IS FLUIDIZATION OF THE PHOSPHOLIPID BILAYER INDUCED BY GENERAL ANESTHETICS THE CAUSE OF GENERAL ANESTHESIA?

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The particular features of the molecular structure of general anesthetics has suggested that their action on nerve cell membranes is nonspecific in nature. From this point of view, it seems logical that the action of general anesthetics is based on fluidization of phospholipid membranes induced by them. This conclusion is shared by most research workers, who have found correlation between the disorderliness of membrane phospholipids induced by general anesthetics and the anesthetizing activity of these substances [1]. It has accordingly been suggested that preparations potentiating the effect of general anesthetics ought to exert an action similar to that of these substances, or at least not reversing it. Among these substances, our attention has been drawn to the phenothiazine derivatives chlorpromazine and pipolfen, for which the partition coefficient in a membrane–water system exceeds 1000 [4]. It is also known that addition of chlorpromazine to a dipalmitoylphosphatidylcholine bilayer leads to an increase in the effective anisotropy of the chemical shift in ^{31}P-NMR spectra and to widening of the line of N-methyl groups of the phospholipid in ^{13}C-NMR spectra [7]. The two last facts are evidence of reduction of mobility of the polar heads of dipalmitoylphosphatidylcholine. The aim of this investigation was to study the effect of chlorpromazine and pipolfen on viscosity of the phospholipid bilayer.

EXPERIMENTAL METHOD

The effect of the test substances on viscosity of the phospholipid membrane was estimated by measuring changes in the EPR spectra of 1-oxyl-2,2,6,6-tetramethyl-4-piperidinol stearate (TEMPO-stearate) and of 7-(1-oxyl-2,2-dimethyl-4-oxazolidinyl)-stearic acid (7-doxylstearic acid) in the composition of unsonicated liposomes from egg phosphatidylcholine. The first of these spin probes enables changes in microviscosity of the polar region of the membrane to be recorded, while the second characterizes the orientation and mobility of the fatty acid chains of the phospholipid close to its glycerol skeleton.

The liposomes were prepared as follows. To a solution of egg phosphatidyl-choline in ethanol spin probes were added in the molar ratio to phospholipid of 1:100. The resulting mixture was dried on a rotary evaporator to dryness, buffer (H_2O, 0.01 M Tris-HCl, pH 6.5; 0.1 M KCl) was added, and the mixture was shaken to produce a homogeneous emulsion. The final concentration of phospholipid in the emulsion was 5%.

EPR spectra were recorded on the RE-1307 spectrometer at a temperature of 20°C. Mobility of TEMPO-stearate is characterized by temporal correlation τ_c, which was calculated in seconds by the equation:

$$\tau_c = 6{,}65 \cdot 10^{-6} \cdot \Delta H_{+1} \cdot [(h_{+1}/h_{-1})^{1/2} - 1] \quad (2)$$

where ΔH_{+1} denotes the width of the low pole component of the spectrum, h_{+1} the amplitude of the low pole component of the spectrum, h_{-1} the amplitude of the high pole component of the spectrum (Fig. 1).

The frequency and amplitude of movement of 7-doxylstearic acid are characterized by a parameter of orderliness S, which was calculated by the equation:

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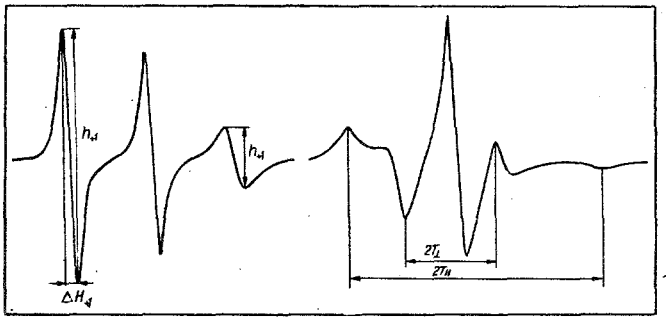

Fig. 1 Fig. 2

Fig. 1. EPR spectrum of tempo-stearate in unsonicated liposomes from egg phosphatidylcholine (molar ratio to phospholipid 1:100). H_{+1}) Width of low-pole component of spectrum, h_{+1}) amplitude of low-pole component of spectrum, h_{-1}) amplitude of high-pole component of spectrum.

Fig. 2. EPR spectrum of 7-doxylstearic acid in unsonicated liposomes from egg phosphatidylcholine (molar ratio to phospholipid 1:100). $T_\perp$ and $T_\parallel$) Perpendicular and parallel components respectively of time-averaged tensor of electron-nuclear superfine interaction.

$$S = \frac{T_\parallel - T_\perp}{T_{zz} - {}^1/_2 \cdot (T_{xx} + T_{yy})} \cdot \frac{a}{a^*} \tag{3}$$

where $a = {}^1/_3 \cdot (T_{zz} + T_{yy} + T_{xx})$; $a^* = {}^1/_3 \cdot (T_\parallel + 2T_\perp)$; T_{xx}, T_{yy}, T_{zz} are the chief components of the tensor of electron-nuclear superfine interaction; $T_\parallel$, $T_\perp$ the parallel and perpendicular components of the tensor of electron-nuclear superfine interaction, averaged in time. For 7-doxylstearic acid, $T_{xx} = T_{yy} = 6$ G, $T_{zz} = 32$ G (Fig. 2).

Liposomes were prepared from egg phosphatidylcholine (lecithin standard) from Khar'khov Bacterial Preparations Factory. The spin probes used were synthesized at the Institute of Organic Chemistry, Bulgarian Academy of Sciences. Drugs were used in the form of pharmacopoieal solutions: chlorpromazine of USSR origin, pipolfen from EGYT (Hungary).

EXPERIMENTAL RESULTS

The piperidine ring of TEMPO-stearate in unsonicated egg phosphatidylcholine liposomes is subjected to rapid isotropic motion with correlation time τ_c of $(1.73 \pm 0.03) \cdot 10^{-9}$ sec. Addition of chlorpromazine and pipolfen, in doses of 5, 10, and 20 mass % relative to phospholipid to the bilayer labeled with TEMPO-stearate, induced an increase in microviscosity of the polar region of the membrane, as shown by the concentration-dependent increase in correlation time, due to a decrease in mobility of the spin probe (Fig. 3).

The use of unsonicated liposomes from egg phosphatidylcholine, labeled with 7-doxylstearic acid, showed that the increase in viscosity of the membrane caused by addition of chlorpromazine extends also to its hydrophobic region. The parameter of orderliness for these liposomes was 0.641 ± 0.002. Addition of chlorpromazine in a dose of 5 and 10% by weight relative to phospholipid caused an increase in the parameter of orderliness up to values of 0.650 ± 0.002 and 0.656 ± 0.002 respectively.

Thus pipolfen and chlorpromazine, which potentiate general anesthesia, increase the viscosity of the phospholipid membrane in the region of the polar heads and in the hydrophobic region close to the glycerol skeleton of the phospholipid. The results (irrespective of the mechanism of potentiation) cast doubts on the role of fluidization of the membrane, induced by general anesthetics, as a mechanism of general anesthesia.

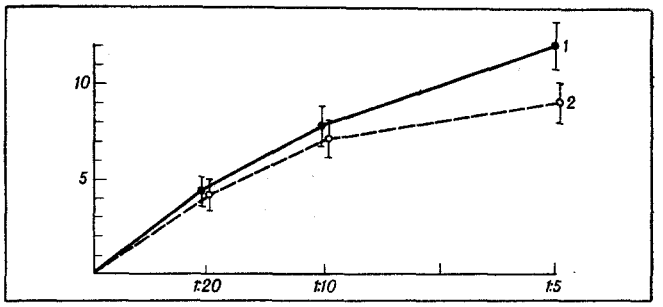

Fig. 3. Effect of pipolfen (1) and chlorpromazine (2) on correlation time of TEMPO-stearate in unsonicated liposomes from egg phosphatidylcholine. Abscissa, concentration of drugs in ratio by weight to phospholipid; ordinate, change in correlation time (in % of background). Graphs of both functions plotted from mean values obtained in four experiments.

The results of the present study are in agreement with those described in [8], in which the authors did not observe correlation between anesthetizing strength and randomizing ability in relation to a homologous series of saturated alcohols. The same conclusion was reached in a review by Dodson and co-workers [6], who consider that molecules of anesthetics have a twofold action: specific on the lipid–protein boundary and nonspecific in the thickness of the lipid phase.

Most investigators have looked for a specific molecular mechanism of the narcotic action of general anesthetics. Perhaps no such mechanism exists. For instance, the view is held that the state of general anesthesia is determined not by the specific physicochemical properties of the anesthetic, but by the property of the brain to organize a nonspecific protective reaction with the basic symptoms of general anesthesia in response to a wide spectrum of aggressive influences of varied nature [5].

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