INTERACTION BETWEEN NEUROTROPIC DRUGS AND THE PHOSPHATIDYLCHOLINE BILAYER. EFFECT ON SORPTION OF POLYVALENT CATIONS

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KEY WORDS: liposomes; local anesthetics; polyvalent cations

The fact that correlation is found between the anesthetizing power of local anesthetics and the affinity of their molecules for the phospholipid bilayer has given rise to the view that the primary acceptor is lipid in nature [5, 7, 10, 11].

The width of the ^{1}H-NMR lines of amphiphilic substances in the composition of the phospholipid membrane, because of a decrease of the mobility of their molecules, is much greater than the width of the lines of molecules located in the aqueous phase. In the case of a rapid exchange between the membrane-bound and free states the molecules of these substances will have a certain intermediate line width, proportional to the coefficient of distribution between membrane and water [1]. The use of this method to determine the binding strength of molecules of neurotropic preparations with the liposomal membrane has been substantially facilitated by the fact that the aromatic region of the ^{1}H-NMR spectra of natural phospholipids is transparent, and molecules of most of the preparations used contain aromatic groups.

The authors of [6, 9], who found correlation between the anesthetizing power of local anesthetics and the ability of their molecules to displace polyvalent cations from the membrane surface put forward the hypothesis that displacement of bound calcium from the membrane surface lies at the basis of the mechanism of action of these preparations.

To test this hypothesis we compared the effect of neurotropic drugs differing in their action on sorption of polyvalent cations by molecules of egg phosphatidylcholine, having first estimated the binding strength of the molecules of these preparations with the phospholipid membrane.

EXPERIMENTAL METHOD

Liposomes were prepared as follows. Egg phosphatidylcholine was dried on a rotary evaporator to dryness, buffer (^2H_2O; 0.01 M Tris-^{2}HCl, p^{2}H 6.5; 0.1 M KCl) was added, and the mixture shaken to obtain a 2% homogeneous emulsion. The resulting emulsion was then aerated for 15 min with argon and treated with ultrasound on the UZDN-1 apparatus with a frequency of 22 kHz for 20 min.

As the polyvalent cation we used Pr^{3+} ions, during sorption of which a pseudocontact chemical shift of protons of the N-methyl groups of the phospholipid, of considerable magnitude [2], is observed on the phosphate groups of phosphatidylcholine. The binding constant with phosphate groups of phosphatidylcholine is higher for Pr^{3+} cations than for Ca^{2+} cations [3] and, consequently, preparations displacing Pr^{3+} cations will displace Ca^{2+} cations even more.

To prepare liposomes we used egg phosphatidylcholine (standard lecithin), from Khar'kov Bacterial Preparations Factory. The drugs were used in the form of pharmacopoeial solutions: chlorpromazine, procaine, trimecaine — Soviet origin; haloperidol, droperidol — from "Gedeon Richter" (Hungar); pipolfen, lidocaine — from EGYT (Hungary). ^{1}H-NMR spectra were recorded under Fourier transform conditions on an FT-80 spectrometer ("Varian, USA) at a frequency of 80 MHz.

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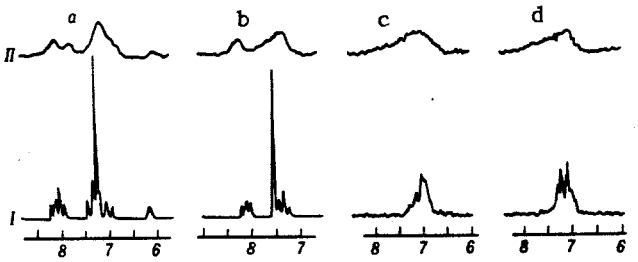

Fig. 1. Aromatic regions of ^{1}H-NMR spectra of droperidol (a), haloperidol (b), chlorpromazine (c), and pipolfen (d) in buffer (^2H_2O, 0.01 M Tris-^{2}HCl, p^{2}H 6.5; 0.1 M KCl), in the absence (I) and presence (II) of sonicated egg phosphatidylcholine liposomes. Here and in Figs. 2-4: ratio of preparations by weight to phospholipid 1:10; abscissa, chemical shift, ppm.

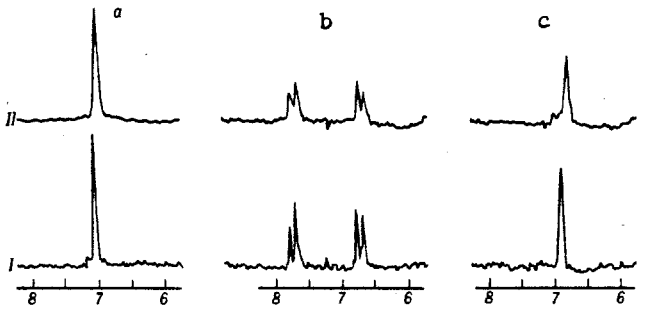

Fig. 2. Aromatic regions of ^{1}H-NMR spectra of lidocaine (a), procaine (b), and trimecaine (c) in buffer (^2H_2O; 0.01 M Tris-^{2}HCl p^{2}H 6.5; 0.1 M KCl) in absence (I) and presence (II) of sonicated egg phosphatidylcholine liposomes.

EXPERIMENTAL RESULTS

Aromatic regions of the ^{1}H-NMR spectra of seven neurotropic drugs in the absence and presence of sonicated liposomes, made from egg phosphatidylcholine, are shown in Figs. 1 and 2. The aromatic lines of the neuroleptics chlorpromazine, haloperidol, and droperidol and of the antihistamine drug pipolfen, in the presence of liposomes, were considerably widened, evidence of the firm binding of their molecules with the liposomal membrane. The spectra of the local anesthetics procaine, trimecaine, and lidocaine, in the absence and presence of liposomes, showed hardly any difference. The very slight widening of the lines in the presence of liposomes was evidently due to sorption of the molecules of these preparations on the surface of the liposomal membrane without their penetration into the hydrophobic region of the bilayer. This difference becomes clear if the fact is recalled that molecules of the first group of substances contain much larger hydrophobic regions than molecules of the second group.

On the addition of 10 mM $Pr(NO_3)_3$ to a 2% solution of vesicles the peak from the N-methyl groups of phosphatidylcholine splits into two: with a chemical shift of 3.73 ppm from the molecules of the outer monolayer, in contact with Pr^{3+} cations, and with a chemical shift of 3.24 ppm from molecules of the inner monolayer, not in contact with Pr^{3+} cations. The addition of haloperidol, droperidol, chlorpromazine, and pipolfen to this solution in the ratio of 1:10 by weight to the phospholipid caused a decrease in the pseudocontact chemical shift to values of 3.59, 3.56, 3.51, and 3.49 ppm respectively (Fig. 3). Similar quantities of procaine, trimecaine, and lidocaine had no effect on the magnitude of the pseudocontact chemical shift (Fig. 4).

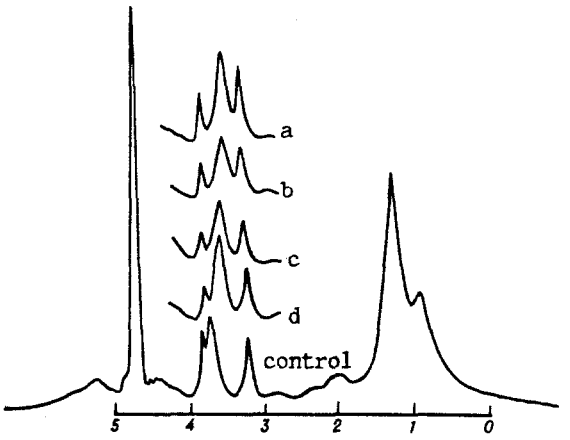

Fig. 3. Effect of pipolfen (a), chlorpromazine (b), droperidol (c), and haloperidol (d) on sorption of Pr^{3+} cations by sonicated egg phosphatidylcholine liposomes.

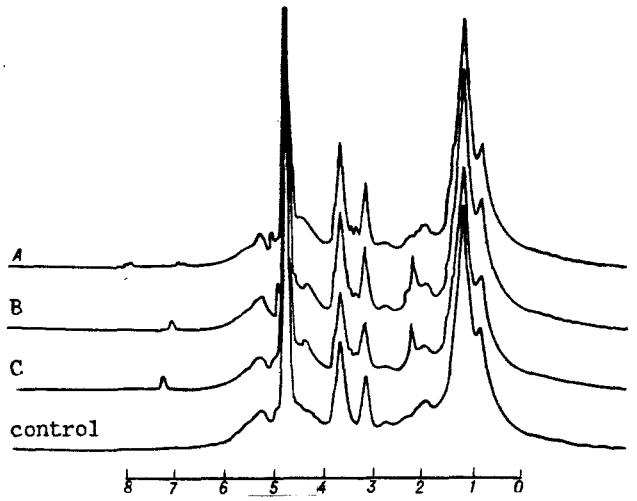

Fig. 4. Effect of procaine (A), trimecaine (B), and lidocaine (C) on sorption of Pr^{3+} cations by sonicated egg phosphatidylcholine liposomes.

The fact will be noted that Pr^{3+} cations are displaced from the surface of the phospholipid membrane only by those preparations whose molecules are firmly bound with the liposomal membrane, for which electrostatic interaction with the membrane surface is evidently insufficient, and hydrophobic interaction with its deeper layers also is required. Under these circumstances, molecules of these preparations carrying a positive charge must change the electrostatic conditions on the surface of the bilayer. This state of affairs may have a significant influence on sorption of peripheral proteins by the membrane surface, and may thereby affect the course of intracellular processes [4].

Preparations displacing Pr^{3+} cations from the membrane surface have much weaker anesthetizing power than local anesthetics which do not affect the sorption of these cations.

These results are in agreement with those of a previous study [8] on membranes of nerve cells. Displacement of Ca^{2+} cations from the membrane surface by molecules of local anesthetics is evidently not directly related to the mechanisms of local anesthesia.

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ANTIFIBROUS ACTION OF ALDEHYDE-DEXTRAN MODIFIED SUPEROXIDE DISMUTASE IN EXPERIMENTAL SILICOSIS

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Applications of modified superoxide dismutase (SOD) are promising in connection with the campaign against lung diseases [3]. For instance, the covalent SOD — Ficoll conjugate possessed prolonged activity in the blood stream. In experimental pulmonary microembolism the permeability of the blood vessels of the lungs for proteins is increased and migration of neutrophils to the endothelium is observed, greatly increasing the risk of involvement of the vascular wall. Prolonged administration of native SOD reduces the pathological increase in permeability of the vessels and demonstrates a protective effect of the preparation. Injection of Ficoll in such a situation reduces adhesion of neutrophils to the endothelium. The above factors mean that the SOD — Ficoll covalent conjugate can be regarded as a promising agent for the treatment of lung diseases that depend on the behavior of neutrophils. In this case both enzyme and carrier (Ficoll) can exhibit a protective effect (preventing accumulation of superoxide radicals and reducing adhesion of neutrophils to the endothelium on account of inhibition of intravascular coagulation respectively) [3]. The useful antifibrotic action of another carrier used to stabilize enzymes, namely dextran, has been described also in lung diseases [4].

The aim of this investigation was to study the action of a preparation of aldehyde-dextran modified SOD (the SOD — AD conjugate) in rats with experimental silicosis. Silicosis is an occupational lung disease which develops as a result of exposure to industrial aerosols containing quartz. Modified enzymes have proved themselves to be effective

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