# MECHANISM OF REINNERVATION OF SKELETAL MUSCLES INJURED BY BOTULINUS TOXIN

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The course of reinnervation of a skeletal muscle, whose own myoneural apparatus has been damaged by botulinus toxin, by an implanted nerve was investigated in experiments on rats in order to determine the essential conditions for regeneration of nerve fibers in an area of paralysis due to botulism. Injection of a sublethal dose of the toxin into the limb was shown to hasten synaptogenesis of the implanted nerve. As a result, restoration of the normal weight of muscle tissue and the level of polarization of its fibers was promoted.

KEY WORDS: paralysis due to botulism; regeneration of nerve fibers; trophic action of nerve.

In botulism restoration of the functions of damaged areas of skeletal muscle takes place very slowly and may extend over many months. Experimental investigations have shown that the recovery process is associated with growth of nerve fibrils from regions of the axon lying proximally to the existing myoneural junctions. These fibrils initially form primitive synaptic connections which, as they mature are converted into normal myoneural synapses. There is a parallel increase in the weight of the muscles and the strength of their contractions is restored [3-6]. However, the causes of this slow recovery of motor functions of the paralyzed skeletal muscles have not yet been explained. The question arises whether the synaptic apparatuses of the damaged muscles are able to inhibit the formation of new synapses and thus to prolong the recovery period. It was accordingly decided to study how skeletal muscles whose own myoneural apparatus has been damaged by botulinus toxin, are reinnervated by a "foreign" nerve.

## EXPERIMENTAL METHOD

Sixty noninbred albino rats of both sexes weighing initially 110-120 g were used. Under pentobarbital anesthesia (5 mg/100 g body weight) and under sterile conditions the right tibial nerve was divided in the lower limb and implanted into the extrasynaptic region of the soleus muscle. Ten days later a sublethal dose of type A botulinus toxin was injected into the posterior group of calf muscles on the side of the operation (0.005 mg dry toxin/100 g body weight in 0.1 ml physiological saline, 1 MLD for mice = $5 \times 10^5$ mg). Paralysis of the muscles of the poisoned limb developed after 48-72 h.

At different times after injection of the toxin the course of reinnervation was investigated by determining the coefficient of synaptic transmission (CST): the ratio of the amplitude of tetanic contraction of the soleus muscle during indirect stimulation of its own and the reimplanted nerves to the amplitude of tetanus developed by the muscle in response to direct stimulation (frequency of stimulation 60 Hz, pulse duration 0.2 msec, above-threshold stimulation, duration of stimulation 1 sec, with intervals of 2.5 sec).

At the same times the resting membrane potential (RMP) of fibers of the soleus muscle was recorded by intracellular glass microelectrodes filled with 2.5 M KCl (resistance 5-20 MΩ). Electrical potentials recorded by the microelectrode were led to a cathode follower with electrometric input. The DC amplifier of vertical deflection of the F1-18 oscilloscope was used as terminal repeater. The RMP was recorded on the N-340 ink-writing potentiometer.

In control series of experiments the nerve was implanted without subsequent injection of toxin or toxin was injected without implantation of the nerve. The data obtained on the experimental limb were compared with data obtained on the contralateral control limb.

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TABLE 1. RMP (in mV) of Soleus Muscle Fibers after Injection of Botulinus Toxin and Implantation of Tibial Nerve

| Series of experiments | Time of investigation | Experimental limb | | Control limb | | |
|---|---|---|---|---|---|---|
| | | n | M±m | n | M±m | P |
| Injection of toxin into intact muscles | 45 | 32 | 57,28±1,24 | 32 | 67,03±1,17 | <0,001 |
| | 75 | 32 | 58,56±1,14 | 15 | 69,67±3,31 | <0,01 |
| Implantation of nerve into intact muscles | 45 | 14 | 69,64±3,26 | 24 | 66,50±2,16 | >0,01 |
| Injection of toxin into muscles after preliminary implantation of nerve | 30 | 27 | 59,67±1,75 | 21 | 69,05±2,16 | <0,001 |
| | 75 | 30 | 66,97±1,87 | 34 | 67,76±1,69 | >0,01 |

Legend. P) Significance of difference between experimental and control limbs; n) number of muscle fibers tested.

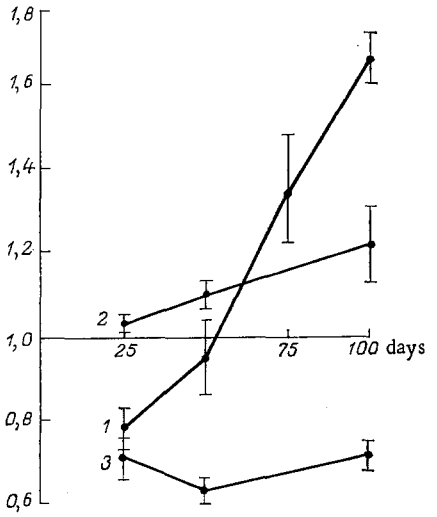


Fig. 1

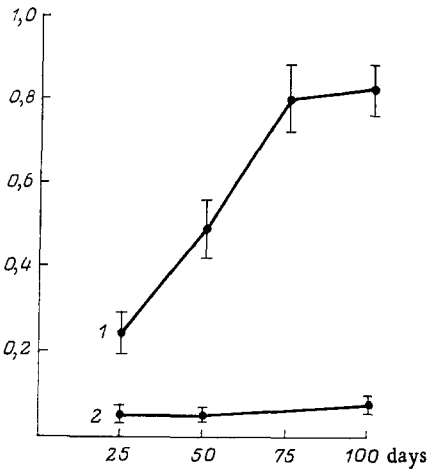


Fig. 2

Fig. 1. Changes in wet weight of soleus muscles at different times of implantation of tibial nerve and injection of botulinus toxin. 1) Dynamics of weight of muscles "poisoned" by botulinus toxin after preliminary implantation of nerves; 2) weight of intact muscles after implantation of additional nerve; 3) weight of muscles poisoned with botulinus toxin without implantation of nerve. Each point on graph represents M ± m (n=5). Abscissa, time (days) after implantation of tibial nerve and injection of botulinus toxin; ordinate, weight of muscles relative to weight of muscles on control side, taken as 1.

Fig. 2. Dynamics of CST of tibial nerve at different times after implantation and injection of botulinus toxin. 1) Dynamics of CST of implanted nerves after preliminary intramuscular injection of botulinus toxin; 2) CST of tibial nerve implanted into intact muscles. Each point on graph represents M ± m (n=5). Amplitude of tetanic response of muscle to direct stimulation taken as 1. Abscissa, time (in days); ordinate, value of CST.

## EXPERIMENTAL RESULTS

The control series of experiments showed that during the first 3-4 days after injection of a sublethal dose of botulinus toxin only repetitive stimulation of the motor nerve to the soleus muscle caused it to give weak contractions. Progressive atrophy of the affected muscles was observed to develop over a period of two months after poisoning. By the end of that time usually partial recovery of neuromuscular transmission took place, but the weight of the muscles on the affected side had not returned to normal even on the 100th day after injection of the toxin (Fig. 1). Parallel with this, the value of RMP for the muscle fibers likewise had not returned to normal (Table 1). Injury to the neuromuscular apparatus following injection even of sublethal doses of botulinus toxin in rats is thus not followed by recovery of functions for a very long time.

To determine the precise conditions essential for regeneration of nerve fibers in an area of paralysis due to botulism the course of reinnervation of the soleus muscle, whose own myoneural apparatus was damaged

by botulinus toxin, with an implanted nerve was studied. As Fig. 1 shows, after implantation of the nerve on the affected side restoration of the normal weight of muscle tissue took place more rapidly than in the previous series of experiments, and marked hypertrophy of the "poisoned" muscles was found after 100 days. Recovery of weight was regularly associated with a considerable increase in CST of the implanted nerve (Fig. 2), in the virtual absence of any significant restoration of transmission of excitation from the "poisoned" proper nerve of the muscle. A similar phenomenon was observed against the background of rapid normalization of RMP of the muscle fibers in the zone of injury on account of an increase in the number chiefly of highly polarized fibers of the reinnervated muscles compared with the control (Table 1).

As regards the phenomenon of reinnervation of the healthy muscle, implantation of the nerve into intact muscles led to slight hypertrophy (Fig. 1) of the muscles but before 45 days it had virtually no effect on the level of polarization of the muscle fibers (Table 1) and was not accompanied by the formation of functioning synapses: even in the late stages after implantation, CST did not reach significant values (Fig. 2).

It can be concluded from the experimental results that growth of additional fibrils in the zone of paralysis due to botulism may be connected with the formation of substances stimulating regeneration of nerve fibers. The synaptic apparatuses damaged by botulinus toxin, do not themselves inhibit the formation of new synapses. However, the formation of completely normal myoneural synapses extends over many months because of profound disturbances of the functions of the trophic centers of the skeletal muscles, i.e., spinal motoneurons [1, 2].

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# RESPONSES OF THE SYSTEMIC CIRCULATION IN THE EARLY PERIOD OF TRAUMATIC SHOCK INDUCED BY CANNON'S METHOD


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Experiments on dogs showed that the erectile phase of shock induced by Cannon's method can be divided into two periods on the basis of changes in parameters of the systemic circulation. The first period has many features in common with "defensive reactions" in response to the appearance of danger and the action of powerful nonspecific stimuli and is characterized by an increase in the return of blood to the heart, an increase in cardiac output, and a decrease in peripheral vascular tone. The second period is characterized by commencing decompensation of functions of the cardiovascular system. The second period ends with the development of the torpid phase of shock.



KEY WORDS: cardiac output; stroke volume of the heart; total peripheral vascular resistance.


Great importance in the system of disturbances of function in shock is attached to circulatory disorders. However, in most investigations changes in the systemic hemodynamics, regional blood flow, and microcirculation have been studied under conditions of marked posttraumatic hypotension when, strictly speaking, the shock process has become established and the primary neuroendocrine and hemodynamic responses have taken effect [2, 8].

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