EFFECT OF ELECTRIC SHOCK AND DIAZEPAM ON OF Na,K-ATPase ACTIVITY IN BRAIN MEMBRANES

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The phenomenon of inactivation of Na,K-ATPase of the unpurified synaptosomal fraction from rat cerebral cortex in electric shock may be connected with "modification" of the potassium combining site of the enzyme. The anticonvulsant diazepam, if injected intramuscularly, also inhibits the Na,K-ATPase of the brain membranes, but diazepam followed by electrical stimulation of the brain in vivo activates Na,K-ATPase by comparison with the control. Diazepam also abolishes clonic convulsions following electrical stimulation of the brain. Meanwhile diazepam does not abolish the compensatory changes in acetylcholinesterase activity of brain and spinal cord synaptosomes of rats with electric shock. The results are discussed in connection with the view that inhibition of Na,K-ATPase activity of the nerve ending membranes may be the pathogenetic mechanism of seizure activity.

KEY WORDS: Na,K-ATPase; acetylcholinesterase; synaptosomes; cerebral cortex; convulsions; diazepam.

It was shown previously [2, 5] that in the stage of clonic convulsions associated with electric shock Na, K-ATPase activity is inhibited in rat brain membranes. Analysis of personal observations and data in the literature on the state of passive and active cation transport through neuronal membranes in the brain of animals with epileptiform activity induced by various methods suggested that the formation of a generator of pathologically enhanced excitation (GPEE) in the brain [3, 4] may be connected with inactivation of Na,K-ATPase in the membranes of neurons and, in particular, membranes of nerve endings [2, 5, 8]. Inactivation of Na,K-ATPase may be the cause of enhanced passive cation transport and secretion of mediators. Inactivation of the Na-pump may also be a cause of the "switching off" of presynaptic inhibition, the mechanism of which in certain cases may be connected with activation of the electrogenic pump.

The outflow of K^+ into the external medium can facilitate depolarization of neighboring neurons and the creation of a focus of hyperactive neurons, i.e., a GPEE. Incidentally, during convulsions induced by electrical stimulation of the brain, the extracellular K^+ concentration rises from 3 to 8-12 mM [12].

The investigation described below was the continuation of the study of the phenomenon of inactivation of Na,K-ATPase in the brain membranes during electric shock; an attempt also was made to correct the "biochemical defect" developing during seizure activity by means of the anticonvulsant diazepam. Acetylcholinesterase (AChE) activity also was studied in the brain membranes during electric shock and after preliminary administration of diazepam.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 200 g. The technique of electrical stimulation and the clinical picture of the seizure were described previously [2]. Immediately after the onset of clonic convulsions induced by electric shock the animals were decapitated and the tissues of the cerebral cortex and spinal cord were removed and used to obtain the unpurified synaptosomal fraction [5]. Diazepam (Seduxen, in ampules, from Hungary) was injected intramuscularly in a dose of 1-2 mg/kg, and the animals were decapitated 20 min later. When diazepam was given, electrical stimulation also was applied 20 min after the injection.

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TABLE 1. Na,K-ATPase Reaction of Membranes of Unpurified Synaptosomal Fraction of Rat Cerebral Cortex during Electric Shock (clonic phase; M ± m)

Enzyme reaction	Activity, μmoles substrate/mg protein/h	
	control	electric shock (clonic phase)
Na,K-ATPase, pH 7.4 (calc. without ouabain)	14,28±0,49 (12)	11,6±0,6 (16)*
Na,K-ATPase, pH 7.4 (calc. with 0.1 mM ouabain)	13,80±0,45 (8)	10,72±0,8 (9)*
Na,K-ATPase pH 5.0 (calc. without ouabain)	5,1±0,6 (5)	8,5±0,7 (5)*
K-dependent NPPase pH 7.4	0,58±0,07 (6)	0,39±0,02 (6)*
Acid phosphatase	1,99±0,03 (5)	2,02±0,05 (5)

Legend. Here and in Table 2: asterisk denotes $P<0.05$ compared with corresponding control. Number of experiments in parentheses.

The method of determining Na,K-ATPase and AChE activity was described previously [5]. In some experiments Na,K-ATPase activity, measured at pH 5.0 (in K-acetate buffer), was determined in the same incubation medium as at pH 7.4 (37°C). Activity of K-dependent paranitrophenyl phosphatase (K-NPPase) was determined [10] from the rate of accumulation of paranitrophenol at 37°C (30 min) in the following incubation medium (1 ml), in millimoles: Na paranitrophenyl phosphate 5, KCl 25, Tris-HCl (pH 7.4) 50, $MgCl_2$ 5; protein 150 μg. Acid phosphatase was determined at pH 5.0 by the use of Na β-glycerophosphate as substrate [1].

EXPERIMENTAL RESULTS

In the first part of the investigation the phenomenon of inhibition of Na,K-ATPase activity in synaptosomal membranes of the rat cerebral cortex was analyzed in the clonic phase of convulsions associated with electric shock. It will be clear from Table 1 that inhibition of Na-K-ATPase activity as a result of electric shock was always found however the enzyme was calculated. It was also shown that activity of K-activated, Mg-dependent NPPase of the synaptosomal membranes also was significantly reduced in the clonic phase of the convulsions of electric shock, whereas acid phosphatase activity was unchanged in electric shock. Considering that K-dependent NPPase imitates the final K-dependent stage of dephosphorylation of Na,K-ATPase intermediates during ATP hydrolysis [15], this suggests that during seizure activity it is the potassium combining site of the Na,K-ATPase molecule, located on the outer surface of the cell membranes, that is disturbed. Another fact points indirectly to the same conclusion. It follows from Table 1, for instance, that Na,K-ATPase activity measured at pH 5.0 was considerably modified during electric shock. At pH 5.0-5.3, Na,K-ATPase is known to function as K-activated ATPase [9, 12], which does not require the participation of Na^+ and is responsible for electroneutral exchange between K^+ and H^+.

In the second part of the investigation the action of diazepam was studied during electric shock. Diazepam [14] is known to depress the excitability of epileptogenic structures in the brain and it is highly effective against generalized seizure discharges. Diazepam limits the spread of paroxysmal activity, depresses focal spike activity only slightly, but quickly abolishes generalized convulsions. Under these circumstances paroxysmal discharges remain in the cortex after abolition of the general convulsions. In large doses diazepam abolishes symmetrical foci of hyperactive neurons [14].

In the present experiments the clinical picture of electrical stimulation was changed under the influence of diazepam. In the control rats the tonic phase of convulsions lasted 7-10 sec, and was quickly followed by the clonic phase (widespread twitches of the limbs and so on). After intramuscular injection of diazepam, starting with a dose of 2 mg/kg, the tonic phase was reduced by a much greater degree than the clonic phase.

It will be clear from Table 2 that diazepam, in a small dose (1 mg/kg) inhibited the Na,K-ATPase of the unpurified synaptosomal fraction of rat cerebral cortex only weakly, but in a larger dose (2 mg/kg) inhibition was moderately strong ($P<0.05$). A similar inhibitory effect also was found with diazepam in doses of 2.5-3 mg/kg. It is important to note that neither electric shock nor diazepam altered Mg-ATPase activity. In experiments in vivo [6] diazepam also moderately inhibited rat brain synaptosomal Na,K-ATPase. On the other hand diazepam, after preliminary electrical stimulation of the brain in vivo in the present experiments, activated (on average by 35% compared with the control without diazepam and electric shock) Na,K-ATPase in the brain

TABLE 2. Changes in Na,K-ATPase and AChE Activity in Unpurified Synaptosomal Fraction Under Influence of Diazepam and Electrical Stimulation (M ± m)

Experimental conditions	AChE activity, μmoles acetylcholine × 10^{-3}/mg protein/min		Na,K-ATPase activity, μmoles P_i/mg protein/h (calc. without ouabain)
	cerebral cortex	spinal cord	cerebral cortex
Control	98±10 (8)	114±80 (11)	14,29±0,49 (12)
Clonic phase of electric shock	97±30 (7)	149±80 (7)*	11,6±0,60 (16)*
Diazepam:			
1 mg/kg	55±0,4 (4)*	88±0,5 (4)*	13,8±1,04 (6)
2 mg/kg	67±0,9 (11)*	79±1,1 (10)*	11,2±1,29 (14)*
1 mg/kg + electrical stimulation	63±2,4 (6)*	136±6,5 (6)*	19,8±2,57 (5)*
2 mg/kg + electrical stimulation	91±7,5 (18)	113±5,7 (18)	18,1±1,34 (17)*

membranes studied. Diazepam thus depressed clonic convulsions induced by electrical stimulation and also abolished the inhibition of Na,K-ATPase activity in electric shock.

The following factors must evidently be taken into account when the results are explained: 1) The mechanisms of inhibition of Na,K-ATPase in electric shock and during the action of an anticonvulsant are different, or otherwise inhibition would be expected to be increased by the combined action of diazepam and electrical stimulation; 2) the inhibitory or activating action of diazepam on Na,K-ATPase of the synaptic membranes depends on the functional state of the potassium combining site of the enzyme molecule. Data on the action of another anticonvulsant – diphenylhydantoin [7, 8, 11] – support the latter possibility. It is clear from the results of the present experiments (Table 1) that during seizure activity the potassium combining site of Na,K-ATPase is modified (although how is not yet clear). This "modification" leads to inactivation of the Na,K-ATPase of the neuron membranes and to the creation of a GPEE. Possibly under these conditions of "modification" of the enzyme (in electric shock, for example) diazepam acts differently on Na,K-ATPase, namely it may activate the enzyme, as will be seen in Table 2. Hence it follows that this anticonvulsant does not remove the cause of the seizure activity, i.e., the GPEE itself, but simply abolishes generalized convulsions. A biochemical solution to this problem can be found only by studying the differential action of anticonvulsants on the primary and secondary (mirror) foci of paroxysmal activity.

It also follows from Table 2 that administration of diazepam depresses AChE activity of unpurified rat brain and spinal cord synaptosomes. However, diazepam followed by electrical stimulation activates AChE (this can be seen particularly clearly in spinal cord tissue), raising its activity to the level observed during the action of electric shock. In other words, compensatory changes in AChE activity of neuronal membranes in electric shock due to increased neuronal activity [2, 5] are in practice not abolished (or are only very slightly reduced) by diazepam. This anticonvulsant does not abolish the after-effects of the GPEE.

The results described in this paper are thus further evidence of the pathogenetic role of changes in Na, K-ATPase activity of neuron membranes during paroxysmal activity.

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