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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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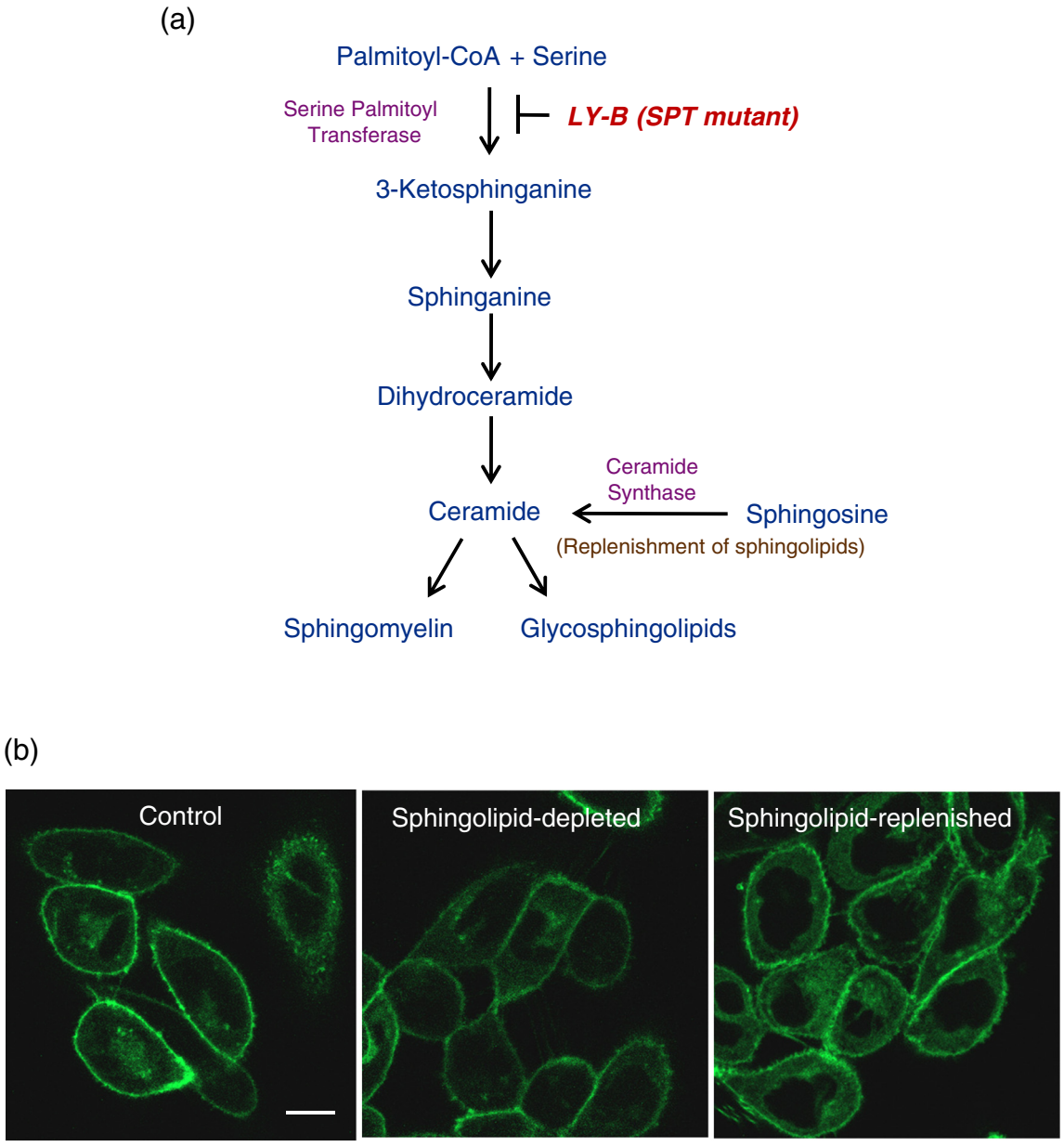

Fig. 1. Biosynthetic pathway of sphingolipids and distribution of serotonin$_{1A}$ receptors in LY-B-5-HT$_{1A}$R-EYFP cells under sphingolipid-modulated conditions. Panel (a) shows the sphingolipid biosynthetic pathway highlighting specific steps that are modulated to achieve sphingolipid depletion 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

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