ROLE OF NEUROTROPHIC REGULATION IN DISTURBANCES OF STRIATED MUSCLE POLARIZATION BY BOTULINUS TOXIN

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Reinnervation of muscles after intramuscular injection of sublethal doses of botulinus toxin was investigated in rats. While not affecting the formation of functioning myoneural synapses, botulinus toxin was shown to lead to prolonged persistence of depolarization of the reinnervated muscle cells without any change in passive biophysical properties of their sarcoplasmic membranes.

KEY WORDS: botulinus toxin; reinnervation of muscle fibers; neurotrophic regulation.

It has been shown experimentally that botulinus toxin (BT), when injected intramuscularly, paralyses skeletal muscles for a long time without causing any direct injury to the muscle cells and nerve fibers [10, 13]. It is transported along nerve trunks from the region of the myoneural junctions to motor nerve cells in the anterior horns of the spinal cord [11, 15] and has a pathogenic action on them [2, 3], and the recovery process after botulinus paralysis, as in other types of spinal motoneuron lesion [7], takes place by terminal regeneration of axons in the region of myoneural synapses [8, 9].

It was accordingly decided to study the course of recovery of function of neuromuscular synapses after artificial stimulation of regeneration of the nerves supplying skeletal muscules paralysed by BT.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male Wistar rats weighing 120-130 g. Under pentobarbital anesthesia and with sterile precautions the nerves to the soleus muscles were crushed bilaterally at a distance of 10 mm from their point of entry into the muscle. A sublethal dose of BT type A (0.005 mg toxin/100 g body weight in 0.1 ml physiological saline, 1 MLD for mice equals 0.00005 mg) was injected into the posterior group of right calf muscles (experimental limb). The soleus muscles of the left calf served as the control. At different times after injection of BT the dynamics of reinnervation of the soleus muscles were studied by calculation of the coefficient of neuromuscular synaptic transmission (CNMST) in relation to tetanic contraction developed by the muscle in response to direct and indirect stimulation for 1 sec with pulses 0.2 msec in duration and with a frequency of 16 Hz. At the same time the resting membrane potentials (RMP) were recorded with glass microelectrodes and the passive electrical constants of the plasma membrane of the reinnervated muscle cells were determined. The radius of the muscle fibers was measured in sections stained with hematoxylin-eosin. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Intramuscular injection of sublethal doses of BT disturbs myoneural transmission for a long period and causes progressive atrophy of the muscles and depolarization of muscle fibers [4, 12, 14]. Preliminary crushing of the nerves to the soleus muscle prevented atrophy of those muscles, and led within relatively short time to recovery of neuromuscular synaptic conduction. This fact was evidence that conditioning injury to the nerves, activating their regeneration, considerably reduced the transmission of BT to spinal α-motoneurons in the motor nuclei of the soleus muscles [11, 15]. As will be clear from Figs. 1 and 2, crushing

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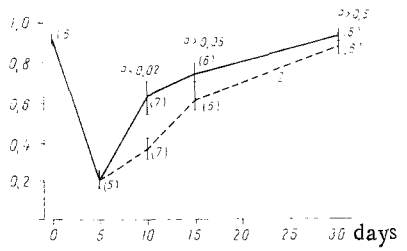

Fig. 1

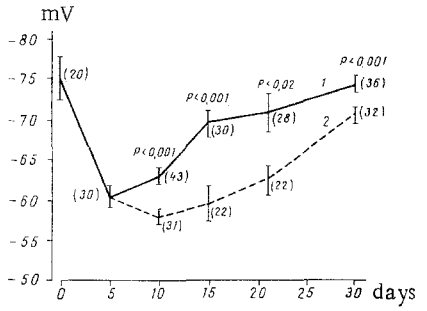

Fig. 2

Fig. 1. Dynamics of recovery of CNMST of reinnervated soleus muscles after intramuscular injection of botulinus toxin into calf muscles. 1) Control limb; 2) experimental limb. Abscissa, time of reinnervation (in days); ordinate, CNMST.

Fig. 2. Dynamics of recovery of RMP of reinnervated soleus muscle fibers after intramuscular injection of botulinus toxin. Ordinate, RMP (in mV). Remainder of legend as in Fig. 1.

the nerves during the first few days led to a decrease in myoneural transmission and polarization of the muscle fibers. Later intensive regeneration of nerves of the control and experimental muscles was observed, accompanied by a progressive increase in CNMST. A significant difference between the value of CNMST in the experimental muscles and its value in the control muscles was observed only in the early periods of reinnervation. Later, recovery of synaptic myoneural conduction took place practically identically in the experimental and control muscles (Fig. 1). By contrast with the identical functional reinnervation of the muscles, restoration and polarization of the myocytes followed a different temporal course. Whereas RMP of the fibers from the control muscles increased significantly until the 15th day of reinnervation, restoration of polarization of the experimental muscle fibers took place much more slowly and their RMP remained low throughout the period of observation (Fig. 2). As will be clear from Fig. 2, the greatest differences in the levels of polarization of the experimental and control muscles were observed on the 15th day of reinnervation. At that time it was interesting to discover whether the greater depolarization of myocytes of the experimental muscles was connected with changes in the passive electrical parameters of their plasma membranes. As Table 1 shows, despite considerable depolarization, the passive electrical constants of the fibers of the experimental muscles, including the specific resistance of the sarcolemma, characterizing its passive ionic permeability, did not differ from the corresponding values for the control muscles. It can accordingly be considered that dissociation between the rapid recovery of CNMST, i.e., the formation of functioning myoneural synapses, and long persistence of depolarization of the muscle fibers of the experimental limb, unconnected with any change in the passive biophysical properties of their sarcolemma, was evidence of a disturbance of neurotrophic regulation of polarization of the reinnervated muscles cells by BT.

In view of the data showing that intramuscularly injected BT reduces the rate of rapid

TABLE 1. Level of Polarization and Passive Electrical Constants of Sarcolemma of Muscle Cells on 15th Day of Reinnervation and 10th Day of Injection of Botulinus Toxin (M±m)

Experimental conditions	RMP, mV	R_{in}, MΩ	r, μ	τ, msec	R_M, Ω·cm²	C_M, μF/cm²
Control muscles	68,90±1,58 (30)	0,372±0,015 (21)	17,21±0,41	2,48±0,10	404±26	6,33±0,62
Experimental muscles	59,14±2,39 (22)	0,369±0,017 (24)	16,94±0,51	2,36±0,09	391±18	6,43±0,54
P	<0,001	>0,5	>0,5	>0,2	>0,5	>0,5

Legend: 1) number of cells shown in parentheses; 2) R_{in}) input resistance; r) radius; τ) time constant; R_M) specific resistance; C_M) specific capacitance. Length constant of fiber taken to be 0.5 mm [5].

axonal transport of labeled protein and reduces the number of macromolecules transported into the muscle [1, 6], it can be tentatively suggested that injury to spinal α-montoneurons by the toxin delays restoration of RMP of the reinnervated muscle fibers probably as a result of disturbance of orthograde axon transport into the muscle of neuronal substances which activate the functioning of the electrogenic potassium-sodium pump of the muscle cell plasmalemma.

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CONDITIONS OF PREPARATION AND MECHANISM OF ACTION OF MACROPHAGAL PYROGEN

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Activation of mononuclear phagocytes by staphylococci *in vitro* leads to the formation of an endogenous pyrogen. The macrophagal pyrogen does not possess specific pyrogenic specificity, and on intracisternal injection sensitivity to it is enhanced by more than 100 times compared with that observed after intravenous injection. An even sharper increase in sensitivity to pyrogen was observed in animals after elevation of the body level of cyclic AMP as a result of preliminary injection of theophylline.

KEY WORDS: fever; pyrogens; macrophages.

The cell system of mononuclear phagocytes plays an important role in the mechanism of nonspecific and specific resistance [2, 5]. According to recently published data, the cells of this system play an active part in the mechanism of the febrile reaction, forming endogenous pyrogens [6-8, 11, 12]. However, some aspects of the conditions of formation and mechanism of action of macrophagal pyrogen have been insufficiently studied, and the investigation described below was accordingly undertaken for this purpose.

To obtain peritoneal macrophages (PM) 200 noninbred albino mice weighing 20-30 g were used, to isolate alveolar macrophages (AM) 11 rabbits were used, and to obtain blood mono-

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