shi, Y.C., Yulyaningsih, E., Aljanova, A., Zhang, L., Macia, L., Nguyen, A.D., Lin, E.J., During, M.J., Herzog, H., and Sainsbury, A. (2009). Critical role of arcuate Y4 receptors and the melanocortin system in pancreatic polypeptide-induced reduction in food intake in mice. PLoS One *4*, e8488.

Lindner, D., Stichel, J., and Beck-Sickinger, A.G. (2008a). Molecular recognition of the NPY hormone family by their receptors. Nutrition *24*, 907–917.

Lindner, D., van Dieck, J., Merten, N., Morl, K., Gunther, R., Hofmann, H.J., and Beck-Sickinger, A.G. (2008b). GPC receptors and not ligands decide the binding mode in neuropeptide Y multireceptor/multiligand system. Biochemistry *47*, 5905–5914.

Lindner, D., Walther, C., Tennemann, A., and Beck-Sickinger, A.G. (2009). Functional role of the extracellular N-terminal domain of neuropeptide Y subfamily receptors in membrane integration and agonist-stimulated internalization. Cell. Signal. *21*, 61–68.

Lu, C., Everhart, L., Tilan, J., Kuo, L., Sun, C.C., Munivenkatappa, R.B., Jonsson-Rylander, A.C., Sun, J., Kuan-Celarier, A., Li, L., et al. (2010). Neuropeptide Y and its Y_2 receptor: potential targets in neuroblastoma therapy. Oncogene *29*, 5630–5642.

Lundell, I., Rabe Bernhardt, N., Johnsson, A.K., and Larhammar, D. (2011). Internalization studies of chimeric neuropeptide Y receptors Y_1 and Y_2 suggest complex interactions between cytoplasmic domains. Regul. Pept. *168*, 50–58.

Magalhaes, A.C., Dunn, H., and Ferguson, S.S. (2012). Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. Br. J. Pharmacol. *165*, 1717–1736.

Marion, S., Oakley, R.H., Kim, K.M., Caron, M.G., and Barak, L.S. (2006). A β-arrestin binding determinant common to the second intracellular loops of rhodopsin family G protein-coupled receptors. J. Biol. Chem. *281*, 2932–2938.

Mentlein, R., Dahms, P., Grandt, D., and Kruger, R. (1993). Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. Regul. Pept. *49*, 133–144.

Merten, N., Lindner, D., Rabe, N., Rompler, H., Morl, K., Schoneberg, T., and Beck-Sickinger, A.G. (2007). Receptor subtype-specific docking of Asp6.59 with C-terminal arginine residues in Y receptor ligands. J. Biol. Chem. *282*, 7543–7551.

Michel, M.C., Beck-Sickinger, A., Cox, H., Doods, H.N., Herzog, H., Larhammar, D., Quirion, R., Schwartz, T., and Westfall, T. (1998). XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol. Rev. *50*, 143–150.

Millar, R.P. and Newton, C.L. (2010). The year in G protein-coupled receptor research. Mol. Endocrinol. *24*, 261–274.

Morales-Medina, J.C., Dominguez-Lopez, S., Gobbi, G., Beck-Sickinger, A.G., and Quirion, R. (2012). The selective neuropeptide Y Y_5 agonist [cPP(1-7),NPY(19-23),Ala31,Aib32,Gln34]hPP differently modulates emotional processes and body weight in the rat. Behav. Brain Res. *233*, 298–304.

Nguyen, A.D., Herzog, H., and Sainsbury, A. (2011). Neuropeptide Y and peptide YY: important regulators of energy metabolism. Curr. Opin. Endocrinol. Diabetes Obes. *18*, 56–60.

Nguyen, A.D., Mitchell, N.F., Lin, S., Macia, L., Yulyaningsih, E., Baldock, P.A., Enriquez, R.F., Zhang, L., Shi, Y.C., Zolotukhin, S., et al. (2012). Y_1 and Y_5 receptors are both required for the regulation of food intake and energy homeostasis in mice. PLoS One *7*, e40191.

Ortiz, A.A., Milardo, L.F., DeCarr, L.B., Buckholz, T.M., Mays, M.R., Claus, T.H., Livingston, J.N., Mahle, C.D., and Lumb, K.J. (2007). A novel long-acting selective neuropeptide Y_2 receptor polyethylene glycol-conjugated peptide agonist reduces food intake and body weight and improves glucose metabolism in rodents. J. Pharmacol. Exp. Ther. *323*, 692–700.

Ouedraogo, M., Lecat, S., Rochdi, M.D., Hachet-Haas, M., Matthes, H., Gicquiaux, H., Verrier, S., Gaire, M., Glasser, N., Mely, Y., et al. (2008). Distinct motifs of neuropeptide Y receptors differentially regulate trafficking and desensitization. Traffic *9*, 305–324.

Pandey, K.N. (2009). Functional roles of short sequence motifs in the endocytosis of membrane receptors. Front. Biosci. *14*, 5339–5360.

Parker, S.L., Kane, J.K., Parker, M.S., Berglund, M.M., Lundell, I.A., and Li, M.D. (2001). Cloned neuropeptide Y (NPY) Y_1 and pancreatic polypeptide Y_4 receptors expressed in Chinese hamster ovary cells show considerable agonist-driven internalization, in contrast to the NPY Y_2 receptor. Eur. J. Biochem. *268*, 877–886.

Parker, M.S., Lundell, I., and Parker, S.L. (2002a). Internalization of pancreatic polypeptide Y4 receptors: correlation of receptor intake and affinity. Eur. J. Pharmacol. *452*, 279–287.

Parker, S.L., Parker, M.S., Lundell, I., Balasubramaniam, A., Buschauer, A., Kane, J.K., Yalcin, A., and Berglund, M.M. (2002b). Agonist internalization by cloned Y_1 neuropeptide Y (NPY) receptor in Chinese hamster ovary cells shows strong preference for NPY, endosome-linked entry and fast receptor recycling. Regul. Pept. *107*, 49–62.

Parker, S.L., Parker, M.S., Buschauer, A., and Balasubramaniam, A. (2003). Ligand internalization by cloned neuropeptide Y Y_5 receptors excludes Y_2 and Y_4 receptor-selective peptides. Eur. J. Pharmacol. *474*, 31–42.

Parker, S.L., Parker, M.S., Wong, Y.Y., Sah, R., Balasubramaniam, A., and Sallee, F. (2008). Importance of a N-terminal aspartate in the internalization of the neuropeptide Y Y_2 receptor. Eur. J. Pharmacol. *594*, 26–31.

Parker, M.S., Sah, R., and Parker, S.L. (2012). Surface masking shapes the traffic of the neuropeptide Y Y_2 receptor. Peptides *37*, 40–48.

Pedragosa Badia, X., Stichel, J., and Beck-Sickinger, A.G. (2013). Neuropeptide Y receptors: how to get subtype selectivity. Frontiers in Endocrinology *4,* 5.

Pheng, L.H., Dumont, Y., Fournier, A., Chabot, J.G., Beaudet, A., and Quirion, R. (2003). Agonist- and antagonist-induced sequestration/internalization of neuropeptide Y Y_1 receptors in HEK293 cells. Br. J. Pharmacol. *139*, 695–704.

Rahmeh, R., Damian, M., Cottet, M., Orcel, H., Mendre, C., Durroux, T., Sharma, K.S., Durand, G., Pucci, B., Trinquet, E., et al. (2012). Structural insights into biased G protein-coupled receptor signaling revealed by fluorescence spectroscopy. Proc. Natl. Acad. Sci. USA *109*, 6733–6738.

Reubi, J.C., Gugger, M., Waser, B., and Schaer, J.C. (2001). Y_1-mediated effect of neuropeptide Y in cancer: breast carcinomas as targets. Cancer Res. *61*, 4636–4641.

Rodriguez, M., Audinot, V., Dromaint, S., Macia, C., Lamamy, V., Beauverger, P., Rique, H., Imbert, J., Nicolas, J.P., Boutin, J.A., et al. (2003). Molecular identification of the long isoform of the human neuropeptide Y Y_5 receptor and pharmacological comparison with the short Y_5 receptor isoform. Biochem. J. *369*, 667–673.

Rose, P.M., Lynch, J.S., Frazier, S.T., Fisher, S.M., Chung, W., Battaglino, P., Fathi, Z., Leibel, R., and Fernandes, P. (1997). Molecular genetic analysis of a human neuropeptide Y receptor. The human homolog of the murine'Y_5' receptor may be a pseudogene. J. Biol. Chem. *272*, 3622–3627.

Salahpour, A., Angers, S., Mercier, J.F., Lagace, M., Marullo, S., and Bouvier, M. (2004). Homodimerization of the β2-adrenergic receptor as a prerequisite for cell surface targeting. J. Biol. Chem. *279*, 33390–33397.

Santos-Cuevas, C.L., Ferro-Flores, G., Arteaga de Murphy, C., and Pichardo-Romero, P.A. (2008). Targeted imaging of gastrin-releasing peptide receptors with ^{99m}Tc-EDDA/HYNIC-[Lys3]-bombesin: biokinetics and dosimetry in women. Nucl. Med. Commun. *29*, 741–747.

Sato, N., Ogino, Y., Mashiko, S., and Ando, M. (2009). Modulation of neuropeptide Y receptors for the treatment of obesity. Expert Opin. Ther. Pat. *19*, 1401–1415.

Schmidt, P.T., Naslund, E., Gryback, P., Jacobsson, H., Holst, J.J., Hilsted, L., and Hellstrom, P.M. (2005). A role for pancreatic polypeptide in the regulation of gastric emptying and short-term metabolic control. J. Clin. Endocrinol. Metab. *90*, 5241–5246.

Schmidt, P., Lindner, D., Montag, C., Berndt, S., Beck-Sickinger, A.G., Rudolph, R., and Huster, D. (2009). Prokaryotic expression, in vitro folding, and molecular pharmacological characterization of the neuropeptide Y receptor type 2. Biotechnol. Prog. *25*, 1732–1739.

Schober, D.A., Van Abbema, A.M., Smiley, D.L., Bruns, R.F., and Gehlert, D.R. (1998). The neuropeptide Y Y_1 antagonist, 1229U91, a potent agonist for the human pancreatic polypeptide-preferring (NPY Y_4) receptor. Peptides *19*, 537–542.

Sheriff, S., Ali, M., Yahya, A., Haider, K.H., Balasubramaniam, A., and Amlal, H. (2010). Neuropeptide Y Y_5 receptor promotes cell growth through extracellular signal-regulated kinase signaling and cyclic AMP inhibition in a human breast cancer cell line. Mol. Cancer Res. *8*, 604–614.

Shi, Y.C., Lin, S., Castillo, L., Aljanova, A., Enriquez, R.F., Nguyen, A.D., Baldock, P.A., Zhang, L., Bijker, M.S., Macia, L., et al. (2011). Peripheral-specific Y_2 receptor knockdown protects mice from high-fat diet-induced obesity. Obesity (Silver Spring) *19*, 2137–2148.

Shimada, K., Ohno, Y., Okamatsu-Ogura, Y., Suzuki, M., Kamikawa, A., Terao, A., and Kimura, K. (2012). Neuropeptide Y activates phosphorylation of ERK and STAT3 in stromal vascular cells from brown adipose tissue, but fails to affect thermogenic function of brown adipocytes. Peptides *34*, 336–342.

Smith, K.R., Muir, J., Rao, Y., Browarski, M., Gruenig, M.C., Sheehan, D.F., Haucke, V., and Kittler, J.T. (2012). Stabilization of $GABA_A$ receptors at endocytic zones is mediated by an AP2 binding motif within the $GABA_A$ receptor β3 subunit. J. Neurosci. *32*, 2485–2498.

Tasan, R.O., Lin, S., Hetzenauer, A., Singewald, N., Herzog, H., and Sperk, G. (2009). Increased novelty-induced motor activity and reduced depression-like behavior in neuropeptide Y (NPY)-Y_4 receptor knockout mice. Neuroscience *158*, 1717–1730.

Thiriet, N., Agasse, F., Nicoleau, C., Guegan, C., Vallette, F., Cadet, J.L., Jaber, M., Malva, J.O., and Coronas, V. (2011). NPY promotes chemokinesis and neurogenesis in the rat subventricular zone. J. Neurochem. *116*, 1018–1027.

Thomas, L., Scheidt, H.A., Bettio, A., Huster, D., Beck-Sickinger, A.G., Arnold, K., and Zschornig, O. (2005). Membrane interaction of neuropeptide Y detected by EPR and NMR spectroscopy. Biochim Biophys Acta Biomembr *1714*, 103–113.

Tough, I.R., Holliday, N.D., and Cox, H.M. (2006). Y_4 receptors mediate the inhibitory responses of pancreatic polypeptide in human and mouse colon mucosa. J. Pharmacol. Exp. Ther. *319*, 20–30.

Vezzani, A. and Sperk, G. (2004). Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? Neuropeptides *38*, 245–252.

Verma, D., Tasan, R.O., Herzog, H., and Sperk, G. (2012). NPY controls fear conditioning and fear extinction by combined action on Y_1 and Y_2 receptors. Br. J. Pharmacol. *166*, 1461–1473.

Voisin, T., Goumain, M., Lorinet, A.M., Maoret, J.J., and Laburthe, M. (2000). Functional and molecular properties of the human recombinant Y_4 receptor: resistance to agonist-promoted desensitization. J. Pharmacol. Exp. Ther. *292*, 638–646.

Wahlestedt, C., Pich, E.M., Koob, G.F., Yee, F., and Heilig, M. (1993). Modulation of anxiety and neuropeptide Y-Y_1 receptors by antisense oligodeoxynucleotides. Science *259*, 528–531.

Walther, C., Nagel, S., Gimenez, L.E., Morl, K., Gurevich, V.V., and Beck-Sickinger, A.G. (2010). Ligand-induced internalization and recycling of the human neuropeptide Y_2 receptor is regulated by its carboxyl-terminal tail. J. Biol. Chem. *285*, 41578–41590.

Walther, C., Lotze, J., Beck-Sickinger, A.G., and Morl, K. (2012). The anterograde transport of the human neuropeptide Y_2 receptor is regulated by a subtype specific mechanism mediated by the C-terminus. Neuropeptides *46*, 335–343.

Wang, G. and Wu, G. (2012). Small GTPase regulation of GPCR anterograde trafficking. Trends Pharmacol. Sci. *33*, 28–34.

Yulyaningsih, E., Zhang, L., Herzog, H., and Sainsbury, A. (2011). NPY receptors as potential targets for anti-obesity drug development. Br. J. Pharmacol. *163*, 1170–1202.

Zhang, L., Bijker, M.S., and Herzog, H. (2011). The neuropeptide Y system: pathophysiological and therapeutic implications in obesity and cancer. Pharmacol. Ther. *131*, 91–113.

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