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Membrane Interactivity of Anesthetic Adjuvant Dexmedetomidine Discriminable from Clonidine and Enantiomeric Levomedetomidine

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Authors' contributions

This work was carried out in collaboration between both authors. Author MM performed experiments, statistically analyzed the data and managed the literature search. Author HT designed the study, wrote the protocol, managed the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Dexmedetomidine, which has been increasingly used as an anesthetic adjuvant, is more lipophilic and more active than another α_2-adrenergic agonist clonidine and enantiomeric levomedetomidine. Lipophilicity and stereostructure affect the clinical effects of α_2-adrenergic agonists. We aimed to compare the membrane interactivity of dexmedetomidine with clonidine and levomedetomidine from a point of view different from the mode of action on α_2-adrenergic receptors.
Methodology: Unilamellar vesicles were prepared with phospholipids and cholesterol to mimic the lipid compositions of peripheral nerve cell, central nerve cell and cardiomyocyte membranes, and lipid rafts. They were subjected to the reactions with dexmedetomidine, clonidine and levomedetomidine at 10-200 μM, followed by measuring fluorescence polarization to determine the membrane interactivity to change membrane fluidity and specify the membrane region for the stereostructure-specific interaction.

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Results: Dexmedetomidine and clonidine acted on lipid bilayers to increase the membrane fluidity with potencies varying by a compositional difference of membrane lipids. Dexmedetomidine showed greater interactivity with neuro-mimetic and cardiomyocyte-mimetic membranes than clonidine, being consistent with their comparative lipophilicity and activity. The effects of α_2-adrenergic agonists on lipid raft model membranes were much weaker than those on other membranes, indicating that lipid rafts are not mechanistically relevant to them. Higher interactive dexmedetomidine was discriminated from lower interactive levomedetomidine in the presence of chiral cholesterol in membranes. An interactivity difference between two enantiomers was largest in the superficial region of lipid bilayers and the rank order of their membrane-interacting potency was reversed by replacing cholesterol with epicholesterol, suggesting that cholesterol's 3β-hydroxyl groups positioned close to the membrane surface are responsible for the enantioselective interaction.

Conclusion: Dexmedetomidine structure-specifically interacts with biomimetic membranes depending on their lipid compositions more potently than clonidine and levomedetomidine. Such membrane interactivity associated with higher lipophilicity and stereostructure characterizes dexmedetomidine in addition to higher affinity for α_2-adrenergic receptors.

Keywords: Dexmedetomidine; membrane interactivity; structure-specific; α_2-adrenergic agonist; clonidine; enantiomeric levomedetomidine.

ABBREVIATIONS

DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; SOPS, 1-stearoyl-2-oleoyl-sn-glycero-3-[phospho-L-serine]; POPS, 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-L-serine]; PI, phosphatidylinositol; SM, sphingomyelin; CL, cardiolipin; CB, cerebroside; DPH, 1,6-diphenyl-1,3,5-hexatriene; 2-AS, 2-(9-anthroyloxy)stearic acid; 6-AS, 6-(9-anthroyloxy)stearic acid; 9-AS, 9-(9-anthroyloxy)stearic acid; 12-AS, 12-(9-anthroyloxy)stearic acid; 16-AP, 16-(9-anthroyloxy)palmitic acid; DMSO, dimethyl sulfoxide.

1. INTRODUCTION

There has been an increasing interest in the use of α_2-adrenergic agonists in anesthesia and intensive care because such drugs exhibit sedative, anesthetic-sparing, analgesic and sympatholytic activity [1]. Among them, clonidine was first clinically introduced to epidural and intrathecal injection and it had been one of the most popular adjuvants for anesthesia. However, the use of long acting clonidine is frequently associated with rebound hypertension following discontinuation [2]. Therefore, newly developed α_2-adrenergic agonist dexmedetomidine has been used for clonidine in sedation and analgesia [3] and as a local anesthetic adjuvant [2,4]. Compared with clonidine, dexmedetomidine is much less likely to cause intraoperative and postoperative adverse effects such as serious hypotension, apnea, hypoxemia or respiratory depression. Dexmedetomidine produces not only sedative and analgesic effects, but also anxiolytic [5], anesthetic-sparing [6], hypotensive and cardiovascular stabilizing [7], and ischemia/reperfusion injury-protecting effects [8]. It also possesses the property to modulate inflammatory response, neurotransmitter release, signaling pathway and ion channel activation [9,10].

The pharmacological profiles of α_2-adrenergic agonists significantly vary by their chemical structures. Compared with clonidine, dexmedetomidine more selectively binds to α_2-adrenergic receptors that are distributed in the central, peripheral and autonomic nervous systems as well as to imidazoline receptors involved in central blood pressure regulation [11]. An advantage of dexmedetomidine is its seven-times higher selectivity for α_2-adrenergic receptors [7,12], which is responsible for a greater effect of dexmedetomidine as dexmedetomidine induces more significant sedation and analgesia than clonidine [13,14]. Dexmedetomidine is more lipophilic than clonidine [15,16]. Increasing lipophilicity influences the analgesic potency of α_2-adrenergic agonists [15,17]. While both dexmedetomidine and clonidine potentially inhibit lipid peroxidation [18,19], such effects are associated with their

action in the lipid phase of membranes and accumulation in membrane lipid bilayers. Antioxidative or lipid peroxidation-inhibitory agents, including drugs acting on adrenergic receptors, commonly interact with lipid membranes to modify their physicochemical properties [20,21].

Dexmedetomidine is a dextrorotatory enantiomer that is an active substance in a racemic mixture medetomidine to show potent clinical effects and high affinity for α_2-adrenergic receptors. On the other hand, its enantiomeric counterpart, levorotatory levomedetomidine is clinically inactive or significantly less active than dexmedetomidine. Therefore, dexmedetomidine has been exclusively introduced to clinical settings as a sedative or analgesic.

In the process of investigating the membrane interactions of different classes of drugs, we found that adrenergic receptor-acting drugs modify membrane physicochemical properties [22]. Our purpose of this study was to determine whether dexmedetomidine potently interacts with biomimetic lipid bilayer membranes consisting of different lipid compositions. The second purpose was to demonstrate the structure-specific membrane interactivity of more lipophilic dexmedetomidine (5-[(1*S*)-1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole) by comparing with less lipophilic clonidine (2-[(2,6-dichlorophenyl)imino]-2-imidazoline) and less active levomedetomidine (5-[(1*R*)-1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole) (Fig. 1). From a point of view different from their conventional interactions with α_2-adrenergic receptors, we characterized the membrane interactivity of dexmedetomidine by comparing with clonidine and levomedetomidine, which may offer a novel insight into the mechanistic pharmacology of anesthetic adjuvants.

2. MATERIALS AND METHODS

2.1 Chemicals

Dexmedetomidine and levomedetomidine (purity of higher than 98% for both) were supplied by Orion Corporation (Espoo, Finland). Clonidine was obtained from Sigma-Aldrich (St. Louis, MO, USA). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoyl-*sn* -glycero-3-phosphocholine (POPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-stearoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine] (SOPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine] (POPS), porcine brain phosphatidylinositol (PI), porcine brain sphingomyelin (SM), bovine heart cardiolipin (CL) and porcine brain cerebroside (CB) were purchased from Avanti Polar Lipids (Alabaster, AL, USA), cholesterol ((3β)-cholest-5-en-3-ol) from Wako Pure Chemicals (Osaka, Japan), and epicholesterol ((3α)-cholest-5-en-3-ol) from Steraloids (Newport, RI, USA). 1,6-Diphenyl-1,3,5-hexatriene (DPH), 2-(9-anthroyloxy)stearic acid (2-AS), 6-(9-anthroyloxy)stearic acid (6-AS), 9-(9-anthroyloxy)stearic acid (9-AS), 12-(9-anthroyloxy)stearic acid (12-AS) and 16-(9-anthroyloxy)palmitic acid (16-AP) were 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 Preparation of Biomimetic Membranes

DPH-labelled liposomal membranes were prepared to be unilamellar vesicles suspended in a buffer as reported previously [23,24]. In brief, the dry film of phospholipids and cholesterol was dissolved with an ethanol solution of DPH. An aliquot (250 µl) of the resulting solution (total lipids of 10 mM and DPH of 50 µM) was injected four times into 199 ml of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4, containing 125 mM NaCl and 25 mM KCl) under stirring above the phase transition temperatures of phospholipids. The membrane lipid compositions were as follows: (1) 100 mol% DPPC for DPPC membranes that have been most frequently used for membrane interaction experiments [23-25], (2) 11 mol% POPC, 16.5 mol% POPE, 11 mol% SOPS, 16.5 mol% SM and 45 mol% cholesterol to mimic peripheral nerve cell membranes [26], (3) 36 mol% POPC, 22 mol% POPE, 3.5 mol% SOPS, 3.5 mol% SM and 35 mol% cholesterol to mimic central nerve cell membranes [27], and (4) 25 mol% POPC, 16 mol% POPE, 3 mol% POPS, 10 mol% CL, 3 mol% PI, 3 mol% SM and 40 mol% cholesterol to mimic cardiomyocyte membranes [28]. Lipid raft model membranes were prepared with 16.7 mol% DOPC, 16.7 mol% POPE, 16.7 mol% SM, 16.7 mol% CB and 33.3 mol% cholesterol [29].

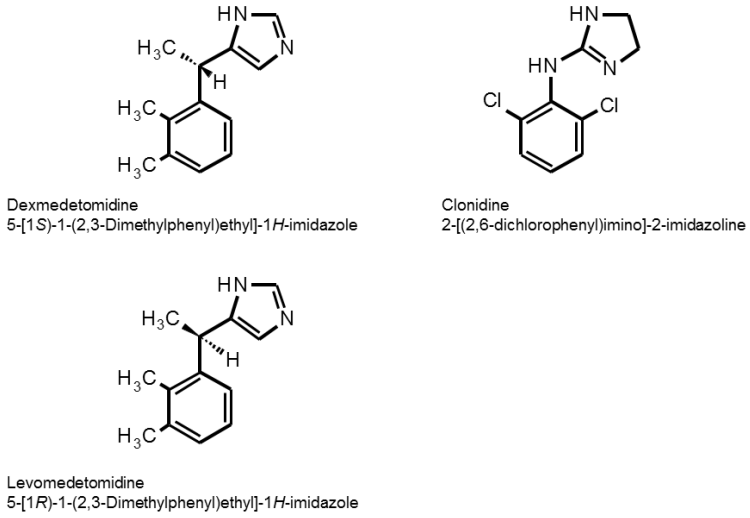

Fig. 1. Chemical structures of drugs tested in this study

In order to evaluate the effect of cholesterol on membrane interaction, neuro-mimetic membranes were prepared with the constant molar ratio of being POPC : POPE : SOPS : SM = 11 : 16.5 : 11 : 16.5 in the absence or presence of 45 mol% cholesterol.

2.3 Determination of Membrane Interactivity

DMSO solutions of drugs were applied to the membrane preparations so that the final concentrations of drugs ranged 10-200 μM. The concentration of DMSO was adjusted to be 0.5% (v/v) of the total volume so as not to affect the fluidity of intact membranes. Control experiments were conducted with the addition of an equivalent volume of DMSO. After reacting at 37°C for 45 min, DPH fluorescence polarization was measured at 360 nm for excitation wavelength and 430 nm for emission wavelength by an RF-540 spectrofluorometer (Shimadzu; Kyoto, Japan) equipped with a polarizer and a cuvette thermo-controlled at 37°C. Polarization values were calculated according to the method of Ushijima et al. [30]. Compared with controls, a decrease of fluorescence polarization means an increase of membrane fluidity. When comparing the interactivity between different membranes, the polarization changes (%) relative to control polarization values were used because the polarization values of control membranes change with varying membrane lipid compositions.

2.4 Specification of Membrane Region for Enantioselective Interaction

Biomimetic membranes were prepared with Avanti's enantio-pure phospholipids of L-isomer as described above, but without the use of DPH. Their membrane lipid composition was 25 mol% POPC, 16 mol% POPE, 3 mol% POPS, 10 mol% CL, 3 mol% PI and 3 mol% SM plus either 40 mol% cholesterol or 40 mol% epicholesterol (a final concentration of 12.5 μM for total lipids). Dexmedetomidine and levomedetomidine were dissolved with DMSO, and the resulting solutions were applied to the membrane preparations at 50 μM for each. The concentration of DMSO was adjusted to be 0.5% (v/v) of the total volume so as not to affect the fluidity of intact membranes. Control experiments were conducted with the addition of an equivalent volume of DMSO. After reacting at 37°C for 45 min, the membranes were labelled with 2-AS, 6-AS, 9-AS, 12-AS or 16-AP (the molar ratio of *n*-AS(P) to total membrane lipids being 1 : 210) as reported previously [31]. Fluorescence polarization was measured at 367 nm for excitation wavelength and 443 nm for emission wavelength. *n*-AS(P) (*n* = 2, 6, 9, 12 and 16) selectively locate at a graded series of levels in lipid bilayers to show the gradient of fluidity from the surface to the center of membranes depending on an increase of *n*. Since the deeper regions of lipid bilayer membranes are more fluid than the superficial

region, *n*-AS(P) polarization values decrease with increasing *n*. The *n*-AS(P) polarization changes (%) relative to control polarization values were used to specify the membrane region for enantioselective interaction.

2.5 Statistical Analysis

The data were statistically analyzed by a one-way analysis of variance (ANOVA) followed by a post hoc Fisher's protected least significant difference (PLSD) test using StatView 5.0 (SAS Institute; Cary, NC, USA). All results are expressed as mean ± S.E.M. (n = 8 for each experiment). Statistical significance was defined as a $P < .05$.

3. RESULTS

3.1 Drug and Membrane Interaction

Dexmedetomidine and clonidine concentration-dependently acted on lipid bilayers to increase the fluidity of DPPC and neuro-mimetic membranes as shown by DPH polarization decreases (Fig. 2A). The interactivity of dexmedetomidine was evident in neuro-mimetic membranes at 10-200 μM, although clonidine was not effective at 10 and 25 μM.

Figs. 2B and 2C show the comparative effects of dexmedetomidine and levomedetomidine on neuro-mimetic membranes in the absence or presence of cholesterol. Both enantiomers interacted with the membranes containing cholesterol to induce smaller polarization changes than with the membranes not containing cholesterol. However, dexmedetomidine was discriminated from levomedetomidine by interacting with cholesterol-containing membranes more potently than levomedetomidine ($P < .01$), whereas dexmedetomidine and levomedetomidine showed no significant difference in interaction with the membranes not containing cholesterol.

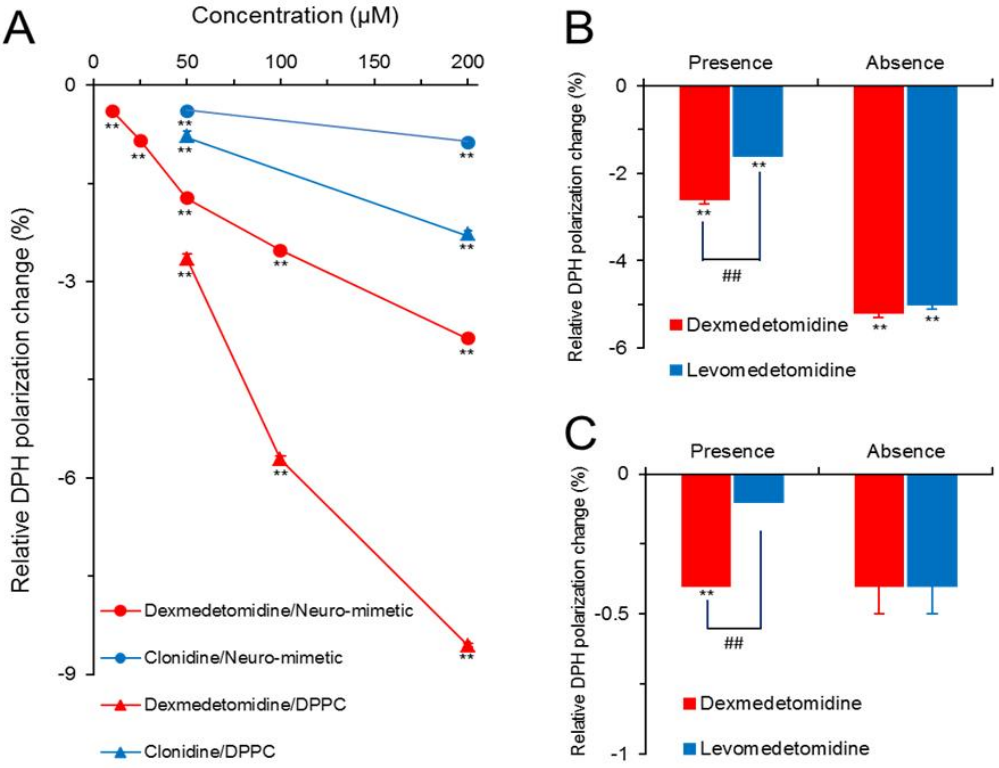

Fig. 2. Concentration-dependent interactions of dexmedetomidine and clonidine with DPPC and neuro-mimetic membranes (A) and interactions of dexmedetomidine and levomedetomidine with neuro-mimetic membranes in the absence or presence of cholesterol (B, 100 μM for each and C, 10 μM for each)

*Data are mean ± S.E.M. (n = 8). **P < .01 compared with control. ##P < .01 compared between dexmedetomidine and levomedetomidine*

3.2 Membrane Interactivity Depending on Lipid Composition

α_2-Adrenergic agonists increased the fluidity of biomimetic membranes with the potency depending on the composition of membrane lipids (Table 1). Based on DPH polarization decreases, dexmedetomidine was 1.8-4.2 times more effective on all the tested membranes than clonidine. The relative polarization changes (%) indicated that the rank order of membrane interactivity of dexmedetomidine was cardiomyocyte-mimetic > DPPC > peripheral nerve cell-mimetic > central nerve cell-mimetic membranes. Dexmedetomidine was superior to levomedetomidine in interactivity with peripheral nerve cell-mimetic, central nerve cell-mimetic and cardiomyocyte-mimetic membranes. The effects of dexmedetomidine and clonidine on lipid raft model membranes were much weaker than those on other membranes. The raft-like membranes did not show so large difference between dexmedetomidine and levomedetomidine.

As shown by DPH polarization values of intact membranes (Fig. 3A), cardiomyocyte-mimetic membranes showing the smallest values were most fluid, followed by DPPC, central nerve cell-mimetic and peripheral nerve cell-mimetic membranes in the decreasing order of membrane fluidity. At 50 μM for each, dexmedetomidine and clonidine induced larger polarization changes (greater membrane fluidization) in more fluid cardiomyocyte-mimetic and DPPC membranes, whereas both of them showed smaller changes in less fluid neuro-mimetic membranes (Fig. 3B). Dexmedetomidine was more effective in decreasing DPH fluorescence polarization of peripheral nerve cell-mimetic membrane than central nerve cell-mimetic membranes.

3.3 Membrane Region for Enantioselective Interaction

Dexmedetomidine induced greater fluidity increases in cholesterol-containing membranes than levomedetomidine as shown by *n*-AS polarization decreases (Fig. 4A). In contrast to dexmedetomidine, levomedetomidine interacted with epicholesterol-containing membranes more potently. Fig. 4B shows the relative *n*-AS(P) polarization changes of dexmedetomidine to levomedetomidine in cholesterol-containing membranes. 2-AS and 6-AS polarization showed the largest difference between two enantiomers, which became smaller with increasing *n*, but not 16-AP polarization.

4. DISCUSSION

Our main findings are: (1) dexmedetomidine interacts with biomimetic membranes consisting of phospholipids and cholesterol more potently than clonidine and levomedetomidine, (2) its interactivity depends on membrane lipid components and their compositions, (3) the effects of dexmedetomidine and clonidine on lipid raft model membranes are significantly weaker compared with those on other membranes, and (4) dexmedetomidine acts preferentially on the superficial region of membranes containing cholesterol with the potency discriminable from that of levomedetomidine and the rank order of such membrane interactivity is reversed by replacing cholesterol with epicholesterol.

Lipophilicity of drug molecules is one of important factors to enhance their membrane interactivity [25], and the receptor action of adrenergic drugs correlates to a high degree of their lipophilicity [32]. The octanol/buffer partition coefficient is 2.8 for dexmedetomidine and 0.8 for clonidine [16]. Dexmedetomidine, which is 3.5-fold more lipophilic than clonidine, should interact with lipid membranes more effectively. Compared with clonidine at an identical concentration of 50 μM, dexmedetomidine induced 3.5-, 4.2- and 3.5-fold larger DPH polarization decreases of DPPC, peripheral nerve cell-mimetic and cardiomyocyte-mimetic membranes, respectively. These results are almost consistent with a difference in lipophilicity between two α_2-adrenergic agonists. The 2,3-dimethylphenylethyl structure is speculated to provide dexmedetomidine with greater membrane interactivity than clonidine without such a structural moiety.

Dexmedetomidine is seven times more selective for α_2-adrenergic receptors than clonidine [7,12]. The receptor selectivity of adrenergic drugs is related to their membrane interactivity [21,33,34]. In the present study, dexmedetomidine was 4.2 and 3.5 times more effective than clonidine in interacting with peripheral nerve cell-mimetic and cardiomyocyte-mimetic membranes, respectively, although the relative potency of membrane interactivity was not quantitatively correlated with the difference of receptor selectivity. When used as an adjuvant to local anesthesia in supraclavicular brachial plexus block, dexmedetomidine makes the duration of analgesia 1.6 times longer than clonidine [14]. In

Table 1. Effects of dexmedetomidine, clonidine and levomedetomidine (50 μM for each) on different membranes

Drugs	DPPC membranes	Peripheral nerve cell-mimetic membranes	Central nerve cell-mimetic membranes	Cardiomyocyte-mimetic membranes	Lipid raft model membranes
DPH polarization change from control					
Dexmedetomidine	–0.0059 ± 0.0002**	–0.0054 ± 0.0001**	–0.0042 ± 0.0001**	–0.0083 ± 0.0002**	–0.0032 ± 0.0001**
Clonidine	–0.0017 ± 0.0002*	–0.0013 ± 0.0001	–0.0023 ± 0.0002**	–0.0024 ± 0.0001**	–0.0009 ± 0.0001**
Levomedetomidine	–0.0060 ± 0.0002**	–0.0038 ± 0.0001**	–0.0030 ± 0.0001**	–0.0064 ± 0.0002**	–0.0026 ± 0.0000**
DPH polarization change (%) relative to control polarization value					
Dexmedetomidine	–2.7 ± 0.1**	–1.8 ± 0.0**	–1.5 ± 0.0**	–3.8 ± 0.1**	–1.2 ± 0.0**
Clonidine	–0.8 ± 0.1*	–0.4 ± 0.0	–0.8 ± 0.0**	–1.1 ± 0.1**	–0.3 ± 0.0**
Levomedetomidine	–2.7 ± 0.1**	–1.3 ± 0.0**	–1.1 ± 0.0**	–3.0 ± 0.1**	–0.9 ± 0.0**

*Data are mean ± S.E.M. (n = 8 for each experiment). *P < .05, **P < .01 compared with control*

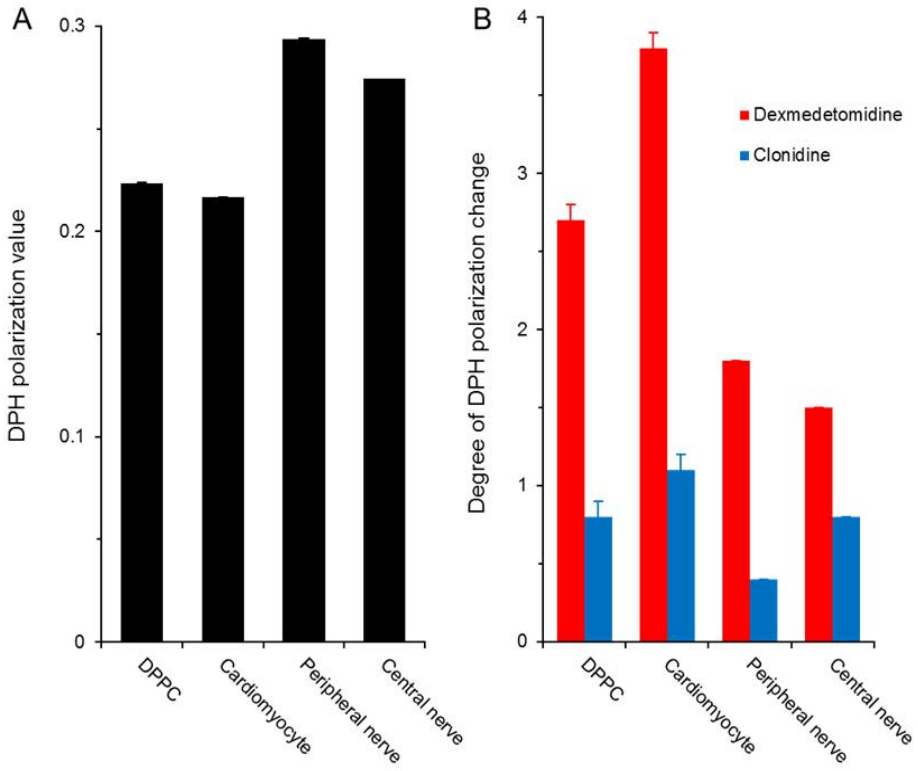

Fig. 3. Comparisons of the fluidity between different membranes and the membrane effect between two α_2-adrenergic agonists. A, DPH polarization values of intact DPPC, cardiomyocyte-mimetic, peripheral nerve cell-mimetic and central nerve cell-mimetic membranes. B, Degrees of DPH polarization changes induced by dexmedetomidine and clonidine (50 μM for each)

Data are mean ± S.E.M. (n = 8)

patients received bupivacaine plus α_2-adrenergic agonists for lower limb surgery, intrathecal dexmedetomidine produces 1.3-1.4 times longer sensory and motor block than clonidine [35]. Epidural anesthesia with ropivacaine and α_2-adrenergic agonists shows higher sedation scores in a dexmedetomidine group than in a clonidine group [13]. The rank orders of potency for dexmedetomidine and clonidine to produce sedation and analgesia are compatible with those of their interactivity with biomimetic membranes.

Dexmedetomidine seems to interact with relatively fluid membranes more effectively. Despite almost the same fluidity, cardiomyocyte-mimetic membranes showed larger changes in fluidity than DPPC membranes. Cardiomyocyte-mimetic membranes exclusively contain anionic CL that could electrostatically interact with a basic imidazole ring of dexmedetomidine to produce more significant membrane fluidization as reported for cationic drugs [36]. Although dexmedetomidine is used as a local anesthetic adjuvant for neuraxial and peripheral nerve block, it induces hypotension and bradycardia [37]. The greater interactivity of dexmedetomidine with cardiomyocyte-mimetic membranes may be responsible for its adverse cardiovascular effects. Dexmedetomidine was more active on peripheral nerve cell-mimetic membranes than central nerve cell-mimetic membranes, which may be accounted for by the compositional difference of negatively chargeable SOPS. Membrane lipids and their compositions vary according to cell and tissue types [38]. Such variations could be linked to the cell-specific membrane interactions of drugs that show different effects between neuronal and cardiovascular cells.

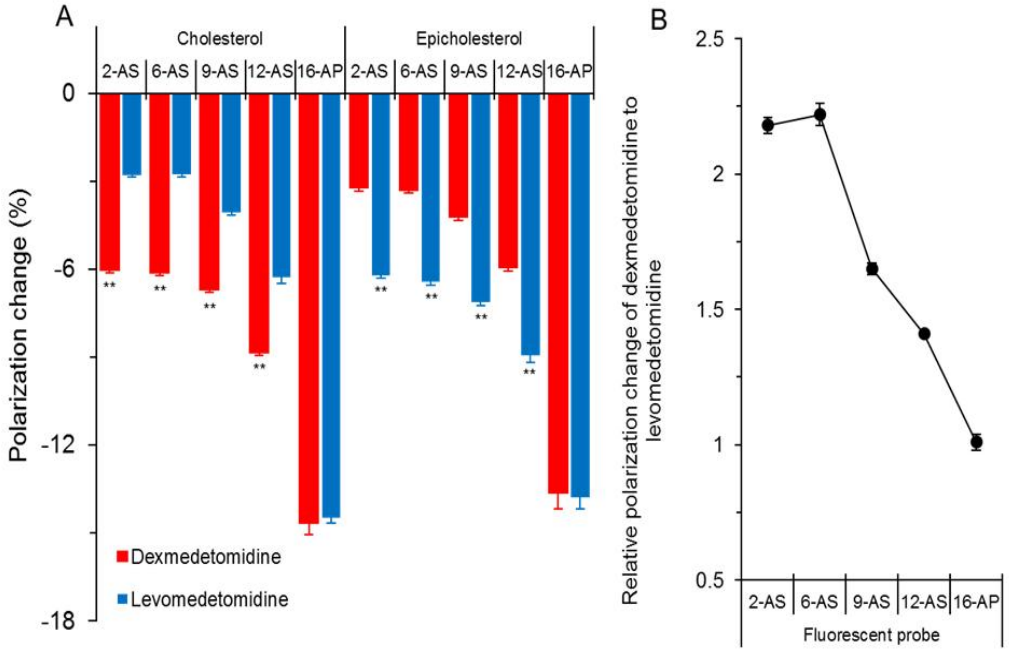

Fig. 4. Interactions of dexmedetomidine and levomedetomidine with biomimetic membranes containing either cholesterol or epicholesterol. A, *n*-AS(P) polarization changes induced by dexmedetomidine and levomedetomidine (50 μM for each). B, *n*-AS(P) polarization changes of dexmedetomidine relative to levomedetomidine in cholesterol-containing membranes

*Data are mean ± S.E.M. (n = 8). **P < .01 compared between dexmedetomidine and levomedetomidine*

Lipid raft microdomains, in which cholesterol and sphingolipids are packed in a highly ordered structure, play an important role as the platform for receptors. It has been suggested that anesthetic agents may target lipid rafts or their action may be mediated by the interaction with lipid raft membranes [39-41]. Since the property of lipid rafts is reproducible in unilamellar vesicles by a defined molar mixture of specific phospholipids and cholesterol [42], we evaluated the interactions of α_2-adrenergic agonists with lipid raft model membranes. However, the effects of dexmedetomidine and clonidine on such membranes were much weaker than those on neuro-mimetic and cardiomyocyte-mimetic membranes. The mechanistic relevance of lipid rafts to α_2-adrenergic agonists is inconclusive at least from their low interactivity with raft-like liquid-ordered membranes. Lipid rafts form caveolae by polymerizing with caveolins to bind to cholesterol. The localization in caveolae/lipid rafts is prerequisite for adrenergic receptor subtypes [43]. α_1-Adrenergic receptor signaling is associated with caveolae considered as a special type of lipid rafts [44], and β_2- and β_1-adrenergic receptors are localized in lipid rafts [45]. By contrast, the specific existence of α_2-adrenergic receptors in lipid rafts has not been reported previously.

When dexmedetomidine and levomedetomidine were subjected to reactions with the membranes consisting of DPPC alone, they were not discriminated in membrane interactivity. However, dexmedetomidine and levomedetomidine interacted differentially with peripheral nerve cell-mimetic, central nerve cell-mimetic and cardiomyocyte-mimetic membranes, all of which contain cholesterol. A crucial role of cholesterol is evident in the discriminable membrane interactions between dexmedetomidine and levomedetomidine only in the presence of cholesterol in membranes. Cholesterol with several chiral centers can endow lipid bilayers with chirality, thereby allowing enantiomers to interact with chiral lipid membranes enantioselectively. Dexmedetomidine showed relatively weak effects on cholesterol-containing membranes, which is attributable to the property of cholesterol to affect membrane fluidity [46].

The detailed location and orientation of dexmedetomidine in membranes are not found in the literature. Because of its amphiphilic structure, however, dexmedetomidine is presumable to locate in lipid bilayers with a dimethylphenylethyl moiety penetrating into the phospholipid acyl chains and a positively chargeable imidazole group positioned closer to the lipid/water interface. Cholesterol orients in membranes with a polar hydroxyl group encountering the aqueous phase, a hydrophobic tetra-ring system buried in the hydrocarbon chains of phospholipids and an isooctyl side-chain reaching the hydrophobic core of lipid bilayers [47]. With respect to the vertical localization in lipid bilayers, a 3β-hydroxyl group of cholesterol is positioned in the region of phospholipid carbonyl groups, while a 3α-hydroxyl group of epicholesterol, in the region of phospholipid phosphate groups [48]. Chiral membrane lipids interact preferentially with molecules of the same chirality [49], possibly increasing selectivity for one enantiomer. *n*-AS(P) polarization differences between dexmedetomidine and levomedetomidine were larger with decreasing "*n*" and the rank order of their membrane interactivity was reversed by replacing cholesterol with epicholesterol. These results suggest that the superficial region of membrane lipid bilayers and cholesterol's 3β-hydroxyl groups positioned there are responsible for the enantioselective interaction. The opposite configuration of a 3-hydroxyl group in cholesterol and epicholesterol would allow two enantiomers to be recognized differently through the interaction with chiral cholesterol and epicholesterol [22,50].

Our study may have some limitations in pharmacological significance of the membrane interactivity. First, the concentrations for dexmedetomidine to interact with biomimetic membranes may be over clinically-relevant ones. When patients received infusions of dexmedetomidine at 2.5-6.0 μg kg^{-1} h^{-1} for 10 min followed by 0.4-0.7 μg kg^{-1} h^{-1}, peak concentrations of dexmedetomidine in plasma range 0.004-0.01 μM [51]. In the present study, dexmedetomidine needed micromolar concentrations to exhibit the significant interactivity with biomimetic membranes. However, lipophilic drugs easily penetrate into lipid bilayers to be concentrated in membranes with high local concentrations. Adrenergic drugs with almost the same partition coefficients as dexmedetomidine show higher concentrations in lipid bilayer membranes than in incubation media [52]. Second, we used unilamellar vesicles or liposomal membranes to focus on the interaction between drugs and lipids. Even if dexmedetomidine-induced changes in such protein-free membranes are not so large, membrane-acting drugs are known to produce much greater effects on cellular membranes containing protein components [53,54]. Third, despite that levomedetomidine is clinically inactive, it induced small but significant changes in membrane fluidity. Although levomedetomidine has the property to enhance bradycardia at remarkably high doses [55], its effects are much weaker or negligible at clinically-relevant concentrations compared with those of dexmedetomidine. Fourth, it remains unclear whether the membrane effects of α_2-adrenergic agonist dexmedetomidine and clonidine are competitively inhibited by α_2-adrenergic antagonists. Burgess et al. treated DPH-labelled plasma membranes prepared from rat liver with noradrenaline in the presence of propranolol to block β-adrenergic receptors, and then measured fluorescence polarization to determine membrane fluidity changes [56]. Consequently, noradrenaline increased the membrane fluidity at 0.1-5 μM but its membrane effects were inhibited by non-selective α-adrenergic blocker phentolamine (1 μM) and phenoxybenzamine (50 μM). This inhibition might be caused through α_2-adrenergic receptors because α_1-adrenergic antagonist prazosin induces the fluidization of phospholipid/cholesterol monolayers [57].

Besides their intrinsic effects induced by binding to the receptors, α_2-adrenergic agonists are able to exert diverse effects, which are not necessarily interpreted by only the mode of drug action on receptor proteins [58,59]. Some of pharmacological properties of dexmedetomidine would not be dependent on the interaction with α_2-adrenergic receptors but derived from the interaction with membrane lipid bilayers. Membrane lipids have been considered not only as a passive component to constitute biological membranes but also as a factor to regulate the functions of membrane-associated proteins. Since integral membrane proteins are not rigid entities, their activities are affected by lipid molecules surrounding them [60]. Membrane-interacting drugs produce changes of membrane physicochemical properties, in particular fluidity, which modulate the localization and activity of membrane-embedded proteins such as receptors [61,62]. While noradrenaline and adrenaline physiologically bind to adrenergic receptors, these neurotransmitters also diffuse into synaptic membranes and influence them biophysically,

shifting the receptor conformational equilibrium [63]. Amphiphilic drugs commonly interact with membrane lipid bilayers, mechanistically underlying their adrenergic, analgesic, sedative, cardio-protective, anti-ischemic, anti-inflammatory and channel-inhibitory effects [25,33,52].

5. CONCLUSION

This is the first study to determine the property of dexmedetomidine to interact with biomimetic lipid bilayer membranes. Dexmedetomidine has been confirmed to increase the fluidity of different biomimetic membranes more potently than clonidine and levomedetomidine, being consistent with the rank orders of their clinical and experimental effects. Dexmedetomidine would modify the physicochemical properties of biomembranes to affect membrane-embedded proteins like receptors and membrane-relevant pathophysiological events such as inflammation, lipid peroxidation, ischemia and channel activation. In addition to higher selectivity for α_2-adrenergic receptors, the structure-specific membrane interactivity characterizes dexmedetomidine by discriminating this anesthetic adjuvant from another α_2-adrenergic agonist and an inactive enantiomeric counterpart.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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