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Impact of G protein-coupled receptor heteromers in endocrine systems

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ABSTRACT

The fine-tuning of endocrine homeostasis is regulated by dynamic receptor mediated processes. The superfamily of G protein-coupled receptors (GPCRs) have diverse roles in the modulation of all endocrine axes, thus understanding the mechanisms underpinning their functionality is paramount for treatment of endocrinopathies. Evidence over the last 20 years has highlighted homo and heteromerization as a key mode of mediating GPCR functional diversity. This review will discuss the concept of GPCR heteromerization and its relevance to endocrine function, detailing *in vitro* and *in vivo* evidence, and exploring current and potential pharmacological strategies for specific targeting of GPCR heteromers in endocrine heath and disease.

1. Introduction

G protein-coupled receptors (GPCRs) are pervasive to most physiological and endocrinological processes. Their necessity in maintaining endocrine homeostasis makes them a lucrative therapeutic target, with approximately 40% of current prescription drugs targeting a GPCR. Resulting pathophysiological disorders caused by GPCR dysfunction drives our need to dissect the basic science underpinning the complex modalities of GPCR regulation. Furthermore, understanding these processes is paramount to more targeted and efficacious next generation pharmaceuticals and to personalized medicine approaches (Ferre et al., 2014).

Homo/heteromerization of GPCR is now a widely accepted modality of how GPCRs regulate their physiological functions (Ferre et al., 2014; Gomes et al., 2016). The current evolved model of GPCR signaling incorporates the ever-increasing complexity in GPCR signal pathways and mechanisms of regulation, to which homo/heteromerization has made a significant contribution. Although most studies have documented homo/heteromerization using heterologous cell lines, several studies have demonstrated the *in vivo* significance of these receptor-receptor interactions. Such complexity in receptor regulation provides key mechanism/s for the multiple and dynamic roles these receptors play *in vivo*.

This review will discuss the functional and *in vivo* evidence for GPCR heteromers in endocrine systems. We will describe the criteria for assessing and classifying GPCR heteromers, review their known functional impact from both *in vitro* and *in vivo* studies of these hetero-complexes on endocrine function.

2. Detecting and classifying GPCR heteromers-an overview

GPCR heteromerization is defined as a macromolecule complex composed of at least two receptor units, with biochemical properties that are demonstrably different from those of its individual components (Gomes et al., 2016). With an increase in the number of reports identifying GPCR heteromers, the resulting challenges in distinguishing the difference between receptors localised to the same cell undergoing functional cross-talk, versus receptors complexed as physiologically relevant heteromers became an important distinction. Thus, three consensus criteria were published by

Abbreviations: α1BR, α1B adrenergic receptors; AT1R, Angiotensin 1 receptor; BRET, Bioluminescence resonance energy transfer; B2R, Bradykinin 2 receptor; CRH, Corticotrophin releasing hormone; D2R, Dopamine D2 receptor; FRET, Fluorescence resonance energy transfer; FSHR, Follicle stimulating hormone receptor; GIPR, Gastric-inhibitory polypeptide receptors; GLP-1, Glucagon-like peptide-1; GPCR, G protein-coupled receptor; GHSR, Growth hormone secretagogue receptor; LHR, Luteinizing hormone receptor; μOR, μ-opioid receptor; MC3R, Melanocortin-3 receptor; PLA, Proximity ligation assay; V1bR, Vasopressin 1b receptor.

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the International Union of Basic and Clinical Pharmacology to facilitate the classification of true GPCR heteromers (Kenakin et al., 2010). The first of these criteria concerns the requirement for evidence of physical receptor-receptor interactions in native or primary tissue. Traditionally, methods such as co-immunoprecipitation and resonance energy transfer techniques (Angers et al., 2000; Hounsou et al., 2015; Albizu et al., 2010; Pfleger et al., 2007; Ramsay et al., 2002) have been used to demonstrate receptor-receptor interactions and hence the proximal existence of heteromers. Recent technological advances in super-resolution and single molecule imaging present an innovative methodology for detecting GPCR heteromers. Techniques such as fluorescent correlation spectroscopy (Mazurkiewicz et al., 2015a; Zakrys et al., 2014), single particle tracking via total internal-reflection fluorescent microscopy (Kasai et al., 2011; Hern et al., 2010; Calebiro et al., 2013) and localization microscopy techniques such as photo-activated localization microscopy (Scarselli et al., 2012; Jonas et al., 2015) have been utilised to identify GPCR heteromers and homomers. Likewise, proximity ligation assays (PLA), also provide a mechanism for identifying heteromers in native tissues (Gonzalez et al., 2012).

The second consensus criteria requires there to be heteromer-specific properties, be it a change in the pharmacology of the receptors via G protein specificity or allosteric binding properties, or ligands that are heteromer-specific. This is demonstrated via classical biochemical, pharmacological and cell signaling techniques to determine changes in ligand binding, G protein-dependent and G protein-independent signal activation. The third criteria necessitates the requirement for the direct physiological evidence for the importance of the identified heteromer. Methodology used to determine this include RNA interference or *in vivo* studies to introduce genetic modifications in receptor protomers participating in the heteromer. If the transmembrane interface is known, expression or incubation of cell permeable peptides corresponding to the transmembrane region have been employed *in vitro* and *in vivo*. Thus, confirming the physiological requirement for the heteromer (Gomes et al., 2016; Kenakin et al., 2010).

In practice, there are very few identified heteromers that fit all three criteria; with criteria three often the hardest to fulfil given that most functional heteromers have been identified using heterologous cell lines. Therefore, fulfilment of two out of three of the criteria are required for the acceptance of a GPCR heteromer. With respect to endocrine systems, most identified GPCR heteromers that fulfil all three inclusion criteria are neuroendocrine in nature. The functional significance of which will be discussed in further detail.

3. Impact of GPCR heteromers on receptor activity in endocrine systems

The canonical view of GPCR signaling has evolved from a ligand binding to a single monomeric receptor that activates a single heterotrimeric G protein, to one of growing complexity. GPCR homo/heteromerization provides a modality whereby receptors can mediate multiple functions via modulating receptor trafficking (both exo- and endocytosis), ligand specificity and functional selectivity. In obligatory heteromers such as the GABAB1 and GABAB2 receptors, (also observed with the sweet and umami taste receptors (Nelson et al., 2001, 2002; Xu et al., 2004)), the functional significance of heteromerization is required for cell surface expression of both receptors, as well as G protein-coupling (Kaupmann et al., 1998; Duthey et al., 2001; Pin et al., 2004), via transactivation of GABAB1-GABAB2 heteromers. However, identifying the functional significance of GPCR heteromers are not always essential to all functions mediated by a specific receptor. That said, heteromer formation can increase the spectrum of ligand recognition and signal outcomes of a receptor. One common mode of regulation within a heteromer is via allosterism, the outcome of which can lead to distinct functional responses to that of the individual receptors. Broadly speaking, allosteric interactions within heteromers can lead to three different functional outcomes:

1. Ligand binding. Ligand binding within a heteromer results in either positive or negative cooperativity exerted on the neighbouring receptor(s) within the heteromer.
2. G protein recruitment. G protein selectivity may change or exert differential preferences between heteromeric and homomeric complexes.
3. G-protein independent mechanisms via β-arrestin recruitment. The heteromer may favour or acquire G protein-independent functionality via β-arrestin recruitment.

Many GPCR heteromers have been shown to have direct roles in endocrine homeostasis, impacting metabolism, reproduction, nutritional status and stress responses (Table 1). The functional significance of such heteromers has largely been dissected using heterologous cell lines, examples of which will be discussed below.

3.1. Metabolism and nutrition

Endocrine-mediated feedback for the control of satiety and appetite is essential for maintaining metabolic homeostasis. Key pathways that regulate appetite stimulation and feeding are mediated via the growth hormone secretagogue receptor (GHSR). GHSR is expressed within various hypothalamic nuclei involved in food intake and reward-seeking behaviour (reviewed by (Wellman and Abizaid, 2015)), and thus has been suggested to be a central player in regulating metabolic homeostasis in both a ligand-dependent (via Ghrelin) and -independent manner, via intrinsic basal GSHR activity (Wellman and Abizaid, 2015; Yin et al., 2014). Several studies have demonstrated GHSR to heteromerize with other GPCRs that also regulate metabolic status (Wellman and Abizaid, 2015). Indeed, heteromerization of GSHR and the melanocortin-3 receptor (MC3R) was identified by fluorescence resonance energy transfer (FRET) (Rediger et al., 2009), with immunohistochemical and in situ studies co-localizing GHSR and MC3R to the arcuate nucleus of the hypothalamus. The functional significance of the GHSR/MC3R heteromers examined *in vitro* via co-expression of GHSR and MC3R in COS-7 and HEK293 cells, showed that GSHR/MC3R heteromers may enhance MC3R-dependent cAMP accumulation yet decrease basal and ligand-induced GHSR activity. Moreover, the enhanced MC3R activity observed was dependent on the decrease in basal GHSR activity, suggesting positive and negative allosteric regulation between MC3R and GHSR protomers to create functionally asymmetric complexes (Rediger et al., 2011).

Heteromers of the Class B GPCR family have been suggested to have roles in glucose homeostasis. Ligand-dependent BRET screening of the glucagon receptor family heteromeric and homomeric interactions in HEK293 cells, showed glucagon-like peptide-1 (GLP-1)-dependent heteromer formation between GLP1 and gastric-inhibitory polypeptide receptors (GIPR). This heteromeric association was GLP-1-specific, with reversal of GLP-1/GIPR association observed with titration of GIPR. Functionally, a change in the activity of the GLP-1R was also observed in terms of calcium response, and β-arrestin recruitment (Schelshorn et al., 2012). There has also been recent evidence for functional cross-talk between Gαs and Gαq-coupled receptors in the regulation of long chain fatty acid mediated incretin secretion. Recent findings have shown that GPR40-mediated GLP-1 release by colonic