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REVIEW

Cholesterol-dependent endocytosis of GPCRs: implications in pathophysiology and therapeutics

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Abstract

G protein-coupled receptors (GPCRs) are the largest family of transmembrane proteins that relay extracellular signals across the plasma membrane and elicit an intricate cascade of cellular signaling events. A significantly large fraction of available drugs target GPCRs in order to exert fine control over functional outcomes from these receptors in pathological conditions. In this context, endocytosis and intracellular trafficking of GPCRs stringently regulate signaling outcomes from GPCRs within physiologically relevant spatiotemporal regimes. The membrane microenvironment around GPCRs has recently emerged as a key player in receptor function. Cholesterol is the single most abundant lipid in the eukaryotic plasma membrane and plays a central role in membrane organization and dynamics, with far-reaching functional implications in cellular physiology. In this review, we discuss current excitements in GPCR endocytosis and trafficking, with an emphasis on the role of membrane cholesterol. We envision that a detailed understanding of the contribution of membrane lipids such as cholesterol in spatiotemporal regulation of GPCR signaling would enable the development of therapeutic interventions fine-tuned to receptors residing in specific membrane microenvironments.

Keywords Cholesterol · GPCRs · Endocytosis · Intracellular Trafficking · Therapeutics

GPCRs: stringently regulated signaling nanomachines in a lipid microenvironment

G protein-coupled receptors (GPCRs) are membrane-resident signal transducers that enable the recognition of chemically and physiologically diverse extracellular cues essential for a repertoire of cellular functions (Chattopadhyay 2014; Pierce et al. 2002; Rosenbaum et al. 2009; Weis and Kobilka 2018). Signaling by GPCRs has been implicated in diverse cellular physiology, ranging from growth and development, to immune responses and pathogen uptake (Gutierrez and McDonald 2018; Hameid et al. 2021; Lämmermann and Kastenmüller 2019; Palczewski and Orban 2013). It is therefore not surprising that GPCRs account for more than one-third of the current drug targets (Chan et al. 2019; Hauser et al. 2017; Sriram and Insel 2018). However, these receptors constitute only ~ 10% of GPCRs encoded in the human genome, leaving a major fraction of *druggable* targets untapped (Cooke et al. 2015; Jacobson 2015; Stockert and Devi 2015). In addition, the GPCRs that are targeted by currently available drugs are primarily aminergic and opioid receptors (Hauser et al. 2017). These statistics suggest that GPCRs offer a largely unexplored, yet potentially rewarding pool of drug targets (Foster et al. 2019; Huang et al. 2017; Sloop et al. 2018).

Owing to the diverse array of intracellular signaling processes elicited by GPCRs, regulation of their activity within physiological regimes and/or toward specific downstream responses assumes relevance. Such regulatory features could intervene at various stages of signal transduction via GPCRs—from exposure to ligands to coupling of effector proteins to receptors (Gurevich and Gurevich 2019; Lane et al. 2013; Magalhaes et al. 2012; Morris and Malbon 1999). Apart from their role in modulating the extent of signaling (desensitization and resensitization), regulatory features of GPCR signaling also contribute to spatiotemporal control over downstream effects emanating from these receptors (Eichel and von Zastrow 2018; Ferguson 2001; Weinberg and Puthenveedu 2019).

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As a direct consequence of their seven transmembrane domain architecture, GPCRs are predisposed to considerable interaction with their membrane lipid microenvironment (Chattopadhyay 2014; Oates and Watts 2011; Sengupta et al. 2018). Several examples of these interactions have been captured in GPCR structures displaying lipids bound to the receptors (Jafurulla et al. 2019; Sarkar and Chattopadhyay 2021a, b; Sejdiu and Tieleman 2020). Importantly, such interactions have been found to be correlated with lipid-sensitive functional readouts in case of several GPCRs (Jafurulla et al. 2019; Sarkar and Chattopadhyay 2021b). Although the molecular underpinnings of signal transduction by GPCRs have been a subject of intense exploration, our understanding of the mechanistic details underlying lipid-mediated modulation of GPCR function is relatively nascent (Kumar et al. 2021). In this review, we highlight the role of the membrane microenvironment in GPCR endocytosis, an important regulatory feature of receptor function, with an emphasis on membrane cholesterol as a modulator of GPCR endocytosis. Using our work on the serotonin$_{1A}$ receptor as an example, we allude to the relevance of the interplay between cholesterol and GPCR endocytosis and its implications in pathophysiology and development of fine-tuned therapeutic interventions.

Endocytosis of GPCRs: desensitization and beyond

Endocytosis is a major regulatory mechanism employed by GPCRs to sustain their downstream signaling within physiological levels under a stringent spatiotemporal regime (Ferguson 2001; Hanyaloglu and von Zastrow 2008; Kunselman et al. 2021b). Endocytosis offers an effective means to decouple a GPCR from its pool of extracellular ligands by sequestering the receptor into intracellular locations. The first observation on internalization of GPCRs dates back to the late 1970s when ligand-induced desensitization of the β-adrenergic receptor was correlated to internalization of a fraction of the plasma membrane–associated receptor pool in frog erythrocytes (Chuang and Costa 1979). Subsequent work led to the identification of regulatory proteins such as G protein-coupled receptor kinases (GRKs) and arrestins that predispose GPCRs to endocytosis as a means of desensitization (Benovic et al. 1987; Ferguson et al. 1996; Lohse et al. 1990). It was further discovered that β-arrestin could act as an adaptor for the assembly of clathrin coats for endocytosis of the β_2-adrenergic receptor (Goodman et al. 1996). The advent of molecular biology and the ability to express fluorescently tagged receptors led to the discovery that phosphorylation of key residues in the intracellular loops of the receptor acts as 'barcodes' (identifiers) for the recruitment of endocytic machinery for GPCR internalization and trafficking (Bahouth and Nooh 2017; Liggett 2011; Yang et al. 2017).

Although GPCR endocytosis was initially identified as a mode of ligand-induced desensitization of receptor-mediated signaling, several other regulatory features associated with their internalization and trafficking have emerged more recently (see Fig. 1). One of the earliest evidence of the role of GPCR endocytosis beyond desensitization implicated β-arrestin–mediated internalization of the β_2-adrenergic receptor as a prerequisite for the activation of MAP kinase signaling (Daaka et al. 1998; Luttrell et al. 1999). A fascinating feature of GPCR endocytosis that has emerged through research over the recent past is the ability of these receptors to signal from intracellular compartments (Crilly and Puthenveedu 2021; Irannejad and von Zastrow, 2014; Jong et al. 2018; Sposini and Hanyaloglu 2017). For example, signaling responses from internalized GPCRs have been reported for the thyroid-stimulating hormone receptor (Calebiro et al. 2009), sphingosine-1-phosphate receptor (Mullershausen et al. 2009), parathyroid hormone receptor (Ferrandon et al. 2009), vasopressin receptor type 2 (Feinstein et al. 2013), and leutinizing hormone receptor (Lyga et al. 2016). More importantly, direct evidences from

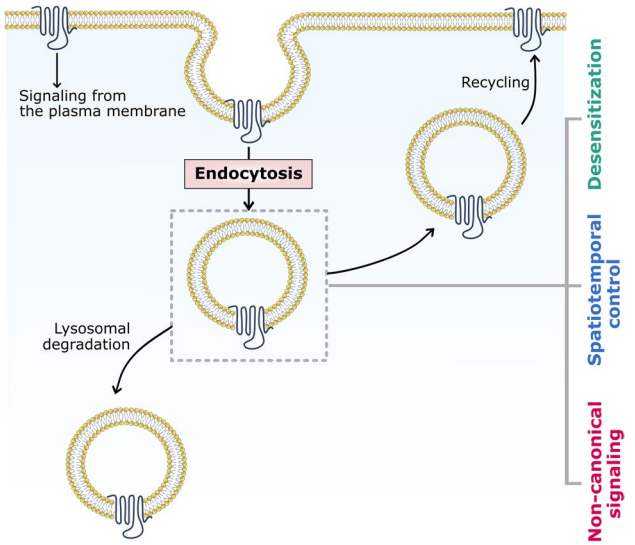

Fig. 1 Regulation of GPCR signaling by endocytosis. A schematic representation depicting various regulatory features associated with GPCR endocytosis. Endocytosis allows regulation of GPCR signaling by spatially decoupling the binding of an extracellular ligand to the receptor (desensitization). In general, upon endocytosis, GPCRs could either recycle back to the plasma membrane or undergo lysosomal degradation within the cell. Such movements are regulated by intricately coordinated intracellular trafficking pathways within cells. Several other regulatory features associated with GPCR internalization and intracellular trafficking have emerged recently. Endocytosis and intracellular trafficking of GPCRs are known to confer spatiotemporal control to receptor-mediated signaling, which is manifested as distinct signals emerging from the plasma membrane and endosomes in case of several receptors

conformation-specific nanobodies that recognize specific activated receptor states have revealed signaling-competent conformations for internalized receptors with distinct cellular signaling consequences (Irannejad et al. 2013, 2017; Kunselman et al. 2021a; Stoeber et al. 2018; Tsvetanova and von Zastrow 2014).

Endocytosis of membrane receptors is a highly complex and stringently regulated phenomenon that involves a wide variety of membrane-associated and cytoplasmic protein machinery that orchestrate the process in a concerted fashion. In this context, several mechanisms facilitating the internalization of membrane receptors have been studied (Doherty and McMahon 2009; Kunselman et al. 2021b). Although a majority of GPCRs have been shown to utilize clathrin-mediated endocytic machinery for internalization (Hanyaloglu and von Zastrow 2008; Wolfe and Trejo 2007), caveolar localization (Bhatnagar et al. 2004; Ostrom and Insel 2004) and caveolin-mediated internalization (Cho et al. 2012; Janoshazi et al. 2007) of GPCRs have also been reported. In addition, multiple possibilities exist in terms of intracellular trafficking routes available for GPCRs upon internalization. Broadly, receptors could either recycle back to the plasma membrane, or they could be sorted to the lysosomal system for degradation (Fig. 1; Hanyaloglu and von Zastrow 2008; Kunselman et al. 2021b; Marchese et al. 2008).

Although much has been explored in terms of the functional consequences of GPCR endocytosis, our mechanistic understanding of this process primarily comprises insights into the protein machinery that orchestrate the GPCR endocytic framework, and details about the role of membrane lipids in this process are rather limited. This is in spite of the fact that GPCRs are polytopic membrane proteins and the polypeptide chain of the receptor crosses the membrane several times. The following sections highlight the importance of cholesterol in various facets of GPCR function. Using examples from the limited body of literature and recent work from our group, we make a case for exploring the role of membrane lipids (particularly cholesterol) in the endocytosis and trafficking of GPCRs.

Cholesterol and GPCRs: an intimate association

Cholesterol is an essential lipid in higher eukaryotic cell membranes that assumes a unique functional role in cellular physiology. It is the single most abundant lipid in the eukaryotic plasma membrane accounting for ~ 30–50% of the total lipid content (Maxfield and van Meer 2010; Mouritsen 2005). Cholesterol assumes a critical role in regulating membrane organization and dynamics, and associated cellular functions such as signaling, sorting, trafficking, and pathogen entry (Kumar and Chattopadhyay 2016; Kumar et al. 2016; Lippincott-Schwartz and Phair 2010; Mouritsen and Zuckermann, 2004; Simons and Ikonen 2000). The unique physicochemical properties of cholesterol that have been fine-tuned over long time scales of evolution are manifested in terms of its nonrandom distribution in the plasma membrane and the ability to interact with membrane lipids and proteins. From a structural standpoint, cholesterol comprises a polar 3β-hydroxyl group, a tetracyclic-fused steroid ring, and a flexible isooctyl chain (see Fig. 2). Each of these features has been suggested to confer distinct interaction profiles to the molecular structure of cholesterol (Chaudhuri and Chattopadhyay 2011; Fantini and Barrantes 2013; Fantini et al. 2019; Paila and Chattopadhyay 2010; Sarkar and Chattopadhyay 2020). The 3β-hydroxyl group allows cholesterol to orient and anchor in the membrane (Villalaín 1996), and facilitates electrostatic interaction with polar

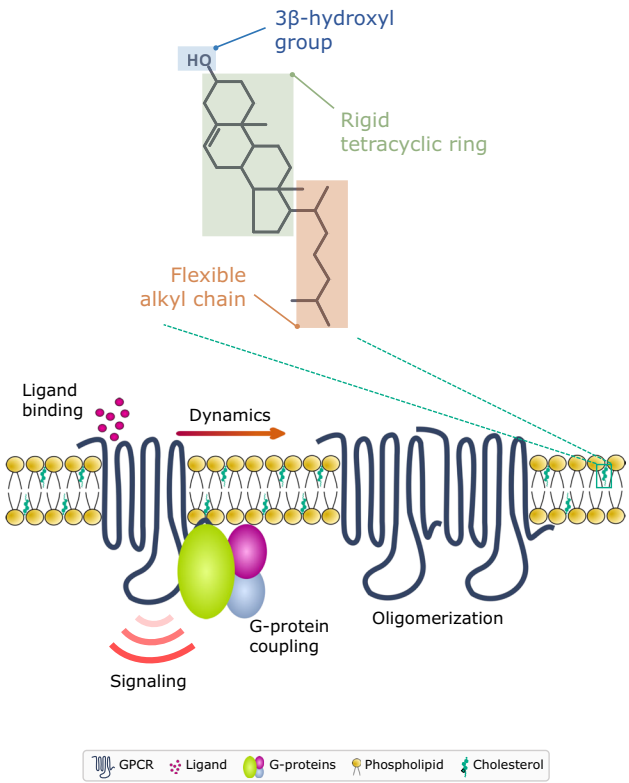

Fig. 2 Multifaceted regulation of GPCR function by cholesterol. The interplay between GPCRs and cholesterol in the membrane milieu has emerged as a major thrust area with far-reaching implications in the pathophysiology mediated by GPCRs. The unique physicochemical properties of cholesterol (encoded in its chemical structure comprising of a polar 3β-OH group, a rigid tetracyclic ring and a flexible isooctyl chain) coupled with its relative abundance in the plasma membrane contribute to the crucial modulatory effects of cholesterol on GPCRs. Extensive work using biochemical, biophysical, and computational approaches has shown that cholesterol affects various features of GPCRs such as ligand binding, G-protein coupling, downstream signaling, dynamics, and oligomerization

residues such as lysine and arginine of membrane proteins. The methyl groups in the α-face of cholesterol have been proposed to exhibit CH-π stacking interactions with aromatic amino acids such as tyrosine and phenylalanine, and the isooctyl chain is believed to intercalate with the side chains of branched amino acids such as valine, leucine, and isoleucine via van der Waals interactions. In addition, cholesterol is a key modulator of membrane physical properties such as fluidity, viscosity, curvature, thickness, and dipole potential, which constitute important features of the membrane microenvironment that influence membrane function (Arora et al. 2004; Bacia et al. 2005; Chen and Rand 1997; Haldar et al. 2012; Nezil and Bloom 1992; Pal et al. 2016; Simon et al. 1982).

The interplay between membrane cholesterol and GPCRs has emerged as a major theme in GPCR biology. The interaction of cholesterol with GPCRs is multifaceted, and has been shown to be implicated in ligand binding, G-protein coupling, signaling, lateral dynamics, and oligomerization of receptors (see Fig. 2; Chattopadhyay 2014; Gimpl 2016; Jafurulla and Chattopadhyay 2013; Jafurulla et al. 2019; Oates and Watts 2011; Pucadyil and Chattopadhyay 2006; Paila and Chattopadhyay 2010; Sengupta and Chattopadhyay 2015; Sengupta et al. 2017, 2018). A careful dissection of the literature on GPCR-cholesterol interaction suggests that the mechanism underlying the cholesterol sensitivity observed by GPCRs is most likely a combination of the direct interaction of cholesterol with GPCRs and its modulatory effects on the membrane microenvironment that houses these receptors (Jafurulla et al. 2019). GPCR structures with cholesterol bound to receptors could suggest direct interaction of cholesterol with GPCRs, although there are some caveats (see Sarkar and Chattopadhyay 2021a, b). In fact, analysis of several GPCR sequences and structures has revealed specific sites for these interactions which have been proposed as cholesterol interaction motifs such as the cholesterol recognition/interaction consensus (CRAC), inverse-CRAC (CARC) and cholesterol consensus motifs (CCM) (recently reviewed in Sarkar and Chattopadhyay 2020). Very recent work from our group has shown that a lysine residue in a CRAC motif in transmembrane helix 2 of the serotonin$_{1A}$ receptor acts as a molecular sensor for changes in membrane cholesterol levels and imparts cholesterol sensitivity to cAMP signaling by the receptor (Kumar et al. 2021). Although the interaction of cholesterol with GPCRs has been shown to be weak and transient (~ μs time scale), such interactions appear to act as functional switches between receptor conformations (Sengupta and Chattopadhyay 2015). On the other hand, the effects of cholesterol on membrane physicochemical properties (such as membrane fluidity, viscosity, hydrophobic mismatch and membrane dipole potential) could modulate receptor conformation(s) facilitating specific functional states.

Cholesterol in endocytosis: why do we care and what do we know?

The motivation behind exploring the role of membrane cholesterol in endocytosis stems from the ability of cholesterol to modulate membrane physical properties and participate in functional interactions with membrane protein cargo as well as the cellular endocytic machinery. The process of endocytosis involves several remodeling events at the plasma membrane that include generation of membrane curvature, recognition of cargo and translocation of cargo into endocytic structures on the membrane. In this context, cholesterol has been shown to impart negative curvature in membranes (Chen and Rand 1997) and accumulate in highly curved regions of bilayers (Wang et al. 2007). The scaffolding protein caveolin-1, involved in caveolin-mediated endocytosis, segregates into cholesterol-rich domains in the membrane (Örtegren et al. 2004; Smart and Anderson 2002). Notably, caveolin-1 contains a stretch of amino acids constituting the CRAC motif (Epand et al. 2005), and a peptide fragment from caveolin-1 containing this motif has been shown to drive the formation of cholesterol-rich domains (Epand et al 2003). In addition, it was recently demonstrated that caveolin-1 can induce higher membrane curvature in cholesterol-rich membranes (Krishna and Sengupta 2019).

One of the earliest studies probing the role of membrane cholesterol on "cellular uptake" reported that metabolic inhibition of cholesterol biosynthesis in mouse fibroblasts using oxygenated derivatives of cholesterol such as 25-hydroxycholesterol and 7-ketocholesterol resulted in reduced internalization of the soluble enzyme horseradish peroxidase (Heinger et al. 1976). Initial insights into the role of cholesterol in the endocytosis of a membrane protein came from studies on transferrin, which demonstrated that acute cholesterol depletion using methyl-β-cyclodextrin (MβCD) significantly reduced the rate of transferrin receptor internalization without affecting recycling. Ultrastructural studies using electron microscopy showed that this was accompanied by accumulation of flat clathrin-coated membranes and decrease in deep-coated pits (Subtil et al. 1999). In another report published around the same time, MβCD was shown to strongly inhibit the endocytosis of transferrin and epidermal growth factor, but not of the general membrane marker, ricin. This effect could be reversed upon replenishment of cholesterol (Rodal et al. 1999). Contrary to this, depletion of cholesterol using MβCD was reported to enhance the rate of internalization of nicotinic acetylcholine receptors (Borroni et al. 2007) via a pathway involving the small GTPase Arf6 (Borroni and Barrantes 2011). Metabolic depletion of cholesterol using mevalonin, an inhibitor of cholesterol

biosynthesis, resulted in reduction in nicotinic acetylcholine receptors in the plasma membrane which accumulated in the intracellular trans-Golgi network (Pediconi et al. 2004).

Cholesterol in GPCR endocytosis: an evolving story

Depletion of membrane cholesterol has been shown to inhibit agonist-induced internalization of GPCRs such as the lysophosphatidic acid receptor 1 (LPA_1; Urs et al. 2005), the δ-opioid receptor (Brejchova et al. 2016), and the formyl peptide receptor 1 (Wang et al. 2019). However, cholesterol depletion did not affect the agonist-induced endocytosis of the M_1 muscarinic acetylcholine receptor (Urs et al. 2005) and the cholecystokinin receptor (Harikumar et al. 2005). In case of LPA_1, cholesterol was shown to be essential for the interaction of the receptor with β-arrestin leading to its internalization via clathrin-mediated endocytosis. On the other hand, the β_2-adrenergic receptor did not exhibit cholesterol dependence in its interaction with β-arrestin (Urs et al. 2005). Constitutive endocytosis and recycling are characteristic features of the melanocortin-4 receptor. Cholesterol depletion from immortalized neuronal cells exogenously expressing these receptors inhibited the constitutive endocytosis of the receptor, resulting in a loss of agonist-induced cAMP generation over time (McDaniel et al. 2012). Such differences in cholesterol-dependent receptor-effector interactions could arise due to partitioning of GPCRs and their endocytic effectors (β-arrestins) into distinct membrane microdomains, and the dynamics of these molecules under various states of receptor activation. For example, although both β_1- and the β_2-adrenergic receptors associate with caveolae under basal conditions, β_2- (but not β_1)-adrenergic receptors were shown to dissociate from these regions upon agonist stimulation (Rybin et al. 2000). The molecular mechanism underlying the interaction of specific GPCRs with the associated endocytic machinery in membrane microdomains with varying cholesterol content is still an important unanswered question.

Extensive work from our group has demonstrated the prominent role of membrane cholesterol in the organization, function, and dynamics of the $serotonin_{1A}$ receptor (Chakraborty et al. 2018; Ganguly and Chattopadhyay 2010; Ganguly et al. 2011; Jafurulla et al. 2014; Paila et al. 2011; Prasanna et al. 2016; Pucadyil and Chattopadhyay 2004, 2007; Sarkar et al. 2020; Shrivastava et al. 2010; Saxena and Chattopadhyay 2012). We recently explored the role of cholesterol in the endocytosis and intracellular trafficking of the $serotonin_{1A}$ receptor using acute and chronic methods of cholesterol depletion (Fig. 3). We observed that under normal conditions, the $serotonin_{1A}$ receptor undergoes agonist-induced clathrin-mediated endocytosis and traffics along the endosomal recycling pathway back to the plasma membrane (Kumar et al. 2019). Acute cholesterol depletion using MβCD resulted in a concentration-dependent inhibition of receptor endocytosis, which could be restored by replenishment of cholesterol (Kumar and Chattopadhyay 2021). Notably, under mild acute cholesterol depletion conditions achieved using a lower concentration of MβCD, the $serotonin_{1A}$ receptor continued to internalize via clathrin-mediated endocytosis, but exhibited a switch in its intracellular trafficking itinerary from recycling to lysosomal degradation (Kumar and Chattopadhyay 2021). Interestingly, when a similar extent of cholesterol was depleted using a chronic approach by treating cells with statin (an inhibitor of the rate limiting step in cellular cholesterol biosynthesis (Istvan and Deisenhofer 2001; Nes 2011)), the $serotonin_{1A}$ receptor exhibited a switch in the mechanism of internalization from clathrin- to caveolin-mediated endocytosis, along with re-routing of intracellular traffic to lysosomes instead of the plasma membrane (Kumar and Chattopadhyay 2020). A similar effect was previously reported for the endothelin receptor type A where oxidation of cholesterol using cholesterol oxidase resulted in a switch in the mechanism of receptor internalization from caveolin- to clathrin-mediated endocytosis (Okamoto et al. 2000). The differential effects of acute and chronic methods of cholesterol depletion on $serotonin_{1A}$ receptor endocytosis point toward the relevance of the actual method used to deplete cholesterol, and not just the extent of cholesterol depletion. The fundamental differences in these methods could arise due to their varied effects on intracellular distribution of cholesterol (and other lipids), physical properties of membranes along intracellular trafficking pathways, function of the protein machinery involved in cargo sorting and trafficking, and other pleiotropic effects of such treatments, all of which constitute essential factors contributing to endocytosis and trafficking in cells (Breusegem et al. 2005; Goodwin et al. 2005; Hilgemann et al. 2020; Liao and Laufs 2005; Sahu et al. 2019; Sarkar et al. 2017; Shvartsman et al. 2006).

Cholesterol in GPCR endocytosis: implications in pathophysiology and therapeutics

Impaired trafficking of GPCRs has been shown to be associated with pathophysiological conditions such as nephrogenic diabetes insipidus (Bernier et al. 2004), retinitis pigmentosa (Hollingsworth and Gross 2012), and cancer (Dorsam and Gutkind 2007). Furthermore, dysfunctional GPCR trafficking could be mapped to defects associated with intracellular trafficking machinery and sorting proteins in disease conditions (Chandra et al. 2021; Wang et al. 2013). Where

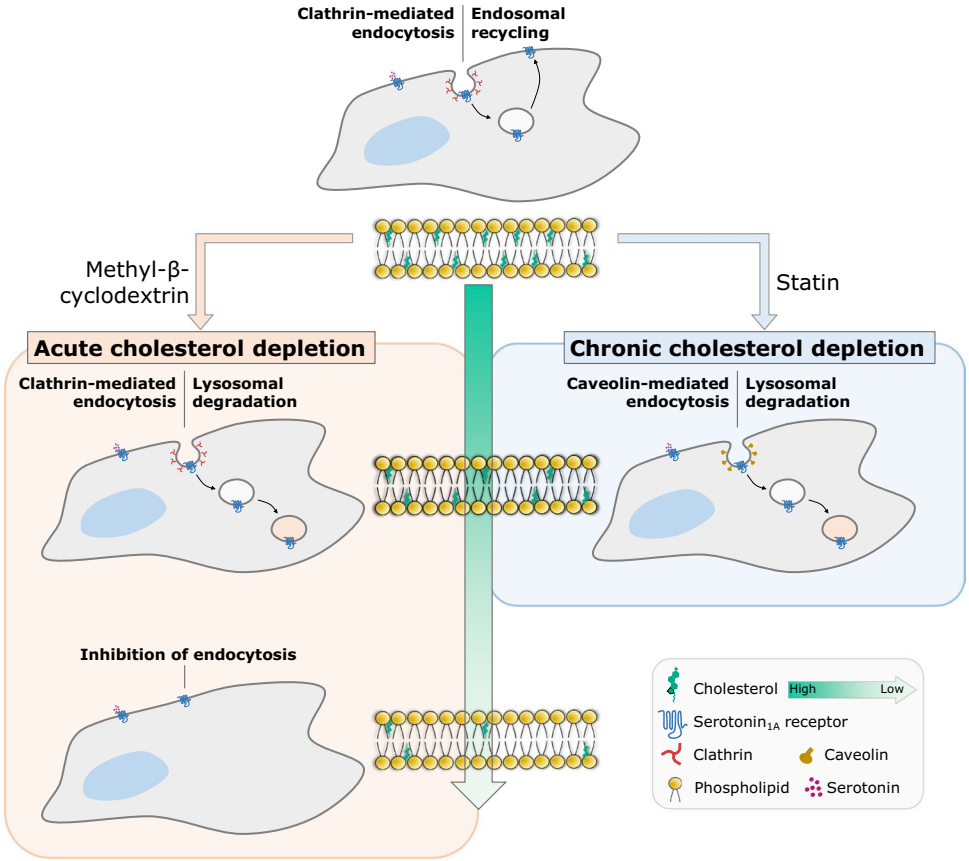

Fig. 3 Cholesterol-induced switch in the endocytosis and intracellular trafficking of the neurotransmitter serotonin$_{1A}$ receptor. A schematic representing the effects of acute and chronic cholesterol depletion on the endocytosis and intracellular trafficking of the serotonin$_{1A}$ receptor. The serotonin$_{1A}$ receptor undergoes agonist (serotonin)-induced internalization via clathrin-mediated endocytosis, and subsequently recycles back to the plasma membrane as its preferred mode of intracellular trafficking. The role of cholesterol in this process was probed using acute (treatment with the soluble sterol-carrier methyl-β-cyclodextrin, MβCD) and chronic (inhibition of cellular cholesterol biosynthesis using statin) approaches of cholesterol depletion. Mild (acute) cholesterol depletion using a lower concentration of MβCD resulted in re-routing of receptors internalized via clathrin-mediated endocytosis toward lysosomal degradation. In addition to an altered intracellular trafficking itinerary, similar extent of cholesterol depletion using statin induced an additional switch in the mechanism of internalization from clathrin- to caveolin-mediated endocytosis. Depletion of higher amounts of cholesterol using MβCD led to complete inhibition in the endocytosis of the serotonin$_{1A}$ receptor. These observations highlight the nuanced role of cholesterol in endocytosis and intracellular trafficking of the serotonin$_{1A}$ receptor and the importance of taking into consideration the approach used to explore such phenomena in interpreting the results. The nuclei are shown in blue

does cholesterol feature in this scenario? As discussed in the previous section, changes in membrane cholesterol levels appear to modulate endocytic and intracellular trafficking pathways for some GPCRs. Notably, membrane cholesterol levels and its biosynthetic pathway exhibit differences across cell and tissue types, and vary with age and development (Dietschy and Turley 2004; Karnell et al. 2005; Mitsche et al. 2015). At subcellular scales, cholesterol content varies across membranes of intracellular organelles (Ikonen 2008), and this distribution exhibits dynamic regulation (Mesmin and Maxfield 2009). In addition, defective cholesterol biosynthesis and metabolism are hallmarks of several pathophysiological conditions (Meng et al. 2020; Platt et al. 2014). These aspects raise the interesting possibility of cholesterol-dependent regulation in trafficking of receptors with consequences in their role in cellular physiology. As an example, recent work from our group has shown that the serotonin$_{1A}$ receptor exhibits late endosomal/lysosomal accumulation accompanied by reduction in the plasma membrane receptor pool in a cellular model of Smith-Lemli-Opitz syndrome, a congenital developmental defect characterized by defective cholesterol biosynthesis (Sharma et al. 2021).

The role of spatiotemporal regulation in GPCR signaling is increasingly recognized in the development of novel drugs targeting these receptors (Nezhady et al. 2020; Retamal et al. 2019; Thomsen et al. 2018). A comprehensive understanding of lipid-mediated effects on GPCR trafficking is therefore essential to tune drug responses based on the membrane environment at the site of drug action

(Payandeh and Volgraf 2021; Wang et al. 2021). The modulatory effects of cholesterol on the endocytosis of the serotonin$_{1A}$ receptor (discussed above) are a relevant case in point. Endocytosis of the serotonin$_{1A}$ receptor has been implicated in the action of a popular class of anti-depressant drugs called selective serotonin reuptake inhibitors (SSRIs). Cohort studies have suggested that SSRIs exhibit enhanced anti-depressant activity when administered to patients as a combination with cholesterol-lowering statin therapy relative to those on SSRI treatment alone (Ghanizadeh and Hedayati 2013; Köhler et al. 2016). These observations provide a potential mechanistic basis for our data showing altered endocytic and intracellular trafficking profiles of the serotonin$_{1A}$ receptor upon statin treatment (Kumar and Chattopadhyay 2020). In another example, the endocytosis of a pH-sensitive peptide-drug conjugate via formyl peptide receptor 1 was insensitive to treatment with pharmacological inhibitors of clathrin- or caveolin-mediated endocytosis, yet exhibited cholesterol dependence pointing toward a crucial role for cholesterol in the drug delivery via cholesterol-sensitive internalization of the receptor (Wang et al. 2019).

Concluding thoughts and future excitements

Our evolving understanding of intracellular landscapes of GPCR signaling has opened up exciting avenues on the role of the membrane lipid milieu in enforcing spatiotemporal control over signaling through modulatory effects on intracellular trafficking and signaling. Differential enrichment of lipids such as cholesterol in various membrane-bound intracellular organelles and endosomal compartments could offer unique microenvironments with distinct effector partners for differential signaling outcomes. We envision that a systematic understanding of lipid dynamics in the spatiotemporal regulation of GPCR signaling would enable the development of therapeutic interventions fine-tuned to precisely target receptors in specific microenvironments.

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Declarations

Conflict of interest The authors declare no competing interests.

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