EFFECT OF PSYCHOTROPIC DRUGS ON BRAIN Ca-ATPases in vitro

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The pharmacological activity of psychotropic drugs (PD) is closely connected with synaptic processes in the brain, including liberation of mediators from synaptic vesicles [2, 7]. Both Ca^{++} ions and the actomyosin-like ATPase which is activated by them (neurosthenin) play a regulatory role in this liberation process [10]. The Ca^{++} ion concentration in the nerve cell is maintained at a comparatively low level because of their active transport, for which Ca^{++}-activated, Mg^{++}-dependent ATPase (Ca,Mg-ATPase) is responsible [3, 12]. There is virtually no information in the literature on the role of brain Ca-ATPases in the mechanism of action of PD. In the investigation described below the effect of members of different classes of PD on activity of actomyosin-like and Ca,Mg-ATPases was studied.

EXPERIMENTAL METHOD

Brain synaptosomes obtained from guinea pigs by the method of Whittaker et al. [15] were used as ATPase preparations. The presence of synaptosomes in the preparations was verified electron-microscopically. Ca,Mg-ATPase activity was determined in medium containing 37.5 mM imidazole-HCl buffer, pH 7.4, 6 mM $MgCl_2$, 1.5 mM ATP, 0.1 mM ouabain, 100 mM KCl, and 0.01 mM $CaCl_2$. In the control the medium contained 1 mM EGTA instead of $CaCl_2$. Activity of actomyosin-like ATPase was determined in medium containing 30 mM imidazole-HCl buffer, pH 7.4, 3 mM ATP, 100 mM KCl, 0.1 mM ouabain, and 3 mM $CaCl_2$. No $CaCl_2$ was present in the control. Incubation began on addition of the enzyme preparation to the corresponding mixtures and continued for 20 min at 37°C in the presence or absence of different concentrations of PD, and was stopped by the addition of 20% TCA. Inorganic phosphate (P_{in}) liberated by the action of the enzymes was determined by Fiske and Subbarow's method using thiourea as reducing agent [5]. PD interfering with the determination of P_{in} were extracted beforehand with chloroform [14]. Protein was determined by Lowry's method [11]. Specific activities of actomyosin-like and Ca,Mg-ATPases averaged 0.1 and 0.09 units respectively (in μmoles P_{in}/min/mg protein).

EXPERIMENTAL RESULTS

The values of I_{50} given in Table 1 were obtained graphically from the experimental data. They show that all PD studied inhibit brain Ca,Mg-ATPase activity. The strongest inhibitory properties were possessed by neuroleptics (haloperidol, perphenazine, chlorpromazine, levomepromazine). The inhibitory effects of the antidepressants (trazodone, imipramine) and also of benactyzine, which has a tranquilizing action, were weaker. Inhibition of Ca,Mg-ATPase by the psychostimulant amphetamine was manifested only in high concentrations.

Compared with Ca,Mg-ATPase, actomyosin-like ATPase was much less sensitive to the action of PD. The neuroleptics inhibited the latter enzyme to a greater degree than antidepressants. Neither benactyzine (in concentrations of 1 to 4.5 mM) nor amphetamine (in concentrations of 1 to 20.0 mM) had any inhibitory action.

Comparison of the results with the effect of PD on brain Na,K-ATPase demonstrated previously [6, 8] shows that Ca-ATPases are less sensitive to PD than Na,K-ATPase. In turn, the degree of inhibition of acto-

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TABLE 1. Inhibition of Activity of Brain Actomyosin-like and Ca,Mg-ATPases by PD in vitro (M ± m)

PD	I_{50}, mM	
	Ca, Mg-ATPase	actomyosin-like ATPase
Haloperidol	0,48±0,01	1,39±0,02
Perphenazine	0,59±0,02	1,16±0,02
Chlorpromazine	0,69±0,01	1,10±0,02
Levomepromazine	0,73±0,02	1,11±0,01
Trazodone	1,28±0,03	2,71±0,06
Imipramine	1,33±0,01	2,76±0,03
Benactyzine	3,12±0,08	No action
Amphetamine	18,52±0,22	"

myosin-like ATPase by PD was much weaker than that of the transport ATPases (Na,K-ATPase, Ca,Mg-ATPase). The essential point is that both Ca- and Na,K-ATPases are particularly sensitive to the action of neuroleptics. However, these enzymes evidently play different roles in the mechanism of the central effects of neuroleptics. Na,K-ATPase, which lies at the basis of the sodium pump in cells [1, 13], was particularly strongly inhibited by neuroleptics, mainly those with sedative properties (levomepromazine, chlorpromazine). On this basis it has been suggested that brain Na,K-ATPase plays a role in the biochemical mechanism of the general inhibitory and, in particular, the sedative action of neuroleptics [6, 8].

The results of the present investigations show that Ca,Mg-ATPase, responsible for the active transport of Ca^{++} ions in cells, is inhibited particularly strongly by neuroleptics, mainly those with antipsychotic activity (haloperidol, perphenazine). Inhibition of active Ca^{++} transport in synapses may perhaps play a definite role in the mechanism of the antipsychotic action of neuroleptics. This view is in harmony with the earlier suggestion of the role of Ca,Mg-ATPase of the sarcoplasmic reticulum in the effects of neuroleptics [4, 9].

The role of actomyosin-like ATPase in specific pharmacological effects of the neuroleptics is evidently inconspicuous. The possibility cannot be ruled out that this enzyme plays a nonspecific role in the effect of neuroleptics on brain synaptic processes.

LITERATURE CITED

1. A. A. Boldyrev, Usp. Biol. Khim., 18, 122 (1978).
2. V. V. Zakusov, Pharmacology of Central Synapses [in Russian], Moscow (1973).
3. S. A. Kudinov and I. R. Alekseenko, Ukr. Biokhim. Zh., No. 2, 68 (1977).
4. É. F. Lavretskaya, V. L. Tat'yanenko, Yu. Sh. Moshkovskii, et al., Farmakol Toksikol., No. 3, 292 (1980).
5. É. Martinson and L. Villako, Lab. Delo, No. 12, 30 (1961).
6. É. I. Paesalu, U. S. Tarve, and L. J. Tahepold (L. Ya. Tyakhepyl'd), Farmakol Toksikol., No. 1, 7 (1979).
7. K. S. Raevskii, Pharmacology of Neuroleptics [in Russian], Moscow (1976).
8. U. S. Tarve, E. I. Paesalu, and L. J. Tahepold (L. Ya. Tyakhepyl'd), Ukr. Biokhim. Zh., No. 3, 326 (1976).
9. L. V. Tat'yanenko, É. F. Lavretskaya, L. M. Raikhman, et al., Vopr. Med. Khim., 22, 406 (1976).
10. S. Berl, S. Puszkin, and W. J. Nicklas, Science, 179, 441 (1976).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. T. Saermark and H. Vilhardt, Biochem. J., 181, 321 (1979).
13. J. C. Shou, Q. Rev. Biophys., 7, 401 (1975).
14. I. Ueda and J. Wada, Anal. Biochem., 37, 169 (1970).
15. V. P. Whittaker, I. A. Michelson, and R. J. A. Kirkland, Biochem. J., 90, 293 (1964).