EFFECT OF ELECTRIC SHOCK ON ACTIVITY OF Na,K-ATPASE AND ENZYMES OF MEDIATOR METABOLISM IN THE RAT BRAIN

A. M. Golenda, G. N. Kryzhanovskii, V. K. Lutsenko, O. P. Sakharova, and R. N. Glebov

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Activity of Na,K-ATPase, acetylcholinesterase (AChE), and glutamate decarboxylase (GDC) was investigated in fractions of the rat brain and spinal cord during and 5 and 30 min after a single electric shock. GDC activity of the brain "synaptosomes" was reduced but not significantly, whereas activity of AChE, Na,K-ATPase and, probably, proteolytic enzymes was increased 5 min after electric shock and returned to normal after 30 min. It is suggested that inhibition of Na,K-ATPase activity in the "synaptosomes" of the rat cerebral cortex may play a role in the pathogenesis of convulsions. KEY WORDS: paroxysmal activity; electric shock; Na,K-ATPase; acetylcholinesterase; glutamate decarboxylase; proteolysis.

An essential role in the origin, maintenance, and termination of paroxysmal activity in brain tissue is played by changes in mechanisms of active transport of cations [2, 3, 7] and in enzymes of mediator metabolism [6]. Paroxysmal activity is also accompanied by significant ultrastructural changes in the nerve cells and their organelles [1]. Accordingly the object of the present investigation was to study the activity of Na,K-ATPase, responsible for active cation transport, glutamate decarboxylase (GDC) – the key enzyme of γ-aminobutyric acid (GABA) biosynthesis, and acetylcholinesterase (AChE) – which regulates the duration of the synaptic action of acetylcholine during convulsions induced by electric shock. Since the distribution of the enzymes

TABLE 1. Protein Content (in mg) in Fractions of Brain and Spinal Cord during and after electric Shock (content in homogenate expressed in mg/g wet weight of tissue)

Experimental conditions	Index	Brain				Spinal cord			
		homogenate	"synaptosomes"	microsomes	cytosol	homogenate	"synaptosomes"	microsomes	cytosol
Control	$M \pm m$	71,3±1,6	14,7±0,6	2,3±0,2	9,7±0,8	68,8±1,7	10,5±0,6	2,7±0,1	10,1±0,5
	n	22	18	18	20	21	23	18	17
	%	100	100	100	100	100	100	100	100
Electric shock	$M \pm m$	72,4±1,8	13,9±0,5	3,1±0,3	11,0±0,5	68,7±1,7	10,7±0,5	3,7±0,1	10,7±0,5
	n	19	20	16	16	15	16	15	16
	%	101	95	135*	113	100	101	138*	106
5 min after electric shock	$M \pm m$	70,9±2,1	5,8±0,5	1,1±0,2	8,3±0,7	62,2±3,2	6,0±0,4	2,0±0,2	9,0±0,8
	n	12	9	10	12	11	9	10	11
	%	99	39*	47*	85	90	57*	73*	90
30 min after electric shock	$M \pm m$	74,6±2,8	13,5±0,8	2,7±0,2	13,4±1,5	68,9±3,0	9,6±0,6	3,1±0,3	11,8±0,7
	n	15	15	14	14	15	15	14	14
	%	104	92	118	138*	110	91	114	116

* $P < 0.05$ compared with control.

Legend: n) number of experiments; %) change in protein content compared with control.

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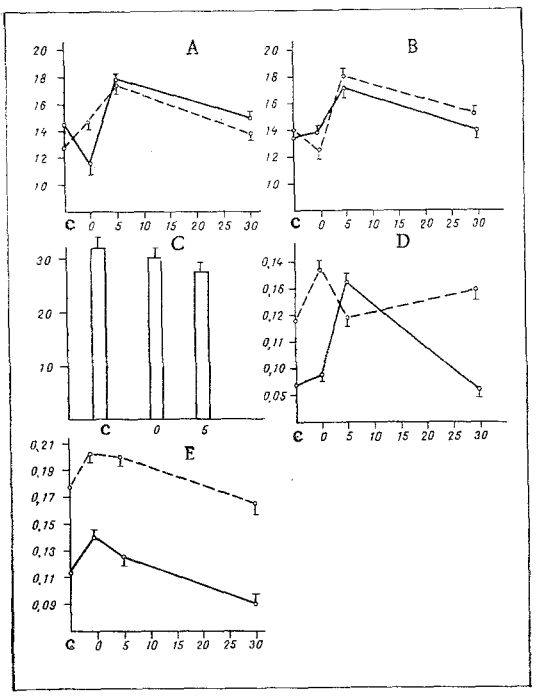

Fig. 1. Changes in Na,K-ATPase, AChE, and GDC activity in subcellular fractions of rat brain tissue during and after convulsions induced by electric shock. Abscissa, time after electric shock (in min): c) control, without electric shock; 0) initial time of electrical stimulation of brain; ordinate, enzyme activity (for Na,K-ATPase, in μmoles P_i/mg protein/h; for AChE, in μmoles acetylcholine/mg protein/min; for GDC, in nanomoles GABA/100 mg protein/h). Continuous line represents "synaptosomes" fraction, broken line fraction of microsomes of brain and spinal cord. A) Na,K-ATPase, brain; B) Na,K-ATPase, spinal cord; C) GDC, brain "synaptosomes"; D) AChE, brain; E) AChE, spinal cord.

among the subcellular fractions of brain tissue differs, and changes in their activity in various functional states may be in the opposite direction [2], the investigation was carried out on homogenates, an unpurified fraction of synaptosomes ("synaptosomes"), microsomes, and cytosol of rat brain and spinal cord tissue.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 200 g. The technique of electrical stimulation and the clinical picture of the convulsion were described previously [3]. Animals were decapitated immediately after the onset of convulsions induced by electric shock, and 5 and 30 min after electric shock. Tissue removed from the cerebral cortex and spinal cord was subjected to subcellular fractionation [3]. Activity of Na,K-ATPase and AChE was determined in the fractions [3]. Activity of GDC also was determined in the fraction of "synaptosomes" by two methods: by measuring the CO_2 output in the course of the enzymic decarboxylation of glutamate and by determining the increase in GABA in the same sample. The GABA concentration was determined by means of an AAA-881 (Czechoslovakia) automatic amino-acid analyzer. The incubation medium (2.5 ml) for determination of GDC activity contained (in mM): pyridoxal phosphate 0.3, glutamic acid 50, K-phosphate buffer (pH 6.4) 100, Triton X-100 0.25% (v/v). The reaction proceeded for 2 h (37°C) in an atmosphere of nitrogen (high purity). The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

As Fig. 1A shows, as a result of electric shock the Na,K-ATPase activity fell significantly only in the brain "synaptosomes." Considering the leading role of the cerebral cortex in the genesis of electroconvulsions, the observed decrease in the efficiency of the Na,K-pump may play a pathogenetic role in the onset of convulsions [3]. The subsequent increase in Na,K-ATPase activity compared with normal in the brain and spinal cord fractions (Fig. 1A, B) was evidently compensatory in character and aimed at restoring ionic gradients. It is important to note that 30 min after the onset of convulsions the Na,K-ATPase activity returned to its initial level (the control, without electric shock). By contrast with changes in Na,K-ATPase activity, the sharp rise in AChE activity, mainly in the "synaptosomes" of the rat cerebral cortex (Fig. 1D, E), was evidently adaptive in character. Like Na,K-ATPase, the cycle of changes in AChE activity in all the fractions studied was complete after 30 min. Functionally speaking, the activation of AChE, localized in the synaptic membranes, may significantly limit the synaptic and extrasynaptic (modulating) effects of extracellular acetylcholine during convulsions and contribute to the active uptake of choline into the nerve endings. Since the depression of brain GDC activity by chemical convulsants correlates well with their ability to induce convulsions [6, 9], the next step was to study GDC activity in the "synaptosomes" fraction of the cerebral cortex (Fig. 1C). Although no statistically significant difference in GDC activity could be found whether during electric shock or 5 min thereafter, a tendency toward inhibition of the enzyme was discovered by two different methods of determination of activity (CO_2 elimination and the increase in GABA). The results for GDC activity in the "synaptosomes" agreed with data on inhibition of the activity of this enzyme in the brain homogenates of rats during electric shock [8] and also during electrical stimulation of a purified fraction of "synaptosomes" [4]. This suggests that changes in the activity of the key enzyme of synthesis of the inhibitory mediator, GABA, do not play an essential role in the formation of the convulsion associated with electric shock. Changes in the activity of hydrolytic and other enzymes in various forms of pathology may lead to ultrastructural changes well known in nerve cells. However, in biochemical investigations of pathological material the possibility of a redistribution of enzymes and proteins in the subcellular fractions is not always taken into account. It follows from Table 1 that the protein concentration in the fractions of the rat brain and spinal cord was regularly reduced 5 min after electric shock in the "synaptosomes" and microsomes, especially in brain tissue, and then recovered after 30 min. This fact may be explained by a substantial increase in intracellular proteolysis, reflecting a disturbance of the state of the intracellular membranes. This reaction also is evidently compensatory in character [5].

This investigation thus showed that active transport of cations is inhibited directly as a result of electric shock, and that it may perhaps be a cause of the paroxysmal activity; changes also were found in the activity of certain enzymes compensating metabolic disturbances due to the increased neuronal activity.

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