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CHLORPROMAZINE EFFECT ON LYSOZYME-LIPID INTERACTIONS

Valeriya M. Trusova
Department of Biological and Medical Physics, V.N. Karazin Kharkov National University, 4 Svobody Sq.
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Despite considerable research efforts, the molecular mechanisms of anaesthetic action still remain the matter of extensive debates. According to one viewpoint, anaesthetics alter the properties of lipid bilayer which, in turn, affects the functions of embedded membrane proteins. In contrast, protein-based theories of anaesthetic action postulate that the drugs modulate the functions of membrane proteins through direct association. To develop a unique conception of anaesthesia further in-depth investigations of drug-membrane interactions are strongly required. In the present work a well-known fluorescent probe pyrene has been employed to gain molecular insights into the interactions between amphipathic phenothiazine derivative chlorpromazine (CPZ) and model membranes composed of cationic globular protein lysozyme (Lz), and lipid vesicles prepared from zwitterionic lipid phosphatidylcholine (PC) and its mixtures with anionic lipid cardiolipin (CL) in the molar ratios 19:1, 9:1 and 4:1. To give unambiguous interpretation of the drug effect on protein-lipid interactions, we first analyzed the changes in pyrene excimerization due to the formation of either CPZ-lipid or Lz-lipid complexes. Pyrene excimer-to-monomer intensity ratio (E/M), a parameter which reflects the alterations in membrane free volume, was found to decrease upon Lz or CPZ binding to the lipid vesicles. Apparently, embedment of the protein and drug molecules into the hydrophobic region of lipid bilayer gives rise to the increase in lipid packing, decrease in the rate of *trans-gauche* isomerization of the lipid acyl chains and, consequently, reduction of membrane free volume. At the next step of the study, we analysed the changes in the rate of pyrene excimerization upon Lz addition to drug-lipid mixtures. In CL-containing liposomes the presence of CPZ does not modify the magnitude and sign of protein effect on membrane free volume. This implies that CPZ is incapable of perturbing Lz structure and exerted no influence on the protein interactions with this kind of liposomes. In contrast, in PC vesicles E/M ratio appeared to increase upon lysozyme binding to CPZ-modified model membranes. This finding may be explained in terms of two possibilities: (i) CPZ induces the formation of the new Lz conformer whose interactions with lipid bilayers are accompanied by the increase in membrane free volume; (ii) CPZ imparts the positive charge to the lipid bilayer thereby preventing Lz penetration into hydrophobic membrane region. Interfacially-located protein molecules are likely to generate structural defects coupled with the increased bilayer free volume. The results presented here clearly demonstrate that membrane composition can modulate the drug action on lipid-protein interactions. The recovered difference between CPZ effect on Lz-lipid binding in PC and CL-containing bilayers provide support to the idea that membrane environment can stabilize certain protein conformations differing in their responsiveness to drug action.

KEY WORDS: chlorpromazine, liposomes, lysozyme, pyrene

Despite the considerable research efforts, the molecular mechanisms of anaesthetic action still remain the matter of extensive debates. The correlation between anaesthetic potency and lipophilicity of the drugs predicted by the century-old Meyer-Overton rule suggests that lipid bilayer represents the prime target for the anaesthetic agents [1,2]. Countering this idea is the hypothesis initially proposed by Moore and Roaf, according to which the uptake of the pharmaceutics by “proteoid” rather than “lipoid” components of cell membranes is responsible for the production of anaesthesia. To overcome the above perplexity, it has been postulated that proteins have the lipophilic portions which could be the site of drug action without violating the Meyer-Overton rule [3]. However, it remains controversial whether anaesthetics alter protein function by direct interaction with proteins or by indirect effects via perturbations of the lipid bilayer, in which membrane proteins are embedded. In view of the ambiguity of aforementioned conjectures an acute need of further investigations of drug-membrane interactions in order to develop a unique conception of

anaesthesia, still exists. In this regard the use of model protein-lipid membranes instead of their biological analogues seems to be very helpful. In the present work a well-known fluorescent probe pyrene has been employed to gain molecular insights into the interactions between amphipathic phenothiazine derivative chlorpromazine (CPZ) and model membranes composed of cationic globular protein lysozyme (Lz), and lipid vesicles prepared from zwitterionic lipid phosphatidylcholine (PC) and its mixtures with anionic lipid cardiolipin (CL).

MATERIALS AND METHODS

Egg yolk phosphatidylcholine and beef heart cardiolipin were purchased from Biolek (Kharkov, Ukraine). CPZ and pyrene were from Sigma (St. Louis, MO, USA). Unilamellar lipid vesicles composed of PC and its mixtures with CL in molar ratios 19:1, 9:1 and 4:1 were prepared by the extrusion method [4]. To incorporate CPZ into the lipid bilayers, liposomal suspensions were incubated with the anaesthetic for 30 min at room temperature to yield a final drug:lipid molar ratio 0.1. Fluorescence measurements were performed with CM 2203 spectrofluorimeter (SOLAR, Belarus). Excitation wavelength was 340 nm, excitation and emission slit widths were set at 2 nm.

RESULTS AND DISCUSSION

To give unambiguous interpretation of the drug effect on protein-lipid interactions, we first analyzed the changes in pyrene excimerization due to the formation either of CPZ-lipid or Lz-lipid complexes. As seen in Fig. 1, lysozyme association with the lipid bilayers resulted in a marked decrease in pyrene excimer-to-monomer intensity ratio (E/M). Alterations in this parameter reflect the changes in the rate of probe lateral diffusion in lipid bilayer which, in turn, depends on membrane free volume available for pyrene movements.

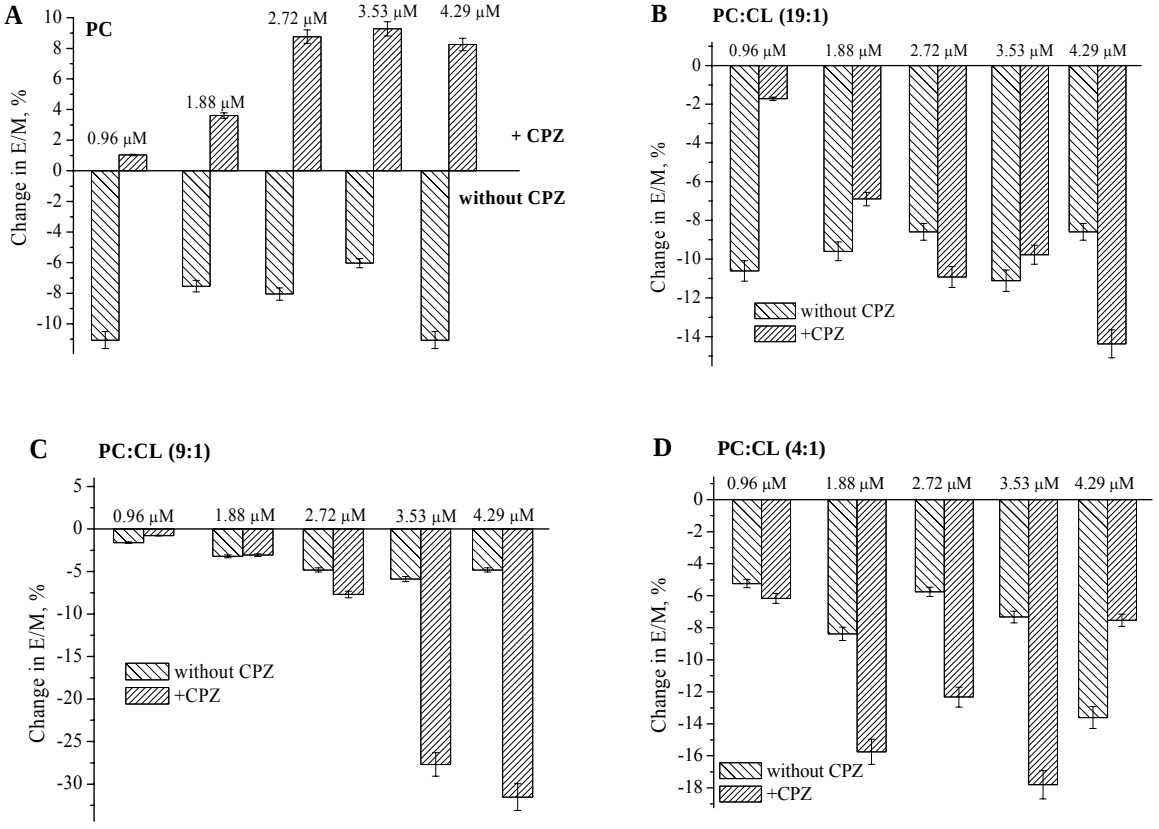

Fig. 1. Lysozyme effect on E/M ratio in intact and CPZ-modified model membranes.

Since pyrene distributes in the nonpolar region of lipid bilayer, the results obtained provide the evidence for Lz insertion into the membrane hydrophobic interior. This process is followed by the reduction of bilayer free volume molecular basis for which is the increase in lipid packing and decrease in the rate of *trans-gauche* isomerization of the lipid acyl chains. [5] The same effect has been observed upon CPZ association to the lipid membranes. More specifically, drug addition to the to liposome suspension resulted in ~1.5-fold decrease in E/M ratio (data not shown) being suggestive of CPZ condensing effect on the lipid bilayer.

At the next step of the study, we analysed the changes in the rate of pyrene excimerization upon Lz addition to drug-lipid mixtures. In CL-containing liposomes the presence of CPZ does not modify the magnitude and sign of protein effect on membrane free volume (Fig. 1). This implies that CPZ exerted no influence on Lz interactions with this kind of liposomes. In contrast, in PC vesicles E/M ratio appeared to increase upon lysozyme binding to CPZ-modified model membranes. This finding may be explained in terms of two possibilities. The first one relates to CPZ ability to cause the alterations in Lz conformation. It was shown that binding centre for the various pharmacological agents in lysozyme molecule involves protein active site [6]. Thus, association of halothane and penicillin was found to occur via Lz cleft and followed by the perturbation of protein secondary structure (namely, increased content of α-helices), and changes in its lytic activity. Analysis of lysozyme crystal structure allowed us to conclude that protein active site represents the most energetically- and sterically-favourable locus for CPZ binding as well. Apparently, electrostatic interactions between negatively charged Glu35, Asp52 and Asp101 located in Lz cleft and cationic drug facilitate the association of CPZ, holding the anaesthetic molecule inside the protein active site. This may result in the formation of new Lz conformer whose interactions with lipid bilayers are accompanied by the increase in membrane free volume. The second possibility suggests that CPZ imparts the positive charge to the lipid bilayer thereby preventing Lz penetration into hydrophobic membrane region. Interfacially-located protein molecules are likely to generate structural defects coupled with the increased bilayer free volume.

The results presented here clearly demonstrate that membrane composition can modulate the drug action on lipid-protein interactions. The recovered difference between CPZ effect on Lz-lipid binding in PC and CL-containing bilayers provide support to the idea that membrane environment can stabilize certain protein conformations differing in their responsiveness to drug action.

CONCLUSIONS

Examining the CPZ effect on lysozyme-lipid interactions with membrane probe pyrene revealed that the drug does not exert influence on protein association with negatively charged lipid vesicles. In contrast, in PC membranes CPZ was found to modify Lz-lipid binding presumably through modifying the protein conformation and membrane electrostatic properties.

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REFERENCES

1. R.S. Cantor // Biochemistry. 1997. V. 36. P. 2339-2344.
2. B.W. Urban // Br. J. Anaesth. 2002. V. 89. P. 167-183.
3. R.G. Eckenhoff // Mol. Pharmac. 1998. V. 54. P. 610-615.
4. B. Mui et al. // Meth. Enzymol. 2003. V. 367. P. 3-14.
5. V.M. Ioffe, G.P. Gorbenko // Biophys. Chem. 2005. V. 114. P. 199-204.
6. A. Morton et al. // Biochemistry. 1995. V. 34. P. 8564-8575.