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Internalization of G-protein-coupled receptors: Implication in receptor function, physiology and diseases

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G protein-coupled receptors (GPCRs) are the largest family of membrane receptors and mediate the effects of numerous hormones and neurotransmitters. The nearly 1000 GPCRs encoded by the human genome regulate virtually all physiological functions and are implicated in the pathogenesis of prevalent human diseases such as thyroid disorders, hypertension or Parkinson's disease. As a result, 30–50% of all currently prescribed drugs are targeting these receptors. Once activated, GPCRs induce signals at the cell surface. This is often followed by internalization, a process that results in the transfer of receptors from the plasma membrane to membranes of the endosomal compartment. Internalization was initially thought to be mainly implicated in signal desensitization, a mechanism of adaptation to prolonged receptor stimulation. However, several unexpected functions have subsequently emerged. Most notably, accumulating evidence indicates that internalization can induce prolonged receptor signaling on intracellular membranes, which is apparently required for at least some biological effects of hormones like TSH, LH and adrenaline. These findings reveal an even stronger connection between receptor internalization and signaling than previously thought. Whereas

Abbreviations: G protein-coupled receptor, (GPCR); protein kinase A, (PKA); cyclic adenosine monophosphate, (cAMP); mitogen-activated protein kinase, (MAPK); thyroid stimulating hormone, (TSH); parathyroid hormone, (PTH); protein kinase A, (PKA); neurokinin, (NK); clathrin-mediated endocytosis, (CME); clathrin-coated pits, (CCPs).

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new studies are just beginning to reveal an important physiological role for GPCR signaling after internalization and ways to exploit it for therapeutic purposes, future investigations will be required to explore its involvement in human disease.

Introduction

G protein-coupled receptors (GPCRs), with a share of almost 4% of the human genome [1], constitute the largest family of receptors that allow cells to sense extracellular stimuli [2,3]. These external stimuli range from sensory cues like light, odorants and tastants to small-molecule neurotransmitters, peptides and hormones [2,3]. This high diversity underscores the fundamental role that GPCRs play in the function of the endocrine, nervous, cardiovascular, sensory and immune systems.

The main initial steps of GPCR activation and signaling have been elucidated in detail [2,4]. These events are initiated by binding of an agonist to a receptor, which triggers a series of conformational changes in the receptor that culminate in its activation. The activated receptor, in turn, binds to and activates heterotrimeric G proteins, which are composed of an α, β and γ subunit and exist in different isoforms. The α and $\beta\gamma$ subunits finally modulate the activity of membrane-localized effectors, including ion channels and enzymes like phospholipase Cβ (PLCβ) and adenylyl cyclase.

A classic example of the role of these receptors in physiology is their involvement in the regulation of heart contractility. β-adrenergic receptors located on the surface of cardiomyocytes mediate the positive ionotropic and chronotropic effects of adrenalin and noradrenalin, released upon sympathetic activation. Binding of adrenalin or noradrenalin to these receptors, which are coupled to the G_s protein, activate adenylyl cyclases to produce cAMP, which stimulates protein kinase A (PKA). PKA, in turn, phosphorylates different molecules involved in cardiac contractility, including L-type Ca^{2+} channels, phospholamban and troponin I, ultimately leading to enhanced cardiomyocyte contractility [5]. In addition, cAMP directly promotes the opening of pacemaker (HCN) channels in the conductive tissue, thus increasing heart rate [6,7]. Parasympathetic activation counteracts these effects via release of acetylcholine, which binds to muscarinic (M2) receptors coupled to $G_{i/o}$ proteins, thus inhibiting adenylyl cyclase activation. In addition, the $\beta\gamma$ subunits released upon $G_{i/o}$ activation stabilize the membrane potential via activating potassium (GIRK) channels in the conductive tissue [8–12]. In the endocrine system, GPCRs play an essential role as receptors for several hormones, hypothalamic releasing factors and local modulators. All major known hypothalamic releasing (TRH, GnRH, CRH, GHRH) and inhibiting (somatostatin, dopamine) hormones act via specific GPCRs [13–17]. With the exception of GH and PRL, anterior (TSH, LH, FSH, ACTH, MSH) and posterior (vasopressin, oxytocin) pituitary hormones also signal through activation of GPCRs [18]. For an extensive discussion of the specific roles of GPCRs and G proteins in human physiology we refer the reader to the comprehensive review by Wettschureck and Offermanns [19].

Mechanisms of GPCR internalization

Like for other types of receptors, prolonged agonist stimulation often leads to GPCR internalization, which can occur via different pathways [2,20–23]. Of these pathways, clathrin-mediated endocytosis (CME) is the best characterized and arguably most relevant one (Fig. 1) [2,20–23]. The first molecular event involved in GPCR internalization is the binding of a family of G protein-coupled receptor kinases (GRKs) to an agonist-occupied receptor, which phosphorylate multiple intracellular serine and threonine residues located in the 3rd intracellular loop or at the C-terminus of the receptor [24–27]. This is followed by binding of arrestins to the phosphorylated receptor, which plays a major role in both fast signal desensitization and receptor internalization [24,26]. On the one hand, arrestins compete with G proteins for binding to the receptor, thus leading to signal desensitization. On the other hand, they promote receptor internalization via interacting with key proteins involved in the assembly of clathrin-coated pits