MEMBRANE Na, K-ATPase AND ACETYLCHOLINESTERASE ACTIVITY OF THE BRAIN AND SPINAL CORD OF RATS DURING SEIZURES

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Activity of ATPases and acetylcholinesterase (ACE) in the fractions of unpurified mitochondria (UMF) and microsomes from the cortex of the brain and spinal cord of rats in the clonic phase of electrically induced seizures and in the postconvulsive period (5 min after the end of the seizures) was investigated. Inhibition of Na,K-ATPase activity in UMF of the brain in the clonic phase of convulsions and increased activity of this enzyme in all fractions of tissues studied in the postconvulsive period were found. Activity of Ca-ATPase in brain UMF increased during seizures but fell in the postconvulsive period, whereas activity of Mg-ATPase was unchanged. ACE activity as a rule increased during seizures; in the postconvulsive period a further increase in ACE activity was observed in the brain tissue but the activation effect was reduced in the spinal cord tissue. The possibility of structural changes in the excitable membranes of neurons during seizure activity is discussed.

KEY WORDS: ATPase; acetylcholinesterase; brain and spinal cord; seizures; structural changes in membranes.

Since seizures are connected with hyperactivity of neurons [4, 5, 9], it is interesting to study the state of transport ATPases in the CNS during seizures of different origin. The mechanism of excitation is known [1] to be connected with the function of Na,K-ATPase, which is responsible for active Na^+ and K^+ transport through neuronal (including synaptic) membranes. Changes in Na,K-ATPase activity in the tissues of the brain [3, 12, 15, 17] and spinal cord [7] of animals during seizures have been described previously. However, membrane Na,K-ATPase activity of the brain and spinal cord has not previously been studied in the course of the seizure process.

The object of this investigation was to study activity of Na,K-ATPase and other ATPases in subcellular fractions of the brain and spinal cord of rats during electrically induced seizures and in the postconvulsive period. Since the production of seizures is connected with the activity of cholinergic structures in the CNS, acetylcholinesterase (ACE) activity in the membrane fractions also was investigated.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 130-140 g were used. Seizures were induced by application of a single electric shock through transcorneal electrodes (ac, 140-150 V, 50 Hz, 0.5 sec). The animals were decapitated in the clonic phase of the seizures or 5 min after their end. Tissue from the cerebral cortex or spinal cord was homogenized in a glass homogenizer in 0.32 M sucrose containing 0.01 M Tris-HCl, pH 7.4. To remove the coarse fraction of nuclei the 4% homogenate was centrifuged at 2000g (10 min, 0-4°C). The supernatant was centrifuged at 10,000g (20 min, 0-4°C) to obtain the fraction of unpurified mitochondria (UMF), containing synaptic structures, which was resuspended and washed once under the same conditions with the isolation medium. The postmitochondrial supernatant was centrifuged at 18,000g (90 min, 0-4°C) in order to obtain the

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TABLE 1. Activity of ATPase (in μmoles P_{inorg}/mg protein/h) and ACE (in μmoles acetylcholine/mg protein/min) during and after Seizures

Enzyme	Statistical index	Cerebral cortex					
		UMF			Microsomes		
		C	CP	PC	C	CP	PC
Na, K-ATPase	$M \pm m$	14,3±0,5	11,6±0,6	18,0±0,8	12,7±1,0	14,1±1,1	17,3±1,2
	n	12	16	10	10	8	9
	%	100	81*	126	100	111	136*
Mg-ATPase	$M \pm m$	17,5±0,6	16,3±0,3	18,4±0,9	25,9±0,9	25,5±0,9	24,3±1,3
	n	15	16	10	10	8	9
	%	100	91	105	100	98	94
Ca-ATPase	$M \pm m$	9,1±1,1	11,5±0,5	5,9±0,2	—	—	—
	n	5	5	5			
	%	100	126*	65*			
ACE	$M \pm m$	0,098±0,011	0,097±0,03	0,137±0,005	0,113±0,004	0,130±0,04	0,141±0,008
	n	8	7	8	10	7	7
	%	100	99	140*	100	115*	125*

Enzyme	Statistical index	Spinal cord					
		UMF			Microsomes		
		C	CP	PC	C	CP	PC
Na, K-ATPase	$M \pm m$	13,6±0,5	13,7±0,7	16,9±1,0	14,0±0,9	12,3±0,8	18,0±1,0
	n	12	10	9	10	10	7
	%	100	101	124*	100	87	128*
Mg-ATPase	$M \pm m$	16,6±0,6	17,0±0,6	15,4±0,7	20,1±0,5	21,7±0,8	23,7±0,5
	n	12	10	10	10	12	7
	%	100	102	93	100	108	118
Ca-ATPase	$M \pm m$	8,7±0,8	11,4±0,8	6,2±0,4	—	—	—
	n	5	5	4			
	%	100	131	71			
ACE	$M \pm m$	0,114±0,008	0,149±0,008	0,127±0,006	0,199±0,011	0,233±0,011	0,216±0,011
	n	11	7	8	9	4	8
	%	100	131*	111	100	112*	108

Legend. CP) Clonic phase of seizures, PC) postconvulsive period. Values differing significantly ($P < 0.05$) from the control (C) are marked by an asterisk.

fraction of heavy microsomes [11]. The freshly obtained UMF and microsome fraction were suspended in deionized water and their protein content (by Lowry's method) and ACE activity were determined [2, 13]. ATPase activity was determined from the rate of accumulation of inorganic phosphate in the course of the reaction (20 min, 37°C) [16]. The composition of the incubation medium (1 ml), in millimoles, was as follows: ATP-Na_2 3, NaCl 100, KCl 20, $MgCl_2$ 5 ($CaCl_2$ 5), Tris–HCl (pH 7.4) 20, protein 150-300 μg. Activity of transport Na,K-ATPase was calculated from the difference between the activities of total and Mg-ATPase. The rate of ATP hydrolysis in the presence of Mg^{++} ions of Ca^{++} ions only was taken as the activity of Mg- or Ca-ATPase respectively. The experimental results were subjected to statistical analysis by the Student–Fisher criterion [10].

EXPERIMENTAL RESULTS AND DISCUSSION

The distribution of activities of the various enzymes studied among the fractions in tissues of the cerebral cortex and spinal cord of the rats was virtually the same with the exception of ACE, whose activity was greater in membrane preparations from the spinal cord than in the corresponding preparations from the cortex (Table 1). Membrane Mg-ATPase activity in the cortex and spinal cord was unchanged both during and after the seizures. In the clonic phase of the seizures, a decrease in Na,K-ATPase activity was observed only in the UMF of the cerebral cortex and in the other fractions changes in its activity were small. In the postconvulsive period in all cases Na,K-ATPase activity was higher than in the control. In UMF from both

the brain and the spinal cord Ca-ATPase activity was increased in the clonic phase of the seizures. In the postconvulsive period Ca-ATPase activity declined (compared with the control) in the tissues of the brain and spinal cord.

Membrane ACE activity in the cerebral cortex and spinal cord was higher in the microsome fraction than in UMF. In the clonic phase of the seizures, in the tissues tested except UMF from the brain tissue, ACE activity increased. In the postconvulsive period ACE activity was increased in both fractions of the brain tissue, but it was reduced in both fractions of the spinal cord tissue compared with its activity in the clonic phase.

Activation of ACE in the clonic phase of the convulsions is evidence of a state of hyperactivity of the cholinergic structures of the brain and spinal cord. Hyperactivity of excitatory synapses may be the result of inhibition of Na,K-ATPase of the synaptic membranes which, as was suggested previously [1], is the structural-functional cause of depolarization. This explains the inhibition of Na,K-ATPase in the brain UMF in the clonic phase of the seizures. It has been shown [12] that after injection of inhibitors of Na,K-ATPase (ouabain, $ZnCl_2$, $CuCl_2$) into the cerebral ventricles of rats, the animals developed convulsions, and under these circumstances maximal inhibition of Na,K-ATPase activity was observed in the hypothalamus. It has been postulated that the disturbances during epileptiform seizures are based on a sudden disturbance of membrane permeability for cations [14]. Increased functional activity of the contractile system of nerve endings responsible for the mechanism of mediator secretion may also be a manifestation of neuronal hyperactivity during seizures [1]. Evidence in support of this view is given by the increase in Ca-ATPase activity in UMF from the brain and spinal cord. Incidentally, morphologically speaking the UMF contained up to 40-50% of the synaptic structures.

It can be postulated on the basis of the analysis of these data that rapid structural changes can take place in the excitable membranes of neurons. Convulsive agents are a factor maintaining this structural and functional shift which can be initiated by a trigger mechanism. Changes of this sort must also take place under normal conditions during the usual excitation of neurons, but in that case they are balanced, reversible, and coordinated during the alternation of states of rest and excitation, during the convergence of excitatory and inhibitory influences. During seizures, however, a relatively stable structural shift characteristic of increased neuronal excitation may arise in the synaptic membranes. Another feature distinguishing the seizure process is that this shift in the synaptic membranes may be initiated by a large population of neurons (for example, as a result of a considerable increase in the extracellular concentration of K^+ ions during seizures [14] the membranes of neighboring neurons are also depolarized), so that the activity of a certain reserve of neurons as excitation generators is facilitated [6, 8].

The scheme described above can serve to explain the increase in Na,K-ATPase activity (and the decrease in Ca-ATPase activity) observed in the present experiment in certain brain structures in the postconvulsive period, i.e., during disinhibition of particular neurons and during a load decrease in hyperactivity of the excitable neurons. Investigation of structural and functional changes in excitable membranes of neurons (and of their regulation) under normal conditions and during and after seizures may thus be a promising approach to the study of the mechanisms of seizure activity in the CNS.

LITERATURE CITED

1. R. N. Glebov and G. N. Kryzhanovskii, Usp. Fiziol. Nauk, No. 4, 3 (1975).
2. R. N. Glebov, G. M. Dolganov, and G. N. Kryzhanovskii, Dokl. Akad. Nauk SSSR, 218, 1464 (1974).
3. R. N. Glebov, V. V. Shevtsov, et al., Byull. Éksp. Biol. Med., No. 10, 36 (1971).
4. R. I. Kruglikov, M. S. Myslobodskii, and V. L. Ézrokhi, Paroxysmal Activity [in Russian], Moscow (1970).
5. G. N. Kryzhanovskii, Tetanus [in Russian], Moscow (1966).
6. G. N. Kryzhanovskii, F. D. Sheikhon, and M. B. Rekhtman, Neirofiziologiya, No. 6, 608 (1975).
7. G. N. Kryzhanovskii, R. N. Glebov, et al., Byull. Éksp. Biol. Med., No. 1, 45 (1974).
8. G. N. Kryzhanovsky (G. N. Kryzhanovskii) et al., Exp. Neurol., 50, 387 (1976).
9. K. I. Pogodaev, The Biochemistry of the Epileptic Fit [in Russian], Moscow (1964).
10. P. F. Rokitskii, Fundamentals of the Statistics of Variations for Biologists [in Russian], Minsk (1961).
11. V. V. Shevtsov, O. M. Pozdnyakov, et al., Byull. Éksp. Biol. Med., No. 1, 94 (1972).
12. J. Donaldson, T. St.-Pierre, J. Minnich, et al., Canad. J. Biochem., 49, 1217 (1971).
13. G. Ellman et al., Biochem. Pharmacol., 7, 88 (1961).
14. H. Hillman, Lancet, 2, 23 (1970).

15. E. Lowin et al., Neurology (Minneapolis), 19, 421 (1969).
16. O. H. Lowry and J. A. Lopez, J. Biol. Chem., 162, 421 (1946).
17. E. Riedel and E. Wamsiedler, Z. Naturforsch., 26, 1158 (1971).

MECHANISM OF INHIBITION OF MYOCARDIAL GLYCOLYTIC ACTIVITY IN THE EARLY POSTNATAL PERIOD

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The reduction in the activity of glycolysis and glycogenolysis that is regularly observed in developing heart muscle is unconnected with reduction of the glycolytic system but is due to inhibition of the phosphofructokinase stage of the glycolytic chain by its regulator. This is shown by an increase in the ratio between the active masses of the phosphofructokinase by 4.5 times in the rat heart during the first 2 weeks of postnatal development.

KEY WORDS: heart; ontogeny; glycolysis; phosphofructokinase.

High glycolytic activity is found in the myocardium of newborn rats [3] and puppies [4] but it falls sharply soon after birth. This dynamics may be due to an increase in the power of the oxidative phosphorylation system in the developing heart [3].

In this investigation the dynamics of the activity of phosphofructokinase (PFK), the main regulator of the glycolytic chain, and the ratio between the active masses of the PFK reaction (G_{PFK}), characterizing the state of the enzyme, were determined in early ontogeny.

EXPERIMENTAL METHOD

Albino rats were used immediately after birth and at the age of 5, 10, and 15 days. The glycolytic activity of heart homogenates was determined in relation to the conversion of glucose, glycogen, and fructose-1,6-diphosphate (F1,6DP) in concentrations of 10 mM [2]. PFK was extracted from the heart muscle homogenate by a solution containing 0.05 M Tris, pH 8.2, 0.005 M $MgCl_2$, 0.001 M EDTA, and 0.002 M mercaptoethanol. The incubation medium included (in mM): Tris (pH 8.0) 50, $MgCl_2$ 4, ATP 2, NADH 0.17, and fructose-6-phosphate (F6P) 0.6; other constituents were aldolase, 0.2 i.e., triose phosphate isomerase 0.3 i.e., and glycerophosphate dehydrogenase 0.3 i.e.,; PFK activity was judged from the decrease in NADH. To determine the concentration of the metabolites the heart was frozen in liquid nitrogen. Adenine nucleotides were determined with the aid of special kits, F6P and F1,6DP by the method described by Kochetov [1].

TABLE 1. Increase in Lactate (in μmoles/mg protein/h) on Incubation of Heart Homogenates (M ± m)

Substrate	Day after birth			P_{1-15}
	1-st	5-th	15-th	
Glucose	1,7±0,2	1,2±0,2	0,8±0,1	<0,01
Glycogen	1,9±0,2	1,7±0,2	1,2±0,1	<0,01
F1, 6DP	4,2±0,5	3,9±0,4	3,8±0,4	—

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