MECHANISM OF DISTURBANCES OF THE CONTRACTILE FUNCTION OF THE MASSETER MUSCLES IN RATS WITH EXPERIMENTAL BOTULISM

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UDC 616.981.553-092.9-07:616.742.7-009.1-072.7

Injection of a sublethal dose of botulinus toxin into one masseter muscle leads to unilateral paralysis of that muscle, accompanied by the development of biting. Membrane polarization and the amplitude of the action potential mainly of highly polarized muscle fibers are depressed in the paralyzed region, but the function of fibers with a low level of polarization is only slightly affected.

KEY WORDS: electrogenesis in muscle fibers; botulinus toxin; masseter muscle; biting.

A disturbance of chewing is one of the earliest symptoms of development of botulism in man and animals. Such disturbances are found not only in the acute phase of the disease, but also in the period of convalescence [7-9]. This suggests that mainly the phasic muscle fibers are damaged in the muscles of mastication, as they also are in the limb muscles [3, 6].

It was accordingly decided to study how the functions of the various types of fibers composing the masseter muscles are changed at different stages of experimental botulism.

EXPERIMENTAL METHOD

Noninbred albino rats of both sexes weighing 150-200 g were used. A local form of botulism of the masseter muscles, the mechanism of development of which was identical with that of the generalized form of the disease, was produced in the animals. Botulinus toxin (BT) of type A (1 MLD for mice = 0.0005 mg) was injected into the left masseter muscle of the rat in a dose of 100 mouse MLD/100 g body weight. Weakness of the muscles of mastication developed on the side of injection of the toxin after 3-4 days and the characteristic erythema of the conjunctiva appeared. The unilateral lesion of the muscles of mastication in most animals did not affect biting, but in some rats biting was so disturbed that after 15-20 days unrestrained growth of the upper and lower incisors was observed (Fig. 1). At different times after injection of BT the animals were used in acute experiments under pentobarbital anesthesia (40 mg/kg). The masseter muscle was dissected on the side of poisoning and the rat was placed in a frame for electrophysiological investigations.

By means of a standard microelectrode technique the resting membrane potential (RMP) and also the parameters of excitability of the cytoplasmic membrane of the muscle fibers (rheobase currents, critical depolarization level, action potential – AP – and overshoot) were investigated. For a more detailed analysis of changes in the microphysiological properties of the muscle fibers depending on the depth at which they were situated, the masseter muscle was conventionally divided into eight layers, each 370 μ thick, in which RMP were recorded, and the parameters of excitability were studied over a wider range of intervals, each measuring 1 mm [1]. The experimental results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Depending on the clinical symptoms, the functional state of the different types of fibers was analyzed both in the absence and in the presence of changes in biting. As Fig. 2 shows, the change in biting depended on the number of damaged highly polarized fibers, located mainly in the upper and middle layers of the masseter. For instance, a disturbance of biting was not present when a considerable number of muscle fibers

Department of Pathological Physiology, N. A. Semashko Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Adо.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 1, pp. 10-12, January, 1980. Original article submitted December 1, 1978.

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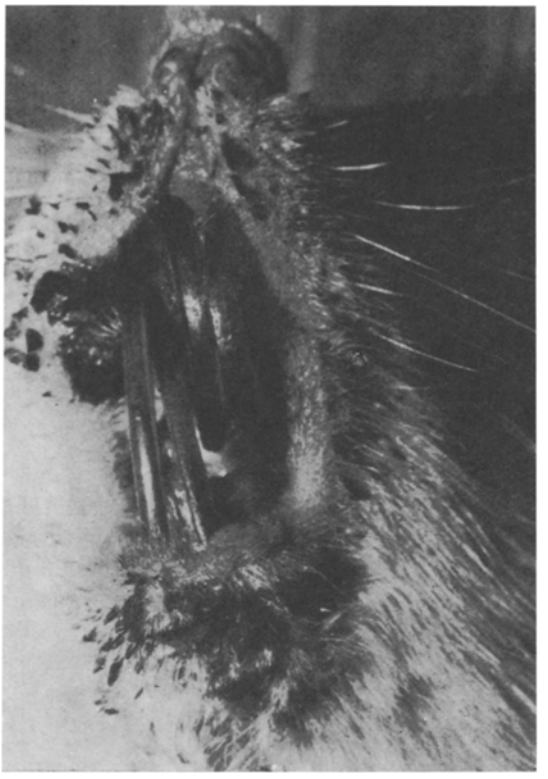

Fig. 1. Disturbance of biting in rat during development of unilateral paralysis (atony) of masseter muscle.

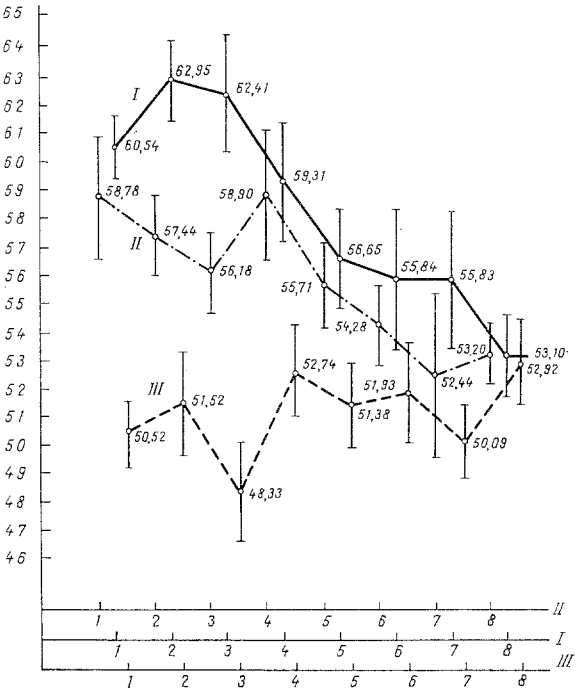

Fig. 2. Layer by layer changes in level of polarization of rat muscle fibers in zone of local botulism. I) Control; II) with no disturbance of biting; III) with biting disturbed. Abscissa, layer of muscle by depth; ordinate, RMP (in mV).

with high RMP was still demonstrable in the masseter. If, however, fibers with a low level of polarization predominated in the poisoned muscle, biting was disturbed.

The next step was to determine whether the development of muscular atony was connected with disturbance of excitability of the muscle fibers. Using intracellular stimulation of the muscle fibers it was found that in the affected masseter muscle a decrease in the amplitude of AP and an increase in the rheobase currents were found mainly in the surface layers of the muscle, where highly polarized fibers are usually found. In the deeper layers, where the number of fibers with a low level of polarization is greater, the amplitude of the overshoot was reduced the most.

Comparison of the character of changes in the functional properties of the muscle fibers in the animals with and without a disturbance of biting showed that the difference was purely quantitative, i.e., when biting was disturbed the changes in excitability of the muscle fibers were most profound and fibers with a low level of polarization which did not generate AP despite rheobase currents twice or three times higher than the control values were frequently discovered.

Disturbance of biting and of the strength of contraction of the masseter muscles in experimental botulism are thus connected with changes in electrogenesis and electrical excitability chiefly of highly polarized fibers. By analogy with the mechanisms of injury of other skeletal muscles it can be postulated that partial blockade of synaptic conduction develops in the masseter muscles [2], and under these conditions a phenomenon of partial denervation, differing in certain indices from total denervation [5], may arise. As regards the duration of recovery of highly polarized fibers in the masseter muscles, we know that in botulism the trophic influence of phasic motoneurons on the corresponding muscle fibers is considerably depressed [4] and repair processes in the affected muscle are severely impaired.

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