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Biophysical Chemistry 129 (2007) 82–91

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Does propofol alter membrane fluidity at clinically relevant concentrations? An ESR spin label study

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Received 7 March 2007; received in revised form 15 May 2007; accepted 17 May 2007
Available online 24 May 2007

Abstract

General anesthetics have been shown to perturb the membrane properties of excitable tissues. Due to their lipid solubility, anesthetics dissolve in every membrane, penetrate into organelles and interact with numerous cellular structures in multiple ways. Several studies indicate that anesthetics alter membrane fluidity and decrease the phase-transition temperature. However, the required concentrations to induce such effects on the properties of membrane lipids are by far higher than clinically relevant concentrations. In the present study, the fluidizing effect of the anesthetic agent propofol (2,6-diisopropyl phenol: PPF), a general anesthetic extensively used in clinical practice, has been investigated on liposome dimyristoyl-L-α phosphatidylcholine (DMPC) and cell (erythrocyte, Neuro-2a) membranes using electron spin resonance spectroscopy (ESR) of nitroxide labeled fatty acid probes (5-, 16-doxyl stearic acid). A clear effect of PPF at concentrations higher than the clinically relevant ones was quantified both in liposome and cell membranes, while no evident fluidity effect was measured at the clinical PPF doses. However, absorption spectroscopy of merocyanine 540 (MC540) clearly indicates a PPF fluidizing capacity in liposome membrane even at these clinical concentrations. PPF may locally influence the structure and dynamics of membrane domains, through the formation of small-scale lipid domains, which would explain the lack of ESR information at low PPF concentrations.

Keywords: ESR; Microviscosity; Membrane; Propofol; Neuro-2a

1. Introduction

Several drugs can be solubilized inside the membrane bilayer and some of them induce lipid perturbations such as a decrease in membrane fluidity, order and permeability. Propofol (PPF) is a nonbarbiturate intravenous anesthetic agent currently used for the induction and maintenance of general anesthesia in clinical practice [1]. It is characterized by a unique phenolic structure not present in any other conventional anesthetic. Due to its lipophilic property, PPF is presumed to penetrate into membranes and to interact with lipids inducing changes in membrane fluidity [2]. Electron spin resonance (ESR), fluorescence spectroscopy or differential scanning calorimetry techniques have allowed to qualitatively demonstrate that PPF modifies membrane fluidity and reduces the phase-transition temperature [3,4]. Although, the $GABA_A$ receptor is now identified as a key target for propofol [5], lipid theory of anesthesia is not totally discarded and a correlation between its action and lipid perturbations is possible. Indeed, Cantor in his lateral pressure profile model

Abbreviations: DMPC, Dimyristoyl-L-α phosphatidylcholine; *n*-DSA, *n*-doxyl stearic acids spin probe; ESR, Electron spin resonance; MC540, Merocyanine 540; PPF, 2,6-diisopropylphenol (propofol); 5-DSA, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxy; 16-DSA, 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxy; τ_c, correlation time parameter; *S*, order parameter.

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doi:10.1016/j.bpc.2007.05.011

connects general anesthesia with anesthetic anisotropic effects on the lateral pressure profile in membranes which are very large even at low anesthetic concentrations [6–8]. The membrane fluidizing effect of PPF has been seen as a concept enclosing a major quantitative problem [5]. Indeed, anesthetic concentration necessary to cause changes in the properties of membrane lipids has been reported to be of several orders of magnitude higher than the clinically relevant concentrations [9,10]. Blood PPF concentrations in the range of 10^{-6} M are commonly used in clinical practice and assumed as effective concentrations for providing anesthesia [4]. Modern techniques for delivering intravenous anesthesia use perfusion systems that include pharmacokinetic models and allow the practitioner to target effect-site concentrations of a given drug, defined as the concentration supposed to be measured at the site of action in the central nervous system. It has been shown that, PPF free aqueous concentrations about 0.4 μM for the effective concentration 50% (EC_{50}) are appropriate benchmarks for quasi steady state concentration required for anesthesia [5,11]. However, given the nature of the different media in the central nervous system which do not share the same biophysical properties, one can reasonably assume that the concentrations of PPF are not necessary similar inside and outside the lipid membranes. Unfortunately, there is no technique available to measure such effective concentrations at the cellular level. Therefore, quantifying the change in membrane fluidity induced by PPF especially at clinically relevant concentrations could be important for a better understanding of the molecular mechanism of its anesthetic action. Hence, this study covers a wide range of PPF concentrations which should include real concentrations at the cellular membrane scale corresponding to effective blood concentration ranges.

Based on one previous work [12], ESR with nitroxide spin probes was used as a method to probe the membrane microviscosity. Indeed, the relative anisotropy observed in an ESR spectrum is directly related to the rotational mobility of the probe, and can be correlated with the probe microenvironment. The change in probe mobility allows to study the membrane fluidity and often yields useful information on the dynamic state of membrane phospholipids [13]. In particular, nitroxide labeled fatty acid probes (*n*-doxyl stearic acid) have been shown to be useful to determine these parameters within lipids and cell membranes [14]. Absolute values of microviscosity can be obtained after prior calibration of the ESR spectra of nitroxide probes in mixtures of known viscosities [12]. In these experiments, the microviscosity is defined as the homogenous solution viscosity, which results in a spectrum identical to the one recorded in the explored microenvironment [15]. Moreover, absorption spectroscopy of merocyanine 540 (MC540) was also used as a second method to probe the fluidity change on membranes. Indeed, the lipophilic dye MC540 has been used as a sensor of molecular events either in model membrane systems or in biological membrane [16] because its absorption spectrum is very sensitive to the solubilizing surrounding properties. MC540 is shown to be located at the membrane interfacial region under dimeric and monomeric forms [17,18]. It preferentially binds to membranes with loosely packed lipids and binds to fluid-phase vesicles rather than to gel-state vesicles. Indeed, in the fluid-state vesicles, the dye molecules have an absorption spectrum characteristic of molecules in a hydrophobic environment. In contrast, at a similar concentration in the gel-state vesicles, the absorption spectrum changes and reflects molecules in aqueous solvent [19]. Thus, any change, even small, on membrane fluidity state could be seen as a spectrum evolution towards one of the above-sited configurations. Moreover, neither the PPF nor the DMPC liposome absorbs in the MC540 absorption wavelength range (300–600 nm).

In the present study, the effects of PPF (diluted in ethanol) and its commercial form Diprivan® have been investigated on liposome and cell membrane fluidity, using the ESR technique with two *n*-doxyl stearic acid spin probes. Microviscosity values at two different depths inside membranes were monitored as a function of PPF concentration in the range 0–10^{-2} M. PPF fluidizing potency was also demonstrated qualitatively by the use of the MC540 as a probe of the DMPC lipid bilayer microviscosity.

2. Materials and methods

2.1. Liposome preparation

Stearic acid derivatives (Aldrich, USA) labeled by stable doxyl radical ring at the C-5 (5-DSA) or at the C-16 (16-DSA) position (counted from the carboxylic group of the stearic acid derivative) were added to a chloroform solution (Merck, Germany) of 5 mg/mL DMPC (Sigma, USA). The mixture was stirred for 5 min and the solvent was evaporated under vacuum. The formed lipid film was suspended in phosphate buffer solution pH 7.0 (1/15 M; Na_2HPO_4–KH_2PO_4, from Riedel-de Haën, Belgium) at a temperature above the DMPC phase-transition temperature (23.9 °C), and stirred by a vortex mixer in order to obtain large multilamellar vesicles (MLV) [20]. After this hydratation, five freeze–thaw cycles using liquid nitrogen were carried out to allow a better incorporation of the labeled stearic acid into the liposome phospholipid bilayer. The MLV suspensions were transferred into an extruder (Lipex Biomembrane, Canada), in which the unilamellar liposomes were formed by passing the suspensions through polycarbonate filters (0.1 μm pore size, Nucleopore, CA), under a pressure up to 6895 Pa of nitrogen. The procedure was repeated ten times at a temperature above the phase-transition temperature of the phospholipids and resulted in unilamellar liposomes with a mean size of about 90 nm and a very low polydispersity [21,22]. Propofol (PPF) (Acros, Belgium) was first dissolved in ethanol (Merck, Germany) in order to obtain stock solutions. Two μL of the stock solutions was added to 250 μL of liposome solution in order to achieve the desired concentration of PPF. The final percentage of ethanol was less than 1% in all experiments. The commercial form of PPF (Diprivan®: PPF 10 mg/mL) is formulated in intralipid, a lipid vehicle emulsion (10% soya bean emulsion, egg phosphatides and glycerol). Diprivan® (Zeneca, Belgium) or equivalent volumes of intralipid (IL) (Kabi Pharmacia, Belgium) were added to DMPC liposomes