

Cholesterol depletion modulates detergent resistant fraction of human serotonin_{1A} receptors

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Abstract

Insolubility of membrane components in non-ionic detergents such as Triton X-100 at low temperature is a widely used biochemical criterion to identify, isolate and characterize membrane domains. In this work, we monitored the detergent insolubility of the serotonin_{1A} receptor in CHO cell membranes and its modulation by membrane cholesterol. The serotonin_{1A} receptor is an important member of the G-protein coupled receptor family. It is implicated in the generation and modulation of various cognitive, behavioral and developmental functions and serves as a drug target. Our results show that a significant fraction (~ 28%) of the serotonin_{1A} receptor resides in detergent-resistant membranes (DRMs). Interestingly, the fraction of the serotonin_{1A} receptor in DRMs exhibits a reduction upon membrane cholesterol depletion. In addition, we show that contents of DRM markers such as flotillin-1, caveolin-1 and GM₁ are altered in DRMs upon cholesterol depletion. These results assume significance since the function of the serotonin_{1A} receptor has previously been shown to be affected by membrane lipids, specifically cholesterol. Our results are relevant in the context of membrane organization of the serotonin_{1A} receptor in particular, and G-protein coupled receptors in general.

Keywords: serotonin_{1A} receptor, detergent resistant membranes, cholesterol

Introduction

The G-protein coupled receptor (GPCR) superfamily is the largest and most diverse protein family in mammals, involved in signal transduction across membranes (Pierce et al. 2002, Rosenbaum et al. 2009). GPCRs are prototypical members of the family of seven transmembrane domain proteins and include >800 members which together constitute ~ 5% of the human genome (Zhang et al. 2006). GPCRs regulate physiological responses to a diverse array of stimuli, and mediate multiple physiological processes. GPCRs have therefore emerged as major targets for the development of novel drug candidates in all clinical areas (Heilker et al. 2009). It is estimated that ~ 50% of clinically prescribed drugs act as either agonists or antagonists of GPCRs (Schlyer and Horuk 2006).

The major paradigm in GPCR signaling is that their stimulation leads to the recruitment and activation of heterotrimeric GTP-binding proteins (G-proteins). These initial events, fundamental in GPCR signaling, occur at the plasma membrane via protein-protein

interactions and are controlled by lateral dynamics (diffusion) of the GPCR (Pucadyil et al. 2004). An important consequence is that the organization of molecules such as receptors and G-proteins in the membrane represents an important determinant in GPCR signaling (Ostrom and Insel 2004, Pucadyil and Chattopadhyay 2006). In this regard, the observation that GPCRs are not uniformly present on the plasma membrane, but are concentrated in specific membrane domains that are enriched in cholesterol assumes significance (Ostrom and Insel 2004). The role of membrane domains in GPCR function therefore represents a challenging aspect of GPCR signaling.

Current understanding of the organization of biological membranes involves the concept of lateral heterogeneities in the membrane, collectively termed membrane domains (Mukherjee and Maxfield 2004, Jacobson et al. 2007, Lingwood and Simons 2010). Many of these domains (sometimes termed as 'lipid rafts') are believed to be important for the maintenance of membrane structure and function, although

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characterizing the spatiotemporal resolution of these domains has proven to be challenging (Jacobson et al. 2007, Ganguly and Chattopadhyay 2010). These specialized regions are believed to be enriched in specific lipids and proteins, and facilitate processes such as trafficking, sorting, signal transduction and pathogen entry (Mukherjee and Maxfield 2004, Jacobson et al. 2007, Pucadyil and Chattopadhyay 2007a). Insolubility of membrane components in non-ionic detergents such as Triton X-100 is a widely used biochemical criterion to identify, isolate and characterize membrane domains (particularly 'rafts') (Brown and Rose 1992, Hooper 1999, Chamberlain 2004).

The serotonin_{1A} receptor is an important member of the GPCR family and is implicated in the generation and modulation of various cognitive, behavioral and developmental functions (Pucadyil et al. 2005, Kalipatnapu and Chattopadhyay 2007b, Müller et al. 2007). The serotonin_{1A} receptor agonists and antagonists have been shown to possess potential therapeutic effects in anxiety or stress-related disorders (Pucadyil et al. 2005). As a consequence, the serotonin_{1A} receptor serves as an important target in the development of therapeutic agents for neuropsychiatric disorders such as anxiety and depression. Previous work from our laboratory comprehensively demonstrated the requirement of membrane cholesterol (Pucadyil and Chattopadhyay 2004, Paila et al. 2008, Shrivastava et al. 2010) and sphingolipids (Paila et al. 2010) in the function of the serotonin_{1A} receptor (reviewed in Pucadyil and Chattopadhyay 2006, Paila and Chattopadhyay 2010).

In this work, we have explored detergent insolubility of the serotonin_{1A} receptor and its modulation by membrane cholesterol. For this, we used DRM specific marker proteins (such as flotillin-1 and caveolin-1) and lipids (GM₁ and cholesterol) to characterize DRM fractions isolated from CHO cell membranes and explored the effect of cholesterol depletion on the distribution of serotonin_{1A} receptors in DRM fractions. Our results show that ~ 28% of serotonin_{1A} receptors are present in DRM fractions and cholesterol depletion leads to a decrease in DRM localization of serotonin_{1A} receptors.

Methods

Materials

Gentamycin sulfate, BSA, penicillin, sodium bicarbonate, streptomycin, M β CD, Triton X-100 and DMPC were obtained from Sigma Chemical Co. (St Louis, MO, USA). Fetal calf serum, DMEM/F-12 (Dulbecco's modified Eagle's medium: nutrient

mixture F-12 (Ham) (1:1) and geneticin (G 418) were from Invitrogen Life Technologies (Grand Island, NY, USA). Amplex Red cholesterol assay kit was from Molecular Probes (Eugene, OR, USA). BCA reagent for protein estimation was obtained from Pierce (Rockford, IL, USA). Protease inhibitor cocktail was from Roche Applied Science (Mannheim, Germany). All other chemicals used were of the highest purity available. Water was purified through a Millipore (Bedford, MA, USA) Milli-Q system and used throughout. Rabbit polyclonal antibodies raised against GFP and rabbit polyclonal antibody to transferrin receptor were from Abcam (Cambridge, MA, USA). Mouse anti-flotillin and rabbit anti-caveolin polyclonal antibodies were obtained from BD-Transduction Laboratories (Sparks, MD, USA).

Cells and cell culture

Chinese hamster ovary (CHO) cells stably expressing the serotonin_{1A} receptor tagged to enhanced yellow fluorescent protein (referred to as CHO-5-HT_{1A}-R-EYFP) were used. Cells were grown in D-MEM/F-12 (1:1) supplemented with 2.4 g/l of sodium bicarbonate, 10% fetal calf serum, 60 μ g/ml penicillin, 50 μ g/ml streptomycin, and 50 μ g/ml gentamycin sulfate in a humidified atmosphere with 5% CO₂ at 37°C. Cells were maintained with 300 μ g/ml geneticin and grown up to 90% confluency before being used for membrane preparation.

Cell membrane preparation

Membranes from CHO cells were prepared as described previously (Kalipatnapu et al. 2004). Total protein was determined using the BCA assay (Smith et al. 1985) and membranes were stored at -70°C until further use.

Cholesterol depletion from control membranes

Cholesterol from membranes prepared from CHO cells was depleted using 30 mM methyl- β -cyclodextrin (M β CD) as described previously (Pucadyil and Chattopadhyay 2007b). Membranes were then spun down at 40,000 g for 5 min at 4°C, washed and suspended in 50 mM Tris, pH 7.4 buffer.

Isolation of detergent resistant membrane fraction

Detergent resistant membranes were isolated from control or cholesterol-depleted membranes (CDMs), as described previously by Brown and Rose with modifications (Brown and Rose 1992). Cell membranes or CDMs were resuspended in 50 mM Tris, pH 7.4 buffer

containing protease inhibitor cocktail and 1% (v/v) Triton X-100 at 2 mg/ml (referred to as solubilization mixture). The solubilization mixture was incubated at 4°C for 30 min with gentle mixing every 5 min. After incubation, the resulting mixture was mixed with an equal volume of 80% sucrose (prepared in 50 mM Tris, pH 7.4 buffer) to give 40% final sucrose concentration in the samples. The solubilization mixture containing 40% sucrose was layered successively with equal volumes of 30% and 5% sucrose in 50 mM Tris, pH 7.4 buffer to obtain a discontinuous sucrose density gradient. Gradients were centrifuged at 160,000 *g* for 22 h at 4°C in a Beckman SW41 rotor. Fractions (1 ml each) were harvested from the top of the gradient (12 fractions; see Figure 1) and proteins in each sample were precipitated by trichloroacetic acid (TCA). Fractions 4–7 were designated as DRM fractions based on their distinctively higher flotillin-1 and caveolin-1 contents. The rest of the fractions (8–12) were designated as DSM fractions. For lipid analysis, DRM (fractions 4–7) and DSM (fractions 8–12) fractions were pooled together, diluted 5 times and centrifuged at 250,000 *g* for 2 h at 4°C to get rid of sucrose. The membrane pellets were then resuspended in 50 mM Tris, pH

7.4 buffer and used for estimation of phospholipid, cholesterol and GM₁.

TCA precipitation of proteins

Fractions collected from the sucrose density gradient were diluted 5 times with 50 mM Tris, pH 7.4 buffer to lower sucrose concentration in samples. Proteins in each fraction were precipitated with 10% TCA for 1 h at 4°C. The precipitate was pelleted at 20,000 *g* for 5 min, washed with ice-cold acetic acid, air dried and dissolved in 50 mM Tris, pH 7.4 buffer containing 2% SDS. Protein content of each fraction was determined by micro-BCA method (Smith et al. 1985) and proteins were probed by immunoblotting.

Immunoblotting and protein quantitation

Serotonin_{1A} receptor, flotillin-1, caveolin-1 and transferrin receptor from control membranes, DRM, DSM and CDM were probed by immunoblotting. Total protein used for detection of flotillin-1, caveolin-1 and transferrin receptor was 20 µg while that for the serotonin_{1A} receptor was 30 µg. Total protein from

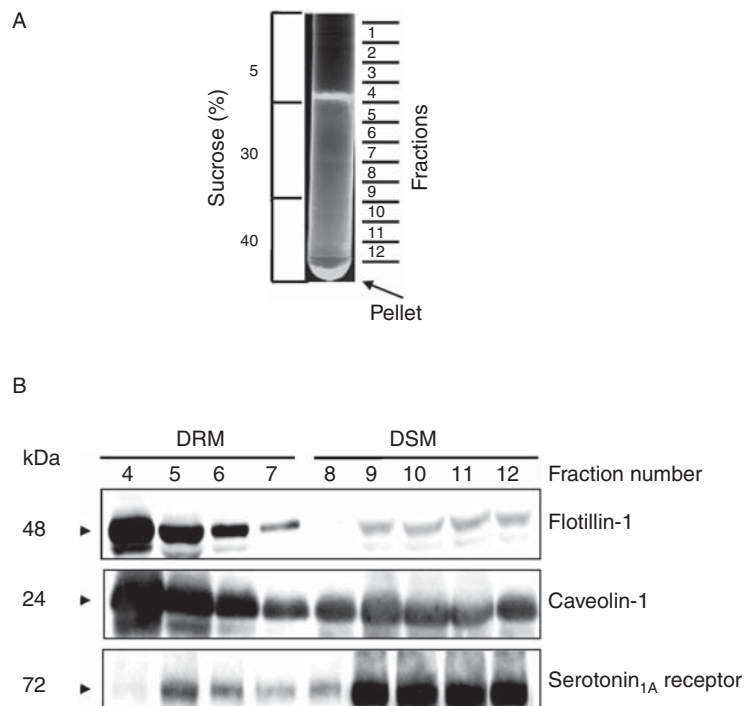


Figure 1. Characterization of DRM and DSM fractions of membranes from CHO cells stably expressing the serotonin_{1A} receptor tagged to EYFP (5-HT_{1A}R-EYFP). (A) Typical pattern of cell membrane fractions on sucrose density gradient. In order to isolate DRM and DSM fractions, cell membranes were treated with 1% Triton X-100 at 4°C and fractionated on a discontinuous sucrose density gradient. (B) 1 ml fractions were collected from top of the tube (total 12 fractions) and immunoblotted for marker proteins of DRM (flotillin-1 and caveolin-1) and the serotonin_{1A} receptor. No protein was detected in fractions 1–3 (not shown). Fractions 4–7 were designated as DRM due to the presence of distinctively higher contents of flotillin-1 and caveolin-1. The rest of the fractions (8–12) were termed as DSM. See *Methods* for other details.

each fraction was mixed with electrophoresis sample buffer and incubated for 30 min at 37°C. Samples were loaded and separated on SDS-PAGE. Proteins were electrophoretically transferred to a nitrocellulose membrane (Hybond ECL, Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) using a semidry transfer apparatus (Amersham). Non-specific binding sites were blocked with 3% BSA in TBST (137 mM NaCl, 20 mM Tris, 0.1% Tween-20, pH 7.6 buffer) for 2 h at room temperature (~ 23°C). Serotonin_{1A} receptors tagged to EYFP (5-HT_{1A}R-EYFP) were probed with rabbit polyclonal antibodies raised against GFP (1:1000 dilution). Flotillin-1 and caveolin-1 were probed with mouse anti-flotillin and rabbit anti-caveolin polyclonal antibodies (1:1000 for flotillin-1 and 1: 4000 for caveolin-1). Transferrin receptor was probed with rabbit polyclonal antibody to transferrin receptor (1:1000). Binding of primary antibodies to respective proteins was carried out by incubating membranes overnight with their respective antibodies in TBST at 4°C. After incubation, membranes were washed with TBST three times. Membranes were then incubated with 1:4000 dilution of respective secondary antibodies (horseradish peroxidase [HRP]-conjugated anti-rabbit antibody for 5-HT_{1A}R-EYFP, caveolin-1 and transferrin receptor and anti-mouse antibody for flotillin-1) in TBST for 45 min at room temperature (~ 23°C). Membranes were then washed and developed using enhanced chemiluminescence detection reagents (Amersham Biosciences, Bucks, UK). Serotonin_{1A} receptor, flotillin-1, caveolin-1 and transferrin receptor were detected using the chemiluminescence detection system (Chemi-Smart-5000, Vilber Loumat). Respective protein levels were estimated using Bio-2D⁺ software (BioRad).

Estimation of membrane cholesterol and phospholipid contents

The cholesterol content in cell membranes was estimated using the Amplex Red cholesterol assay kit (Amundson and Zhou 1999). The phospholipid content in these membranes was determined after total digestion with perchloric acid as described previously (McClare 1971) using Na₂HPO₄ as a standard.

Dot blotting

To detect GM₁ content in control membranes, CDMs and DRMs, 0.2 µg of protein from each membrane sample was dot blotted on nitrocellulose membrane, air dried and blocked for 2 h with 3% BSA in PBS (128 mM NaCl, 2 mM KCl, 8 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4 buffer) at room temperature.

Membranes were incubated with HRP-conjugated cholera toxin β-subunit (1:5000) and detected with enhanced chemiluminescence detection reagents (Amersham Biosciences, Buckinghamshire, UK). GM₁ in dot blot was detected using the chemiluminescence detection system (Chemi-Smart-5000, Vilber Loumat). Respective GM₁ levels were estimated using Bio-2D⁺ software (BioRad).

Results

Membrane organization of the serotonin_{1A} receptor, a pharmacologically important GPCR, represents an important determinant in its function (Kalipatnapu and Chattopadhyay 2007b). We previously reported the membrane organization of the serotonin_{1A} receptor using both detergent insolubility and detergent-free approaches (Kalipatnapu and Chattopadhyay 2004, 2005, 2007a). However, domains monitored in these studies were not characterized based on the presence of specific marker proteins for DRMs and DSMs. Since composition and properties of membrane domains are often influenced by the method used (Banerjee et al. 1995, Schuck et al. 2003), it is important to characterize them based on the presence of specific marker proteins and lipids. In the present study, we have explored the localization of the human serotonin_{1A} receptor in DRMs, based on the presence of specific marker proteins such as caveolin-1 and flotillin-1 and lipids (GM₁) and its modulation by membrane cholesterol content. For this, cell membranes isolated from CHO cells stably expressing 5-HT_{1A}R-EYFP were treated with 1% cold Triton X-100 and fractionated on a discontinuous sucrose density gradient. It is important to mention here that we have previously characterized CHO-5-HT_{1A}R-EYFP and shown that the EYFP-tagged receptor is essentially similar to the native receptor (Pucadyil et al. 2004). Specifically, we previously showed that EYFP fusion to the serotonin_{1A} receptor does not affect receptor function, i.e., ligand binding, G-protein coupling and signaling (Pucadyil et al. 2004). A typical pattern of membrane fractions on a sucrose density gradient is shown in Figure 1A. A total of 12 fractions were collected from top of the centrifuge tube and analyzed for the presence of flotillin-1 and caveolin-1 by immunoblotting. Fractions containing DRMs or DSMs were characterized by the presence or absence of specific marker proteins such as flotillin-1 and caveolin-1. Since no detectable amounts of protein were found in fractions 1–3, these fractions were discarded. Figure 1B shows that fractions 4–7 are characterized by distinctively higher flotillin-1 and caveolin-1 contents and were therefore termed as DRMs. The remaining fractions

(8–12) were assigned as DSMs. We therefore pooled fractions 4–7 and 8–12 for DRMs and DSMs, respectively, for further analysis. It should be noted that DRMs are typically low-density membrane fractions and DSMs are mostly the high-density membrane fractions. Interestingly, we observed a distribution of the human serotonin_{1A} receptor in different fractions, although the receptor was found to be predominantly present in DSM fractions.

In order to estimate the serotonin_{1A} receptor content in DRMs and DSMs, we performed densitometric analysis of immunoblots of the serotonin_{1A} receptor and marker proteins of DRMs and DSMs (Figure 2A). We observed that ~ 28% of serotonin_{1A} receptors are localized in DRMs. This is in excellent agreement with previous reports on the localization of the serotonin_{1A} receptors in DRMs (Kalipatnapu and Chattopadhyay 2004, Renner et al. 2007). In addition, ~ 95% flotillin-1 and ~ 83% caveolin-1 were found to be present in DRMs. It is known from earlier literature that the transferrin receptor (a single transmembrane domain) exhibits solubility in Triton X-100 and therefore is often

used as a control for DSM (Mayor and Maxfield 1995). Figure 2B shows that ~ 96% transferrin receptor was found to be present in DSMs. The presence of higher contents of flotillin-1 and caveolin-1 in pooled fractions for DRM and transferrin receptor in pooled fractions for DSM reinforces our analysis. Importantly, the glycosphingolipid GM₁ and cholesterol are commonly enriched in DRMs and frequently used as markers to characterize DRMs (Brown and London 2000). Cholera toxin B (CTXB) is known to bind specifically to GM₁ with high affinity (Fishman 1982). In order to quantitate GM₁ level, we performed dot blot analysis of DRM fractions on nitrocellulose membrane and probed GM₁ with CTXB (Figure 3A). We observed ~ 2-fold enrichment of GM₁ in DRM fractions relative to control membranes (see Figure 3B). In addition, both cholesterol and phospholipids exhibited ~ 3-fold increase in DRMs relative to control membranes (Figure 3B). Cholesterol, GM₁ and phospholipids were not estimated in DSM fractions due to their poor recovery during centrifugation.

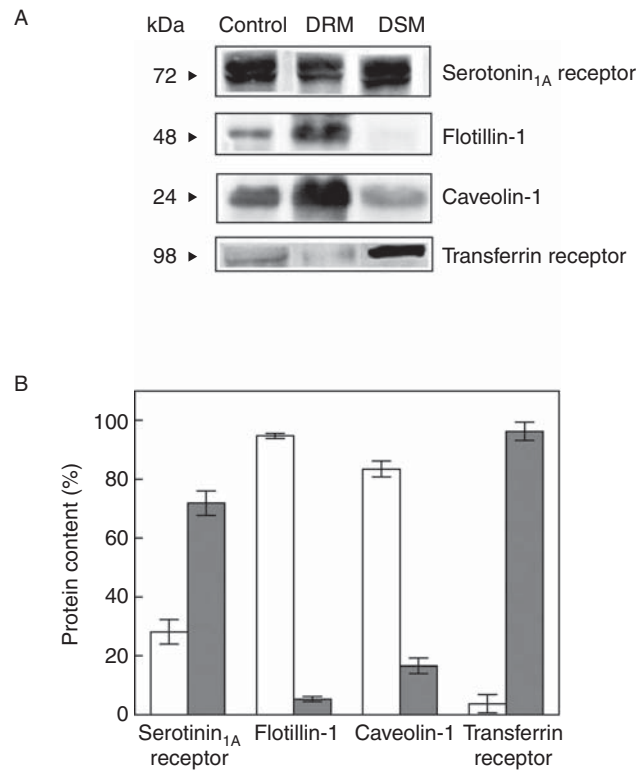


Figure 2. Quantitative analysis of serotonin_{1A} receptors in DRM and DSM fractions of cell membranes. (A) Representative immunoblots showing distribution of serotonin_{1A} receptors, flotillin-1, caveolin-1 and transferrin receptors in control membranes, and DRM and DSM fractions. (B) Contents of serotonin_{1A} receptors, flotillin-1, caveolin-1 and transferrin receptors in DRM (white bars) and DSM (gray bars). Values are expressed as percentages of total protein content in DRM and DSM fractions. Protein contents were estimated by densitometric analysis of their respective bands on immunoblots using Bio-2D⁺ software (Bio-Rad). Data represent means \pm SE of at least three independent experiments. See *Methods* for other details.

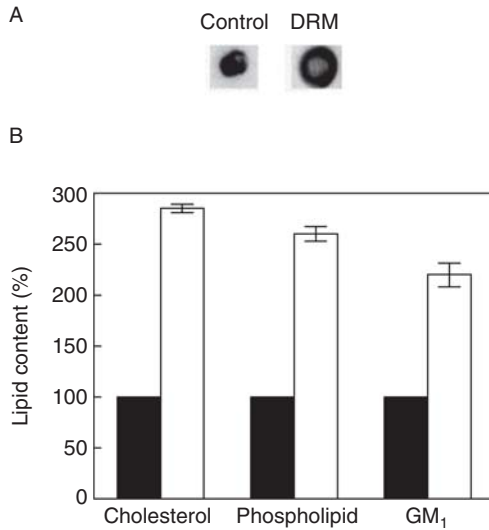


Figure 3. Quantitative analysis of lipid contents in DRM fractions of cell membranes. (A) Representative dot blots of GM₁ in control membranes and DRMs. (B) Lipid contents in control membranes (black bars) and DRM fractions (white bars). Values are expressed as percentages of lipid contents in control membranes, normalized to total protein content of respective membranes. GM₁ was estimated by densitometric analysis of dot blots using Bio-2D⁺ software (Bio-Rad). Data represent means \pm SE of at least three independent experiments. See *Methods* for other details.

We (Pucadyil and Chattopadhyay 2007b, Shrivastava et al. 2010) and others (Sjögren et al. 2008) previously reported that membrane cholesterol is crucial for the function of serotonin_{1A} receptor. It has been reported earlier that DRMs are enriched with cholesterol (Brown and London 1998). We therefore explored the distribution of serotonin_{1A} receptors in DRMs and DSMs of CDMs and the results are shown in Figure 4A. In order to deplete cholesterol, we treated cell membranes with M β CD. M β CD is a water-soluble cyclic oligosaccharide, and has earlier been shown to extract cholesterol from membranes in a selective and efficient manner by including it in a central nonpolar cavity (Zidovetzki and Levitan 2007). We observed \sim 90% of cholesterol was depleted from cell membranes upon M β CD treatment (data not shown). On the other hand, there was negligible loss of phospholipids and GM₁ contents from these membranes under these conditions (not shown). The effect of cholesterol depletion on DRM localization of serotonin_{1A} receptors was monitored by immunoblotting the receptor and marker proteins for DRMs and DSMs, followed by densitometric analysis. Representative immunoblots of the serotonin_{1A} receptor and marker proteins in CDMs, and in DRMs and DSMs isolated from these membranes are shown in Figure 4A. As shown in Figure 4B, cholesterol depletion resulted in a decrease in the pool of serotonin_{1A} receptors in DRMs

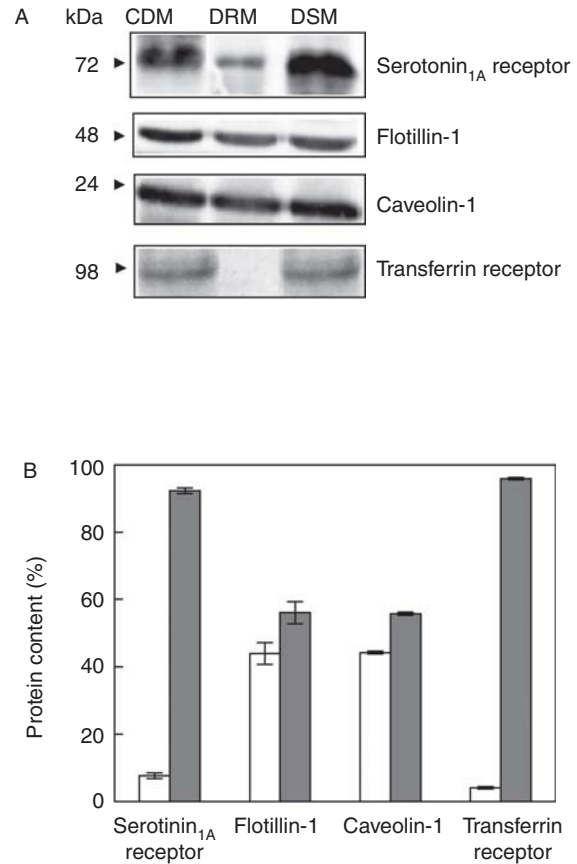


Figure 4. Estimation of serotonin_{1A} receptors in DRM and DSM fractions of cholesterol-depleted membranes. (A) Representative immunoblots showing the distribution of serotonin_{1A} receptors, flotillin-1, caveolin-1 and transferrin receptors in cholesterol-depleted membranes and the corresponding DRM and DSM fractions. (B) Contents of serotonin_{1A} receptors, flotillin-1, caveolin-1 and transferrin receptors in DRM (white bars) and DSM (gray bars). Values are expressed as percentages of total protein contents in DRM and DSM fractions. Estimation of proteins was performed by densitometric analysis of respective bands on immunoblots using Bio-2D⁺ software (Bio-Rad). Data represent means \pm SE of at least three independent experiments. See *Methods* for other details.

isolated from CDMs in comparison to DRMs isolated from control membranes (*cf.* Figure 2B). Figure 4B shows that serotonin_{1A} receptor content was reduced to \sim 7% in DRMs accompanied by a concomitant increase in DSM fractions. Similarly, levels of flotillin-1 and caveolin-1 also displayed reduction to \sim 45% in DRM fractions and their corresponding levels were increased in DSM fractions. Such reduction in the localization of specific marker proteins from DRMs upon cholesterol depletion has earlier been reported (Troost et al. 2004, Hajduch et al. 2008). Since glycosphingolipids are known to preferentially interact with cholesterol (Ramstedt and Slotte 2006), we monitored the effect of cholesterol depletion on lipids contents, particularly GM₁ in

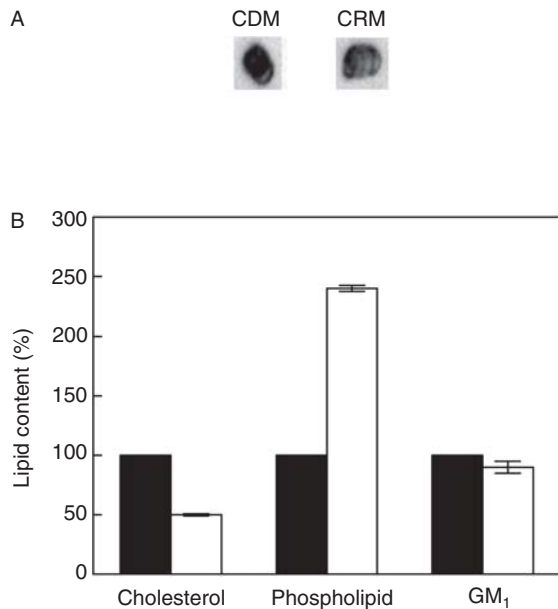


Figure 5. Estimation of lipid contents in DRM and DSM fractions of cholesterol-depleted membranes. (A) Representative immunoblots showing distribution of GM₁ in cholesterol-depleted membranes and corresponding DRM fractions. (B) Lipid contents in cholesterol-depleted membranes (black bars) and DRM fractions (white bars). Values are expressed as percentages of lipid contents in cholesterol-depleted membranes, normalized to total protein content of respective membranes. GM₁ was estimated by densitometric analysis of dot blots using Bio-2D⁺ software (Bio-Rad). Data represent means \pm SE of at least three independent experiments. See *Methods* for other details.

DRMs isolated from CDMs. Figure 5A shows representative dot blots of GM₁ in CDMs and DRMs isolated from them. Cholesterol level in DRMs was reduced to \sim 50% of its level in CDMs (see Figure 5B). Interestingly, we observed a decrease in GM₁ content in DRMs isolated from CDMs relative to DRMs isolated from control membranes. In addition, the level of phospholipids in DRMs remained more or less invariant with cholesterol depletion (Figures 3B and 5B).

Discussion

Insolubility of membrane components in non-ionic detergents such as Triton X-100 at low temperature represents an extensively used biochemical criterion to identify, isolate and characterize certain types of membrane domains (Brown and Rose 1992, Hooper 1999). Evidence from model membrane studies shows that enrichment with lipids such as sphingolipids (with high melting temperature) and cholesterol serves as an important determinant for the phenomenon of detergent resistance (Schroeder et al. 1998). The tight acyl chain packing in cholesterol-sphingolipid-rich membrane regions is thought to confer detergent resistance

to membrane regions enriched in these lipids and to the proteins which reside in them. Several GPI-anchored proteins, few transmembrane proteins and certain G-proteins have been found to reside in DRMs (Brown and Rose 1992, Chamberlain 2004). In spite of reported concerns on the possibility of membrane perturbation due to the use of detergents (Heerklotz 2002), resistance to detergent extraction continues to be a principal tool to study membrane domains since the need for relatively simple and straightforward biochemical methods for detecting membrane domains persists. Information obtained from this extensively used biochemical approach can often form the basis for a more detailed analysis of membrane domains utilizing other specialized approaches.

In this work, we monitored the detergent in solubility of the serotonin_{1A} receptor in CHO cell membranes and its modulation by membrane cholesterol. Our results show that a significant fraction (\sim 28%) of the serotonin_{1A} receptor resides in DRMs and this distribution is altered upon membrane cholesterol depletion. In addition, we show that contents of DRM markers such as flotillin-1, caveolin-1 and GM₁ are altered in DRMs upon cholesterol depletion. These results gain relevance in the context of our previous results that the function of the serotonin_{1A} receptor is affected by membrane cholesterol and sphingolipids (Pucadyil and Chattopadhyay 2004, Paila et al. 2008, 2010, Shrivastava et al. 2010). Our results assume broader significance since membrane organization of the serotonin_{1A} receptor is crucial for a comprehensive understanding of its function (Björk et al. 2010; Saxena and Chattopadhyay 2011).

We report here the change in the membrane organization of the serotonin_{1A} receptor upon cholesterol depletion. The organization of the serotonin_{1A} receptor under low cholesterol condition is relevant since reduced membrane cholesterol results in manifestation of several physiological effects. For example, it has been previously shown that cholesterol depletion affects sorting (Hansen et al. 2000), distribution (Pike and Casey 2002), endocytosis (Subtil et al. 1999) and trafficking (Pediconi et al. 2004) of membrane proteins. Importantly, we recently reported that chronic cholesterol depletion impairs the function of the serotonin_{1A} receptor, which could have important implications in mood disorders (Shrivastava et al. 2010).

We have previously reported, utilizing a green fluorescent protein-based microscopic approach, that the detergent insoluble fraction of the serotonin_{1A} receptor in CHO cells exhibits a small increase upon cholesterol depletion using M β CD (Kalipatnapu and Chattopadhyay 2005). In the present work, our results

show that the detergent insoluble fraction of the serotonin_{1A} receptor is reduced upon membrane cholesterol depletion. Although these results appear contradictory, it can be rationalized as follows. In the case of detergent insolubility using green fluorescent protein-based microscopic approach, the results referred to live cells and cholesterol depletion was carried out in intact cells (Kalipatnapu and Chattopadhyay 2005). In the present case, we have biochemically isolated cell membranes and depleted cholesterol from the isolated membranes. A possible reason for this difference could be due to reorganization of membrane cholesterol upon M β CD treatment in live cells which is clearly absent when isolated cell membranes were used for M β CD treatment. We conclude that caution should be exercised in interpreting results of Triton X-100 insolubility experiments, specifically in cases where cholesterol depletion is involved.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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