POSSIBILITY OF PHARMACOLOGIC CORRECTION OF SPINAL CORD Na,K-ATP-ase ACTIVITY DURING POISONING WITH BOTULINUM TOXIN

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A characteristic feature of the pathogenic action of botulinum toxin (BT) is its marked neurotropic effect [8]. The nature of the metabolic disorders arising in different parts of the nervous system has not yet been adequately studied. In particular, the state of active energy-dependent transport in neurons in the cervical region of the spinal cord has not been elucidated. Yet it is this zone of the spinal cord which plays an important role in the regulation of breathing: the nuclei of the phrenic nerves and also the decussation of the reserve reticulospinal pathways to the respiratory muscles are located here [3].

The aim of this investigation was to study the character and mechanisms of changes in synaptosomal Na,K- and Mg-ATPases from the cervical region of the spinal cord, which would not only clarify existing ideas on the causes of development of paralysis of the respiratory muscles in botulinum poisoning (BP), but would also reveal any possible opportunities for pharmacologic correction of disturbances of the Na,K-pump.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing about 250 g. BP of type C was induced by intramuscular injection of BT in a dose of 0.04 mg/kg (1 MLD for mice — 0.0005 mg of the dry toxin), and the animals were decapitated 1 day later, when they had developed pareses of their skeletal musculature. Some of the experiments were done 2 h after intraperitoneal injection of BT, in the absence of any manifestations of BP. The cervical region of the spinal cord was removed from the decapitated rats in the cold, the tissue was homogenized in 10 volumes of 0.32M sucrose and in 0.01M Tris-HCl, pH 7.4, and then subjected to differential centrifguation at 10,000g in order to isolate the fraction of unpurified synaptosomes. Activity of Na,K- and Mg-ATPases was determined by the usual method [2]. To study the kinetics of the enzyme reaction, the Michaelis constant (K_m) for ATP and the maximal rate of hydrolysis of the substrate (V) were determined by the use of straight-line graphs [4].

EXPERIMENTAL RESULTS

The experiments showed that competitive inhibition of Na,K-ATPase, coupled with activation of Mg-ATPase, appeared even in the preclinical period of BP in synaptosomes in the cervical region of the spinal cord (Table 1). During the development of pareses of the skeletal musculature, noncompetitive inhibition of synaptosomal Na,K-ATPase was discovered. Parallel activation of Mg-ATPase was observed.

To discover the degree of damage caused by BT to the ATPase system of the spinal cord synaptosomes and the possibility of pharmacologic correction of this effect of BT, an attempt was made to prevent inhibition of the Na,K-ATPase of the synaptosomal membranes by means of serotonin, gutimin, and parmidine.*

The depotentiating action of serotonin on the lethal effect of BT is known to be observed in experiments on animals of different species, provided that the serotonin is injected be-

*Alternative names: Prodectin, pyridinol carbamate.

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TABLE 1. State of Activity of Na,K- and Mg-ATPases of Synaptosomal Fraction of Cervical Region of Spinal Cord in the Course of BP Accompanied by Pharmacologic Correction (M ± m)

Series of experiments	Na, K-ATPase		Mg-ATPase	
	V	K_M	V	K_M
Control	8,00±0,17	1,40±0,10	22,9±0,38	0,53±0,04
Preclinical period of BP	8,20±0,15	2,10±0,09	24,70±0,42	0,38±0,02
P_1	>0,05	<0,001	<0,002	<0,01
Preclinical period of BP with injection of serotonin immediately after BT	8,10±0,20	1,30±0,09	19,20±0,40	0,35±0,07
P_1	>0,5	>0,2	<0,001	<0,05
P_2	>0,5	<0,001	<0,001	>0,5
The same, with pharmacologic correction by gutimin	8,00±0,43	0,95±0,13	15,80±0,53	0,90±0,14
P_1	>0,5	<0,01	<0,001	<0,02
P_2	>0,5	<0,001	<0,001	<0,001
The same, with pharmacologic correction by parmidine	6,80±0,37	0,71±0,08	20,00±0,66	0,62±0,06
P_1	<0,01	<0,001	<0,001	>0,2
P_2	<0,001	<0,001	<0,001	<0,001
Stage of pareses of skeletal musculature	7,10±0,18	1,10±0,10	24,80±0,31	0,70±0,04
P_1	<0,001	<0,02	<0,001	<0,1
The same with injection of serotonin immediately after BT	5,70±0,25	1,00±0,13	25,90±0,50	0,62±0,05
P_1	<0,001	<0,02	<0,001	>0,1
P_2	<0,001	>0,5	>0,05	>0,1
The same, with injection of serotonin before BT	7,40±0,28	0,74±0,11	24,20±0,52	0,86±0,10
P_1	<0,05	<0,001	<0,05	<0,01
P_2	>0,2	<0,02	>0,2	>0,1
The same with injection of gutimin immediately after BT'	8,00±0,33	0,59±0,07	22,50±0,51	0,50±0,06
P_1	>0,5	<0,001	>0,5	>0,5
P_2	<0,02	<0,001	<0,001	<0,01
The same, with injection of parmidine immediately after BT	7,10±0,37	0,88±0,13	23,60±0,49	0,59±0,04
P_1	<0,05	<0,01	>0,2	>0,2
P_2	>0,5	>0,2	<0,05	<0,05

Legend. P_1) Relative to control, P_2) relative to corresponding stage of BP without pharmacologic correction, K_m) calculated in millimoles ATP, V) in micromoles P_i/mg protein/h. Four to six animals were used in each series of experiments.

fore the BT [7, 9]. At the same time, it has been shown that serotonin injections given immediately after the injection of BT have a marked potentiating action on the lethal effect of BT [7]. The mechanism of these opposite effects of serotonin on the severity of the course of BP have not yet been explained. Considering data in the literature on the regulating action of serotonin on transport ATPases [6], an attempt was made to discover whether the change in severity of the course of BP after injection of serotonin by different methods correlate with its effect on Na,K-ATPase. It was found that intraperitoneal injection of serotonin in a daily dose of 5 mg/kg immediately after BT prevented the development of competitive inhibition of synaptosomal Na,K-ATPase in the preclinical period of BP. This effect was accompanied by noncompetitive inhibition of Mg-ATPase. The appearance of pareses of the skeletal musculature under these experimental conditions was combined with noncompetitive inhibition of synaptosomal Na,K-ATPase activity, which was much more marked than in the group of animals not receiving the drug. Meanwhile activation of Mg-ATPase was observed, just as in the experiments without serotonin. In the modification of the experiments with intraperitoneal injection of serotonin in a dose of 5 mg/kg 40 min before injection of BT, noncompetitive inhibition of Na, K-ATPase activity was observed against the background of development of pareses of the skeletal musculature, just as in experiments without the use of any drugs. Mg-ATPase activity changed in the same way as in the corresponding experiments without serotonin. The data described above can be summed up on the conclusion that the character of the effect of serotonin on synaptosomal Na,K-ATPase activity largely depends on the method of injection of the drug and the stage of development of BP. Injection of serotonin immediately after injection of BT aggravates the inactivation of Na,K-ATPase against the background of development of pareses of the skeletal musculature. Meanwhile, injection of serotonin before BT did not prevent the development of noncompetitive inhibition of synaptosomal Na,K-ATPase against a background of clinical manifestations of BP.

A characteristic feature of BP is the development of progressive external respiratory failure and of corresponding metabolic disorders. The possibility therefore cannot be ruled out that a definite role in the genesis of the changes discovered in Na,K-ATPase activity in

the course of BP must be played by the hypoxic factor, leading to activation of lipid peroxidation in biological membranes and to changes in their functional activity [5]. This state of affairs necessitated experiments with the use of the antihypoxants gutimin and parmidine, which also have a membrane-stabilizing action [1, 5]. Gutimin, in a daily dose of 100 mg/kg, was injected intraperitioneally [1] immediately after BT. Under these circumstances gutimin prevented exhibition of the inhibitory effect of BT on synaptosomal Na,K-ATPase activity, both in the preclinical period of BP and against the background of marked clinical manifestations of it. Meanwhile Mg-ATPase activity was appreaciably depressed in the preclinical period compared with the corresponding stage of BP without pharmacologic correction, and it was completely restored to normal when pareses of the skeltal musculature developed.

In the next series of experiments, in which parmidine was injected intraperitoneally immediately after injection of BT in a daily dose of 200 mg/kg, a change was discovered in the mechanisms of inhibition of Na,K-ATPase, which produced noncompetitive inhibition of this system in the preclinical period of BP. Parallel inhibition of Mg-ATPase was observed instead of its activation in "poisoned" animals without injection of the drug. As BP developed and pareses of the skeletal musculature appeared, noncompetitive inhibition of synaptosomal Na, K-ATPase was preserved, whereas the parameters of the Mg-ATPase kinetics were completely restored to normal.

Consequently, the maximal opportunities for reactivation of Na,K-ATPase of the synaptosomal membranes in the course of BP are observed against the background of gutimin administration. The ability of serotonin to prevent the inhibitory effect of BT on Na,K-ATPase activity was exhibited only in the preclinical period of BP, irrespective of whether it was given before or after injection of BT. Meanwhile parmidine does not lead to activation of energy-dependent transport processes through the synaptosomal membrane in the cervical region of the spinal cord.

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