MC4R Dimerization in the Paraventricular Nucleus and GHSR/MC3R Heterodimerization in the Arcuate Nucleus: Is There Relevance for Body Weight Regulation?

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Key Words

Weight regulation • G protein-coupled receptor • Dimerization • Melanocortin 3 receptor • Melanocortin 4 receptor • Growth hormone secretagogue receptor

Abstract

The worldwide obesity epidemic is increasing, yet at this time, no long-acting and specific pharmaceutical therapies are available. Peripheral hormonal signals communicate metabolic status to the hypothalamus by activating their corresponding receptors in the arcuate nucleus (ARC). In this brain region, a variety of G protein-coupled receptors (GPCRs) are expressed that are potentially involved in weight regulation, but so far, the detailed function of most hypothalamic GPCRs is only partially understood. An important and underappreciated feature of GPCRs is the capacity for regulation via di- and heterodimerization. Increasing evidence implicates that heterodimerization of GPCRs results in profound functional consequences. Recently, we could demonstrate that interaction of the melanocortin 3 receptor (MC3R) and the growth hormone secretagogue receptor (GHSR)-1a results in a modulation of function in both receptors. Although the physiological role of GPCR-GPCR interaction in the hypothalamus is yet to be elucidated, this concept prom-

ises new avenues for investigation and understanding of hypothalamic functions dependent on GPCR signaling. Since GPCRs are important targets for drugs to combat many diseases, identification of heterodimers may be a prerequisite for highly specific drugs. Therefore, a detailed understanding of the mechanisms and their involvement in weight regulation is necessary. Fundamental to this understanding is the interplay of GPCR-GPCR in the hypothalamic nuclei in energy metabolism. In this review, we summarize the current knowledge on melanocortin receptors and GHSR-1a in hypothalamic weight regulation, especially as they pertain to possible drug targets. Furthermore, we include available evidence for the participation and significance of GPCR dimerization.

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Regulation of Food Intake

A healthy adult is able to maintain his energy reserve, and thereby his body weight, on a constant level for years and even decades. This maintenance of a constant physi-

A.R., C.L.P., and H.B. contributed equally to this study.

ological state was firstly described in the 19th century [1]. Accordingly, the term homeostasis was introduced at the beginning of the 20th century [2] and describes the delicate and meticulous regulation of an active metabolic process that results in a stable condition. Body weight maintenance is realized by a very complex regulatory system that is on one hand evolutionary, constrained to protect us from starving, and on the other hand from overeating (hyperphagia). In the long-term, this regulation produces a balanced state in which energy intake through food consumption is in accordance with the basal energy rate of the body.

A multitude of different peripheral (e.g. stomach elasticity, circulating nutritive signals, and hormones) and central factors are needed for a short- or long-term balance between food intake and energy requirements. However, the vast majority of this information on energy intake and storage is transmitted to and processed by a complex control system involving a number of GPCRs localized in the hypothalamus. In this review, we focus on the role of melanocortin receptors (MC3R and MC4R) and ghrelin receptor on weight regulation and their possible role of therapeutic intervention in the treatment of obesity and cachexia.

The Leptin-Melanocortin Pathway

Since the identification of a mutation in the leptin gene [3] in the *ob/ob* mouse (obese mouse), our current knowledge on the leptin-melanocortin pathway has increased tremendously. Potentially, the most important pathway in energy homeostatic regulation, this pathway allows for peripheral information on the filling status of energy stores to be communicated to the central nervous system by a variety of hormones and cytokines, such as leptin or ghrelin. These communication pathways are integrated in hypothalamic nuclei stimulating adaptation to the current metabolic status.

Leptin is an adipocyte-derived hormone that is secreted into the bloodstream in proportion to the energetic content of fat cells [4]. In the hypothalamic arcuate nucleus (ARC), high levels of leptin cause an anorexigenic response. In addition to leptin, other peripheral signals like insulin, pancreatic peptide YY3–36, or plasma cholecystokinin exert central effects to reduce food intake and enhance energy expenditure [5].

To date, ghrelin is the only known food intake-promoting hormone from the periphery. In 1999, stomach-derived ghrelin was discovered and described as a ‘hun-

ger-hormone’, because it was secreted under fasting conditions [6]. Further investigation has shown that ghrelin is secreted periodically showing high levels preprandially and low levels after meals [7–10]. Furthermore, ghrelin promotes positive energy balance by inducing food intake and reducing energy expenditure [11] via activation of a GPCR, the growth hormone secretagogue receptor (GHSR)-1a, also known as ghrelin receptor. Ghrelin is a hormone comprised of 28 amino acids and is only an active ligand for GHSR following the introduction of an octanonyl moiety at serine 3. The enzyme responsible for this important step is Ghrelin-o-acyl-transferase (GOAT) [12]. Interestingly, GOAT is expressed in proportion to the triglyceride content of nutrient supply, and therefore, ghrelin may serve as a nutrient sensor.

Appetite regulation by the ARC is modulated by two groups of hypothalamic neurons, the NPY/AgRP-neuron (neuropeptide Y/Agouti-related protein) and the POMC/CART-neuron (proopiomelanocortin/cocaine amphetamine related transcript). These neurons project, in parallel, into other regions of the hypothalamus, acting to stimulate opposing effects on food intake and energy homeostasis [13]. Several peripheral signals have been shown to modulate the expression of orexigenic neuropeptides of the NPY/AgRP-neuron as well as anorexigenic neuropeptides of the POMC/CART-neurons (fig. 1) [14], resulting in coordinated regulation of these signals in response to changing energy balance.

In normal-weight individuals, neuroendocrine circuits maintain a constant body weight [15]. In such situations, leptin signals through NPY/AgRP neurons as an indication of sufficiently filled energy stores. This signal transduction results in repression of the expression of orexigenic neuropeptides. In addition to orexigenic repression, leptin activates POMC/CART neurons leading to POMC expression and processing. As a result, levels of the anorexigenic peptides alpha- and beta-MSH are enhanced. These neuropeptides were axonally transported via the so-called ‘second order neurons’ to the paraventricular nucleus (PVN) where they activate the melanocortin 4 receptor (MC4R), a family A GPCR. This process leads to reduction of food intake and enhancement of energy expenditure (fig. 1). During times of reduced energy stores, leptin levels decrease, which aborts activation of anorexigenic neurons. Additionally, repression on NPY/AgRP neurons is released, resulting in stimulation of orexigenic signals and subsequent food intake. This central system of energy homeostasis is complemented by a variety of peripheral signals, including ghrelin, to ensure a rapid and consistent adaptation to environmental changes.

Fig. 1. GPCRs in hypothalamic weight regulation. Anorexigenic system: adipocytes secrete leptin in proportion of body fat (long-time regulation). This peripheral hormone downregulates the release of orexigenic peptides through NPY/AgRP-neurons and upregulates the expression of POMC. Cleavage products of POMC – alpha- and beta-MSH – stimulate MC4R in the PVN to inhibit food intake and activation in other hypothalamic sites, like the lateral hypothalamic area or the dorsomedial hypothalamus, which lead to energy expenditure. Orexigenic system: NPY and AgRP expression by ghrelin stimulation results in increased appetite and the initiation of food intake. Due to the association

of the MC3R at both ARC neurons, the stimulation of these GPCRs with alpha-, beta-, or gamma-MSH results in the constitution of a kind of bridge between appetite-suppressing and appetite-stimulating signals. Furthermore, a selection of GPCRs involved in the hypothalamic appetite regulation which were investigated in regard to the formation of heterodimers either with MC3R or MC4R is listed up. CB1R: Cannabinoid 1 receptor; GPR7: G protein-coupled receptor 7; NPY2R: neuropeptide Y2 receptor; MCHR: melanin-concentrating hormone receptor (sub-type 1); OX1R: orexin receptor 1, PRPR: prolactin-releasing hormone receptor; μ-OPR: μ-opioid receptor.

Disturbances in Energy Homeostasis

Although the leptin-melanocortin system is highly effective in maintaining a constant body weight, obesity has become one of the major health problems of the last decades [16]. Furthermore, lifestyle changes have been found to be largely ineffective to counteract or combat this epidemic. This phenomenon might be due to the fact

that genomic stability prevents genetic adaptation to environmental changes like sedentary lifestyle or high-energy diets in a short period of time. Individuals who are prone to be overweight or obese are often carriers of genetic constellations that facilitate efficient energy storage and decompensation of normal regulatory systems [17].

So far, interference at the level of leptin and its receptor is complicated by leptin resistance and subsequent hyper-

leptinemia in obese patients. Therefore, one therapy for body weight modulation could be the targeting of GPCRs in the hypothalamic system that act leptin independent.

GPCRs in Hypothalamic Weight Regulation

GPCRs play important roles in nearly every physiological function. Therefore, detailed understanding of structure and function of this largest class of integral membrane proteins is of major importance for the elucidation of physiological processes as well as for the development of novel therapeutic agents. Currently, 40–60% of drugs target GPCRs [18]. The brain, and especially areas of the hypothalamus involved in energy homeostasis like ARC and PVN, are regions with the highest expression rate of GPCRs [19, 20]. GPCRs (with the exclusion of orphan GPCRs) with a connection to energy homeostasis, their expression pattern, ligands and signaling properties are summarized in online supplementary table 1 (www.karger.com/doi/10.1159/000334903). In addition to the aforementioned melanocortin receptors, a total of more than 40 GPCRs [21] expressed in hypothalamic nuclei are involved in energy homeostasis; however, the role of many of these GPCRs in energy homeostasis is not fully understood yet.

MC4R-related signaling in the PVN comprises the majority of research on hypothalamic expressed GPCRs involved in weight regulation. This receptor is activated by POMC-derived alpha- and beta-MSH and its signal is propagated through activation of the Gs/adenylyl cyclase pathway [22]. Confirming its roles in the regulation of energy homeostasis, targeted deletion of MC4R results in late-onset obesity in mice [23]. Interestingly, heterozygous female mice display an intermediate phenotype compared to knockout and wild-type littermates, suggesting a gene-dose effect. To date, heterozygous MC4R mutations are the most frequent genetic cause of obesity in humans [24]. Due to the prominent role of MC4R in hypothalamic weight regulation, intense studies on structure-function relationships have been performed [summarized in 25]. In addition to MC4R, another melanocortin receptor, MC3R, is expressed in the ARC and has been shown to play a role in energy homeostasis. Mice lacking the MC3R are obese but hypophagic. The obese phenotype in these mice is attributed to decreased locomotor activity and aberrant food partitioning into fat [26, 27]. There is evidence that on POMC neuron the MC3R functions as a so-called autoreceptor [28]. In this context, activation of MC3R by POMC neu-

rons inhibits the anorexic action of POMC-derived peptides. Furthermore, peripheral injection of MC3R-specific ligands results in food intake [29]. Together these findings indicate that in the ARC the MC3R may exert an orexigenic function.

Structure and Function of Melanocortin and Ghrelin Receptors

MC4R and MC3R are characterized by common structural features of the rhodopsin/beta2-adrenergic receptor family A, including highly conserved amino acid motifs at the transmembrane helices (TMH), e.g. the so-called DRY motif at TMH3, or the NPxxY motif at TMH7 (fig. 2a). Interestingly, melanocortin receptors lack typical family A GPCR features, such as a conserved cysteine residue in extracellular loop (ECL) 2 and a proline in TMH5. Additionally, these receptors have specific features such as disulphide-bridged cysteines in ECL3 [30] and an extremely short ECL2 comprised by four amino acids. For rational drug development, knowledge concerning binding determinants and properties of the binding sensitive region/s is crucial. It is known for MC3R and MC4R that specific amino acids in the TMH region are essential to the interaction with the core-binding motif of alpha- and beta-MSH (His-Phe-Arg-Trp motif). The N-terminal receptor region, as well as the extracellular loops (excluding ECL3) [30], have been shown to be of less importance for ligand binding [31, 32]. The N-terminal part of the MC4R was found to be involved in the slight constitutive activity of the receptor [33].

Within the TMHs, ionic and aromatic residues are mainly responsible for high affinity ligand binding [reviewed in 34]. Specifically, residues important for ligand binding at MC3R (fig. 2b) are localized in TMH2 (Glu131), in TMH3 (Asp154 and Asp158), and in TMH6 (Trp292, Phe295, His298) [35]. Residues of significance for binding at MC4R were also found in TMH2 (Glu100), in TMH3 (Asp122 and Asp126), and in TMH6 (Trp258, Phe261 and His264) [32, 36]. In mutagenesis studies, TMHs 1, 3, 6, 7 and the peripheral intracellular helix 8 were found to be involved in conformational rearrangement after ligand binding to induce signaling [37]. In MC4R, some motifs in the ECLs were reported to be responsible for AgRP 87–132 binding affinity, but not for AgRP 110–117; moreover, amino acid side chains in TMH3 and 4 are important for binding of both AgRP subtypes [38].

In addition to MC3R and MC4R, other GPCRs have been shown to be crucial for energy homeostasis, as

Fig. 2. Structural features and ligand-binding determinants of MC3R. **a** This MC3R homology model shows structural features that are common to family A GPCRs or specific for MC3R. Conserved in family A GPCRs are particular amino acids (green sticks) in the TMH, like the N(D)PxxY motif in TMH7, a proline in TMH6, the DRY motif in TMH3, or an aspartate in TMH2. These residues are of high functional and structural importance and are numbered according to the unified family A GPCR numbering scheme of Ballesteros and Weinstein. Specific MC3R characteristics are an extremely short extracellular loop 2 (red), a cysteine bridge in extracellular loop 3 (yellow), and a methionine at a position in TMH5 (blue stick) where in most other GPCRs a proline induces a distortion of the helix. **b** The top view from the extracellular side with a clipped surface of MC3R shows a pocket-like shape between the extracellular ends of the helices (TMH) and the extracellular loops (ECL). Amino acids (blue sticks) known to be of importance for binding of peptidic ligands covering this pocket between helices 2, 3 and 6. These amino acids are responsible for binding with specific ligand residues and for effective justification of the ligand. Colors refer to the online version only.

ghrelin was demonstrated to be a potent stimulation for food intake in rodents and humans [39]. In the hypothalamus, GHSR is only expressed on ARC NPY/AgRP neurons. Ghrelin challenge leads to activation of the Gq/11 phospholipase C pathway. One of the most prominent functional features of GHSR is a high ligand-independent (constitutive) basal signaling activity, which is approximately 50% of the ghrelin-induced activity [40]. This constitutive activity is likely to be due to hydrophobic amino acids in TMH6 and aromatic counterparts in TMH7 [41]. For activation of GHSR and as a general concept of GPCR activation, a tryptophan residue in TMH6, the so-called tryptophan rotamer switch located in the Cys-Trp-X-Pro motif, is involved in the activation process [42]. The ligand-binding domain is characterized by a cluster of aromatic amino acids in TMHs 3, 6 and 7 [43, 44]. So far, the physiological relevance of this high constitutive activity is not fully understood, but it was re-

cently shown that the high constitutive activity of GHSR leads to a ligand-independent activation of transcription factor CREB (cAMP-responsible element-binding protein). CRE-binding proteins (CREB) are phosphorylated via Ca^{2+} /calmoduline-dependent kinase IV. At present, it is assumed that the constitutive activity of GHSR establishes a so-called set point which regulates the sensation of hunger, and that several inhibitory regulatory systems (e.g. leptin, insulin and PYY_{3-36}) counteract against this activity [45].

Targeted deletion of GHSR results in mice that are normal in growth and food intake [46]. However, when exposed to a high-fat diet these mice are protected from diet-induced obesity [47]. A rare number of GHSR mutations have been reported in humans [48–51] and most were identified in patients with short stature. Currently, only two of the reported GHSR mutations (F279L and A204E) are connected to a higher body weight.

GPCRs as Targets for Treatment of Cachexia and Obesity

There are two possibilities to target GPCRs for obesity treatment: (1) development of highly potent ligands stimulating an anorexigenic acting receptor like MC4R, or (2) the usage of antagonists or inverse agonists to block effects of an orexigenic acting receptor like GHSR. Conversely, the opposing ligands could be used in the treatment of cachexia.

The first study to reduce body weight in humans via targeting of the MC4R was performed with ACTH4–10, and was found to be effective in healthy adults [52]. However, in patients with POMC mutations, this substance did not have any influence on body weight [53]. Artificial alpha-MSH analogs were developed to enhance affinity and efficacy at MC4R such as NDP-alpha-MSH or the cyclic melanotan II. These substances were effective in vitro as well as in rodents; however, they are not selective for MC4R and also activate MC3R [54].

Several alpha-MSH analogs were developed with the purpose to selectively activate MC4R and to pass the blood-brain barrier. On the basis of the core His-Phe-Arg-Trp-binding motif substance, THIQ and other small molecule MC4R agonists were developed [55, 56]. However, these substances also influence sexual behavior and are therefore not useful for anti-obesity treatment at least in children. Additional approaches were performed and tested in vitro and in vivo. Specifically, it was demonstrated that analogs based on spiroindane amide are suitable for oral application [57–59], and recently, it was shown that two MC4R agonists, IRC-022493 and IRC-022511, are able to efficiently activate loss-of-function MC4R mutations [60].

A new substance that was highly MC4R selective and effective in mice to reduce energy intake and to induce weight loss, MK-0493 [61], was used to reduce body weight in normal-weight to obese healthy men. Unfortunately, in humans MK-0493 was not effective to reduce energy intake and to induce weight loss [62]. So far, these studies point to the difficulty in transferability of rodent model systems to the human situation.

For cachexia treatment, antibodies [63] or MC4R antagonists [64] were developed and are effective in rodents. Up to now, no studies of their effectiveness in humans exist.

The ghrelin/GHSR system is another potential target for treatment of disturbed weight regulation due to its stimulation of appetite as well as growth hormone release [65, 66]. For GHSR, two different strategies to block signaling are favored: antagonizing ghrelin induced signal-

ing function or blockage of the high basal activity by an inverse agonist [40].

Currently, a huge variety of different mostly low molecular weight agonists and antagonists of GHSR were designed in order to serve as therapeutic agents to treat obesity and diabetes as well as cachexia and growth deficiency disorders. These substances were quinazolinone derivatives [67], ligands based on tri-substituted 1,2,4-triazole structure [68, 69], tetralin carboxamide [70, 71] isoxazole carboxamide [72], 2,4-diaminopyrimidines [73], oxindole [74], piperazine-bisamide [75] GHSR ligands as well as R-prolinol-derivate agonists [76].

Antagonists that interrupt the effect of ghrelin to stimulate the release of growth hormone were developed, e.g. BIM-28163 [77]. Surprisingly, BIM-28163 stimulates feeding which leads to the speculation that a second GHSR subtype exists. At high concentrations, the GHSR antagonists [D-Lys-3]-GHRP-6 seem to be effective to reduce food intake in rats [78]. In the same setting, the effects of substance P, an inverse agonist of GHSR, is more effective [78]. Further possibilities to modulate GHSR function in vitro and in vivo is the interference of ghrelin action by bio-stable RNA-based compound (so-called Spiegelmers). L-NOX-B11 was found to inhibit the action of ghrelin at the GHSR in vitro in a low nanomolar range, and intravenous injection suppresses ghrelin-induced growth hormone release in vivo [79]. Additionally, vaccination with ghrelin immunoconjugates has been shown to slow weight gain in rats by decreasing feed efficiency [80].

A ghrelin mimetic (MK-677) was developed and found to effectively induce weight-gain and enhance growth-hormone secretion in healthy adults [81]. However, these findings have been controversial [82]. Furthermore, obese individuals were found to be more sensitive to ghrelin administration when compared to lean individuals [83].

In summary, initially promising approaches to modulate the activity of MC4R or GHSR have failed to yield effective therapies in the treatment for diseases of disturbed energy balance so far. One possibility to specifically interfere MC4R or GHSR function might be the ability of these receptors to form receptor homo- and/or heterodimers [84].

Heterodimerization of GPCRs Involved in Weight Regulation

In 1980, Agnati et al. [85] were one of the first to express the possibility of GPCR dimerization. This hypothesis greatly expanded on the prevailing picture of a linear

Fig. 3. Spatial localization of naturally occurring mutations of the ghrelin receptor. Positions of pathogenic mutations (red sticks) are highlighted on the structural GHSR homology model. They are distributed on the entire receptor structure: the extracellular loops (ECL), transmembrane helices (TMH), and the intracellular loops (ICL). Shown are the receptor backbone as ribbon and a translucent protein surface. The arrows indicate preferred and

reported intermolecular GPCR-PCR contact areas (also at the top view from the extracellular site, figure part 2), which are localized between TMH5–6, between TMH1–2, and between TMH3–4, including the connecting loops of these helices. Naturally occurring mutations might indicate by their functional effects positions which are directly or indirectly involved in GHSR dimer contacts. Colors refer to the online version only.

regulatory system of one receptor encoded by one single gene and activated by binding of a specific ligand. The first proof of the functional necessity of GPCR-GPCR interaction was shown for GABA_B receptor subtypes [86–88]. The GABA_B receptor dimer consists of GABA_{B1} and GABA_{B2}, which ensure ligand binding [89], transport to the plasma membrane, and activation of G protein.

The formation of dimers, as well as larger oligomerization, is now established for a variety of homo- and heteroforms of GPCR [90–92] (for references on GPCR dimerization and functional relevance, see also <http://data.gpcr-okb.org/gpcr-okb/>).

Naturally occurring mutations in GPCRs are an excellent tool to obtain deeper insights into receptor functionalities. Previous investigation of a heterozygous complete loss-of-function mutation in MC4R elucidated a dominant-negative effect on the wild-type receptor. Further characterization of this effect revealed a dimerization between the mutated and wild-type MC4R receptor [93]. To understand how MC4R homodimers are or-

ganized, we first excluded extracellular cysteine residues as intermolecular contact points between the protomers [94]. Recent studies point to an important role of the transmembrane portion of the receptor as a mediator of receptor-receptor interactions [Biebermann et al., unpubl. data]. The identification of MC4R homodimerization raised the question as to whether the MC4R or other GPCRs involved in weight regulation are able to form receptor di- or oligomers. Subsequently, we have shown that the MC4R expressed in the hypothalamic PVN is able to heterodimerize with other GPCRs involved in weight regulation like G protein-coupled receptor 7 [84]. The identification of MC4R interaction partners opens the possibility to specifically modulate the function of MC4R in the PVN. Moreover, we tested for heterodimerization of MC3R expressed in the hypothalamic ARC and found evidence for interaction of MC3R with GHSR [84]. The identification of GPCR interaction in vitro, however, is meaningless unless this interaction is also observed in vivo. Recently, we could demonstrate colo-

Fig. 4. Heterodimerization might modulate the function of the interacting partners. Focusing on ligand-binding and G protein-coupling potential functional variances of the heterodimer in contrast to the protomers resulting from interaction of two GPCRs (receptor 1 shown in green and receptor 2 shown in red). Heterodimerization of two GPCRs could cause modulations of the ligand-binding cooperative effects (**a**) and signaling capacity (**d**) of both receptors or enable the binding of new ligands (**c**, **f**).

Bivalent ligands composed of modified ligand-variants of two interacting receptors which were linked through a spacer allow co-stimulation of the resulting heterodimer and activate downstream pathways (**b**, **f**). Furthermore, the selective coupling of a G-protein change initiates a characteristic signaling pathway. Cross talks between activated signaling pathways could change intracellular effects (**e**). Colors refer to the online version only.

calization of MC3R and GHSR in vivo [95]. Moreover, we were able to demonstrate that the functional characteristic of the MC3R to activate the Gs/adenylyl cyclase signaling system is enhanced due to coexpression with GHSR. On the other hand, signaling properties of GHSR to activate Gq/11 signaling are reduced after coexpression with MC3R [95]. This mutual opposite influence of signaling properties of both receptors could be explained by physical interaction and is most likely due to the high basal activity of GHSR-1a and a constrained conformation of MC3R. By usage of naturally occurring mutation in MC3R and GHSR, we could confirm these hypotheses. On the other hand, such knowledge on dimer contact partners may open new perspectives for molecular

mechanisms of natural mutations and causes of diseases (fig. 3). Pathogenic mutations may also be responsible for disturbance of dimer-dependent receptor-mediated signaling effects.

Concluding Remarks

The identification of GPCR heterodimerization provides a promising tool for direct targeting of a GPCR of interest due to specific protein-protein interactions [96, 97]. Diversification of receptor pharmacology, a fine-tuning of ligand binding and G-protein specificity, as well as regulation of receptor maturing and internaliza-

tion are discussed as possible functions of dimerization [98, 99]. Furthermore, bivalent ligands that interact with the receptor of interest and an interacting receptor are addressed as a specific modulation of receptor signaling capacity (fig. 4).

In the context of novel and evolving therapies, GPCR heterodimerization has already been shown to influence the effectiveness of known treatments. A current example of this interaction is that of the EP₁R (prostaglandine receptor E) and β_2 AR (β_2 -adrenergic receptor) in cells of the smooth musculature [100, 101]. Here, interaction between β_2 AR and EP₁R results in a decoupling of the β_2 AR from its signal transduction pathway. This uncoupling diminishes the effectiveness of β_2 -adrenergic agonists and complicates the treatment of asthma.

For MC4R and GHSR, the possibility of dimerization as an important feature of in vivo function is a poorly understood phenomenon that merits further investigation. Furthermore, this newly described interaction could facilitate the development of highly specific and

effective ligands. For patients suffering from obesity or cachexia, targeting of dimerized MC4R in the PVN and of GHSR in the ARC could represent a valuable tool to modulate food intake. However, a prerequisite for this purpose is detailed knowledge on every interaction partner of these receptors. These interaction partners could not only be GPCRs, but also other membrane-bound or cytoplasmic proteins. Finally, mutual modulation of signaling capabilities by direct GPCR-GPCR interaction should be characterized by several facets including effects on selectivity, biased or promiscuous signaling, and the impact of basal ligand-independent signaling activity.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) BI893/2-1, KL2334/2-1; GRK 1208, TP1, and the Bundesministerium für Bildung und Forschung, NGFN Plus 01GS0825.

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