INHIBITION OF SEROTONIN UPTAKE BY SYNAPTOSOMES AND GLIAL CELLS INDUCED BY CERTAIN DRUGS

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Uptake of [^{14}C]serotonin by glial cells and synaptosomes in the rabbit cerebral cortex was studied. The value of K_m for serotonin uptake was the same (0.083 ± 0.02 μM) for both synaptosomes and glial cells. Cortical synaptosomes took up serotonin twice as fast as glial cells (the rates of uptake were compared as protein). Of the psychotropic drugs tested, the most active inhibitors of both synaptosomal and glial serotonin uptake were the tricyclic antidepressant imipramine and the psychostimulant cocaine which, in concentrations of 50 μM, inhibited uptake of [^{14}C]serotonin in synaptosomes by 90% and in glial cells by 75-80%.

KEY WORDS: synaptosomes; glia; serotonin uptake; imipramine; cocaine.

Many investigations of serotonin uptake by various structures and tissues have recently been published. The uptake of serotonin by nerve endings [10, 12, 13, 15], platelets of man and animals [7, 14], and slices from different parts of the brain [12] has been studied in fair detail. Yet there is an almost complete absence of information on serotonin uptake by glial cells.

Considering the possible role of the neuroglia in specific nervous processes, it is interesting to compare serotonin uptake by isolated nerve endings and glial cells and also to investigate the effect of some psychotropic drugs on these processes.

METHODS

Fractions enriched with glial cells were obtained from the rabbit cerebral cortex by Rose's method [11] in our modification described in [1]. The total synaptosomal fraction was obtained by the method of Gray and Whittaker [8] in the modification of Shevtsov et al. [6]. The uptake of [^{14}C]serotonin by glial cells or synaptosomes (0.25 mg protein/ml) was determined in incubation medium containing 100 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 3 mM $MgCl_2$, 10 mM glucose, 10 mM sucrose, and 30 mM Tris-phosphate buffer, pH 7.4, with continuous shaking for 20 min at 37°C. [^{14}C]serotonin (Amersham) with specific radioactivity of 58 Ci/mole was used. The reaction was stopped by cooling the samples to 0-4°C. After centrifugation (20,000g, 15 min, 0-4°C) the residues were washed twice with cold incubation medium and dissolved in 0.3 ml of 10% Triton X-100. A sample of 0.2 ml was taken from the resulting solution and added to 10 ml scintillation fluid containing 7 ml toluene, 3 ml ethanol, 0.5% PPO and 0.01% POPOP. Radioactivity was measured by the Nuclear Chicago Mark-I scintillation counter. Protein was determined by Lowry's method [9].

In a parallel series of control experiments with both synaptosomes and glial cells absorption of serotonin was studied in 0.32 M sucrose at 0°C without incubation.

RESULTS

At 0°C absorption of serotonin was found to be a linear function of its concentration. By subtracting the quantity of serotonin absorbed as a result of diffusion (absorption at 0°C) from the total quantity of serotonin accumulated at 37°C the true curve of serotonin uptake at 37°C over a period of 20 min was obtained.

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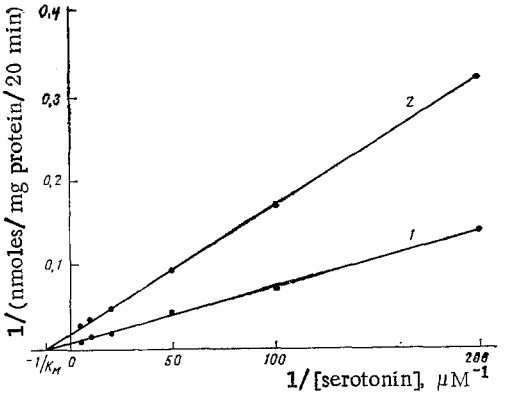

Fig. 1. Kinetics of serotonin uptake by synaptosomes and glial cells in rabbit cerebral cortex (Lineweaver—Burk plot). Curves: 1) synaptosomes, 2) glial cells.

TABLE 1. Effect of Drugs on [^{14}C]Serotonin Uptake by Rabbit Cortical Synaptosomes and Glial Cells (M ± m)

Substance	Concentration, μM	Uptake of [^{14}C]serotonin (in % of control)	
		synaptosomes	glial cells
Control	—	100±10	100±10
Chlorpromazine	50	24±3	27±2
	500	4±1	11±1
Trifluperidol	50	36±8	59±5
	500	8±4	13±4
Imipramine	50	9±2	20±3
	500	4±1	12±4
Cocaine	50	12±2	26±1
	500	8±1	14±3

Legend. Mean values of uptake and confidence intervals (P = 0.05) relative to control (results of five to seven measurements). 100% control: 50 nM serotonin/mg protein bound in 20 min in synaptosomes, 25 nM in glia respectively.

Analysis of these data by the "double reciprocals" method showed that serotonin uptake by the synaptosomes and glial cells obeys the Michaelis—Menten kinetics, i.e., that is an enzymic process of active transport. The Michaelis constant (K_m), found by constructing Lineweaver—Burk plots (Fig. 1), was 8.3×10^{-6} M, for both synaptosomal and glial uptake. This value agrees well with those obtained by Tuomisto and Tuomisto [15], Ross and Renyi [12], and Pugsley and Lippmann [10], for synaptosomes of the brain stem, midbrain, and cerebral cortex of rats ($7.6 \cdot 10^{-6}$, $8.0 \cdot 10^{-6}$, and $8.9 \cdot 10^{-6}$ M respectively).

It will be clear from Fig. 1 that rabbit cortical synaptosomes took up serotonin twice as fast as the glial cells, if the rate of uptake was compared as protein. No significant differences were found in glial and synaptosomal uptake of serotonin, as is confirmed by data in Table 1 showing the effect of psychotropic drugs on serotonin uptake by glial cells and synaptosomes when a standard concentration (100 nM) of serotonin was used (the approximate value of K_m).

Table 1 shows that the strongest inhibiting effect on both synaptosomal and glial serotonin uptake was given by the tricyclic antidepressant imipramine and the psychostimulant cocaine, which in a concentration of 50 μM inhibited serotonin uptake by synaptosomes by 90% and by glial cells by 75-80%. These results agree with those of investigations which showed that imipramine is a selective inhibitor of serotonin uptake by synaptosomes [10, 12], rat brain slices [7, 12], and human platelets [2].

On the whole the synaptosomal system of serotonin uptake proved to be more sensitive to the action of psychotropic drugs than the glial system. The exception was the experiments with chlorpromazine, which inhibited both synaptosomal and glial uptake equally (by 75%) in a concentration of 50 μM. This is in agreement with data in the literature on the membranotropic effect of chlorpromazine [3], which probably lies at the basis of the inhibition of uptake of mediators of different chemical nature by this drug [4, 5].

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