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# MECHANISM OF CHANGES IN POLARIZATION OF SECRETORY CELLS OF THE SUBMANDIBULAR SALIVARY GLANDS IN EXPERIMENTAL BOTULISM

V. V. Mikhailov and A. G. Rusanova

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Nearly all patients with botulism develop a syndrome of xerostomia. This is connected with the development of insufficiency of the secretory function of the salivary glands as a result of injury to their parasympathetic innervation [5, 6].

In view of data showing that parasympathetic decentralization of tissues for a long time is not followed by trophic disturbances, whereas denervation leads to the rapid development of trophic disturbances in them [7], it was decided to study changes in polarization of the acinar and duct cells at different stages of poisoning with botulinus toxin in order to shed light on the problem of the dynamics of injury to the parasympathetic innervation of the submandibular salivary glands.

## EXPERIMENTAL METHOD

Noninbred rats of both sexes weighing 50-70 g at different stages of botulinus poisoning were used. To reproduce a general form of botulism, type A botulinus toxin was injected into the region of the left submandibular salivary gland in a dose of 0.2 ml/100 g body weight, and to reproduce the local form of botulism, a dose of 0.09 mg/100 g body weight was given by the same route (1 MLD for an albino mouse in 0.5 μg of the dry toxin). Some animals received two injections of pilocarpine in a dose of 1 mg/kg (24 and 18 h before the acute experiment).

Acute experiments were carried out on animals previously deprived of food for 24 h. The rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneally), the left submandibular salivary gland was isolated, and an immobilizing disk of transparent plastic was inserted beneath it to ensure that the gland did not move when the microelectrode was inserted into its tissue. The temperature of the gland was kept between 37 and 38°C by irrigation with warm Ringer-Locke solution. The resting membrane potential (MP) was investigated by a standard microelectrode technique. Cells with MP of under 36 mV were classed as acinar and those with MP of above 36 mV were classed as duct cells [10].

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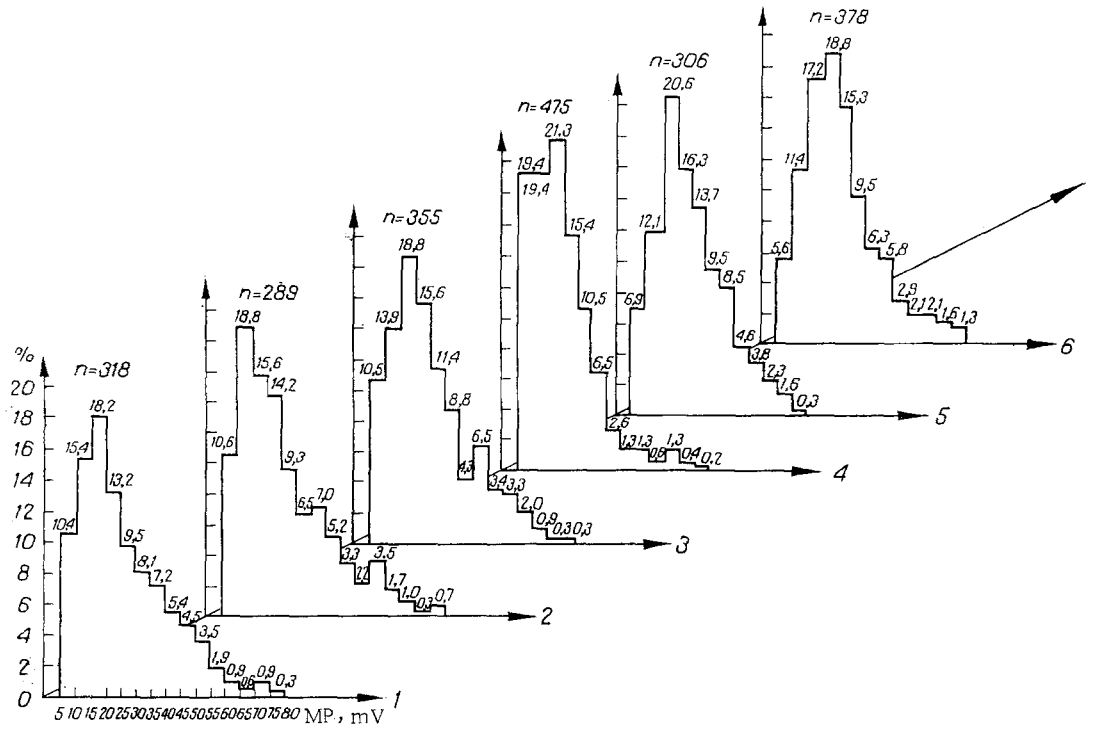


Fig. 1. Histograms of distribution of MP in left submandibular salivary gland at different stages of experimental botulism. 1) Control, 2) general form of botulism (first 2 days), 3) local form of botulism (14 days), 4) local form of botulism (30 days), 5) stimulation by pilocarpine (control), 6) local form of botulism (30 days) with stimulation by pilocarpine; n) number of cells recorded.

## EXPERIMENTAL RESULTS

In botulism, development of neurodystrophic changes and inhibition of the neurotrophic influences of cholinergic nerves on the effector take place in the late stages of poisoning [4]. Three periods of poisoning were accordingly studied: early (before 2 days), middle (14 days), and late (30 days).

It will be clear from Fig. 1 that in the initial period of development of the paralytic syndrome, when the picture is dominated by disturbances of transmission in synapses of the CNS and in parasympathetic ganglia [3, 8], no changes were observed in MP in the different types of secretory cells in the submandibular salivary glands. In these experiments, after the microelectrode had punctured the epithelial cells a marked increase was observed in the number of depolarized acinar cells, but the rate of hyperpolarization was somewhat slower than in the control (Table 1).

On the 14th day after poisoning, i.e., at the beginning of development of neurodystrophic changes in the soma of the cholinergic neuron [1], none of the indices studied yet differed significantly from normal. Finally, in the late period, when besides the soma, activity of the axon of cholinergic neurons was disturbed [2], MP in a group of acinar and duct cells was significantly reduced (Fig. 1). After puncture of the cells by the microelectrode, an increase was observed in the number of hyperpolarized cells, with an amplitude of maximal hyperpolarization a little higher than the normal initial level (Table 1). This suggested that in the late stages of development of botulinus poisoning the lowering of MP of the salivary gland cells was associated with prolonged inhibition of secretory activity of the cells and not with their injury. It was accordingly decided to study the effect of pilocarpine, which stimulates secretion of salivary glands and potentiates the activity of the sympathetic nervous system [9].

It was clear from Fig. 1 that injection of pilocarpine into the animals 30 days after poisoning led to a marked increase in the MP level of the acinar and duct cells. The ratio between hyperpolarized and depolarized cells was restored close to normal under these circumstances, although the rate of hyperpolarization developing after puncture of the cells remained low.

TABLE 1. MP of Secretory Cells of Submandibular Salivary Glands at Different Stages of Botulinus Poisoning

| Experimental conditions | Maximal hyperpolarization of first group of cells, mV | Maximal hyperpolarization of second group of cells, mV | Rate of hyperpolarization, mV/sec | Ratio of hyperpolarized to depolarized cells of first group | Ratio of hyperpolarized to depolarized cells of second group |
|---|---|---|---|---|---|
| Control | 5,15±0,27 (56) | 6,52±1,36 (5) | 1,50±0,14 (23) | 1:0,55 (278) | 1:2,0 (75) |
| Botulism, general form (first 48 h) | 5,07±0,27 (48) | 8,04±0,72 (48) | 1,07±0,11 (19) $P<0,05$ | 1:1,23 (210) $P<0,001$ | 1:4,0 (56) |
| Botulism, local form (14 days) | 5,44±0,21 (63) | 6,05±1,23 (8) | 1,37±0,11 (28) | 1:0,47 (271) | 1:1,95 (65) |
| Botulism, local form (30 days) | 6,50±0,41 (70) $P<0,01$ | 5,36±1,1 (5) | 1,47±0,08 (58) | 1:0,28 (357) $P<0,001$ | 1:0,87 (28) $P<0,05$ |
| Stimulation by pilocarpine (control) | 4,90±0,37 (44) | 7,30±0,81 (6) | 1,0±0,1 (27) $P<0,01$ | 1:0,55 (286) | 1:1,26 (65) |
| Botulism, local form (30 days) with stimulation by pilocarpine | 5,10±0,29 (63) $P_1<0,01$ | 5,7±1,8 (6) | 1,01±0,08 (40) $P<0,01$ $P_1<0,001$ | 1:0,57 (200) | 1:1,23 (83) |

Legend: 1) Number of cells given in parentheses; 2) P denotes significance of difference from control; $P_1$) significance of difference from local form of botulism (30 days); 3) first group consists of cells with MP of between 6 and 36 mV (acinar), second group of cells with MP of between 36 and 80 mV (duct cells).

In botulism the preganglionic parasympathetic neuron is thus evidently injured whereas the postganglionic neuron preserves its trophic influence on the tissues of the gland for a long time. This explains the fact that artificial stimulation of the parasympathetic innervation by pilocarpine can maintain the normal level of electrogenesis in the salivary gland cells of the poisoned animals even at the very late stages of poisoning.

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