LITERATURE CITED

1. A. A. Dmitriev, O. P. Shevchenko, and D. D. Petrunin, Problems in Equipment for and the Clinical Use of Hemoperfusion [in Russian], Gor'kii (1984), pp. 70-74.
2. A. A. Dmitriev, O. P. Shevchenko, and D. D. Petrunin, Ter. Arkh., No. 5, 81 (1984).
3. A. A. Dmitriev, O. P. Shevchenko, A. S. Fomin, and D. D. Petrunin, Ter. Arkh., No. 10, 76 (1985).
4. D. D. Petrunin, Yu. M. Lopukhin, M. N. Molodenkov, and G. A. Olefirenko, Byull. Éksp. Biol. Med., No. 2, 66 (1982).
5. D. D. Petrunin and Yu. M. Lopukhin, Gematol. Transfuziol., No. 11, 3 (1985).
6. O. P. Shevchenko, V. F. Kirsanov, A. A. Ovcharov, et al., Transplantation of Organs [in Russian], Kiev (1985), pp. 145-146.
7. O. P. Shevchenko, L. V. Beletskaya, V. F. Kirsanov, et al., Byull. Éksp. Biol. Med., No. 7, 53 (1986).
8. W. Straube, B. Klausch, and R. Hofmann, Immunological Methods [Russian translation], Moscow (1979), pp. 64-77.
9. W. Bernhard, H. E. Keplin, R. Reinards, et al., Artif. Organs, 9(A), 46 (1985).
10. W. Glinski, D. Barszcz, Z. Zanczura-Zarebska, et al., Br. J. Dermatol., 111, 147 (1984).
11. L. S. Kaplow, Blood, 26, 215 (1965).
12. H. L. Meier, L. W. Heck, E. S. Schulman, et al., Int. Arch. Allergy, 77, 179 (1985).
13. M. R. Moreno, C. Goulu, M. C. Fondaneche, et al., Immunol. Lett., 9, 215 (1985).
14. K. Ohlsson and I. Ohlsson, Eur. J. Biochem., 42, 519 (1974).
15. H. R. Wenzel, S. Engelbrecht, H. Reich, et al., Hoppe-Seylers, Z. Physiol. Chem., 361, 1413 (1980).

EFFECT OF PHENOTHIAZINES ON VISCOSITY AND ELECTRICAL STABILITY OF MODEL PHOSPHOLIPID MEMBRANES

O. M. Parnev, O. G. Naumov, N. N. Ivkov, and Yu. A. Vladimirov

UDC 615.214.22:547.869.2].44:612.397.7.014.2:576.314

KEY WORDS: phenothiazines; membrane viscosity; breakdown voltage

Derivatives of the phenothiazine series are nowadays widely used in many different branches of medicine, but in particular in clinical psychiatry as neuroleptic drugs and antipsychotic agents. Although the history of the use of pentothiazines in medical practice goes back more than 30 years and the mechanism of their action on the CNS has been intensively studied, there are as yet no unanimously held views on the molecular mechanisms of their action. There is evidence that derivatives of the penothiazine series can affect the physical properties of the lipid bilayer of membranes [13, 15] and change the osmotic resistance of closed membrane formations [8-10, 12], and this is largely determined by the viscous properties of the phospholipid bilayer of membranes [14, 15] and also, under certain conditions, the surface charge on the membrane. However, this problem has not been systematically studied.

In the investigation described below the effect of derivatives of the phenothiazine series on the viscosity and electrical stability of model lipid membranes was studied.

EXPERIMENTAL METHOD

Monolayer liposomes for fluorescence investigations were obtained by the method in [6] from hen egg yolk phospholipids [7].

Multilayered unified liposomes were obtained by the method in [5]. The relative lateral viscosity of the lipid bilayer was determined by recording excimerization of pyrene [1]. The kinetics of excimerization of pyrene obeys the Stern–Volmer equation:

$$\frac{1}{\tau}=\frac{1}{\tau_0}+K_e\cdot C,$$

N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii I Meditsiny, Vol. 103, No. 5, pp. 555-557, May, 1987. Original article submitted May 26, 1986.

 0007-4888/87/0005- 0628$12.50

TABLE 1. Change (in %) of Viscosity of Lipid Bilayer of Monolayer Liposomes in the Presence of Phenothiazine Derivatives

Phenothiazine derivative	Concentration, M				
	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Chlorpromazine	100	107±3	117±4	126±3	250±4
Chloracizine	100	97±3	94±4	93±5	91±4
Promazine	100	100±4	103±2	106±3	300±17
Trifluoperazine	100	99±3	100±3	99±4	284±18
Nonachlazine	100	117±3	127±2	141±6	156±8

where K_e denotes the excimerization constant, τ_0 the life of the excited state of the monomers in the absence of excimerization, and τ the life of the excited state of the monomers in the presence of a concentration C of pyrene molecules.

Since excimerization takes place through a mechanism of dynamic quenching [16]:

$$F_\mu = \tau \cdot C \cdot p,$$

where p is a constant independent of excimerization, we obtain:

$$C/F_\mu = A + B \cdot C,$$

where F_μ denotes the intensity of fluorescence of the monomers in fluorescence with a wavelength of 595.5 nm, A is a parameter proportional to the life of the excited state of pyrene, and B a coefficient of proportionality to the diffusion constant.

Thus the tangent of the angle of slope $C/F_\mu = f(C)$, i.e., B, is the characteristic velocity of lateral diffusion of pyrene.

The electrical stability of liposomal membranes was estimated from the critical concentration (C_{cr}) of potassium acetate (KAc), added continuously to the suspension, at which the barrier properties of the liposomal membranes, recorded as a change in light transmission [3], took place. The value of the breakdown voltage (φ^*) of the liposomal membranes was calculated by the equation [3]:

$$\varphi^* = \frac{RT}{2F}(\lg C_{cr} + \mathrm{pH}),$$

where R, T, and F are generally accepted constants, pH the hydrogen ion concentration in the liposome suspension, and C_{cr} the "critical" concentration of KAc at which electrical breakdown of the liposomal membrane takes place.

Of all the derivatives of the phenothiazine series we used chloropromazine, chloracizine, promazine, trifluoperazine, and nonachlazine — all of USSR manufacture [2].

EXPERIMENTAL RESULTS

Data on the relative viscosity of the lipid bilayer of the liposomes (in per cent) in the presence of various concentrations of phenothiazines are given in Table 1. It follows from Table 1 that, with the exception of one phenothiazine derivative, namely chloracizine, all the rest significantly increased the viscosity of the phospholipid bilayer only in a concentration of 10^{-4} M, whereas chlopromazine and nonachlazine had this effect, although significantly weaker, in a concentration of 10^{-5} M.

The greatest increase in relative viscosity of the lipid bilayer of the liposomes was produced by promazine in a concentration of 10^{-4} M, whereas chloracizine, in the same concentration, had a tendency to reduce the viscosity of the phospholipid bilayer of the liposomes. The reason for this difference may be that the promazine molecule has a more positive charge, and thus prevents lateral diffusion of the molecules on account of the strong electrostatic interactions.

As was shown previously cholesterol, by increasing the viscosity of the lipid bilayer, increases the electrical stability of liposomal membranes [4]. It can be postulated that phenothiazine derivatives will affect the breakdown voltage of the phospholipid layer in a similar way. The results of this investigation are evidence that all the phenothiazine de-

TABLE 2. Effect of Phenothiazines on Breakdown Voltage of Lipid Bilayer of Liposomes (in mV, M ± m)

Phenothiazine derivative	Concentration, M				
	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}
I. Chlorpromazine	149,5±0,3	149,5±0,3	146,0±0,3	139,6±0,6	134,9±0,1
Chloracizine	150,0±0,2	147,6±0,3	145,1±0,3	141,1±0,5	130,5±0,5
Promazine	150,1±0,2	147,8±0,1	146,0±0,1	143,2±0,2	137,4±0,3
Trifluoperazine	150,0±0,2	148,2±0,2	145,9±0,4	141,9±0,4	132,4±0,4
Nonachlazine	149,7±0,3	147,5±0,4	146,3±0,4	144,4±0,9	142,1±1,2
II. Chlorpromazine	14,3±0,3	14,3±0,3	10,8±0,3	6,5±0,3	4,5±0,1
Chloracizine	14,9±0,4	12,3±0,3	10,1±0,2	7,3±0,3	3,2±0,1
Promazine	15,0±0,3	12,5±0,1	10,8±0,1	8,7±0,1	5,6±0,3
Trifluoperazine	14,9±0,2	12,9±0,2	10,7±0,3	7,8±0,3	3,7±0,3
Nonachlazine	14,5±0,2	12,2±0,3	11,1±0,3	9,5±0,8	7,9±1,0

Legand. I) Breakdown voltage; II) critical concentration of KAc (in mM).

rivatives without exception lowered the breakdown voltage of the liposomes (Table 2). This effect was seen particularly clearly with the phenothiazines in concentrations of 10^{-4} M. However, it will be clear from the data in Table 2 that even in a concentration as low as 10^{-7} M the phenothiazines affected the electrical stability of the phospholipid bilayer of the liposomal membranes. No correlation could be found between the breakdown voltage and the change in viscous properties of the liposomal membrane. Thus unlike cholesterol, derivatives of the phenothiazine series, which increase the viscosity of the lipid bilayer, reduce the electrical stability of the liposomal membranes. Lowering of the breakdown voltage of the phospholipid membranes is due in all probability to an increase in the positive surface charge, such as is found when positively charged detergents are added to liposomes [3], and it was shown previously [11, 15] that the phenothiazines increase the positive surface charge of cellular and model membranes.

Thus phenothiazine derivatives significantly enhance the viscous properties of phospholipid membranes, with the exception of chloracizine, and they also lower the breakdown voltage of phospholipid membranes. Taking into account the fact that viscous characteristics and the surface membrane potential play an important role in the functioning of electrically excitable membranes, when the mechanism of the psychotropic action of the phenothiazines is discussed, the possible effect of these drugs on the physical properties of the lipid layer must be taken into account.

LITERATURE CITED

1. G. E. Dobretsov, V. A. Petrov. T. A. Borshchevskaya, et al., Vopr. Med. Khim., No. 7, 812 (1977).
2. M. D. Mashkovskii, Therapeutic Substances [in Russian], Vol. 1, Moscow (1985).
3. O. M. Parnev, T. V. Puchkova, A. V. Putvinskii, and Yu. A. Vladimirov, Abstract Lodged at the All-Union Institute of Scientific and Technical Information, No. 5391-80 (1980).
4. T. V. Puchkova, A. V. Putvinskii, Yu. A. Vladimirov, and O. M. Parnev, Biofizika, No. 2, 265 (1981).
5. V. I. Sorokovoi, A. V. Putvinskii, D. I. Roshchupkin, et al., Compendium of Inventions and Efficiency Suggestions from Medical Schools and Research Institutes of the RSFSR [in Russian], Ivanovo (1974), pp. 223-225.
6. S. Batzzi and E. D. Korn, Biochim. Biophys. Acta, 298, 1015 (1973).
7. E. J. Bligh and W. J. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
8. R. E. Eckel, Fed. Proc., 19, 128 (1960).
9. J. G. R. Elfernic, Biochem. Pharmacol., 26, 511 (1977).
10. Q. R. Freeman and M. A. Spirtes, Biochem. Pharmacol., 11, 161 (1962).
11. R. Gruener and T. Narahashi, J. Pharmacol. Exp. Ther., 181, 167 (1972).
12. Y. Kanaho, Mol. Pharmacol., 20, 704 (1981).
13. R. R. Lew and R. M. Spanswick, Biochim. Biophys. Acta, 731, 421 (1983).
14. T. Ogiso, M. Iwaki, and K. K. Mori, Biochim. Biophys. Acta, 649, 325 (1981).
15. D. F. Sears and K. K. Brandes, Agents Actions, 1, 28 (1969).
16. J. M. Vanderkooi and J. B. Callis, Biochemistry (Washington), 13, 4000 (1974).