EFFECT OF TRIFLUOPERAZINE ON ENZYME ACTIVITY IN THE TISSUES OF THE HIPPOCAMPUS

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The effect of trifluoperazine on the activity of glutamate-aspartate aminotransferase and of pyruvate and α-ketoglutarate dehydrogenases in subcellular fractions of the rabbit hippocampus was studied. Whereas more than half of the aminotransferase activity was concentrated in fractions of the synaptic complex, the maximal dehydrogenase activity was found in the mitochondria. Trifluoperazine lowered the activity of all the enzymes studied. The changes were most marked in glutamate-aspartate aminotransferase activity localized in the synaptosomes.

It has been concluded from the results of electrophysiological investigations that the hippocampus has an important role in the manifestation of the pharmacological role of neuroleptics. It has been shown, in particular, that trifluoperazine affects synaptic transmission in the hippocampus [3].

It was accordingly decided to investigate the effect of trifluoperazine on metabolism in the hippocampus. Considering the manner in which neurotropic drugs act on synapses [1], metabolic processes were investigated in isolated synaptic structures. Activity of glutamate-aspartate aminotransferase (GAA) and of pyruvate and α-ketoglutarate dehydrogenases, enzymes catalyzing the most important reactions of glutamate metabolism and of the Krebs cycle, was determined in fractions isolated from hippocampal tissue.

EXPERIMENTAL METHOD

Trifluoperazine was injected intravenously into adult rabbits in a dose of 5 mg/kg. The choice of this dose was based on electrophysiological observations. The animals were decapitated 1 h later and all subsequent manipulations of the brain were carried out in a cold room at 2-4°C. The hippocampus was quickly removed, homogenized in 0.25 M sucrose solution with 0.01 M tris-HCl, pH 7.4, and "crude" mitochondria were obtained by centrifugation at 12,000 g. The suspension of mitochondria was layered on a continuous 0.8-1.4 M sucrose gradient and fractionation was then carried out by De Robertis' method [6]. As a result five fractions were obtained from the original fraction ("crude" mitochondria): myelin fragments, light synaptosomes, heavy synaptosomes, and "pure" mitochondria. The synaptosome fractions, which consist mainly of free presynaptic nerve endings, preserving their fine structure under these conditions and containing mediators [9], were especially important for this particular investigation. The purity of the fractions was tested biochemically by determining the activity of succinate dehydrogenase (SDH) [4], an enzyme with a strictly mitochondrial localization. GAA activity was determined by the method of Reitman and Frankel [7], by measuring the increase in the quantity of oxaloacetic acid or, more exactly, of pyruvic acid formed from it during incubation for 1 h. A mixture of α-ketoglutaric and L-aspartic acids was used as the substrate. Activity of pyruvate and α-ketoglutarate dehydrogenases was determined by the method of Severin et al. [5]. The activity of these enzymes was calculated per milligrams protein, the content of which was determined by Lowry's method. The results were subjected to statistical analysis. Mean values (from 7-8 experiments) and standard errors are given in Table 1.

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TABLE 1. Localization of Enzymes in Subcellular Fractions of the Rabbit Hippocampus (M ± m)

Subcellular fractions	Succinate dehydrogenase (in conventional units, ΔE in 1 mg protein/5 min	Pyruvate dehydrogenase	α-ketoglutarate dehydrogenase	GAA (in μg/mg protein/h
"Crude" mitochondria (original fraction	30,3±14,8	8,41±2,64	8,10±1,73	62,0±5,7
Myelin fragments	0,25±0,15	2,21±0,51	1,63±0,63	110,0±2,1
Synaptic membranes	0,15±0,07	1,23±0,36	1,47±0,51	72,8±10,4
Light synaptosomes	0,10±0,03	1,09±0,53	0,97±0,62	101,8±16,1
Heavy synaptosomes	16,0±2,07	0,90±0,53	0,85±0,51	57,9±6,1
Pure mitochondria	64,0±8,14	9,70±2,48	9,91±2,31	86,8±3,0

EXPERIMENTAL RESULTS AND DISCUSSION

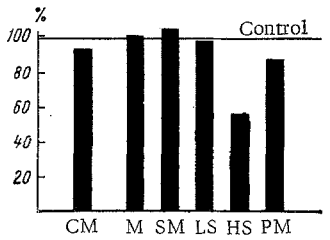

Fig. 1. Effect of trifluoperazine on GAA activity in subcellular fractions of the hippocampus (in % of the control): CM) crude mitochondria; M) myelin fragments; SM) synaptic membranes; LS) light synaptosomes; HS) heavy synaptosomes; PM) pure mitochondria.

The distribution of the enzymes investigated in the subcellular fractions of the hippocampus is given in Table 1. The character of the distribution of SDH shows that the fractions isolated were sufficiently pure. Highest activity was found (79.5%) in the pure mitochondria, while activity was relatively low (18.8%) in the heavy synaptosomes, and the remaining fractions contained no SDH activity. Other workers [8] have obtained similar results for the content of the marker enzyme.

The yield of protein after fractionation was about the same in the control and experimental samples (73.3 and 72.4%, respectively, of its content in the original mitochondrial fraction). About 50% of the protein was accounted for by pure mitochondria, 15% by the fraction of myelin fragments, and the rest was uniformly divided among the fractions of the synaptic structures (synaptic membranes, light and heavy synaptosomes).

The GAA activity was high in all fractions: more than half (54.2%) of it was concentrated in the synaptic fractions, 20.2% in the pure mitochondria, and 25.5% in the myelin fraction. The total enzyme activity after fractionation was higher than its activity in the original fraction, as other investigators also have observed, evidently as a result of liberation of a latent form of the enzyme [8].

The distribution of the dehydrogenases was different in principle. The pure mitochondria possessed the highest dehydrogenase activity (64%). The myelin fraction and the fractions of the synaptic complex were much less able to oxidize pyruvic and α-ketoglutaric acids. Their activity was 14.5 and 21.5%, respectively, of the total enzyme activity. By contrast with GAA, the dehydrogenases tested were thus mainly mitochondrial in their localization.

The results of these experiments showed that trifluoperazine decreases the activity of the enzymes investigated in the hippocampal tissues. The localization of its action differs, however, for GAA and for the dehydrogenases.

It will be clear from Fig. 1 that GAA activity was reduced by 45.5% in the synaptosomal fraction (heavy synaptosomes). In the other fractions, including the mitochondria, this enzyme was resistant to the action of the drug. This effect of trifluoperazine is evidence of marked inhibition of the transamination reaction, in which glutamic and aspartic acids, which play an important role in the metabolism of the CNS and, in particular, in its mediator function, are participants [9]. The precise localization of this change in the synaptosomes indicates that trifluoperazine may act on the biochemical components of synaptic transmission.

As regards the dehydrogenases, the experiments showed that the action of trifluoperazine is aimed mainly at the mitochondrial enzymes. The decrease in the activity of pyruvate and α-ketoglutarate dehydrogenases in the fractions of crude and pure mitochondria was about the same (30-35%). This result is in a measure of agreement with the moderate inhibitory action of trifluoperazine on other NAD-dependent

dehydrogenases in the rat hippocampus revealed by histochemical methods [2]. In the present investigation a very low content of dehydrogenases was found in the synaptosomes, so that it is impossible to draw any reliable conclusions regarding the action of trifluoperazine on them in these structures. Pyruvate and α-ketoglutarate dehydrogenases play an important role in energy formation, and for that reason the decrease in their activity in the mitochondria under the influence of trifluoperazine may be the cause of certain disturbances in the energy metabolism of the brain tissue and, in particular, in ATP formation.

From the analysis of these results and their comparison with the results of electrophysiological investigations it can be postulated that the observed effects of trifluoperazine in the region of the hippocampus and, in particular, its effect on synaptic transmission may be due, to a certain extent, to metabolic changes in this part of the brain.

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