CHAPTER SIX

Solubilization of G Protein-Coupled Receptors: A Convenient Strategy to Explore Lipid–Receptor Interaction

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Contents

Abstract

G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across cell membranes and are major drug targets. Since GPCRs are integral membrane proteins, their structure and function are modulated by membrane lipids. In particular, membrane cholesterol is an important lipid in the context of GPCR function. Solubilization of integral membrane proteins is a process in which the proteins and lipids in native membranes are dissociated in the presence of a suitable amphiphilic detergent. Interestingly, solubilization offers a convenient approach to monitor lipid–receptor interaction as it results in differential extents of lipid solubilization, thereby allowing to assess the role of specific lipids on receptor function. In this review, we highlight how this solubilization strategy is utilized to decipher novel information about the structural stringency of cholesterol necessary for supporting the function of the serotonin$_{1A}$ receptor. We envision that insight in GPCR–lipid interaction would result in better understanding of GPCR function in health and disease.

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1. G PROTEIN-COUPLED RECEPTORS

The G protein-coupled receptors (GPCRs) represent the largest and most diverse group of proteins in mammals, involved in information transfer (signal transduction) from outside the cell to the cellular interior (Chattopadhyay, 2014; Perez, 2003; Pierce, Premont, & Lefkowitz, 2002; Rosenbaum, Rasmussen, & Kobilka, 2009). GPCRs are seven transmembrane domain proteins and include more than 800 members which are encoded by approximately 5% of human genes (Zhang, DeVries, & Skolnick, 2006). They transmit extracellular signals to the cellular interior by concerted changes in the transmembrane domain structure (Deupi & Kobilka, 2010; Nygaard et al., 2013). GPCRs respond to a variety of physiological stimuli that include endogenous ligands (such as biogenic amines) and exogenous ligands (e.g., odorants, pheromones, and photons) for sensory perception. As a consequence, GPCRs regulate a large number of physiological processes such as neurotransmission, secretion, cellular differentiation, growth, entry of pathogens into host cells, and inflammatory and immune responses. For this reason, GPCRs represent major drug targets in all clinical areas (Ellis & The Nature Reviews Drug Discovery GPCR Questionnaire Participants, 2004; Heilker, Wolff, Tautermann, & Bieler, 2009; Insel, Tang, Hahntow, & Michel, 2007; Jacoby, Bouhelal, Gerspacher, & Seuwen, 2006). It is estimated that approximately 50% of clinically prescribed drugs and 25 of the 100 top-selling drugs target GPCRs (Schlyer & Horuk, 2006; Thomsen, Frazer, & Unett, 2005).

2. MEMBRANE LIPIDS IN GPCR ORGANIZATION AND FUNCTION

GPCRs are integral membrane proteins with seven passes across the membrane, and as a result, a considerable portion of GPCRs remains in contact with the membrane lipid environment. Membrane lipids therefore act as important modulators of GPCR structure and function. Cells possess the ability to vary their membrane lipid composition in response to a variety of stress and stimuli, thereby changing the environment and the activity of the membrane receptors. Such interplay between the function of a given GPCR and its immediate lipid environment in the membrane is physiologically relevant. Results from our laboratory and others have shown that the interaction of GPCRs with membrane lipids is crucial for their structure and

function (Burger, Gimpl, & Fahrenholz, 2000; Jafurulla & Chattopadhyay, 2013a; Oates & Watts, 2011; Paila & Chattopadhyay, 2010; Pucadyil & Chattopadhyay, 2006; Soubias & Gawrisch, 2012). It has recently been reported that even the interaction between GPCRs and G-proteins could be modulated by membrane lipids (Inagaki et al., 2012). Interestingly, the membrane lipid environment of GPCRs has been implicated in disease progression during aging (Alemany et al., 2007). The most studied lipid in the context of GPCR–lipid interaction is cholesterol.

3. CHOLESTEROL: AN IMPORTANT MODULATOR OF GPCR FUNCTION

Cholesterol is a crucial membrane lipid in higher eukaryotes. It plays an important role in membrane organization, dynamics, function, and sorting (Mouritsen & Zuckermann, 2004; Simons & Ikonen, 2000). Typically, membrane cholesterol is distributed in a nonrandom fashion in domains in biological and model membranes (Chaudhuri & Chattopadhyay, 2011; Lingwood & Simons, 2010; Mukherjee & Maxfield, 2004; Xu & London, 2000). These membrane domains are believed to play a key role in membrane sorting and trafficking (Simons & van Meer, 1988), signal transduction (Simons & Toomre, 2000), and the entry of pathogens into host cells (Chattopadhyay & Jafurulla, 2012; Pucadyil & Chattopadhyay, 2007; Roy, Kumar, Jafurulla, Mandal, & Chattopadhyay, 2014).

The role of membrane cholesterol in the organization and function of membrane proteins in general, and GPCRs in particular is an exciting and contemporary area of research (Burger et al., 2000; Jafurulla & Chattopadhyay, 2013a; Oates & Watts, 2011; Paila & Chattopadhyay, 2010; Pucadyil & Chattopadhyay, 2006; Soubias & Gawrisch, 2012). The mechanism underlying the effect of membrane cholesterol on the structure and function of membrane receptors is not straightforward and still emerging (Lee, 2011; Paila & Chattopadhyay, 2009, 2010). Membrane cholesterol could modulate the function of membrane proteins by direct (specific) interaction, which could induce local conformational change(s) in the receptor. Another mechanism proposes an indirect effect by altering the physical properties of the membrane in which the protein is embedded. A third possibility could be a combination of both types of effects.

As stated above, membrane cholesterol has been reported to influence the function of a number of GPCRs. A representative GPCR in the context

of cholesterol sensitivity of receptor organization, dynamics, and function is the serotonin$_{1A}$ receptor (Jafurulla & Chattopadhyay, 2013a; Paila & Chattopadhyay, 2010; Pucadyil & Chattopadhyay, 2006). The serotonin$_{1A}$ receptor is an important neurotransmitter receptor, which acts as a drug target for neuropsychiatric disorders (Celada, Bortolozzi, & Artigas, 2013; Kalipatnapu & Chattopadhyay, 2007; Müller, Carey, Huston, & De Souza Silva, 2007; Pucadyil, Kalipatnapu, & Chattopadhyay, 2005; Savitz, Lucki, & Drevets, 2009). The receptor is implicated in the generation and modulation of various cognitive, behavioral, and developmental functions. Previous work from our laboratory has shown that the organization, dynamics, and function of the serotonin$_{1A}$ receptor are critically dependent on membrane cholesterol (reviewed in Jafurulla & Chattopadhyay, 2013a, 2013b; Paila & Chattopadhyay, 2010; Pucadyil & Chattopadhyay, 2006). Utilizing a number of approaches, we showed that membrane cholesterol plays an important role in the ligand-binding activity and G-protein coupling of the receptor. These approaches include: (i) physical depletion of membrane cholesterol using MβCD; (ii) treatment with agents such as nystatin and digitonin, which complex cholesterol and modulate the availability of membrane cholesterol without physically depleting it; (iii) oxidation of cholesterol to cholestenone (chemical modification) using cholesterol oxidase; and (iv) metabolic inhibition of cholesterol biosynthesis using inhibitors such as statin and AY 9944. Another important approach used by us to monitor the effect of specific lipids on receptor function was solubilization of the receptor using suitable detergents. Solubilization offers a convenient way to explore lipid–receptor interaction as it results in differential extents of lipid solubilization, thereby allowing to assess the role of specific lipids on receptor function (see below).

4. MEMBRANE PROTEIN SOLUBILIZATION: AN ESSENTIAL STEP TOWARD PURIFICATION

Biological membranes represent a complex milieu of a large variety of lipids and proteins, the organization of which allows the membrane to carry out its function. A commonly used approach to study membranes is to dissociate the membrane into its components. An important step in this direction is purification of membrane proteins, an area of considerable experimental challenge (Anson, 2009). Experiments performed using purified and reconstituted membrane receptors have helped significantly in our current understanding of the function of membrane receptors. An essential

criterion for purification of a transmembrane protein is that the protein must be carefully removed from the native membrane environment and dispersed in solution. This is carried out using suitable amphiphilic detergents and the process is known as solubilization (Duquesne & Sturgis, 2010; Helenius & Simons, 1975; Hjelmeland & Chrambach, 1984; Jones, Earnest, & McNamee, 1987; Kalipatnapu & Chattopadhyay, 2005; Kubicek, Block, Maertens, Spriestersbach, & Labahn, 2014; Madden, 1986; Privé, 2007; Seddon, Curnow, & Booth, 2004).

Solubilization of membrane proteins could be defined as a process in which proteins and lipids, held together in native membranes, are suitably dissociated in a buffered solution containing an appropriate detergent. The dissociation of the native membrane leads to the formation of small clusters of protein, lipid, and detergent that remain dissolved in the aqueous solution (see Fig. 1). An important criterion for effective solubilization and purification of membrane proteins is that the function of the protein should be retained to the maximum possible extent. This poses a considerable challenge since many detergents irreversibly denature membrane proteins (Garavito & Ferguson-Miller, 2001), which is responsible for the modest list of membrane proteins solubilized with retention of function. In case of GPCRs, solubilization and purification from natural sources is still rare due to low amounts of the receptor present in the native tissue. Since solubilization constitutes the crucial first step toward purification of any transmembrane receptor, it is important to identify factors responsible for achieving successful solubilization. We outline below some crucial aspects of membrane receptor solubilization.

4.1 Choice of an appropriate detergent

Efficient solubilization of functional GPCRs utilizing a suitable detergent constitutes the first step in their molecular characterization. Detergents are soluble amphiphiles with critical micelle concentrations (CMCs) typically in the range of millimolar. The ability of a detergent to solubilize membranes is related to its hydrophile–lipophile balance (HLB), especially for solubilization by nonionic detergents (Helenius & Simons, 1975; Neugebauer, 1990). This principle has been utilized earlier in order to achieve optimum solubilization of membrane proteins (Slinde & Flatmark, 1976). HLB is an empirical parameter and is a measure of the hydrophilic character of a detergent. It is calculated as the weight percentage of hydrophilic versus lipophilic groups present in a detergent. Detergents

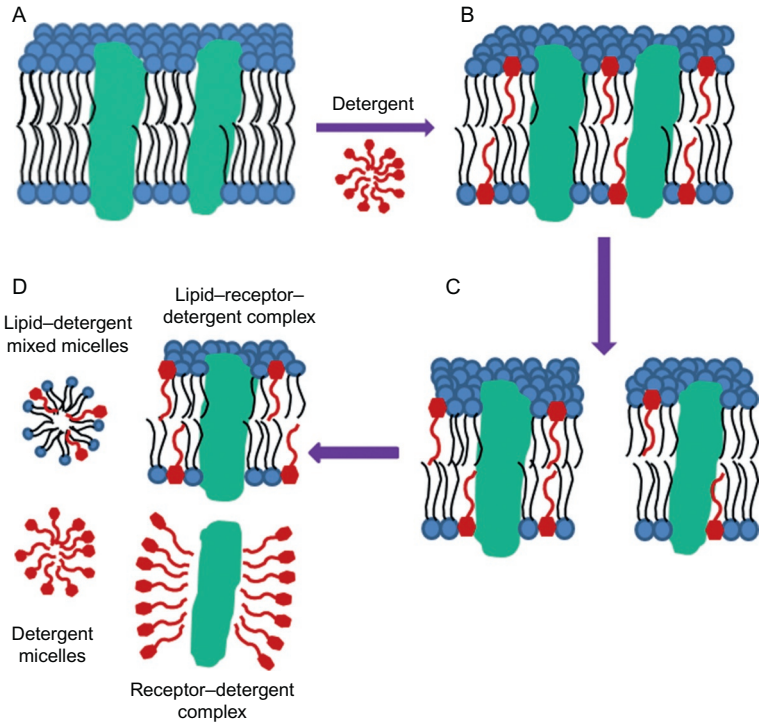

Figure 1 A schematic representation of different stages of solubilization of biological membranes by detergents. When detergents are added to biological membranes (shown in (A)), the detergent monomers (shown in maroon (dark gray in the print version) with single tails) bind to the membrane and cause minimum perturbation at low concentrations (B). With increasing detergent concentration, the membrane bilayer gets further perturbed (C). At even higher detergent concentrations, complexes of detergent, lipid, and receptor of varying compositions are formed. These complexes include lipid–detergent mixed micelles, lipid–receptor–detergent complex, receptor–detergent complex, and detergent micelles (D).

with a relatively high HLB value of 12–20 are recommended for efficient solubilization of membrane proteins without denaturation (Bhairi & Mohan, 2001).

Detergents that belong to the class of nonionic and zwitterionic detergents are particularly popular for their ability to solubilize membrane proteins with retention of function. An important member of this class of detergents is CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; see Fig. 2A), which is a mild, nondenaturing, and zwitterionic detergent (Hjelmeland, 1980). CHAPS is a synthetic detergent that combines useful features of both the bile salt hydrophobic group and the

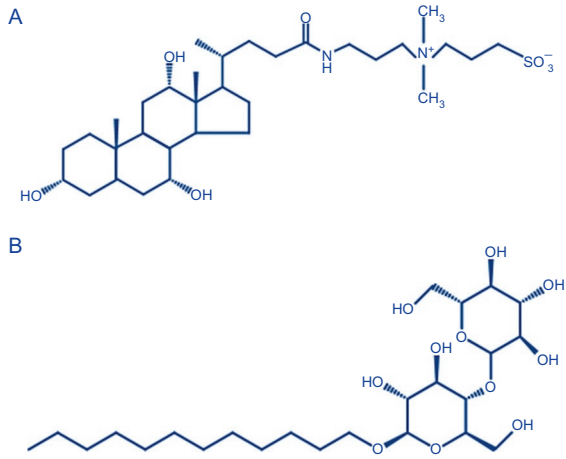

Figure 2 Chemical structures of detergents commonly used for solubilization of GPCRs: (A) CHAPS and (B) DDM. See text for more details.

N-alkyl sulfobetaine-type polar group. It is more efficient in solubilizing membrane proteins than its parent bile acid (such as cholate, which is anionic) due to the absence of net charge. The advantages of using CHAPS for solubilization include its low absorbance at 280 nm and lack of circular dichroic signature in the far-UV region, thereby making it ideal for studies of membrane proteins using optical spectroscopy. For these reasons, CHAPS is widely used in solubilization of membrane proteins and receptors (Banerjee, Joo, Buse, & Dawson, 1995; Chattopadhyay & Harikumar, 1996; Chattopadhyay, Harikumar, & Kalipatnapu, 2002; Cladera, Rigaud, Villaverde, & Duñach, 1997; Kline, Park, & Meyerson, 1989; Locatelli-Hoops, Gorshkova, Gawrisch, & Yeliseev, 2013; Talmont, Moulédous, Mollereau, & Zajac, 2014; Vukoti, Kimura, Macke, Gawrisch, & Yeliseev, 2012; White et al., 2012).

Another detergent that has been used extensively in the last few years to solubilize and crystallize GPCRs is DDM (*n*-dodecyl-β-D-maltoside) (see Fig. 2B). DDM is a mild, nonionic detergent and has been found to be effective in solubilizing several membrane proteins, due to its gentle nature and favorable properties for maintaining the function of aggregation-prone membrane proteins in solution (Privé, 2007). It forms large micelles, which offer the advantage of preventing membrane protein aggregation. However, this could be a limitation in structural studies since DDM masks the protein to a large extent in protein–detergent complexes (Privé, 2007). DDM is known to occlude hydrophilic regions of the protein that are essential to form crystal

contacts, which is not conducive for crystallization of GPCRs (Tate, 2012). This was avoided in later studies by increasing the hydrophilic regions of the GPCRs using antibodies or fusion proteins. DDM has been used to effectively solubilize GPCRs such as β_2-adrenergic receptor (Cherezov et al., 2007; Rasmussen et al., 2011), A_{2A} adenosine receptor (Liu et al., 2012), μ-opioid receptor (Manglik et al., 2012), κ-opioid receptor (Wu et al., 2012), β_1-adrenergic receptor (Huang, Chen, Zhang, & Huang, 2013), serotonin$_{1B}$ receptor (Wang et al., 2013), serotonin$_{2B}$ receptor (Wacker et al., 2013), and metabotropic glutamate type 1 receptor (Wu et al., 2014). Some GPCRs such as neurotensin receptor (White et al., 2012) and CB_2 cannabinoid receptor (Locatelli-Hoops et al., 2013; Vukoti et al., 2012) have been solubilized utilizing a combination of DDM and CHAPS.

It should be noted that the choice of a suitable detergent for optimal solubilization of a given membrane protein has to be worked out on an individual basis (Privé, 2007). For example, efficient solubilization of the IgE receptor has been shown to occur with the anionic detergent cholate but not with the nonionic detergent octyl glucoside (Rivnay & Metzger, 1982). Compatibility of the detergent in biochemical assays is another important factor to be considered.

4.2 CMC of detergents

Detergents are soluble amphiphiles and above a critical concentration (strictly speaking, a narrow concentration range), referred to as the CMC, they self-associate to form thermodynamically stable, noncovalent aggregates called micelles (Tanford, 1978). The concept of micelle formation is important in the context of solubilization and reconstitution of membrane receptors. There is a certain correlation between micelle formation and detergent concentration necessary for solubilization (Rivnay & Metzger, 1982). In case of receptors such as the insulin receptor, opioid receptor, and angiotensin II receptor, efficient solubilization is achieved only with high (>1 m*M*) CMC detergents such as CHAPS and octyl glucoside at concentrations below the CMC (Hjelmeland & Chrambach, 1984). Detergents used at concentrations above their CMC invariably resulted in loss of receptor function. The mechanism by which detergents solubilize membranes at concentrations below the CMC, and the related loss of function above the CMC is not clear. This has given rise to the useful concept of "effective CMC" (Chattopadhyay & Harikumar, 1996; Chattopadhyay et al., 2002; Jones et al., 1987; Rivnay & Metzger, 1982; Schürholz, 1996), which is the concentration of detergent existing as monomers at a given condition

(such as lipids, proteins, ionic strength, pH, and temperature). Solubilization could therefore be carried out below the CMC if the effective CMC is lower than literature CMC. Another key parameter is the critical solubilization concentration (CSC), which is the minimal detergent concentration required to disrupt a given membrane into micellar dispersion (Privé, 2007). Selective solubilization of membrane proteins at detergent concentrations below CSC could be an effective purification strategy.

4.3 Detergent–lipid–protein ratio

Membrane solubilization by detergents is a multistep process (Helenius & Simons, 1975; Hjelmeland & Chrambach, 1984; Jones et al., 1987; le Maire, Champeil, & Møller, 2000; see Fig. 1). The relative detergent–lipid–protein ratio is an important factor for optimal solubilization of membrane proteins. At a given protein or lipid concentration, with increasing detergent concentration, an increase in solubilized lipid (Pucadyil & Chattopadhyay, 2004) or protein (Demoliou-Mason & Barnard, 1984) is observed until saturation is reached. However, it is not advisable to use high detergent concentrations since membrane protein function is often compromised under such conditions. To overcome this, a mild concentration of detergent could be used which may balance these two aspects, that is, maximize solubilization yet preserve protein function. Arriving at an optimal detergent, lipid, and protein ratio involves trial and error by carrying out solubilization over a wide range of detergent–lipid ratios.

An empirical relationship between these experimental parameters was developed in which the parameter (ρ) was defined as the molar ratio of detergent to lipid optimal for functional solubilization (Rivnay & Metzger, 1982).

$$\rho = \frac{[\text{Detergent}] - \text{CMC}_{\text{eff}}}{[\text{Phospholipid}]}$$

where CMC_{eff} represents the effective CMC determined under specific experimental conditions (as mentioned above). An increase in solubilization is expected with increase in the value of the ρ parameter (generally up to $\sim$2).

5. SOLUBILIZATION AS A STRATEGY TO MONITOR LIPID–PROTEIN INTERACTIONS

As mentioned earlier, solubilization provides a convenient approach to explore lipid–receptor interaction since it results in differential extents

of lipid solubilization, thereby allowing to assess the role of specific lipids on receptor function. A common feature often associated with membrane solubilization is delipidation (loss of lipids). This results in loss of protein function since lipid–protein interactions play a crucial role in maintaining the structure and function of integral membrane proteins and receptors (Lee, 2003). For example, displacement of annular lipids from the receptor was shown to be an integral feature of detergent-induced inactivation in case of the nicotinic acetylcholine receptor (Jones, Eubanks, Earnest, & McNamee, 1988). Interestingly, the phenomenon of delipidation caused by solubilization and the subsequent loss of membrane protein function has been effectively utilized to gain molecular insight into the specific lipid requirements of membrane proteins (Jones et al., 1988; Kirilovsky & Schramm, 1983).

This strategy has been successfully utilized for exploring lipid–GPCR interaction. It was previously reported that solubilization of the native hippocampal serotonin$_{1A}$ receptors using CHAPS results in loss of receptor activity and membrane cholesterol (Banerjee, Buse, & Dawson, 1990; Banerjee et al., 1995; Chattopadhyay, Jafurulla, Kalipatnapu, Pucadyil, & Harikumar, 2005). We previously demonstrated that specific ligand binding of the serotonin$_{1A}$ receptor could be restored upon replenishment of cholesterol into solubilized membranes (Chattopadhyay et al., 2005). Utilizing this experimental strategy, we were able to examine the degree of stringency required by closely related analogs of cholesterol, necessary for restoring receptor activity. In order to explore the structural stringency of cholesterol necessary for supporting receptor function, we replaced cholesterol with its close structural analogs with minor differences (see Fig. 3). In one set of experiments, solubilized membranes were replenished with 7-dehydrocholesterol (7-DHC) and desmosterol, which are immediate biosynthetic precursors of cholesterol in the Kandutsch–Russell and Bloch pathways, respectively, both of which differ with cholesterol merely in an additional double bond. While 7-DHC differs with cholesterol only in a double bond at the seventh position in the sterol ring, desmosterol differs with cholesterol only in a double bond at the 24th position in its flexible alkyl side chain (see Fig. 3). Accumulation of either 7-DHC or desmosterol due to defective sterol biosynthesis has been shown to result in fatal neurological disorders (Porter & Herman, 2011). Figure 4A shows that while desmosterol could support receptor function, 7-DHC could not restore receptor activity (Chattopadhyay et al., 2007; Singh et al., 2011). This brings out the fine stringency of cholesterol requirement for receptor function

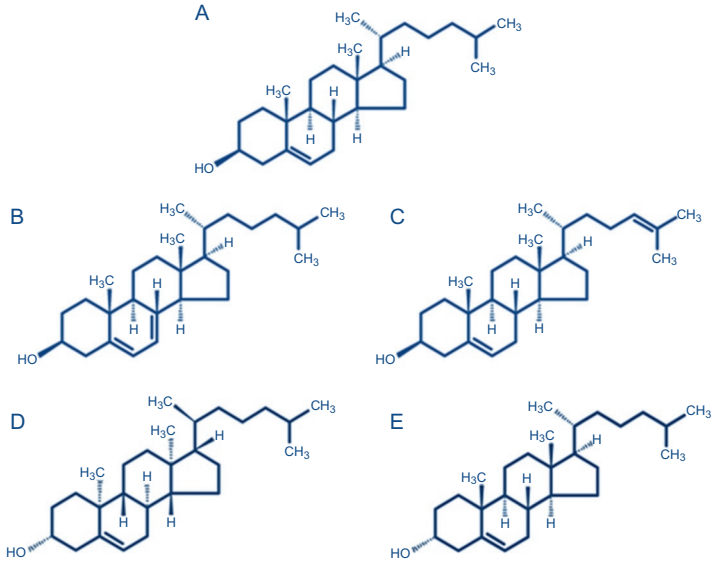

Figure 3 Chemical structures of (A) cholesterol, (B) 7-dehydrocholesterol, (C) desmosterol, (D) *ent*-cholesterol, and (E) *epi*-cholesterol. Both 7-dehydrocholesterol (7-DHC) and desmosterol are immediate biosynthetic precursors of cholesterol in Kandutsch–Russell and Bloch pathways, respectively, differing with cholesterol only in a double bond. While 7-dehydrocholesterol differs with cholesterol only in a double bond at the 7th position in the sterol ring, desmosterol differs with cholesterol only in a double bond at the 24th position of the flexible alkyl side chain. Patients with mutations in enzymes that catalyze the final step in these pathways exhibit low levels of serum cholesterol and accumulation (high levels) of the respective immediate precursor (7-DHC or desmosterol) leading to diseases such as the Smith–Lemli–Opitz syndrome (SLOS) and desmosterolosis. Both *ent*-cholesterol and *epi*-cholesterol are stereoisomers of cholesterol. *ent*-Cholesterol is the enantiomer of cholesterol and is a nonsuperimposable mirror image of cholesterol, whereas *epi*-cholesterol is a diastereomer of cholesterol which differs with cholesterol in the orientation of hydroxyl group at carbon-3 position. *ent*-Cholesterol shares similar physicochemical properties with cholesterol but *epi*-cholesterol does not.

since the presence of an additional double bond in the sterol ring (7-DHC) appears more detrimental to receptor function than the presence of an extra double bond in the alkyl side chain (desmosterol).

The degree of structural stringency was explored further by examining whether stereoisomers of cholesterol (*ent*-cholesterol and *epi*-cholesterol) could support receptor function. While *ent*-cholesterol is the enantiomer of cholesterol and is a nonsuperimposable mirror image of cholesterol, *epi*-cholesterol is a diastereomer (not a mirror image of cholesterol) (see

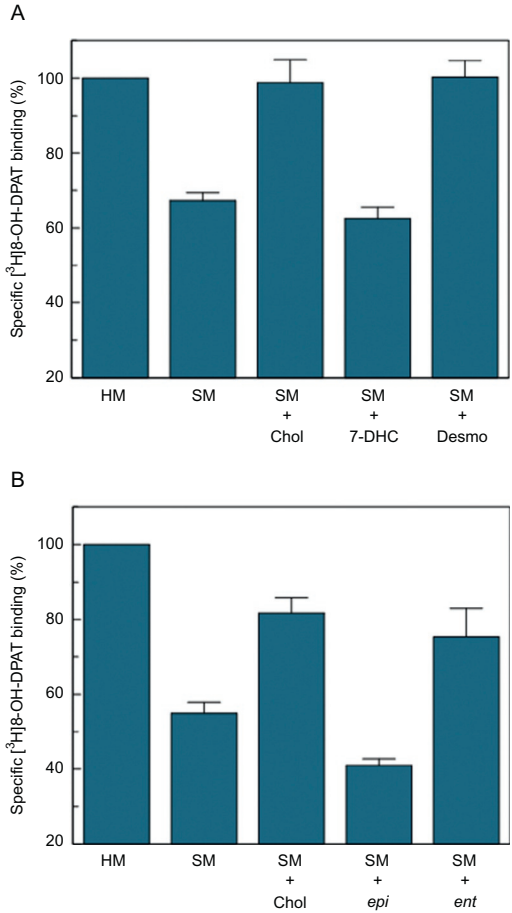

Figure 4 Effect of replenishment of (A) 7-DHC and desmosterol, and (B) *ent*-cholesterol and *epi*-cholesterol into solubilized membranes (SM) on specific binding of the agonist [^{3}H]8-OH-DPAT to the serotonin$_{1A}$ receptor. Data for solubilized membranes replenished with cholesterol are shown in all cases for comparison. Native hippocampal membranes (HM) without any treatment served as control. See text for more details. *Data shown in panel (A) are from Chattopadhyay et al. (2007) and Singh, Jafurulla, Paila, and Chattopadhyay (2011). Panel (B) is adapted and modified from Jafurulla et al. (2014).*

Fig. 3). Figure 4B shows that *ent*-cholesterol could replace cholesterol in supporting the function of the serotonin$_{1A}$ receptor, but *epi*-cholesterol could not (Jafurulla et al., 2014). These results clearly show that the requirement of membrane cholesterol for the serotonin$_{1A}$ receptor function is *diastereospecific*, yet not *enantiospecific*. Taken together, we were able to

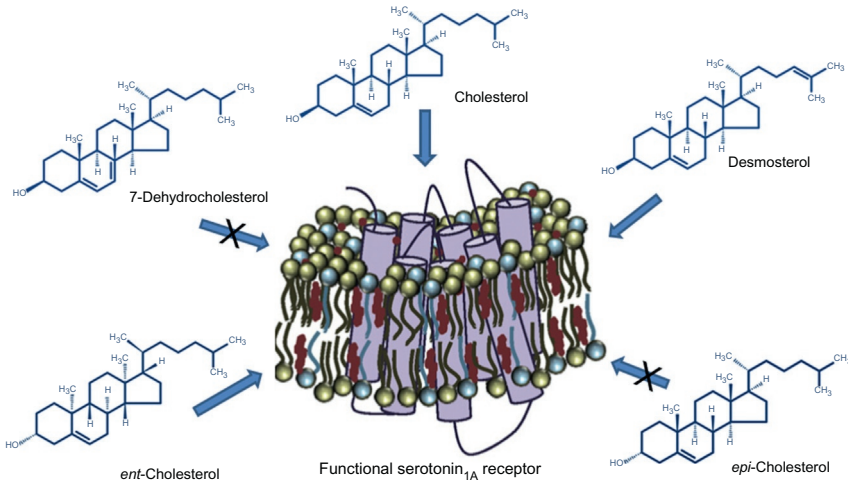

Figure 5 A schematic representation showing the role of various sterols in supporting the function of the reconstituted serotonin$_{1A}$ receptor. The serotonin$_{1A}$ receptor is shown in purple, and the replenished sterol molecules are shown in maroon. The ligand-binding activity of the reconstituted serotonin$_{1A}$ receptor was supported upon replenishment with cholesterol, desmosterol, and *ent*-cholesterol, whereas replenishment with 7-DHC and *epi*-cholesterol could not support the function of the receptor. This brings out the important message that even subtle changes in sterol structure could be detrimental for receptor function, thereby implying stringent receptor–lipid interaction.

decipher the subtle details of structural stringency of cholesterol necessary for serotonin$_{1A}$ receptor function, utilizing solubilization strategy. A comprehensive representation of the role of various cholesterol analogs on serotonin$_{1A}$ receptor activity is shown in Fig. 5.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

GPCRs represent one of the evolutionarily conserved families of membrane receptors dating back more than a billion years (Schöneberg, Hofreiter, Schulz, & Römpler, 2007). GPCRs occupy a unique position in contemporary biology due to their ability to transduce a variety of information across the cell membrane and as drug targets (Chattopadhyay, 2014). Interaction of membrane lipids with GPCRs leading to functional modulation of the receptor is an increasingly emerging area of interest. The recent reports of high-resolution crystal structures of several GPCRs with cholesterol bound to various positions of the receptor (reviewed in

Chattopadhyay, 2014; Jafurulla & Chattopadhyay, 2013a) have provided more impact to this field. Unfortunately, a majority of GPCRs are not available in purified form from native sources. This is a severe limitation in attempts to reconstitute the purified receptor into a defined lipid environment, thereby preventing lipid–receptor studies using established approaches. In this overall scenario, solubilization using suitable detergents which allow selective depletion of membrane lipids offers a window of opportunity to assess the lipid specificity of GPCRs. The knowledge gained from these studies will provide a better understanding of specific lipid dependence of receptor function. Such advances in deciphering molecular details of receptor–lipid interaction would lead to better understanding of GPCR function in health and disease.

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