LITERATURE CITED

1. N. V. Darinskii, in: New Cardiovascular Drugs [in Russian], Moscow (1979), pp. 43-44.
2. N. V. Kaverina, G. A. Markova, G. G. Chichkanov, et al., Kardiologiya, No. 7, 43 (1975).
3. N. V. Kaverina, I. N. Pidevich, L. M. Demina, et al., in: New Cardiovascular Drugs [in Russian], Moscow (1979), pp. 46-47.
4. E. V. Lukoshkova, in: Physiology of Man and Animals [in Russian], Vol. 23, Moscow (1979), pp. 136-192.
5. D. A. Kvachadze and É. A. Margvelashvili, in: Nonachlazine in Clinical and Experimental Medicine [in Russian], Tbilisi (1976), pp. 13-14.
6. D. D. Matsevskii, Byull. Éksp. Biol. Med., No. 9, 119 (1970).
7. O. S. Medvedev, Fiziol. Zh. SSSR, No. 9, 1473 (1974).
8. O. S. Medvedev, Patol. Fiziol., No. 4, 69 (1979).
9. Yu. B. Rozonov, in: New Cardiovascular Drugs [in Russian], Moscow (1979), pp. 31-33.
10. M. Lavallee, J. de Champlain, R. A. Nadeau, et al., Can. J. Physiol. Pharmacol., 56, 642 (1978).
11. M. N. Levy and B. Blattberg, Circulat. Res., 38, 81 (1976).
12. G. Preiss and S. Polosa, Am. J. Physiol., 226, 724 (1974).

MEMBRANE PERMEABILITY FOR CHLORPROMAZINE AND NONACHLAZINE

G. E. Dobretsov and M. M. Spirin

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An important role in the mechanism of action of drugs is played by their ability to penetrate through cell membranes and tissue–blood and blood–brain barriers. Investigation of the permeability of biomembranes for each substance is a difficult task. It is much easier to study such problems on model membranes, which can reproduce many of the properties of biomembranes.

The writers compared the ability of two drugs, chlorpromazine and nonachlazine, to penetrate through model membranes, namely liposomes. The two drugs are tranquilizers of the phenothiazine series but they differ in the spectrum of their pharmacological action: whereas chlorpromazine is a psychotropic agent, nonachlazine is an effective antianginal drug of Soviet origin, with a basically new type of action [1, 6, 7].

It was shown previously by the fluorescent probe method that chlorpromazine [3] and nonachlazine [2] interact with model phospholipid membranes.

The object of this investigation was to use the fluorescent probe method to study permeability of model membranes for these substances.

EXPERIMENTAL METHOD

Phospholipid model membrane bubbles (liposomes) were obtained by two methods: 1) by rapid injection of a solution of total egg phospholipids, containing the fluorescent probe p-terphenyl (molar ratio of probe to lipid 1:40) in ethanol into a buffer solution (0.12 M KCl, 0.01 M Tris-HCl buffer, pH 7.4) [10] — these liposomes were named LPS-1; 2) by slow mixing of the same solutions in the same proportions — these were named LPS-2. The LPS-1 have one bilayer lipid membrane [10, 12], whereas LPS-2 have many such membranes [9].

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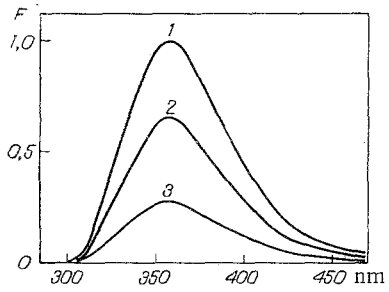

Fig. 1

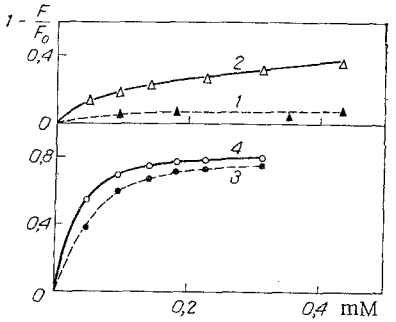

Fig. 2

Fig. 1. Fluorescence spectra of p-terphenyl in a suspension of LPS-1 (0.1 mg/ml) in the absence (1) and presence (2) of 0.5 mM nonachlazine or 0.1 mM chlorpromazine (3). F) Intensity of fluorescence.

Fig. 2. Extinction of fluorescence of p-terphenyl in liposomes by chlorpromazine and nonachlazine. Continuous lines represent LPS-1, broken lines LPS-2, Quenchers: nonachlazine (1 and 2) and chlorpromazine (3 and 4). F_0 and F) Intensity of fluoresence of p-terphenyl in absence and presence of quencher, respectively. Abscissa, concentration of quencher (in mM).

Solutions of chlorpromazine and nonachlazine (5 mM in ethanol and in water, respectively were added to the suspension of liposomes from a microsyringe. Fluorescence was measured 15 min after the addition of these substances on an apparatus described previously [4]. Fluorescence of p-terphenyl was excited at 280 nm and recorded at 370 nm. The results were given with a correction for absorption of the exciting light by chlorpromazine and nonachlazine. Absorption spectra were measured on a Hitachi-124 spectrophotometer in a solution of polyethylene glycol-400 (Ferak, Berlin). Crystalline preparations of chlorpromazine and nonachlazine were generously provided by N. V. Kaverina (Institute of Pharmacology, Academy of Medical Sciences of the USSR).

EXPERIMENTAL RESULTS

It was shown previously that during interaction between chlorpromazine or nonachlazine and membranes, fluorescence of an MBA probe is extinguished by 47% by chlorpromazine in a concentration of 0.1 mM [3] and by 8% by nonachlazine in a concentration of 0.5 mM [1, 2]. In the present investigation a different probe was used, namely p-terphenyl, fluorescence of which in the presence of the same concentrations of chlorpromazine and nonachlazine was extinguished by 73 and 35%, respectively (Fig. 1). This probe was thus far more sensitive to binding of phenothiazine with membranes than MBA.

Chlorpromazine extinguished fluorescence of p-terphenyl about equally in LPS-1 and LPS-2 (the plateau of the extinction curves in Fig. 2). Meanwhile, nonachlazine extinguished fluorescence of LPS-1 4.5 times more effectively than that of LPS-2 (Fig. 2). These observations suggested that chlorpromazine passes freely through membranes of liposomes, but nonachlazine does not pass through.

To verify this hypothesis, the following experiments were undertaken. Chlorpromazine and nonachlazine were added to the buffer solution either before addition of the solution of phospholipids in ethanol or after its addition. In the first case LPS-2 were formed in the presence of these substances, and the quenchers thus were located not only outside the liposomes, but also inside them. In the second case, the drug was initially outside the liposomes, but later it either penetrated inside or did not penetrate. The results showed (Table 1) that the order of addition of chlorpromazine made no difference: In both cases extinction was very high. For nonachlazine, however, extinction was high only if the drug entered the liposomes during their formation.

In the next series of experiments LPS-2 were formed in the presence of 1 mM nonachlazine or chlorpromazine, after which the whole suspension was diluted tenfold; the external concentration of the drug in this way was reduced tenfold, whereas inside the concentration would still remain high if the membranes were impermeable to the drug, or it would fall tenfold if

TABLE 1. Effect of Conditions of Addition of Chlorpromazine or Nonachlazine to LPS-2 on Extinction by These Drugs of Fluorescence of p-Terphenyl Probe Present in LPS-2 (M±m)

Substance	Conditions of addition of substance	Concentration of substance, mM	Fraction of extinction of p-terphenyl, %
Chlorpromazine	Before formation of LPS-2	0.3	83±2
	After formation of LPS-2	0.3	84±3
Nonachlazine	Before formation of LPS-2	0.3	39±2
	After formation of LPS-2	0.3	3±1
Chlorpromazine	Before formation of LPS-2, followed by dilution 1:10	0.1	53±3
Nonachlazine	Before formation of LPS-2, followed by dilution 1:10	0.1	16±2

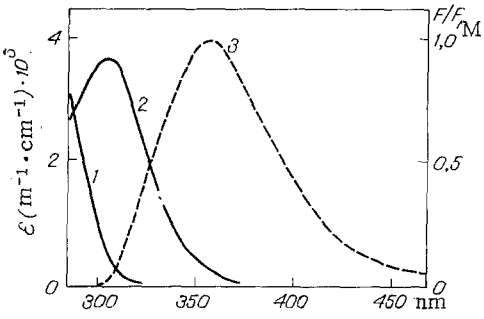

Fig. 3. Absorption spectra of nonachlazine (1) and chlorpromazine (2) and normalized fluorescence spectrum of p-terphenyl (3) in polyethylene glycol-400. ε) Molar extinction, F_M) intensity of fluorescence at maximum of spectrum.

they were permeable. As will be clear from Fig. 2 (curve 1), extinction by nonachlazine after dilution still remained 5 times higher than if it had penetrated through the LPS-2 membranes. In the case of chlorpromazine, however, extinction fell to 53%, which corresponds to its level when chlorpromazine was added externally to LPS-2, namely 61% (Fig. 2, curve 3).

The results of all these experiments thus demonstrate that model phospholipid membranes (liposomes) are permeable for chlorpromazine but impermeable for nonachlazine. If these substances behave relative to biomembranes in the same way as they behave relative to model liposomes, it can be tentatively suggested that chlorpromazine penetrates inside the cells and acts on subcellular structures (and, in particular, on mitochondria [8]), whereas nonachlazine interacts mainly with the cell surface.

The difference in permeability is evidently due to differences in the structure of the substituent at the nitrogen atom of the phenothiazine ring. An uncharged molecule is carried through the membrane, but the probability of the uncharged state of the molecule is considerably lower in the case of nonachlazine than in the case of chlorpromazine, for nonachlazine has two nitrogen atoms capable of binding H^+, whereas chlorpromazine has only one such atom.

The physical mechanism of extinction of fluorescence of p-terphenyl in the membrane by phenothiazine derivatives may perhaps consist of the resonance transfer of energy [5, 11]. The critical transfer distance, calculated by the method in [11] for the spectra in Fig. 3, was 1.95 nm for chlorpromazine and 0.85 nm for nonachlazine. This corresponds to the more effective extinction of fluorescence of p-terphenyl by chlorpromazine than by nonachlazine.

LITERATURE CITED

1. E. K. Grigor'eva, Byull. Éksp. Biol. Med., No. 11, 1345 (1976).
2. E. K. Grigor'eva and G. E. Dobretsov, Byull. Éksp. Biol. Med., No. 9, 1084 (1976).
3. A. I. Deev, E. A. Yarova, and G. E. Dobretsov, Farmakol. Toksikol., No. 3, 351 (1977).
4. G. E. Dobretsov, V. A. Petrov, A. I. Deev, et al., Biofizika, 20, 1014 (1975).
5. V. L. Ermolaev, E. N. Bodunov, E. B. Sveshnikova, et al., Nonradiative Transfer of Energy of Electronic Excitation [in Russian], Leningrad (1977).
6. N. V. Kaverina and G. A. Markova, Farmakol. Toksikol., No. 2, 173 (1973).
7. S. G. Kobaladze (editor), Nonachlazine in Clinical and Experimental Medicine [in Russian], Tbilisi (1976).
8. N. S. Kovaleva, A. I. Tentsova, G. A. Ladygina, et al., Farmatsiya, No. 1, 31 (1978).
9. A. F. Poglazov, M. V. Shubin, Yu. G. Skotselyas, et al., Biofizika, 20, 69 (1975).
10. S. Batzri and E. D. Korn, Biochim. Biophys. Acta, 298, 1015 (1973).
11. T. Förster, Disc. Faraday Soc., No. 27, 7 (1959).
12. A. K. Soutar, H. J. Pownall, A. S. Hu, et al., Biochemistry (Washington), 13, 2828 (1974).
13. A. Waggoner and L. Stryer, Proc. Natl. Acad. Sci. USA, 67, 579 (1970).