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The changes of capacitance relaxation of bilayer lipid membranes induced by chlorpromazine

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Chlorpromazine (CHP) belongs to the class of neuroleptics of low potency. Although both the existence of binding sites of CHP near the ion channel of the acetylcholine receptor [1] and the obtained activation of chloride currents in oocytes [2] might suggest a direct effect of CHP on membrane proteins, the indirect, lipid-mediated effect of CHP on biomembranes should not be excluded [3]. CHP is known to influence the shape of red blood cell membranes [4], and it can mediate the interaction of some drugs (e.g. *cis*-flupentixol) with phospholipids [5]. CHP decreases the membrane fluidity although it does not significantly influence the rotation relaxation time of 1,6-diphenyl 1,3,5-hexatriene (DPH) fluorescence probe.

In our recent work [6] we showed that a non-specific interaction of an amphiphilic drug – the local anesthetic tetracaine (TTC) – with bilayer lipid membranes (BLM) resulted in a more positive membrane surface potential. The surface potential increased with increasing concentration of TTC and was higher for the less charged from (at pH 9) than for the more highly charged one (pH 6). We proved that the main contribution to the change of surface potential comes from a dipole contribution. The 33 μ M TTC concentration induced changes of dipole potential of about (5.3 ± 2.0) mV and (29.8 ± 3.0) mV at pH 6 and pH 9 respectively, which corresponds to a change of surface dipole moment of 8.1 ± 3.0 and 34.0 ± 3.5 Debye, respectively. Unmodified BLMs were characterized by a single relaxation time of about 5 μ s that correspond to reorientation of individual molecular dipoles. Addition of TTC (final concentration 0.1 mM) resulted in the appearance of an additional, slower relaxation component ($\tau = 50$ μ s) at electrolyte pH 9, while at pH 6 no changes of relaxation time occurred. We assumed that due to its more neutral form at pH 9 TTC could penetrate more deeply into the lipid bilayer. Interaction of TTC with BLM probably induces heterogeneity of phospholipid environment and perturbs the bilayer dynamics. CHP, like TTC, is an amphiphilic molecule, and the drugs have similar ionization constants (pK_a 9.3 for CHP and pK_a 9.5 for TTC). While the polar parts of TTC and CHP are similar, the differences in the structure of the non polar parts might result in different abilities of their neutral forms to penetrate into the membrane and thus might result in dif-

Table: Relaxation times (τ , μ s) of reorientation of molecular dipoles in polar region of BLM of egg PC + cholesterol with and without chlorpromazine (CHP) (final concentration 0.1 mM)

Egg PC + cholesterol		Egg PC + cholesterol + CHP	
pH 5.5	pH 9.5	pH 5.5	pH 9.5
4.5 \pm 0.31	4.1 \pm 0.12	4.83 \pm 0.14	4.47 \pm 0.31
		8.16 \pm 0.63	

Means \pm S.D. were obtained by averaging of 64 current relaxation curves from one

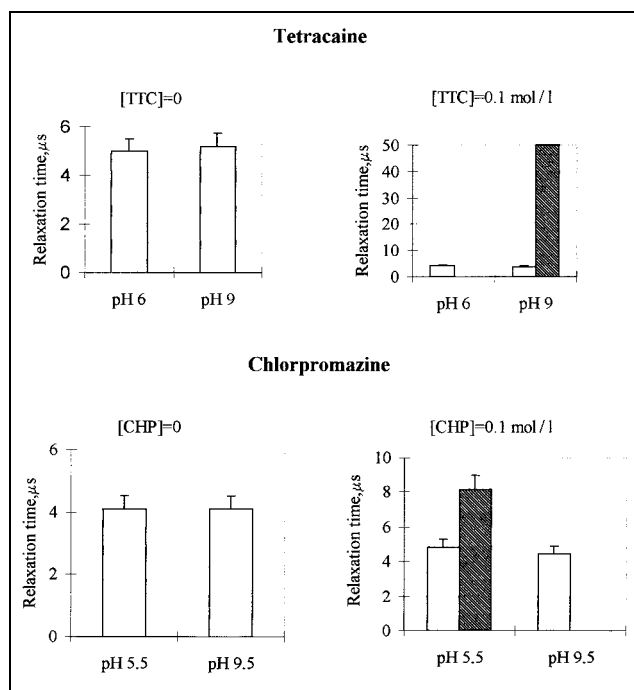


Fig. Comparison of relaxation times of reorientation of molecular dipoles in polar region of BLM of egg PC + cholesterol with and without chlorpromazine and tetracaine respectively. Means \pm S.D. were obtained by averaging of 64 current relaxation curves from one membrane.

ferent influences on membrane physical properties. In this work we therefore studied how the interactions of both the charged and more neutral form of CHP with BLM will affect the capacitance relaxation of a lipid bilayer. The results are compared with those for TTC.

The results of capacitance relaxation experiments are shown in the Table. One can see that unmodified membranes are characterized by one relaxation component corresponding to the reorientation time of dipole moments of phospholipid molecules following application of a voltage jump (-100 mV to $+100$ mV) to the membrane. For unmodified BLM formed at pH 5.5 and pH 9.5, this relaxation time was partially the same. Addition of CHP resulted in a slight increase of the relaxation time for BLM at both pH 5.5 and pH 9.5 and in the appearance of an additional relaxation component at pH 5.5 with a two-fold longer relaxation time of 8.16 ± 0.63 μ s. This effect correlates well with the weak effect of CHP on dipole potential (preliminary results). These changes are considerably different from those found with TTC for which practically no changes took place at the lower pH (pH 6), but an additional relaxation component (49.9 ± 10.1 μ s) was observed at pH 9 (Fig.). Whereas the measured changes of the fast relaxation time constant may or may not be significant, the appearance of a slower component at lower pH suggests that at this pH CHP hinders the reorientation of neighboring lipid molecules most probably through mechanical or electrostatic interactions. With TTC this effect is considerably stronger, but it is observed at pH 9.5 rather than at pH 5.5. The decrease of mobility of lipids and protein induced by CHP has been observed also by time-resolved fluorescence spectroscopy [7].

The appearance of an additional relaxation component for the reorientation of dipole moments at pH 5.5 shows that CHP affects the order of the lipid head groups at this pH. Thus drugs like CHP and TTC can have at least three modes of action: i) influencing the mobility in the lipid phase (the "fluidity concept" of a non-specific action of

amphiphilic drugs [8]), ii) influencing ionic transport by means of changes in the surface concentrations of ions due to changes of surface charge density, and iii) influencing the potential barriers within the channels by changing the dipole potential in their immediate surroundings.

Experimental

1. Formation of BLM and chemicals

Bilayer lipid membranes were formed according to the method of Mueller et al. [9] on a circular hole (diameter ~ 0.7 mm) in a wall dividing a Teflon cup into two identical compartments of 3 ml volume each. The lipid solution used was egg yolk phosphatidylcholine (egg PC) and cholesterol (4:1, w:w) dissolved in n-heptane (20 mg/ml). Egg PC was from Sigma (USA), cholesterol and n-heptane were from Fluka (Switzerland). The cup was filled with 10 mM KCl + 2 mM HEPES or 0.1 M KCl + 10 mM HEPES at pH 5.5 or pH 9.5. KCl and HEPES (analytical grade) were from Merck (Germany) and Sigma (USA), respectively. Chlorpromazine (CHP) was from Sigma (USA). All experiments have been performed at $T \sim 20^\circ\text{C}$.

2. Capacitance relaxation method

The capacitance relaxation method is based on analysis of the time course of changes in the capacitance following sudden changes in the voltage applied across the bilayer [10]. Using this method one may obtain information about, e.g. reorientation of molecular dipoles and cluster formation. Capacitance relaxation was determined by measuring the time course of the displacement current, I , following a step change of applied voltage from -100 to $+100$ mV. A detailed description of the construction and operation of the apparatus is given elsewhere [10]. Capacitance relaxation curves, that had quasi exponential shape (i.e. $I = I_0 \sum \exp(-\tau_i/t)$) were digitalized and recorded using a Biomation (USA) 805 waveform recorder. Calculation of relaxation times (τ_i) was performed using the program DIS-CRETE [11].

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