Chemico-Biological Interactions 183 (2010) 19–24

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Local anesthetics structure-dependently interact with anionic phospholipid membranes to modify the fluidity

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ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form 4 October 2009
Accepted 12 October 2009
Available online 22 October 2009

Keywords:
Local anesthetics
Membrane interaction
Anionic phospholipids
Fluidity modification
Structure-dependence
Cardiotoxicity

ABSTRACT

While bupivacaine is more cardiotoxic than other local anesthetics, the mechanistic background for different toxic effects remains unclear. Several cardiotoxic compounds act on lipid bilayers to change the physicochemical properties of membranes. We comparatively studied the interaction of local anesthetics with lipid membranous systems which might be related to their structure-selective cardiotoxicity. Amide local anesthetics (10–300 μM) were reacted with unilamellar vesicles which were prepared with different phospholipids and cholesterol of varying lipid compositions. They were compared on the potencies to modify membrane fluidity by measuring fluorescence polarization. Local anesthetics interacted with liposomal membranes to increase the fluidity. Increasing anionic phospholipids in membranes enhanced the membrane-fluidizing effects of local anesthetics with the potency being cardiolipin ≫ phosphatidic acid > phosphatidylglycerol > phosphatidylserine. Cardiolipin was most effective on bupivacaine, followed by ropivacaine. Local anesthetics interacted differently with biomimetic membranes consisting of 10 mol% cardiolipin, 50 mol% other phospholipids and 40 mol% cholesterol with the potency being bupivacaine ≫ ropivacaine > lidocaine > prilocaine, which agreed with the rank order of cardiotoxicity. Bupivacaine significantly fluidized 2.5–12.5 mol% cardiolipin-containing membranes at cardiotoxicologically relevant concentrations. Bupivacaine is considered to affect lipid bilayers by interacting electrostatically with negatively charged cardiolipin head groups and hydrophobically with phospholipid acyl chains. The structure-dependent interaction with lipid membranes containing cardiolipin, which is preferentially localized in cardiomyocyte mitochondrial membranes, may be a mechanistic clue to explain the structure-selective cardiotoxicity of local anesthetics.

1. Introduction

Local anesthesia is accompanied by the potential risk such as cardiovascular disorders which is a rare but fatal complication. Local anesthetics display cardiotoxic capacities when their concentrations in blood are elevated by an accidental intravenous injection and an absolute overdose. Great concerns over local anesthetic cardiotoxicity include profound bradycardia, arrhythmia, myocardial depression and eventually cardiovascular collapse [1]. While local anesthetics show the pharmacotoxicological diversity, especially the cardiotoxic potency is known to be different between them. Bupivacaine, a long-acting amide anesthetic, has been widely used for cutaneous infiltration, regional nerve block, epidural anesthesia and spinal anesthesia in surgery and obstetrics. However, this agent is more toxic than other commonly used ones. In humans, bupivacaine is noted to exert a cardiotoxic effect at much lower serum levels than those required for other local anesthetics. Serious cardiac arrhythmias develop with the use of bupivacaine, but not with a shorter-acting amide local anesthetic lidocaine [2]. The rank order of cardiotoxic potency has been estimated to be bupivacaine > ropivacaine > lidocaine > prilocaine [3]. However, the detailed mechanism(s) for structure-selective cardiotoxicity is still unclear.

Although the primary target of local anesthetics is referred to as ion channels of cardiomyocytes, the action on other sites has been presumed to contribute to the cardiotoxic discrimination between structurally different drugs [3]. Local anesthetics have the ability to change membrane physicochemical properties such as fluidity or lipid packing order, and their induced membrane fluidization or disordering influences not only directly the functions

Abbreviations: POPA, 1-palmitoyl-2-oleoylphosphatidic acid; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; POPI, 1-palmitoyl-2-oleoylphosphatidylinositol; POPS, 1-palmitoyl-2-oleoylphosphatidylserine; SM, sphingomyelin; DPH, 1,6-diphenyl-1,3,5-hexatriene; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMSO, dimethyl sulfoxide.

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doi:10.1016/j.cbi.2009.10.006

of biomembranes but also indirectly the activities of membrane-embedded channels, receptors and enzymes through alterations of the membranous lipid environment and the protein conformation [4]. The generation of ionic currents affected by anesthetics also requires the molecular interplay of ion channel proteins and membrane lipids [5]. Since local anesthetics are present in cationic and non-ionic form under physiological conditions, they would show both electrostatic and hydrophobic interaction with cardiomyocyte membranes. Local anesthetics show different potencies to interact with membrane lipid bilayers depending on the lipid composition. In particular, they strongly interact with the membranes consisting of anionic phospholipids [6,7].

Several cardiotoxic compounds affect the permeability of mitochondrial membranes [8]. While local anesthetics increase membrane permeability by acting on lipid bilayers, such an effect of bupivacaine is associated with the seriousness of cardiotoxicity [9]. Önyüksel et al. [10] presumed membrane cardiolipin to be the possible determinant for local anesthetics to produce their different cardiotoxicity. In their study, bupivacaine, but not lidocaine, increased the permeability of 7.5 mol% cardiolipin-containing liposomes at 200 and 400 μM. However, they found that bupivacaine and lidocaine were inactive at 100 and 400 μM, respectively, despite that these concentrations are higher than their plasma concentrations to produce cardiac collapse and depress myocardial function [11,12].

Membrane permeability is influenced by the change in membrane fluidity [13]. Local anesthetics induce membrane fluidization which is linked to the mode of pharmacotoxicological action. The aim of this study was to find the drug and membranous system interaction which might be related to the structure-selective cardiotoxicity of local anesthetics. Based on the hypothesis that bupivacaine may fluidize anionic phospholipid membranes more intensively than other local anesthetics at cardiotoxicologically relevant concentrations, we addressed whether local anesthetics interact differently with liposomal and biomimetic membranes containing anionic phospholipids and which specific component(s) is responsible for the structure-selective membrane fluidization.

2. Materials and methods

2.1. Materials

Bupivacaine, lidocaine and prilocaine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ropivacaine was supplied by AstraZeneca (Södertälje, Sweden). Phospholipids: 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), cardiolipin, 1-palmitoyl-2-oleoylphosphatidylserine (POPS), 1-palmitoyl-2-oleoylphosphatidylinositol (POPI), sphingomyelin (SM), 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG) and 1-palmitoyl-2-oleoylphosphatidic acid (POPA) were obtained from Avanti Polar Lipids (Alabaster, AL, USA), and cholesterol and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) from Wako Pure Chemicals (Osaka, Japan). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased 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 grade available commercially.

Table 1
Preparation of 40 mol% cholesterol-containing liposomal membranes of varying phospholipid compositions.

Anionic phospholipids (mol%)	Other phospholipids (mol%)			
	POPC	POPE	POPI	SM
Cardiolipin				
0	32	20.4	3.8	3.8
5	29.3	18.7	3.5	3.5
10	26.6	17	3.2	3.2
20	21.3	13.6	2.55	2.55
POPS				
0	32	20.4	3.8	3.8
5	29.3	18.7	3.5	3.5
10	26.6	17	3.2	3.2
20	21.3	13.6	2.55	2.55
POPG				
0	32	20.4	3.8	3.8
5	29.3	18.7	3.5	3.5
10	26.6	17	3.2	3.2
20	21.3	13.6	2.55	2.55
POPA				
0	32	20.4	3.8	3.8
5	29.3	18.7	3.5	3.5
10	26.6	17	3.2	3.2
20	21.3	13.6	2.55	2.55

2.2. Preparation of anionic phospholipid liposomal membranes

DPH-labeled unilamellar vesicles were prepared with 60 mol% phospholipids and 40 mol% cholesterol according to the method of Okimoto et al. [14] with some modifications as follows: phospholipids and cholesterol (total lipids of 10 mM), and DPH (50 μM) were dissolved in ethanol. An aliquot (250 μl) of the ethanol solution was injected four times into 199 ml of 10 mM HEPES buffer (pH 7.4, containing 125 mM NaCl and 25 mM KCl) under stirring above the phase transition temperatures of phospholipids. Phospholipid compositions other than 40 mol% cholesterol are shown in Table 1. Whereas the relative contents of anionic phospholipids (cardiolipin, POPS, POPG and POPA) were varied, the other phospholipids were adjusted to have the constant molar ratio of being POPC:POPE:POPI:SM = 25:16:3:3 for all preparations.

2.3. Preparation of biomimetic membranes

Biomimetic membranes were prepared as described in Section 2.2 to have the composition of major lipids in human cardiomyocyte mitochondrial membranes [15]. They consisted of 25 mol% POPC, 16 mol% POPE, 10 mol% cardiolipin, 3 mol% POPS, 3 mol% POPI, 3 mol% SM and 40 mol% cholesterol.

Table 2
Relative polarization changes of liposomal membranes containing 10 mol% cardiolipin, POPS, POPG or POPA which were treated with local anesthetics of 200 μM for each.

Local anesthetics	Relative polarization changes of membranes containing			
	Cardiolipin	POPS	POPG	POPA
Bupivacaine	1.00 ± 0.03	0.36 ± 0.02	0.40 ± 0.02	0.49 ± 0.02
Ropivacaine	1.00 ± 0.05	0.47 ± 0.03**	0.53 ± 0.04**	0.62 ± 0.05**
Lidocaine	1.00 ± 0.09	0.59 ± 0.05**	0.67 ± 0.05**	0.71 ± 0.03**
Prilocaine	1.00 ± 0.07	0.70 ± 0.07**	0.74 ± 0.08**	0.76 ± 0.06**

Data are presented as mean ± S.E. ($n = 7$).
** $p < 0.01$ compared with bupivacine.