The Influence of Local Anesthetics on the Gel-Liquid Crystal Phase Transition in Model Dipalmitoylphosphatidylcholine Membranes

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Abstract. The influence of local anesthetics (LA): tetracaine, lidocaine, cocaine, dibucaine and heptacaine derivatives on the gel to liquid crystalline phase transition temperature (T_c) of model dipalmitoylphosphatidylcholine (DPPC) membranes was studied using electron spin resonance (ESR) and polarization microscopy methods. The decrease of T_c in the presence of anesthetics (δT_c) was found to be dependent on the [DPPC]/[H_2O] molar ratio at constant [LA]/[DPPC] molar ratio. Hence, the parameter $\alpha = \delta T_c/([LA]/[DPPC])$ in dependence on [H_2O]/[DPPC] was extrapolated to zero concentration of water and compared with biological efficiency.

Key words: Local anesthetics — Model membranes — Phase transition — Polarization microscopy — ESR spectroscopy

Introduction

Local anesthetics affect neural conduction, both at the synapse and along the axon; the mechanism of their action remains however to be elucidated. Various investigators have presented evidence favouring either protein or lipid as sites of action. The isolation and purification of a protein which specially binds tertiary amine local anesthetics, from mammalian axonal membranes, strongly supports the hypothesis according to which a protein should be a local anesthetic receptor (Greenberg and Tsong 1982, 1984). On the other hand, the variability of the chemical structure of various local anesthetics (amines, alkanes, alkohols, steroids, etc.) supports the idea that local anesthetics act rather nonspecifically. Futhermore, the correlations of biological activities with oil/water partition coefficients (Seeman 1972) strongly suggest that it is the lipid part of the membrane that is the site of action of LA. For example, it has been proposed

that local anesthetics might cause physical pertubations of the lipidic part of the nerve membranes due to which sodium channels get closed and the propagation of nerve impulses is blocked (Lee 1976). One indication for such a possibility is the correlation of the local anesthetic activities of several drugs with their effect on gel-liquid crystal phase transition temperature in model phosphatidylcholine membranes (Ueda et al. 1977; Lee 1978; Ondriaš et al. 1984). However, local anesthetic are amphiphilic compounds: consequently the phase transition temperature decrease should be a function of both the pertubation effect of the drug on the membrane and the lipid/water partition coefficient in the sample. To distinguish between these two contributions, we studied the effects of several local anesthetics on the phase transition temperature in dipalmitoylphosphatidylcholine model membranes as a function of the water/lipid ratio at low water content in the samples.

Materials and Methods

L-α,β-dipalmitoyl-γ-phosphatidylcholine (DPPC puriss.) was obtained from Fluka AG (Switzerland) and used after TLC test of purity without further purification. Tetracaine (Te), dibucaine (Di), cocaine (Co) and procaine (Pro) were purchased from Medika (Czechoslovakia). Lidocaine (Li) was a commercial product of Astra (Sweden) and derivatives of heptacaine (compounds XIX -ortho, XX -meta, XXI -para) were provided by Dr. J. Čižmárik from Faculty of Pharmacy, Bratislava. Preparation and properties of heptacaine derivatives have been extensively described elsewhere (Čižmárik et al. 1975; 1976). All the above anesthetics were used as obtained (hydrochloride salts); lidocaine was used as both hydrochloride (Li_{Cl}) and base (Li_{Ba}).

The phase transition temperatures of the DPPC model membranes in the presence of anesthetics (T_c) were determined by two physical methods. The first one, introduced by Shimshick and McConnel (1973), uses changes in spin label partitioning between lipid and water in the phase transition region. The spin label, 2,2,6,6-tetrametylpiperidin-1-oxyl (TEMPO), was purchased from Technika (Bulgaria). Electron spin resonance (ESR) spectra were measured using an ERS 230 spectrometer (ZWG, Berlin, GDR). The spectra were evaluated as described by Shimshick and McConnel (1973). The other method measured changes in the sample birefringence as a function of temperature using polarization microscopy. A home-built apparatus (Račanský and Balgavý 1987) was used and the records were evaluated as described by Petrov et al. (1982) and Gawrisch et al. (1980).

The same samples were used for both ESR and microscopy measurements. Dry microcrystals of DPPC and the respective drug were filled into a glass capillary (internal diameter 0.7 mm). Redistilled water with dissolved TEMPO (10^{-4} mol.l^{-1}, see Table 1) was added and the capillary was sealed. The amount of each constituent in the sample was determined by gravimetry using Mettler (Switzerland) microbalances. Samples were homogenized by repeated forth-back centrifugation (1600G) at temperature over T_c. Before the measurements, the samples were stored in a refrigerator. After each measurement, the samples were opened, another weighted amount of water was added, and the whole procedure was repeated. The heating rate in microscopy measurements was 1 K/min. In ESR experiments, the samples were equilibrated at each temperature for 5 min, the accuracy of the temperature setting was $\pm$ 0.5 K.

Results and Discussion

With both methods used, the phase transition temperature of model DPPC membranes in absence of drugs was $T_c = 314.7 \pm 0.5$ K. All the local anesthetics examined decreased the phase transition temperature. The depression of the phase transition temperature ($\delta T_c = T_c - T_{c_i}$) was found to be a linear function of the local anesthetic molar ratio at $0.1 \leq [LA]/[DPPC] \leq 0.5$. The efficiency of i-th water concentration of local anesthetic can be thus expressed as the parameter $\alpha_i = \delta T_c/[LA]/[DPPC])$ (Račanský et al. 1984). The phase transition temperature increases with the increasing $[H_2O]/[DPPC]$ molar ratio in the sample. Both experimental methods used gave consistent T_c values within the experimental error. Some of the experimental results are presented in Table 1.

The dependence of parameter α_i on the $[H_2O]/[DPPC]$ molar ratio suggests that the drug partition between lipid and water plays a role in the phase transition. This effect can be eliminated by extrapolating the value of α_i to zero concentration of water. As clearly seen in Table 1, the values of α_0 are different for different drugs pointing to the significance of the chemical structure of a particular drug for its disordering effect on the membrane.

Table 1. Effect of local anesthetics on the structural disordering parameter α_i; c_i — amount of water in the samples in μmol. The standard error of α_0 was about $\pm$ 0.5.

	Li_{Ba}	Li_{Cl}	Te	Co	Pro	Di	XIX	XX	XXI
α_1[K]	3.17	1.82	0.67	2.40	0.64	18.51	23.2	20.01	18.87
c_1[μmol]	105	155	145	140	120	141	138	161	182
α_2[K]	1.82	1.81	5.62	1.39	0.49	13.16	21.74	18.52	13.89
c_2[μmol]	383	485	468	583	454	519	511	532	462
α_3[K]	1.75	1.79	5.35	1.26	0.26	12.21	18.52	16.67	13.31
c_3[μmol]	662	955	891	1013	878	941	939	880	750
α_0[K]	2.22	1.82	6.67	2.56	1.00	19.95	25.01	20.32	19.22

It was of interest to correlate the values of α_0 with the local anesthetic activities of the drugs investigated. The activity coefficients for surface and infiltration anesthesia were taken from the literature (Čižmárik et al. 1976; Wenke et al. 1983). For the sake of comparison, we also took values of partition coefficients of the drugs examined (log P′) for the octanol/water partitioning system (Kozlovský et al. 1982; Hansch and Leo 1979; Rekker 1977), as these values are frequently in various structure-activity relationships. A simple linear regression analysis showed that the structural disordering parameters (α_0) of the investigated drugs correlate better with the respective biological activity than the partition coefficients (see Table 2).

Table 2. Correlation coefficients, r^2, and t-test values for linear correlation of structural disordering parameter α_0 and partition coefficient, log P′, with local anesthetic activity (LAA)

α_0		log P′		LAA
r^2	t	r^2	t	$n = 8$
0.625	3.41	0.357	1.97	surface
0.591	3.18	0.287	1.68	infiltration

Our results point to the importance of disturbing effects of drugs on the lipid part of the membrane for the local anesthetic action. The structural disordering parameter α_0 introduced in the present paper is more suitable for the characterization of these properties than the partition coefficient for the octanol/water system. The parameter α_0 can be determined using various physical methods. According to our experience, satisfactory results can be obtained with the described microscopic technique, which is simple, sensitive and cheap.

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