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DIFFERENCES AND SIMILARITIES IN THE INFLUENCE OF TRIFLUOPERAZINE ON THE PHASE TRANSITIONS OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE

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Influence of trifluoperazine, phenothiazine derivative known to reverse multidrug resistance of cancer cells, on the thermal properties of dimyristoylphosphatidylcholine and dimyristoylphosphatidyl-ethanolamine was studied by means of microcalorimetry. Main phase transition of both lipids was affected by the drug in a concentration-dependent manner. In case of dimyristoylphosphatidylcholine we observed additionally the effect of trifluoperazine-induced phase separation. This phenomenon was observed for drug/lipid molar ratios higher than 0.06. From the experimental results we conclude that trifluoperazine incorporates into both dimyristoylphosphatidylcholine and dimyristoylphosphatidyl-ethanolamine bilayers. The phase separation is presumably induced by the different mode of drug-bilayer interactions of protonated and unprotonated form of trifluoperazine. Only phosphatidylcholine, which polar heads are not so densely packed in bilayer as phosphatidylethanolamine ones, is able to distinguish between the different protonation forms of trifluoperazine.

INTRODUCTION

Trifluoperazine (TFP), as well as better known chlorpromazine (CPZ) are both structurally related phenothiazine derivatives. Apart of their use in psychiatry these compounds have recently been found to display also some anticancer properties (Seydel, Velasco, Coats, Cordes, Kunz & Wiese, 1992). Multidrug resistance (MDR) reversal properties of TFP have been demonstrated by Molnár, Szabo, Mándi, Musci, Fischer, Varga, König and Motohashi (1998). TFP (and also CPZ) share the properties characteristic for vast group of MDR modulators – they are usually hydrophobic cations able to penetrate and pass biological membranes (Ramu and Ramu, 1992). The correlation between the lipophilicity of the drugs and their ability to reverse MDR (Eytan, Regev, Oren & Assaraf, 1996) suggest that interaction with membrane lipids should play some role in the mechanism of their activity.

The membranes of eucariotic cells are composed of different classes of lipids among which phosphatidylcholines (PC) and phosphatidylethanolamines (PE) belong to the most numerous and important ones. The properties of these two lipid classes are different. To call one of the best known difference: PCs spontaneously form bilayers in polar environment while PEs should form both bilayer and inverted hexagonal structures. The aim of this study was to investigate the interaction of trifluoperazine with chosen PC and PE species. Since these lipids differ in the structure of polar head groups, molecules of the same hydrocarbon chain length and saturation were chosen for the study.

MATERIALS AND METHODS

1,2–Dimyristoyl–*sn*–glycero–3–phosphatidylcholine (DMPC) and 1,2–dimyristoyl–*sn*–glycero–3–phosphatidylethanolamine (DMPE) were purchased from Sigma (St. Louis, MO, USA). Lipids were used without further purification. Trifluoperazine was purchased from ICN biomedicals Inc. (Costa mesa, CA, USA). All other chemicals used in experiments were of analytical grade.

Samples were prepared using method described elsewhere Hendrich, Wesołowska and Michalak (2001). It is worth to emphasise that in experimental conditions both lipids used form bilayer structures. Microcalorimetric measurements were performed using Rigaku microcalorimeter equipped with measuring head constructed in our laboratory. Samples were scanned at 1.25°C/min, data were collected on a hard disc and analysed off-line using software developed in our laboratory.

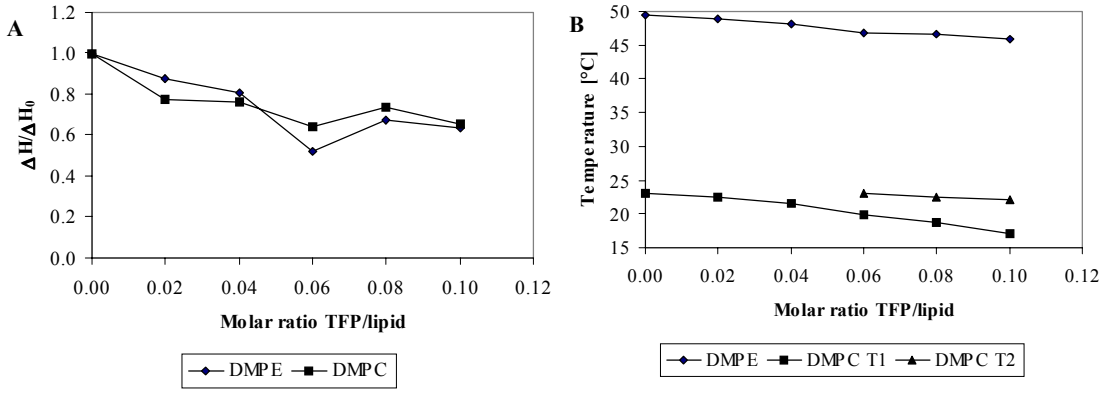

Fig. 1. The influence of trifluoperazine on the DMPC and DMPE phase transition parameters: A – relative transition enthalpy (ΔH transition enthalpy of mixture, ΔH_0 transition enthalpy of pure lipid) and B – transition temperature.

RESULTS AND DISCUSSION

Pretransition of dimyristoylphosphatidylcholine was abolished by TFP in the whole range of studied concentrations (data not shown). Main phase transitions of both DMPC and DMPE were influenced by trifluoperazine in a concentration-dependent manner. We observed TFP-induced decrease of main transition enthalpies (Fig. 1A) and temperatures (Fig. 1B). Transition peaks of drug/lipid mixtures were broadened in the relation to the pure lipid ones. All the above effects confirm that trifluoperazine interacts with both studied lipids and penetrates bilayers formed by these lipids. Since TFP is structurally similar to CPZ we assume that like this drug (Frenzel, Arnold & Nuhn, 1978; Nerdal, Gundersen, Thorsen, Hoiland & Holmsen, 2000) after incorporation into the membrane it is located in the vicinity of polar/apolar interface. Such location should perturb both polar heads and hydrocarbon chains packing and result in observed phase transition modifications. Alterations in lipid molecules packing induced by TFP should be responsible for effects such as change in membrane permeability (Driori, Eytan & Assaraf, 1995) or, according to the "vacuum cleaner" hypothesis to the modulation of MDR-related proteins (Ferte, 2000).

In case of the TFP/DMPC mixtures, for molar ratios higher than 0.06 two distinct transition peaks were recorded — corresponding transition temperatures are denoted as T1 and T2 in Fig. 1B. Appearance of separate peaks is usually a result of phase separation occurring in the sample. In case of TFP/DMPC mixtures, like in TFP/DPPC (Hendrich *et al*., 2001), this domain formation is caused presumably by the slightly different interaction of protonated and unprotonated forms of TFP with zwitterionic lipid. More tightly bound by hydrogen bonds and more densely packed DMPE bilayers are not sensitive to the protonation of TFP.

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