INTERACTION BETWEEN PSYCHOTROPIC DRUGS AND CEREBRAL CORTICAL SYNAPTIC MEMBRANES IN RATS

A. V. Eremenko, N. A. Avdulov, and A. V. Val'dman*

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Results obtained by the writers previously indicate differences in the arrangement of psychotropic drugs in lipid bilayer membranes formed from phosphatidylcholine. In particular, antidepressants with different chemical structure and neuroleptics of the phenothiazine series were bound in the zone of the polar heads of the lipids; neuroleptics, moreover, also exhibited affinity for the deeper regions of phosphatidylcholine liposomes [2]. Data obtained by other investigators also indicate a different distribution of tricyclic antidepressants and neuroleptics in biological membranes [5, 6].

The aim of this investigation was to study interaction between psychotropic drugs (antidepressants, neuroleptics, tranquilizers) and synaptic membranes of the rat cerebral cortex, and also to compare the binding characteristics of these drugs with synaptic and model membranes.

EXPERIMENTAL METHOD

Synaptic membranes were obtained from the coarse synaptosomal fraction [1] after osmotic and cold shock. The synaptosomal fraction in 0.32 M sucrose was layered on a gradient of 1.2 and 0.8 M sucrose and centrifuged on a Beckman L8-80 ultracentrifuge (USA) at 100,000 g for 60 min. The fraction of synaptic membranes, located between layers of 1.2 and 0.8 M sucrose, was withdrawn by means of a syringe and a solution of 50 mM Tris-HCl buffer was added to it in order to obtain a final sucrose concentration of 0.32 M. The purified fraction of synaptic membranes thus obtained was frozen at -20°C. Protein was determined as described previously [4].

TABLE 1. Characteristics of Interaction of Psychotropic Drugs with Synaptic Membranes of Rat Cerebral Cortex, Determined by Their Influence on Fluorescence of ANS and MBA (M ± m)

Probe	Drug	K_b, m^{-1} ($\times 10^5$)	N, M/mg protein ($\times 10^{-1}$)	$K_b \cdot N$ ($\cdot 10^3$)	Δf, %	r
ANS	Imipramine	6,5±0,2	0,20±0,01	13,1±0,1	1,6	0,920
	Desimipramine	0,4±0,1	0,56±0,08	1,9±0,3	1,75	0,998
	Clomipramine	0,4±0,1	1,40±0,10	5,0±0,3	4,95	0,997
	Pirlindol	0,5±0,1	0,56±0,05	2,5±0,2	2,3	0,996
	(+)-Viloxazine	0,03±0,01	0,07±0,01	0,02±0,002	0,17	0,994
	(−)-Viloxazine	0,07±0,003	0,03±0,003	0,02±0,002	0,15	0,963
	Zimelidine	0,13±0,02	0,08±0,008	0,1±0,01	0,18	0,978
	Norzimelidine	0,02±0,002	0,4±0,04	0,08±0,002	0,18	0,999
	Trazodone	0,3±0,1	0,3±0,1	0,74±0,03	4,35	0,995
	Nomifensine	0,14±0,01	0,10±0,02	0,14±0,01	0,25	0,996
	Chlorpromazine	1,3±0,1	0,63±0,03	8,20±0,3	5,4	0,993
	Trifluoperazine	4,95±0,8	0,28±0,03	13,0±0,8	6,6	0,995
	Haloperidol	0,1±0,03	0,56±0,03	0,91±0,03	1,1	0,981
MBA	Chlorpromazine	(1,5±0,3)·10^5	(1,3±0,2)·10^{-5}	1,9±0,2	—	0,897
	Trifluoperazine	(0,5±0,1)·10^5	(1,3±0,1)·10^{-5}	0,7±0,2	—	0,990

Legend. K_b) Binding constant, N) number of binding sites, $N \cdot K_b$) total affinity, Δf) change in density of surface change of membranes, r) coefficient of correlation.

*Academician of the Academy of Medical Sciences of the USSR

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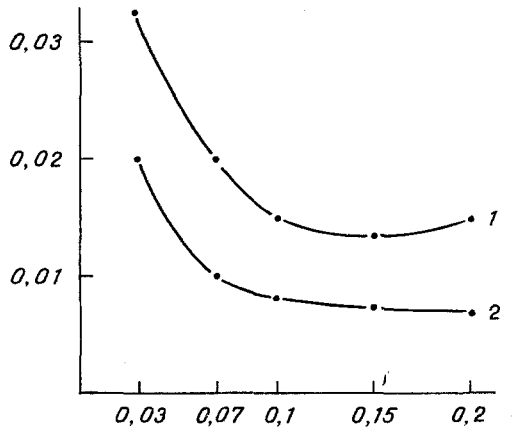

Fig. 1. Effect of tranquilizers on intensity of fluorescence of ANS in cerebral cortical synaptic membranes of rats. Abscissa, concentration of drugs (in mM); ordinate, reciprocal of intensity of fluorescence (F). 1) Diazepam, 2) phenazepam.

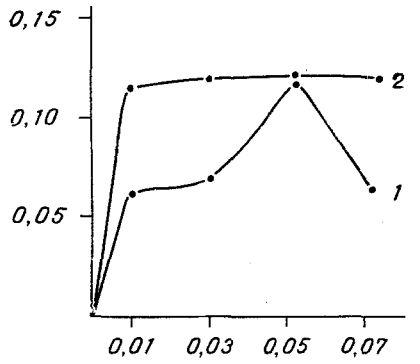

Fig. 2. Effect of neuroleptics on polarization of fluorescence of MBA in cerebral cortical synaptic membranes of rats. Abscissa, concentration of drugs (in mM); ordinate, polarization of fluorescence of MBA (in conventional units). 1) Chlorpromazine, 2) trifluoperazine.

Fluorescence of the probes: 1-anilinonaphthalene-8-sulfonic acid — ANS (λ_{exc} = 380 nm; λ_{flu} = 480 nm) and 3-methoxybenzanthrone — MBA (λ_{exc} = 436 nm; λ_{flu} = 537 nm), and also polarization of fluorescence of MBA were recorded on a Hitachi-850 spectrofluorometer (Japan). A suspension of synaptic membranes was incubated with 10 μM ANS or 0.5 μM MBA at room temperature for not more than 5 min, then titrated with aqueous solutions of the test preparations (for phenazepam and diazepam, their solutions in ethanol were used). The following psychotropic drugs were used in the experiments: imipramine, desimipramine, clomipramine, pirlindol, (+)-(—)-isomers of viloxazine, zimelidine, norzimelidine, trazodone, nomifensine, chlorpromazine, trifluoperazine, haloperidol, diazepam, and phenazepam. All the reagents were analytically pure.

EXPERIMENTAL RESULTS

Data relating to interaction between psychotropic drugs and synaptic membranes in zones where the fluorescent probes ANS and MBA were located are given in Table 1. Just as in the case of phosphatidylcholine liposomes [2], the antidepressants and neuroleptics in a concentration of 10^{-7}–10^{-6} M exhibited affinity for the surface regions of the synaptic membranes. The most active drugs were imipramine, trifluoperazine, and chlorpromazine, which exhibited high total affinity for synaptic membranes — ($K_{b.n}$) for the rat cerebral cortex was (13.1 ± 0.1)·10^3, (13.0 ± 0.8)·10^3, and (8.2 ± 0.3)·10^3, respectively — as well as chlomipramine

(Table 1). The density of the surface charge was actively affected by trifluoperazine, chlorpromazine, clomipramine, and trazodone (6.6, 5.4, 4.95, and 4.35%, respectively). On the whole these experiments showed a similarity between the interaction of tricyclic structures of psychotropic drugs and artificial and synaptic membranes. However, the clear difference observed between the (+)- and (−)-isomers of viloxazine in binding with liposomes [2] correlates only with binding constants of these isomers on synaptic membranes, which could indicate a difference in the affinity of (+)- and (−)-viloxazine for lipid domains of varied composition.

The tranquilizers of the benzodiazepine series studied, namely diazepam and phenazepam, unlike the other groups of psychotropic drugs, quenched fluorescence of ANS (Fig. 1); this effect was stronger in the case of phenazepam than of diazepam. It was shown previously that phenazepam and diazepam are unable to bind with phosphatidylcholine liposomes [2]. However, the fluorescent probe ANS is known to be capable of interacting not only with the lipid, but also with the protein surface of biological membranes [3]. The decrease in the intensity of fluorescence of ANS in evidence of competitive interaction between diazepam (phenazepam) and ANS for the polar groups of the protein molecules.

With an increase in concentration up to 10^{-5} M only phenothiazine derivatives (chlorpromazine, trifluoperazine) can penetrate into the deeper regions of the lipid phase of rat cerebral cortical synaptic membranes, increasing the viscosity of the lipid bilayer, as is shown by increased polarization of fluorescence of MBA (Fig. 2). We obtained similar results for phosphatidylcholine liposomes [2], and they were also obtained by other workers with platelets [6] and erythrocytes [5]. An increase in viscosity of the membranes due to phenothiazine derivatives is considered to be determined by the structure of the ring and not of the side chain [6].

The psychotropic drugs studied in this investigation (antidepressants, neuroleptics, and tranquilizers) can thus interact with cerebral cortical synaptic membranes of rats in the zone of the fluorescent probe ANS. A distinguishing feature of interaction of phenothiazine neuroleptics with synaptic membranes was their ability to influence actively not only the state of the surface of the membranes, but also the order of the hydrophobic anisotropic zone of the membranes, limiting mobility of the fatty acid chains of the lipid molecules, in good agreement with data obtained previously on liposomes [2].

LITERATURE CITED

1. N. A. Avdulov and N. I. Maisov, Byull. Éksp. Biol. Med., No. 11, 564 (1981).
2. N. A. Avdulov, A. V. Eremenko, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 10, 440 (1985).
3. Yu. A. Vladimirov and G. E. Dobretsov, Flurescent Probes in the Study of Biological Membranes [in Russian], Moscow (1980).
4. G. L. Peterson Anal. Biochem., 83, 346 (1977).
5. G. Zimmer and P. Shulze, Arzneim.-Forsch., 31, 1389 (1981).
6. G. S. Zubenko and B. M. Cohen, Psychopharmacology (Berlin), 84, 289 (1984).