Review

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Neuropeptide Y receptors: ligand binding and trafficking suggest novel approaches in drug development

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NPY, PYY and PP constitute the so-called NPY hormone family, which exert its biological functions in humans through YRs (Y_1, Y_2, Y_4 and Y_5). Systematic modulation of YR function became important as this multireceptor/multiligand system is known to mediate various essential physiological key functions and is involved in a variety of major human diseases such as epilepsy, obesity and cancer. As several YRs have been found to be overexpressed on different types of malignant tumors they emerge as promising target in modern drug development. Here, we summarize the current understanding of YRs function and the molecular mechanisms of ligand binding and trafficking. We further address recent advances in YR-based drug design, the development of promising future drug candidates and novel approaches in YR-targeted tumor diagnostics and therapy opportunities. Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: GPCR; NPY; YR; structure-activity relationship; internalization; drug development; receptor trafficking

Introduction

The neuropeptide Y hormone family, comprising NPY, PYY and PP, is involved in the regulation of a large number of physiological effects by interacting with a set of different GPCR subtypes known as Y_1, Y_2, Y_4, Y_5 and y_6 in mammals [1]. Based on their influence on fundamental processes such as food intake, regulation of blood pressure and memory retention, these neuropeptides are known to be associated with diseases such as obesity, inflammatory, gastrointestinal and cardiac complications and mood disorders [2–5]. The lifestyle of the western world leads to increasing numbers of patients who suffer from obesity, which emerged as one of the greatest public health challenges in the modern world [2,6]. Moreover, cancer and cardiovascular complications are severe human diseases which at present cause the death of approximately 60% of the human population worldwide. Consequently, therapeutic research and development in order to treat such human health risks is currently the major focus of researches worldwide and accordingly, the multireceptor/multiligand system of the NPY family has been part of intense investigations over the past decades [7,8]. With respect to YR-targeted drug discovery, several aspects of signal transduction, mediated by the NPY peptide family in context with their receptors, have to be considered (Figure 1). Starting at the cell surface, the peptide ligand has to interact with one of the receptors in order to initiate a corresponding signal. In case of a multireceptor/multiligand system, different combinations of ligand and receptor subtypes will have the pivotal influence on the subsequent mediated intracellular response. Thus, it is necessary to investigate and conceive the structure of the peptide ligand, its receptors and the bound ligand–receptor complex, including subtype specific differences, structure–affinity and structure–activity relationships. To interfere with signaling pathways and subsequently alter the cellular response specifically, agonist- and antagonist-based drugs can be applied as pharmacological tools, e.g. in the treatment of epilepsy and obesity.

Once the receptor is activated by the peptide, GPCR signaling is mediated and modulated by two general mechanisms: G protein activation and β-arrestin signaling [9]. As the cellular responses of GPCR-targeted drugs are determined by the interplay of distinct signaling pathways, the major pathway has to be elucidated. Biased agonists, accordingly, have been identified as novel pharmacological tool to contribute to the better understanding of GPCRs functionality. Those compounds might provide a new approach for the design of therapeutics [9,10]. Another further approach to treat YR related diseases takes advantage of pathological receptor subtype expression on tumor cells. This allows selective targeting of tumor cells by shuttling a medicinal therapeutic inside the target cancer cell due to receptor internalization. However, interference with receptor internalization or degradation could modify the strength and the duration of the signaling process. To address this issue, understanding the detailed intracellular trafficking circuitries constitutes the fundamental prerequisite for successful YR-targeted

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Abbreviations used: *NPY, neuropeptide Y; PYY, peptide YY; PP, pancreatic polypeptide; YR, neuropeptide Y receptor; GPCR, G protein-coupled receptor; CNS, central nervous system; TM; transmembrane helix; ECL, extracellular loop; GRK, G protein-coupled receptor kinase; BRET, bioluminescence resonance energy transfer; BIFC, bimolecular fluorescence complementation; ICL, intracellular loop; EYFP, enhanced yellow fluorescent protein.*

Biography

Cornelia Walther was born 1983 in Karl-Marx-Stadt, Germany. She studied biochemistry at Martin Luther University Halle/Wittenberg and at the University of Leipzig where she obtained her diploma in 2007 under guidance of Prof. Dr Annette G. Beck-Sickinger. After a 3-month research internship with Indraneel Ghosh at the University of Arizona in Tucson in 2007, she returned to the University of Leipzig and joined the group of Prof. Dr Annette G. Beck-Sickinger for her Ph.D. with her main research interest in the molecular mechanism underlying YR trafficking pathways.

Karin Mörl was born in 1967 in Munich, Germany. She studied Biology at the Ludwig-Maximilians -University in Munich. After graduating as Dr. rer.nat. at the Max-Planck-Institute for Neurobiology in Martinsried, Germany in the group of Prof. Dr Hans Thoenen she continued with two postdoctoral positions with Dr Michael Meyer in Martinsried and Prof. Dr Volker Bigl in Leipzig. Since 2001 she is working as staff scientist in the group of Prof. Dr Annette G. Beck-Sickinger at the Institute of Biochemistry at the University of Leipzig.

Annette G. Beck-Sickinger studied chemistry (diploma in 1986) and biology (diploma in 1990) at the University of Tübingen (Germany). She graduated under the supervision of G. Jung (Organic Chemistry, University of Tübingen) and was working as research fellow with R. A. Houghten (Scripps Clinic & Research Foundation, La Jolla, USA), E. Carafoli (Laboratory of Biochemistry, ETH Zürich) and T. W. Schwartz (Univ. Copenhagen, Denmark). She was appointed as assistant professor of Pharmaceutical Biochemistry at ETH Zürich (1997–1999) and since 1999 she is a full-time professor of Biochemistry and Bioorganic Chemistry at the University of Leipzig. In 2009 she spent a semester as visiting professor at Vanderbilt University, Nashville, TN. In 2009 she was awarded with the gold medal of the Max-Bergmann-Kreis which honored her contribution to field of neuropeptides. Her major interests include peptide–receptor interaction of GPCRs, protein expression and modification, biomedical therapeutic and diagnostic approaches in cancer, obesity and regenerative medicine as well as novel biomaterials.

tumor therapy. In this review, we discuss current knowledge on YRs functions, their involvement in severe human diseases and how recent studies provide opportunities for the development of novel drug candidates in clinical application for diagnostics and therapy.

The Neuropeptide Y Hormone Family

The neuropeptide Y hormone family comprises the three closely related peptides, NPY, PYY and PP. These peptides consist of 36 amino acids, are *C*-terminally amidated (Table 1) [1] and are formed by proteolytic processing of preprohormones. X-ray crystallography, NMR and FRET studies revealed different structural features for the members of the NPY hormone family: X-ray crystallography for avian PP revealed a structure, which comprises a type II polyproline helix (residues 1–8), followed by a turn and a *C*-terminal amphiphatic α-helix (residues 15–32). This three-dimensional hairpin-like structure is also referred to as PP-fold [11]. pPYY in solution displays the PP-fold likewise. In contrast, pPYY, in the presence of lipid mimetic dodecylphosphocoline (DPC), displays the characteristic *C*-terminal amphipathic α-helix but revealed an unstructured freely diffusing *N* terminus [12]. Similarly, the solution structure of NPY in the presence of DPC displays an unstructured flexible *N*-terminal region followed by a well-defined *C*-terminal amphipathic α-helix in contrast to the suggested PP-fold [13–15].

Table 1. Amino acid sequences of pNPY, hPYY and hPP

Peptide	Sequence
pNPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY-NH_2
hPYY	YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-NH_2
hPP	APLEPVYPGDNATPEQMAQYAADLRRYINMLTRPRY-NH_2

The first member of the NPY family was identified and sequenced in 1975 when PP was isolated from chicken pancreas [16]. The expression of PP is restricted to endocrine cells, predominantly present in the pancreas [17]. Consequently, its physiological effects comprise the inhibition of pancreatic secretion and intestinal motility, but PP is also suggested to play a role in body weight regulation by inhibition of food intake [18,19]. PYY was the next identified NPY family member due to its isolation from porcine intestinal mucosa by Tatemoto and Mutt in 1980 [20]. Primarily, PYY is synthesized and released from L-cells in the intestinal mucosa of the ileum and large intestine, thus, the highest tissue concentrations are found within the gastrointestinal tract. Besides its expression in gut endocrine cells, also peripheral neurons express PYY although at lower concentrations, e.g. hypothalamus [17]. Two major endogenous forms of PYY exist, PYY(1–36) and PYY(3–36). The cleavage of the *N*-terminal amino acids Tyr and Pro is mediated by the ubiquitously expressed enzyme di-peptidyl peptidase IV to sustain the Y_2R selective PYY(3–36) [21], which is the predominant form being released in the circulation. The most pronounced effect of PYY is its involvement in the regulation of food intake and energy homeostasis [18,22–25]. The third member of the NPY family, NPY, is a peptide hormone which was first isolated from porcine brain in 1982 [26]. NPY is processed from the 97 amino acids precursor protein pre-pro-NPY, directed to the endoplasmic reticulum by a signal peptide sequence. After cleavage of the 28-amino acid signal sequence, the precursor protein pro-NPY is submitted to successive endoproteolytic processing during its further transport along the secretory pathway. Pro-NPY is cleaved by prohormone converting enzymes PC1/3 and/or PC2 at a single dibasic site (Lys^{38}-Arg^{39}), Arg^{39} being important to determine the cleavage efficiency. This cleavage results in a 39-amino acid form of NPY [NPY(1–39)] and a 30-amino acid carboxyl terminal peptide, the *C*-terminal flanking peptide of NPY (CPON). NPY(1–39) has to be subsequently processed by carboxypeptidase H and peptidylglycine α-amidating monoxygenase to yield the

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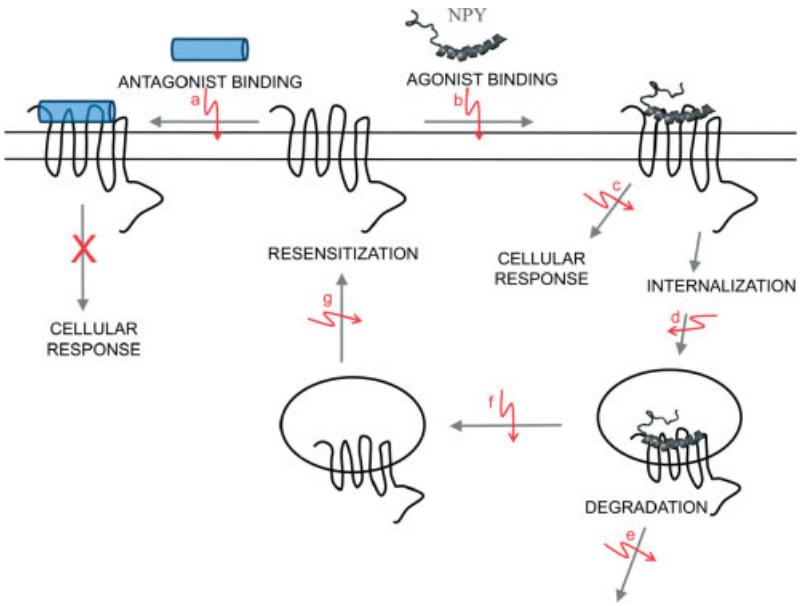

Figure 1. *Schematic illustration of possible therapeutic interventions in the NPY system.* Modulation of YR functionality and subsequent cellular consequences at different stages in the life of YRs: (a) antagonist treatment blocks the receptor and prevents cellular signaling; (b) agonist binding activates the receptor and mediates cellular consequences by distinct signaling pathways (c), e.g. G protein and β-arrestin signaling. The development of biased ligands enables specific modulation of intracellular signals. (d) Agonist stimulation also provokes receptor internalization which can be enhanced or retarded, thus prolonging or shortening intracellular signaling cascades. Furthermore, receptor mediated internalization can be exploited to shuttle specific pharmaceutical compounds into a target cell. (e) Subsequent intracellular receptor fate usually is determined by intracellular receptor domains targeting for degradation or resensitization (f). The development of drugs which specifically bind these domains would lead to specific interference with down-regulation or resensitization processes, thus regulating receptor cell surface density (g) and in turn receptors responsiveness.

mature 36-amino acid, *C*-terminally amidated peptide [27,28], with the amide group as essential requirement for receptor binding and biological activity [29,30]. NPY can be characterized as one of the most potent orexigenic peptides [31] and as one of the most abundant neuropeptides in the brain [1]. It shows a widespread distribution within the peripheral and CNS with high expression levels in the brain, particularly in brain regions including hippocampus, thalamus, hypothalamus, cerebral cortex and brainstem, suggesting a major role of NPY in the regulation of CNS functions [32]. Here it acts as a neurotransmitter which is synthesized and released by neurons [27]. Additionally, NPY is also found in peripheral nerves more precisely in sympathetic neurons where it co-exists with noradrenalin and ATP [32–34]. There it is located in nerve plexuses which surround blood vessels [35], thus being involved in the regulation of blood pressure [27,32,36]. Evidently, NPY is involved in a variety of physiological processes, e.g. the regulation of feeding [37], axon guidance, neurogenesis [38], alcohol consumption, dependence and withdrawal [39,40], anxiety, stress, mood disorders [41,42], circadian rhythm, memory retention [34,43,44], vasoconstriction [45], pain [46], aggression [47], endocrine and cardiovascular functions [27] as well as inflammation and immune responses [48].

Accordingly, all three members of the NPY family are attractive pharmacological tools to target YRs and modulate their functionality for therapeutic purposes.

Neuropeptide Y Receptors

The large family of GPCRs, comprising more than 800 members, became an important therapeutic target as evidence emerged reporting on their involvement in the regulation of various fundamental physiological processes and their dysfunction in diseases. Currently, more than 30% of the available pharmaceuticals in clinical use act on GPCRs [49,50]. Within this large receptor family prominent members are the YRs which can be classified into the group of rhodopsine-like GPCRs and are activated by the peptides of the NPY hormone family. Up to date, five different receptor subtypes have been cloned from mammals (Y_1R, Y_2R, Y_4R, Y_5R and y_6R). In humans, only four YRs are functionally expressed (hY_1R, hY_2R, hY_4R and hY_5R). Although y_6 is present in mice and rabbit, it is the only so far known YR subtype which displays no functionality in human [8]. Surprisingly, the YR family shows an unexpected low overall sequence identity [34]. YR subtypes can be distinguished by their different affinities for their endogenous ligands NPY, PYY and PP: NPY preferably binds to Y_1R and Y_5Rs, Y_4Rs have a very high affinity to PP, and Y_2R binds NPY and PYY with similar affinities [7]. All YRs are mainly distributed in hypothalamic brain regions [51], but can also be found in many peripheral tissues. After agonist-mediated receptor activation, YRs signal via pertussis toxin-sensitive G proteins, e.g. members of the G_i and G_o family, thus mediating the inhibition of adenylyl cyclases and consequently, the inhibition of cyclic adenosine monophosphate (cAMP) synthesis. Furthermore, depending on the cell type, additional signaling responses are associated with activated YRs, e.g. modulation of calcium and potassium channels [7,8,52].

Y_1 Receptor

The human Y_1R consists of 384 amino acids and is mainly expressed in the CNS in the cerebral cortex, thalamus and the amygdala [1], but is also found in adipose tissue and in vascular smooth muscle cells [44,53]. Y_1R is considered to be postsynaptic and mediates vasoconstriction because this receptor subtype, in the periphery, is mainly localized in blood vessels [54]. Moreover, Y_1R is involved in mediating the anxiolytic effects

Table 2. Selected peptide-derived agonists with selective binding affinity to the NPY receptor subtypes Y_1, Y_2 and Y_5

NPY receptor subtype	Peptide-derived selective agonists	Reference
Y_1R	[Phe^7,Pro^{34}]pNPY	[58]
	[D-Arg^{25}]NPY	[59]
	[D-His^{26}]NPY	[59]
	[Leu^{31},Pro^{34}]pNPY	[60]
	[Pro^{30},Nle^{31},Bpa^{32},Leu^{34}]NPY(28–36)	[61]
Y_2R	NPY(3–36) up to NPY(22–36)	[1,7]
	PYY(3–36)	[21,22]
	(Ahx$^{5-24}$)NPY	[1,7,62]
Y_5R	[Ala^{31},Aib^{32}]NPY	[63]
	[D-Trp^{34}]NPY	[64]
	[cPP$^{1-7}$,pNPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP	[65]

of NPY [55] and, together with Y_5R, it plays an important role in the circuitries of energy homeostasis [7] and controls alcohol consumption [56]. Y_1R displays high affinity for NPY, PYY and the analogs with the substitution Pro^{34} and low affinities for *N*-terminally truncated analogs and for PP [8,57]. Furthermore, variation of Asn^7 to Phe in the NPY peptide ([Phe^7,Pro^{34}]NPY), as well as substitution of Arg^{25} to D-Arg^{25} and His^{26} to D-His^{26} and [Leu^{31},Pro^{34}]NPY/PYY give Y_1 preference [58–60]. Recently, [Pro^{30},Nle^{31},Bpa^{32},Leu^{34}]NPY(28–36) was identified as the first small size Y_1R selective peptide with agonistic properties (Table 2) [61].

Y_2 Receptor

The 381 amino acid human Y_2R is expressed in a variety of brain regions, including hippocampus, thalamus, hypothalamus and brain cortex. In the peripheral nervous system Y_2R is found in parasympathetic, sympathetic and sensory neurons, and also in intestine and certain blood vessels [1,32,66]. Y_2R is presynaptically localized in neurons thus mediating its effects by suppression of neurotransmitter release [8]. There is evidence that Y_2R has effects on the regulation of memory retention, circadian rhythm and angiogenesis and it is considered to be involved in epilepsy [44,67]. NPY and PYY are high affinity ligands for Y_2R, but in contrast to the Y_1R, also *C*-terminal fragments [NPY(3–36) up to NPY(22–36) and PYY(3–36)], as well as centrally truncated analogs ([Ahx$^{5-24}$]NPY), show high affinity binding (Table 2). In contrast, Pro^{34}-substituted NPY/PYY analogs and PP show only low affinity binding [1,62].

Y_4 Receptor

Y_4R is the only subtype with a very high affinity for PP in a picomolar range, while NPY and PYY are still able to activate Y_4R with moderate affinities [1]. Due to the high selectivity for PP this receptor subtype is also referred to as PP-preferring receptor. The 375 amino acid protein is mainly expressed in the periphery like the gastrointestinal tract including colon, pancreas and intestine, moreover in the heart, skeletal muscle and thyroid gland. It is also found in the CNS at low expression levels, including hypothalamus, hippocampus, cerebellum, spinal cord and medulla [44,68,69]. The most pronounced effects transmitted by this receptor subtype is the inhibition of gall bladder contraction [70], pancreatic secretion [68] and stimulation of LH secretion [71]. Besides PP, as the most potent endogenous agonist for the Y_4R subtype, NPY, PYY, [Pro^{34}]PYY/NPY still bind the Y_4R although with lower affinity compared to PP [1,27,72].

Y_5 Receptor

There are two isoforms of this receptor which are encoded by two splice variants. The Y_5R isoforms, 455 amino acids (long isoform) and 445 amino acids (short isoform), differ in an *N*-terminal extension by 10 amino acids but display a comparable pharmacological profile [73]. Y_5Rs are mainly expressed in the CNS, particularly in the hypothalamus, where receptor activation is suggested to induce food intake [74]. Furthermore, hippocampal Y_5Rs were linked to trigger seizures [75]. In contrast to the other YR subtypes, Y_5R is only rarely found in peripheral tissues [76]. The pharmacological profile of Y_5R displays equal affinities for NPY, PYY, Pro^{34}-substituted analogs, (2–36)NPY and (3–36)NPY. Nevertheless, PP still binds with a fairly good affinity [8,74]. Y_5 selective peptides include [Ala^{31},Aib^{32}]NPY, [D-Trp^{34}]NPY and [cPP$^{1-7}$,pNPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP (Table 2) [63–65].

Structure–Activity and Structure–Affinity Relationship Studies

The existence of a complicated network of different homologous peptides binding to a subset of homologous cell surface receptors and in addition the broad range of physiological actions influenced by these peptides and receptors, makes it of intriguing interest, not only to understand expression patterns of different peptides and receptors, but also to understand differential binding and binding modes.

For the proper recognition and subsequent binding of the peptide agonist by its receptors the amido-Tyr^{36}, or at least an aromatic residue at position 36, is required for binding to all receptor subtypes [7,44]. In addition, it appears that not only specific residues are involved in high affinity receptor binding, but rather overall structural requirements are necessary to form the bioactive conformation [77]. Although, there are many more significant residues involved in high affinity binding, the two conserved residues Arg^{33} and Arg^{35} are essential for NPY/PP binding to all YR subtypes [1].

Complementarily, receptor mutagenesis studies revealed detailed insights into receptor binding sides. In all known YRs, the residue Asp$^{6.59}$ on top of TM6 is fully conserved and obviously plays a fundamental role in agonist binding and signal transduction at all YRs. Nevertheless, substitution of Asp$^{6.59}$ by either Ala, Glu, Asn, and Arg revealed a subtype-specific binding pocket apparently due to different ligand recognition patterns [78]. With respect to the Y_1R, besides Asp$^{6.59}$ (end of TM6) a second residue, namely Asp$^{2.68}$ (first extracellular loop, ECL1), is implicated in electrostatic interactions with the identified important Arg residues in the ligands. In addition Tyr$^{2.64}$ (TM2), Phe$^{6.58}$ (TM6) and His$^{7.31}$ (ECL3) have been identified to be involved in peptide interactions [79,80]. Regarding the Y_2R subtype further putative residues which might be involved in ligand binding, e.g. Tyr$^{2.64}$ and Glu$^{5.24}$, were identified by a mutagenesis approach [81]. Compared to the other YR subtypes, binding of NPY to the Y_5R is likewise dictated by the conserved residue Asp$^{6.59}$ (ECL3). Strikingly, two other acidic

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residues came up as potential recognition sites for ligand binding to the Y_5R: $Glu^{5.27}$ and $Asp^{2.68}$. Mutation of both residues to Ala led to a dramatic loss of affinity; hence both residues are possible interaction partners for NPY. In the Y_5R subtype the ligand recognition might also be influenced by three aromatic residues, which can interact with the Arg residues in the ligands via π–cation interactions. All of them are located in the three ECLs or extracellular domains of the TMs: $Trp^{2.70}$, $Phe^{4.63}$ and $Tyr^{7.35}$ [78,82]. Besides the ECLs, the *N* terminus might be a potential structural domain involved in building up the ligand binding pocket. However, *N*-terminal mutagenesis studies and chimeric receptors revealed that the *N*-terminal residues do not contribute to receptor subtype selectivity [83].

Such ligand and receptor mutagenesis studies are fundamental prerequisites to further identify and characterize subtype-specific ligand–receptor interaction points. Recent studies by Merten *et al*. and Lindner *et al*., applying a complementary mutagenesis approach, revealed first insights to direct contact points between NPY and its receptors [78,82]. Interestingly, differences in determinants participating in binding were identified between YR subtypes. $Asp^{6.59}$ in Y_1R binds through polar attractions to Arg^{35} of the ligand. In contrast to the Y_1R, $Asp^{6.59}$ in Y_2R interacts with Arg^{33} through ionic interactions. For the Y_4R the interaction is comparable to the Y_1R subtype as the same residues are involved in the interaction: Arg^{35} in the ligand interacts electrostatically with $Asp^{6.59}$ in the receptor. For Y_5R two interaction points were identified so far: Arg^{25}-$Asp^{2.68}$ and Arg^{33}-$Asp^{6.59}$. Whereas the Arg^{25}-$Asp^{2.68}$ interaction is of electrostatic nature (is designated by ionic attractions, with a notable influence of polar attractions), no polar attraction or steric limitations could be identified for Arg^{33}-$Asp^{6.59}$. Notably, the Arg^{25}-$Asp^{2.68}$ interaction site is unique to the Y_5R subtype [44,78,82]. Furthermore, a ligand-based mutagenesis approach clearly demonstrates that all three members of the NPY hormone family (NPY, PYY and PP) share the same contact points with Y_5R which strikingly differ from those in the Y_1R and Y_4R. Consequently, it is evident that the binding mode within the multireceptor/multiligand system of the NPY family is dictated by the receptors and not the agonists [82]. In sum, ligand binding to YRs can be delineated by two different binding modes: Y_1/Y_4Rs are characterized by the binding of Arg^{35}-$Asp^{6.59}$ whereas Y_2/Y_5Rs are considered to interact via Arg^{33}-$Asp^{6.59}$. These data are in good agreement with the evolutionary relationships and structural similarities [78,82].

Internalization of YRs

The endocytic trafficking of many GPCRs has been studied extensively. With respect to YRs, intracellular trafficking pathways are still poorly understood. However, for several therapeutic interventions it is of tremendous significance to understand the underlying molecular mechanism of GPCRs removal from the cell surface and the subsequent pursued complex intracellular trafficking networks. Based on many studies from several groups, with a variety of different GPCRs over the past decades, a classical view of GPCRs signaling and trafficking has been established. This involves receptor activation through agonist binding, which in turn leads to the activation of heterotrimeric G proteins. Upon persistent stimulation, the receptor gets specifically phosphorylated at Ser/Thr residues by GRKs thereby uncoupling the receptor from the G protein (desensitization) which subsequently leads to the binding of arrestin proteins. The desensitization process is considered to be a crucial physiological process to maintain the cellular homeostasis [84]. Receptor phosphorylation usually occurs at specific disposed Ser/Thr clusters which are also referred to as phosphorylation barcode or fingerprint. Subsequently, the clathrin-binding protein arrestin attaches to the phosphorylated receptor. Thereby, arrestin acts as adaptor to link the arrestin/receptor complex to the endocytic machinery, more precisely to the clathrin-coated endocytic vesicles, to get internalized [85]. In doing so, further signaling through G proteins is prevented. Once internalized, the receptor gets dephosphorylated and can either recycle back to the plasma membrane (resensitization) where the functional receptor can promote signaling again or is targeted to lysosomes for degradation (down-regulation). The internalization process itself is best characterized by the removal of functional receptors from the cell surface to control GPCRs signal termination and transmission and subsequently reduce responsiveness [86–89]. Thus, GPCRs functional activity can be strongly influenced due to the processes of de- and resensitization.

As information about YR endocytosis and the regulation of the complex intracellular causality was rather limited, the work of many groups in the past focused on unraveling these open issues, e.g. desensitization, internalization, subcellular trafficking, recycling and down-regulation. Recently, the internalization properties of the human YRs have been elucidated. Although the Y_1, Y_2 and Y_4R subtypes show fast internalization properties the Y_5R displays a rather slow internalization which was attributed to structural differences within ICLs and the *C*-terminal tails (Figure 2) [90].

Y_1 Receptor

Y_1R is the best studied subtype among the YRs. It has been reported by several groups that this subtype is rapidly internalized upon agonist exposure, either in transfected cells as well as in cells endogenously expressing the Y_1R (human neuroblastoma cell line SK-N-MC) [84,90–96]. The internalization mechanism is considered to be clathrin-dependent [92,96], suggesting interactions with arrestins. Fluorescence microscopy, BRET2 and BIFC studies reveal strong evidence that arrestin-3 (also referred to as β-arrestin-2) is recruited to the plasma membrane after agonist-induced receptor activation, which is in agreement with the high internalization rate [95–98]. Nevertheless, arrestin-independent events were not strictly excluded. Holliday *et al*. also addressed phosphorylation events that might occur at Y_1R. Usually, phosphorylation of GPCRs takes place within the third ICL and/or the *C* terminus. The *C*-terminal tail of the Y_1R contains multiple potential phosphorylation sites among which several key residues ^{352}STxxTxxSxTS^{362} were identified to be phosphorylated by GRK2 [97,98]. Obviously, the phosphorylation of those residues is a prerequisite for further downstream events such as arrestin binding, as shown by Kilpatrick *et al*. [98]. Further, resensitization studies revealed rapid receptor recycling back to the cell membrane [84,92,94]. The Y_1R recycling process might be regulated by the specific *C*-terminal consensus sequence motif [ϕ-H-(S/T)-(E/D)-V-(S/T)-X-T] (ϕ, aromatic or hydrophobic residue), which has been identified by Ouedraogo *et al*. [96]. Recently, a second the *C*-terminal tyrosine-based YXXϕ (YETI) motif was found to be involved in trafficking processes of internalized Y_1Rs and particularly to contribute to fast recycling properties. Apart from that this motif was also shown to be involved in agonist-independent constitutive internalization of a truncated Y_1R variant ($Y_1\Delta32$), missing the last 32 *C*-terminal amino acids. As wild type Y_1Rs do

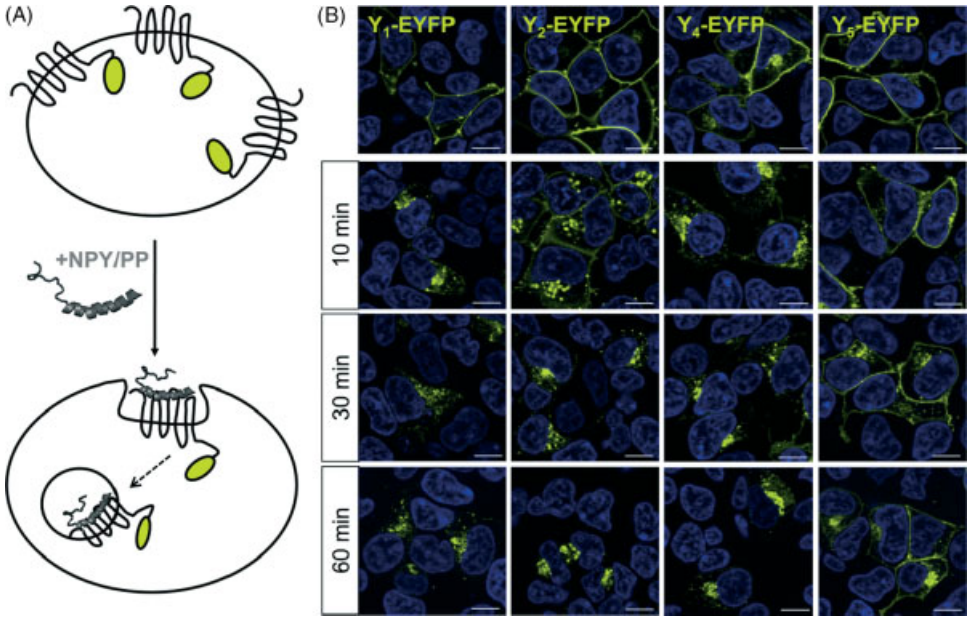

Figure 2. *Internalization properties of the human YR subtypes.* (A) schematic illustration of the localization of YR-EYFP fusion proteins prior to and upon ligand stimulation. Prior to stimulation receptors are theoretically localized exclusively in the plasma membrane. Stimulation with NPY (Y_1R, Y_2R, Y_5R)/PP (Y_4R) leads to receptor internalization. Depending on the duration of stimulation receptors are localized in the membrane only to a minor extend. In addition, internalized receptors are found in intracellular compartments, e.g. endosomes. (B) Representative images showing HEK293 cells transiently expressing hYRs *C*-terminally fused to EYFP. According to the scheme in (A), YR-EYFP fusion proteins (yellow) are mainly found in the plasma membrane prior to stimulation (upper panel). In response to 10-min ligand stimulation [1 μM NPY (Y_1R, Y_2R, Y_5R) or 1 μM PP (Y_4R)] the fluorescence is additionally distributed in intracellular compartments for Y_1R, Y_2R and Y_4R subtypes, e.g. endosomes. Only the Y_5R still displays membrane localization to a major extend (10 min). Prolonged stimulation leads to Y_1R, Y_2R and Y_4Rs internalization. These receptors are then mainly found in intracellular compartments (30 and 60 min). In contrast, the Y_5R is still predominantly localized in the plasma membrane with only few yellow spots in the intracellular compartments bearing the internalized receptor. The nuclei were visualized with Hoechst33342 (blue). Scale bar represents 10 μm.

not constitutively internalize, it has been proposed that this motif is masked in the wild type and truncations lead to conformational changes thus unmasking trafficking determinants such as YXXϕ [99]. Surprisingly, there is evidence that Y_1R can be internalized also by antagonists, although receptor activation is generally supposed to be a prerequisite for internalization events. Studies with a peptidic Y_1R antagonist GR231118 revealed internalization properties comparable to agonist stimulation which lead to a long-lasting receptor disappearance [92]. However, BIFC studies indicate an arrestin-independent mechanism [98]. The major difference though, is the endocytic pathway chosen upon either agonist/antagonist stimulation. Although agonist treatment forces the receptor to a classic endocytic/recycling pathway (clathrin- and arrestin-dependent), receptors internalized by the antagonist mainly proceeded through a clathrin-independent endocytic pathway. This strongly indicates that antagonist-mediated down-regulation may have important therapeutic implications [92].

Y_2 Receptor

In contrast to Y_1R, the Y_2R internalization process was a matter of controversy over the past years. For a long time it was assumed, and has been reported by several groups independently, that Y_2Rs neither internalize nor desensitize, or only to a little extent with extremely slow internalization rates, after persistent agonist stimulation [84,94,96]. So far, only very weak arrestin association was demonstrated which was in good agreement with the reported lack of internalization [95,96]. However, mutagenesis studies revealed recently that substitution of either His^{155} or His^{159} by Pro in the ICL2 can lead to an accelerated internalization and in consequence to an enhanced arrestin association [100]. This is based on the hypothesis that amino acids in the conserved DRY motif area provide binding determinants for arrestin recognition. Notably, the postulated regulation of the Y_2R internalization and subsequent protein interactions by its ICL2 is inconsistent with the most recent findings, reporting on the regulation by its *C*-terminal tail and to a minor extend by the ICL3 [90]. Strikingly, in 2008 Böhme *et al*. reported for the first time on rapid Y_2R internalization in response to agonist stimulation, which was comparable to the Y_1R internalization rate. In the meanwhile, these findings were also confirmed by others [83,90,98,101]. In addition, studies with Y_5/Y_2 chimeric receptors strongly pinpointed on the *C*-terminal tail as structural requirement for a sufficient internalization [90], but also arrestin-3 association [101]. The most recent findings confirmed the involvement of the Y_2R *C*-terminal tail in all kinds of endocytic events, due to the location of various regulatory motifs within this domain, which were proven to be essential for internalization, arrestin-3 association, recycling and also the overall regulation via an inhibitory sequence, independent of the cellular environment. However, arrestin-mediated internalization was shown for wild-type Y_2Rs, but also arrestin-independent events have been verified [101]. Whereas the distal $^{374}SxTxxT^{379}$ motif mediates GRK2 dependent hY_2R/arrestin-3 interaction and subsequently internalization, the proximal $^{347}DxxxSExSxT^{356}$ motif promotes GRK2- and arrestin-3-independent internalization. Moreover the identified conserved motif [ϕ-H-(S/T)-(E/D)-V-(S/T)-X-T] within the Y_1R was shown to contribute to Y_2R recycling processes too, as the proximal region $^{347}DAIHSEVSVT^{356}$ strongly influences the recycling pattern [101]. Besides intracellular domains that are obviously involved in direct protein–protein interactions, thus

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necessary to promote endocytic processes, studies on extracellular domains set up an additional point of view. From very recent mutagenesis studies, it is evident that the receptors' *N* terminus does not contribute to internalization processes. Partial or full *N*-terminal deletion as well as single residue mutations revealed no general requirement of the *N* terminus, as it participates not actively in the internalization process. Only its complete deletion strongly reduces internalization rates which might be due to overall structural requirements. Hence, it is likely that the extension of the first TM domain is necessary to obtain the proper receptor structure, indicating that internalization events are not necessarily dictated by intracellular domains, but moreover require the correctly folded receptor structure [83].

Y_4 Receptor

Initial reports about Y_4R internalization were as contradicting as for the Y_2R. This was due to the different methods applied. Although pharmacological studies with the human Y_4R in CHO cells revealed neither desensitization nor internalization [102], radioligand binding studies with the rat Y_4R in CHO cells revealed good internalization properties. Little later, it became evident that the internalization properties of the Y_4R are comparable to those of the Y_1R subtype which was characterized by fast internalization rates [94]. The mechanism seems to be the same as the internalization process was found to be sensitive to selective inhibitors, as e.g. sucrose and alkylators like the vicinal cysteine-bridging arsenical phenylarsine oxide [94]. Thus, also arrestin association would be expected and not surprisingly, the interaction of the Y_4R with arrestin-3 was verified by BRET2 with an intermediate tendency for arrestin-3 association when compared to Y_1R [95]. With respect to receptor restoration it has to be noted that it evidently occurs, but with a recovery percentage much lower compared to the Y_1R [94].

Y_5 Receptor

The internalization of the Y_5R has not been investigated as intensively and therefore is still not well understood. Our knowledge about Y_5R endocytic processes is up to now restricted to the single fact that this subtype internalizes to an extremely slower extend than the other YR subtypes [90,103]. Furthermore, it is suggested that Y_5R also internalizes via clathrin-dependent pathways [103]. A surprising finding, reported by Berglund *et al*. was the rapid association of Y_5R with arrestin-3 [95], which is inconsistent with the observed slow internalization rate. This is also in contrast to our findings that no arrestin recruitment to this subtype was observed at any time (unpublished data). A possible explanation might be the significant differences in terms of structural features as the length of intracellular domains like the ICL3 and the *C*-terminal tail. In comparison to the other subtypes, Y_1R, Y_2R and Y_4R, its ICL3 is about 100 amino acids longer whereas the *C*-terminal tail is with 17 amino acids much shorter than the *C*-termini of the other subtypes (60 amino acids for the Y_1R). These structural differences might account for the slower internalization rates. Although the ICL3 bears a quadruple Ser motif, which might be a potential phosphorylation and arrestin binding site, it is more likely that the extraordinary length contributes to an inhibitory effect and for conformational reasons the Ser motif is hidden from the GRKs. As the Y_5R internalization is really slow, it might indicate a degradative removal rather than a recycling pathway [103].

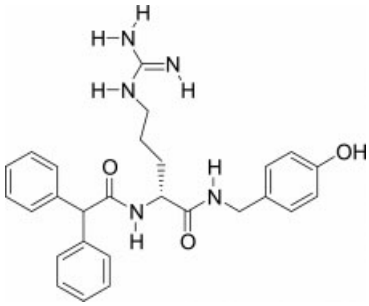
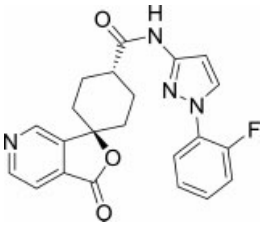
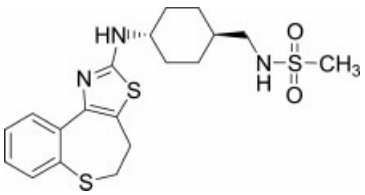
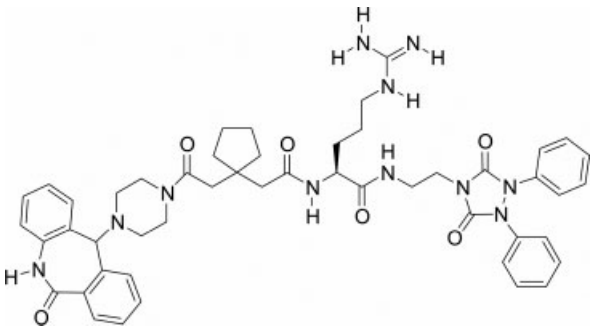
Taken together, although the internalization pathways appear to be mechanistically similar, the rates and the subsequent various subcellular fates, either degradation or resensitization, differ substantially. This might contribute to the diverse physiological functions of YRs. Understanding the regulation of these complex networks and taking advantage of the ascertained subtype-specific differences, but also similarities, can provide an applicable platform for selective therapeutic interventions.

YRs as Targets in Drug Development

The development of YR-targeting drugs yet remains a strong focus in modern drug research due to the involvement of YRs, together with their peptides, in various serious health problems. Moreover, there is still a lack of clinical approved receptor therapeutics available. Accordingly, there is a severe need to explore and develop potential NPY hormone family related small ligands as promising drug candidates for future clinical utility [2]. With respect to therapeutic/diagnostic applications of NPY/PYY/PP-derived drugs preconditions such as size, solubility and bioavailability constitute basic important features for drug development [104]. In addition, receptor activation and internalization are often prerequisites for peptides used in many aspects of clinical practice. Consequently, various laboratories have been working on the establishment of selective agonists and antagonist acting on GPCRs as potential pharmaceuticals for therapeutic interventions. Over the past decades some compounds have been developed which to date might be promising future therapeutics. Among them many potential analogs and compounds derived from the NPY hormone family have been developed to study physiological effects and their approach in clinical applications. The Y_1R antagonist GR231118 (Table 3) (also referred to as GW1229 or 1229U91) is one of these compounds which was identified more than a decade ago. Initially, this modified nonapeptide was thought to bind only to Y_1R with high selectivity but later on was also found to be a potent Y_4R agonist [105,106]. Therefore, the use of GR231118 in further studies was clearly limited. Nevertheless, further modifications of GR231118 improved the selectivity of the compound and moreover, its structure provided the basis for the development of further potent Y_1R selective agonists [107,108]. Based on this knowledge, the first selective peptidic agonist for Y_1R with reduced size [Pro^{30},Nle^{31},Bpa^{32},Leu^{34}]NPY(28–36) was developed, recently. This agonist displays promising characteristics for NPY-mediated tumor diagnosis and therapy [61]. Not only the interest in research on cancer is growing but also brain diseases like epilepsy are in the focus of researchers worldwide. Because the role of Y_2R in epilepsy is evident, the development of selective Y_2R peptides became highly interesting. Potent Y_2R selective agonists have been successfully used in *in vivo* studies where it has been shown that those selective agonists reduce epileptic seizures in rats and wild-type mice. Therefore, Y_2R is a promising target for new therapeutic approaches in epilepsy treatment using selective Y_2R agonists [67].

The identification of potential candidates for diagnostic and therapeutic purposes as major focus in the treatment of human diseases led to the development also of nonpeptidic-NPY analogs. Among them, the most prominent compound is BIBP3226 (R)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-arginin-amide (Table 3), which was the first nonpeptidic Y_1R antagonist [109]. Administration of BIBP3226 inhibits ethanol-induced sedation, presumably by acting via Y_1R or Y_5R subtypes [113]. Latest studies using BIBP3226 furthermore showed that NPY can directly

Table 3. Sequences and structures of selected specific YR antagonists

Antagonist	Structure/sequence	Receptor subtype	Reference
GR231118 (1229U91)	IleGluProDprTyrArgLeuArgTyr-NH_2 | | H_2N-TyrArgLeuArgTyrDprProGluIle	Y_1	[105,106]
BIBP3226		Y_1	[109]
MK-0557		Y_1, Y_5	[110]
Lu AA33810		Y_5	[111]
BIIE0246		Y_2	[112]

regulate human adrenal cortisol production [114]. In the field of food intake regulation as well as for diagnostic approaches further modified compounds were identified as promising antagonists. A novel carbazole derivative was developed as potent antagonist. Its oral bioavailability and its potential to penetrate the blood–brain barrier make this compound attractive for pharmacological purposes [115]. A 2,4-diaminopyridine-based Y_1 antagonist turned out to be a highly promising compound, as it was shown to inhibit food intake after intraperitoneal administration in rodents. In addition to being a potential PET tracer candidate, this Y_1R antagonist is suitable for diagnostic approaches [116,117]. Also for the Y_2R a nonpeptidic antagonist has been developed. The peptidomimetic BIIE0246 (S)-*N*(2)-[[1-[2-[4-[(R,S)-5,11-dihydro-6(6h)-oxodibenz[b, e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl] cylopentyl] acetyl]-*N*-[2-[1,2-dihydro-3,5(4H)-dioxo-1,2-diphenyl-3H-1,2,4-triazol-4-yl]ethyl]-argininamide (Table 3) was the first selective antagonist reported [112]. BIIE0246 was shown to regulate transmitter release in the brain and to exhibit anxiolytic effects in rats in the elevated pulse maze model [118,119]. Unfortunately, this compound has relevant drawbacks with respect to therapeutic application. It is known to be an insurmountable antagonist, with the capacity to block the receptor completely and therefore might lead to the prevention of further receptor activation and may consequently result in a long-term loss of the receptor. Moreover, it is big in size with a large polar surface which makes this compound unable to cross the blood–brain

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barrier, which would be the prerequisite for successful therapeutic interventions [120,121]. Thus, it was necessary to generate novel selective antagonists, which are substantially different from BIIE0246, with improved brain permeability to make them suitable for pharmacological studies not only in the periphery but also in the brain. Recently, high throughput screenings with a series of indolylpiperidin- and diamide-based substances revealed compounds which act as selective Y_2R antagonists and are to date promising future therapeutic tools and are consequently part of further investigations [121–123]. Lately, the Y_5R was moreover identified to be a target in the treatment of mood disorders. Studies with the Y_5R antagonist LuAA 33810 (*N*-[[*trans*-4-[(4,5-dihydro[1]benzothiepino[5,4-*d*]thiazol-2-yl)amino]cyclohexyl]methyl]methanesulfonamide) (Table 3) discovered the Y_5R as being part of an endogenous stress sensing system with anxiolytic- and antidepressant-like effects [111].

Dual YR Agonists and Antagonists as Anti-obesity Drug Candidates

Obesity, one of the most serious major human health concerns, is a result of an imbalance of food intake and energy expenditure [124]. To date, only few anti-obesity drugs are approved for long-term administration thus there exists a serious need for novel therapeutic agents treating obesity [125]. Generally, energy homeostasis is physiologically controlled by numerous receptor/ligand systems, among them the NPY system. The highly selective Y_1R agonist [Phe^7,Pro^{34}]NPY, but also Y_5R agonist was reported to provoke food intake and weight gain in rats [58,126]. It is still a matter of debate, if the regulation of feeding and energy expenditure is governed by the Y_1R or the Y_5R. To date, the prevalent perspective is still the involvement of both subtypes, yet unknown to what extent [127]. Nonetheless, several potent and selective Y_5R antagonists have been developed, but not all of them could show an effect on NPY mediated food intake. Therefore, it has been suggested that Y_5R is not the major feeding receptor to regulate NPY-induced feeding in rodents but maintains the pronounced orexigenic effect induced by NPY [128]. Promising compounds are novel imidazoline derivatives which exhibit excellent brain permeability and pharmacokinetic properties. Unfortunately, the first clinical trials using the highly selective, orally available antagonist MK-0557 (*trans*-*N*-[1-(2-fluorophenyl)-3-pyrazolyl]-3-oxospiro[6-azaisobenzofuran-1(3*H*),1′-cyclohexane]-4′-carboxamide) (Table 3) in 2006 could not clarify the receptors role in regulation of feeding, as MK-0557 targets not only the Y_5R, but also the Y_1R subtype which made this compound not clinically meaningful as expected [110]. Recently, a novel analog of MK-0557, the spironolactone Y_5R antagonist, was shown to maintain anti-obesity effects in diet-obese animals and is part of further investigations [129]. Based on the ongoing interest in obesity treatment and feeding responses, many further NPY antagonists were established within the last years, among them benzimidazole derivatives [130], ureido derivatives [131] or spirolindoline class compounds [132] and many more [133], all of them with good prospects to be future candidates in anti-obesity therapies. Moreover, besides its impact on neurological diseases, the Y_2R emerged as interesting target in obesity treatment as numerous studies reported on the Y_2R-selective agonist PYY(3–36) being capable to reduce hunger and food intake in humans [134]. Based on these findings, PYY and PYY(3–36) [135] and also numerous other novel Y_2R agonists identified by 7TM Pharma (under patents WO2005089789, WO2007038943 and WO2008132435) are currently part of further investigations in clinical studies. The development of novel anti-obesity drugs by targeting the PYY system would include either blocking the Y_2R by potent antagonists thus inhibiting feeding or benefit from selective agonists such as PYY(3–36) and improved PYY variants with anorectic effects [22,136]. Due to its tissue distribution and in addition to its functions within the gastrointestinal tract, PP is involved in energy homeostasis primarily as a satiety factor. Hence, Y_4R agonism comprises a critical step in successful anti-obesity drug development and therapy. Various selective Y_4R agonists are currently part of clinical trials and displayed promising preclinical data (7TM Pharma under patents WO2005089786, WO2007038942 and WO2008132435), among them the Y_4R-selective PP-based agonist TM30339 showed promising anti-obese effects in preclinical studies [137]. As Y_2R and Y_4R agonists reduce/inhibit food intake and Y_1R and Y_5R agonists stimulate feeding, the development of potent Y_2/Y_4R agonists and selective Y_1/Y_5R antagonists would represent potential anti-obesity drug candidates. Recent studies illustrated synergistic interactions of multiple YRs, suggesting dual therapies as most promising approach in anti-obesity treatment. Moriya *et al*. investigated the impact of the Y_2R-selective agonist PYY(3–36) and the recently developed spironolactone Y_5R antagonist either alone or both combined, with respect to anti-obese effects in diet-induced obese mice. Interestingly, combined administration resulted in an additive anti-obesity effect caused by decreased food intake upon PYY(3–36) treatment and body weight reduction in response to Y_5R antagonist treatment [138]. Synergistic effects were also documented for Y_1R and Y_5R antagonists thus confirming the interaction and the role of both receptor subtypes in the regulation of energy homeostasis as the blockade of both receptors produced greater anti-obesity effects than the blockade of each receptor separately [139]. However, the most pronounced effect with respect to reduced food intake and long-term body weight regulation can be attributed to obinepitide, a Y_2/Y_4R dual peptide agonist, developed by 7TM Pharma (under patent WO2005089790) that has been successfully tested in clinical phase I/II trials and is recently under further investigation [137]. Apparently, the regulatory mechanisms of energy homeostasis in humans is an extremely complex network and mono-therapy is not sufficient to obtain appropriate anti-obesity effects. Thus, combination therapies provide new therapeutic potential with respect to the NPY system as target in anti-obesity treatment [137–140].

YRs in Cancer

Besides its physiological implications and potential in diverse malregulated physiological processes, YRs attracted strong attendance on its involvement in oncogenesis and have recently been predicted as tumor markers [141]. Evidently, YR subtypes have been reported to be overexpressed on various cancer cells and therefore comprise one of the most interesting targets in cancer therapy. The significance of the NPY system in cancer progression has been extensively reviewed elsewhere [4], therefore only prominent examples are mentioned here: Y_1Rs are mainly overexpressed on breast cancer cells, in primary human sarcomas, cortical adenomas, prostate cancer and ovarian cancer (in concert with Y_2Rs) [142–144]. A remarkable high expression of Y_2R was recently identified in human brain tumors, such as neuroblastomas [145] and glioblastomas [141]. Thus, the Y_2R is a potential therapeutic target in neuroblastoma therapy. Up to now the most

pronounced effect could be obtained by blocking Y_2Rs which led to an inhibition of tumor cell proliferation and consequently to an inhibition of neuroblastoma growth *in vivo*. As exogenous NPY stimulates neuroblastoma proliferation and blocking Y_2Rs on these tumor cells significantly inhibits tumor growth, the development of selective and potent Y_2R antagonists might constitute the most promising therapeutic approach [145]. As the Y_4R is predominantly distributed in the gastrointestinal tract, this receptor subtype is mainly related to those types of cancer, e.g. colonic adrenocarcinoma [146]. The activation of the tumoral peptide receptors by their peptide hormones substantially contributes to the tumor cell proliferation, hormone release, metastatization and tumor angiogenesis [147]. Therefore, the appropriate peptides and more importantly selective analogs acting on the specific YR subtypes can be used as therapeutic tools. For diagnostic and therapeutic approaches this can be achieved by covalent coupling of chemotherapeutics or either radioisotope labeling [104]. This would allow selective receptor targeted tumor therapy *in vivo*. However, a fundamental prerequisite for the *in vivo*-targeted receptor radiotherapy is for many aspects receptor internalization: (i) to cargo the therapeutic peptide inside the cell to selectively destroy the tumor cell and thereby preventing major systemic side effects as e.g. the damage of healthy tissue, (ii) the regulation of receptor densities on the cell surface of the tumor cell and therefore the down-regulation of the receptors responsiveness and (iii) the labeling efficiency for diagnostic purposes can increase significantly. As NPY and its related peptides appear to be suitable for *in vivo* tumor targeting, many efforts have been made to develop new promising NPY conjugates. To date, some compounds have been already developed, e.g. a Y_2-selective ^{99m}Tc-labeled NPY compound which might be a potential agent to be applied in tumor imaging or a daunorubicin-coupled NPY analog potentially suitable for chemotherapy purposes [148,149]. The most prominent compounds have been established very recently. These conjugates are ^{99m}Tc labeled, NPY-derived Y_1R ligands which have been successfully used in preclinical and first clinical studies for breast cancer treatment. These studies clearly verify a significant uptake of the labeled Y_1R selective ligands into breast cancer cells overexpressing the Y_1R subtype. Up to now, this is the first study reporting on successful clinical application of NPY-derived ligands in breast cancer imaging [150].

Perspectives

The NPY multireceptor/multiligand system plays a critical role in numerous important physiological functions, and its involvement in the etiology of human pathologies has made it an interesting target for clinical therapies. Thus, the NPY system has been a major focus in the research over the last decades. Many efforts have been made to characterize the receptor–ligand interactions and to elucidate the structure of the bioactive receptor–ligand complex which constitutes the basis for successful development of clinical relevant agonists and antagonists treating human diseases related to the NPY hormone family and its receptors. Thus, the identification of YR subtype specific receptor–ligand interaction points represents a fundamental achievement in the field of future drug design. Such novel potential drugs would provide new therapeutic opportunities to treat severe YR associated diseases such as cancer and obesity. To date, only few new approaches exist, such as the dual agonism/antagonism, which resulted in several promising compounds as anti-obesity drugs that are now under further investigation in clinical trials. But also improved selective agonists emerged as potential future anti-obesity drugs, due to auspicious results in pre-clinical studies and Phase I/II clinical trials. Unfortunately, YR-targeted cancer treatment is currently still restricted to diagnostics rather than therapy. Successful receptor-targeted tumor diagnosis and therapy presumes not only suitable peptide drugs which target the corresponding receptor, but requires receptor internalization as basic necessity to shuttle the receptor bound drug into the tumor cell. Recently, the first positive results in the field of cancer diagnostics have been achieved with the Y_1R where it has been possible to selectively label breast cancer cells in patients by using NPY analogs that specifically bind this receptor subtype. These studies clearly emphasize the importance of the receptor internalization process which accordingly represents a key step in cancer treatment. However, with regard to any YR-related disease there is still a lack of therapeutic compounds available for clinical use. Consequently, the identification of new peptidic or nonpeptidic small molecule YR ligands as pharmaceutical tools is pivotal to assess the role of YRs in human pathologies and to explore novel medication strategies.

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