Influence of chlorpromazine and its derivatives on the dynamics of lipid membranes

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Perturbation effect of the tranquilizers chlorpromazine, chlorprothixene, levopromazine, thioridazine, and perfenazine on lipid membranes was studied using the electron spin resonance spectroscopy of stearic acid spin labeled at the position 16. The order parameter S of the spin probe in the lipid membranes depending either on the membrane order and/or dynamics of the hydrophobic membrane part was used to estimate the perturbation effect of the drugs. Chlorpromazine increased the order parameter S of the probe in lecithin liposomes, and decreased the parameter S in the liposomes prepared from the rat brain total lipid/lecithin mixtures. The disordering effect of chlorpromazine increased with the increase of the total lipid/lecithin mass ratio in the liposomes. The tranquilizers showed different propensities to decrease the S parameter of the probe in the liposomes prepared from the total lipids. The propensities with the individual compounds increase in the order: perfenazine, levopromazine ~ chlorprothixene ~ chlorpromazine, thioridazine. The different propensities of the drugs may be explained by their structure, depending on the hydrogen bonds spanned between the headgroups of lipids and by their incorporation into the membranes.

С помощью спектроскопии электронного спинового резонанса стеариновой кислоты, спиново меченной в положении 16, изучалось нарушающее действие транквилизаторов хлорпромазина, хлорпротиксена, левопромазина, тиоридазина и перфеназина на липидные мембраны. Параметр упорядоченности S спиновой пробы в липидных мембранах, зависящий от упорядоченности мембраны и/или от ди-

намики гидрофобной части мембраны, был использован для оценки нарушающего действия лекарственных препаратов. Хлорпромазин повышал параметр упорядоченности S пробы в лецитиновых липосомах и понижал его в липосомах, полученных из смесей полных липидов из мозга крыс с лецитином. Нарушающее влияние хлорпромазина увеличивалось с возрастанием отношения полный липид : лецитин в липосомах. Транквилизаторы обладали различной способностью снижать величину параметра S пробы в липосомах, полученных из полных липидов. Порядок изменения этой способности был следующий: перфеназин < левопромазин ~ хлорпротиксен ~ хлорпромазин < тиоридазин. Это различие в свойствах препаратов может объясняться их строением, зависящим от водородных связей между головными группами липидов, а также их инкорпорацией в мембраны.

Chlorpromazine and its derivatives chlorprothixene, levopromazine, thioridazine, and perfenazine are amphiphilic drugs clinically useful as tranquilizers. Their mode of action is not understood so far. Chlorpromazine (CPZ) influences various membrane-related processes, such as the activity of variety of phospholipases [1] and phospholipid biosynthetic enzymes [2], inhibits ATP-ase and F_1-ATP-ase activities [3, 4], effects hemolysis of biomembranes [5]. The drug—membrane interaction can be important in these processes. The drug in biological membranes can interact with membrane proteins, lipids and/or lipid/protein interface. Chlorpromazine was found to decrease the phase transition temperature of various single phospholipids [6—8], it influences the membrane order in lecithin liposomes [9], perturbs proteins [4], and effects lipid—protein interface in erythrocyte membranes [10, 11]. Up to now, mostly only the chlorpromazine effect on single phospholipids has been studied. However, the single phospholipids have different physical properties in comparison to the total lipids in biological membranes. Therefore the aim of the present work was both to investigate the effect of CPZ on the liposomes prepared at various mass ratios of lecithin to the total rat brain lipids and simultaneously to compare the effect of CPZ and its derivatives on the dynamics of liposomes prepared from the total lipids isolated from rat brain.

Experimental

Chemicals

Chlorpromazine, chlorprothixene, levopromazine, thioridazine, and perfenazine were offered by the Centre for Research of Mental Health in Pezinok (Fig. 1). Stearic acid spin labeled at the position 16 with the 2,2-dimethyl-*N*-oxyl-oxazolidinyl group (SA(1, 14)) was from Syva (Palo Alto, CA). Total lipids (TL) were extracted from rat brain according

to the method of *Folch et al.* [12]. *Macala et al.* [13] reported the following composition of rat brain total lipids in mass %: cholesterol 18.6, sphingomyelin 2.8, lecithin 22.6, phosphatidyl ethanolamine 24.1, phosphatidyl inositol 2.4, phosphatidyl serine 14.6, cerebrosides 11.3, and sulfatides 3.6. Yolk egg lecithin (PC), isolated according to the method of *Singleton et al.* [14], was provided by courtesy of Dr. P. Balgavý (Faculty of Pharmacy, Comenius University, Bratislava). Abbreviation PC means the yolk egg lecithin and it does not include lecithin which is present in the rat brain total lipids.

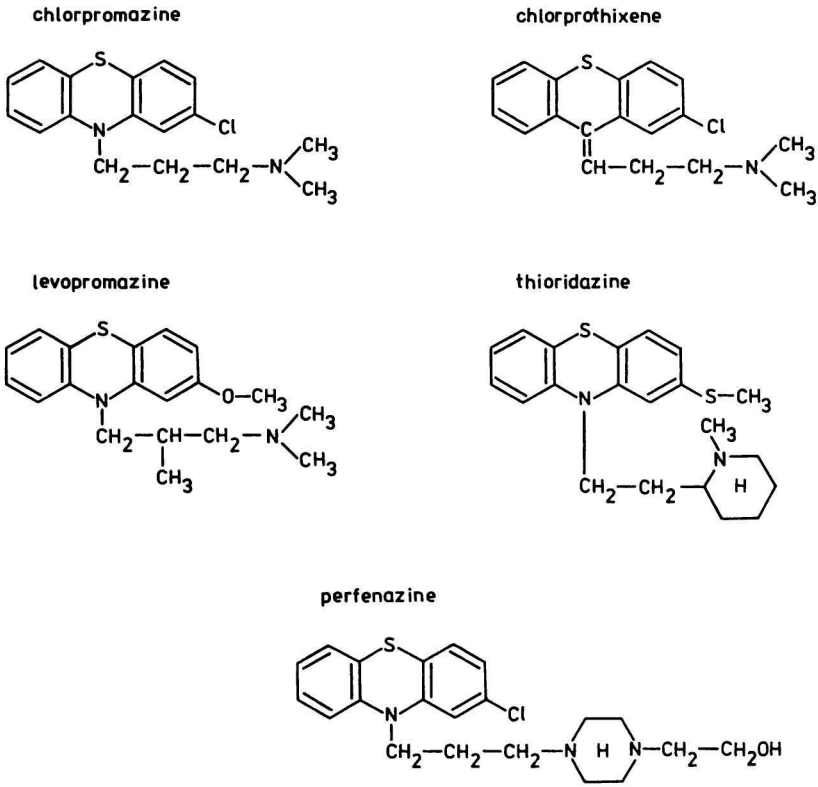

Fig. 1. Structural formulas of chlorpromazine (CPZ), chlorprothixene (CPT), levopromazine (LPZ), thioridazine (TRZ), and perfenazine (PFZ).

Method

PC and TL (together 5 mg) in the mixture chloroform—methanol (φ_r = 1 1) were mixed in the mass ratios of 4 : 0; 3 1; 2 : 2; 1 : 3; and 0 : 4. The solvent was evaporated in a stream of nitrogen, followed by evacuation. The dry lipids were hydrated with the buffer (in mmol dm^{-3}) NaCl 145, KCl 5, $MgCl_2$ 1.4, $CaCl_2$ 1, [4-(2-hydroxyethyl)-1--piperazinyl]ethanesulfonic acid (Hepes HCl) 20, pH = 7.4. The lipid/buffer mass ratio

in the sample was 0.1 (lipid concentration 117.3 mmol dm^{-3}). In order to prepare liposomes the samples were sonicated in a bath at 25 °C and subjected to freeze-thaw-vortex cycles several times. 55 mm^3 of the liposomes were added to the desired amount of powder drug, containing 20 μg of the spin probe. In order to attain equilibration of the drug in liposomes the samples were again sonicated in the bath and subjected to the freeze-thaw-vortex cycles from −70 °C to 25 °C several times. The lipid/drug mole ratio was calculated assuming the average lipid molar mass to be 775 g mol^{-1}

ESR measurements

55 mm^3 of the liposomes were filled into a glass capillary and ESR spectra of the spin probe in liposomes were recorded by a Bruker ER 200 D-SRC spectrometer with 9.9 mW microwave power and 0.05 mT modulation amplitude. To evaluate the relative propensities of the drugs in perturbing of the lipid liposomes the splitting constants $A_{\parallel}$ and $A_{\perp}$ of ^{14}N nucleus were evaluated from the spectra according to [15] (Fig. 2) and the order parameter S was calculated according to *Marsh* [16]. When the $A_{\perp}$ splitting constant was the only available, the order parameter $S_{\perp}$ was calculated according to *Sauerheber et al.* [17]. The order parameter S (or $S_{\perp}$) of the spin probe in the lipid membranes evaluated from the $A_{\parallel}$ and $A_{\perp}$ splitting constants in this study depended either on the order and/or dynamics of the hydrophobic membrane part [18]. These two membrane properties were not distinguished in this study.

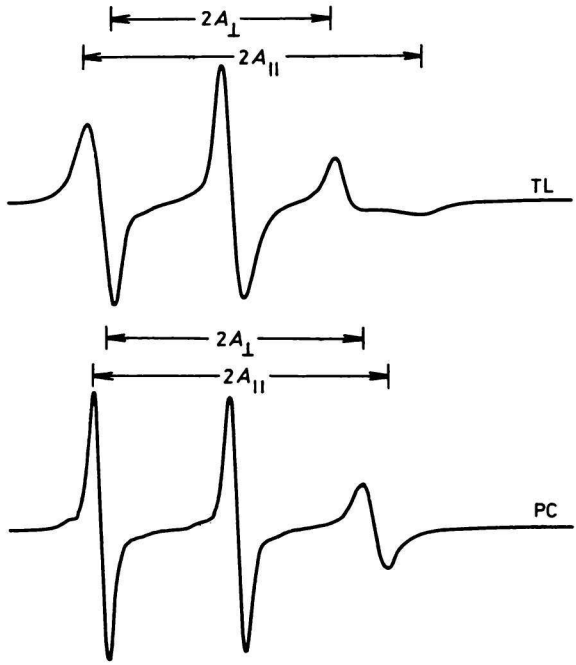

Fig. 2. ESR spectra of the spin probe at 37 °C in liposomes prepared from rat brain total lipids (TL) and lecithin (PC).

Results and discussion

The typical ESR spectra of the spin probe SA(1, 14) in liposomes prepared from TL or PC are shown in Fig. 2. Comparing the splitting constants $A_{\parallel}$ and $A_{\perp}$ for the TL and PC spectra, PC liposomes showed lower S parameter at the hydrophobic membrane part than TL liposomes. The order parameter $S_{\perp}$ increased proportionally to the increasing amount of TL in TL/PC liposomes (Fig. 3). Similar results were obtained for cholesterol/PC liposomes by *Pang et al.* [9] where the membrane order increased with the increasing cholesterol/PC mole ratio in the samples. The order parameter $S_{\perp}$ of the probe was significantly higher (Fig. 3) in TL liposomes than in PC ones. This may be a result of higher electrostatic interaction and/or presence of more hydrogen bonds between TL headgroups than between PC headgroups in the liposomes. *Boggs et al.* [19] found that intermolecular hydrogen binding interactions between lipid headgroups increased the phase transition temperature of the lipids. The hydrogen bonds were found in TL-headgroup regions particularly between acidic lipids [19], glycolipids [20], and sphingolipids [21]. However, the higher value of the $S_{\perp}$ parameter in TL liposomes in comparison to PC ones, may be a result of vertical shift of the spin probe in the membrane induced by different lipids [22].

The effect of CPZ on the S parameter depended on the mass ratio of TL/PC in the liposomes (Fig. 3). CPZ slightly increased the S parameter of the probe in the PC liposomes, but decreased the S parameter, in comparison to the

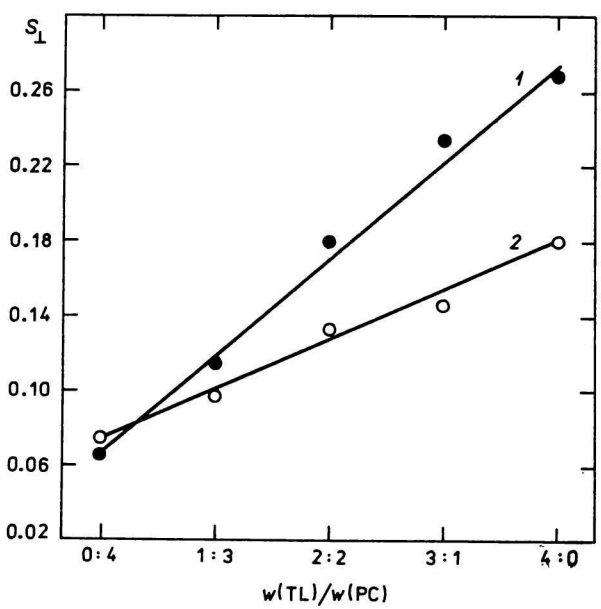

Fig. 3. Dependence of the order parameter $S_{\perp}$ of the spin probe on the total lipid/lecithin mass ratio in the liposomes at 37 °C. *1.* Control sample; *2.* sample containing lipid and CPZ in the 3 1 mole ratio.

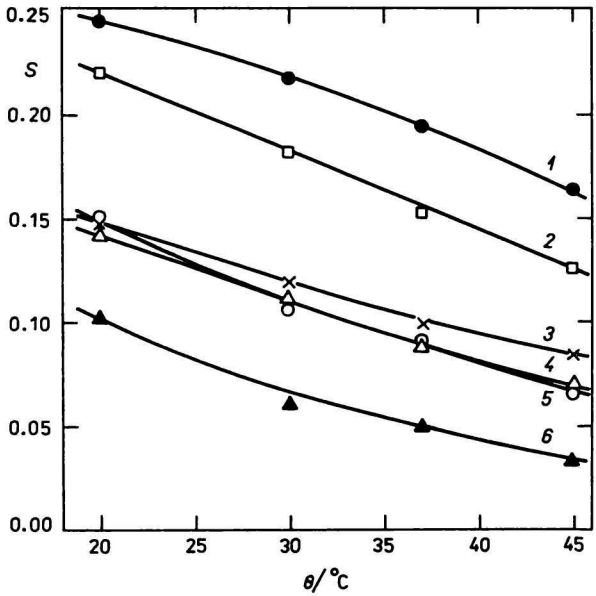

Fig. 4. Temperature dependence of the parameter S of the spin probe in total lipid liposomes for various drugs (drug concentration 30 mmol dm^{-3}). *1.* Control sample; *2.* perfenazine; *3.* levopromazine; *4.* chlorprothixene; *5.* chlorpromazine; *6.* thioridazine.

control sample, in the liposomes containing an increasing mass ratio of TL/PC in the sample. The highest CPZ effect was found in TL liposomes. Similar observation was described by *Pang et al.* [9], where CPZ decreased membrane order parameter in cholesterol—PC liposomes and the effect was pronounced with the increasing amount of cholesterol in the liposomes, whereas at low cholesterol/PC mole ratio (less than 20 % of cholesterol) CPZ increased the order parameter of lipid membrane.

The observed CPZ effect in our study can be explained by the influence of cholesterol which is present in TL on the PC liposomes as was found by *Pang et al.* [9]. However, we suppose that the dependence of the CPZ effect on liposomes with different PC/TL mass ratios can be also mediated by different lipids. That is, CPZ can specifically interact with some of the TL by rearrangement of electric interactions and/or hydrogen bond in the polar membrane part [8, 23] and by perturbation of hydrocarbon membrane part by CPZ specific incorporation into the membrane. The involvement of the hydrogen bonds in described CPZ effect is supported by findings of *Hanpft* and *Mohr* [8]. The authors suggested that intercalation of the drug molecules (including CPZ) between the polar headgroups of phospholipids can interrupt specific hydrogen bond arrangement of a headgroup region and so decrease phase transition temperature of the lipids.

Since the highest effect of CPZ was found in TL liposomes, only the TL liposomes were used in further investigations.

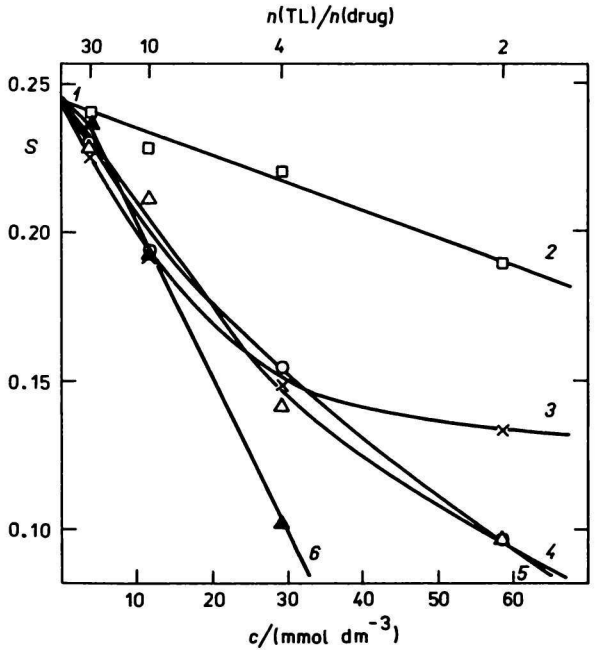

Fig. 5. Influence of drug concentration on the order parameter S of the spin probe in total lipid liposomes at 20 °C. For denotation see Fig. 4.

The influence of CPZ and its derivatives on the order parameter at various temperatures is compared in Fig. 4. The concentration of the drug in the samples was 30 mmol dm^{-3}. Reported value of the molar partition coefficient of CPZ between PC and buffer was 4.4×10^5, at 37 °C and pH = 7.4 [24]. Approximately similar partition coefficient was also supposed for CPZ derivatives. Therefore the most of the drug molecules in the samples were intercalated in the membrane phase, where the drug/total lipid mole ratio was practically 1 : 4.

A reduction of the ESR signal amplitude was observed in the samples containing CPZ after 24 h. This was less pronounced with thioridazine and perfenazine and nonsignificant in the samples with levopromazine and chlorprothixene. A similar behaviour of the tranquilizers was found investigating their ability to form radical products in the reaction with dibenzoyl peroxide [25]. The highest concentration of radical products was observed with CPZ, less with thioridazine and perfenazine and no radical products were found with levopromazine and chlorprothixene. The origin and nature of the radicals was not investigated. The free radicals generated from the drugs represent reactive species which destroy the nitroxide free radicals, probably by a reduction reaction [26, 27]. Thus CPZ and its derivatives can influence the amount of free radicals in biological tissues.

All the drugs decreased the S parameter within the studied temperature range of 20—45 °C (Fig. 4). The drugs showed different propensities to decrease S. The

propensities of the drugs increase in the following order: perfenazine, levopromazine ~ chlorprothixene ~ chlorpromazine, thioridazine. Similar disordering effect of the drugs at different concentrations is shown in Fig. 5. The drugs decreased the S parameter within the studied concentrations (3.9—58.6 mmol dm^{-3}) in the samples. The decrease of the S parameter may result from direct interaction of positively charged drugs with negatively charged —COO^- group of the spin probe. This is less probable in our case, since in the neutral PC liposomes the interaction of the positively charged drug with the negatively charged probe should be more pronounced than in negatively charged total lipid liposomes. In the latter case the positively charged drug can interact with the negatively charged lipids instead of the spin probe. Nevertheless, we observed that the drugs had more pronounced effect in the total lipid liposomes in comparison to the PC liposomes.

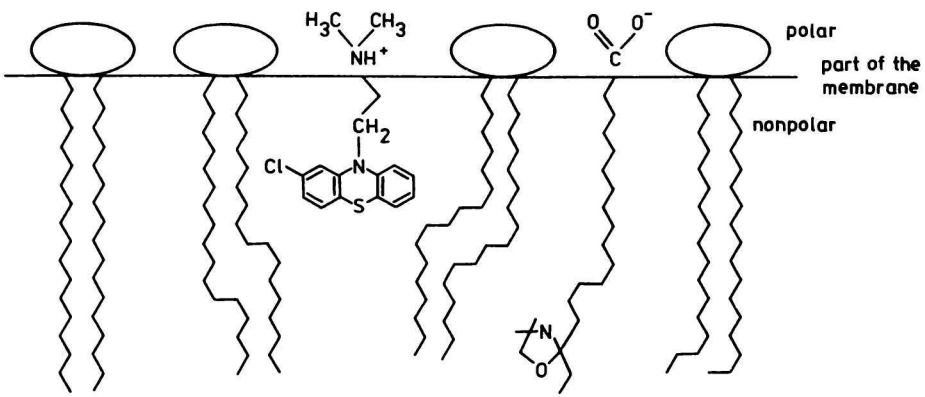

Fig. 6. Scheme of incorporation of chlorpromazine and spin probe between lipids in membrane.

The decrease of the order parameter S can be explained by spatial incorporation of the drugs into lipid membrane as it is shown for CPZ in Fig. 6, similarly as described in [28]. The amphiphile molecule is located in the membrane with the polar part located at the membrane surface and the hydrophobic part intercalated between the lipid acyl chains. Such drug incorporation can create more molecular freedom for the lipid acyl chain at the 16th carbon depth, as detected by SA(1, 14). A similar drug incorporation into lipid membrane was found for local anesthetics and β-adrenoceptor blocking drugs [29, 30].

All of the studied drugs have similar heterocyclic skeleton and different aliphatic substitutes. Chlorpromazine, chlorprothixene, and levopromazine, which had approximately the same disordering effect, possess similar aliphatic substitutes, whereas the more potent thioridazine has N-methylpiperidine in the

aliphatic chain, and the least potent perfenazine has piperazine with polar 2-hydroxyethyl moiety.

These structural differences can modify the influence of the drugs on the hydrogen bonds spanned between the headgroups of lipids at the membrane surface [8] and also modify the incorporation of the drugs into the membrane (Fig. 6) leading to different degree of molecular freedom of lipid acyl chains. Creation of more molecular freedom for the lipid acyl chain can indicate the changes in the membrane elastic energy [31, 32], with consequent influence on membrane proteins [33]. Since the pharmacological concentrations of the drugs in target tissue or membrane are unknown, the comparison of the pharmacological potency of the drugs *in vivo* and in our model system cannot be done.

In conclusion, the drugs were found to possess different propensities to perturb lipid membrane, with their effect being more pronounced in total lipid liposomes than in lecithin liposomes.

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