EFFECT OF NEUROLEPTICS AND ANTIDEPRESSANTS ON UPTAKE OF γ-AMINOBUTYRIC ACID-H^3 BY ISOLATED RAT BRAIN NERVE ENDINGS

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The effect of phenothiazine neuroleptics (chlorpromazine, trifluoperazine, perphenazine, fluophenazine and antidepressants (imipramine and fluacizine) on the uptake of GABA-H^3 by synaptosomes of the rat cerebral cortex was studied. All the neuroleptics proved to be active inhibitors of the accumulation of neuromediator by the synaptosomes. The antidepressants were less active. The most active inhibitor of GABA uptake was chlorpromazine and the least active was the new Soviet phenothiazine antidepressant fluacizine. It is suggested that their effect on GABA uptake may play a role in the mechanism of the synaptic action of neuroleptics.

KEY WORDS: GABA-uptake; synaptosomes; psychotropic drugs.

The use of synaptosomes (the fraction of isolated nerve endings [14]) makes it possible to study the effect of neurotropic drugs on synaptic processes. Synaptosomes are known to be able to accumulate γ-aminobutyric acid (GABA) with the aid of a specific process of active transport [7, 10] which, it is assumed, participates in the regulation of the inhibitory mediator function of GABA [6, 13].

Since GABA uptake by presynaptic endings may be one point of application of depriming agents [8] it was decided to study the effect of several neuroleptics and antidepressants on this process.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-250 g. Synaptosomes were obtained from the cerebral cortex by centrifugation of the unpurified mitochondrial fraction in a stepwise sucrose density gradient (0.8-1.2 M) at 132,000 g for 40 min on the MSE-65 ultracentrifuge [2, 14]. The fraction of synaptosomes was collected at the boundary between the 1.0-1.1-1.2 M sucrose levels, diluted with 0.32 M sucrose at 0-2°C, and centrifuged at 20,000 g for 20 min. The resulting residue was suspended in 1.5 ml 0.32 M sucrose per gram of the original weight of brain.

An incubation medium [11] containing 100 mM NaCl, 6 mM KCl, 10 mM glucose, 100 mM sucrose, and 30 mM Tris-phosphate buffer, pH 7.4, was used. Incubation (20 min, 37°C) was carried out with continuous shaking by the following scheme: simultaneous addition of 10 μM GABA-H^3 and the pharmacological agent in the test concentration, followed by 50 μl of suspension of synaptosomes, equivalent on the average to 0.25 mg protein/ml. Protein was determined by Lowry's method [9]. Binding of GABA by the synaptosomes was stopped by cooling to 0-4°C. Isolation of the synaptosomes from the incubation medium and recording of the activity of bound GABA-H^3 were carried out by a modified method of Synder and Coyle [12]. For this purpose the incubation medium containing synaptosomes was centrifuged at 20,000 g for 15 min at 0-4°C. The resulting residues were twice washed with ice-cold incubation medium and dissolved in 1 ml 5% Triton X-100. Samples of 0.2 ml were taken from the resulting solution and added to 10 ml of scintillation fluid containing 3 ml ethanol, 7 ml toluene, 0.4% 2,5-diphenyloxazole (PPO), and 0.01% 1,4-di[2-(5-phenyl)-oxazolyl] benzene (POPOP). Activity of the GABA-H^3 was measured with the Mark I Nuclear Chicago (USA) scintillation counter. GABA-H^3 (New England Nuclear) with a specific activity of 10 mCi/mmole was used and was

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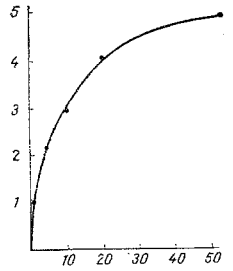

Fig. 1. Binding of GABA-H^3 by synaptosomes as a function of GABA concentration (results of 3-6 experiments). Abscissa, GABA concentration (in μM); ordinate, uptake of GABA (in μmoles/mg protein during incubation for 20 min at 37°C).

TABLE 1. Effect of Neuroleptics and Antidepressants on Uptake of GABA-H^3 by Isolated Synaptosomes of Rat Cerebral Cortex (M ± m)

Substance	Concentration* (in μM)	Uptake of GABA-H^3 (in % of control)
GABA-H^3	10	100±9
Chlorpromazine + GABA-H^3	50	29±3
	100	9±2
	500	3±1
Trifluoperazine + GABA-H^3	50	27±3
	100	17±2
	500	10±1
Perphenazine + GABA-H^3	50	39±4
	100	27±3
	500	16±2
Fluophenazine + GABA-H^3	50	30±5
	100	23±3
	500	13±2
Imipramine + GABA-H^3	50	88±10
	100	47±5
	500	9±2
Fluacizine + GABA-H^3	50	90±10
	100	65±7
	500	30±3

*In all experiments GABA-H^3 was added in concentration of 10 μM.

added to nonradioactive GABA in the molar ratio of 1:100. A sample containing 0.2 ml of a 1 μM solution of this GABA mixture gave on the average 10,000 counts/min. The counting efficiency was checked by the external standard method.

EXPERIMENTAL RESULTS

Binding of GABA by synaptosomes is an enzymic process of active transport that obeys the Michaelis–Menten kinetic equation (Fig. 1), in agreement with other observations [6, 10, 11]. The Michaelis constant (K_m) i.e., the GABA concentration at which the rate of its uptake by synaptosomes is half of the maximal value (V_{max}), was 7.0 ± 1.2 μM in the present experiments. According to Martin [10], K_m for GABA uptake by synptosomes is 4.0 ± 1.0 μM, in satisfactory agreement with the results now obtained by the use of brain slices or homogenates instead of synaptosomes [8]. On the basis of these data the effect of the neurotropic drugs on GABA uptake by synaptosomes was studied in the region close to the K_m value with a standard GABA concentration of 10 μM.

The neuroleptics (chlorpromazine, trifluoperazine, perphenazine, and fluophenazine inhibited the GABA uptake by synaptosomes in concentrations of 50-500 μM (Table 1). The most active inhibitor of GABA uptake was chlorpromazine, followed shortly in a concentration of 100 μM by trifluoperazine, fluophenazine, and perphenazine, the latter being less active in all concentration tested.

The antidepressants (imipramine and fluacizine) had a weaker inhibitory effect on GABA uptake than the neuroleptics, and this was particularly marked in low concentrations. The results obtained for chloropromazine and imipramine are in agreement with those obtained by other workers [8] who, unlike the present writers, used brain slices.

It is interesting to compare the results of the present investigation with those showing that antidepressants selectively inhibit the uptake of noradrenalin (NA) by brain slices, but without any effect on dopamine uptake [4]. Unlike the antidepressants, chlorpromazine in experiments in vivo does not inhibit the reassimilation of NA [5], but exhibits this action only in experiments in vitro [3].

These observations are in agreement with the fact, observed in the present experiments, that the new antidepressant with phenothiazine structure, fluacizine, which, as was shown previously, can inhibit the uptake of exogenous NA by peripheral adrenergic neurons [1], is weaker than that of the neuroleptics. It can accordingly be suggested that ability to inhibit GABA uptake by the presynaptic nerve endings of the brain is a characteristic property of neuroleptics that may play an important role in the realization of the synaptic effect of these substances.

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