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Research Article

Solubilization of the serotonin$_{1A}$ receptor monitored utilizing membrane dipole potential

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ABSTRACT

Solubilization of membrane proteins by amphiphilic detergents represents a crucial step in studies of membrane proteins in which proteins and lipids in natural membranes are dissociated giving rise to mixed clusters of proteins, lipids and detergents in the aqueous dispersion. Although solubilization is a popular method, physicochemical principles underlying solubilization are not well understood. In this work, we monitored solubilization of the bovine hippocampal serotonin$_{1A}$ receptor, a representative member of the GPCR family, using membrane dipole potential measured by a dual fluorescence ratiometric approach with a potential-sensitive fluorophore. Our results show that membrane dipole potential is a good indicator of solubilization and reflects the change in dipolar environment upon solubilization due to dipolar reorganization associated with solubilization. To the best of our knowledge, these results constitute the first report linking membrane dipole potential with solubilization. We envision that these results are potentially useful in providing a molecular mechanism for membrane protein solubilization.

1. Introduction

Solubilization of membrane proteins constitutes an important step in their purification (Kalipatnapu and Chattopadhyay, 2005; Duquesne and Sturgis, 2010; Kubicek et al., 2014; Chattopadhyay et al., 2015). Solubilization enables proteins and lipids in natural membranes to be dissociated by use of a suitable amphiphilic detergent. The dissociation of the natural membrane components results in the formation of small mixed clusters of proteins, lipids and detergents in aqueous dispersion. A hallmark of effective solubilization is that the function of a given membrane protein be retained to a considerable extent. This could prove to be tricky, since many detergents induce irreversible denaturation of membrane proteins (Garavito and Ferguson-Miller, 2001). In case of G protein-coupled receptors (GPCRs), an important class of membrane receptors that act as signaling hubs and major drug targets (Rosenbaum et al., 2009; Chattopadhyay, 2014; Jacobson, 2015), solubilization and purification from natural sources poses considerable challenge due to low amounts of receptor present in the native tissue.

Dipole potential represents an important electric potential in organized molecular assemblies (such as membranes or micelles) and it originates due to the nonrandom orientation of electric dipoles (lipids, detergents, proteins, water molecules) inside the assembly (Brockman, 1994; Clarke, 2001; O'Shea, 2005; Wang, 2012; Sarkar and Chattopadhyay, 2015). The increasing application of dipole potential to problems related to biological and model membranes is evident from a growing body of literature in this area. Membrane dipole potential has been shown to provide novel information on the nature of membrane lipids (Starke-Peterkovic et al., 2006; Starke-Peterkovic and Clarke, 2009; Haldar et al., 2012; Bandari et al., 2014), function and lipid interactions of membrane proteins (Cladera and O'Shea, 1998; Duffin et al., 2003; Starke-Peterkovic et al., 2005; Singh et al., 2013; Chaudhuri and Chattopadhyay, 2014; Clarke, 2015; Richens et al., 2015; Sarkar et al., 2017).

With an overall goal to explore lipid specificities in GPCR function, in our laboratory, we have focused on lipid interactions of the serotonin$_{1A}$ receptor. The serotonin$_{1A}$ receptor is a representative GPCR that serves as a crucial neurotransmitter receptor, and is implicated in behavior, learning, development and cognition (Pucadyil et al., 2005; Müller et al., 2007). Importantly, the serotonin$_{1A}$ receptor represents an important drug target for neuropsychiatric disorders such as anxiety and depression and in neuronal developmental defects (Fiorino et al., 2014). In our previous work, we demonstrated the requirement of

Abbreviations: BCA, bicinchoninic acid; BSA, bovine serum albumin; 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; CMC, critical micelle concentration; di-8-ANEPPS, 4-(2-(6-(dioctylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-pyridinium inner salt; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; GPCR, G protein-coupled receptor; HLB, hydrophile-lipophile balance; PMSF, phenylmethylsulfonyl fluoride

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membrane cholesterol (Pucadyil and Chattopadhyay, 2006; Paila and Chattopadhyay, 2010; Jafurulla and Chattopadhyay, 2013) and sphingolipids (Jafurulla and Chattopadhyay, 2015) in the function of the serotonin$_{1A}$ receptor. In the process, we utilized solubilization of the receptor and the lipid loss associated with it as a convenient strategy to explore lipid-receptor interaction (Chattopadhyay et al., 2015). The process of solubilization involves reorganization of membrane components and lipid-protein interaction (Valpuesta et al., 1986; de Foresta et al., 1989). Solubilized membranes are composed of heterogeneous complexes of detergent, lipid and protein (Kalipatnapu and Chattopadhyay, 2005; Singh et al., 2011; Chattopadhyay et al., 2015). They are more disordered (loosely packed) relative to native membranes, thereby inducing increased water penetration. The dielectric environment in solubilized membranes could therefore be considerably different from natural membranes. Keeping this in mind, we monitored membrane dipole potential along with solubilization (as monitored by specific ligand binding to the serotonin$_{1A}$ receptor) using a potential-sensitive fluorescent probe by a dual wavelength ratiometric approach. Our results show that membrane dipole potential is well correlated with the extent of solubilization.

2. Materials and methods

2.1. Materials

BSA, CHAPS, DMPC, EDTA, EGTA, iodoacetamide, $MgCl_2$, $MnCl_2$, NaCl, Na_2HPO_4, PEG, PMSF, polyethylenimine, serotonin, sucrose, sodium azide, and Tris were obtained from Sigma Chemical Co. (St. Louis, MO). Di-8-ANEPPS was purchased from Molecular Probes/Invitrogen (Eugene, OR). BCA reagent for protein estimation was from Pierce (Rockford, IL). [^{3}H]8-OH-DPAT (sp. activity 141 Ci/mmol) was purchased from MP Biomedicals (Santa Ana, CA). GF/B glass micro fiber filters were from Whatman International (Kent, U.K.). All other chemicals used were of the highest available purity. 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 −80 °C till further use.

2.2. Methods

2.2.1. Preparation of native hippocampal membranes

Native hippocampal membranes were prepared as described previously (Harikumar and Chattopadhyay, 1999). Bovine hippocampal tissue (~50 g) was homogenized as 10% (w/v) in a polytron homogenizer in 2.5 mM Tris, 0.32 M sucrose, 5 mM EDTA, 5 mM EGTA, 0.02% sodium azide, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4 buffer. The homogenate was centrifuged at 900 × *g* for 10 min at 4 °C. The resultant supernatant was filtered through four layers of cheesecloth and centrifuged at 50,000 × *g* for 20 min at 4 °C. The pellet obtained was suspended in 10 vols of 50 mM Tris, 1 mM EDTA, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4 buffer using a hand-held Dounce homogenizer and centrifuged at 50,000 × *g* for 20 min at 4 °C. This procedure was repeated until the supernatant was clear. The final pellet (native hippocampal membranes) was suspended in a minimum volume of 50 mM Tris, pH 7.4, homogenized using a hand-held Dounce homogenizer, flash frozen in liquid nitrogen and stored at −80 °C. The protein concentration was assayed using the BCA reagent (Smith et al., 1985).

2.2.2. Solubilization of native membranes

Hippocampal membranes were solubilized as described previously using the zwitterionic detergent CHAPS (Chattopadhyay and Harikumar, 1996; Chattopadhyay et al., 2002, 2005; Jafurulla et al., 2014). Native hippocampal membranes were incubated with 5 mM CHAPS and 1 M NaCl in buffer A (50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, pH 7.4) at a final protein concentration of ~2 mg/ml for 30 min at 4 °C with occasional shaking. The membranes were briefly sonicated (~5 s) using a Branson model 250 sonifier at the beginning of the incubation period, and mildly homogenized using a hand-held Dounce homogenizer at the beginning and end of the incubation period. After incubation for 30 min at 4 °C, the contents were centrifuged at 100,000 × *g* for 1 h at 4 °C. The clear supernatant containing CHAPS-solubilized membrane was carefully removed from the pellet, and was reconstituted using PEG (termed solubilized membranes). PEG precipitation was performed to remove NaCl from the solubilized extract, since the agonist binding of the serotonin$_{1A}$ receptor is inhibited by NaCl (Harikumar and Chattopadhyay, 1998). This procedure is believed to remove detergent and salt (Gal et al., 1983; Kremenetzky and Atlas, 1984). PEG precipitation was carried out by diluting the extract with equal volume of 40% (w/w) PEG-8000 in buffer A. Following vigorous vortexing and incubation for 10 min on ice, the samples were centrifuged at 15,000 × *g* for 10 min at 4 °C. The pellet was carefully rinsed twice with buffer A, resuspended in buffer A and used for radioligand binding assays or dipole potential measurements.

2.2.3. Radioligand binding assays

Receptor binding assays were carried out as described earlier (Pucadyil and Chattopadhyay, 2004) with some modifications. Tubes in duplicate with ~1 mg native hippocampal membrane protein (or ~0.5 mg for solubilized membranes) in a total volume of 1 ml of 50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM $MnCl_2$, pH 7.4 buffer were incubated with the radiolabeled agonist [^{3}H]8-OH-DPAT (final concentration in assay tube being 0.5 nM) for 1 h at 25 °C. Nonspecific binding was determined by performing the assay in the presence of 10 μM serotonin. The binding reaction was terminated by rapid filtration under vacuum in a Millipore multiport filtration apparatus through Whatman GF/B 2.5 cm diameter glass micro fiber filters (1 μm pore size), which were presoaked in 0.3% polyethylenimine for 1 h (Bruns et al., 1983). Filters were then washed three times with 3 ml of cold water (4 °C) and dried. The retained radioactivity was measured in a Packard Tri-Carb 2900 liquid scintillation counter using 5 ml of scintillation fluid.

2.2.4. Estimation of cholesterol

Cholesterol content in native and solubilized membranes was estimated using Amplex Red cholesterol assay kit (Amundson and Zhou, 1999).

2.2.5. Estimation of inorganic phosphate

The concentration of lipid phosphate was determined subsequent to total digestion by perchloric acid (McClare, 1971) using Na_2HPO_4 as standard. DMPC was used as an internal standard to assess lipid digestion. Samples without perchloric acid digestion produced negligible readings.

2.2.6. Sample preparation for dipole potential measurements

Di-8-ANEPPS was added from a methanolic stock solution to native or solubilized hippocampal membranes containing 100 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 (Le Goff et al., 2007). The final di-8-ANEPPS concentration was 0.66 μM in all cases and methanol content was always low (0.02%, v/v). This ensures optimal fluorescence intensity with negligible membrane perturbation. Control experiments showed that at this concentration of methanol, ligand binding properties of the receptor are not altered (Pucadyil and Chattopadhyay, 2004). Di-8-ANEPPS was added to membranes while being vortexed for 1 min at room temperature (~23 °C). Background

samples were prepared the same way except that di-8-ANEPPS was not added to them. Samples were incubated in dark for 30 min at room temperature (~23 °C) for equilibration before measuring fluorescence. Experiments were performed with at least three sets of samples in triplicates at room temperature (~23 °C).

2.2.7. Measurement of dipole potential

Measurements were carried out by the dual wavelength ratiometric approach using the voltage-sensitive fluorescence probe di-8-ANEPPS (Gross et al., 1994; Clarke and Kane, 1997; Starke-Peterkovic et al., 2005, 2006; Haldar et al., 2012; Sarkar and Chattopadhyay, 2016). Steady state fluorescence measurements were performed with a Hitachi F-7000 (Tokyo, Japan) spectrofluorometer using 1 cm path length quartz cuvettes at room temperature (~23 °C). Excitation and emission slits with a 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 scattering artifacts. 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 (Starke-Peterkovic et al., 2006), which is a measure of membrane dipole potential. 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 (Clarke and Kane, 1997). Dipole potential (ψ_d) in mV was calculated from R using the linear relationship (Starke-Peterkovic et al., 2005, 2006):

$$\psi_d = (R + 0.3)/(4.3 \times 10^{-3}) \quad (1)$$

2.2.8. Statistical analysis

Significance levels were estimated using Student's two-tailed unpaired *t*-test using Graphpad Prism version 4.0 (San Diego, CA). The correlation between agonist binding activity of the serotonin$_{1A}$ receptor with membrane dipole potential was analyzed using the same software with 99% confidence interval. Plots were generated using Microcal Origin version 8.0 (OriginLab, Northampton, MA).

3. Results

We carried out the process of solubilization utilizing CHAPS, a mild, nondenaturing, and zwitterionic detergent (see Fig. 1a; Hjelmeland, 1980). CHAPS is a synthetic derivative of the naturally occurring bile salts and combines useful features of both the bile salt hydrophobic group and N-alkyl sulfobetaine-type polar group. CHAPS has been shown to be a more efficient detergent for solubilization than its parent bile acid (such as cholate) since it is effective in breaking protein-protein interactions, possibly due to lack of the negative charge of cholate. The other advantages of using CHAPS for solubilization are its low absorbance at 280 nm (unlike neutral detergents such as Triton X-100) and lack of strong circular dichroic signature in the far-UV region, thereby making it an ideal choice for studies of membrane proteins. For these reasons, CHAPS has been widely used for solubilization of membrane proteins and receptors. Use of CHAPS at high concentrations could be detrimental for effective solubilization of GPCRs in a functionally active form (Bayewitch et al., 2000). Keeping this in mind, we have previously fine-tuned and optimized solubilization of serotonin$_{1A}$ receptors using CHAPS at low (pre-micellar) concentration in the presence of NaCl followed by PEG precipitation (Chattopadhyay et al., 2002). PEG precipitation of the CHAPS-solubilized membranes helps to efficiently remove detergents and NaCl from the solubilized membrane (Medrano et al., 1989) since the agonist binding activity of the serotonin$_{1A}$ receptor is inhibited by NaCl (Harikumar and Chattopadhyay, 1998).

We have previously shown that CHAPS at a concentration of 5 mM

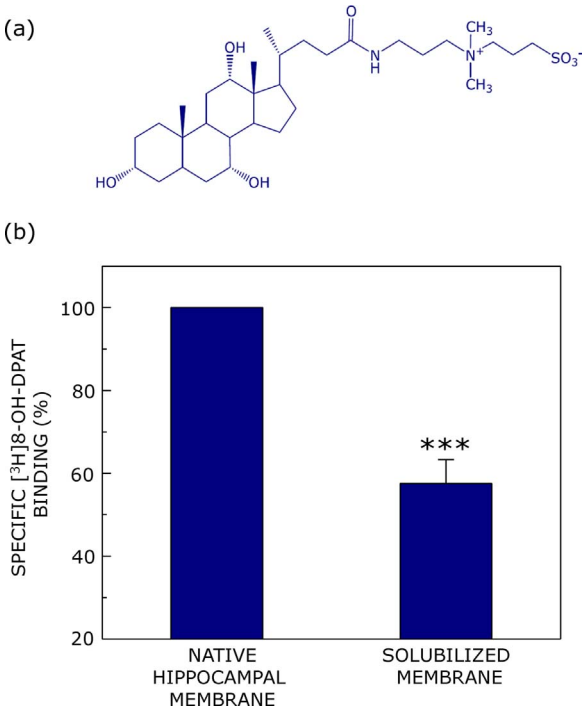

Fig. 1. (a) Chemical structure of the zwitterionic detergent CHAPS used for solubilization of serotonin$_{1A}$ receptors in this study. (b) Effect of solubilization of hippocampal membranes on the specific binding of the agonist [^{3}H]8-OH-DPAT to the serotonin$_{1A}$ receptor. Solubilization is expressed as a percentage of specific binding obtained in native hippocampal membranes. The figure shows mean ± S.E. of at least three independent experiments of samples in triplicate (*** corresponds to significant ($p < 0.001$) difference in specific ligand binding to solubilized membranes relative to native membranes). See Section 2 for more details.

in the presence of 1 M NaCl is optimal in solubilizing serotonin$_{1A}$ receptors from bovine hippocampal membranes (Chattopadhyay and Harikumar, 1996; Chattopadhyay et al., 2002, 2005). In this study, we monitored the solubilization of native hippocampal membranes containing the serotonin$_{1A}$ receptor by the specific agonist [^{3}H]8-OH-DPAT binding. The specific agonist binding of native and solubilized bovine hippocampal membranes are shown in Fig. 1b. The specific [^{3}H]8-OH-DPAT binding of the serotonin$_{1A}$ receptor exhibits a reduction (~43%) upon solubilization by CHAPS, relative to that obtained using native membranes. This is accompanied by a corresponding reduction (~55%) in cholesterol content of hippocampal membranes upon solubilization (see Fig. 2). These results are in agreement with previous work from us and others in which it was previously reported that solubilization of native hippocampal serotonin$_{1A}$ receptors using CHAPS results in loss of receptor activity and membrane cholesterol (Banerjee et al., 1990, 1995; Chattopadhyay et al., 2005; Singh et al., 2011).

In order to monitor changes in dipole potential associated with solubilization of hippocampal membranes, we carried out dipole potential measurements by a dual wavelength ratiometric approach using the voltage-sensitive probe, di-8-ANEPPS (see inset in Fig. 3b for chemical structure) (Gross et al., 1994; Clarke and Kane, 1997; Starke-Peterkovic et al., 2005, 2006; Sarkar et al., 2017). The dual wavelength ratiometric approach utilizing di-8-ANEPPS represents a convenient method to monitor membrane dipole potential. We have previously showed, using the parallax method (Chattopadhyay and London, 1987), that the fluorescent styrylpyridinium group in di-8-ANEPPS is localized at the membrane interface, at a distance of ~12 Å from the center of the bilayer (Haldar et al., 2012). The excitation spectrum of di-8-ANEPPS is sensitive to alterations in membrane dipole potential. Representative normalized fluorescence excitation spectra of di-8-ANEPPS in native and solubilized hippocampal membranes are shown in Fig. 3a. The useful parameter in this method is the fluorescence ratio (R) which is

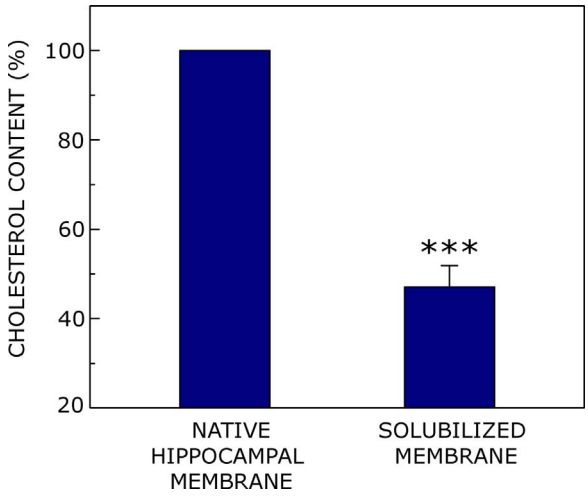

Fig. 2. Quantitation of cholesterol in native hippocampal membranes (control) and solubilized membranes. The figure shows mean ± S.E. of at least three independent experiments of samples in triplicates (*** corresponds to significant ($p < 0.001$) difference in cholesterol content in solubilized membranes relative to native membranes). See Section 2 for more details.

the ratio of fluorescence intensities at an excitation wavelength of 420 nm to that at 520 nm (indicated by arrows in Fig. 3a), keeping emission constant at 670 nm. The intensity ratio of di-8-ANEPPS is sensitive to changes in the dipolar field due to an electrochromic mechanism, resulting in a shift of di-8-ANEPPS excitation spectrum that could be correlated to the electric field strength (Loew et al., 1979; Le Goff et al., 2007). Interestingly, it has previously been shown that the ratio R for di-8-ANEPPS is sensitive to only dipole potential and is independent of specific molecular interactions (Gross et al., 1994; Robinson et al., 2011). Fig. 3a shows that solubilization of hippocampal membranes results in a red shift of di-8-ANEPPS fluorescence excitation spectra resulting in reduction of fluorescence intensity at 420 nm and increase in fluorescence intensity at 520 nm (marked by arrows). To obtain a quantitative estimate of R, we plotted R (averaged over three different experiments) for native and solubilized hippocampal membranes (see Fig. 3b). The figure shows that the dipole potential of native hippocampal membranes corresponds to ~335 mV and is reduced to ~297 mV upon solubilization, which corresponds to ~11% reduction in dipole potential. This is in agreement with previous results in which it was shown that membrane dipole potential decreases with reduction in membrane cholesterol content in model membranes (Starke-Peterkovic et al., 2006; Haldar et al., 2012). The reduction in membrane dipole potential with decrease in cholesterol could be due to the condensing ability of cholesterol and its ability to change water penetration in the membrane, both of which would contribute to the resultant dipolar environment of the membrane interface.

As stated above, our overall objective was to extend dipole potential measurements to complex natural membranes such as the hippocampal membrane and to explore the usefulness of membrane dipole potential as an indicator of the process of membrane protein solubilization. In order to gain insight into membrane dipole potential change and solubilization, we plotted the change in dipole potential upon solubilization, against the corresponding change in receptor activity. For this, we plotted specific agonist binding (values taken from Fig. 1b) with corresponding membrane dipole potential (from Fig. 3b). This is shown in Fig. 4 in which each data point represents a measurement of specific [^{3}H]8-OH-DPAT binding and the corresponding value of membrane dipole potential measured using the potential sensitive probe di-8-ANEPPS (as mentioned, the values shown in Figs. 1b and 3b are average of three measurements, each in triplicate). A linear regression analysis yielded a correlation coefficient (r) ~0.96. The significance of the correlation is apparent from the 99% confidence band (plotted as

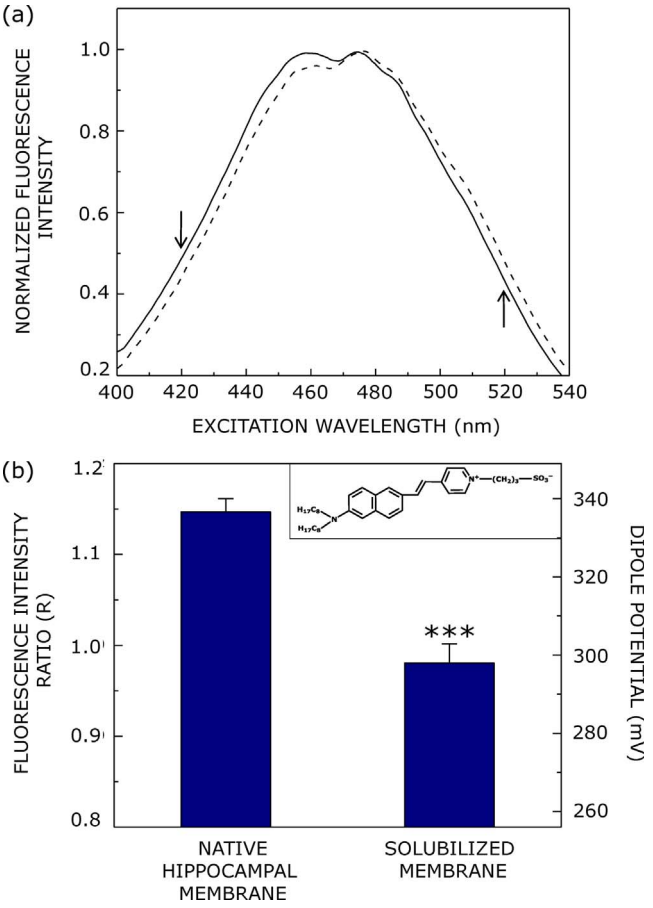

Fig. 3. Solubilization of hippocampal membranes monitored using the potential-sensitive probe di-8-ANEPPS. (a) Representative normalized excitation spectra of di-8-ANEPPS in native (—) and solubilized (—) hippocampal membranes. The ratio of di-8-ANEPPS to membrane phospholipids was 1:100 (mol/mol), and the concentration di-8-ANEPPS was 0.66 μM in both cases. The arrows indicate changes in di-8-ANEPPS fluorescence intensity upon solubilization corresponding to excitation wavelengths of 420 and 520 nm. (b) Effect of solubilization on dipole potential of hippocampal membranes. The fluorescence ratio (R) is 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). Measurements were carried out at room temperature (~23 °C). Data shown are means ± S.E. of three independent measurements of samples in triplicate (*** corresponds to significant ($p < 0.001$) difference in fluorescence intensity ratio (R) to solubilized membranes relative to native membranes). The inset shows the chemical structure of di-8-ANEPPS. See Section 2 for other details.

dashed lines) which contained all the data points. This points out to the intrinsic relationship between the dielectric environment of the membrane interior (monitored by dipole potential) and solubilization (followed by specific agonist binding). We would like to add here that although we mentioned above the dielectric environment in the membrane interior, in reality, it could be the dielectric environment in a mixture of membrane bilayer and micelles (sometimes called co-micelles).

As mentioned above, previous work from us and others showed that solubilization of native hippocampal serotonin$_{1A}$ receptors using CHAPS results in loss of membrane cholesterol (Banerjee et al., 1990, 1995; Chattopadhyay et al., 2005; Singh et al., 2011). Along with this, we and others previously reported that membrane dipole potential increases with increase in cholesterol content in model membranes containing binary lipid mixtures (Starke-Peterkovic et al., 2006; Haldar et al., 2012). A minor concern therefore arises from the trivial possibility that the reduction in dipole potential upon solubilization could be solely due to loss of cholesterol. To address this possibility, we plotted membrane cholesterol content upon solubilization against the corresponding value of dipole potential for each measurement (taking data

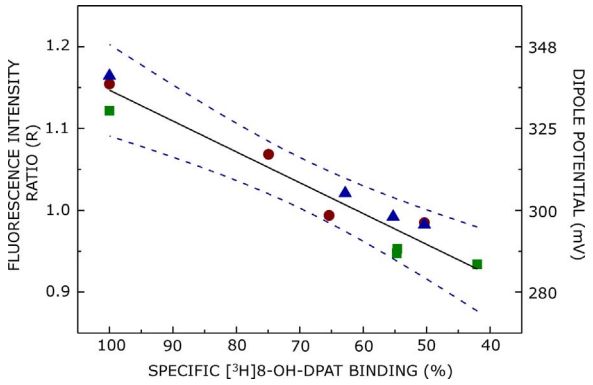

Fig. 4. Correlation of specific agonist binding activity of the serotonin$_{1A}$ receptor with membrane dipole potential. Every data point represents measurements of specific [^{3}H]8-OH-DPAT binding and the corresponding value of membrane dipole potential from three independent experiments each carried out in triplicate (please note that Figs. 1 and 3b represent means ± S.E. of all these data points). Each independent experiment is characterized by a combination of a specific color (maroon, blue and green) and shape (circle, triangle and square). A linear regression analysis yielded a correlation coefficient (r) ~0.96. The significance of the correlation is apparent from the 99% confidence band (plotted as dashed lines). The influence of membrane dipole potential on serotonin$_{1A}$ receptor activity is noteworthy. See Section 3 for more details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

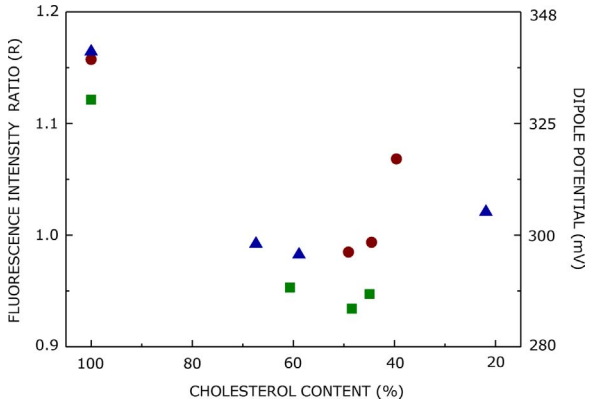

Fig. 5. Lack of correlation of membrane cholesterol content with dipole potential. Each data point represents a measurement of cholesterol content (normalized to native membranes; an arbitrary value of 100 was assigned to cholesterol content in native membranes) and the corresponding value of membrane dipole potential measured using the potential sensitive probe di-8-ANEPPS (data from Figs. 2 and 3b). For explanation on the color and shape of the data points, please see the legend to Fig. 4. The lack of correlation of cholesterol content with dipole potential implies that dipole potential depends on other factors in a complex heterogeneous natural membrane, besides cholesterol. See Section 3 for more details.

from Figs. 2 and 3b; the values shown in Figs. 2 and 3b are average of three measurements, each in triplicate). This is shown in Fig. 5 in which each data point represents a measurement of cholesterol content and the corresponding value of membrane dipole potential measured using the potential sensitive probe di-8-ANEPPS. Fig. 5 shows lack of correlation between membrane cholesterol content with dipole potential. The lack of correlation (scatter) of cholesterol content with dipole potential implies that dipole potential depends on a number of factors in a complex heterogeneous natural membrane. This is in contrast to model membranes composed of binary lipids (Starke-Peterkovic et al., 2006; Haldar et al., 2012). We conclude that adequate caution should be exercised in interpreting change in dipole potential in complex natural membranes, and linear extrapolation of results from model membranes could be misleading.

4. Discussion

Efficient solubilization resulting in functional GPCRs requires a suitable detergent. The ability of a detergent to solubilize membranes depends on the choice of detergent in terms of its hydrophile-lipophile balance (HLB), an empirical parameter that provides a measure of the hydrophilic character of a detergent (Helenius and Simons, 1975; Neugebauer, 1990). Detergents with a relatively high HLB value (~12–20) are generally recommended for efficient solubilization of membrane proteins without loss of function (Bhairi and Mohan, 2001). The concept of micellization of detergents above a critical concentration, termed as critical micelle concentration (CMC), plays an important role in solubilization and reconstitution of membrane receptors. Use of detergents at concentrations above their CMC is often accompanied by a loss of membrane receptor function. Interestingly, the mechanism by which detergents solubilize membranes at concentrations below CMC remains speculative (Chattopadhyay and Harikumar, 1996; Chattopadhyay et al., 2002). This has given rise to the interesting concept of 'effective CMC' (Rivnay and Metzger, 1982; Jones et al., 1987; Chattopadhyay and Harikumar, 1996; Schürholz, 1996) which is the concentration of detergent existing as monomers at a given experimental condition and may vary from literature CMC values since the latter are measured in standard conditions. Another important parameter is the critical solubilization concentration (CSC), which is the minimal detergent concentration required to disrupt a given membrane into micellar dispersion (Privé, 2007). Yet another important parameter in membrane solubilization is the relative detergent-lipid-protein ratio. An empirical relationship between these experimental parameters has been developed in which the parameter (ρ) is defined as the molar ratio of detergent to lipid optimal for functional solubilization (Rivnay and Metzger, 1982).

$$\rho = \frac{[\text{Detergent}] - \text{CMC}_{\text{eff}}}{[\text{Phospholipid}]}$$

where CMC_{eff} represents the effective CMC determined under specific experimental conditions. An increase in solubilization is expected with increase in the value of ρ parameter. How all these parameters relate to the membrane dipolar field will be interesting to explore in future studies.

In this work, we report that the process of solubilization involves profound dipolar reorganization which gets manifested as changes in membrane dipole potential. To the best of our knowledge, the present results constitute the first report correlating membrane protein solubilization with dipole potential. We should note here that our dipole potential measurements are carried out in organized assemblies that are strictly not membranes, but represent co-micelles (mixture of membranes and micelles) due to the solubilization process. While this may have some caveat, we believe that the general feature of our results are not affected by it. We therefore envision that extending this type of studies using various combinations of detergents and a variety of GPCRs in different lipid environments would help arrive at a comprehensive understanding of solubilizing abilities of various detergents in different environments of membrane lipids. In addition, a comprehensive understanding of membrane solubilization in physicochemical terms would help in understanding the reverse process of assembly of membranes (reconstitution) and preparation of crystals of membrane proteins for x-ray crystallographic studies, since the underlying physical chemistry would be overlapping. In summary, our results imply that dipolar reorganization is an important molecular aspect of the process of membrane protein solubilization. An understanding of these processes could lead to more effective strategies of solubilization, along with a better understanding of lipid-protein interaction in biological membranes since solubilization often results in partial or complete loss of protein activity.

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