Differential effect of sterols on dipole potential in hippocampal membranes: Implications for receptor function

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Dipole potential is the potential difference within the membrane bilayer, which originates due to the nonrandom arrangement of lipid dipoles and water molecules at the membrane interface. In this work, we have explored the possible correlation between functional modulation of a G protein-coupled receptor (the serotonin1A receptor) and membrane dipole potential, under conditions of altered membrane sterol composition. We have previously shown that the ligand binding activity of the hippocampal serotonin1A receptor is reduced upon cholesterol depletion and could be restored upon replenishment with cholesterol. Interestingly, when the replenishment was carried out with an immediate biosynthetic precursor of cholesterol (7-DHC), differing with cholesterol merely in a double bond, the ligand binding activity of the receptor was not restored. In order to understand the mechanistic framework of receptor–cholesterol interaction, we carried out dipole potential measurements of hippocampal membranes under these conditions, by the dual wavelength ratiometric approach using an electrochromic probe (di-8-ANEPPS). We show here that dipole potential of hippocampal membranes is reduced upon progressive depletion of cholesterol and is restored upon replenishment with cholesterol, but not with 7-DHC. Our results show that the recovery of ligand binding activity of the serotonin1A receptor upon replenishment with cholesterol (but not with 7-DHC) could be related to the differential ability of these closely related sterols to modulate membrane dipole potential. We conclude that subtle changes in membrane dipole potential could be crucial in understanding the complex interplay between membrane lipids and proteins in the cellular milieu.

1. Introduction

Dipole potential is an internal potential of biological membranes and is generated due to the nonrandom orientation of electric dipoles of lipid and water molecules at the membrane interface [1–4]. The magnitude of dipole potential has been estimated to be 200–1000 mV, depending on membrane composition and this results in enormous electric field strength in the range of 108–109 Vm−1 within the membrane [1,2]. Dipole potential has been implicated to influence the function of membrane proteins and peptides such as Na+/K+ ATPase [5] and the ion channel gramicidin [6,7]. Modulation of dipole potential has been reported to affect the membrane insertion and folding of amphiphilic peptides such as p25 (the signal sequence of subunit IV of cytochrome c oxidase) [8], and simian immunodeficiency viral fusion peptide [9]. Importantly, it has been proposed that the dipole potential may play a crucial role in the structure and function of proteins associated with cholesterol-rich domains in the membrane [4]. Interestingly, cholesterol has been shown to increase membrane dipole potential [10,11].

Cholesterol is an essential component of eukaryotic membranes and plays a crucial role in membrane organization, dynamics, function and sorting [12,13]. Cholesterol is the end product of a long, multi-step and exceedingly fine-tuned sterol biosynthetic pathway involving more than 20 enzymes. According to the “Bloch hypothesis”, proposed by Konrad Bloch, the sterol biosynthetic pathway parallels sterol evolution [14]. It essentially means that cholesterol is selected over a very long time scale of natural evolution for its ability to optimize certain physical properties of eukaryotic cell membranes with regard to biological functions. Cholesterol precursors should therefore have properties that gradually support cellular function of higher organisms as they progress along the pathway toward cholesterol. Defects in cholesterol biosynthetic pathway have been identified with several inherited metabolic disorders [15]. For example, the Smith–Lemli–Opitz Syndrome (SLOS), a congenital and developmental malformation syndrome, is clinically diagnosed by reduced plasma levels
of cholesterol along with elevated levels of 7-dehydrocholesterol (7-DHC) [15]. 7-DHC is an immediate biosynthetic precursor of cholesterol in the Kandutsch–Russell pathway [16]. It differs with cholesterol only in a double bond at the 7th position in the sterol ring (see Fig. 1).

The G protein-coupled receptor (GPCR) superfamily is the largest and most diverse protein family in mammals, involved in signal transduction across membranes [17,18]. There are more than 800 members of the GPCR superfamily and typically they are seven transmembrane domain proteins. Due to their role in regulating multiple physiological processes, GPCRs have emerged as major drug targets in all clinical areas [19]. It is estimated that ~50% of currently prescribed drugs act as either agonists or antagonists of GPCRs [20]. The serotonin1A receptor is an important neurotransmitter receptor of the GPCR family and is implicated in the generation and modulation of various cognitive, behavioral and developmental functions [21–23]. Agonists [24] and antagonists [25] of the serotonin1A receptor represent major classes of molecules with potential therapeutic applications in anxiety- or stress-related disorders.

Cholesterol plays an important role in the function and organization of membrane proteins and receptors [26–30]. The exact mechanism of the interaction of cholesterol with membrane proteins and receptors is not clear. It has been proposed that such effects of cholesterol on integral membrane proteins could occur either through specific molecular interaction, or due to alterations in membrane physical properties, or by a combination of both [31,32]. Earlier work from our laboratory comprehensively demonstrated the requirement of membrane cholesterol in the organization, dynamics and function of the serotonin1A receptor ([33–36]; recently reviewed in [28,30]). We showed that the function of the serotonin1A receptor is impaired in SLOS-like condition, i.e., in presence of 7-DHC [34,37]. This implies that the interaction between cholesterol and the serotonin1A receptor is considerably stringent since 7-DHC, an immediate biosynthetic precursor of cholesterol differing with cholesterol in a double bond, was not able to maintain receptor function.

With an overall objective of broadening the mechanistic framework of receptor–cholesterol interaction, we have explored here the correlation between functional changes in the serotonin1A receptor induced by alterations in sterol composition and membrane dipole potential. We show here that dipole potential of hippocampal membranes is reduced upon progressive depletion of cholesterol and is restored upon cholesterol replenishment. Interestingly, replenishment with 7-DHC could not restore dipole potential in hippocampal membranes, thereby providing novel insight into the loss of receptor activity under these conditions.

2. Materials and methods

2.1. Materials

Cholesterol, 7-DHC, Mj/CD, DMPC, EDTA, EGTA, MgCl2, MnCl2, Na2HPO4, iodoacetamide, PMSF, sucrose, sodium azide and Tris were obtained from Sigma Chemical Co. (St. Louis, MO). BCA reagent for protein estimation was from Pierce (Rockford, IL). DPPC was from Avanti Polar Lipids (Alabaster, AL). Di-8-ANEPPS was purchased from Molecular Probes (Eugene, OR). Pre-coated silica gel 60 thin layer chromatography plates were from Merck (Darmstadt, Germany). The purity of lipids was checked by thin layer chromatography on silica gel precoated plates (Merck) in chloroform/methanol/water (65:35:5, v/v/v) and was found to give only one spot with a phosphate-sensitive spray and on subsequent charring [38]. All other chemicals and solvents used were of the highest purity available. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout. Fresh bovine brains were obtained from a local slaughterhouse within 10 min of death and the hippocampal region was carefully dissected out. The hippocampi were immediately flash frozen in liquid nitrogen and stored at −70 °C until further use.

2.2. Methods

2.2.1. Preparation of native hippocampal membranes

Native hippocampal membranes were prepared as described earlier [33]. Native membranes were suspended in a minimum volume of 50 mM Tris, pH 7.4 buffer, homogenized using a hand-held Dounce homogenizer, flash frozen in liquid nitrogen and stored at −70 °C. Protein concentration was determined using the BCA reagent with bovine serum albumin as a standard [39].

2.2.2. Cholesterol depletion of native membranes

Native hippocampal membranes were depleted of cholesterol using Mj/CD as described previously [33,37]. Briefly, membranes with a total protein concentration of ~2 mg/ml were treated with varying concentrations (10–40 mM) of Mj/CD in 50 mM Tris, pH 7.4 buffer at 25 °C in a temperature controlled water bath with constant shaking for 1 h. Membranes were spun down at 50,000 × g for 10 min, washed with the Tris buffer and resuspended in the same buffer. Membrane cholesterol was estimated using the Anthoplex Red cholesterol assay kit [40].

2.2.3. 7-DHC and cholesterol replenishment of cholesterol-depleted membranes

Cholesterol-depleted hippocampal membranes were replenished with 7-DHC or cholesterol using 7-DHC–Mj/CD or cholesterol–Mj/CD complex as described earlier [37]. 7-DHC–Mj/CD or cholesterol–Mj/CD complex was prepared by dissolving the required amounts of sterols and Mj/CD in a ratio of 1:10 (mol/mol) in 50 mM Tris, pH 7.4 buffer by constant shaking at 25 °C. Stock solutions (typically 2 mM 7-DHC or cholesterol: 20 mM Mj/CD) of this complex were freshly prepared before each experiment. Sterol (7-DHC or cholesterol) replenishment was carried out at a protein concentration of 2 mg/ml by incubating the cholesterol-depleted membranes with 1 mM sterol:10 mM Mj/CD complex for 1 h in 50 mM Tris, pH 7.4 buffer at 25 °C under constant shaking. Membranes were spun down at 50,000 × g for 10 min at 4 °C, washed with Tris buffer and resuspended in the same buffer.

2.2.4. Estimation of 7-DHC and cholesterol by thin layer chromatography

Lipid extraction from native, cholesterol-depleted, and sterol (cholesterol or 7-DHC)-replenished membranes was carried out according to Bligh and Dyer [41]. Lipid extracts were dried under a stream of nitrogen at 45 °C. The dried extracts were dissolved in a mixture of chloroform/methanol (1:1, v/v). 7-DHC and cholesterol were resolved by thin layer chromatography. TLC plates were impregnated with a 3% (w/v) silver nitrate solution in methanol, allowed to dry briefly and activated at 120 °C for 15 min. Cholesterol and 7-DHC were separated from total lipid extracts using n-heptane/ethyl acetate (2:1, v/v) as the solvent system [42]. The separated lipids were visualized on the TLC plate by charring with a solution containing cupric sulfate (10%, w/v) and phosphoric acid (8%, v/v) at 150 °C for ~5 min. 7-DHC and cholesterol bands were identified with the help
of standards. TLC plates were scanned and sterol band intensities were analyzed using the Adobe Photoshop software version 5.0 (Adobe Systems, San Jose, CA). Intensities of sterols from all samples on TLC plates were normalized to intensity of the cholesterol band obtained from the native membrane.

2.2.5. Estimation of phospholipids

Concentration of lipid phosphate was determined subsequent to total digestion by perchloric acid [43] using Na₂HPO₄ as standard. DMPC was used as an internal standard to assess lipid digestion. Samples without perchloric acid digestion produced negligible readings. The phospholipid content of native membranes is typically ~960 nmol/mg of total protein [44].

2.2.6. Sample preparation

Di-8-ANEPPS from a methanolic stock solution was added to hippocampal membranes containing 200 nmol total phospholipid in 1.5 ml of 50 mM Tris, pH 7.4 buffer. The amount of di-8-ANEPPS added was such that the final probe concentration was ~1 mol% with respect to total phospholipid content. The concentration of the stock solution of di-8-ANEPPS in methanol was estimated from its molar absorption coefficient of 37,000 M⁻¹ cm⁻¹ at 498 nm [45]. The final di-8-ANEPPS concentration was 1.3 μM in all cases and methanol content was always low (0.33%, v/v). This ensures optimal fluorescence intensity with negligible membrane perturbation. Di-8-ANEPPS probe was added to membranes while being vortexed for 1 min at room temperature (~23 °C).

For experiments with model membranes, large unilamellar vesicles (LUVs) of 100 nm diameter of DPPC containing increasing concentrations (0–40 mol%) of cholesterol or 7-DHC were prepared. All samples contained 1 mol% di-8-ANEPPS. For this, 640 nmol of total lipid (DPPC and sterol) and 6.4 nmol of di-8-ANEPPS were mixed well and dried under a stream of nitrogen while being warmed gently (~35 °C). After further drying under a high vacuum for at least 3 h, the lipid mixture was hydrated (swelled) by addition of 1.5 ml of 30 mM Tris, 1 mM EDTA, 150 mM sodium chloride, pH 7.4 buffer, and each sample was vortexed for 3 min to uniformly disperse the lipids and form homogeneous multilamellar vesicles. The buffer was always maintained at a temperature (~60 °C) well above the phase transitions of DPPC. LUVs of 100 nm diameter were prepared by the extrusion technique using an Avestin Liposofast Extruder (Ottawa, Ontario, Canada) as previously described [46]. Briefly, the multilamellar vesicles were freeze–thawed five times using liquid nitrogen to ensure solute equilibration between trapped and bulk solutions and then extruded through polycarbonate filters (pore diameter of 100 nm) mounted in an extruder fitted with Hamilton syringes (Hamilton Company, Reno, NV). The samples were subjected to 11 passes through the polycarbonate filters to give the final LUV suspension. Background samples were prepared in the same way except that di-8-ANEPPS was not added to them. The optical density of the samples measured at 420 and 520 nm were less than 0.15 in all cases, which rules out any possibility of scattering artifacts. Samples were incubated in dark for 12 h at room temperature (~23 °C) for equilibration before measuring fluorescence. All experiments were done with multiple sets of samples at room temperature (~23 °C).

2.2.7. Membrane dipole potential measurement

Membrane dipole potential measurements were carried out by dual wavelength ratiometric approach using voltage sensitive fluorescence probe di-8-ANEPPS [5,10,11,47,48]. Steady state fluorescence measurements were performed with a Hitachi F-4010 spectrofluorometer using 1 cm path length quartz cuvettes at room temperature (~23 °C). Excitation and emission slits with a nominal bandpass of 5 nm were used for all measurements. Background intensities of samples were subtracted from each sample to cancel any contribution due to the solvent Raman peak and other scattering artifacts. Steady state fluorescence intensities were recorded at two excitation wavelengths (420 and 520 nm). Emission wavelength was fixed at 670 nm. The fluorescence ratio (R), defined as the ratio of fluorescence intensities at an excitation wavelength of 420 nm to that at 520 nm (emission at 670 nm in both cases) was calculated [10]. The choice of the emission wavelength (670 nm) at the red edge of the fluorescence spectrum has previously been shown to rule out membrane fluidity effects [47]. Dipole potential (ψd) in mV was calculated from R using the linear relationship [5,10]:

\[ \psi_d = (R - 0.3) / \left(4.3 \times 10^{-3}\right) \]  

R values remained invariant after dilution of membrane samples, indicating the absence of any scattering artifacts [49].

2.2.8. Statistical analysis

Significance levels were estimated using Student’s two-tailed unpaired t-test using Graphpad Prism software version 4.0 (San Diego, CA).

3. Results and discussion

3.1. Effect of cholesterol depletion on the dipole potential of hippocampal membranes

Membrane cholesterol content can be modulated using MβCD. MβCD is a water-soluble cyclic polyosaccharide with a nonpolar central cavity. It is known to efficiently extract cholesterol from cellular membranes [27,50,51] and has been used previously to extract cholesterol from hippocampal membranes [33,37]. Cholesterol content of hippocampal membranes shows progressive reduction upon treatment with increasing concentrations of MβCD (see Fig. 2a). The cholesterol content is reduced to ~78% of the initial (control) level when native membranes were treated with 10 mM MβCD. The extent of cholesterol depletion was maximal when 40 mM MβCD was used. Cholesterol was reduced to ~17% of the control under these conditions (see Fig. 2a). Importantly, total phospholipid content remained unaltered upon MβCD treatment (not shown), confirming the selective extraction of cholesterol by MβCD under these conditions.

Dipole potential of hippocampal membranes was monitored utilizing the dual wavelength ratiometric approach with the electrochromic probe di-8-ANEPPS. This method for the measurement of dipole potential has previously been used in a number of studies [10,11,47,48]. We recently showed, using the parallax method [52], that the fluorescent styrlypyridinium group in di-8-ANEPPS is localized at the membrane interface, at a distance of ~12 Å from the center of the bilayer [11]. The fluorescence ratio (R) of the potential-sensitive styrlypyridinium probe, di-8-ANEPPS, is sensitive to any change in the dipolar field at the membrane interface where the probe is localized. This is believed to be due to electrochromic mechanism [45]. According to this mechanism, the spectral shift displayed by a charge transfer fluorescent probe such as di-8-ANEPPS is related to the electric field strength. It should be mentioned that the fluorescence ratio (R) of di-8-ANEPPS has been shown to be sensitive to only dipole potential and is independent of specific molecular interactions [48,53]. Fig. 2b shows the dipole potential of hippocampal membranes with increasing cholesterol depletion. The figure shows that the dipole potential of native hippocampal membranes is ~287 mV. The membrane dipole potential displays progressive reduction with increasing cholesterol depletion (i.e., decreasing membrane cholesterol content, see Fig. 2a) and attains a value of ~227 mV when 40 mM MβCD was used (corresponding to membrane cholesterol content ~17% of control value). This is in agreement with previous results in which it was shown that membrane dipole potential increases with increasing cholesterol content [10,11]. Fig. 2c shows a plot of membrane dipole potential vs. cholesterol.
content. A linear regression analysis between dipole potential and cholesterol content yielded a positive correlation of ~0.99. Such a strong correlation between dipole potential and cholesterol content could have functional implications measured in terms of receptor activity (see later).

3.2. Effect of cholesterol and its immediate biosynthetic precursor on receptor activity and dipole potential in hippocampal membranes

Hippocampal membranes are isolated from the hippocampal region of the brain. They are neuronal in origin and have been established as a rich source of GPCRs such as the serotonin1A receptor. Although the membrane lipid composition of bovine hippocampus is not known, the phospholipid composition of rat hippocampus has earlier been reported [54–56]. Analysis of the phospholipid composition of the rat hippocampus shows phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine as the predominant headgroups, while the fatty acid composition shows enrichment with 16:0, 18:0, 18:1, 18:2, 20:4, and 22:6 fatty acids. In addition, plasmalogens have been reported in rat hippocampus. Hippocampal membranes are rich in protein and cholesterol [44]. Previous work from our laboratory has established native hippocampal membranes as a convenient natural source for exploring the interaction of the serotonin1A receptor, an important member of the GPCR family, with membrane lipids [33,57].

We have previously demonstrated the requirement of membrane cholesterol in modulating ligand binding function of the hippocampal serotonin1A receptor [33,37,58–61]. The effect of cholesterol and its immediate biosynthetic precursor 7-DHC on the ligand binding activity of the hippocampal serotonin1A receptor is shown in Fig. 3. Fig. 3a shows the extents of replenishment of cholesterol and 7-DHC into cholesterol-depleted hippocampal membranes replenished with MβCD-sterol complex. Treatment of membranes with 40 mM MβCD resulted in ~61% reduction in the cholesterol content (Fig. 3a). This is accompanied by a corresponding reduction (~56%) in the specific [3H]8-OH-DPAT binding (see Fig. 3b). Replenishment with cholesterol-MβCD complex resulted in recovery of specific [3H]8-OH-DPAT binding to ~65% of native membranes (Fig. 3b) when ~86% of the cholesterol could be replenished (Fig. 3a). We have previously reported by saturation binding analysis that the binding of the agonist [3H]8-OH-DPAT to

![Fig. 2. Effect of membrane cholesterol content on dipole potential of hippocampal membranes. (a) Cholesterol content in hippocampal membranes upon treatment with increasing concentrations of methyl-β-cyclodextrin (MβCD). (b) Dipole potential of hippocampal membranes upon cholesterol depletion with increasing concentrations of MβCD. (c) Correlation between dipole potential and cholesterol content in hippocampal membranes.](image-url)

![Fig. 3. Effect of 7-DHC and cholesterol on receptor activity in hippocampal membranes. (a) Sterol content of hippocampal membranes upon cholesterol depletion and sterol replenishment. The concentration of MβCD used for cholesterol depletion was 40 mM and the ratio of sterol: MβCD used for sterol replenishment was 1:10. Cholesterol (crisscrossed bar) and 7-DHC (hatched bar) were separated on thin layer chromatogram and estimated by densitometry. Values are expressed as percentages of the cholesterol content in native membranes (in the absence of any treatment). Data represent means ± S.E. of five independent experiments. (b) Specific [3H]8-OH-DPAT binding to serotonin1A receptors in hippocampal membranes replenished with cholesterol and 7-DHC. Values for specific [3H]8-OH-DPAT binding under these conditions are from [37].](image-url)
the hippocampal serotonin₁A receptors is characterized by a dissociation constant ($K_d$) of ~0.4 nM and maximum binding sites ($B_{max}$) of ~125 fmol/mg. The reduction in specific agonist binding, upon cholesterol depletion using 40 mM MβCD, could be attributed to a reduction in the number of total binding sites with a marginal reduction in the affinity of ligand binding ($B_{max}$ ~90 fmol/mg and $K_d$ ~0.5 nM in case of cholesterol-depleted membranes) [33].

In order to monitor whether replenishment with 7-DHC could restore the specific $[^{3}H]8$-OH-DPAT binding, cholesterol-depleted membranes were replenished with 7-DHC using 7-DHC–MβCD complex. Interestingly, the specific agonist binding could not be restored upon replenishment with 7-DHC (Fig. 3b) in spite of the fact that the extent of loading of 7-DHC was higher than what was obtained with cholesterol replenishment (Fig. 3a). This is attributed to the inability of 7-DHC in restoring the specific agonist binding activity of the hippocampal serotonin₁A receptor [37].

With an overall goal of correlating these sterol-dependent functional changes with membrane properties, we measured dipole potential of hippocampal membranes under these conditions. Fig. 4 shows that dipole potential of native hippocampal membranes is ~287 mV and is reduced to ~227 mV upon cholesterol depletion with 40 mM MβCD. Interestingly, cholesterol replenishment into hippocampal membranes resulted in an increase of dipole potential to ~254 mV with a concomitant recovery of specific agonist binding (see Fig. 3b). In contrast to this, the increase in dipole potential of hippocampal membranes upon replenishment with 7-DHC is not significant and was found to be ~232 mV. These results show that replenishment with 7-DHC does not restore dipole potential in hippocampal membranes. This is in agreement with our recent results that the ability of a sterol to modulate membrane dipole potential could vary considerably depending on sterol structure. Even a subtle difference such as a double bond (as is the case with cholesterol and 7-DHC) in the sterol structure can have drastic effect on the ability of the sterol to influence membrane dipole potential [11].

### 3.3. Effect of cholesterol and its immediate biosynthetic precursor on dipole potential in gel and fluid phase membranes

Natural membranes such as the hippocampal membrane are characterized by complex composition and co-existing phases [62,63]. In order to explore the role of membrane phase on the sterol-induced modulation of membrane dipole potential, we chose to examine the effect of cholesterol and 7-DHC on dipole potential in representative fluid (liquid-disordered), and gel (ordered) phase membranes. The effect of these sterols on membrane dipole potential in fluid phase 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membranes has recently been reported [11] and is shown in Fig. 5a. The figure shows that the dipole potential of POPC membranes in the fluid state exhibits a progressive increase with increase in cholesterol concentration. This results in an increase of dipole potential from ~363 mV (in absence of sterol) to ~580 mV in presence of 40 mol% cholesterol. The corresponding change in dipole potential with 7-DHC was rather modest, resulting in a membrane dipole potential of ~430 mV in presence of 40 mol% 7-DHC (this amount to an increase of ~18% in contrast to ~60% increase in case of cholesterol). The dipole potential of gel phase DPPC membranes in absence of sterols was estimated to be ~334 mV (see Fig. 5b). Interestingly, the effect of cholesterol on membrane dipole potential in the gel phase appears to be more pronounced, possibly due to less involvement of water dipoles (since water penetration is less in the gel phase due to tighter packing of lipids). The dipole potential of DPPC membranes displays a concentration-dependent increase with increasing cholesterol content and reaches a value of ~862 mV (~158% increase) when the cholesterol concentration was 30 mol%. Upon further increase in cholesterol concentration, a small reduction in dipole potential is observed. In case of 7-DHC, the membrane dipole potential for DPPC membranes shows a modest increase up to 30 mol%, followed by a small reduction. Again, the increase in dipole potential with increasing 7-DHC is rather less (~27%) compared to the corresponding value for cholesterol (~158%, see above). An important message from these results is that the effect of 7-DHC on the membrane dipole potential is much less than that of cholesterol, independent of the phase state of the membrane.

As stated earlier, our overall objective of extending dipole potential measurements to complex natural membranes such as the hippocampal membrane and its sterol content was to explore the role of dipole potential in the mechanism of receptor–cholesterol interaction and its functional correlates. We have previously shown that the interfacial dielectric constant of hippocampal membranes increases upon cholesterol depletion [64]. The reduction in dipole potential in
hippocampal membranes with cholesterol depletion could be attributed in part to this increase in dielectric constant, since an increase in dielectric constant would reduce dipole potential [2,10,11]. Fig. 6 shows the relevance of membrane dipole potential in the context of receptor activity, as measured by specific $[^{3}H]$8-OH-DPAT binding. The apparent correlation observed between membrane dipole potential change and receptor activity under conditions of cholesterol depletion is indeed encouraging.

In summary, we show here that the recovery of ligand binding activity of the serotonin1A receptor upon replenishment with cholesterol but not with 7-DHC could be related to the differential ability of these closely related sterols to modulate membrane dipole potential. A lower value of membrane dipole potential in presence of 7-DHC can possibly be attributed to factors such as lower dipole moment of 7-DHC (1.42 D) relative to cholesterol (1.87 D) due to possible shortening of bond length, and difference in tilt angle in the membrane [11]. This could be further due to the differential ability of cholesterol and 7-DHC to polarize water molecules at the membrane interface [10,65].

In the context of hippocampal membranes, which are of neuronal origin, cholesterol represents an important lipid since it is known to regulate the function of neuronal receptors [27,28,66], thereby affecting neurotransmission and giving rise to mood and anxiety disorders [67]. In a broader perspective, our results are significant in understanding the complex organization of neuronal membranes, and could have functional implications in neuronal diseases such as the Smith–Lemli–Opitz syndrome [15,34] characterized by low cholesterol (and high 7-DHC) condition due to defective cholesterol biosynthesis.

As mentioned earlier, membrane dipole potential has been reported to influence the function of membrane proteins and peptides such as Na$^+$/K$^+$-ATPase [5], the ion channel gramicidin [6,7], and the membrane insertion and folding of the amphiphilic peptides such as p25 [8], and the simian immunodeficiency virus with membranes. Role of the membrane dipole potential, J. Biol. Chem. 274 (1999) 29951–29959.


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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2012.11.022

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