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EFFECT OF METRAZOL-INDUCED EPILEPTIC ACTIVITY ON TRANSPORT Ca-ATP-ASE ACTIVITY IN RAT BRAIN SYNAPTIC MEMBRANES

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According to the membrane hypothesis of the pathogenesis of epileptic activity (EA) of the brain, the creation of a generator of pathologically enhanced excitation (GPEE) of neurons may be attributed to specific disturbances of the structure of neuronal membranes, leading to the opening of additional ionic channels or to the longer existence of these channels in the open state [3, 5, 7]. Under these conditions the accumulation of intracellular Ca^{++} (probably up to 10 μM) facilitates prolonged secretion of excitatory mediators by hyperactive neurons, i.e., the conditions are created for long-term, persistent depolarization of the neurons. It was shown previously [2, 6] in the writer's laboratory that inactivation of electrogenic Na,K-ATPase of synaptic membranes is an important (evidently the trigger) factor in epileptization of neurons. This phenomenon corresponds in principle to the fact that inactivation of the electrogenic Na,K-pump may be the trigger factor coupling depolarization with transmitter secretion by nerve endings [4].

It can be tentatively suggested that pathological hyperactivity of neurons may also be maintained by the fact that the systems for the outflow of Ca^{++} which accumulates in the synaptoplasm are "switched off" because of structural changes in the membrane of nerve endings. In other words, inactivation of the Ca-pump and (or) the direct Na^{+}/Ca^{++} exchange system leads to increased efficiency of the secretory process of hyperactive neurons.

The aim of the present investigation was accordingly to study the state of Ca-ATPase activity of rat brain synaptic membranes at different stages of development of generalized EA induced by injection of metrazol.

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TABLE 1. Transport Ca-ATPase Activity (in μmoles P_i/mg protein/h) in Osmotically Destroyed Synaptosomes and in the SJ Fraction (M ± m)

Test object	Ca-ATPase	Mg-ATPase
Destroyed synaptosomes	9,9±1,4 (5)	18,2±3,2 (5)
SJ fraction	27,1±1,5 (3)	1,2±1,0 (3)

TABLE 2. Transport Ca-ATPase Activity (in μmoles P_i/mg protein/h) in Osmotically Destroyed Rat Brain Synaptosomes at Different Stages of EA Induced by Injection of Metrazol (M ± m)

Series of expts.	Control		Experiment	
	Ca-ATPase	Mg-ATPase	Ca-ATPase	Mg-ATPase
I	2,8±0,7 (3)	10,0±1,5 (3)	2,4±0,8 (3)	5,8±0,5 (3)
II	4,7±0,8 (3)	6,9±0,8 (3)	1,8±0,3 (4) —62,7 %*	7,8±1,0 (4)
III	6,5±0,7 (8)	18,8±1,8 (8)	1,0±0,5 (6) —84,6 %*	21,0±2,8 (6)
IV	3,5±1,4 (3)	16,8±5,9 (3)	3,6±1,3 (3)	12,5±3,4 (3)

Legend. Inhibitory effect shown as a percentage. Control and experiment in each series relate to one batch of animals. *$p < 0.05$ compared with control of corresponding series.

EXPERIMENTAL METHOD

Experiments were carried out on 65 control and 68 experimental male Wistar rats weighing 200-220 g, kept under standard conditions (temperature 22 ± 2°C, alternation of light and darkness every 12 h, food and water ad libitum). The preliminary operation (implantation of electrodes) was performed under hexobarbital anesthesia (100 mg/kg, intraperitoneally) supplemented by local procaine anesthesia. Electrical activity in the region of the sensomotor cortex of the rat brain (electrocorticogram) was recorded with monopolar silver ball electrodes (diameter 0.5 mm) on a P84-01 polygraph. The active electrode was inserted subdurally into the cranial vault in the region of the sensomotor cortex. The reference electrode was inserted into the nasal bones. The experiments were carried out on the day after the operation on conscious, unrestrained animals. EA was induced by intraperitoneal injection of 10% metrazol solution in a dose of 75 mg/kg body weight. This dose of metrazol caused death of the animals in 25% of cases 7-25 min after injection.

The electrophysiological manifestations of EA were evaluated by recording the latent period (LP) of the first seizure responses and the duration of EA, which in these experiments were 30-55 sec and 9-11 h after injection of metrazol respectively (Fig. 1). Control animals were given an injection of the same volume of physiological saline.

For the biochemical investigations the animals were decapitated 15 and 30 sec (series I and II respectively – LP) and 12 h (series IV – the recovery period) after injection of metrazol or physiological saline, and also after the development of clonico-tonic convulsions with the animal on its side, and with a marked tonic extension phase (series III), which began 1.5-2.5 min after injection of metrazol depending on the animal's individual sensitivity to the drug.

The synaptosomal fraction was isolated from the cerebral cortex of the rats [8]. Protein was determined by Lowry's method and transport Ca-ATPase activity by pH-metry [1]. Freshly isolated synaptosomes were subjected to osmotic shock by suspension in 8 volumes of

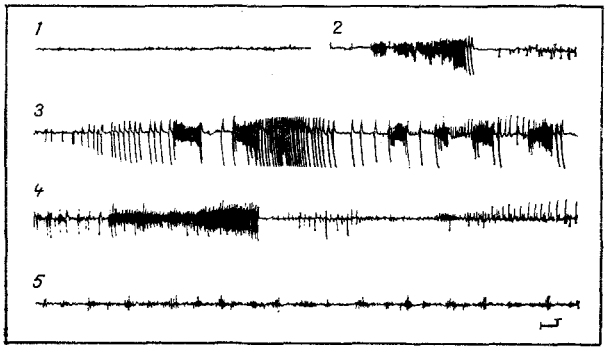

Fig. 1. Electrical activity in rat cerebral cortex after intraperitoneal injection of metrazol in a dose of 75 mg/kg. 1) Before injection of metrazol; 2, 3) 20 and 75 sec, 4, 5) 18 and 480 min after injection of metrazol respectively. Calibration: 200 μV, 5 sec.

medium containing 5 mM Tris-HCl, 0.1 mM EDTA, pH 7.4 (4°C), followed by freezing and thawing. The product was centrifuged at 15,000g for 30 min. The residue was suspended in medium containing 2.5 mM Tris-HCl, 100 mM KCl, pH 7.4 (4°C). The potentiometric cell, with a capacity of 3 ml, contained the following medium: 2.5 mM Tris-HCl, 100 mM KCl, 4 mM $MgCl_2$, 2 mM ATP, 25-30 μM $CaCl_2$, 0.1 mM EGTA, 5 mM NaN_3, and 1 mM ouabain, pH 7.2 (37°C). Transport Ca-ATPase activity was calculated as the difference between total activity and Mg-ATPase activity (in the absence of Ca^{++}).

The synaptic junction (SJ) fraction was isolated from whole brain (without the cerebellum) [9] and kept at −20°C in medium containing 0.25 mM sucrose, 20 mM Tris-HCl, pH 7.4 (4°C).

EXPERIMENTAL RESULTS

The Ca-pump (or its biochemical equivalent, Ca-ATPase) in the nerve endings was located in two places: in the plasmalemma of the terminals and in membranes of the endoplasmic reticulum, present in nerve endings. Under these circumstances hydrolysis of ATP in those cases takes place in the synaptoplasm: in the first case, on the inner surface of the plasmalemma, and in the second on the outer surface of membranes of the endoplasmic reticulum, facing the synaptoplasm.

In this investigation we used the fraction of osmotically destroyed synaptosomes, which contains mainly plasma membranes with small traces of membranes of the endoplasmic reticulum.

It will also be noted that molecules of the Ca-pump were evidently unevenly distributed in the plasmalemma of the nerve endings. The experiments showed (Table 1) that Ca-ATPase activity in the SJ fraction of the brain (SJ is the active zone of synapses, i.e., the region of the junction between the pre- and postsynaptic membranes) is almost 3 times higher than activity of the enzyme in the fraction of osmotically destroyed synaptosomes, whereas Mg-ATPase activity was sharply reduced. This fact, discovered by us for the first time, probably indicates that the Ca-ATPase molecule in membranes of SJ is much larger than at other sites on the neuronal plasmalemma, and it is important to take this into account when synaptic function is examined. During the development of EA, transport Ca-ATPase activity at the beginning of LP (series I) was virtually unchanged, at the end of LP (series II) Ca-ATPase activity had fallen sharply (by 62.7%), at the height of EA (series III) inactivation reached a maximum (84.6%), and after cessation of EA, normal Ca-ATPase activity was restored. Mg-ATPase activity at all stages of EA was virtually the same as in the control (Table 2). Thus Ca-ATPase, like the Na,K-ATPase of synaptic membranes, is inactivated as early as during LP of EA [6], i.e., when epileptization of the neurons has begun but the focus of hyperactive neurons has not yet been formed, nor has their "work" become synchronized (under these conditions EA is not manifested electrographically).

The development of EA was thus accompanied by inactivation of the Ca-pump of the synaptic membranes, the functioning of which may be disturbed first in the course of epileptization of the neurons. Hence it follows that during epileptization of the neurons a comparatively high Ca^{++} ion concentration is created in the cytoplasm. Inactivation of one of the systems for outflow of Ca^{++} from the cell, namely the Ca-pump of the membranes of nerve endings, facilitates a "longer" action of Ca^{++} ions on the secretory process, i.e., it helps to maintain

epileptization for a comparatively long period of time. Consequently, inactivation of the Ca-pump of the membranes of nerve terminals may be a factor in the development and maintenance of pathological hyperactivity of neurons. Possible inhibition of transport Ca-ATPase activity in membranes of the endoplasmic reticulum, which were present in small numbers in our preparation, during the development of EA may also promote prolonged accumulation of Ca^{++} in the synaptoplasm, with all the consequences which that entails.

The study of regulation of systems for Ca^{++} inflow and outflow in neurons and nerve endings during the appearance and disappearance of EA is thus of great importance. The next step must now be to study the effect of known anticonvulsants on function of the electrically excitable Ca-channel. It can also be postulated that specific inhibitors of the Ca-channel may be potential anticonvulsants.

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EFFECT OF ADAPTATION TO PHYSICAL EXERCISE ON REACTIVITY OF THE ISOLATED RAT ATRIUM TO NORADRENALIN

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Adrenoreceptor reactivity of the heart during adaptation to physical exercise has been studied in numerous investigations [1, 5, 8, 10, 13-15], most of them devoted to β-adrenoreceptors. Some workers [1, 5, 8, 13] found that the number and activity of β-adrenoreceptors decrease as a result of adaptation to physical exercise, whereas others found no change [10, 15] or an increase [14]. These contradictions are evidently due to differences in the conditions of adaptation, and also to the fact that in some experiments adrenoreceptor reactivity was assessed in relation to the chronotropic effect of agonists, and in others, to the inotropic effect. The effect of adaptation to physical exercise on α-adrenoreceptor reactivity has not been seriously studied.

The aim of this investigation was to compare the effect of adaptation to physical exercise on the inotropic and chronotropic effects of α- and β-adrenoreceptor agonists.

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