CORRELATION BETWEEN AFFINITY OF ANTIDEPRESSANTS FOR MEMBRANES AND THEIR EFFECT ON NEUROMEDIATOR REASSIMILATION

N. A. Avdulov, G. E. Dobretsov,
and A. V. Val'dman

UDC 615.214.32.015.44:612.8.018

KEY WORDS: antidepressants; mediator uptake; rat brain synaptosomes; liposomes.

An important component of the mechanism of action of the tricyclic antidepressants is their ability to inhibit reassimilation of neuromediators by nerve endings. In recent years new effective antidepressants have been produced which differ significantly from the tricyclic compounds, not only in chemical structure, but also in pharmacological properties, including their effect on neuromediator uptake [6]. The molecular mechanisms of mediator uptake have not been adequately studied. The writers have suggested that inhibition of reassimilation by antidepressants is connected with the affinity of the lipophilic molecules of these compounds for biological presynaptic membranes. According to the liquid-mosaic model of the biological membrane [9], its principal structural determinants are the asymmetrical phospholipid bilayer and granular proteins. Activity of the Na,K-ATPase, with which the transport (uptake) of neuromediators is coupled, is essentially modulated by changes in the state of the membrane lipids [8].

The object of this investigation was to study interaction between antidepressants with different chemical structure and model phospholipid membranes and to discover correlation between the affinity of these compounds for membranes and their effect on reassimilation of neuromediators by rat brain synaptosomes.

EXPERIMENTAL METHOD

Phospholipid model membrane bubbles (liposomes) were obtained by spraying an ethanol solution of total egg phospholipids into 0.01 M Tris-HCl buffer, pH 7.4 [7]. The resulting liposomes had a single bilayer membrane [7, 10]. The substance 1-anilinonaphthalene-8-sulfonic acid (ANS) was used as the probe (molar ratio probe:lipid = 1:19). Aqueous 1 mM solutions of antidepressant with bi-(befuralin), tri-(imipramine, desmethylimipramine, chlorimipramine), and tetracyclic (pirazidol) structure were added to the liposome suspension from a microsyringe. Fluorescence was measured 5 min after addition of the drug on an Opton (West Germany) spectrofluorometer. Fluorescence of ANS was excited at 360 nm and recorded at 480 nm. Absorption spectra of the test antidepressants were recorded on a dual-beam Aminco (USA) spectrophotometer. The effect of the antidepressants in concentrations of 50 and 500 μM on reassimilation of ^{3}H-serotonin and ^{3}H-GABA was studied on fractions of unpurified rat brain synaptosomes [1]. The crystalline preparation of pirazidol was generously supplied by Academician of the Academy of Medical Sciences of the USSR M. D. Mashkovskii.

EXPERIMENTAL RESULTS

It was shown previously that during interaction between imipramine and the membranes fluorescence of the 3-methoxybenzanthrone (MBA) probe is extinguished by 60% by imipramine in a concentration of 0.2 mM [2, 3]. We used a different probe, namely ANS, fluorescence of which increased on the addition of the antidepressants, in some cases (tricyclic compounds) by over 100%. This probe was thus more sensitive to binding of antidepressants with membranes than MBA.

Values of binding constants of the preparations with liposome membranes (K_b), and the products of K_b and the specific number of binding sites (N_{sp}), characterizing the total affinity of the antidepressants for lipid membranes, and values of $\Delta F/\Delta C$ (Fig. 1), are shown in Table 1.

It will be clear from Table 1 that the bicyclic antidepressant befuralin binds with liposome membranes better than imipramine. The least active compound was the tetracycline drug pirazidol. The presence of a halogen substituent in the tricyclic system (chlorimipramine) considerably improved binding of the preparation with membranes. Demethylation at the nitrogen atom in the side chain (secondary amine compared with the tertiary amine) was not reflected in binding of the antidepressant with the membranes.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 44-46, August, 1981. Original article submitted March 13, 1981.

 0007-4888/81/9208-1054$07.50

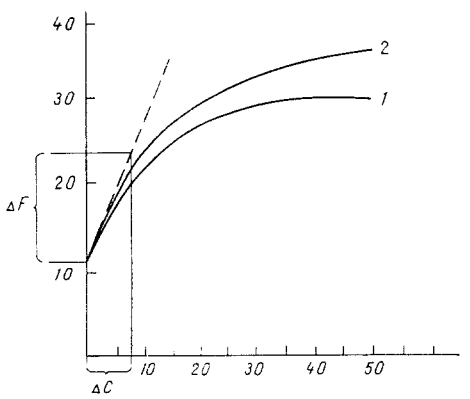

Fig. 1

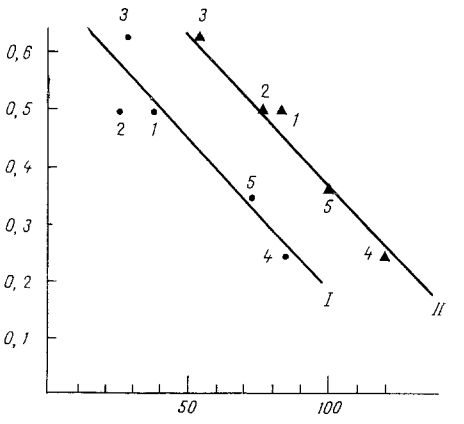

Fig. 2

Fig. 1. Effect of some antidepressants on fluorescence: 1) imipramine, 2) chlorimipramine. Abscissa, concentration (in μM); ordinate, intensity of fluorescence (in relative units).

Fig. 2. Comparison of affinity of antidepressants of different chemical groups for membranes and effects of these compounds on neuromediator reassimilation. Inhibition of uptake of serotonin (I) and GABA (II) by antidepressants in concentrations of 50 μM and affinity for membranes: 1) imipramine, 2) desmethylimipramine, 3) chlorimipramine, 4) befuralin, 5) pirazidol, γ_I −0.91 ± 0.07; γ_{II} −0.98 ± 0.02. Abscissa, mediator uptake (in percent), ordinate, value of $\Delta F/\Delta C$.

TABLE 1. Some Characteristics of Interaction of Antidepressants with Liposome Membranes

Preparation	K_b, μM^{-1}	$K_b \cdot N_{sp}$, μM^{-1}	$\Delta F/\Delta C$
Imipramine	0,137	9,069	0,5
Desmethylimipramine	0,116	6,692	0,5
Chlorimipramine	0,234	18,78	0,625
Befuralin	0,279	6,182	0,25
Pirazidol	0,041	4,641	0,375

Comparison of the results with the effect of the test antidepressant on reassimilation of neuromediators by rat brain synaptosomes [1] revealed correlation between affinity of the drugs for the membranes (as reflected in the index $\Delta F/\Delta C$) and inhibition of neuromediator reassimilation by them (Fig. 2).

The mechanism of intensification of ANS fluorescence by antidepressants of the different chemical groups may perhaps consist of an increase in binding of the negatively charged ANS probe with the membrane, which becomes positively charged on interaction with the cations of the antidepressants. If, in fact, the binding constant and number of binding sites for ANS with the membrane are counted in the presence of antidepressants, the values of $K_b \cdot N_{sp}$ correlate well with changes in the intensity of ANS fluorescence ($\Delta F/\Delta C$) [2]. Meanwhile there was no correlation between changes in K_b and $\Delta F/\Delta C$. The effect of the antidepressant on neuromediator uptake was thus due mainly to the number of antidepressant molecules bound with the membrane (which is proportional to $K_b \cdot N_{sp}$).

Activity of Na, K-ATPase is largely dependent on the physical state of the membrane lipids [8]. That is why accumulation of antidepressants in a lipid bilayer (especially in the case of befuralin and chlorimipramine) and the changes in the charge on the membrane connected with this may be the cause of changes in the functional activity of the system transporting mediators through the presynaptic membrane.

Pirazidol, which is a selective type A monoamine oxidase inhibitor, has negligible effect on noradrenalin and serotonin uptake. In high concentrations (500 μM) pirazidol reduces Na,J-ATPase activity [4, 5], there by disturbing neuromediator uptake also. However, this is a reflection of the nonspecific action of antidepressants on membrane affinity and it correlates with the manifestation of the general sedative action of the drug.

LITERATURE CITED

1. N. A. Abdulov and N. I. Maisov, in: Abstracts of Proceedings of the 4th All-Union Symposium on the Goal-Directed Search for Physiologically Active Substances [in Russian], Riga (1981), p. 58.

2. Yu. A. Vladimirov and G. E. Dobretsov, Fluorescence Probes in Investigations of Biological Membranes [in Russian], Moscow (1980).
3. E. K. Grigor'eva, and G. E. Dobretsov, Byull. Éksp. Biol. Med., No. 9, 1084 (1976).
4. M. D. Mashkovskii, K. S. Raevskii, N. I. Maisov, et al., Khim. Farm. Zh., No. 8, 5 (1976).
5. M. D. Mashkovskii and N. I. Andreeva, Farmakol. Toksikol., No. 1, 5 (1975).
6. M. D. Mashkovskii, A. I. Polezhaeva, and N. I. Andreeva, Khim. Farm. Zh., No. 6, 19 (1979).
7. S. Batzri and E. D. Korn, Biochim. Biophys. Acta, 198, 1015 (1973).
8. C. M. Grisham and R. E. Barnett, Biochemistry (Washington), 12, 2635 (1973).
9. S. J. Singer and G. L. Nicholson, Science, 175, 720 (1972).
10. A. K. Soutar, H. J. Pownall, A. S. Hu, et al., Biochemistry (Washington), 13, 2828 (1974).

POSSIBLE DISPARITY BETWEEN ADEQUACY OF BEHAVIOR AND MOTOR AUTOMATISMS IN AMPHETAMINE STEREOTYPY IN CATS

B. A. Tolpyshev, L. E. Smirnova, and É. B. Arushanyan

UDC 616.8-009.29-092.9-02:615.214.3

KEY WORDS: amphetamine stereotypy; adequacy of behavior; haloperidol; clozapine; metoclopramide.

Stereotyped behavior arising in animals receiving large doses of amphetamine is nowadays regarded as one of the most adequate models of psychopathology [3, 7, 8]. This state, because of high sensitivity to neuroleptics, is widely used for the screening of new antipsychotic drugs. The criterion of specificity of action of the drugs in this case is limitation of motor automatisms accompanying the stereotypy. Meanwhile, as the authors cited above have shown, in some cases disparity may arise between the intensity of the motor disturbances in amphetamine stereotypy and the adequacy of the animals' behavior.

EXPERIMENTAL METHOD

Experiments were carried out on 17 cats of both sexes weighing 2-4 kg. Stereotyped behavior induced by amphetamines was assessed by two methods. In animals kept in a chamber measuring 60 × 60 × 60 cm external indices of stereotypy were considered first. The number of automatized movements (turning the head from side to side, nodding it up and down, movements around the chamber, sniffing) were counted three times in the course of 1 min at different times after injection of the drug. Immediately after this the cats were allowed to move freely around the experimental room, where the adequacy of their behavior was determined by means of a series of natural test stimuli (calling by name, response to stroking, playing with a paper "butterfly"). The response to each test was assessed by a 5-point system. For example, for play behavior it appeared as follows: 0 points – no response to the "butterfly," 1 point – having seen the "butterfly," the cat at once lost interest, 2 points – the cat watched the butterfly for a long time but without starting to play, 4 points – the cat started to play, stretched out its paw, but did not pursue the object, 5 points – the cat pursued the "butterfly," and tried to catch it. The averaged total of points for different tests constituted the index of adequacy.

Altogether there were four series of experiments (six experiments on six animals in each series). In series I the cats received only amphetamine, in increasing doses (from 0.25 to 2.0 mg/kg) with an interval of 25 min, and in the next three series, against the background of a dose of amphetamine at the threshold level for stereotypy, one of the drugs inhibiting amphetamine stereotypy was given repeatedly (every 15 min) until this was completely abolished: haloperidol – 0.125, 0.25, and 0.5 mg/kg; clozapine – 0.25, 0.5 and 1.0 mg/kg; metoclopramide – 0.5, 1.0, and 2.0 mg/kg. In the experiments of series V the adequacy of the animals' behavior was assessed during administration of gradually increasing doses of haloperidol only. The drugs were given intraperitoneally, and the numerical results were subjected to statistical analysis by Student's t-test (at the $P < 0.05$ level).

Department of Pharmacology and Central Research Laboratory, Chita Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 46-48, August, 1981. Original article submitted February 13, 1981.

 0007-4888/81/9208-1056$07.50