CHANGES IN THE STATE OF NEURONAL MEMBRANE Na,K-ATPase IN EPILEPTIC FOCI INDUCED BY PENICILLIN IN THE RAT CORTEX

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Correlation between electrophysiological changes and the state of neuronal membrane Na,K-ATPase in the rat cerebral cortex was studied in epileptic foci induced by application of the sodium salt of penicillin. During the latent period and at the stage of formation of the epileptic foci, Na,K-ATPase activity was shown to be inhibited both in the primary focus and in the contralateral symmetrical region. In the stage of marked paroxysmal activity Na,K-ATPase activity was inhibited only in the primary focus. In concentrations of $2 \cdot 10^{-6}$-$2 \cdot 10^{-3}$ M, penicillin was shown not to affect Na,K-ATPase of unpurified rat cerebral cortical synaptosomes. It is suggested that inactivation of Na,K-ATPase may be a pathogenetic factor in the development of epileptic activity.

KEY WORDS: epileptic focus; penicillin; synaptosomes; Na,K-ATPase; inhibition.

An essential condition for the formation of generators of pathologically enhanced excitation (GPEE) in the CNS is insufficiency of inhibitory influences in neuron ensembles forming the generator, which may be due either to a direct disturbance of inhibitory mechanisms or to the enhanced maintained excitation of the neurons, overcoming inhibitory control [3]. The formation of a focus of hyperactivity, created experimentally by various methods in a given part of the brain in animals, is largely determined by the molecular pathology of the neuronal membranes. During hyperactivity of a neuron, characterized by depolarization shifts of membrane potential, passive cation transport and secretion of mediators, chiefly excitatory [6], are intensified. The appearance of K^+ in the extracellular medium depolarizes neighboring neurons and a focus of synchronously functioning hyperactive neurons, i.e., a GPEE, is created. The molecular mechanisms of the GPEE are thus based on a disturbance of states of passive and active cation transport through neuronal and, in particular, synaptic membranes.

The functioning of the Na,K pump, i.e., of Na,K-ATPase, is directly connected with the process of excitation. Depolarization of isolated nerve endings (synaptosomes) has been shown to be accompanied by inactivation of Na,K-ATPase [1]. On this basis it is suggested that reversible inactivation of Na,K-ATPase may be the cause of depolarization of the nerve endings. Such inactivation could cause an increase in passive transport of the cations Ca^{++}, Na^+, and K^+ and subsequent triggering of the apparatus for mediator secretion [2].

Hence it follows that one of the main causes of genesis of paroxysmal activity in the CNS and, in particular, of the appearance of a GPEE may be inactivation of the Na,K-ATPase of the neuronal membranes of the brain [2, 6]. In the clonic phase of generalized convulsions induced by electrical stimulation of the rat brain, activity of Na,K-ATPase in unpurified cerebral cortical synaptosomes has been shown [4] to be inhibited. The study of biochemical changes in the whole brain during generalized convulsions cannot be used to judge what takes place in foci of epileptic activity, for under these conditions hypoxia may have a substantial role. It is more useful to study foci of epileptic activity, for in that case combined investigations can be made of biochemical changes and electrical activity in the foci, so that some idea may be obtained of the possible pathogenetic role of these changes.

The object of this investigation was a simultaneous study of electrical activity and the

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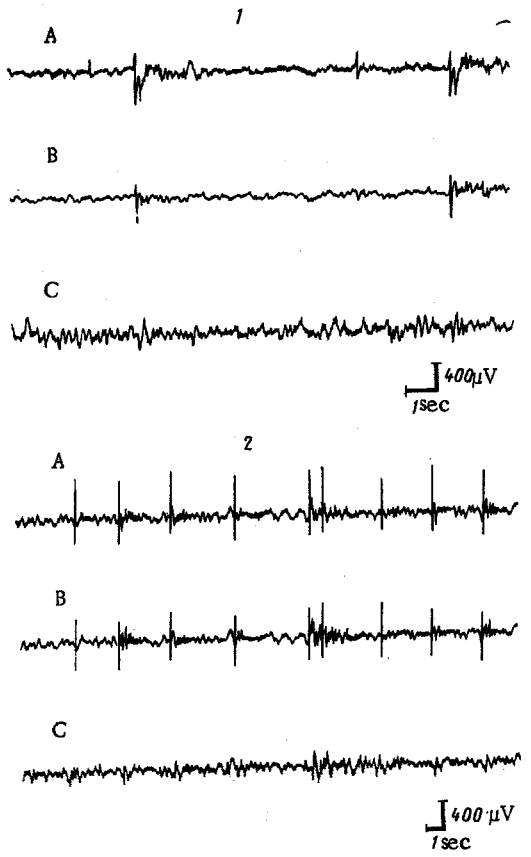

Fig. 1. Development of paroxysmal activity after penicillin application. 1) Stage of formation of foci of hyperactivity; 2) stage of marked paroxysmal activity. A) Left sensomotor cortex; B) right sensomotor cortex; C) right visual cortex.

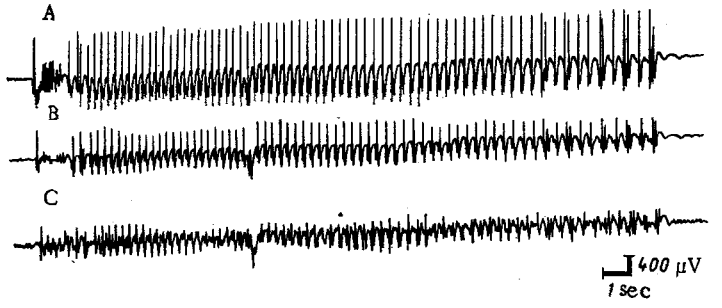

Fig. 2. ECoG of rat during epileptic fit after application of penicillin. Derivations as in Fig. 1.

TABLE 1. Changes in Na,K-ATPase Activity (in μmoles ADP/mg protein · h) in Primary and Secondary Foci of Hyperactivity (M ± m)

Object	Control		Latent period (3 min after penicillin application)		Stage of formation of foci of hyperactivity (4 min after penicillin application)		Stage of marked period activity (6 min after penicillin application)	
	Na, K-ATPase	Mg-ATPase	Na,K-ATPase	Mg-ATPase	Na, K-ATPase	Mg-ATPase	Na,K-ATPase	Mg-ATPase
Sensomotor cortex, left hemisphere (primary focus)	5,7±0,54 (n=11)	8,5±0,64 (n=11)	3,7±0,63* (n=6)	5,7±0,30* (n=6)	2,2±0,50* (n=6)	4,6±0,72* (n=6)	3,4±0,33* (n=10)	8,4±0,63 (n=10)
Sensomotor cortex, right hemisphere (secondary focus)	5,5±0,36 (n=10)	8,3±0,79 (n=10)	3,6±0,43* (n=7)	6,0±0,54* (n=7)	3,4±0,61* (n=6)	4,5±0,51* (n=6)	5,6±0,64 (n=9)	8,0±1,01 (n=9)
Visual cortex, right hemisphere	4,2±0,53 (n=11)	6,4±0,37 (n=11)	2,9±0,44 (n=7)	5,7±0,59 (n=7)	4,1±0,64 (n=6)	5,4±0,37 (n=6)	4,1±0,51 (n=10)	7,9±0,94 (n=10)

Legend. 1. Composition of incubation medium (in mM): NaCl 100, KCl 20, $MgCl_2$ 5, ATP 4, Tris-HCl 50, pH 7.6, 20°C. 2. Mg-ATPase activity measured in presence of 0.8 mM ouabain. 3. Number of experiments given in parentheses. 4.*$P < 0.05$.

TABLE 2. Action of Penicillin on Activity of Na,K- and Mg-ATPase in Fraction of Unpurified Rat Cerebral Cortical Synaptosomes (in % of control)

Enzyme	Control	Penicillin concentration, M			
		$2 \cdot 10^{-6}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-4}$	$2 \cdot 10^{-3}$
Na, K-ATPase	100±3 (8)	104±6 (6)	108±8 (8)	99±7 (7)	111±5 (7)
Mg-ATPase	100±3 (8)	98±3 (7)	96±8 (8)	96±4 (7)	99±4 (7)

Legend. Number of determinations in parentheses.

state of neuronal membrane Na,K-ATPase in a GPEE in the rat cerebral cortex in epileptic foci created by penicillin application.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 180-200 g were used. Under hexobarbital anesthesia three burr holes measuring 2 × 4 mm were drilled in the skull above the sensomotor cortex of both hemispheres and above the visual cortex of the right hemisphere and the dura was removed from these areas. To record the electrocorticogram (ECoG) silver electrodes (diameter of ball point 0.5 mm) were applied to the dura at a distance of 0.2-0.3 mm anteriorly to the burr hole. The indifferent electrode was introduced into the subcortical structures at the junction of the frontal, temporal, and parietal bones of the right hemisphere. To prevent drying, the exposed brain surface, moistened with physiological saline, was covered with adhesive tape in such a way that the brain was not touched. Experiments were carried out on unanesthetized animals on the day after the operation. The rat was fixed in a special jacket which allowed movement of the limbs. The head was fixed by the incisors. After adaptation for 10-20 min the background ECoG was recorded. The sodium salt of penicillin was applied by means of wide-pore filter paper measuring 1.5 × 3.5 mm, soaked in penicillin solution in a concentration of 15,000-20,000 iu/ml and applied to the surface of the left sensomotor cortex. Pieces of filter paper soaked in 0.85% NaCl were applied to the surface of the right sensomotor cortex and visual cortex. The ECoG was recorded on an RM-85 polygraph (Nihon Kohden, Japan). Before tissue sampling the pieces of filter paper were removed from the brain and the sample taken in the course of 1 min by means of a special cold spoon measuring 1.5 × 3.5 mm, with sharply ground edges. The samples of 2-3 mg cortical tissue obtained from each region were at once homogenized in glass microhomogenizers manually at 0°C, the fraction of unpurified synaptosomes was then isolated, and Na,K-ATPase activity was then determined by the fluorescence micromethod described previously [7]. Na,K-ATPase activity in experiments to study the action of the sodium salt of penicillin *in vitro* was measured by the method of Lowry and Lopez [12]. The fraction of unpurified synaptosomes was isolated by the standard method [15], and subjected to osmotic shock in Tris-HCl solution (pH 7.6, 20°C) with a single freezing and thawing. The solution of the sodium salt of penicillin in a final concentration of $2 \cdot 10^{-6}$ to $2 \cdot 10^{-3}$ M was made up in distilled water.

EXPERIMENTAL RESULTS

Between 4 and 6 min after application of penicillin to the surface of the left sensomotor cortex of the rats epileptiform discharges (200-300 V) began to appear in the left hemisphere; after 10-15 sec their amplitude increased and spike potentials also began to appear in the right hemisphere (Fig. 1, 1). This stage of the process can be described as the stage of formation of foci of hyperactivity. As a rule, 2-4 min after the beginning of epileptiform activity a clear electrophysiological picture was established: The amplitude of the spike potentials remained constant. After 4-7 min, for each spike potential on the ECoG there were corresponding twitches of the forelimbs and the muscles of mastication of the rat, more marked on the side contralateral to the focus. Tonico-clonic convulsions developed after 15-30 min (Fig. 2). The duration of the tonic phase was 1-1.5 sec, and this was followed by multiple paroxysmal discharges with a frequency of 2-4/sec; the structure of the individual discharge corresponded to that of the classical spike—wave complex [14]. As the fit developed, discharges consisting of a double spike and wave were observed. At the end of the fit, interictal discharges were frequently absent in the ECoG for 1-4 min. It must be pointed out that after unilateral application of penicillin spontaneous bursts of spike—wave complexes 1-5 sec in duration were never observed on the ECoG, in agreement with data in the literature [11].

Correlation between changes in the ECoG and Na,K-ATPase activity in the primary focus (GPEE), in the contralateral symmetrical region of the sensomotor cortex (the secondary dependent, or "mirror" focus), and also in the visual cortex contralateral to GPEE was studied (Table 1). During the latent period and in the stage of formation of the foci of hyperactivity, both Na,K-ATPase and Mg-ATPase activity was found to be reduced in both the left and the right sensomotor cortex. However, when the primary and secondary foci were formed (the stage of marked paroxysmal activity, Fig. 1, 2), Na,K-ATPase activity was significantly reduced only in the left sensomotor cortex (GPEE).

Experiments *in vitro* showed that the sodium salt of penicillin, in a concentration of $2 \cdot 10^{-6}$-$2 \cdot 10^{-3}$ M, did not affect Na,K- or Mg-ATPase activity in the fraction of unpurified

rat cerebral cortical synaptosomes subjected to osmotic shock (Table 2). These results show that the inactivation of Na,K-ATPase in penicillin convulsions cannot be attributed to the direct action of penicillin on the enzyme. It is perhaps the ability of penicillin as an antagonist of GABA receptors to abolish inhibition and, consequently, to facilitate the secretion of exitatory mediators, which lies at the basis of creation of the GPEE through the action of penicillin.

Differences in the metabolism of the primary epileptic focus and of the secondary focus dependent on it have been observed previously [8]. Amino acid metabolism in the dependent secondary focus of epileptic activity has been shown to remain within normal limits, whereas in independent secondary foci the changes in the levels of amino acids studied were the same as in the primary focus. At the stage of repetitive interictal discharges following injection of penicillin into the region of the sensomotor cortex in rats a decrease in the concentration of high-energy phosphates has been described only in the region of the primary focus of epileptic activity [9]. It has also been shown that at the stage of interictal discharges after application of penicillin to the cat cerebral cortex [13] the cyclic GMP concentration in the focus of hyperactivity increases, whereas in the contralateral symmetrical region the cyclic GMP level corresponds to the control. An increase in the cyclic GMP concentration in certain structures of the rat brain is observed as early as 30 sec after injection of metrazol and before the beginning of paroxysmal activity [10] and it continues to rise as the convulsions develop. The increase in the cyclic GMP concentration in the brain may perhaps lead to inactivation of Na,K-ATPase through phosphorylation of cyclic GMP-dependent protein kinase.

There are two possible explanations of the fact that Na,K-ATPase is inactivated during the latent period: 1) inactivation of the enzyme always precedes the onset of electrical activity; 2) inactivation of the enzyme reflects existing pathological hyperactivity of single neurons, which is not synchronized in the latent period.

The absence of inactivation of Na,K-ATPase in the secondary focus at the stage of marked paroxysmal activity is perhaps connected with the fact that neurons of the "mirror" focus remain capable of overcoming the metabolic disturbances, whereas GPEE neurons are affected to a far greater degree. The electrophysiological data point to this possibility [5].

The results of these experiments thus confirm the hypothesis that inactivation of Na,K-ATPase of the brain neuronal membranes may be a pathogenetic factor in the development of convulsions.

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