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Stereostructure-based differences in the interactions of cardiotoxic local anesthetics with cholesterol-containing biomimetic membranes

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ABSTRACT

Amide-type pipecoloxylidide local anesthetics, bupivacaine, and ropivacaine, show cardiotoxic effects with the potency depending on stereostructures. Cardiotoxic drugs not only bind to cardiomyocyte membrane channels to block them but also modify the physicochemical property of membrane lipid bilayers in which channels are embedded. The opposite configurations allow enantiomers to be discriminated by their enantiospecific interactions with another chiral molecule in membranes. We compared the interactions of local anesthetic stereoisomers with biomimetic membranes consisting of chiral lipid components, the differences of which might be indicative of the drug design for reducing cardiotoxicity. Fluorescent probe-labeled biomimetic membranes were prepared with cardiolipin and cholesterol of varying compositions and different phospholipids. Local anesthetics were reacted with the membrane preparations at a cardiotoxically relevant concentration of 200 μM. The potencies to interact with biomimetic membranes and change their fluidity were compared by measuring fluorescence polarization. All local anesthetics acted on lipid bilayers to increase membrane fluidity. Chiral cardiolipin was ineffective in discriminating *S*(−)-enantiomers from their antipodes. On the other hand, cholesterol produced the enantiospecific membrane interactions of bupivacaine and ropivacaine with increasing its composition in membranes. In 40 mol % and more cholesterol-containing membranes, the membrane-interacting potency was *S*(−)-bupivacaine < racemic bupivacaine < *R*(+)-bupivacaine, and *S*(−)-ropivacaine < *R*(+)-ropivacaine. Ropivacaine (*S*(−)-enantiomer), levobupivacaine (*S*(−)-enantiomeric), and bupivacaine (racemic) interacted with biomimetic membranes in increasing order of intensity. The rank order of membrane interactivity agreed with that of known cardiotoxicity. The stereoselective membrane interactions determined by cholesterol with higher chirality appears to be associated with the stereoselective cardiotoxic effects of local anesthetics. The stereostructure and membrane interactivity relationship supports the clinical use and development of *S*(−)-enantiomers to decrease the adverse effects of pipecoloxylidide local anesthetics on the cardiovascular system.

1. Introduction

Bupivacaine is an amide-type long-acting local anesthetic which has been widely used for cutaneous infiltration, regional nerve block, epidural anesthesia and spinal anesthesia in surgery and obstetrics. Bupivacaine is also known to exert a greater toxic effect on the cardiovascular system compared with other local anesthetics. This drug potentially induces bradycardia, arrhythmia, myocardial depression and eventually cardiovascular collapse especially when its concentration in blood is elevated by an accidental intravenous injection or an absolute overdose.[1]

Due to its three dimensional structure, bupivacaine has two enantiomer molecules which are differentiated by their effects on the rotation of the plane of a polarized light into dextrorotatory (*R*(+)-bupivacaine or dextrobupivacaine) and levorotatory (*S*(−)-bupivacaine or levobupivacaine) stereoisomers (see Fig. 1 for their structures). Bupivacaine contains an equal amount of these two enantiomers to be called racemic bupivacaine. Since the cardiotoxicity of bupivacaine was found to originate mostly from its *R*(+)-enantiomer, the pure *S*(−)-enantiomeric drugs like levobupivacaine and its structurally analogous pipecoloxylidide local anesthetic ropivacaine (*S*(−)-ropivacaine or levoropivacaine) were developed as a possible alternative to racemic bupivacaine.[2]

The adverse effects of local anesthetics on the cardiovascular system have been exclusively related to the action on sodium, potassium, and calcium channels.[3,4] The blockade of cardiac sodium and potassium channels by bupivacaine is stereoselective with an *R*(+)-enantiomer being more potent than its *S*(−)-enantiomeric counterpart and racemic mixture.[3,5] Ropivacaine also shows a difference in channel blockade between *S*(−)-ropivacaine and

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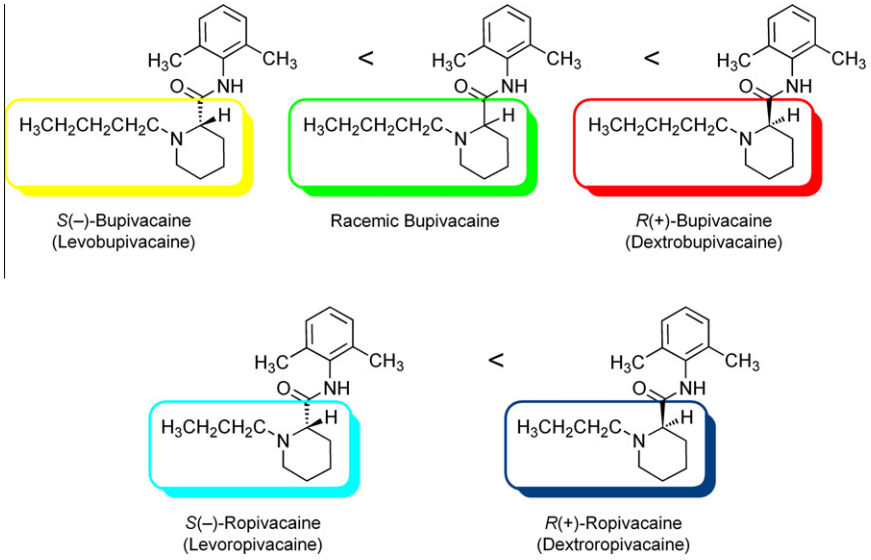

Figure 1. Structures of bupivacaine and ropivacaine stereoisomers. Inequality signs show the relative cardiotoxicity.

R(+)-ropivacaine (dextroropivacaine).[6] However, the comparative effects of bupivacaine stereoisomers on ion channels are not necessarily consistent with their relative cardiotoxic effects. *S*(−)-Bupivacaine was reported to be more effective in blocking potassium channels than racemic bupivacaine.[7] No differences were indicated between racemic and *S*(−)-bupivacaine.[4] Although the stereostructure-specific binding of local anesthetics to membrane-embedded functional proteins is emphasized for explaining the stereoselectivity of cardiotoxic local anesthetics, the potential capacity of membrane lipids to discriminate chiral drug molecules has not been fully explored.[8,9] Different hydrophobic (lipophilic) properties determine the stereoselective effects of bupivacaine and ropivacaine on human Kv1.1 potassium channels.[10] The action on membrane lipid bilayers is not excluded from the toxic mechanism of local anesthetics.

Drug-induced changes in membrane physicochemical property influence the functions of ion channels and enzymes by altering the lipid environments surrounding such proteinous drug targets, which is referred to as one of mechanisms of anesthetics.[11,12] The generation of ionic currents affected by anesthetics requires a molecular interplay of channel proteins and membrane lipids.[13] Local anesthetics also act on lipid bilayers to disturb membrane permeability, which is related to their cardiotoxic effects.[14] The good correlation between local anesthetic potency and hydrophobicity (lipid solubility) suggests that the site of pharmacotoxicological action includes membrane lipids.

The opposite configurations allow enantiomers to be discriminated by their enantiospecific interactions with another chiral molecule in membranes.[15] While both bupivacaine and ropivacaine have a chiral carbon atom (Fig. 1), biomembrane-constituting phospholipids and cholesterol also show the chirality which could possibly contribute to the discrimination between enantiomers.[16] Since sodium channels embedded in plasma membranes are influenced by membrane fluidity changes,[11,17,18] it is not unreasonable to assume that bupivacaine and ropivacaine might interact enantiospecifically with lipid bilayers to modify membrane fluidity differently.

In the present study, we verified the hypothesis that *S*(−)-bupivacaine, racemic bupivacaine, *R*(+)-bupivacaine, *S*(−)-ropivacaine, and *R*(+)-ropivacaine exert different effects on the lipid membranous system, the stereoselectivity of which is determined by chiral membrane components. We addressed whether bupivacaine and ropivacaine show stereostructure-based differences in the interactions with biomimetic membranes prepared with phospholipids and cholesterol, and which lipid component(s) is responsible for the stereoselective membrane interactivity. The comparative results would not only give a mechanistic clue to understand the cardiotoxic stereoselectivity of local anesthetics but also indicate a structural requirement to reduce their cardiotoxicity.

2. Materials and methods

2.1. Chemicals

S(−)-Bupivacaine, *R*(+)-bupivacaine, and racemic bupivacaine, and *S*(−)-ropivacaine and *R*(+)-ropivacaine were supplied by Maruishi Pharmaceuticals (Osaka, Japan) and AstraZeneca (Södertälje, Sweden), respectively. For clarity, *S*(−)-ropivacaine is referred to as clinically used 'ropivacaine'. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), cardiolipin (CL), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine] (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol) (POPI), and sphingomyelin (SM) were purchased from Avanti Polar Lipids (Alabaster, AL, USA), and cholesterol from Wako Pure Chemicals (Osaka, Japan). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was obtained from Molecular Probes (Eugene, OR, USA). Dimethyl sulfoxide (DMSO) and ethanol of spectroscopic grade (Kishida, Osaka, Japan) and water of liquid chromatographic grade (Kishida) were used for preparing reagent solutions. All other chemicals were of the highest analytical grade available commercially.

2.2. Chromatography of local anesthetics

The purity of tested drugs was confirmed by reversed-phase HPLC reported previously.[19] The HPLC system (Shimadzu, Kyoto, Japan) consisted of an LC-10ADVP liquid chromatograph connected to an SIL-10ADVP auto-sampler, a DGU-4A degasser, a CTO-6A column oven and an SPD-M10AVP diode-array detector. An aliquot (5 μL) of the aqueous solutions of *S*(−)-bupivacaine, *R*(+)-bupivacaine, racemic bupivacaine, *S*(−)-ropivacaine, and *R*(+)-ropivacaine (1 mM for each) was injected onto a Shim-pack CLC-ODS column (length of 150 mm × internal diameter of 6.0 mm; Shimadzu). Chromatographic conditions were essentially the same as previous ones.[19] Column eluates were detected at absorption wavelengths

of 203 and 220 nm, and the absorption spectra of peak fractions were measured.

2.3. Preparation of biomimetic membranes

For investigating the influence of CL on membrane interaction, DPH-labeled liposomal membranes were prepared with 0 and 20 mol % CL in the presence or absence of 40 mol % cholesterol by an injection method as reported previously.[20] In brief, an aliquot (250 μL) of the ethanol solutions of phospholipids and cholesterol (total lipids of 10 mM) and DPH (50 μM) was injected four times into 199 mL of 10 mM HEPES (pH 7.4, containing 125 mM NaCl and 25 mM KCl) under stirring above the phase transition temperatures of phospholipids. Membrane phospholipids other than CL had the constant molar ratio of POPC/POPE/POPS/POPI/SM to be 25:16:3:3:3 for all preparations.

For investigating the influence of cholesterol on membrane interaction, liposomal membranes suspended in HEPES buffer were prepared with phospholipids and cholesterol as described above, but by varying the cholesterol composition from 0 to 45 mol %. Membrane lipids other than cholesterol had the constant molar ratio of POPC/POPE/CL/POPS/POPI/SM to be 25:16:10:3:3:3 for all preparations.

DPH-labeled biomimetic membranes were also prepared with a mixture of 25 mol % POPC, 16 mol % POPE, 10 mol % CL, 3 mol % POPS, 3 mol % POPI, 3 mol % SM, and 40 mol % cholesterol to have the composition of major lipids in human cardiomyocyte membranes.[20] For determining the effect of cholesterol, the membranes were also prepared without cholesterol.

2.4. Determination of membrane interactivity

The comparative analyses of a pair of local anesthetic enantiomers were performed on the same experimental day using the membranes prepared freshly as described in Section 2.3. All bupivacaine and ropivacaine dissolved in DMSO were applied to membrane preparations so that a final concentration was 200 μM for each. When open-chest dogs were subjected to the incremental overdosage with local anesthetics, free and total plasma concentrations to produce cardiovascular collapse were 10–38 and 39–100 μM for racemic bupivacaine, 17–62 and 49–126 μM for *S*(−)-bupivacaine, and 36–142 and 64–163 μM for *S*(−)-ropivacaine.[21] Based on these values, a concentration of 200 μM which should definitely exert cardiotoxic effects for all drugs was chosen for comparing their membrane interactions. The concentration of DMSO was adjusted to be 0.5% (v/v) of the total volume so as not to affect the fluidity of membranes. Control experiments were conducted with the addition of an equivalent volume of DMSO vehicle. The membrane-interacting potencies of bupivacaine and ropivacaine stereoisomers were compared by their induced changes in membrane fluidity.[22] The reaction mixtures were incubated at 37 °C for 5 min, followed by measuring DPH fluorescence polarization using an RF-540 spectrofluorometer (Shimadzu) equipped with a polarizer and a cuvette thermo-controller as reported previously.[23] Compared with controls, a decrease of fluorescence polarization means an increase of membrane fluidity (membrane fluidization). Since the DPH polarization values of control membranes change with varying membrane lipid components and their compositions, the polarization changes (%) relative to control polarization values were used for comparing the potencies of local anesthetics to interact with different membranes.

2.5. Statistical analysis

The data were statistically analyzed by one-way ANOVA followed by post hoc Fisher's PLSD test using StatView 5.0 (SAS Institute, Cary, NC, USA). Results are expressed as the mean ± standard error mean (SEM), $n = 7$ for each experiment and values of $p < 0.01$ were considered statistically significant.

3. Results

3.1. Methodological evaluation

The HPLC separation of *S*(−)-bupivacaine, *R*(+)-bupivacaine, racemic bupivacaine, *S*(−)-ropivacaine, and *R*(+)-ropivacaine provided each analyte with a single peak. The absorption spectra of their peak fractions agreed with those of authentic standards.

The analytical precision of an experimental method used for comparing the membrane interactions was evaluated by duplicate seven analyses of different membrane preparations containing 0–45 mol % cholesterol (Table 1). Intra-assay coefficients of variation were 0.33–1.04% in all analyses.

3.2. Influence of CL on membrane interaction

S(−)-Bupivacaine, racemic bupivacaine, and *R*(+)-bupivacaine interacted with liposomal membranes and increased their fluidity as shown by polarization decreases (Fig. 2). All membrane-fluidizing effects were enhanced when CL was contained in the membranes. The polarization changes by bupivacaine stereoisomers in the presence of 20 mol % CL were 3.7–3.8 times larger than those in the absence of CL. However, CL showed no stereostructure-based differences in membrane fluidization, whereas biomimetic membranes with cholesterol were fluidized differently by bupivacaine stereoisomers.

3.3. Influence of cholesterol on membrane interaction

S(−)-Bupivacaine, racemic bupivacaine, and *R*(+)-bupivacaine were discriminated with increasing cholesterol in biomimetic membranes, but not without cholesterol (Fig. 3). *R*(+)-Bupivacaine was more effective than its antipode in fluidizing the membranes which contained 5 mol % and more cholesterol. When 40 mol % and more cholesterol were contained in biomimetic membranes, bupivacaine fluidized the membranes with the potency being

Table 1
Intra-assay coefficients of variation in local anesthetic and membrane interaction analyses

	Coefficient of variation (%) in analysis of membrane preparations containing cholesterol of					
	0 mol %	5 mol %	10 mol %	20 mol %	40 mol %	45 mol %
S(−)-Bupivacaine	0.61	1.03	1.03	0.60	0.61	0.44
Racemic bupivacaine	0.56	0.82	0.97	0.64	0.68	0.55
R(+)-Bupivacaine	0.45	0.88	0.79	0.89	0.65	0.60
S(−)-Ropivacaine	0.83	1.03	0.81	0.43	0.66	0.33
R(+)-Ropivacaine	1.04	0.95	0.41	0.37	0.58	0.44

All values were obtained from seven duplicated membrane preparations.

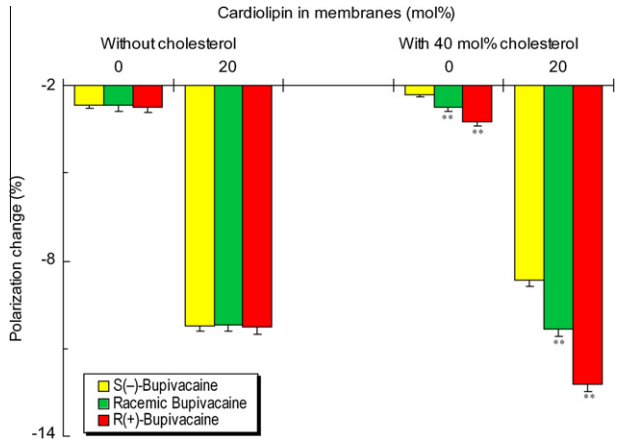

Figure 2. Influence of CL on the membrane interactions of bupivacaine stereoisomers. *S*(−)-Bupivacaine, racemic bupivacaine and *R*(+)-bupivacaine were reacted with 0 and 20 mol % cardiolipin-containing membranes without or with 40 mol % cholesterol, followed by measuring fluorescence polarization. Values represent mean ± SEM, $n = 7$. $^{**}p < 0.01$ compared with *S*(−)-bupivacaine.

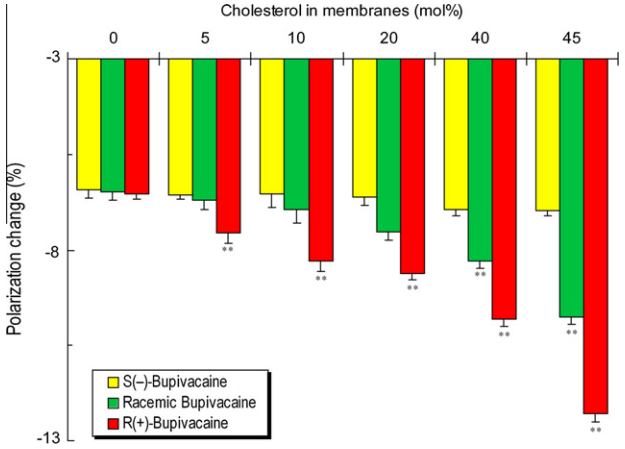

Figure 3. Effects of bupivacaine stereoisomers on biomimetic membranes of varying cholesterol compositions. *S*(−)-Bupivacaine, racemic bupivacaine, and *R*(+)-bupivacaine were reacted with the membranes containing cholesterol of the indicated compositions, followed by measuring fluorescence polarization. Values represent mean ± SEM, $n = 7$. $^{**}p < 0.01$ compared with *S*(−)-bupivacaine.

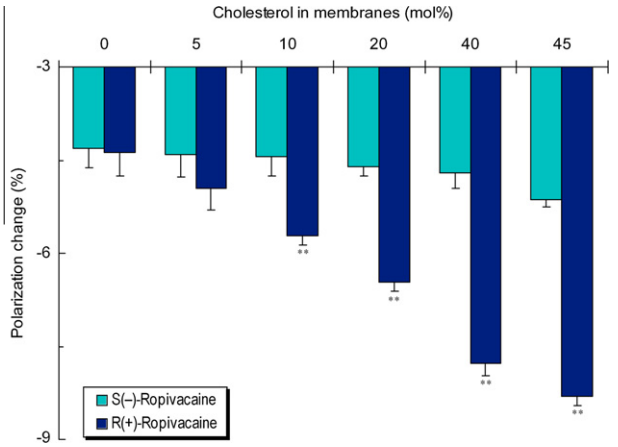

Figure 4. Effects of ropivacaine enantiomers on biomimetic membranes of varying cholesterol compositions. *S*(−)-Ropivacaine and *R*(+)-ropivacaine were reacted with the membranes containing cholesterol of the indicated compositions, followed by measuring fluorescence polarization. Values represent mean ± SEM, $n = 7$. $^{**}p < 0.01$ compared with *S*(−)-ropivacaine.

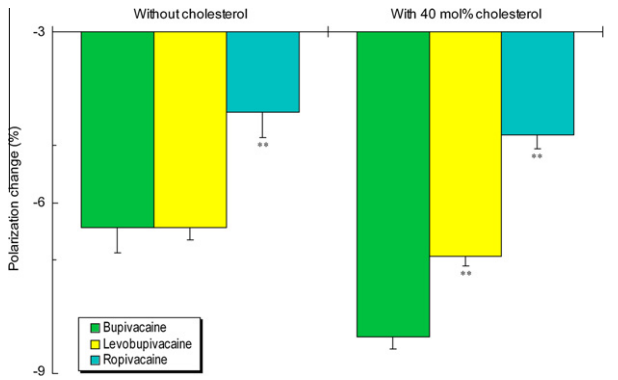

Figure 5. Effects of bupivacaine, levobupivacaine, and ropivacaine on biomimetic membranes without or with 40 mol % cholesterol. Local anesthetics were reacted with different membrane preparations, followed by measuring fluorescence polarization. Values represent mean ± SEM, $n = 7$. $^{**}p < 0.01$ compared with bupivacaine.

S(−)- < racemic < *R*(+)-bupivacaine. The polarization changes of *R*(+)-bupivacaine were 1.4–1.8 times larger than those of *S*(−)-bupivacaine.

Ropivacaine enantiomers also interacted differently with biomimetic membranes containing 10 mol % and more cholesterol, although they were not discriminated in 0 and 5 mol % cholesterol-containing membranes (Fig. 4). When the membranes contained 40 mol % and more cholesterol, *R*(+)-ropivacaine showed 1.6–1.7 times larger polarization changes than *S*(−)-ropivacaine.

3.4. Comparative effects on biomimetic membranes

Bupivacaine (racemic) and levobupivacaine (*S*(−)-enantiomeric) interacted differently with biomimetic membranes, whereas they were not discriminated in the absence of cholesterol (Fig. 5). Both of them were more interactive than ropivacaine (*S*(−)-enantiomer). These anesthetics fluidized 40 mol % cholesterol-containing membranes at 200 μM with the potency being ropivacaine < levobupivacaine < bupivacaine, which showed the relative polarization changes to be 1.0:1.4:1.7. Stereoselective membrane interactions were also found at lower concentrations of 10–100 μM (data not shown).

4. Discussion

HPLC analyses with the spectral measurements of peak fractions showed that all the used drugs were highly pure. The method used for membrane interactivity determination was confirmed to possess high analytical reproducibility. The comparisons between a pair of local anesthetic enantiomers were performed on the same experimental day. Therefore, the different polarization changes obtained are attributable to the differences in inherent membrane interactivity of local anesthetic stereoisomers, but not to the experimental artifacts derived from impurity contamination and/or polarization measurement.

The opposite absolute configurations allow enantiomers to be discriminated by the interaction with another chiral molecule.[15] Biomembrane-constituting phospholipids have a chiral center at the C-2 position of their glycerol moiety. However, a primary membrane component phosphatidylcholine interacts identically with enantiomeric compounds and behaves as an achiral molecule in biophysical property.[24,25] CL is referred to as a dimmer of phosphatidylglycerol and phosphatidic acid with one chiral carbon

atom in each glycerol backbone, so this phospholipid characteristically has two chiral centers which are favorable for enantiomeric discrimination.

However, CL has been revealed to be ineffective in discriminating between bupivacaine *S*(−)- and *R*(+)-enantiomer despite its higher chirality than other phospholipids, although it is effective in enhancing the membrane interactivity of all bupivacaine stereoisomers. Amphiphilic local anesthetics cause not only the hydrophobic interaction with phospholipid acyl chains but also the electrostatic interaction with phospholipid head groups. Bupivacaine with the pK_a value of 8.10 (at 36 °C) is positively charged at the physiological pH,[26] which enables bupivacaine to interact electrostatically with anionic CL more intensively. CL is preferentially localized in mammalian heart mitochondria in the range of 10–20% of total phospholipids.[27] Local anesthetics are able to intracellularly diffuse to mitochondria rapidly even when applied on the outside of cells.[28] The CL-dependent action on cardiomyocyte mitochondrial membranes underlies the cardiotoxic intensity of local anesthetics by potentiating their membrane interactions, but not the cardiotoxic stereoselectivity of local anesthetics.[14,20]

Since cholesterol has more chiral centers than phospholipids including CL, it was assumed as a membrane component contributable to the discrimination of enantiomers. The membrane interactions of local anesthetic stereoisomers were compared in the presence and absence of cholesterol. Consequently, cholesterol has been found to provide membrane lipid bilayers with the stereoselective interactivity being *S*(−)-bupivacaine < racemic bupivacaine < *R*(+)-bupivacaine. Cholesterol is also effective in discriminating *S*(−)-ropivacaine from *R*(+)-ropivacaine. Such stereoselectivity depends on the content of membrane cholesterol. Cholesterol is an important constituent of animal cell membranes, where it accounts for up to 50 mol % of the membrane lipids.[29] When membranes contain cholesterol of 40 mol % and more, the rank order of membrane fluidization by local anesthetic stereoisomers agrees with that of their cardiotoxicity.[1,2] In human cardiomyocytes, cholesterol shows the composition of 40 mol % in membrane lipids.[20]

Bupivacaine enantiospecifically blocks cardiac sodium channels with *R*(+)-bupivacaine being 1.2-fold more inhibitory on guinea pig sodium currents and 1.7-fold more interactive with sodium channels than *S*(−)-bupivacaine.[3] Human cardiac sodium channels show 1.5-fold higher affinity for *R*(+)-bupivacaine compared with *S*(−)-bupivacaine.[5] When membrane preparations contain cholesterol of 40–45 mol % which correspond to the typical composition in biological membranes, the relative polarization change of *R*(+)-bupivacaine to *S*(−)-bupivacaine is consistent with their relative inhibition of cardiac sodium channels. While the polarization changes by *R*(+)-ropivacaine are 1.6–1.7 times larger than *S*(−)-ropivacaine, *R*(+)-ropivacaine also blocks human cardiac potassium channels 2.5-fold more intensively than its antipode.[6]

DPH, a fluorescent probe used, readily penetrates into membrane lipid bilayers and aligns with phospholipid acyl chains. Since DPH is subject to the rotational restriction imparted by membrane fluidity (lipid order), its rotation is reduced in less fluid (ordered) membranes to show a higher degree of fluorescence polarization, while being enhanced in fluidized (disordered) membranes to show lower polarization. If local anesthetics change the fluorescence lifetime of DPH, fluorescence polarization should be affected to underestimate their membrane interactions.[8] In the present study, the polarization was measured under the conditions in which the DPH lifetime reached a plateau.[30] Therefore, membrane-fluidizing bupivacaine and ropivacaine are considered to increase the rotational mobility of membrane lipids with a resultant decrease of DPH polarization values.[31] The membrane interactions of local anesthetics also produce the rearrangement of an intermolecular hydrogen-bonded network among phospholipid molecules and the change of a P–N dipole orientation of phospholipid molecules, resulting in the fluidization of membrane lipid bilayers.[32]

While CL increases the binding of local anesthetics to membranes,[32] both bupivacaine and ropivacaine stereoselectively interacted with cholesterol-containing biomimetic membranes in the absence of CL. Cholesterol changes the partition coefficients of local anesthetics in lipid bilayers,[33] although it does not differently influence their enantiomers. Since the pK_a and partition coefficient of *S*(−)-bupivacaine are the same as those of racemic bupivacaine,[34] the discriminated membrane interactions are due to the stereostructural differences of local anesthetics.

A question arises as to how cholesterol mediates the stereostructure-dependent membrane fluidization by bupivacaine and ropivacaine. Local anesthetics are assumed to penetrate into membrane lipid bilayers and align between phospholipid acyl chains using a butyl chain for bupivacaine and a propyl chain for ropivacaine, although the space to be occupied by such alkyl chains varies by *S*(−)- and *R*(+)-configurational differences. Cholesterol is also oriented in membranes with a 3β-hydroxyl moiety anchoring to phospholipid polar head groups, a steroid ring adjoining fatty acyl chains and a flexible alkyl chain extending into hydrophobic membrane cores.[35] In membrane lipid bilayers, the adjacently aligning cholesterol with higher chirality may interact differently with bupivacaine and ropivacaine enantiomers to influence their lipid rotation-increasing effects depending on *S*(−)- or *R*(+)-configuration. A crucial role of cholesterol in enantiospecific membrane interaction is supported by studies in distinct fields. Pesek et al.[36] resolved a pair of enantiomers by reversed-phase liquid chromatography using a cholesterol-bonded stationary phase, showing the property of chiral cholesterol to separate stereoisomers. Lalitha et al.[37] found that the absolute configuration of cholesterol influenced the biophysical property of membranes to cause the enantiospecific interactions of membrane components. This enantiospecificity was enhanced with increasing membrane cholesterol to 30 mol %,[38] which is almost comparable to the cholesterol composition to produce the stereoselective membrane interactions of *S*(−)-, racemic, and *R*(+)-bupivacaine.

In the comparison of clinically used local anesthetics, their stereoisomers have been revealed to interact with biomimetic membranes at cardiotoxically relevant concentrations with the potency being ropivacaine (*S*(−)-enantiomer) < levobupivacaine (*S*(−)-bupivacaine) < bupivacaine (racemic). Their relative membrane effects agree with experimental and clinical features of their cardiovascular effects.[1,2] Ropivacaine acts on the membranes less intensively than both levobupivacaine and bupivacaine even in the absence of cholesterol. This is due to different *N*-substituents of pipecoloxylidide: a propyl group for ropivacaine but a butyl group for levobupivacaine and bupivacaine.[39] An increase of membrane fluidity is related to the blockade of cardiac sodium currents.[11,17,18] Membrane-fluidizing drugs also influence the permeability of membranes and the activity of cardiac receptors embedded in plasma membranes.[40,41] The membrane fluidity changes induced by bupivacaine and ropivacaine stereoisomers are likely to be sufficient to affect cell functions.[18,42] The stereoselective membrane interactions determined by chiral cholesterol are considered to be responsible for the stereostructure-based cardiotoxic effects of local anesthetics.

5. Conclusions

Bupivacaine and ropivacaine stereoselectively interact with the membranous system containing a substantial content of cholesterol to show the stereostructure-based differences in membrane fluidization, which are consistent with the relative cardiotoxic

effects of local anesthetic enantiomers. The stereoselectivity does not depend on the overall membrane lipid composition, but on the presence of cholesterol. The lower membrane interactivity of ropivacaine and levobupivacaine appears to be mechanistically associated with the lower cardiotoxicity of an *S*(−)-enantiomer than an *R*(+)-enantiomer and a racemic mixture. The stereostructure and membrane interactivity relationship supports the clinical use of *S*(−)-enantiomers to decrease the adverse effects of pipecoloxylidide local anesthetics on the cardiovascular system.

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