



Short communication

Molecular rheology of neuronal membranes explored using a molecular rotor: Implications for receptor function



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ARTICLE INFO

Article history:

Received 14 December 2015

Received in revised form 23 January 2016

Accepted 10 February 2016

Available online 12 February 2016

Keywords:

Molecular rotor

Hippocampal membrane

Viscosity

Cholesterol

Neuronal receptor

ABSTRACT

The role of membrane cholesterol as a crucial regulator in the structure and function of membrane proteins and receptors is well documented. However, there is a lack of consensus on the mechanism for such regulation. We have previously shown that the function of an important neuronal receptor, the serotonin_{1A} receptor, is modulated by cholesterol in hippocampal membranes. With an overall objective of addressing the role of membrane physical properties in receptor function, we measured the viscosity of hippocampal membranes of varying cholesterol content using a *meso*-substituted fluorophore (BODIPY-C₁₂) based on the BODIPY probe. BODIPY-C₁₂ acts as a fluorescent molecular rotor and allows measurement of hippocampal membrane viscosity through its characteristic viscosity-sensitive fluorescence depolarization. A striking feature of our results is that specific agonist binding by the serotonin_{1A} receptor exhibits close correlation with hippocampal membrane viscosity, implying the importance of global membrane properties in receptor function. We envision that our results are important in understanding GPCR regulation by the membrane environment, and is relevant in the context of diseases in which GPCR signaling plays a major role and are characterized by altered membrane fluidity.

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1. Introduction

Biological membranes are complex, highly organized, two-dimensional, supramolecular assemblies of a diverse variety of lipids and proteins. The function of membranes is to allow cellular compartmentalization, and impart an identity to individual cells and organelles, besides providing an appropriate environment for proper functioning of membrane proteins. Interestingly, cellular

membranes in the nervous system are characterized by very high concentration and remarkable diversity of lipids, and these are correlated with increased complexity in the function of the nervous system (Sastry, 1985; Wenk, 2005). In this context, cholesterol represents an important lipid since brain cholesterol has been implicated in a number of neurological disorders (Chattopadhyay and Paila, 2007; Martín et al., 2014), some of which share a common etiology of defective cholesterol metabolism in the brain (Porter and Herman, 2011). More importantly, the function of neuronal receptors depends on cholesterol (Pucadyil and Chattopadhyay, 2006; Allen et al., 2007; Paila and Chattopadhyay, 2010; Jafurulla and Chattopadhyay, 2013), which affects neurotransmission, resulting in mood and anxiety disorders (Papakostas et al., 2004). In spite of these important functional correlates, the organization and dynamics of neuronal membranes as a consequence of alterations in membrane cholesterol is only beginning to be understood.

We have earlier established native hippocampal membranes as a convenient natural source for exploring the interaction of

Abbreviations: 2-AS, 2-(9-anthroyloxy)stearic acid; 12-AS, 12-(9-anthroyloxy)stearic acid; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; GPCR, G protein-coupled receptor; 8-OH-DPAT, 8-hydroxy-2-(di-N-propylamino)tetralin; 5-PC, 1-palmitoyl-2-(5-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; 12-PC, 1-palmitoyl-2-(12-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; M β CD, methyl- β -cyclodextrin; Tempo-PC, 1,2-dioleoyl-*sn*-glycero-3-phosphotempocholine.

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neuronal receptors such as the serotonin_{1A} receptor, with membrane lipids of neuronal origin (Pucadyil and Chattopadhyay, 2004). In this overall context, we have demonstrated the requirement of membrane cholesterol in modulating the function of the serotonin_{1A} receptor (Pucadyil and Chattopadhyay, 2004, 2006; Paila et al., 2008; Paila and Chattopadhyay, 2010; Shrivastava et al., 2010; Jafurulla and Chattopadhyay, 2013). A continuing effort in our laboratory has been to explore how to correlate these cholesterol-dependent functional changes of the serotonin_{1A} receptor with alterations in membrane organization and dynamics (Mukherjee et al., 2007; Saxena et al., 2008; Singh et al., 2012).

In this work, we have monitored the change in viscosity associated with cholesterol depletion in hippocampal membranes using viscosity-dependent fluorescence depolarization of BODIPY-C₁₂ (Levitt et al., 2009), a *meso*-substituted molecular rotor based on the BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) structure (see Fig. 1a). BODIPY is a popular fluorescent probe and is characterized by high extinction coefficient, quantum yield and photostability (Johnson et al., 1991). Fluorescent molecular rotors are interesting reporter molecules that are generally characterized by an excited state process that is dependent on an intramolecular rotational motion (Haidekker et al., 2010; Levitt et al., 2011; Kuimova, 2012). A fluorescent molecular rotor typically consists of an electron donor moiety covalently linked to an electron acceptor unit *via* a spacer that conjugates the donor and the acceptor units, and facilitates electron movement between the two. The donor and acceptor groups assume a planar or near-planar conformation (with respect to each other) in the ground state. Electronic excitation triggers an intramolecular charge transfer from the donor to the acceptor *via* a twisting of the bond connecting them. This leads to a non-planar excited state conformation termed as twisted internal charge transfer (TICT)

state characterized by a lower energy. The radiative relaxation from the TICT state is associated with a red-shifted fluorescence emission. The relaxation of these rotors on photoexcitation is therefore coupled to rotations of the donor and acceptor units relative to each other. The rotation of the donor and acceptor groups in the excited state is extremely sensitive to the viscosity of the environment. This property makes molecular rotors superior viscosity probes.

A major application of molecular rotors is to measure microviscosity in biological systems since the intramolecular rotation depends on viscosity of the immediate environment in which the molecular rotor is localized (Haidekker and Theodorakis, 2007, 2010; Kuimova et al., 2008; Levitt et al., 2009; Wu et al., 2013). Our results using BODIPY-C₁₂ show that the viscosity of hippocampal membranes exhibits reduction under conditions of cholesterol depletion. More importantly, receptor activity (measured as specific binding of radioligand) exhibits a close correlation with hippocampal membrane viscosity, thereby implying the importance of global membrane properties in receptor activity.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphotempocholine (Tempo-PC), 1-palmitoyl-2-(5-doxyl)stearoyl-*sn*-glycero-3-phosphocholine (5-PC), and 1-palmitoyl-2-(12-doxyl)stearoyl-*sn*-glycero-3-phosphocholine (12-PC) were obtained from Avanti Polar Lipids (Alabaster, AL). 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), methyl- β -cyclodextrin (M β CD), EDTA, EGTA, MgCl₂, MnCl₂, iodoacetamide, phenylmethylsulfonyl fluoride (PMSF), sucrose, Na₂HPO₄, sodium azide, Tris, and sodium acetate were obtained from Sigma Chemical Co. (St. Louis, MO). [³H]8-OH-DPAT (sp. activity 134.1Ci/mmol) was purchased from MP Biomedicals (Santa Ana, CA). Bicinchoninic acid (BCA) reagent for protein estimation was from Pierce (Rockford, IL). *n*-AS probes (2- and 12-(9-anthroyloxy) stearic acid) and Amplex Red cholesterol assay kit were from Molecular Probes/Invitrogen (Eugene, OR). BODIPY-C₁₂ was synthesized as described previously (Levitt et al., 2009). DOPC was checked for purity by thin layer chromatography on silica gel precoated plates obtained from Merck (Darmstadt, Germany) in chloroform/methanol/water (65:35:5, v/v/v) and was found to give only one spot with a phosphate-sensitive spray and upon subsequent charring (Baron and Coburn, 1984). Concentrations of stock solutions of *n*-AS probes and BODIPY-C₁₂ in methanol were estimated using the molar extinction coefficients (ϵ_m) of 8000 and 82100 M⁻¹cm⁻¹ at 361 nm (Haugland, 1996) and 492 nm (López Arbeloa et al., 1999), respectively. Solvents used were of spectroscopic grade. 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. Estimation of phospholipids

The concentration of DOPC was determined by phosphate assay subsequent to total digestion by perchloric acid (McClare, 1971). DMPC was used as an internal standard to assess lipid digestion. Samples without perchloric acid digestion produced negligible readings. The phosphate content of native and cholesterol-depleted hippocampal membranes was estimated by phosphate assay.

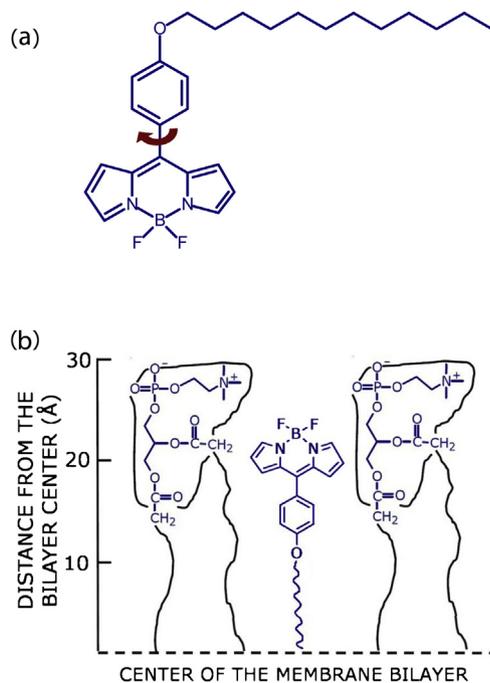


Fig. 1. (a) The chemical structure of the fluorescent molecular rotor BODIPY-C₁₂ used in this study. The free rotation of the C—C single bond connecting the BODIPY moiety with the benzene ring (marked by a curved arrow) depends on viscosity of the medium and this phenomenon modulates the photophysical properties of the probe. The hydrophobic C₁₂ chain ensures that the molecule partitions into the membrane. See text for other details. (b) A schematic representation of one-half of the membrane bilayer showing the localization of the BODIPY fluorophore in phosphatidylcholine membranes. The horizontal line at the bottom indicates the center of the bilayer. See Table 1 for other details.

2.2.2. Depth measurements using the parallax method

The actual spin (nitroxide) contents of the spin-labeled phospholipids (Tempo-, 5- and 12-PC) were assayed using fluorescence quenching of anthroyloxy-labeled fatty acids (2- and 12-AS) as described previously (Abrams and London, 1993). For depth measurements, unilamellar vesicles (ULV) of DOPC containing 10% (mol/mol) spin-labeled phospholipids (Tempo-, 5- or 12-PC) labeled with 1% (mol/mol) of BODIPY-C₁₂ were prepared by the ethanol injection method (Kremer et al., 1977). This method produces predominantly unilamellar vesicles of ~100 nm diameter. For this, 160 nmol of lipid (90% DOPC/10% Tempo- or 5- or 12-PC, mol/mol) and 1.6 nmol of BODIPY-C₁₂ was co-dried under a steady stream of nitrogen with gentle warming (~35°C), followed by further drying under a high vacuum for at least 3 h. The dried lipids were then dissolved in ethanol to give a final concentration of 40 mM. The ethanolic lipid solution was then injected into 1 ml of 10 mM sodium acetate, 150 mM sodium chloride, pH 5 buffer while vortexing to give a final concentration of 0.16 mM lipid in the buffer. Duplicate samples were prepared in each case except samples lacking the quencher for which triplicates were prepared. Background samples lacking BODIPY-C₁₂ were prepared in all experiments, and their fluorescence intensity was subtracted from the respective sample fluorescence intensity in each case. Samples were kept in the dark for 12 h before fluorescence measurements.

2.2.3. Steady state fluorescence measurements

Steady state fluorescence measurements was carried out at room temperature (~25°C) with a Hitachi F-7000 spectrofluorometer (Tokyo, Japan) using 1 cm path length quartz cuvettes. Fluorescence anisotropy measurements were performed at room temperature (~25°C) with a Hitachi F-7000 spectrofluorometer (Tokyo, Japan). Fluorescence anisotropy values were calculated using the following equation (Lakowicz, 2006):

$$r = \frac{I_{VV} - GI_{VH}}{I_{W+} + 2GI_{VH}} \quad (1)$$

where I_{VV} and I_{VH} are the measured fluorescence intensities (after appropriate background subtraction) with the excitation polarizer vertically oriented and emission polarizer vertically and horizontally oriented, respectively. G is the grating correction factor and is the ratio of the efficiencies of the detection system for vertically and horizontally polarized light, and is equal to I_{HV}/I_{HH} . The excitation wavelength was set at 490 nm, and emission was monitored at 510 nm. Excitation and emission slits with slit widths of 3 nm were used for all measurements. The optical density of the samples measured at 490 nm was less than 0.15. Data shown in Fig. 2b represent means ± S.E. of at least three independent measurements.

2.2.4. Generation of calibration curve for fluorescence anisotropy with viscosity

The calibration curve for fluorescence anisotropy with viscosity was generated by measuring fluorescence anisotropy of BODIPY-C₁₂ (~2 μM) in glycerol-methanol mixtures of varying ratio and plotting anisotropy as a function of viscosity. The viscosity values of glycerol-methanol mixtures were taken from previous literature (Levitt et al., 2009, 2011). The following hyperbolic equation was fitted to the data points:

$$r = \frac{a - b}{(1 + c\eta)^{1/d}} \quad (2)$$

where r is fluorescence anisotropy and η is viscosity, while a , b , c and d are fitting parameters.

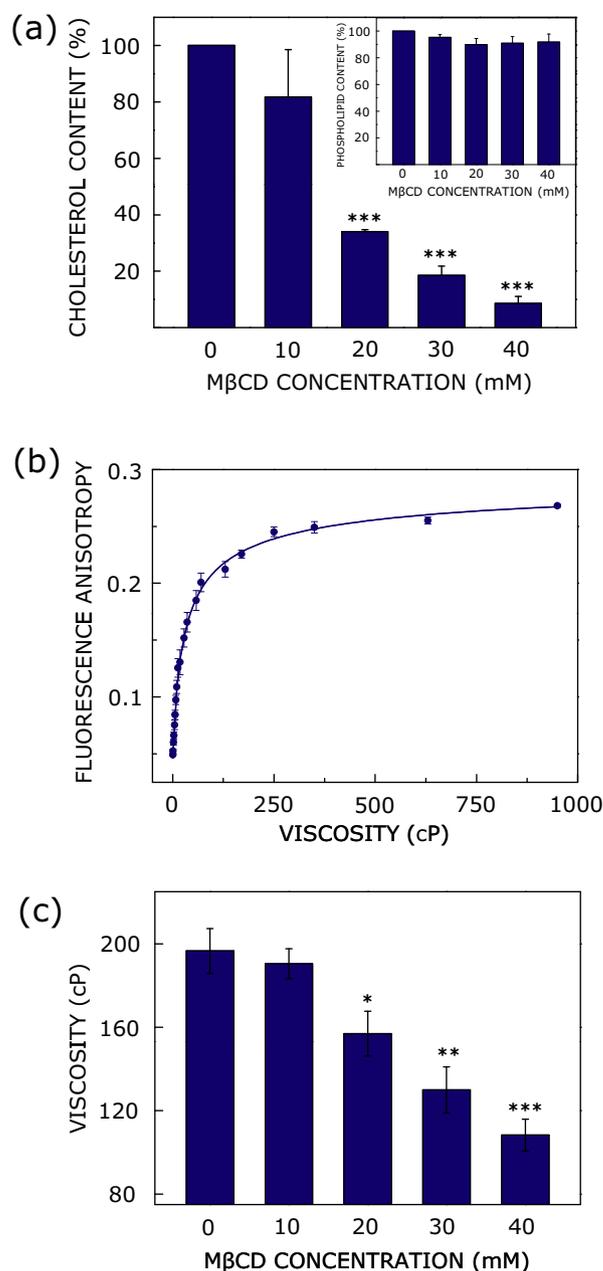


Fig. 2. (a) Effect of increasing concentration of MβCD on cholesterol content of bovine hippocampal membranes. Values are expressed as percentage of cholesterol content for native hippocampal membranes in the absence of MβCD treatment. Data represent means ± S.E. of at least three independent measurements (*** corresponds to significant ($p < 0.001$) difference in cholesterol content of MβCD treated hippocampal membranes relative to native membranes). The inset shows phospholipid content of hippocampal membranes upon cholesterol depletion. (b) Steady state anisotropy of BODIPY-C₁₂ in methanol-glycerol mixtures with increasing viscosity. The viscosity values corresponding to various compositions of methanol and glycerol were taken from Levitt et al., 2009, 2011. Data represent means ± S.E. of at least three independent measurements. See Section 2 for other details. (c) Effect of cholesterol depletion on viscosity of hippocampal membranes. Data represent means ± S.E. of at least three independent measurements (*, ** and *** correspond to significant ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) difference in viscosity of cholesterol-depleted hippocampal membranes relative to native membranes). See Section 2 for more details.

2.2.5. Hippocampal membrane preparation and cholesterol depletion

Native hippocampal membranes were prepared from frozen hippocampal tissue, as described previously (Jafurulla et al., 2014). The final pellet (native membranes) was suspended in a minimum volume of buffer C (50 mM Tris, pH 7.4), homogenized using a

hand-held Dounce homogenizer, flash frozen in liquid nitrogen and stored at -80°C . Protein concentration was assayed using the BCA assay (Smith et al., 1985). Native hippocampal membranes were depleted of cholesterol using M β CD as described previously (Pucadyil and Chattopadhyay, 2004). Briefly, membranes resuspended at a protein concentration of ~ 2 mg/ml were treated with different concentrations of M β CD in buffer C at room temperature ($\sim 25^{\circ}\text{C}$) with constant shaking for 1 h. Membranes were then spun down at $50,000 \times g$ for 5 min, washed once with buffer C and resuspended in the same buffer. Cholesterol was estimated using the Amplex Red cholesterol assay kit (Amundson and Zhou, 1999). Radioligand binding assays were carried out as described previously (Jafurulla et al., 2014).

Native and cholesterol-depleted hippocampal membranes containing 50 nmol of total phospholipids were suspended in 1.5 ml of buffer C and used for fluorescence anisotropy experiments. The amount of probe added was such that the final probe concentration was 0.25 mol% with respect to the total phospholipid content. This ensures that the fluorescence anisotropy is not affected due to probe-probe interaction (Wu et al., 2013). Membranes were vortexed for 1 min after addition of the probe and kept in the dark overnight before measurements. Background samples were prepared the same way except that the probe was omitted. The final probe concentration was $0.08 \mu\text{M}$ in all cases and the methanol content was always low (0.01%, v/v). Control experiments showed that at this concentration of methanol, the ligand binding properties of the receptor are not altered (Pucadyil and Chattopadhyay, 2004). Viscosity values for native and cholesterol-depleted hippocampal membranes were obtained by extrapolation from the calibration plot (Fig. 2b).

2.2.6. 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 viscosity was analyzed using the same software with 95% confidence interval. Plots were generated using Microcal Origin version 6.0 (OriginLab, Northampton, MA).

3. Results and discussion

Knowledge of the penetration depth of the fluorescent moiety in a membrane probe is important because it allows to correlate the fluorescence parameters of the probe with a defined location in the membrane (Haldar et al., 2012; Saxena et al., 2014). The penetration depth of the BODIPY fluorophore of BODIPY-C₁₂ in DOPC membranes was determined by the parallax method (Chattopadhyay and London, 1987) using the equation:

$$z_{\text{CF}} = L_{\text{cl}} + \left\{ \frac{[(-1/\pi C)\ln(F_1/F_2) - L_{21}^2]}{2L_{21}} \right\} \quad (3)$$

where z_{CF} is the depth of the fluorophore from the center of the bilayer, L_{cl} is the distance of the center of the bilayer from the shallow quencher (Tempo-PC), L_{21} is the difference in depth between the two quenchers (*i.e.*, the vertical distance between the shallow and deep quenchers), and C is the two-dimensional

quencher concentration in the plane of the membrane (molecules/ \AA^2). Here, F_1/F_2 is the ratio of F_1/F_0 and F_2/F_0 , in which F_1 and F_2 are the fluorescence intensities in the presence of the shallow quencher (Tempo-PC) and the deep quencher (5-PC), respectively, both at the same quencher concentration (C); F_0 is the fluorescence intensity in the absence of any quencher. All the bilayer parameters used were the same as described previously (Chattopadhyay and London, 1987). Analysis of quenching data using Eq. (3) shows that the depth of penetration of the BODIPY group in BODIPY-C₁₂ is $\sim 20 \text{\AA}$ from the center of the bilayer (see Table 1 and Fig. 1b). These results suggest that the BODIPY moiety of the probe is localized at the interfacial region of the membrane, a region characterized by unique dynamics and dielectric properties (Haldar et al., 2011).

We have previously shown that cholesterol content in hippocampal membranes can be efficiently modulated using M β CD as it forms a selective inclusion complex with cholesterol by including it in the central nonpolar cavity (Pucadyil and Chattopadhyay, 2004). Fig. 2a shows that cholesterol content in hippocampal membranes gets considerably reduced upon treatment with increasing concentrations of M β CD. The extent of cholesterol depletion was highest when 40 mM M β CD was used, which resulted in cholesterol content being reduced to $\sim 10\%$ of the control. The membrane phospholipid level did not display significant change under these conditions (see inset in Fig. 2a).

We utilized the viscosity-sensitive fluorescence depolarization of BODIPY-C₁₂, a fluorescent molecular rotor, for estimating viscosity of native and cholesterol-depleted hippocampal membranes. For this purpose, we generated a calibration curve (see Fig. 2b) by plotting the variation in steady state fluorescence anisotropy of BODIPY-C₁₂ as a function of viscosity using methanol-glycerol mixtures of varying composition (viscosity values of glycerol-methanol mixtures were taken from the literature) (Levitt et al., 2009, 2011). The figure shows that at the higher end of the viscosity range, the fluorescence anisotropy approaches r_0 , *i.e.*, the limiting (fundamental) anisotropy of the BODIPY in the absence of any depolarizing processes such as rotational diffusion (Karolin et al., 1994). An advantage of fluorescence anisotropy measurements is that anisotropy is generally independent of fluorophore concentration.

We measured fluorescence anisotropy of BODIPY-C₁₂ in hippocampal membranes and utilized the calibration plot (shown in Fig. 2b) to obtain an estimate of apparent membrane viscosity. The apparent viscosity of native hippocampal membranes was found to be ~ 197 cP, which is in the same range as previously reported for natural membranes from the nervous system (Cone, 1972; Poo and Cone, 1974). The apparent viscosity of native and hippocampal membranes with progressive cholesterol depletion (corresponding to increasing concentrations of M β CD) was estimated from the calibration plot (see Fig. 2c). The figure shows that there was an overall decrease in membrane viscosity with cholesterol depletion. For example, viscosity was reduced by $\sim 45\%$ (to ~ 108 cP), relative to control, when 40 mM M β CD was used.

Membrane cholesterol has previously been shown to be a crucial modulator of the function of membrane receptors (Burger et al., 2000; Pucadyil and Chattopadhyay, 2006; Paila and Chattopadhyay, 2010; Oates and Watts, 2011; Jafurulla and

Table 1
Average membrane penetration depth of BODIPY-C₁₂^a.

Spin-labeled PC pair used for quenching analysis	Calculated distance from the bilayer center z_{CF} (\AA)
Tempo-PC/5-PC	~ 20

^a Depths were calculated from fluorescence quenching obtained with samples containing 10 mol% of Tempo- and 5-PC and using Eq. (3). Samples were excited at 490 nm and emission was collected at 510 nm. The ratio of BODIPY-C₁₂ to total lipid was 1:100 (mol/mol) and the concentration of BODIPY-C₁₂ was $1.6 \mu\text{M}$ in all cases. See Section 2 for more details.

Chattopadhyay, 2013). In this context, we have previously shown that the specific ligand binding activity of the serotonin_{1A} receptor is dependent on hippocampal membrane cholesterol concentration (Pucadyil and Chattopadhyay, 2004). The specific agonist (³H] 8-OH-DPAT) binding was reduced to ~60% of the original binding observed (in control membranes in the absence of MβCD) when 40 mM MβCD was used for cholesterol depletion (see Fig. 3a). In order to gain insight into cholesterol-dependent changes in the function of the serotonin_{1A} receptor in terms of changes in hippocampal membrane viscosity, we plotted the specific agonist binding of the serotonin_{1A} receptor (taken from Fig. 3a) as a function of hippocampal membrane viscosity (from Fig. 2c). This plot is shown in Fig. 3b. Interestingly, a linear regression analysis between specific agonist binding and hippocampal membrane viscosity resulted in a correlation coefficient (*r*) of ~0.84. The 95% confidence intervals contained all the data points, thereby implying that the close correlation among these parameters is significant. Such a close correlation indicates that membrane viscosity plays a crucial role in the function of the serotonin_{1A} receptor in hippocampal membranes. The role of membrane

fluidity in GPCR function has important physiological implication (Escribá et al., 2007). Certain diseases in which GPCR signaling is known to play a crucial role, such as hypertension and Alzheimer's disease, have been reported to be characterized by altered membrane fluidity (Roth et al., 1995; Zicha et al., 1999).

Although the role of membrane cholesterol in the function of membrane receptors is well documented (Burger et al., 2000; Pucadyil and Chattopadhyay, 2006; Paila and Chattopadhyay, 2010; Oates and Watts, 2011; Jafurulla and Chattopadhyay, 2013), zeroing in on the mechanism underlying the effect of membrane cholesterol on the structure and function of membrane proteins and receptors has proved to be challenging (Paila and Chattopadhyay, 2009, 2010; Paila et al., 2009; Lee, 2011). A possible mechanism is centered on change in membrane physical properties (such as viscosity, thickness and curvature) that would give rise to altered protein function (Brown, 2012; daCosta et al., 2013; Soubias et al., 2014). An alternate mechanism could involve a direct (specific) interaction between cholesterol and the given membrane protein that triggers subtle conformational changes in the receptor, giving rise to altered function. An emerging theme in this area is that these mechanisms need not be mutually exclusive, and the possibility of such cholesterol-dependent membrane protein function could be due to a combination of both.

In case of the serotonin_{1A} receptor, previous work from our laboratory has comprehensively demonstrated the requirement of membrane cholesterol in the function of the receptor (reviewed in Pucadyil and Chattopadhyay, 2006; Paila and Chattopadhyay, 2010; Jafurulla and Chattopadhyay, 2013). An important aspect of our results is that the interaction between cholesterol and the serotonin_{1A} receptor appears to be stringent, since immediate biosynthetic precursors of cholesterol (differing with cholesterol merely in a double bond) were not able to maintain receptor function (Paila et al., 2008; Singh et al., 2009). In addition, we recently showed that the requirement of cholesterol for receptor function is diastereospecific, but not enantiospecific (Jafurulla et al., 2014). This difference in sterol stereospecificity for receptor function appears to be related to membrane dipole potential (Bandari et al., 2014). We have also shown by coarse-grain molecular dynamics simulations that membrane cholesterol has higher occupancy in certain sites on the serotonin_{1A} receptor (Sengupta and Chattopadhyay, 2012). Taken together, these results tend to support a specific mechanism for the requirement of membrane cholesterol for the function of the serotonin_{1A} receptor.

On the other hand, we have shown that the serotonin_{1A} receptor is more compact (Paila et al., 2011) and stable (Saxena and Chattopadhyay, 2012; Patra et al., 2015) in the presence of membrane cholesterol. More importantly, we previously reported that the agonist binding of the serotonin_{1A} receptor in hippocampal membranes was reduced in the presence of capsaicin, which changes the physical properties of membranes by increasing the membrane elasticity (Prasad et al., 2009). In the overall context of these results, our present observation of the close correlation between membrane viscosity and receptor activity assumes relevance. We conclude that lipid effects on membrane protein structure and function are complex and multifaceted, and adequate caution should be exercised while interpreting experimental results.

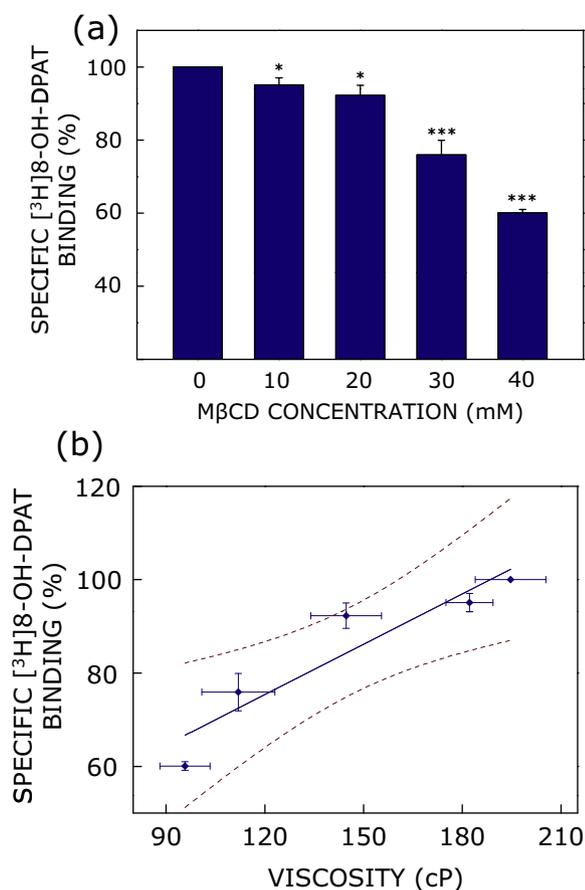


Fig. 3. (a) Effect of increasing concentration of MβCD on specific [³H]8-OH-DPAT binding to serotonin_{1A} receptors in hippocampal membranes. Values are expressed as percentage of specific binding for native hippocampal membranes in the absence of MβCD treatment. Data represent means ± S.E. of at least three independent measurements (* and *** correspond to significant (*p* < 0.05 and *p* < 0.001, respectively) difference in specific [³H]8-OH-DPAT binding in MβCD treated hippocampal membranes relative to native membranes). (b) Correlation of agonist binding activity of the serotonin_{1A} receptor with membrane viscosity. Specific [³H] 8-OH-DPAT binding to serotonin_{1A} receptors (values taken from Fig. 3a) and corresponding values of membrane viscosity (from Fig. 2c) are plotted. Linear regression analysis yielded a correlation coefficient (*r*) ~0.84. The significance of the correlation is apparent from the 95% confidence band (plotted as dashed lines). The close correlation between membrane viscosity and receptor activity is noteworthy. See text for more details.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the Council of Scientific and Industrial Research, Govt. of India (A.C.). S.P. thanks the University

Grants Commission for the award of a Junior Research Fellowship. H.C. acknowledges the Council of Scientific and Industrial Research (Govt. of India) for the award of Senior Research Associateship and the University Grants Commission for UGC-Assistant Professor position. A.C. is an Adjunct Professor of Tata Institute of Fundamental Research (Mumbai), RMIT University (Melbourne, Australia), Indian Institute of Technology (Kanpur), and Indian Institute of Science Education and Research (Mohali). A.C. gratefully acknowledges J.C. Bose Fellowship (Department of Science and Technology, Govt. of India). We thank Md. Jafurulla, Sandeep Shrivastava and Pavan Chodiseti for help with experiments and useful discussion, and members of the Chattopadhyay laboratory for critically reading the manuscript.

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