

Membrane Lipids in the Function of Serotonin and Adrenergic Receptors

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Abstract: G-protein coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major targets in the development of novel drug candidates in all clinical areas. Since GPCRs are integral membrane proteins, interaction of membrane lipids such as cholesterol and sphingolipids with GPCRs constitutes an emerging area of research in contemporary biology. Cholesterol and sphingolipids represent important lipid components of eukaryotic membranes and play a crucial role in a variety of cellular functions. In this review, we highlight the role of these vital lipids in the function of two representative GPCRs, the serotonin_{1A} receptor and the adrenergic receptor. We believe that development in deciphering molecular details of the nature of GPCR-lipid interaction would lead to better insight into our overall understanding of GPCR function in health and disease.

Keywords: Adrenergic receptor, adenosine receptor, CRAC, cholesterol, G-protein coupled receptor, SBD, serotonin_{1A} receptor, sphingolipids.

1. INTRODUCTION

The G-protein coupled receptor (GPCR) superfamily is the largest and most diverse protein family in mammals, involved in signal transduction across membranes [1, 2]. GPCRs are typically seven transmembrane domain proteins and include >800 members which are encoded by ~5% of human genes [3]. Since GPCRs regulate multiple physiological processes, they have emerged as major targets for the development of novel drug candidates in all clinical areas [4]. It is estimated that ~50% of clinically prescribed drugs act as either agonists or antagonists of GPCRs [5].

Since GPCRs are integral membrane proteins with multiple transmembrane domains, the interaction of membrane lipids with GPCRs is an important determinant in their structure and function [6-8]. Interestingly, it has been recently reported that the interaction between GPCRs and G-proteins could be modulated by membrane lipids [9]. Importantly, the membrane lipid environment of GPCRs has been implicated in disease progression during aging [10]. In this review, we have focused on the role of membrane lipids in the structure and function of two representative GPCRs, the serotonin_{1A} receptor and the adrenergic receptor.

2. MEMBRANE CHOLESTEROL IN GPCR FUNCTION

Cholesterol is an essential and representative lipid in higher eukaryotic cellular membranes and is crucial in the organization, dynamics, function, and sorting of membranes [11, 12]. Cholesterol plays a vital role in the function and organization of membrane proteins and receptors. The effect of cholesterol on the structure and function of integral membrane proteins and receptors has been a subject of intense investigation [6-8]. The role of membrane cholesterol in the function of a number of G-protein coupled receptors (GPCRs) has been reported [13]. The mechanism underlying the effect of cholesterol on the receptor structure and function appears to be complex and there is a lack of consensus on the manner in which cholesterol modulates receptor function [6-8, 13]. Cholesterol has been proposed to modulate the function of GPCRs either by a direct/specific interaction with the GPCR, which could induce a conformational change in the receptor or through an indirect way by altering the membrane physical properties in which the receptor is embedded or due to a combination of both. In addition, cholesterol could affect structure and function of membrane proteins by another mechanism that invokes the concept of 'nonannular' binding sites of membrane lipids [8] (see later). An important feature observed in recently solved high resolution crystal structures of

GPCRs (such as rhodopsin [14], β_1 -adrenergic receptor [15], β_2 -adrenergic receptor [16, 17] and A_{2A} adenosine receptor [18]), is the close association of cholesterol molecules to the receptor see Fig. (1).

Fig. (1a) shows the crystal structure of a photo-stationary state, highly enriched in metarhodopsin I with a cholesterol molecule between two rhodopsin monomers, which may represent a nonannular site for cholesterol binding ([14]; see Fig. 1a). More recently, high resolution crystal structures of the A_{2A} adenosine receptor ([18]; see Fig. 1b) and β_2 -adrenergic receptor ([16, 17]; Figs. 1c and d) have revealed structural evidence of tightly associated cholesterol molecules. Fig. (1b) shows the structure of the human A_{2A} adenosine receptor with three bound cholesterol molecules. All the three cholesterol molecules in this structure are found to be bound to the extracellular half of the receptor. Two of these cholesterol molecules have extensive contacts with Phe255 in transmembrane helix VI, and the aromatic ring of Phe255^{6,57} is found to be sandwiched between these cholesterol molecules [18]. In addition, one of the cholesterol molecules form a hydrogen bond with Ser263, and another show polar interaction with sulfur of Cys259. From the observed specific interactions of cholesterol molecules, it is proposed that the conformational stabilization of the transmembrane helix VI region by cholesterol may play a functional role in ligand binding of the A_{2A} adenosine receptor [18]. Moreover, such specific interaction of cholesterol molecules with the receptor is consistent with the observation that the cholesterol hemisuccinate (CHS) increases the thermostability of the purified receptor [18].

Figs. (1c and d) show the structure of the human β_2 -adrenergic receptor with bound cholesterol molecules. In one of these crystal structures, a specific cholesterol binding site has been reported ([17]; see Fig. 1d). As shown in the figure, the crystal structure of the receptor shows a cholesterol binding site between transmembrane helices I, II, III and IV with two cholesterol molecules bound per receptor monomer. In addition, cholesterol appears to be important in the crystallization of the β_2 -adrenergic receptor [16], and in improving receptor stability [17, 19]. These recent advancements in structure analysis of GPCRs have enhanced our understanding of the role of membrane cholesterol in GPCR structure and function.

(a). Role of Cholesterol in the Serotonin_{1A} Receptor Function

The serotonin_{1A} receptor is an important neurotransmitter receptor which belongs to the GPCR superfamily and is implicated in the generation and modulation of various cognitive, behavioral and developmental functions [20-22]. The serotonin_{1A} receptor is the first among all types of serotonin receptors to be cloned as an intronless genomic clone (G-21) of the human genome which cross-hybridized with a full length β -adrenergic receptor probe at reduced

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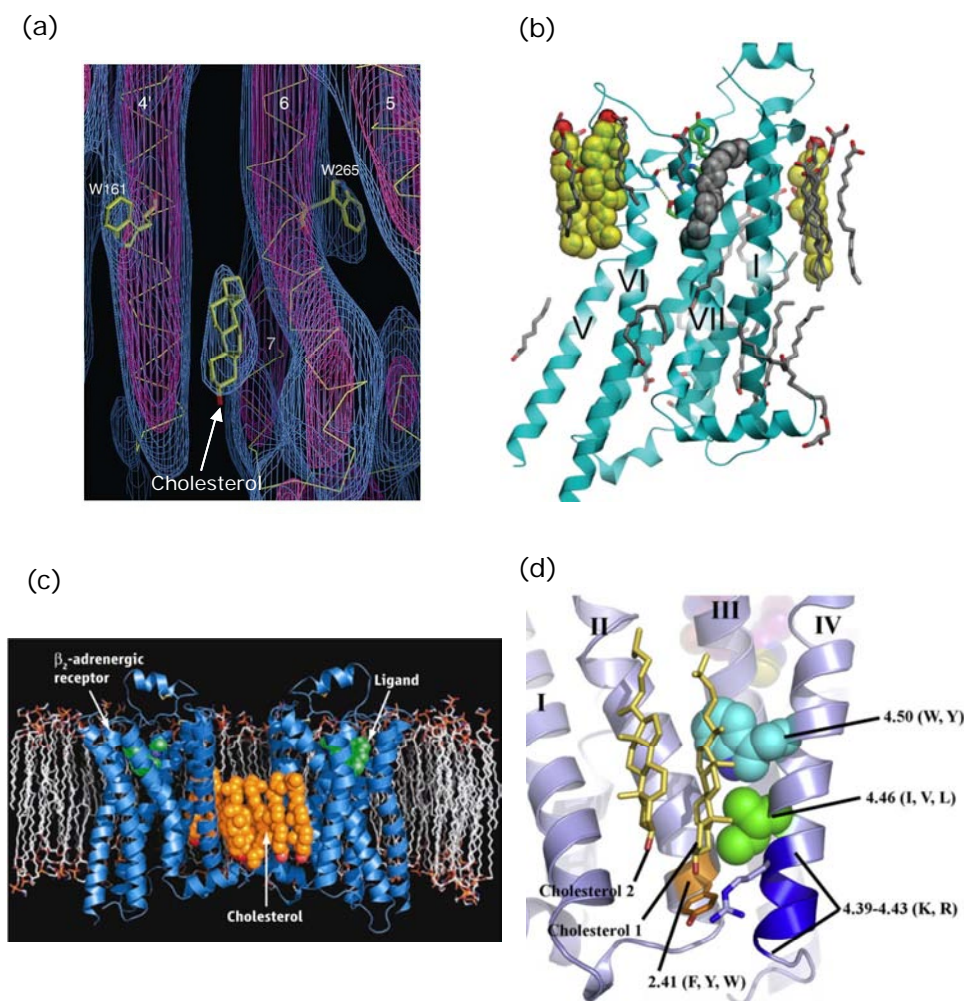


Fig. (1). Closely associated cholesterol molecules in GPCR crystal structures. Panel (a) shows metarhodopsin I with cholesterol between its transmembrane helices. Panel (b) shows the structure of the human A_{2A} adenosine receptor (shown in light blue), with three bound cholesterol molecules (shown in yellow). Reproduced from [18], with permission from AAAS, license number 2961270 786589. Panel (c) depicts the structure of the human β_2 -adrenergic receptor (shown in blue) with a bound partial inverse agonist carazolol (in green) embedded in a lipid bilayer. Cholesterol molecules between two receptor molecules are shown in orange. Panel (d) shows the Cholesterol Consensus Motif (CCM) in the β_2 -adrenergic receptor (bound to the partial inverse agonist timolol) crystal structure. The side chain positions of the β_2 -adrenergic receptor and two bound cholesterol molecules are shown. Panels (a), (c) and (d) are reproduced from ref. [58]. See text for more details.

stringency [20]. Sequence analysis of this genomic clone (later identified as the serotonin_{1A} receptor gene) showed ~43% amino acid similarity with the β_2 -adrenergic receptor in the transmembrane domain. The serotonin_{1A} receptor was therefore initially discovered as an 'orphan' receptor and was identified ('deorphanized') later [8]. Serotonin_{1A} receptor agonists [23] and antagonists [24] represent major classes of molecules with potential therapeutic applications in anxiety- or stress-related disorders.

Earlier work from our laboratory has comprehensively demonstrated the requirement of membrane cholesterol in the organization, dynamics and function of the serotonin_{1A} receptor (reviewed in [7, 8]). We showed that the membrane cholesterol plays a crucial modulatory role in the ligand binding activity and G-protein coupling of the hippocampal serotonin_{1A} receptor utilizing a number of approaches. These approaches include: (i) physical depletion of membrane cholesterol using M β CD; (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. The common message from the

results of these experiments was that it is the non-availability of membrane cholesterol, rather than the manner in which its availability is modulated, is crucial for the function of the serotonin_{1A} receptor [7, 8]. As mentioned earlier, cholesterol has been proposed to affect receptor function by altering either the membrane physical properties or specific local molecular interaction with the receptor. Our results using digitonin and cholesterol oxidase, in which the change in membrane order and the effect on ligand binding function of the serotonin_{1A} receptor did not show any correlation, gave us a lead to a possible specific interaction between cholesterol and the receptor [7, 8].

A critical assessment of cholesterol specificity could be performed by replacing cholesterol with its close analogs, followed by monitoring receptor function. Utilizing this approach, we explored the stringency of the requirement of membrane cholesterol in supporting the function of the serotonin_{1A} receptor by replacing it with its immediate biosynthetic precursor, 7-dehydrocholesterol (7-DHC), differing with cholesterol in an extra double bond. Toward this effect, we generated a cellular model of the Smith-Lemli-Opitz Syndrome (SLOS), a congenital and developmental malformation

syndrome associated with defective cholesterol biosynthesis in which the immediate biosynthetic precursor of cholesterol (7-dehydrocholesterol or 7-DHC) is accumulated [25]. This was achieved by metabolically inhibiting the biosynthesis of cholesterol by utilizing a specific inhibitor (AY 9944) of the enzyme required in the final step of cholesterol biosynthesis [26]. In addition, we performed physical depletion of membrane cholesterol and replaced it with 7-DHC utilizing M β CD in native hippocampal membranes [27]. In both these conditions, the serotonin_{1A} receptor function was compromised although the overall membrane order was unaltered. These results therefore suggested that the requirement for maintaining ligand binding activity appears to be more stringent than the requirement for maintaining membrane order. Importantly, these results indicate that the molecular basis for the requirement of membrane cholesterol in maintaining the ligand binding activity of serotonin_{1A} receptors could be specific interaction, although global bilayer effects may not be ruled out [28].

In addition, cholesterol appears to be important in improving serotonin_{1A} receptor stability under conditions of thermal deactivation, extreme pH, and proteolytic digestion [29]. Importantly, receptor modeling studies showed that the serotonin_{1A} receptor is more compact in the presence of tightly bound cholesterol [30], and such a compact conformation could contribute to receptor stability [29]. In the light of these results, it is interesting to note the close association of cholesterol in the recently solved high resolution crystal structures of GPCRs (see above and Fig. 1) and the reported cholesterol binding sites (possibly nonannular) in the crystal structure of a closely related receptor *i.e.*, the β_2 -adrenergic receptor Fig. (1d; also see later).

(b). Membrane Cholesterol in the Function of Adrenergic Receptors

Adrenergic receptors are one of the important members of the GPCR superfamily, and are endogenously expressed in cardiac myocytes. In adult heart, terminally differentiated myocytes are susceptible to discrete pathological consequences such as hypertrophy, apoptosis and autophagy, each with immense clinical implications [31, 32]. Adrenergic receptors are broadly classified into alpha (α) and beta (β) subtypes. Catecholamines are known to induce vasoconstriction through α -adrenergic receptors, and increased heart rate and contraction through β -adrenergic receptors [33]. Among the adrenergic receptors identified so far, the β_2 -adrenergic receptor has been the most studied. The early availability of specific ligands for the β_2 -adrenergic receptor led to its purification and subsequent cloning, which allowed identification of domains involved in ligand binding and G-protein coupling utilizing various approaches [34, 35]. These factors allowed extensive biochemical, physiological and pharmacological characterization and provided early insight into the structure of the β_2 -adrenergic receptor [33].

As mentioned earlier, cholesterol has been shown to be important in improving the stability of the β_2 -adrenergic receptor [17, 19], and appears to be necessary in the receptor crystallization [16]. In spite of significant enhancement of our understanding of adrenergic signaling, our knowledge of the role of membrane lipids such as cholesterol in these processes is limited. Such knowledge is crucial in a better understanding of cardiovascular pathobiology, particularly because of the close relationship between cholesterol, adrenergic signaling and heart failure [36]. Keeping this in mind, we recently explored the role of membrane cholesterol in differential (subtype-specific) adrenergic signaling in cardiac myocytes, as a paradigm for understanding how cellular cholesterol dictates cells to choose hypertrophic or apoptotic responses [37].

It has been reported earlier that depending on the concentration used, agonists such as angiotensin II and norepinephrine may elicit hypertrophic and apoptotic responses [38-41]. Norepinephrine released from the sympathetic nervous system and its cognate recep-

tors (α and β with several subtypes) play a critical role in cardiac performance and homeostasis [42, 43]. A gene-specific framework involved in differential signaling pathways of eliciting hypertrophic and apoptotic responses has been previously demonstrated [39]. These are demarcated by the induction of FosB and Fra-1, two distinct members of the AP-1 family of transcription factors [39]. In our work, we utilized *fosB* and *fra-1* genes as the end-point targets for analyzing signaling pathways for hypertrophy and apoptosis, and monitored the effect of cellular cholesterol on respective pathways. Our results showed that cholesterol depletion from cardiac myocytes induced the promoter activities of *fosB* and *fra-1* genes both in the absence (control) and presence of the agonist norepinephrine, with induction being much higher in the presence of norepinephrine see Fig. (2). The figure shows that this induction in promoter activities of *fosB* and *fra-1* genes, reduces to more or less the original level upon replenishment of cellular cholesterol. This demonstrates that the observed changes in promoter activities of *fosB* and *fra-1* genes are cholesterol-specific.

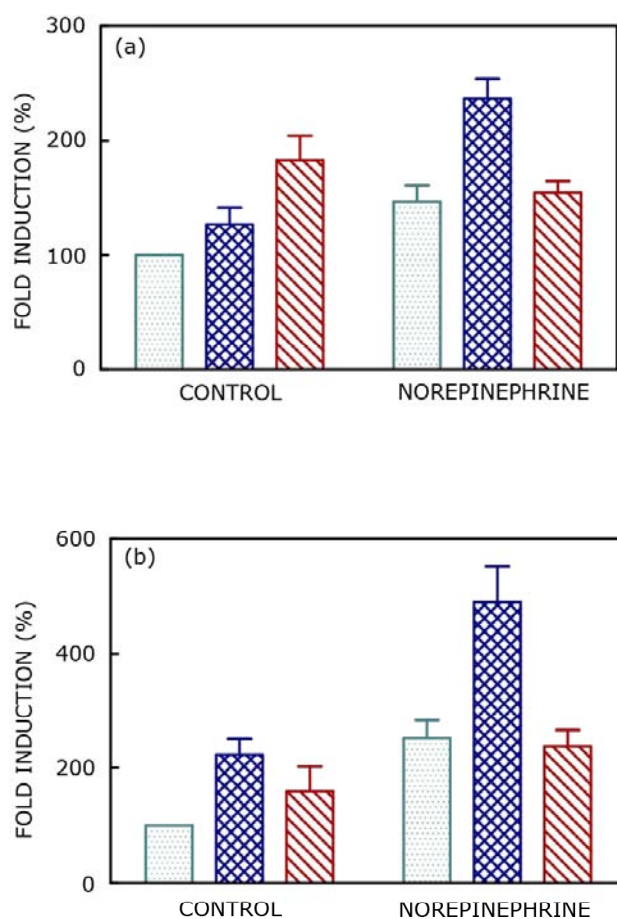


Fig. (2). Role of membrane cholesterol in cellular signaling: effect of cholesterol on the activity of *fosB* and *fra-1* promoters, end-point targets of differential adrenergic signaling in cardiac myocytes. H9c2 cells transfected with (a) *fosB* and (b) *fra-1* promoter-reporter constructs, were stimulated with 2 and 100 μ M norepinephrine, respectively. Induction of promoter activity with or without (control) ligand stimulation is measured in the absence of M β CD treatment (dotted bar), cholesterol-depleted (crisscrossed bar), and cholesterol-replenished (hatched bar) cells by quantitating luciferase activity in respective cell lysates. The ordinate represents luciferase activities of respective constructs, normalized to control (without M β CD and agonist treatment) cells. Reproduced from ref. [37]. See text and ref. [37] for more details.

We further monitored the subtype specificity of adrenergic receptors (α and β) endogenously expressed in cardiac myocytes in eliciting such effect on promoter activities of *fosB* and *fra-1* genes under conditions of varying cholesterol levels. Induction of these promoters upon stimulation with adrenergic receptor subtype-specific ligands (phenylephrine and isoproterenol) was monitored under cholesterol-depleted conditions see Fig. (3). The figure shows that cholesterol depletion enhances the induction of *fosB* and *fra-1* promoter activities upon treatment with both phenylephrine and isoproterenol, suggesting the lack of subtype specificity. Taken together, these results demonstrated that adrenergic signaling is enhanced upon cholesterol depletion in cardiac myocytes, irrespective of subtype specificity [37].

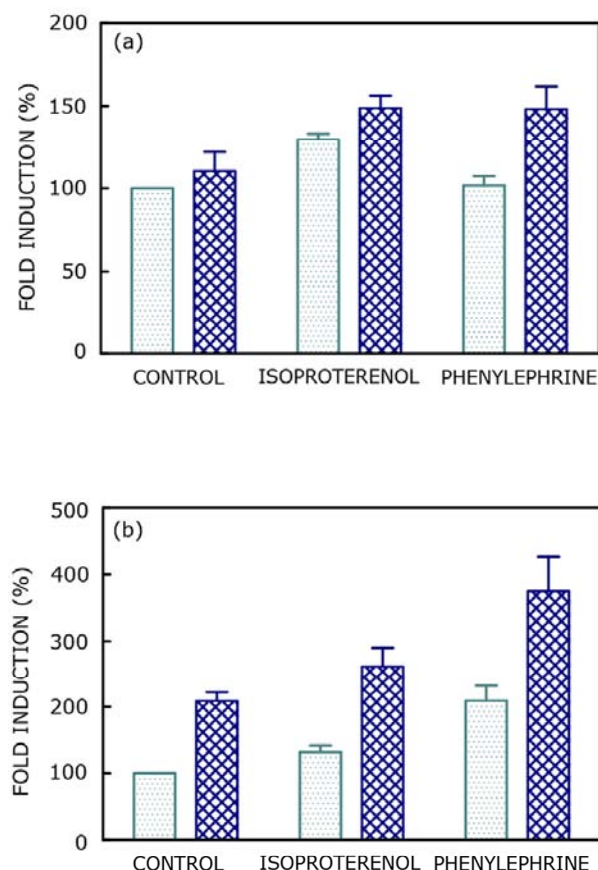


Fig. (3). Monitoring the role of membrane cholesterol in adrenergic receptor subtype-specific signaling. Effect of cholesterol on *fosB* and *fra-1* promoter activity upon stimulation with specific agonists of α - and β -adrenergic receptors. H9c2 cells transfected with (a) *fosB* and (b) *fra-1* promoter-reporter constructs were stimulated with isoproterenol or phenylephrine at different concentrations. Induction of promoter activity with or without (control) ligand stimulation is measured in the absence of M β CD treatment (dotted bar) and cholesterol-depleted (crisscrossed bar) cells, by quantitating luciferase activity in respective cell lysates. The ordinate represents luciferase activities of respective constructs, normalized to control (without M β CD and agonist treatment) cells. Reproduced from ref. [37]. See text and ref. [37] for more details.

(c). Specific Cholesterol Binding Motifs in GPCRs

As mentioned earlier, an important feature observed in recently solved high resolution crystal structures of GPCRs is the close association of cholesterol molecules to the receptor [14-18] see Fig. (1). Recent studies have also showed several structural features of proteins such as CRAC (cholesterol recognition/interaction amino

acid consensus) motif [44, 45], CCM (cholesterol consensus motif) [17], SSD (sterol-sensing domain) [46, 47] and CARC (inverse CRAC) motif [48], that are believed to result in preferential association with cholesterol (reviewed in [8, 49]). It has been proposed that cholesterol binding sequence or motif should contain at least one aromatic amino acid, which could interact with ring D of cholesterol [17] and a positively charged residue [45, 50], capable of participating in electrostatic interactions with the 3 β -hydroxyl group of cholesterol.

It has been reported earlier that many proteins that interact with cholesterol have a characteristic amino acid sequence, termed the cholesterol recognition/interaction amino acid consensus (CRAC) motif in their juxtamembrane region [45, 51]. The CRAC sequence is defined by the presence of the pattern -L/V-(X)₁₋₅-Y-(X)₁₋₅-R/K-, in which (X)₁₋₅ represents between one and five residues of any amino acid [45]. We have recently reported the presence of CRAC motifs in three representative GPCRs, namely, rhodopsin, the β_2 -adrenergic receptor, and the serotonin_{1A} receptor [51]. This report, along with the report of occurrence of CRAC motif in human type-1 cannabinoid receptor [52], constituted the first reports of the presence of CRAC motifs in GPCRs. Importantly, all these receptors have been shown to have cholesterol dependence for their function [8]. Interestingly, we have shown that CRAC motifs are inherent characteristic features of the serotonin_{1A} receptor and are conserved over natural evolution. This motif has also been shown to be present in caveolin-1 [53], the peripheral-type benzodiazepine receptor [44, 54], the HIV-1 transmembrane protein gp41 [55], and the mammalian seminal plasma protein PDC-109 [56]. In addition, the presence of CRAC sequence in membrane proteins has been related to their propensity to be incorporated into cholesterol-rich lipid domains [57]. Recently, a new potential cholesterol recognizing domain with the putative CRAC sequence oriented in the opposite direction along the polypeptide chain (*i.e.*, “inverted CRAC” domain), termed as “CARC” sequence is reported. It is shown to be present in several GPCRs, and the nicotinic acetylcholine receptor and is found to be conserved over natural evolution among the members of the acetylcholine receptor family [48].

Sterol-sensing domain (SSD) is another important cholesterol interacting domain which is relatively large (consists of five transmembrane segments) and reported to be involved in cholesterol biosynthesis and homeostasis [46, 47]. In addition, a specific cholesterol binding site consisting of four amino acids has been identified in a recent crystal structure of the β_2 -adrenergic receptor and is termed as cholesterol consensus motif (CCM) ([17]; see Fig. 1d). As mentioned earlier, the crystal structure of the β_2 -adrenergic receptor shows a cholesterol binding site between transmembrane helices I, II, III and IV with two cholesterol molecules bound per receptor monomer Fig. (1d). Importantly, we have recently shown that CCM is present in the serotonin_{1A} receptor and is conserved over natural evolution [58]. Interestingly, pairwise alignment of the human serotonin_{1A} receptor with the human β_2 -adrenergic receptor and rhodopsin exhibited conservation of the motif in all sequences.

In this context, we have recently proposed that cholesterol binding sites in GPCRs could represent ‘nonannular’ binding sites whose possible locations could be inter or intramolecular (interhelical) protein interfaces [58]. Integral membrane proteins are surrounded by a shell or annulus of lipid molecules, termed as ‘annular’ lipids [59]. The rate of exchange of lipids between the annular lipid shell and the bulk lipid phase was shown to be approximately an order of magnitude slower than the rate of exchange of bulk lipids [8, 59]. In addition, it has been proposed earlier that the cholesterol binding sites could be ‘nonannular’ for Ca²⁺/Mg²⁺-ATPase [60, 61] and the nicotinic acetylcholine receptor [62]. Nonannular sites are characterized by lack of accessibility to the annular lipids, *i.e.*, these sites cannot be displaced by competition with annular lipids [19]. This was apparent from the analysis of fluorescence quenching of intrinsic tryptophans of membrane proteins by bromi-

nated phospholipids or cholesterol [61, 62]. The exchange of lipid molecules between nonannular sites and bulk lipids is proposed to be relatively slow compared to the exchange between annular sites and bulk lipids although this has not yet been shown experimentally. Binding to the nonannular sites is considered to be more specific compared to annular binding sites [8, 58, 59].

3. ROLE OF SPHINGOLIPIDS IN THE SEROTONIN_{1A} RECEPTOR FUNCTION

Sphingolipids are ubiquitous constituents of eukaryotic cell membranes and constitute ~10-20% of membrane lipids, and are abundant in the plasma membrane compared to intracellular membranes [63]. Sphingolipids are believed to be essential components and are recognized as diverse and dynamic regulators of a multitude of cellular processes. They are implicated in the regulation of cell growth, differentiation, and neoplastic transformation through participation in cell-cell communication, and possible interaction with receptors and signaling systems [64]. The basic building block of sphingolipids is sphingosine, which upon acylation forms ceramide. Based on the headgroup attached to ceramide, sphingolipids are classified as phosphosphingolipids or glycosphingolipids. Sphingomyelin with a phosphatidylcholine headgroup is a major phosphosphingolipid in eukaryotes. In glycosphingolipids, a variety of monosaccharides linked by glycosidic bonds could form the headgroup [63].

Sphingolipids are abundant in the plasma membrane compared to intracellular membranes. Their distribution in the bilayer appears to be heterogeneous, and it has been postulated that sphingolipids along with cholesterol localize in laterally segregated lipid domains (sometimes termed as 'lipid rafts') [65-67]. Many of these domains are believed to be important for the maintenance of membrane structure and function, although analyzing the spatiotemporal resolution of these domains is proving to be challenging [68, 69]. The idea of such membrane domains gains significance since physiologically important functions such as cellular membrane sorting, trafficking [70], signal transduction [71], and the entry of pathogens into cells [72, 73] have been attributed to these domains. As discussed above, we have comprehensively demonstrated the requirement of membrane cholesterol in the function of the serotonin_{1A} receptor [7, 8]. In the overall context of the role of sphingolipids (along with cholesterol) in the formation and maintenance of membrane domains [65-67], and keeping in mind the relevance of sphingolipids in the nervous system [74, 75], we monitored the role of sphingolipids in the organization, dynamics and signaling of the serotonin_{1A} receptor, an important neurotransmitter GPCR [76-80].

(a). Role of Sphingomyelin

Sphingomyelin is a major sphingolipid in mammals and is the most abundant sphingolipid in the nervous system. It is reported to be ~25% of total lipids in the myelin sheath [81]. In order to address the role of sphingomyelin in the serotonin_{1A} receptor function, we utilized sphingomyelinase (SMase) [82], an enzyme that specifically catalyzes the hydrolysis of sphingomyelin into ceramide and phosphorylcholine [79]. This results in the release of water soluble phosphorylcholine leaving a hydrophobic ceramide backbone of sphingomyelin in the membrane. Fig. (4a) shows that the treatment of hippocampal membranes with sphingomyelinase results in considerable reduction of sphingomyelin content [79]. Specific binding of the agonist [³H]8-OH-DPAT to the receptor in control and sphingomyelinase-treated membranes is shown in Fig. (4b). As evident from the figure, the specific agonist binding to serotonin_{1A} receptors is reduced upon treatment with increasing concentrations of sphingomyelinase. Further analysis revealed that there is no significant change in overall membrane order upon sphingomyelinase treatment [79]. These observations show specific structural requirement of sphingomyelin in the function of sero-

tonin_{1A} receptors. Importantly, we have recently proposed a putative sphingolipid binding domain (SBD) in serotonin_{1A} receptors [83] (see later).

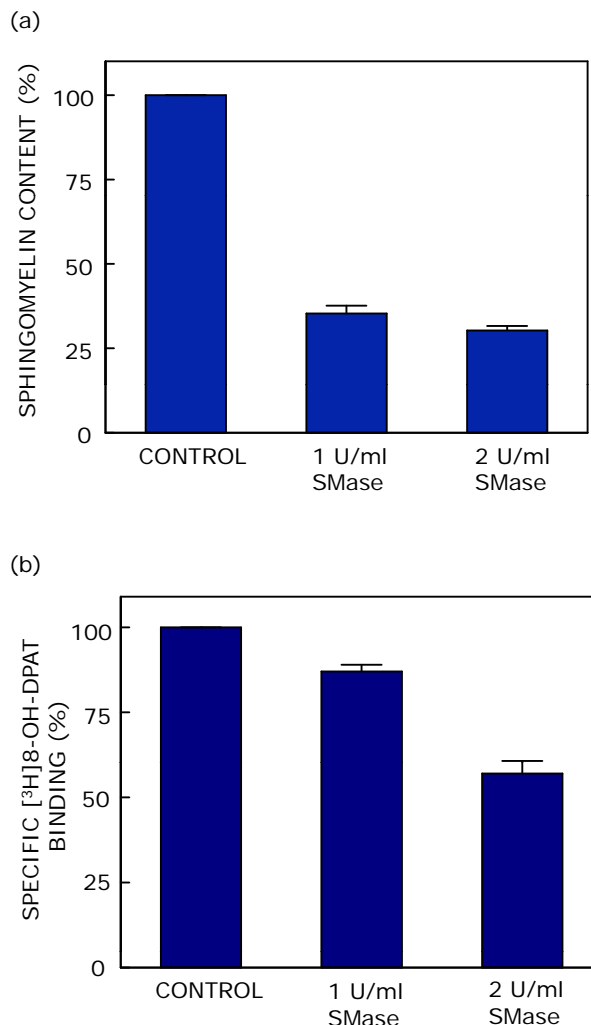


Fig. (4). Effect of sphingomyelinase treatment on sphingomyelin content and ligand binding of serotonin_{1A} receptors in hippocampal membranes. **(a)** Sphingomyelin levels in hippocampal membranes with or without (control) sphingomyelinase treatment. Values are expressed as percentages of sphingomyelin content in control membranes. **(b)** Specific binding of the agonist [³H]8-OH-DPAT to serotonin_{1A} receptors in membranes treated with or without (control) sphingomyelinase. Values are expressed as percentages of specific agonist binding obtained in control membranes. Adapted and modified from ref. [79]. See text and ref. [79] for more details.

(b). Effect of Metabolic Depletion of Sphingolipids

Cellular sphingolipid levels can be modulated using biosynthetic inhibitors such as fumonisins. Fumonisins are a group of naturally occurring mycotoxins, which are ubiquitous contaminants of corn and other grain products, produced by *Fusarium verticillioides* and several other *Fusarium* species [84]. In the family of fumonisins, fumonisin B₁ (FB₁) is the most abundant and is structurally similar to sphingoid bases such as sphinganine and sphingosine, which are intermediates in sphingolipid metabolism. FB₁ is known to reduce sphingolipid levels in cells by specifically inhibiting the reaction catalyzed by sphinganine *N*-acetyltransferase (ceramide synthase) [84], an enzyme catalyzing one of the early steps in sphingolipid biosynthesis. In order to explore the role of sphing-

golipids in the function of serotonin_{1A} receptors, cells stably expressing the serotonin_{1A} receptor were treated with FB₁ and receptor function was monitored [77]. This resulted in a steady reduction in sphingomyelin levels in cell membranes. Figs. (5a and c) show a steady reduction in the specific agonist and antagonist binding of the receptor upon treatment with increasing concentrations of FB₁. The change in specific agonist and antagonist binding of the receptor with respect to the corresponding change in sphingomyelin content is shown in Figs. (5b and d). In addition, it was found that the G-protein coupling and downstream signaling of the serotonin_{1A} receptor were significantly affected under these conditions [77]. Interestingly, there was no significant change in overall membrane order under these conditions. These results, along with the results using sphingomyelinase (see above), highlight the possibility of specific requirement of sphingolipids for the functioning of serotonin_{1A} receptors. In addition, the effect of sphingolipids on ligand binding function of the serotonin₇ receptor has been reported [85].

(c). Sphingolipid Binding Domain (SBD)

In this context, it is important to mention that recently a common sphingolipid binding domain (SBD) has been identified in a number of proteins such as HIV-1 gp120, Alzheimer's beta amyloid peptide and the prion protein [86-89]. In order to monitor whether

the observed sphingolipid sensitivity of the serotonin_{1A} receptor function (discussed above) [77, 79], could be induced by SBD(s), we recently examined the presence of SBD motif in the serotonin_{1A} receptor. By applying an algorithm based on the systematic presence of key amino acids [90], we showed that the human serotonin_{1A} receptor contains a putative SBD motif (LNKWTLG QVTC), corresponding to amino acids 99 to 109 (see Fig. 6; [83]). This specific sequence contains the characteristic combination of basic (Lys-101), aromatic (Trp-102) and turn-inducing residues (Gly-105), representative of SBDs [88, 91]. Interestingly, our analysis showed that the SBD motif appears to be an inherent characteristic feature of the serotonin_{1A} receptor and is conserved over natural evolution across various phyla [83].

4. CONCLUSION AND FUTURE PERSPECTIVES

GPCRs are involved in a multitude of physiological functions and represent important drug targets in all clinical areas [4]. Although the pharmacological and signaling features of GPCRs have been extensively studied, their interaction with membrane lipids is addressed in very few cases. In view of the enormous implications of GPCR function in human health [5] and several diagnosed diseases being attributed to altered lipid-protein interactions [92, 93], a comprehensive understanding of the role of membrane lipid envi-

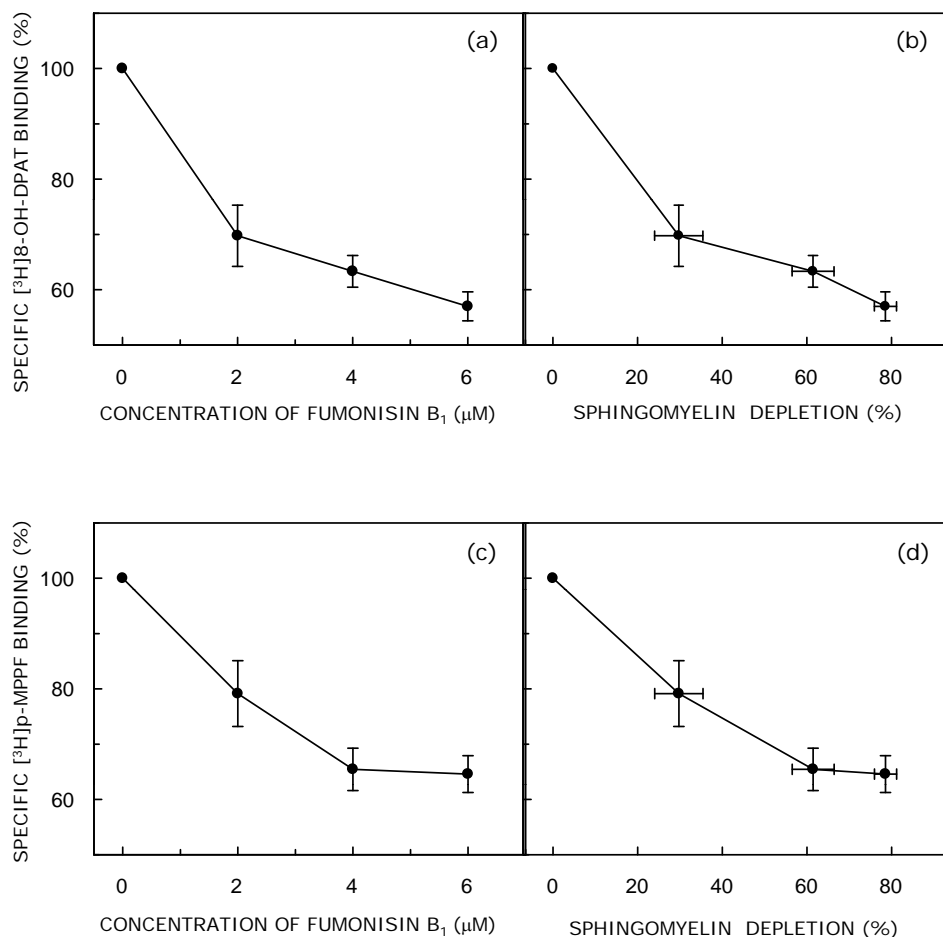


Fig. (5). Specific ligand binding of the human serotonin_{1A} receptor upon metabolic depletion of sphingolipids using the inhibitor fumonisins B₁. Panels (a) and (c) show specific agonist ([³H]8-OH-DPAT) and antagonist ([³H]p-MPPF) binding to serotonin_{1A} receptors in membranes isolated from CHO cells stably expressing the receptor (CHO-5-HT_{1A}R cells), upon treatment with increasing concentrations of fumonisins B₁. The change in specific binding of [³H]8-OH-DPAT and [³H]p-MPPF with respect to change in sphingomyelin content upon fumonisins B₁ treatment is shown in panels (b) and (d), respectively. Values are expressed as percentages of specific binding for control cell membranes without fumonisins B₁ treatment. Reproduced from ref. [77]. See text and ref. [77] for more details.

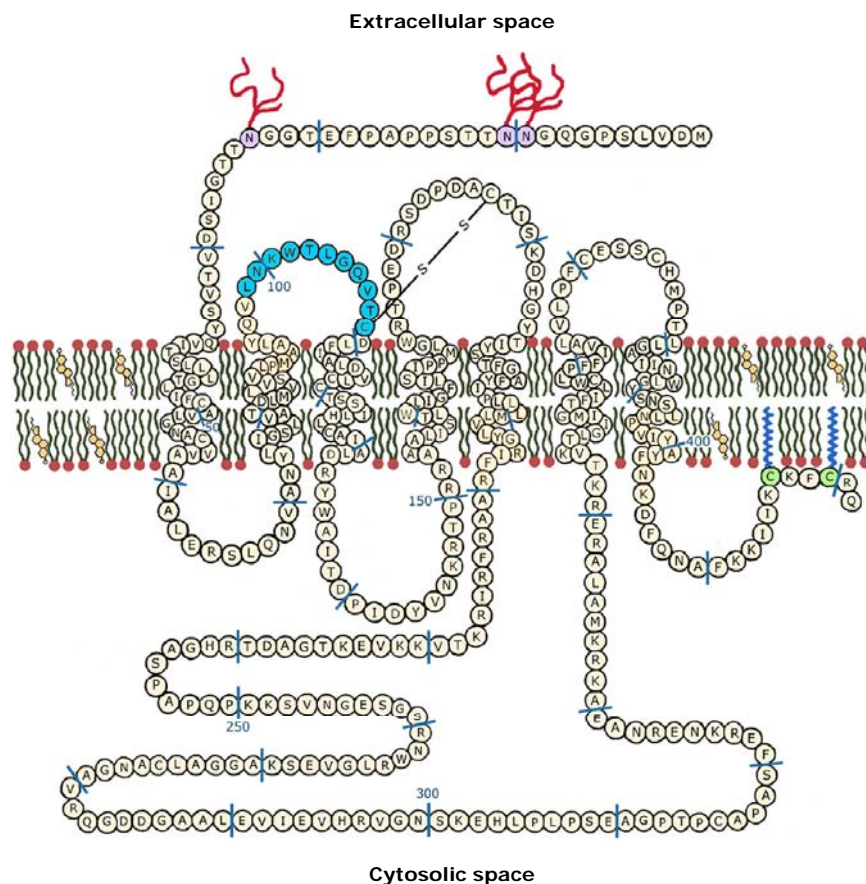


Fig. (6). A schematic representation of the membrane embedded human serotonin_{1A} receptor in a typical eukaryotic membrane with phospholipids and cholesterol. Seven transmembrane stretches of the receptor depicted as putative α -helices (each composed of ~22 amino acids) were predicted using the program TMHMM2. The location of the residues relative to the membrane bilayer is putative, as the exact boundary between the membrane and the aqueous phase is not known. The putative sphingolipid-binding domain (SBD) is highlighted (in cyan). The amino acids in the receptor sequence are shown as circles. Adapted and modified from ref. [83]. See text and ref. [83] for more details.

ronment in GPCR function is important. In this context, the realization that membrane lipids such as cholesterol and sphingolipids could influence the function of GPCRs has remarkably transformed our understanding of the function of this important class of membrane proteins. With increasing evidence of specific lipid binding sites in GPCRs [51, 83], mutational analysis of the amino acid residues involved in such interactions, followed by functional and organizational analyses of the receptor, are likely to provide a better understanding on specific lipid dependence of the receptor function. Such progress in deciphering molecular details of the nature of this interaction in the membrane would lead to better insight into our overall understanding of GPCR function in health and disease. This would enhance our ability to design better therapeutic strategies to combat diseases related to malfunctioning of these receptors.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

7-DHC	=	7-dehydrocholesterol
8-OH-DPAT	=	8-hydroxy-2-(di- <i>N</i> -propylamino)tetralin
CCM	=	Cholesterol Consensus Motif
CHS	=	Cholesterol Hemisuccinate
CRAC	=	Cholesterol Recognition/Interaction Amino Acid Consensus
FB ₁	=	Fumonisin B ₁
GPCR	=	G-Protein Coupled Receptor
M β CD	=	Methyl- β -cyclodextrin
<i>p</i> -MPPF	=	4-(2'-methoxy)-phenyl-1-[2'-(<i>N</i> -2"-pyridinyl)- <i>p</i> -fluorobenzamido]ethyl-piperazine

SBD = Sphingolipid Binding Domain
 SMase = Sphingomyelinase
 SSD = Sterol-Sensing Domain

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