EFFECT OF PSYCHOTROPIC DRUGS ON SYNAPTOSOMAL UPTAKE OF H^3-γ-AMINOBUTYRIC ACID AND ON Na,K-ATPase ACTIVITY

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Neurotropic drugs belonging to various groups (fluphenazine, nonachlazine, trifluperidol fluacizine, imipramine, diazepam, apomorphine, fentanyl, diphenylhydantoin) in experiments in vitro showed an inhibitory effect on active transport of γ-aminobutyric acid (GABA) by synaptosomes of the rat cerebral cortex. In most cases a parallel decrease in the activity of synaptosomal Na,K-ATPase was observed. Substances not changing GABA uptake as a rule had no effect on the activity of the enzyme (carbidine and morphine). Some substances inhibiting Na,K-ATPase were ineffective with respect to GABA uptake (azabuperon, tetrabenazine). It is suggested that the drugs tested have at least two possible points of application: Na,K-ATPase and the hypothetical transmembrane carrier of GABA.

KEY WORDS: GABA uptake; Na,K-ATPase; psychotropic drugs; rat brain synaptosomes.

Chlorpromazine and imipramine have been shown to inhibit the uptake of γ-aminobutyric acid (GABA) by isolated nerve endings [7, 11]. The mechanism of active transport of mediators including GABA) with high carrier affinity functions in low concentrations of the mediator in the incubation medium [7, 13], it is sensitive to metabolic inhibitors [9], and it leads mainly to accumulation of the mediators by synaptic vesicles. Active uptake takes place in medium with a high Na^+ (50-150 mM) and low K^+ (1-10 mM) concentrations and is inactivated in medium with a high K^+ concentration (20-50 mM). This mechanism, it has been suggested, is linked with the function of Na,K-ATPase and is inhibited by the specific inhibitor, ouabain [9].

With these facts in mind, it was decided to compare the effects of psychotropic drugs of different classes on the active uptake of H^3-GABA by synaptosomes, on the one hand, and on Na,K-ATPase activity of the synaptosomes on the other hand.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-250 g. After decapitation the cerebral cortex was removed and the subcellular fractions were obtained by fractionation of the total synaptosomal fraction in a sucrose density gradient [8]. The residue of synaptosomes was suspended in 0.32 M sucrose. Active uptake of H^3-GABA by synaptosomes was studied by the method described previously [7]. The composition of the incubation medium was as follows (in mM): NaCl 100, KCl 6, sucrose 100, glucose 10, tris-phosphate buffer (pH 7.4) 30, drug 0.5, together with 0.2 μM GABA (a mixture of H^3-GABA, New England Nuclear, USA, with specific radioactivity 10 mCi/mmole, and nonradioactive GABA in a molar ratio of 1:100). The particular sample, without protein, had an emission rate of 10^4 counts/min. The counting efficiency was verified by the external standard method. The reaction was stated by the addition of 50 μl of synaptosomal suspension (200-250 μg protein) to 1 ml of medium. After incubation (20 min, 37°C) the reaction was stopped by cooling the samples to 0-4°C. After centrifugation (20,000 g, 15 min, 0-4°C) the

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TABLE 1. Effect of Psychotropic Drugs of Different Types on Uptake of H^3-GABA by Synaptsosomes and on Activity of Synaptosomal Na,K-ATPase (M±m)

Substance, 0.5 mM	H^3-GABA uptake (in % of control)	Na,K-ATPase activity (in % of control)	Mg-ATPase activity (in % of control)
Ouabain	11±4	0	100±10
Dimethylsulfoxide (DMSO)	100±8	100±9	100±8
Fluphenazine	3±1	67±7	65±8
Nonachlazine	10±2	16±4	14±4
Tetrabenazine	17±3	25±5	38±6
Azabuperon	15±3	72±11	73±7
Carbidine	102±14	41±6	46±6
Tetrabenazine	98±15	106±10	107±11
Diazepam*	89±10	52±8	95±9
Imipramine	60±10	75±14	88±4
Fluacizine	15±5	82±8	81±8
Amphetamine	30±3	41±7	73±7
Apomorphine	98±12	73±7	106±9
Diphenyl	39±8	17±4	87±6
Diphenylhydantoin*	72±10	88±7	84±7
Carbamazepine*	63±8	96±8	108±7
Morphine	102±10	97±9	96±9
Fentanyl	40±8	45±6	70±6

Legend. Drugs dissolved in DMSO, the final concentration of which in the samples was 0.5%, are marked by an asterisk. Results of 4 to 5 experiments are given. Na,-K- and Mg-ATPase activities were 15 and 18 μmoles P_i/mg protein/h respectively. Values for ATPase activity and degree of H^3-GABA uptake taken as 100% for samples not containing drugs.

residues were washed twice with cold incubation medium (without isotope) and dissolved in 1 ml 10% Triton X-100. Of the resulting solution a sample of 0.2 ml was taken and treated with 10 ml scintillation fluid containing 3 ml ethanol and 7 ml toluene with 0.5% 2,5-diphenyloxazole (PPO) and 0.01% diphenyloxazolylbenzene (POPOP). Radioactivity was measured by the Mark 1 (Nuclear Chicago) scintillation counter. Protein was determined by Lowry's method. Activity of Na,K-ATPase was determined by measuring the degree of accumulation of inorganic phosphate in the course of the reaction (20 min, 37°C). The composition of the incubation medium was as follows (in mM): NaCl 100, sucrose 100, glucose 10, KCl 6, $MgCl_2$ 5, ATP-Na_2 3, tris-HCl (pH 7.4) 30, with or without ouabain 0.5. The reaction was started by the addition of 50 μl of synaptosomal suspension (200-250 μg protein/ml) and stopped with 10% TCA (1:1). Phosphate was determined by the method of Lowry and Lopez [12].

EXPERIMENTAL RESULTS AND DISCUSSION

To obtain a broader view of the action of the drugs of each group, not only neuroleptics (chlorpromazine, fluphenazine, trifluperidol, azabuperon), but also representatives of other classes of compounds were used: antidepressants (imipramine, fluacizine), carbidine* which combine the features of neuroleptics and antidepressants [2], the tranquilizer diazepam, the antiepileptic drugs diphenylhydantoin and carbamazepin, the narcotic analgesics morphine and fentanyl, the reserpine-like preparation tetrabenazine, and stimulants (amphetamine and apomorphine). For comparison, the new antianginal preparation nonachlazine [4], a phenothiazine derivative, was used.

The experimental results are summarized in Table 1, which shows satisfactory correlation for most of the drugs used between their effect on H^3-GABA uptake and the activity of synaptosomal transport Na, K-ATPase of rat brain. For instance, substances blocking GABA uptake by synaptosomes (fluphenazine,

*3,6- dimethyl-1,2,3,4,4a,9a-hexahydro-γ-carboline dihydrochloride – Consultants Bureau.

nonachlazine, fluacizine, fentanyl) inhibited Na,K-ATPase by about the same degree. Ouabain, in these experiments, completely blocked Na,K-ATPase and inhibited the uptake of labeled GABA by 89%. For some substances (chlorpromazine, trifluperidol, diazepam, imipramine, diphenylhydantoin) the block of GABA uptake by synaptosomes was more marked than the degree of inhibition of Na,K-ATPase. Substances not affecting GABA uptake (morphine, carbidine) had no effect on Na,K-ATPase activity. The strongest blocking action on active GABA transport through the presynaptic membranes was shown by chlorpromazine, imipramine, nonachlazine, and trifluperidol; the strongest inhibitory effect on transport ATPase was shown by fluphenazine, nonachlazine, and apomorphine. It is also interesting to note that some drugs (tetrabenazine, apomorphine and, to some extent, amphetamine) acted specifically on Na,K-ATPase. In a concentration of 0.5 mM the blocking action of these drugs was not accompanied by any change in the activity of Mg^{++}-ATPase. No correlation was found for four substances between the degree of inhibition of uptake and the change in Na,K-ATPase activity. For instance, tetrabenazine and azabuperon inhibited Na,K-ATPase activity by 27, 48, and 58% respectively but did not affect H^3-GABA uptake by the synaptosomes. On the other hand, carbamazepine moderately inhibited uptake but had no appreciable effect on Na,K-ATPase activity.

These factors indicate the presence of a complex relationship between the systems for active mediator transport and for the transport of Na^+ and K^+. At least two possible points of application of the drugs affecting active GABA uptake must evidently be distinguished: Na,K-ATPase directly and the hypothetical GABA carrier in the presynaptic membrane, the function of which is coupled with that of the Na, K-pump. An inhibitory effect on Na,K-ATPase has been demonstrated for chlorpromazine [5, 6], imipramine, amphetamine [6], and diphenylhydantoin [5, 10], in agreement with the present results. The similarity between the drugs of the different classes in the general direction of their action (neuroleptics, antidepressants, tranquilizers, anticonvulsants, narcotic analgesics) suggests that the effects observed do not determine the specific character of action of the substances of each group.

LITERATURE CITED

1. V. A. Arefolov, L. V. Panasyuk, K. S. Raevskii, et al., Byull. Éksperim. Biol. Med., No. 3, 76 (1974).
2. N. K. Barkov, Farmakol. Toksikol., No. 2, 154 (1973).
3. Yu. I. Vikhlyaev, G. Ya. Avrutskii, and S. V. Zhuravlev, in: Current Problems in Psychopharmacology [in Russian], Kemerovo (1970), pp. 271-274.
4. N. V. Kaverina and G. A. Markova, Farmakol. Toksikol., No. 2, 173 (1975).
5. É. F. Lavretskaya, Yu. Sh. Moshkovskii, and L. M. Raikhman, in: The Action of Physiologically Active Compounds on Biological Membranes [in Russian], Moscow (1974), pp. 288-292.
6. É. M. Paésalu, U. S. Tarve, É. K. Taigimyaé, et al., in: Advances in Neurochemistry [in Russian], Leningrad (1974), pp. 107-118.
7. K. S. Raevskii and N. I. Maisov, Byull. Éksperim. Biol. Med., No. 6, 63 (1975).
8. V. V. Shevtsov, O. M. Pozdnyakov, I. I. Musin, et al., Byull. Éksperim. Biol. Med., No. 1, 94 (1972).
9. J. Davies and G. Johnston, J. Neurochem., 22, 931 (1974).
10. B. Formby, J. Pharm. Pharmacol., 22, 81 (1970).
11. L. L. Iversen and G. Johnston, J. Neurochem., 18, 1939 (1971).
12. O. Lowry and S. Lopez, J. Biol. Chem., 162, 421 (1946).
13. J. Simon and D. Martin, Arch. Biochem., 147, 348 (1973).