UPTAKE OF GABA-^{3}H BY CORTICAL GLIAL CELLS AND SYNAPTOSOMES OF RATS RECEIVING PSYCHOTROPIC DRUGS

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UDC 615.214.2.015.4:612.8.014.46

Chlorpromazine noncompetitively inhibits the uptake of GABA-^{3}H by both glial cells and synaptosomes, synaptosomal uptake being more sensitive to certain inhibitors. β-Alanine competitively inhibits only the low-affinity GABA uptake system in the glial cells. Correlation is observed between inhibition of GABA uptake by psychotropic drugs in glial cells and synaptosomes. It is postulated that two different systems of GABA uptake with high and low affinity function in nerve endings and glial cells.

KEY WORDS: GABA uptake; psychotropic drugs; glial cells and synaptosomes.

γ-Aminobutyric acid (GABA) is actively taken up by neurons and by glial cells, the ratio between the neuronal and glial uptakes being 30:1 [10]. It is suggested that inactivation of GABA, as the mediator of inhibition, takes place mainly by uptake of the amino acid by nerve endings [4]. The neuronal uptake of GABA takes place on account of a high-affinity enzyme system, besides which a system of low-affinity GABA uptake also functions in brain tissue [5, 8].

It was decided to study the kinetics of the two systems of GABA uptake and also to compare their sensitivity to psychotropic drugs with an inhibitory action on synaptosomal uptake of GABA [1, 2, 6].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-250 g. The fraction of glial cells was isolated from the rat cerebral cortex by Rose's method [9] with certain modifications. After removal of the blood vessels the brain tissue was washed two or three times with physiological saline and was cut into small pieces. The minced tissue was suspended in 10% Ficoll containing 100 mM NaCl and 0.1 M sodium phosphate buffer, pH 7.4. The suspension was rubbed successively through nylon sieves with a mesh size of 1000, 500, 100, 75, and 50 μ at 0-4°C. The resulting fine suspension was centrifuged in a density gradient (40% sucrose–30% Ficoll suspension) for 90 min at 53,000g. The solutions of sucrose and Ficoll contained 100 mM NaCl and 0.1 M sodium phosphate buffer. After centrifugation samples of the glial cell fraction were taken from the upper boundary of the 30% Ficoll layer, diluted in 0.32 M sucrose, and sedimented at 1500g (30 min). The yield of glia as protein was 10% and the purity of the fraction as shown by examination under the phase-contrast microscope was 85%. The glial cells were astrocytes and oligodendrocytes. The combined fraction of synaptosomes was obtained by Whittaker's method in the modification of Shevtsov et al. [3]. The uptake of GABA-^{3}H was determined by incubating the synaptosomes or glial cells (0.25 mg protein in 1 ml) in medium containing 100 mM NaCl, 6 mM KCl, 10 mM glucose, 100 mM sucrose, and 30 mM Tris–phosphate buffer, pH 7.4, with continuous agitation (20 min, 37°C). A mixture of GABA-^{3}H from New England Nuclear (USA) with specific radioactivity of 10 Ci/mmole with nonradioactive GABA in the molar ratio of 1:1000 was used. The reaction was stopped by cooling the sample to 0-4°C. After centrifugation (20,000g, 15 min, 0-4°C) the residues were washed twice with cold incubation medium (without the isotope) and dissolved in 1 ml Triton X-100. A

Laboratory of Neurochemical Pharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Department of Biochemistry, Tbilisi University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 561-563, May, 1977. Original article submitted November 17, 1976.

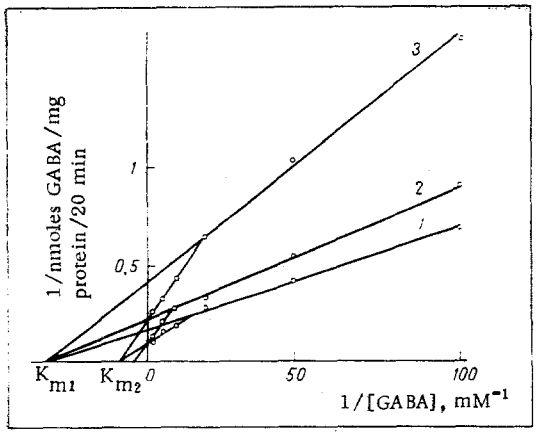

Fig. 1

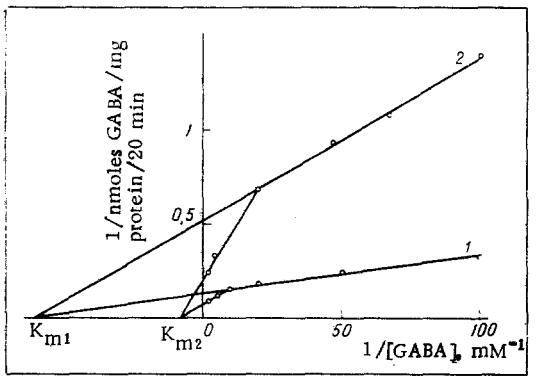

Fig. 2

Fig. 1. Effect of chlorpromazine and β-alanine on uptake of GABA by glial cells (Lineweaver–Burke plot, results of 5-6 experiments): 1) control; 2) 500 μM β-alanine; 3) 100 μM chlorpromazine.

Fig. 2. Effect of chlorpromazine on GABA uptake by synaptosomes (Lineweaver–Burke plot, results of 5-6 experiments): 1) control; 2) 100 μM chlorpromazine.

0.2-ml sample was taken from the resulting solution and added to 10 ml of scintillation fluid containing 3 ml ethanol and 7 ml toluene with 0.5% 2,5-diphenyloxazole and 0.01% 1,4-bis-[2-(5-phenyl)oxazole]-benzene. The radioactivity was measured with a Mark I Nuclear Chicago (USA) scintillation counter. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Kinetic analysis of the GABA uptake by the glial cells, the results of which are shown in Fig. 1, revealed that there are two systems of uptake: one with high (K_{m1} = 31 ± 7 μM) and one with low (K_{m1}* = 123 ± 10 μM) affinity. As Fig. 1 shows, β-alanine did not change the maximal rate of GABA uptake with low affinity, i.e., it behaved as a competitive inhibitor of that system. The apparent value of the Michaelis constant (K_m) in the presence of β-alanine was 210 μM, i.e., it differed appreciably from the value of K_{m2}. The constant of inhibition (K_i) calculated on the basis of these data for β-alanine was 441 ± 38 μM. β-Alanine had no significant effect on the GABA uptake system with high affinity. Meanwhile chlorpromazine (Fig. 1, curve 3) noncompetitively inhibited both the high-affinity (K_i = 80 ± 10 μM) and the low-affinity (K_i = 81 ± 12 μM) systems. Uptake of β-alanine itself by glial cells is known to be competitively inhibited by GABA [10]. It could be postulated that the low-affinity system of uptake is a feature of glial cells, whereas the high-affinity system is found on account of contamination with synaptosomes. Two systems of GABA uptake with different affinities are known to exist in the synaptosomes also [8].

It was therefore considered important to compare the kinetic characteristics of GABA uptake by the synaptosomes, on the one hand, and by glial cells on the other. As Fig. 2 shows, the kinetic characteristics of the synaptosomal uptake of GABA point to the existence of two systems with high (K_{m1} = 12 ± 5 μM) and low (K_{m2} = 115 ± 15 μM) affinity, and that both systems are inhibited noncompetitively by chlorpromazine; K_i for the system of synaptosomal uptake with high affinity was lower than for the system with low affinity (Table 1).

As Table 1 shows, K_i of chlorpromazine for both uptake systems in the glial cells also had a higher value. Chlorpromazine, according to these observations, thus behaved as a noncompetitive inhibitor of both uptake systems both in glial cells and in synaptosomes. The synaptosomal uptake, moreover, was more sensitive to the inhibitory influence of chlorpromazine than the glial, and the high-affinity uptake system was more sensitive than the low-affinity system. This is in agreement with the writers' earlier findings of the high sensitivity of synaptosomal uptake to phenothiazine neuroleptics [1, 2]. Unlike chlorpromazine, β-alanine, a competitive inhibitor of the low-affinity GABA uptake system in glial cells, in a concentration of 500 μM, had no appreciable effect on either GABA uptake system in the synaptosomes.

For the purpose of comparison, other psychotropic drugs belonging to the groups of neuroleptics, antidepressants, tranquilizers, and stimulants were studied. Data on the effect of these drugs on the uptake of

*As in Russian original; probably K_{m2} – Consultants Bureau.

TABLE 1. Some Kinetic Characteristics of GABA Uptake Systems in Glial Cells and Synaptosomes of Rat Cerebral Cortex (M ± m)

Kinetic constants	Glial cells	Synaptosomes
K_m of high affinity	31±7 μM	12±5 μM
K_m of low affinity	123±10 μM	115±5 μM
K_i of chlorpromazine for high-affinity system	80±10 μM	28±4 μM
K_i of chlorpromazine for low-affinity system	81±12 μM	49±7 μM
K_i of β-alanine for high-affinity system	No inhibition	
K_i of β-alanine for low-affinity system	441±38 μM	The same

TABLE 2. Effect of Psychotropic Drugs on Uptake of GABA-^{3}H by Glial Cells and Synaptosomes of Rat Cerebral Cortex in Experiments in vitro (M ± m)

Drug	Concentration, μM	GABA unptake, % of control	
		glial cells	synaptosomes†
Control*	—	100	100
Fluphenazine	50	52±6	30±5
	500	24±4	13±2
Trifluperidol	50	92±10	72±9
	500	32±4	15±3
Azabuperon	50	98±10	94±12
	500	92±11	102±14
Carbidine	50	104±12	104±13
	500	94±11	98±15
Imipramine	50	84±12	88±10
	500	31±5	15±5
Fluacizine	50	89±11	90±10
	500	29±4	30±3
Diazepam	50	95±12	93±12
	500	57±6	68±12
Cocaine	50	104±11	—
	500	106±11	99±11
Amphetamine	50	103±10	101±10
	500	98±11	98±12

*GABA in experiments with synaptosomes was added in concentration of 10 μM, in experiments with glial cells 200 μM.
†Data taken from Maisov et al. [1].

labeled GABA by glial cells are given in Table 2 where, for comparison, results obtained previously in experiments on synaptosomes are also given [1]. As Table 2 shows, on the whole there was definite correlation between the inhibition of uptake by glial cells and synaptosomes, although the system of neuronal uptake was more sensitive to the action of neuroleptics. Drugs not inhibiting synaptosomal uptake of GABA were also ineffective against glial uptake (azabuperon, carbidine, cocaine, amphetamine).

These results are in agreement with the view that two different systems of GABA uptake function in nerve endings and glial cells [7].

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