# UPTAKE OF TRYPTOPHAN BY GLIAL CELLS AND SYNAPTOSOMES OF THE RABBIT CEREBRAL CORTEX

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The uptake of L-[$^{14}$C]tryptophan by glial cells and synaptosomes of the rabbit cerebral cortex was investigated. The assimilation system of the glial cells exhibits high affinity for tryptophan ($K_m$ = 0.8 $\mu$M). Tryptophan uptake by the synaptosomes has lower affinity ($K_m$ = 50 $\mu$M). The psychotropic drugs chlorpromazine and imipramine inhibit glial uptake. The leading role of the glial cells in the nutrition of the neurons and the normal course of neurodynamic processes is confirmed.

KEY WORDS: glia; synaptosomes; tryptophan uptake.

In recent years many facts indicating that glial cells, through the uptake and inactivation of neurotransmitters [8, 13] and amino acids [9, 11], play a direct part in the mechanisms of metabolic feedback in the neuron–neuroglia system, have accumulated in the literature [1].

It was accordingly decided to study the uptake of tryptophan, a metabolic precursor of serotonin, by glial cells and synaptosomes of the rabbit cerebral cortex.

## EXPERIMENTAL METHOD

Fractions rich in glial cells were obtained from the rabbit cerebral cortex by Rose's method [12] in the modification of Aleksidze et al. [2].

The cerebral hemispheres were minced and suspended in 10% Ficoll, containing 100 mM NaCl and 0.1 M Na-phosphate buffer, pH 7.4. The suspension was filtered with gentle suction through a plastic syringe with pores 1500 $\mu$ in diameter, and then passed successively through nylon gauze with a mesh of 1000, 500, 100, 75, and 50 $\mu$. The resulting suspensions were centrifuged in a density gradient (40% sucrose–30% Ficoll–suspension) for 90 min at 53,000g on the MSE-65 centrifuge (England). After centrifugation the fraction of glial cells was collected from the upper boundary of the 30% Ficoll layer, diluted with 0.32 M sucrose, and sedimented at 1500g (30 min). The yield of glia, calculated as protein, was 10% and the purity of the fraction, according to phase-contrast microscopy, was 85%.

The uptake of L-[$^{14}$C]tryptophan by the glial cells or synaptosomes (0.25 mg protein/ml) was determined in an incubation medium containing (in mM): NaCl 100, KCl 6, $CaCl_2$ 2, $MgCl_2$ 3, glucose 10, sucrose 100, Tris-phosphate buffer 30, pH 7.4, continuous shaking for 20 min at 37°C. A mixture of L-[$^{14}$C]tryptophan (Amersham, USA) with a specific radioactivity of 52 mCi/mmole and nonradioactive L-tryptophan (Sigma) in the molar ratio of 1:1000. The reaction was stopped by cooling the samples at 0-4°C. After centrifugation (20,000g, 15 min, 0-4°C) the residues were twice washed with cold incubation medium (without the isotope) and dissolved in 1 ml of 10% Triton X-100. A 0.2-ml sample was taken from the resulting solutions and added to 10 ml of scintillation fluid, containing 3 ml ethanol and 7 ml toluene, 0.5% 2,5-diphenyloxazole (PPO), and 0.01% 1,4-bis[2-(5-phenyl)oxazolyl]benzene (POPOP). Radioactivity was measured by the Mark-1 Nuclear Chicago (USA) scintillation counter. Protein was determined by Lowry's method [10].

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

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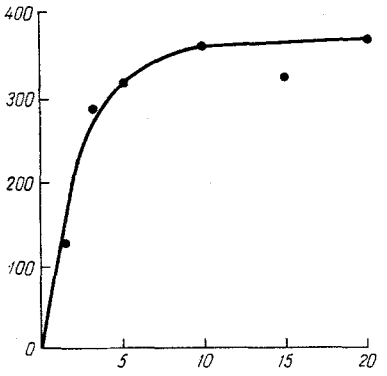


Fig. 1. Uptake of L-tryptophan by glial cells as a function of incubation time. Abscissa, incubation time (in min); ordinate, quantity of tryptophan bound (in cpm/mg protein; 350 cpm is equivalent to 0.25 nmole tryptophan bound with 1 mg protein).

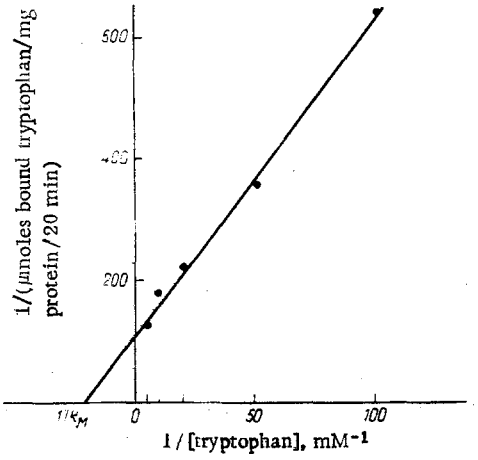


Fig. 2. Kinetic analysis of uptake of L-tryptophan by synaptosomes of rabbit cerebral cortex (Lineweaver–Burk plot).

## EXPERIMENTAL RESULTS

As Fig. 1 shows, on addition of 2 $\mu$M L-[$^{14}$C]tryptophan its uptake by the glial cells rises rapidly to reach a maximum of 0.25 nmole/mg protein after 10-15 min. The glial and synaptosomal uptake of tryptophan was studied in a concentration of added tryptophan of between 0.2 and 500 $\mu$M.

By means of the double reciprocal coordinates method and the construction of Lineweaver–Burk plots it was found that a system of tryptophan uptake with a value of $K_m = 50$ $\mu$M and a maximal velocity ($V_{max}$) of 10 nmoles/mg protein/20 min functions in the synaptosomes ( Fig. 2). A tryptophan uptake system with higher affinity, with a value of $K_m = 0.8$ $\mu$M and $V_{max} = 0.42$ nmole/mg protein/20 min was found in the glial cells (Fig. 3).

Tryptophan uptake by the glial cells took place between concentrations of 0.4 and 5 $\mu$M; a further increase in substrate concentration to 10 $\mu$M was unaccompanied by any increase in the tryptophan uptake. The increase in radioactivity in samples containing 10 $\mu$M tryptophan was due to an increase of absorption.

The tryptophan uptake system found in the synaptosomes of the rabbit cerebral cortex exhibits higher affinity than those described in the literature for synaptosomes [7] and slices [14] of rat cerebral cortex.

Special experiments in vitro were carried out to study the effects of members of different classes of psychotropic drugs (neuroleptics, antidepressants, stimulants) on tryptophan uptake by glial cells and synapto-

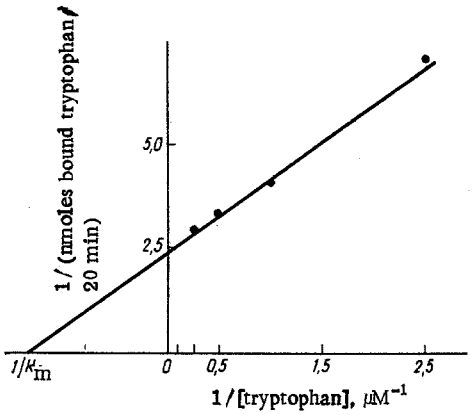


Fig. 3. Kinetics of uptake of L-tryptophan by glial cells of rabbit cerebral cortex (Lineweaver–Burk plot).

TABLE 1. Effect of Psychotropic Drugs on Uptake of L-[$^{14}$C]Tryptophan by Glial Cells and Synaptosomes of Rabbit Cerebral Cortex

| Substance | Concentration, μM | Uptake of L-[$^{14}$C]tryptophan % of control * | |
|---|---|---|---|
| | | glial cells | synaptosomes |
| Control † | — | 100±12 | 100±12 |
| Chlorpromazine | 50 | 59±10 | 83±10 |
| | 500 | 15±5 | 35±6 |
| Trifluperidol | 50 | 72±12 | 115±14 |
| | 500 | 62±8 | 80±9 |
| Imipramine | 50 | 50±7 | 71±8 |
| | 500 | 23±4 | 39±4 |
| Cocaine | 50 | 87±11 | 88±11 |
| | 500 | 114±12 | 77±14 |

* Concentration of added labeled tryptophan was 50 μM in experiments with synaptosomes and 1 μM in experiments with glial cells
† 100% is equivalent to 5 nmoles bound tryptophan/mg protein/20 min for synaptosomes and 0.25 nmole for glial cells.

somes of the rabbit cerebral cortex (Table 1). As this table shows, on the whole there was definite correlation between inhibition of uptake by the glial cells and synaptosomes, although the system of glial uptake was more sensitive to the action of neuroleptics and of imipramine. Cocaine had no effect on either neuronal or glial uptake, whereas trifluperidol was ineffective against synaptosomal uptake.

The experiments thus showed that the system responsible for tryptophan uptake by the glial cells has higher affinity and greater sensitivity to the inhibitory action of chlorpromazine and imipramine than the corresponding system of the synaptosomes of the rabbit cerebral cortex. This conclusion agrees with previous observations showing the high sensitivity of synaptosomal uptake of GABA to the phenothiazine neuroleptics and antidepressants [4, 5]. The mechanism of the inhibitory effect of the psychotropic drugs on tryptophan uptake by synaptosomes and glial cells is not yet clear. Processes of active transport, which include tryptophan uptake are known to be closely linked with the functioning of transport Na,K-ATPase [6]. This suggests that inhibition by psychotropic drugs of the synaptosomal and glial uptake of tryptophan may be based on a decrease in the activity of the transport ATPase, which is known to be sensitive to the action of neuroleptics and antidepressants [3].

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# EFFECT OF DERIVATIVES OF GAMMA-AMINOBUTYRIC ACID ON SLEEP DISTURBANCES IN NEUROSES


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Electropolygraphic investigations revealed a tendency in patients with sleep disturbances associated with various forms of neuroses for the total duration of sleep to increase under the influence of derivatives of γ-aminobutyric acid (GABA), on account of an increase in the principal stages of sleep (second stage, Δ sleep, and fast sleep) and a statistically significant decrease in the number of spontaneous awakenings, in the total duration of wakefulness at night, and in the activation index of movements. Analysis of some of the electrographic indices within the stages of sleep revealed a tendency for the number of sleep spindles to increase in the second stage, an increase in the Δ index in the third and fourth stages of sleep, and an increase in the mean numerical indices of rapid eye movements in the absence of significant changes in their specific occurrence per unit time. GABA derivatives in the doses used cause on the whole similar changes in the structure of sleep in its various disturbances, with sodium hydroxybutyrate having a relatively stronger action.



KEY WORDS: sleep disturbance; stages of sleep; sodium hydroxybutyrate; fenibut.


The wide-spread occurrence of sleep disorders in neuroses and the lack of any sufficiently effective drugs with a soporific action necessitate the search for new ways and means of correcting such disturbances. The attention of research workers has been drawn to biologically active substances and, in particular, to γ-aminobutyric acid (GABA) which, according to experimental data, plays an active role in the regulation of sleep.

Because of the difficulty with which GABA passes through the blood–brain barrier, its analogs sodium hydroxybutyrate and fenibut (beta-phenyl-gamma-aminobutyric acid) are used. According to the information published, in experiments on animals sodium hydroxybutyrate promotes the appearance of phases of slow [5-7] and rapid sleep – RS [1, 3]. The phase of sleep which develops has been shown to depend on the dose of the drug [2].

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