EFFECT OF ELECTRIC SHOCK ON ADENOSINETRIPHOSPHATASE AND MONOAMINE OXIDASE ACTIVITY IN SUBCELLULAR FRACTIONS OF THE RAT BRAIN

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After a single electric shock, the activity of transfer ATPase in the rat brain was increased in the fractions of microsomes and heavy synaptosomes, but reduced in the light synaptosomes and in the synaptic membranes. Monoamine oxidase activity under these conditions was reduced mainly in the mitochondria.

Electrically induced convulsions are a model of hyperexcitation states of the central nervous system. In electric shock the energy metabolism of the brain is severely disturbed and the content of high-energy compounds in the brain is reduced [7]. After epileptogenic injury to the cerebral cortex in rats by freezing with ethyl chloride the transfer ATPase activity of the homogenate is increased [10].

The object of the present investigation was to examine changes taking place at the subcellular level after a single electric shock in the activities of the various ATPases and of monoamine oxidase (MAO), which plays an important role in catecholamine metabolism, for these enzymes are considered to participate in the formation of epileptiform fits [4].

EXPERIMENTAL METHOD

Subcellular fractionation of a homogenate of the sensomotor area of gray matter from the rat (Wistar, weight 120-140 g) brain was carried out by the method of de Robertis [5] modified by the authors. Protein was determined by Lowry's method. Activity of the ATPases was determined from the accumulation of inorganic phosphorus [11], using an incubation (10 min, 37°C) medium of the following composition: 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$ [1]. MAO was determined colorimetrically with p-nitrophenylethylamine as the substrate [2]. The activity of the enzyme was studied after a single freezing and thawing of the fractions, which were kept for 3-5 days at −30°C. Convulsions were induced by applying an alternating current (50 Hz, 90-110 V) to electrodes applied to the animal's head (duration of stimulation 0.5 sec). The rats were decapitated in the clonic phase of electric shock.

EXPERIMENTAL RESULTS

The distribution of the enzymes investigated among the subcellular fractions agreed with that published in the literature [5, 6]. Transfer ATPase (Na, K-ATPase), for instance, was mainly localized in the synaptic membrane fractions, and less was found in the microsomes; Mg-ATPase was mainly detected in the microsomes and mitochondria, while MAO was found mainly in the mitochondria (Table 1).

After a single electric shock the activity of the ATPase systems changed sharply (Table 1). The specific activity of transfer ATPase was increased in the fractions of microsomes and heavy synaptosomes

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TABLE 1. Activity of ATPases and MAO in Subcellular Fractions of Gray Matter of Rat Brain under Normal Conditions and after Single Electric Shock (M ± m)

Fraction	Enzyme	Normal	Electrical shock	%
Myelin	Na, K, Mg-ATPase	36,3±0,4	25,2±0,3	—31
	Mg-ATPase	26,6±0,3	16,5±0,2	—38
	Na, K-ATPase	9,7±0,1	8,7±0,1	—11
	MAO	0	—	
Synaptic membranes	Na, K, Mg-ATPase	57,4±1,7	30,7±2,7	—47
	Mg-ATPase	30,0±2,0	23,5±2,1	—22
	Na, K-ATPase	27,4±1,7	7,2±1,3	—74
	MAO	0	—	
Light synaptosomes	Na, K, Mg-ATPase	63,1±3,2	44,0±3,6	—70
	Mg-ATPase	43,3±2,1	31,0±2,5	—28
	Na, K-ATPase	19,8±1,4	13,0±1,0	—34
	MAO	15,0±2,1	8,8±1,1	—42
Heavy synaptosomes	Na, K, Mg-ATPase	58,0±3,0	52,8±2,2	—10
	Mg-ATPase	48,4±2,0	38,4±1,8	—21
	Na, K-ATPase	9,6±1,0	14,4±1,3	+50
	MAO	21,0±2,2	12,0±0,9	—43
Mitochondria	Na, K, Mg-ATPase	55,3±2,8	45,4±2,4	—18
	Mg-ATPase	49,7±1,8	39,7±1,1	—20
	Na, K-ATPase	5,6±0,3	5,7±0,4	—11
	MAO	59,7±8,7	21,1±2,0	—65
Heavy microsomes	Na, K, Mg-ATPase	55,3±3,8	62,6±2,0	+13
	Mg-ATPase	47,0±2,0	51,0±2,1	+9
	Na, K-ATPase	8,3±1,1	11,6±0,8	+40
	MAO	10,4±1,5	6,5±1,2	—38

Note. Specific activity of ATPase shown in μmoles P_{in}/mg protein per h, MAO activity in nmoles substrate/mg protein per min. Figures in "normal" column represent results of 4 experiments, in "electric shock" column results of 6 experiments, each consisting of 3 repetitions. Tissue obtained from 3 rats used in 1 experiment. Heavy microsomes obtained by centrifuging at 18,000 g for 60 min. % denotes percentage decrease (—) or increase (+) in enzyme activity after electric shock compared with normal. Activity of Na, K-ATPase calculated from difference between activities of Na, K, Mg- and Mg-ATPases.

(noncholinergic in de Robertis' terminology), reduced in the fractions of synaptic membranes and light synaptosomes (described by de Robertis as cholinergic), and showed little change in the fractions of mitochondria and myelin. On the other hand, the Mg-ATPase activity after electric shock showed much smaller changes in the membrane structures: no change in the microsomes, while activity was reduced in the remaining fractions studied. The membrane ATPases of the different synaptic structures and microsomes thus showed different changes in activity in response to the action of electric shock. This fact suggests that the lipoprotein complex of ATPase has specific features which depend on the membranes of the subcellular structures to which they belong.

It also follows from the results given in Table 1 that MAO activity was reduced after a single electric shock in the synaptic structures, microsomes and, in particular, in the mitochondria (by 65%). This correlates completely with the fact that MAO is a typical mitochondrial enzyme. A decrease in the catecholamine content [4, 9] and in the incorporation of noradrenalin-H^3 into homogenates of the rat cerebral cortex have been described during epileptiform convulsions. Ladisich et al., [8] found no changes in MAO activity in rat brain tissues after repeated electrical convulsions. Morphological changes in the epileptogenic foci of the cortex are evidently among the causes of the sharp changes in ATPase and MAO activity in the

subcellular structures after electrical convulsions. These changes include swelling of the mitochondria, an increase in the size of the nerve endings, a decrease in the size of the synaptic vesicles, and changes in the size of the capillary membranes [3].

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