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EFFECT OF CHLORPROMAZINE AND TRIFLUPERAZINE ON SYNAPTOSOMAL MEMBRANES OF THE RAT CEREBRAL CORTEX

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The writers showed previously that psychotropic preparations belonging to various groups differ in their affinity for phosphatidylcholine liposomes [2]. The neuroleptics studied, unlike antidepressants and tranquilizers, penetrated deeper into the bilayer, into the zone where a hydrophobic fluorescent probe, 3-methoxybenzanthrone, was located. Penetration of phenothiazine neuroleptics into the central zone of the lipid bilayer has been demonstrated on erythrocyte membranes [9].

It was accordingly decided to study the effect of the neuroleptics chlorpromazine and trifluperazine and to compare it with that of the tricyclic antidepressant imipramine, on viscosity of the lipid bilayer of synaptosomal membranes of the rat cerebral cortex, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

A pure fraction of cerebral cortical synaptosomal membranes from male rats weighing 180-200 g was obtained by the usual methods of gradient centrifugation of a coarse synaptosomal fraction after cold and osmotic shock, as described previously [5]. As fluorescent probes we used 1, 6-diphenyl-1,3,5-hexatriene (DPHTE, from Fluka, West Germany) and pyrene (Sigma, USA). Luminescence of DPHTE was excited at 350 nm and recorded at 430 nm. The final concentration of the probe in the sample was 10^{-6} M and the insertion time 30 min at 37°C in darkness, with constant mixing. Luminescence of pyrene was excited at 334 nm and recorded at 373 and 480 nm; the excitation filter was 350 nm, the concentration of the probe in the sample $5 \cdot 10^{-6}$ M, and the insertion time 5 min at 37°C in darkness with constant mixing. Luminescence of tryptophan of the membranes and of L-tryptophan (Sigma) was excited at 286 nm and recorded at 330 nm. Fluorescence was recorded using corrected spectra on a Hitachi M-850 fluorescent spectrophotometer (Japan). Next, 25 µl of synaptosomal suspension (average 56 µg protein) was added to 1 ml of incubation medium containing 150 mM NaCl, 6 mM KCl, 0.5 mM ethylenediaminetetra-acetic acid, and 0.01 M Tris-HCl buffer, pH 7.4. Solutions of the probes (10 mM) in dimethylformamide were added to the membranes in the incubation medium from a microsyringe at the rate of 1 µl/min, with constant mixing. The viscosity of the lipid component of the synaptosomal membranes was judged from the ratio of the intensity of fluorescence of pyrene at 373 nm (monomer) and 480 nm (excimer), and also from the change in polarization of the fluorescence of DPHTE, calculated by the equation:

$$P = \frac{(F_{\parallel} - f_{\parallel}) - (F_{\perp} - f_{\perp})}{(F_{\parallel} - f_{\parallel}) + (F_{\perp} - f_{\perp})},$$

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TABLE 1. Effect of Chlorpromazine and Trifluperazine on Microviscosity of Synaptosomal Membranes of the Rat Cerebral Cortex (M ± m)

Concentration of drug, μM	Pyrene	DPHTE
	Trifluperazine	
0	0,674±0,002	4,815±0,018
2,0	0,571±0,005	5,032±0,098
5,0	0,471±0,021	5,410±0,036
7,5	0,376±0,006	5,536±0,037
10,0	0,307±0,001	5,620±0,028
	Chlorpromazine	
0	0,652±0,014	4,796±0,012
2,0	0,523±0,010	5,225±0,008
5,0	0,457±0,030	5,495±0,068
7,5	0,399±0,023	5,603±0,011
10,0	0,344±0,019	5,946±0,080

TABLE 2. Effect of Drugs Tested on Fluorescence of Endogenous Tryptophan (M ± m)

Preparation	$IC_{50} \cdot 10^{-5}$, M
Trifluperazine	1,29±0,01
Chlorpromazine	1,54±0,02
Imipramine	7,27±0,54

Legend. IC_{50} denotes concentration of preparation reducing fluorescence of endogenous tryptophan by 50%.

where $f_{||}$ and $f_{\perp}$ denote the intensity of polarized fluorescence of the membranes without the probe, and $F_{||}$ and $F_{\perp}$ denote the intensity of polarized fluorescence of the membrane suspension with the probe [3]. To calculate the microviscosity of the membranes the equation $\eta = 2P(0.46-P)$, was used, as recommended in [10]. Protein was determined by a modified method in [8]. The data were subjected to correlation analysis on a PC 20 computer (Commodore, England), using specially devised programs.

EXPERIMENTAL RESULTS

The results indicate condensation of the lipid bilayer of the synaptosomal membranes by the neuroleptics studied (Table 1). Control values of microviscosity lay within the limits of known values for the microviscosity of biological membranes: 0.1-10.0 Pa [10]. The decrease in the degree of excimerization of pyrene under the influence of chlorpromazine and trifluperazine also is evidence of condensation of the lipid bilayer of the synaptosomal membranes. Comparison of data obtained with the aid of pyrene and DPHTE showed close correlation between condensation of the synaptosomal membranes under the influence of trifluperazine ($r = 0.96$) and chlorpromazine ($r = 0.98$). It must be emphasized that the tricyclic antidepressant imipramine did not affect fluorescence of the probes used, and it evidently does not affect packing of lipid molecules of the bilayer in the membranes studied.

A very small change in microviscosity of the lipid bilayer of biological membranes may lead to substantial changes in the activity of membrane-bound proteins [4, 7]. It was therefore interesting to study also changes in the state of the proteins of synaptosomal membranes against the background of the action of these drugs. The state of the membrane-bound proteins was judged by changes in fluorescence of endogenous tryptophan, a luminescent amino aeid which is a component of many of the proteins of synaptosomal membranes. The experiments showed that all three drugs studied quench the fluorescence of endogenous tryptophan (Table 2), without shifting the maximum of its fluorescence, but they have no effect on the fluorescence of pure L-tryptophan in solution. Comparison of condensation of the membranes under

the influence of chlorpromazine and trifluperazine and the time course of quenching of the fluorescence of endogenous tryptophan by these preparations revealed close correlation (r = 0.99) between these processes. Since the neuroleptics did not affect the luminescence of pure tryptophan, the results suggest that trifluperazine and chlorpromazine, by condensing the structure of the lipid component of synaptosomal membranes, modify the conformation of membrane-bound proteins, and this is reflected in the arrangement of the tryptophan groups and interferes with the fluorescence of some of them. Changes in the conformation of the membrane-bound proteins may also be reflected in their activity. Imipramine quenched fluorescence of endogenous tryptophan much less strongly than did chlorpromazine and trifluperazine, possibly due to a change in the density of the surface charge of the lipid bilayer of the synaptosomal membranes under the influence of this antidepressant [1].

The data obtained on rat brain synaptosomal membranes are in good agreement with data in the literature obtained on membranes of human platelets [10] and of rat brain homogenate [6]. Meanwhile, the problem of the role of changes in microviscosity of biological membranes under the influence of the neuroleptics tested in the realization of the specific antipsychotic effect of these drugs remains undecided and requires more detailed experimental analysis.

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