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Short communication

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

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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-C$_{12}$) based on the BODIPY probe. BODIPY-C$_{12}$ 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.

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; MβCD, 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-C$_{12}$ (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.

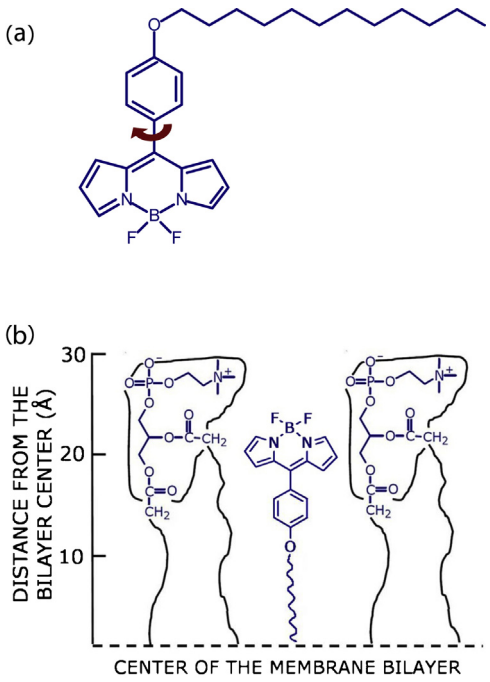

Fig. 1. (a) The chemical structure of the fluorescent molecular rotor BODIPY-C$_{12}$ used in this study. The free rotation of the C—C single bond connecting the BODIPY moiety with the benzene ring (marked by a curved arrow) depends on viscosity of the medium and this phenomenon modulates the photophysical properties of the probe. The hydrophobic C$_{12}$ 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.

A major application of molecular rotors is to measure micro-viscosity 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-C$_{12}$ 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-Dioleyl-*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 (MβCD), EDTA, EGTA, $MgCl_2$, $MnCl_2$, iodoacetamide, phenylmethylsulfonyl fluoride (PMSF), sucrose, Na_2HPO_4, sodium azide, Tris, and sodium acetate were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]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-C$_{12}$ 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-C$_{12}$ in methanol were estimated using the molar extinction coefficients (ε_m) of 8000 and 82100 $M^{-1}cm^{-1}$ 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.