INTERACTION BETWEEN PSYCHOTROPIC DRUGS AND MODEL LIPID MEMBRANES

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A steady flow of data on the role of lipids in the regulation and function of various membrane systems has been published in recent years [13]. The state of membrane lipids is known to influence Na,K-ATPase [11], adenylate cyclase [8], and cytochrome P-450 [7] activity and to determine the degree of aggregation of membrane proteins [6]. It is also considered that many drugs have their effect predominantly on the lipid phase of biological membranes [1, 4]. The writers showed previously that antidepressants of various chemical classes band with liposomes formed from hens' egg phospholipids, and that their ability to change the surface charge density of membrane lipids correlates with inhibition of neurotransmitter reuptake by synaptosomes [1, 2].

Accordingly, in the investigation described below, interaction between psychotropic drugs of various groups and liposomes formed from phosphatidylcholine (PCh), one of the principal phospholipids of the outer surface of most membranes [3, 12], was studied.

EXPERIMENTAL METHOD

Liposomes were obtained by rapid injection of an alcoholic solution of PCh into 0.01M Tris-HCl buffer, pH 4, as described previously [9]. Fluorescence of the probes 1-anilinonaphthalenesulfonic acid—ANS (λ_e = 380 nm, λ_f = 480 nm) and 3-methoxybenzanthrone — MBA

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TABLE 1. Characteristics of Interaction of Psychotropic Drugs with Liposomes, Based on Their Effect on Fluorescence of ANS and MBA (M ± m)

Drug	K_b, M^{-1}	N, moles/mg lipid	$N \cdot K_b$	Δf, %	r
		ANS			
Imipramine	$(7,5\pm1,12)\cdot10^4$	$(1,78\pm0,11)\cdot10^{-1}$	$(1,32\pm0,001)\cdot10^4$	0,6	0,996
Desmethylimipramine	$(8,68\pm0,21)\cdot10^4$	$(1,07\pm0,02)\cdot10^{-1}$	$(9,24\pm0,07)\cdot10^3$	0,4	0,998
Chlorimipramine	$(1,65\pm0,19)\cdot10^5$	$(1,11\pm0,09)\cdot10^{-1}$	$(1,45\pm0,33)\cdot10^4$	0,7	0,996
Amitriptyline	$(4,86\pm0,38)\cdot10^5$	$(2,30\pm0,07)\cdot10^{-2}$	$(1,11\pm0,11)\cdot10^4$	0,7	0,990
(+)-Viloxazine	$(5,97\pm0,35)\cdot10^4$	$(8,83\pm0,69)\cdot10^{-2}$	$(5,23\pm0,11)\cdot10^3$	0,25	0,998
(−)-Viloxazine	$(1,82\pm0,18)\cdot10^5$	$(3,29\pm0,20)\cdot10^{-1}$	$(5,89\pm0,21)\cdot10^4$	0,225	0,997
Zimelidine	$(1,87\pm0,29)\cdot10^6$	$(3,19\pm0,17)\cdot10^{-2}$	$(5,81\pm0,68)\cdot10^4$	1,350	0,962
Norzimelidine	$(1,42\pm0,10)\cdot10^6$	$(2,05\pm0,03)\cdot10^{-2}$	$(2,90\pm0,19)\cdot10^4$	0,750	0,981
Pyrazidol	$(2,43\pm0,40)\cdot10^5$	$(1,38\pm0,14)\cdot10^{-2}$	$(3,20\pm0,17)\cdot10^3$	0,15	0,986
Chlorpromazine	$(3,12\pm0,04)\cdot10^5$	$(7,78\pm0,06)\cdot10^{-2}$	$(2,45\pm0,04)\cdot10^4$	1,2	0,994
Trifluoperazine	$(1,96\pm0,06)\cdot10^5$	$(7,91\pm0,29)\cdot10^{-2}$	$(1,54\pm0,02)\cdot10^4$	0,7	0,980
Azabutyrone	$(3,79\pm0,75)\cdot10^5$	$(3,48\pm0,48)\cdot10^{-2}$	$(1,32\pm0,20)\cdot10^4$	0,475	0,973
Dicarbine	$(1,76\pm0,21)\cdot10^5$	$(5,31\pm1,10)\cdot10^{-2}$	$(7,49\pm0,54)\cdot10^3$	0,3	0,997
Haloperidol	$(1,78\pm0,22)\cdot10^5$	$(9,76\pm0,56)\cdot10^{-2}$	$(1,71\pm0,10)\cdot10^4$	0,75	0,994
		MBA			
Chlorpromazine	$(1,41\pm0,07)\cdot10^4$	$(2,54\pm0,06)\cdot10^{-6}$	$(3,56\pm0,10)\cdot10^{-2}$	—	0,997
Trifluoperazine	$(2,08\pm0,24)\cdot10^4$	$(2,64\pm0,19)\cdot10^{-6}$	$(5,35\pm0,25)\cdot10^{-2}$	—	0,998

Legend. K_b) Binding constants, N) number of binding sites, $N.K_b$) total affinity, Δf) surface charge density of membrane, r) coefficient of correlation. Constants determined as in [1, 2].

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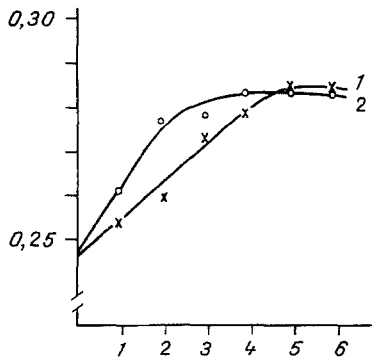

Fig. 1. Effect of neuroleptics on polarization of MBA fluorescence. Abscissa, concentration of drugs (in M); ordinate, polarization of fluorescence (in conventional units). 1) Trifluoperazine; 2) chlorpromazine.

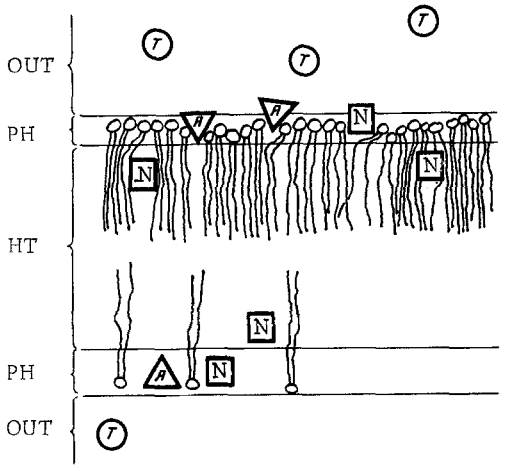

Fig. 2. Scheme of localization of psychotropic drugs in lipid membrane. OUT) Region outside membrane, PH) polar heads, HT) fatty acid residues, A) antidepressants, N) neuroleptics, T) tranquilizers.

(λ_e = 436 nm, λ_f = 537 nm), and polarization of fluorescence were recorded on a Hitachi-850 spectrofluorometer (Japan). The ANS concentration in the sample was chosen on the basis of the results of previous experiments [1]. About half of the probe, under these circumstances, was in the unbound state (molar ratio probe:lipid = 1:25. For MBA conditions were chosen under which the whole probe was bound with the membrane (molar ratio probe:lipid = 1:5). In standard experiments a suspension of liposomes (0.1 mg/ml) was incubated with 5 μM ANS or 1 μM MBA at room temperature, then titrated with aqueous solutions of the test substances. Imipramine, desmethylimipramine, chlorimipramine, (−)-violoxazine, (+)-violoxzine, zimelidine, norzimelidine, pyrazidol, diazepam, phenazepam, lonetil, amitriptyline, befuraline, chlorpromazine, trifluoperazine, dicarbine, azabutyrone, and haloperidol were used in the experiments. All reagents were of the analytical grade of purity. The numerical results were subjected to statistical and regression analysis by HP-33E electronic calculator (USA). The crystalline preparation of pyrazidol was generously provided by Academician of the Academy of Medical Sciences of the USSR M. D. Mashkovskii.

EXPERIMENTAL RESULTS

A study of interaction of the drugs with membrane lipids and their distribution in the lipid bilayer of the membranes, in the light of ever-increasing interest in the lipid components of the membrane, is of great theoretical and practical importance. To study the localization of the psychotropic drugs in model lipid membranes, two fluorescent probes, located at different depths in the bilayer, were used: ANS — in the region of the polar heads of the lipids molecules, MBA — in the zone of the hydrophobic "tails" of the lipids, at the level of the 5th-8th carbon atom of the fatty acid chain.

The results showed that the tranquilizers studied did not bind with PCh liposomes in the zones where ANS and MBA were located. Their targets at the cellular level are evidently mainly the benzodiazepine receptors, for which these drugs have high affinity, while exhibiting no affinity for other receptors [14]. However, the possiblity of interaction between tranquilizers and liposomes formed from other lipids cannot be ruled out.

Neuroleptics and antidepressants in concentration of between 10^{-7} and 10^{-6} M were located in the bilayer of their PCh in the zone of polar heads. The most active of the antidepressants by this test were (—)-viloxazine and zimelidine, which exhibited the greatest total affiniity for the membrane: $(5.89 \pm 0.21) \times 10^4$ and $(5.81 \pm 0.68) \times 10^4$ respectively (Table 1). Zimelidine, norzimelidine, and chlorimipramine had the greatest effect on surface charge density (1.35, 0.75, and 0.7% respectively). Tricyclic antidepressants exhibited high activity. The least active drug was pyrazidol. Incidently, (—)-viloxazine was an order of magnitude more active according to this test than its (+)-isomer; this correlates with their different activity in behavioral tests [5, 10].

Among the neuroleptics it was chlorpromazine which was most lipotropic. The atypical neuroleptics dicarbine and azabutyrone were less active than the classical compounds, namely phenothiazine derivatives. In a concentration of 2×10^{-6} M, imipramine, desmethylimipramine, norzimelidine, and all the neuroleptics except chlorpromazine increased the polarization of ANS fluorescence, possibly indicating condensation of the interphase under the influence of these drugs.

With an increase in concentration up to 10^{-5} M the phenothiazine derivatives penetrate deeper into the liposomal membrane — into the zone of fatty acid residues of the lipid molecules (Table 1). The antidepressants and atypical neuroleptics studied had no such effect. The increase in polarization of MBA fluorescence (Fig. 1) observed during the action of neuroleptics is evidence of condensation of the membrane.

The results indicate differences in affinity of the psychotropic drugs tested for lipid PCh bilayers. For instance, tranquilizers did not bind with liposomes, whereas antidepressants and neuroleptics did so bind and were located: the first, in the zone of the polar heads of the lipids, the second in the zone of the polar heads and of the 5th-8th carbon atom of the fatty acid chains of the lipid molecules (Fig. 2). These data on the depth of penetration of the neuroleptics and antidepressants into the membrane agree with results obtained previously on erythrocyte membranes [15]. The tricyclic neuroleptics, in the concentrations studied, signficiantly affected the orderliness of both the interphase and the hydrophobic anisotropic zone of the membrane, causing an increase in viscosity of the bilayer, whereas tricyclic antidepressants, in the same concentrations, had no such action on the isotropic zone.

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