Short communication

Local Anesthetic [2-(Decyloxy)phenyl]-2-(1-piperidinyl)ethyl Ester of Carbamic Acid Perturbs Phosphatidylcholine Bilayer

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Abstract. Monohydrochloride of [2-(decyloxy)phenyl]-2-(1-piperidinyl)ethyl ester of carbamic acid (C10A) has a biphasic effect on the fluidity of egg yolk phosphatidylcholine (EYPC) model membranes as detected by the methyl ester of stearic acid spin probe, with the paramagnetic doxyl group bound to C-16. The fluidity increases up to a molar ratio of C10A:EYPC=0.5 with a subsequent decrease. This decrease in fluidity may be due to interdigitation of hydrocarbon chains in the bilayer.

Key words: Local anesthetic — Spin probe — ESR spectroscopy — Drug-lipid interaction — Phosphatidylcholine

Many surface-active drugs have biphasic effects on cell and subcellular membranes upon increasing their concentration. For example, low surfactant concentrations protect membranes from osmotic, mechanical or acid lysis, whereas higher concentrations potentiate or directly cause lysis. Probably, biphasic effects are connected with changes in the membrane structure. In our recent paper (Gallová et al. 1992) we have observed, using the stearic acid spin probes labeled at C-5 and C-16 (5-DSA and 16-DSA) and ESR spectroscopy, that the influence of surface-active local anesthetic carbisocaine (monohydrochloride of [2-(heptyloxy)phenyl]-2-(diethylamino)-1-methylethyl ester of carbamic acid) on the structure of egg yolk phosphatidylcholine (EYPC) model membrane is biphasic. In the present work, we studied the effect of monohydrochloride of [2-(decyloxy)phenyl]-2-(1-piperidinyl)ethyl ester of carbamic acid (abbreviation C10A), on EYPC bilayer fluidity using the methyl ester of stearic acid spin probe labeled at C-16 (16-MDSA). C10A displays local anesthetic activities (Čižmárik et al. 1976; Račanská et al. 1990).

C10A was prepared as described by Čižmárik and Borovanský (1975). EYPC was isolated and purified according to Singleton et al. (1965). 16-MDSA was obtained from Sigma (St. Louis, USA). The other chemicals were analytically pure and were purchased from Lachema (Brno, Czech Republic). Solvents were redis-

tilled before experiments. EYPC, 16-MDSA and C10A were mixed in chloroform – methanol and deposited on ground glass powder in a sample tube. The molar ratio of 16-MDSA:EYPC was 1:100 or less to avoid spin-spin interaction. The organic solvents were removed by evaporation in a stream of gaseous nitrogen and, finally, by diffusion pump evacuation. The resulting mixture on ground glass powder was dried over P_2O_5 and hydrated over saturated NaCl solution (76% relative humidity).

Electron spin resonance spectra were recorded with an ERS 230 X-band ESR spectrometer (ZWG AdW Berlin, Germany) using the 100 kHz modulation technique. The sample temperature was maintained at 22.0 ± 1.0 °C. The bilayer lipid fluidity was characterized by the spin probe rotational correlation times τ_B and τ_C calculated as described by Schreier et al. (1978), Marsh (1981) and Hemminga (1983). The principal values of the g-factor tensor and nitrogen hyperfine splitting tensor of spin probes used in these calculations were those obtained by Lange et al. (1985). At a constant temperature, the value of τ is a measure of the bilayer microviscosity, i.e. the reciprocal value of the fluidity. This approach is valid for the isotropic fast probe motion in an isotropic medium where $0.05 \text{ ns} < \tau < 2.5$ ns (Schreier et al. 1978; Hemminga 1983). Our data are within this limit (see Fig. 1). The values of $\tau_B \neq \tau_C$ indicate an anisotropy in the probe motion or/and an anisotropy in the surrounding medium.

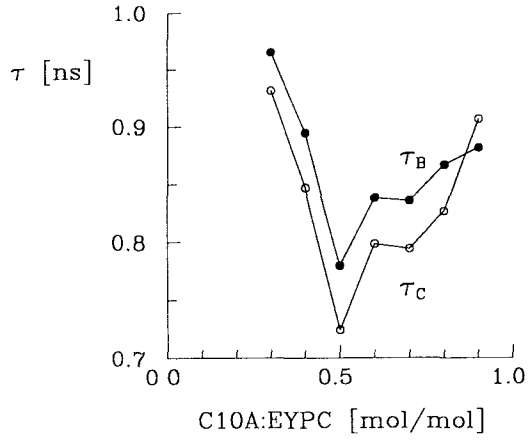

Figure 1. The dependencies of the correlation times τ_B (closed circles) and τ_C (open circles) for the 16-MDSA spin probe in EYPC bilayers, on the C10A: :EYPC molar ratio. The error in τ is ± 0.04 ns.

The dependencies of τ_B and τ_C on C10A:EYPC molar ratio for the 16-MDSA spin probe clearly display biphasic behavior (Fig. 1). The values of correlation times decrease up to a molar ratio of C10A:EYPC=0.5:1, and increase at higher molar ratios. This means that close to the center of the hydrophobic region the bilayer fluidity initially increases with the increase in C10A concentration and,

after reaching a critical concentration, a decrease in the fluidity is observed. The values of the τ_B/τ_C ratio are within the limit of 0.97:1.07, and this indicates that the spin probe motion is only slightly anisotropic.

The biphasic course of the fluidity of the 16-MDSA spin probe in dependence on the C10A concentration is in accord with our previous observation (Gallová et al. 1992): at first, correlation time of the 16-DSA spin probe and order parameter of the 5-DSA spin probe decreased, and increased for molar ratios of carbisocaine (intercalated in EYPC bilayer):EYPC higher than $\simeq$ 0.6. The turning point is near to the value observed in the present study for C10A which indicates a similar mechanism of the drug-EYPC interaction. Biphasic changes in the fluidity were also observed (using the *m*-DSA spin probes) in pigeon red cell membranes in the presence of tetracaine, chlorpromazine, methochlorpromazine, octanol and octanoic acid (Salesse et al. 1981). These findings indicate that the membrane fluidity, as detected by the spin probe method, displays biphasic changes in different membrane preparations in the presence of structurally different drugs irrespective if charged or not. The common feature of these drugs is that they contain a polar (charged or not charged) part and a hydrophobic fragment consisting of aromate and/or a hydrocarbon chain. In lipid bilayers the polar part will interact with the lipid polar fragments and the hydrophobic part will insert in the hydrophobic region of the bilayer consisting of lipid acyl chains. This will cause lateral expansion of the lipid bilayer and will affect the packing of the fatty acyl chains. As a result, the lipid acyl chains will bend cooperatively, and/or eliminate the structural defects by a changed rate of *trans-gauche* isomerization. We suggest that this causes the increase in the EYPC bilayer fluidity at low C10A:EYPC molar ratios. At higher molar ratios, the defects in the bilayer hydrophobic region may be eliminated by interpenetration or interdigitation of hydrocarbon chains from apposing monolayers in the hydrophobic center of the bilayer. Interdigitation is typical for the gel phase of lipid. Full interdigitation was observed e.g. in phosphatidylcholine in the presence of chlorpromazine and tetracaine (McIntosh et al. 1983), mixed interdigitation in fully hydrated phospholipid bilayers with asymmetric acyl chains (Hui et al. 1984; Boggs and Mason 1986; Zhu and Caffrey 1993), partially interdigitated bilayers are formed e.g. by asymmetric phospholipids at low hydration (Boggs and Mason 1986) and cerebroside sulfate with long acyl chain (Stinson and Boggs 1989). The ESR experiments showed that the 16-DSA spin probe in the interdigitated bilayers gave spectra typical of the 5-DSA spin probe, as a result of a drastic change in the doxyl group localization towards a region with high order (Boggs and Rangaraj 1985). No such qualitative change in the 16-DSA spin probe spectrum was observed in our experiments. Partial interdigitation, where the shorter chain of lipid on one side of the bilayer is packed end to end with the longer chain of lipid on the other side of the bilayer, is believed to be present in the liquid-crystaline phase, too (Boggs and Mason 1986; Stinson and Boggs 1989; Zhu and Caffrey 1993). Maulik et al. (1986)

have supposed that in the liquid-crystaline state, the order in the central region increased in partial interdigitated bilayers of long chain sphingomyelins. Dörfler et al. (1990) ascribed the decrease in bilayer thickness of dipalmitoyl phosphatidylcholine in the liquid-crystaline state in the presence of equimolar concentration of heptacaine to partial interdigitation.

On the basis of these facts we suggest that the decrease in membrane fluidity observed at high drug concentrations in the present and previous work (Gallová et al. 1992) may be due to partial drug-induced hydrocarbon chain interdigitation. However, this hypothesis needs further verification.

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