Destabilization of raft-mimetic structure on liposomal membrane induced by local anesthetics

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Introduction

Local anesthetics (LAs) are often used as pain killer among the surgical or dental operation. Despite of long time after development of LAs, mechanism of pain suppression by LAs have not been fully explained yet. Pain sensation is transmitted through neural membranes as the electric signal: the repetition of membrane potential inversion due to the cross-membrane permeation of cation [1–3]. LAs are known to suppress the function of the protein: voltage-dependent sodium channel which is controlling the influx of sodium ion into neural cell [4]. Until recently, LAs are thought to interact between channel proteins by direct bonding [5,6] (Fig.1, left). However, these studies have not been fully explained the interaction between LAs and whole ion channels which can be influenced by LAs. Recently, some studies indicated the correlation between efficacy and hydrophobicity of LAs [7,8] (Fig.1, right). These facts indicates the indirect interaction between LAs and channels due to the physical property change of biomembranes.

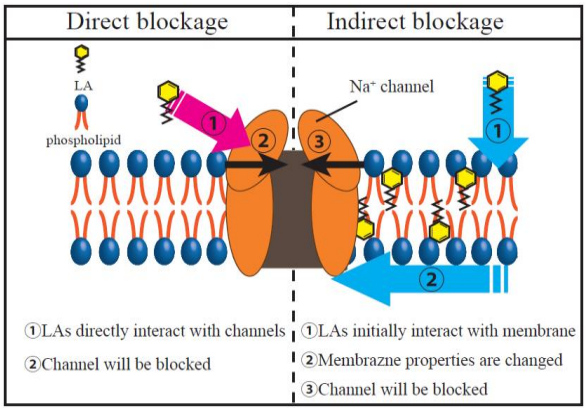

Fig.1 Direct (left) and indirect (right) mechanisms of sodium-channel blockage.

Biomembranes are composed of bilayer structure mainly contains phospholipids and cholesterol (Chol) [9–13]. Membrane molecules are oriented with weak attraction derives from hydrophobic interaction, and they are forming fluidic and flexible structure. On the surface of biomembranes, there are several of integral molecules such as sugar chains and proteins are exist. Functions of these molecules can be influenced by the behaviors of biomembranes. Furthermore, recently, Ikonen *etal*. suggested the specific lipid region lipid raft [14–16]. Lipid raft is known as a region rich with saturated phospholipid and Chol, and various integral proteins such as ion channels are localized. Therefore, biomembranes and lipid raft on them are regarded as important platform, and their physical properties and behaviors should strongly influenced to the cross-cellular signal transduction. However, biomembranes are composed with diverse components such as lipid, sugar, and proteins. Moreover, behaviors of biomembranes are corresponds to the other organelle such as cytoskeleton. As above, to discuss the properties of biomembranes, we need consider plenty of factors.

In recent research, biomimetic membrane: liposome is widely used as a simplified experimental system excluding such unnecessary factors. Liposome is the aggregation of amphiphilic phospholipid which has spherically-closed bilayer structure. Furthermore, we can realize raft-mimetic structure in multi-components liposome as phase-separated structure. Therefore, many studies discuss the fundamental phenomena in biomembrane with analyzing property of liposomal membranes.

Research objective

Through our study, we investigated the effect of LAs on the stability of raft-mimetic structure; phase-separated structure in liposomal membrane composed with unsaturated phospholipid (DOPC), saturate phospholipid (DPPC), and cholesterol (Chol). To discuss the effects of LAs on membrane physical property more precisely, we measured membrane fluidity with GP value of Laurdan fluorescence, and line tension at boundary of 2-phase liposomes. We also performed DSC experiment to understand the affinity and localization of LAs in different phases.

Materials

Liposomal membranes were prepared with Natural swelling method (Fig.2). Lipids (DOPC, DPPC, Chol) and LAs (lidocaine, tetracaine), fluorescents (Rhod-DHPE, Laurdan) were dissolved in chloroform, and their concentrations were 2mM, 0.5mM, and 10μM, respectively. 10mM Glucose dissolved in methanol. These stock solutions were put into glass test tubes, and then mixed all of them. These solutions were dried under vacuum for least 3 hours to form thin lipid films. The films were then hydrated overnight with deionized water at 55 ºC to produce unilamellar liposomes. The final concentrations were 0.2mM lipids and LAs, 0.6mM glucose, 0.01mM Rhod (10μM in case of GP value measurement), and 0.25μM Laurdan.

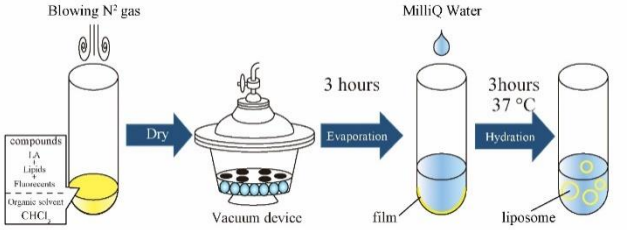

Fig.2 Schematic of liposomal preparation

Result and discussion

In chaptor 2, initially, we observed formation of phase separation on LAs presented DOPC/DPPC (solid ordered: S_o/liquid disordered: L_d), and DOPC/DPPC/Chol (liquid ordered: L_o/L_d) membranes. This experiment revealed that the presence of LAs suppressed L_o/L_d phase separation in DOPC/DPPC/Chol (Fig.3). On the other hand, S_o/L_d phase separation on DOPC/DPPC liposome was not influenced by LAs. We also clarified the fluidity decrease of DOPC-rich region (L_d) in DOPC/DPPC/Chol liposomes by presence of LAs (Fig.4). Fluidity decrease of L_d phase should make the fluidity difference between DOPC-rich phase and DPPC/Chol-rich phase. This action of LAs should decrease the line energy between Lo and L_d phases, and suppressed phase separation.

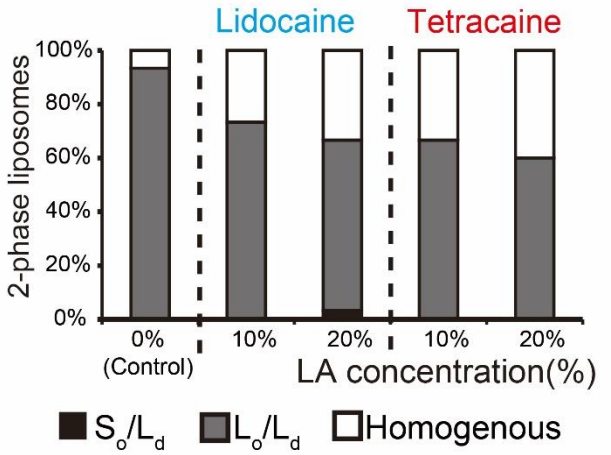

Fig.3 The ratio of heterogeneous LA containing DOPC/DPPC/Chol membranes in each different concentration of presented LAs.

In chapter 3, we observed decrease of miscibility temperature of L_o/L_d phase separation in DOPC/DPPC/Chol membrane. Furthermore, based on image analysis, we revealed the line tension decrease at L_o/L_d phase boundary of LA added DOPC/DPPC/Chol liposomes (Fig.5). On the other hand miscibility temperature of S_o/L_d phase separation in DOPC/DPPC liposomes was not influenced by LAs. Finally, we performed Differential Scanning Calorimetry (DSC) experiments with LA added DPPC and DPPC/Chol membrane to discuss the effect on membrane thermal-stability and localization of LAs in multi-lipid liposomes (Fig.6). Through the DSC experiment with DPPC membrane, addition of LAs decreased the transition temperature of both S_o and L_o membranes in concentration dependent manner.

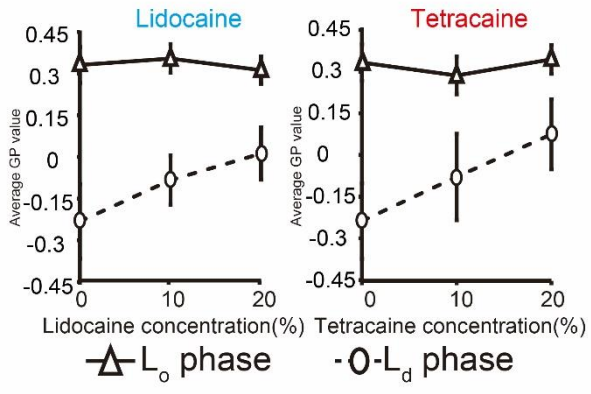

Fig.4 Fluidity of different phases in LA containing DOPC/DPPC/Chol liposomes.

At the same time, in the experiment of DPPC (S_o) membrane with higher LA concentration (5~10 mol%), we observed the shoulder on the lower temperature side on the thermal peaks. On the other hand, we could not observe similar asymmetric shoulder of thermal peaks on DPPC/Chol (S_o/L_o coexist) membranes despite the

presence of higher LAs concentration. This phenomena indicates relatively stronger affinity of LAs with L_o phase rather than with S_o phase. Therefore, we discussed localization LAs into DPPC-rich region was enhanced by the contribution of Chol. This localization change of LAs result into decrease of the line tension on phase-boundary, and finally destabilized phase-separated structure.

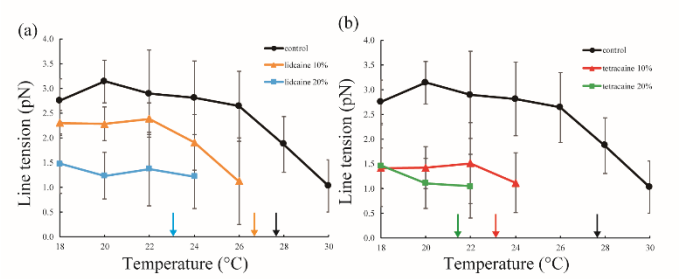

Fig.5 Line tension at L_o/L_d phase boundary in DOPC/DPPC/Chol/LAs mixtures as a function of temperature. Miscibility temperatures measured by microscopic observations are shown as arrows. The colors of arrows correspond to those of lipid compositions.

Results of our study indicates correlation between LA function and phase behavior of biomembrane. Lacking of heterogeneity on membranes could change various membrane properties like membrane thickness, bending or expansion rigidities, spontaneous curvature, etc. These physical property changes can effect on gaiting or binding abilities of membrane proteins such as ion-channels. Our results are suggesting some correlation between raft destabilization and protein disordering by LAs

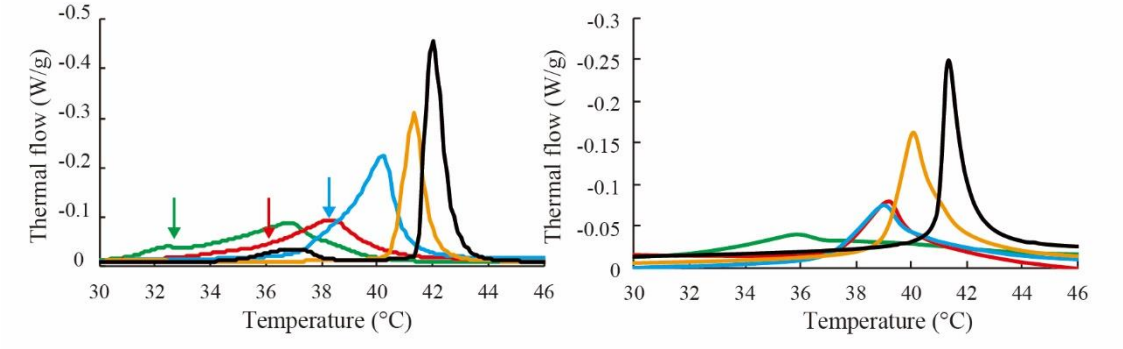

Fig.6 Thermograph of tetracaine containing DPPC (left), and DPPC/Chol (right) membranes. The color of lines are corresponds to different LA concentrations (LA 0% black, 2.5% yellow, 5% blue, 7.5% red, 10% green). Location of small shoulder derives from LA-rich phase in DPPC membranes are shown as arrows. The colors of arrows are corresponds to those of LA concentration.

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Table of contents:

Achievement

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