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Sphingolipids modulate the function of human serotonin$_{1A}$ receptors: Insights from sphingolipid-deficient cells☆

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ABSTRACT

Sphingolipids are essential components of eukaryotic cell membranes and are known to modulate a variety of cellular functions. It is becoming increasingly clear that membrane lipids play a crucial role in modulating the function of integral membrane proteins such as G protein-coupled receptors (GPCRs). In this work, we utilized LY-B cells, that are sphingolipid-auxotrophic mutants defective in sphingolipid biosynthesis, to monitor the role of cellular sphingolipids in the function of an important neurotransmitter receptor, the serotonin$_{1A}$ receptor. Serotonin$_{1A}$ receptors belong to the family of GPCRs and are implicated in behavior, development and cognition. Our results show that specific ligand binding and G-protein coupling of the serotonin$_{1A}$ receptor exhibit significant enhancement under sphingolipid-depleted conditions, which reversed to control levels upon replenishment of cellular sphingolipids. In view of the reported role of sphingolipids in neuronal metabolism and pathogenesis of several neuropsychiatric disorders, exploring the role of serotonin$_{1A}$ receptors under conditions of defective sphingolipid metabolism assumes relevance, and could contribute to our overall understanding of such neuropsychiatric disorders. This article is part of a Special Issue entitled: Lipid order/lipid defects and lipid-control of protein activity edited by Dirk Schneider.

1. Introduction

G protein-coupled receptors (GPCRs) constitute a superfamily of seven transmembrane domain proteins that respond to a variety of physical, chemical and biological stimuli [1–3] and serve as important drug targets [3,4]. GPCRs are primarily involved in transducing signals of extracellular stimuli across the plasma membrane to the cellular interior. It is now becoming increasingly clear that membrane lipids play a crucial role in modulating GPCR function [5–10], either through direct interaction or by indirect effects on membrane physical properties, or both [11,12]. In addition, membrane lipids have been shown to modulate the interaction between GPCRs and G-proteins [13].

Sphingolipids are essential components of eukaryotic cell membranes that constitute ~10–20% of total membrane lipids [14,15]. Sphingolipids are recognized as diverse regulators of a number of cellular processes and have been implicated in cellular signaling, growth, differentiation and neoplastic transformation. Sphingolipids are found to be abundant in the plasma membrane relative to intracellular membranes, and their distribution in the bilayer appears to be heterogeneous. It has been postulated that sphingolipids together with cholesterol form ordered lipid domains that laterally segregate from the bulk membrane [16–18], although this view has been recently questioned [19–21]. Importantly, sphingolipids have been shown to modulate the function of several membrane proteins, including GPCRs and ion channels [9,22,23]. One of the best studied GPCRs in the context of membrane lipid effects on its organization, dynamics and function is the serotonin$_{1A}$ receptor [24–26]. The serotonin$_{1A}$ receptor is an important neurotransmitter receptor and is implicated in behavior, learning, development and cognition. As a result, the serotonin$_{1A}$ receptor serves as an important drug target for neuropsychiatric disorders such as anxiety and depression as well as in neuronal developmental defects [27].

We have previously shown that membrane cholesterol [5,6] and sphingolipids [8,9] play an important role in the function and dynamics of the serotonin$_{1A}$ receptor. A popular approach used to explore the role of sphingolipids in cellular functions is by modulating membrane sphingolipid content utilizing inhibitors that target enzymes catalyzing specific steps in the biosynthetic pathway [9]. Sphingolipid biosynthesis is initiated by condensation of L-serine with palmitoyl CoA (see Fig. 1a), that is catalyzed by the enzyme serine palmitoyl transferase (SPT). Sphinganine formed this way is converted to ceramide (by acylation of sphinganine or sphingosine) by ceramide synthase (*N*-acetyltransferase).

Abbreviations: 5-HT$_{1A}$ receptor, 5-hydroxytryptamine-1A receptor; BCA, bicinchoninic acid; GTP-γ-S, guanosine-5′-*O*-(3-thiotriphosphate); 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; Nutridoma-BO, nutridoma-oleic acid-albumin complex; PMSF, phenylmethylsulfonyl fluoride; SPT, serine palmitoyl transferase; TLC, thin layer chromatography.

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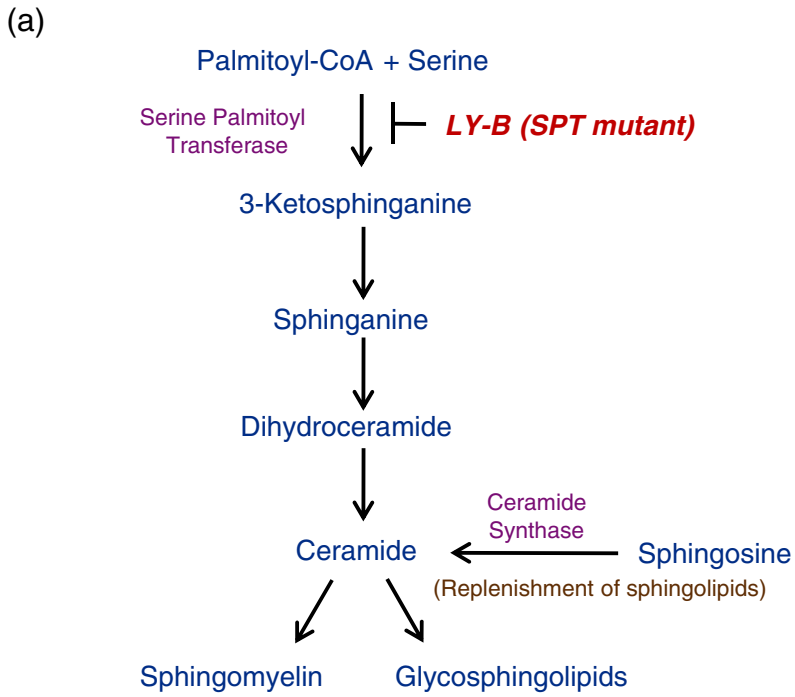

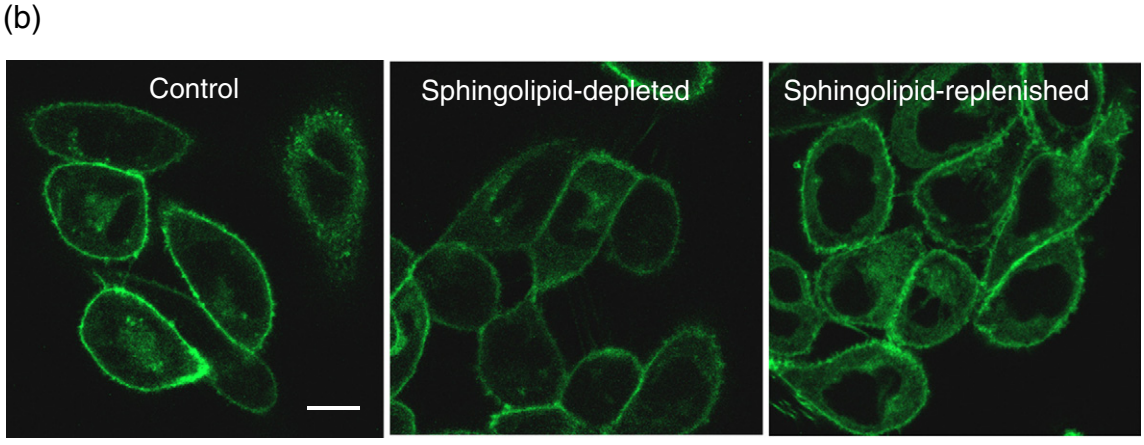

Fig. 1. Biosynthetic pathway of sphingolipids and distribution of serotonin$_{1A}$ receptors in LY-B-5-HT$_{1A}$R-EYFP cells under sphingolipid-modulated conditions. Panel (a) shows the sphingolipid biosynthetic pathway highlighting specific steps that are modulated to achieve sphingolipid depletion and replenishment. Sphingolipid biosynthesis is initiated by condensation of L-serine with palmitoyl CoA, which is catalyzed by the enzyme serine palmitoyl transferase (SPT). Sphinganine thus formed is converted to ceramide by ceramide synthase. Ceramide is either converted to sphingomyelin or glucosylceramide which gets further converted to complex glycosphingolipids. CHO mutant cells that lack the LCB1 subunit of the serine palmitoyl transferase enzyme, that catalyzes the first committed step in sphingolipid biosynthesis, is termed LY-B. Sphingolipids in LY-B-5-HT$_{1A}$R-EYFP cells were depleted by growing cells under sphingolipid-deficient conditions and were replenished by supplementation of complete medium with sphingosine (a metabolic intermediate). Panel (b) shows representative confocal microscopic images of LY-B-5-HT$_{1A}$R-EYFP cells depicting distribution of serotonin$_{1A}$ receptors under control, sphingolipid-depleted and sphingolipid-replenished conditions. Receptors appear predominantly localized at the plasma membrane. The images shown represent midplane confocal sections and the scale bar represents 10 μm. See text for more details.

Ceramide is either converted to sphingomyelin or glucosylceramide which gets further converted to complex glycosphingolipids. Chemical inhibitors such as myriocin, fumonisin B$_1$, PDMP and PPMP target enzymes which catalyze specific steps in sphingolipid biosynthesis and have been extensively utilized to monitor effect of sphingolipids on protein function. These inhibitors act as useful tools for exploring the role of sphingolipids in a variety of cellular processes and in the function of membrane receptors [28–30]. However, a limitation of this approach is that besides disrupting sphingolipid metabolism, they exhibit (non-specific) effects on cellular components. For example, fumonisin B$_1$, a commonly used competitive inhibitor of sphingolipid biosynthesis, has been shown to inhibit protein phosphatases [31] and induce oxidative stress [32]. Similarly, PDMP, an inhibitor of glycosphingolipid synthesis, has been reported to perturb cellular cholesterol homeostasis [33].

To overcome these limitations, a convenient and powerful approach for understanding the biological functions of membrane sphingolipids has been developed in terms of temperature sensitive or sphingolipid-auxotrophic cell mutants defective in functioning of enzymes involved in sphingolipid biosynthesis [34–36]. Use of mutant cell lines that exhibit defective sphingolipid biosynthesis, not only avoids non-specific effects

of chemical inhibitors, but also provides flexibility of conditionally modulating the sphingolipid content without accumulation of metabolic intermediates [37].

One such CHO (Chinese hamster ovary) mutant cell line that lacks the LCB1 subunit of the serine palmitoyl transferase (SPT) enzyme, which catalyzes the first committed step in sphingolipid biosynthesis, is termed LY-B [35,36; see Fig. 1a]. LY-B cells are conditional sphingolipid-biosynthesis mutant cells (lipid-auxotrophic mutants) that cannot synthesize sphingolipids on their own. Since sphingolipids are essential for a large number of cellular processes, LY-B cells have to be continuously supplied with sphingolipid supplement for maintaining normal cellular functions [35–37]. This property allows LY-B cells to serve as a convenient system for metabolically modulating sphingolipid content and studying sphingolipid dependence of the function of membrane proteins. In this work, we stably expressed serotonin$_{1A}$ receptors in LY-B cells and monitored the role of sphingolipids in the function of the receptor by modulating cellular sphingolipids.

2. Materials and methods

2.1. Materials

D-*erythro*-Sphingosine, DMPC, oleic acid-albumin complex, penicillin, streptomycin, gentamycin sulfate, polyethylenimine, PMSF, primuline, serotonin, sodium bicarbonate, and Tris were obtained from Sigma Chemical Co. (St. Louis, MO). D-MEM/F-12 [Dulbecco's modified Eagle's medium/nutrient mixture F-12 (Ham) (1:1)], fetal calf serum, Geneticin (G 418) and lipofectamine were from Invitrogen Life Technologies (Carlsbad, CA). GTP-γ-S and Nutridoma-SP were from Roche Applied Science (Mannheim, Germany). Porcine brain sphingomyelin was purchased from Avanti Polar Lipids (Alabaster, AL). BCA reagent for protein estimation was from Pierce (Rockford, IL). [^{3}H]-8-OH-DPAT (specific activity of 135.0 Ci/mmol) was purchased from DuPont New England Nuclear (Boston, MA). GF/B glass microfiber filters were from Whatman International (Kent, U.K.). Precoated silica gel 60 thin layer chromatography plates were from Merck (Darmstadt, Germany). All other chemicals and solvents used were of the highest available purity. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

2.2. Methods

2.2.1. Cells and cell culture

LY-B cells are CHO-K1 cell mutants which lack the LCB1 subunit of the serine palmitoyl transferase enzyme that catalyzes the first committed step in sphingolipid biosynthesis [35,36]. These cells were a generous gift from Dr. Kentaro Hanada (National Institute of Infectious Diseases, Tokyo, Japan). LY-B cells were stably transfected with the human serotonin$_{1A}$ receptor tagged to enhanced yellow fluorescent protein using 5-HT$_{1A}$R-EYFP fusion expression vector as described earlier [38]. Briefly, LY-B cells were maintained in D-MEM/F-12 complete medium (D-MEM/F-12 (1:1) medium supplemented with 2.4 g/l of sodium bicarbonate, 10% fetal calf serum, 60 μg/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml gentamycin sulfate) in a humidified atmosphere with 5% CO_2 at 37 °C. Cells after reaching ~80% confluency were transfected as per manufacturer's instructions (1 μg of receptor construct and 6 μl of lipofectamine). Cells were trypsinized after 48 h of transfection and were plated in low density in D-MEM/F-12 complete medium containing 1 mg/ml geneticin. After the cells were cultured for a week, a colony of cells was picked on the basis of fluorescence intensity and were cultured under selection pressure with geneticin. LY-B cells selected this way stably expressing the receptor (referred to as LY-B-5-HT$_{1A}$R-EYFP) were maintained in D-MEM/F-12 complete medium with 300 μg/ml geneticin in a humidified atmosphere with 5% CO_2 at 37 °C.

2.2.2. Modulation of sphingolipid content and confocal microscopy

Metabolic sphingolipid depletion in LY-B-5-HT$_{1A}$R-EYFP cells was carried out as described earlier [35,36] with some modifications. Briefly, cells were grown in D-MEM/F-12 complete medium for 48 h, washed with PBS to remove serum components and were then cultured in Nutridoma-BO medium (D-MEM/F-12 medium containing 1% Nutridoma-SP, 0.1% fetal calf serum, and 10 μM oleic acid-albumin complex supplemented with 12 μg/ml penicillin, 10 μg/ml streptomycin, 10 μg/ml gentamycin sulfate) for 24 h. Metabolic replenishment of sphingolipids into sphingolipid-depleted cells was carried out by culturing cells in D-MEM/F-12 complete medium supplemented with 1 μM sphingosine for 24 h [29].

Receptor distribution in control, sphingolipid-depleted and sphingolipid-replenished cells was monitored by acquiring confocal images with an inverted Zeiss LSM 510 Meta confocal microscope (Jena, Germany) as described earlier [39] with some modifications. Briefly, cells were grown on Lab-Tek chamber slides (Nunc, Denmark) and the images were acquired at room temperature (~23 °C) in the presence of PBS containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$. Images were acquired using the 514 nm line of an argon laser, and a 535–590 nm emission filter for the collection of EYFP fluorescence. Images of *z*-sections were acquired with a 63×/1.4 NA oil immersion objective under 1 airy condition.

2.2.3. Cell membrane preparation

Cell membranes were prepared as described earlier [40]. The total protein concentration in the isolated membranes was determined using the BCA reagent [41].

2.2.4. Radioligand binding assay

Receptor binding assays with membranes isolated from control and sphingolipid-modulated LY-B-5-HT$_{1A}$R-EYFP cells were carried out as described previously [40] with ~100 μg of total protein and 0.5 nM of radiolabeled agonist [^{3}H]-8-OH-DPAT.

2.2.5. GTP-γ-S sensitivity assay

The efficiency of G-protein coupling to the receptor in control and sphingolipid-modulated conditions was measured utilizing GTP-γ-S sensitivity assays as described earlier [40]. The concentrations of GTP-γ-S leading to 50% inhibition of specific agonist binding (IC_{50}) were calculated by nonlinear regression fitting of the data to a four parameter logistic function [42]:

$$B = a\left[1 + (x/I)^s\right]^{-1} + b \quad (1)$$

where, B is the specific binding of the agonist in presence of GTP-γ-S normalized to binding observed at lowest concentration of GTP-γ-S used, *x* denotes concentration of GTP-γ-S, *a* is the range ($y_{max} - y_{min}$) of the fitted curve on the ordinate (y-axis), I is the IC_{50} concentration, *b* is the background of the fitted curve (y_{min}) and s is the slope factor.

2.2.6. Quantitation of sphingomyelin by thin layer chromatography

Total lipids were extracted from cells grown in control and sphingolipid-modulated conditions according to Bligh and Dyer [43]. The lipid extracts from ~3 mg total protein were dried under a stream of nitrogen at 45 °C and were then dissolved in a mixture of chloroform/methanol (1:1, *v*/v). The extracted lipids were separated by thin layer chromatography (TLC) on pre-coated silica gel TLC plate using chloroform/methanol/water (65:25:4, *v*/v/v) as a solvent system. A sphingomyelin standard was run along with the lipid extracts. The TLC plate was sprayed with a solution of 0.01% (*w*/*v*) primuline [44] and the lipid bands were visualized under ultraviolet light. Sphingomyelin thus separated from total cellular lipids was quantitated as described previously [29]. The lipid bands corresponding to sphingomyelin on the TLC plate were scraped out and the lipids were re-extracted. The extract was dried, and the concentration of lipid phosphate of the extract was determined

subsequent to total digestion by perchloric acid [45] using Na_2HPO_4 as standard. DMPC was used as an internal standard to assess lipid digestion.

2.2.7. Statistical analysis

Student's two-tailed unpaired *t*-test was performed to estimate significance levels using Graphpad Prism software, version 4.0 (San Diego, CA). The half-maximal inhibition concentration (IC_{50}) was calculated by nonlinear regression analysis of binding data using the GRAFIT program version 3.09b (Erithacus Software, Surrey, U.K.).

3. Results and discussion

3.1. Characterization of LY-B-5-HT$_{1A}$R-EYFP cells

LY-B cells were stably transfected with the construct for serotonin$_{1A}$ receptor fused with enhanced yellow fluorescent protein (EYFP) at its C-terminus (termed as LY-B-5-HT$_{1A}$R-EYFP). We have earlier optimized the conditions for expression of the serotonin$_{1A}$ receptor utilizing this construct in CHO cells [38] and have shown that the receptor tagged to EYFP exhibited characteristics of ligand binding and G-protein coupling similar to native receptors. The fluorescence intensity and specific ligand binding characteristics of the receptor in LY-B-5-HT$_{1A}$R-EYFP cells were retained over several passages confirming stable integration of the receptor-EYFP construct. Fluorescence imaging of LY-B-5-HT$_{1A}$R-EYFP cells showed greater fluorescence intensity at the cell periphery, implying that the receptors were predominantly localized at the plasma membrane (see Fig. 1b).

3.2. Modulation of sphingolipid content of LY-B-5-HT$_{1A}$R-EYFP cells

LY-B cells lack the LCB1 subunit of the serine palmitoyl transferase enzyme that catalyzes the first committed step in sphingolipid biosynthesis and therefore cannot synthesize sphingolipids on their own. In order to metabolically deplete cellular sphingolipid content, we cultured LY-B-5-HT$_{1A}$R-EYFP cells under sphingolipid-deficient conditions. Since sphingomyelin constitutes the major fraction of total cellular sphingolipids (~80%) [15,46], we quantitated cellular sphingomyelin content as a representative indicator of total cellular sphingolipids. We monitored the change in sphingomyelin by separating it from total cellular lipids by TLC. A representative chromatogram showing sphingomyelin in LY-B-5-HT$_{1A}$R-EYFP cells grown under control and sphingolipid-deficient conditions (Nutridoma-BO medium) is shown in Fig. 2a. We observed a reduction in the intensity of the sphingomyelin band in the chromatogram and quantitative estimation subsequent to extraction from TLC plates showed a significant (~55%) reduction in sphingomyelin content under these conditions (Fig. 2b).

In order to replenish cellular sphingolipids, we cultured sphingolipid-depleted cells in complete medium supplemented with sphingosine (an intermediate of sphingolipid biosynthesis). Our results show that by this means we could replenish cellular sphingomyelin levels to ~113% of control cells (Fig. 2). We utilized these conditions of sphingolipid modulation to explore the role of sphingolipids in the function of the serotonin$_{1A}$ receptor.

3.3. Specific agonist binding to the serotonin$_{1A}$ receptor is enhanced under sphingolipid-deficient conditions

To monitor the effect of sphingolipid deficiency on specific agonist binding activity of the serotonin$_{1A}$ receptor, we isolated membranes from cells grown under control and sphingolipid-modulated conditions. Fig. 3 shows that the binding of specific agonist to the receptor exhibits significant enhancement (~50%) under sphingolipid-depleted conditions. It is interesting to note that we have earlier reported that serotonin$_{1A}$ receptors show enhanced ligand binding activity upon depletion of cell surface sphingomyelin utilizing sphingomyelinase [47]. The present results are therefore in agreement with our earlier observations. Although we

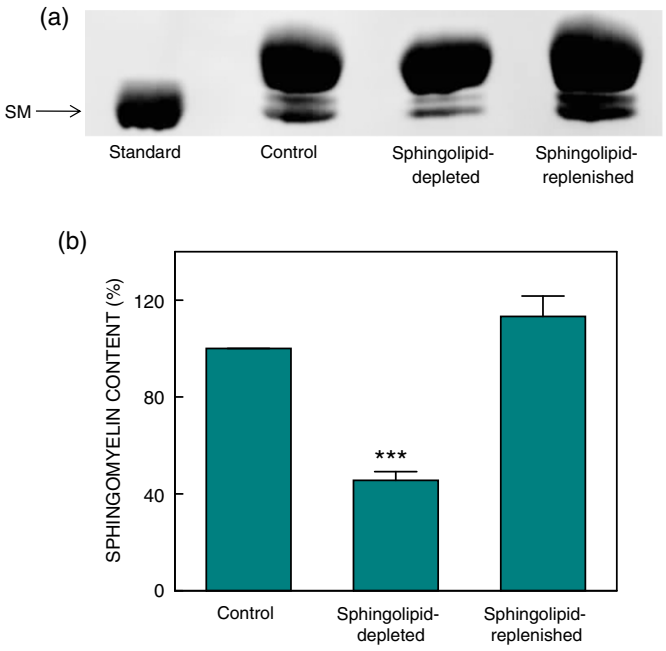

Fig. 2. Quantitation of sphingomyelin in LY-B-5-HT$_{1A}$R-EYFP cells. Sphingolipids in LY-B-5-HT$_{1A}$R-EYFP cells were depleted by growing cells under sphingolipid-deficient conditions (Nutridoma-BO medium) and were replenished by supplementation of sphingosine (a metabolic intermediate) in complete medium. Panel (a) shows a representative thin layer chromatogram of total cellular lipids from LY-B-5-HT$_{1A}$R-EYFP cells under varying sphingolipid conditions. Total cellular lipids were extracted from control, sphingolipid-depleted and sphingolipid-replenished cells and were separated by thin layer chromatography. Lipid bands that correspond to sphingomyelin standard are highlighted. Sphingomyelin content was quantitated by extracting lipids from respective bands (as described in Materials and methods) and is shown in panel (b). Data represent means ± S.E. of duplicate points from at least four independent experiments (*** corresponds to significant ($p < 0.001$) difference in mean sphingomyelin content in cells grown under sphingolipid-deficient conditions relative to control cells). See Materials and methods section for more details.

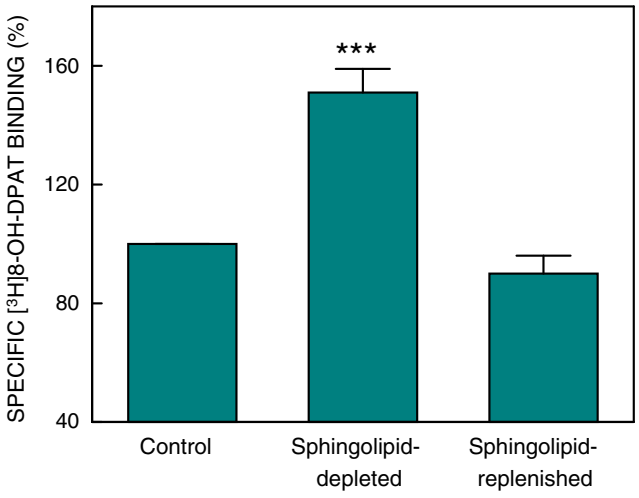

Fig. 3. Metabolic depletion of sphingolipids enhances specific agonist binding to serotonin$_{1A}$ receptors. Binding of specific agonist [^{3}H]8-OH-DPAT to serotonin$_{1A}$ receptors was monitored with membranes isolated from control, sphingolipid-depleted and sphingolipid-replenished LY-B-5-HT$_{1A}$R-EYFP cells. Values are expressed as percentages of specific agonist binding obtained in membranes isolated from control cells. Data represent means ± S.E. of duplicate points from at least six independent experiments (*** corresponds to significant ($p < 0.001$) difference in agonist binding under sphingolipid-deficient conditions compared to control). See Materials and methods section for more details.

could not differentiate in our previous work whether the observed effect was either due to the reduction in membrane sphingomyelin levels or the accumulation of ceramide in the membranes (upon hydrolysis of sphingomyelin by sphingomyelinase), our present results indicate that this effect could be independent of ceramide accumulation. Interestingly, the observed enhanced ligand binding activity of the receptor under sphingolipid-deficient conditions could be reversed (to ~90% of control) upon metabolic replenishment of sphingolipids (Fig. 3). These results, along with our previous observations, demonstrate the specific requirement of sphingolipids for maintaining the function of the serotonin$_{1A}$ receptor.

3.4. G-protein coupling efficiency of the serotonin$_{1A}$ receptor is enhanced under sphingolipid-deficient conditions

The major signaling mechanism of GPCRs involves activation of G-proteins upon stimulation with specific agonists, and guanine nucleotides have been shown to modulate agonist binding. GTP-γ-S, a non-hydrolyzable analog of GTP, that uncouples the normal cycle of guanine nucleotide exchange, has been extensively utilized to explore the efficiency of G-protein coupling to GPCRs. Previous studies have explored the cell-specific G-protein coupling of the serotonin$_{1A}$ receptor [48] and have shown that the receptor specifically activates the G_i/G_o class of G-proteins upon stimulation with its specific agonist in CHO cells [49]. In case of the serotonin$_{1A}$ receptor, we have previously shown that the receptor exhibits a shift from a high affinity state to a low affinity state upon dissociation of G-proteins utilizing GTP-γ-S [50].

In agreement with our previous findings, Fig. 4 shows a characteristic reduction in the binding of specific agonist 8-OH-DPAT to serotonin$_{1A}$ receptors with increasing concentration of GTP-γ-S under control and sphingolipid-modulated conditions. The half-maximal inhibition concentration (IC_{50}) derived from nonlinear regression analysis of such inhibition plots would reflect the efficiency of the interaction between the receptor and G-proteins. The respective IC_{50} values of GTP-γ-S for specific agonist binding to the serotonin$_{1A}$ receptors under control and sphingolipid-modulated conditions are shown in Table 1. The inhibition curve shown in Fig. 4 for the agonist binding to the receptor under sphingolipid-depleted condition displays a significant shift toward lower concentrations of GTP-γ-S compared to control. This implies that under sphingolipid-depleted condition, the binding of agonist to the receptor is more sensitive to GTP-γ-S than in control, which would in turn indicate that the efficiency of G-protein coupling is enhanced under these conditions. Analysis of these inhibition curves show that the efficiency of G-protein coupling to the receptor is significantly high (with an IC_{50} of ~2.3 nM; $p < 0.05$) under sphingolipid-depleted conditions relative to control (IC_{50} of ~4.4 nM).

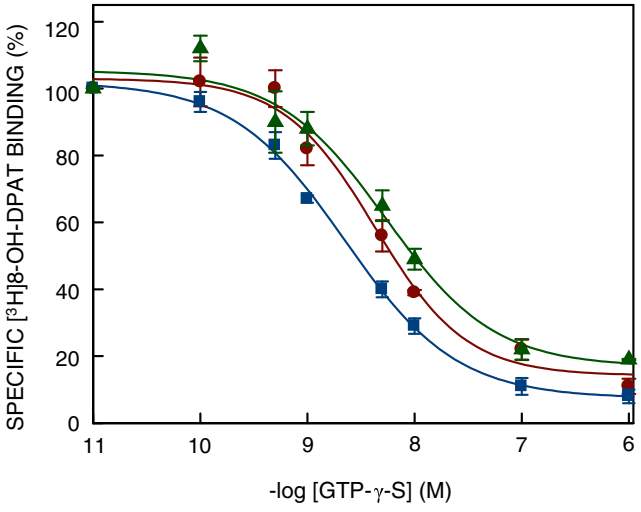

Fig. 4. Efficiency of G-protein coupling to the serotonin$_{1A}$ receptor under sphingolipid-modulated conditions. The efficiency of G-protein coupling to serotonin$_{1A}$ receptors was monitored by the sensitivity of specific binding of the agonist [^{3}H]-8-OH-DPAT to the receptor in the presence of GTP-γ-S, a non-hydrolyzable analog of GTP. In the presence of increasing concentrations of GTP-γ-S, specific binding of [^{3}H]-8-OH-DPAT to serotonin$_{1A}$ receptors in membranes isolated from control (●), sphingolipid-depleted (■), and sphingolipid-replenished (▲) LY-B-5-HT$_{1A}$R-EYFP cells show characteristic inhibition pattern. Values are expressed as percentages of specific agonist binding obtained at the lowest concentration of GTP-γ-S. The curves shown are nonlinear regression fits to the experimental data obtained using Eq. (1). The respective half maximal inhibition concentrations (IC_{50}) of GTP-γ-S, reflecting the efficiency of G-protein coupling to the receptor, are shown in Table 1. Data represent means ± S.E. of duplicate points from at least three independent experiments. See Materials and methods section for more details.

Our results showing an increase in efficiency of G-protein coupling accompanied with ~50% increase in agonist binding to the receptor (see Fig. 3) under sphingolipid-deficient conditions are in agreement with our previous observations that showed shift in the receptor to a high affinity agonist binding state with increase in efficiency of G-protein interaction [50]. Interestingly, replenishment of sphingolipids into sphingolipid-depleted cells resulted in a significant reduction in the efficiency of G-protein coupling to the receptor (IC_{50} of ~5.2 nM) which is also reflected in the reversal of agonist binding to control levels (see Fig. 3). The reversibility of agonist binding and G-protein coupling to the receptor upon specific modulation of sphingolipids highlight the specific requirement of sphingolipids for maintaining the function of the serotonin$_{1A}$ receptor.

It is of interest to note here that membrane lipids have been reported to play a role in the interaction of G-proteins with the membrane, which could modulate their interaction with the receptors [13,51–54]. Interestingly, sphingolipids have been previously shown to affect the membrane distribution and function of the G-protein $G\alpha_s$ in LY-B cells [55]. The observed change in G-protein coupling to the receptor under conditions of sphingolipid modulation (Fig. 4) could therefore be also due to the interaction of sphingolipids with G-proteins.

The effect of sphingolipids on the conformation and function of membrane proteins could be attributed to specific interaction. Sphingolipids have been reported to interact directly with several membrane proteins involved in diverse physiological processes such as endocytosis, protein sorting, ion conductance and GPCR function [9,23,56]. In this context, it is interesting to note that several structural motifs for sphingolipid interactions have been identified, such as the 'sphingolipid binding domain' (SBD) in proteins such as HIV-1 gp120, Alzheimer's beta amyloid peptide and the prion protein that are known to interact with (glyco)sphingolipids [57,58], and a specific sphingomyelin binding motif with the signature sequence VXXTLXXIY in a COPI machinery protein p24 [59]. We previously identified a characteristic sphingolipid binding domain [60] and sphingomyelin binding motif (S. Shrivastava, M. Jafurulla and A. Chattopadhyay, unpublished observations) in serotonin$_{1A}$ receptors that are conserved over natural evolution across various phyla among serotonin receptors. Recent results from our group, using coarse-grain molecular dynamics simulations, show that GM1 interacts with

Table 1
Effect of sphingolipids on the sensitivity of specific [^{3}H]8-OH-DPAT binding to GTP-γ-S[a].

Condition	IC_{50} (nM)
Control	4.4 ± 0.5
Sphingolipid-depleted	2.3 ± 0.2
Sphingolipid-replenished	5.2 ± 0.9

[a] Sensitivity of [^{3}H]8-OH-DPAT binding to GTP-γ-S was analyzed by calculating the half maximal inhibition concentration (IC_{50}) values for inhibition of specific ligand binding in presence of increasing concentrations of GTP-γ-S. Data shown in the table represent means ± S.E. of at least three independent experiments. See Materials and methods section for other details.

the serotonin$_{1A}$ receptor predominantly at the extracellular loop 1 and specifically at the SBD [61].

It is important to note that sphingolipids are enriched within neural tissue [46] and have been shown to play a crucial role in the general metabolism, survival and regeneration of nervous system [62,63]. Sphingolipids have been implicated in the pathogenesis of several neurological disorders [64]. In particular, recent evidence has shown the involvement of sphingolipids in the pathogenesis of Alzheimer's disease [65,66] and Parkinson's disease [67,68]. Imbalance in serotonergic signaling is implicated in several neuropsychiatric disorders such as Parkinson's disease, schizophrenia, Alzheimer's disease, anxiety and depression [69,70]. In particular, serotonin$_{1A}$ receptors have been shown to play a crucial role in the pharmacotherapy of schizophrenia and Parkinson's disease [71,72]. In this context, our present results highlighting the role of membrane sphingolipids in the function of the serotonin$_{1A}$ receptor, an important neurotransmitter receptor, assume relevance. These results could contribute to our overall understanding of the molecular mechanisms of such neuropsychiatric disorders.

Conflict of interest

The authors declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

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