EFFECTS OF PATHOGENIC ACTION OF BOTULINUS TOXIN OF SPINAL MOTONEURONS OF VARIOUS TYPES

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Motoneurons of the lumbosacral region of the spinal cord were studied during local botulinus paralysis. The development of paralysis of the poisoned limb was accompanied by lowering of the membrane potential, the amplitude of the antidromic action potentials and of mono- and polysynaptic EPSPs, and the input resistance, and by an increase in the critical depolarization level of the soma membrane of phasic motoneurons in the affected segments of the spinal cord. The excitability of the tonic motoneurons was substantially unchanged in the course of development of local botulism.

KEY WORDS: botulism; spinal cord; motoneurons.

In local botulism disturbance of neuromuscular transmission is preceded by disappearance of simple and complex spinal reflexes and depression of electrical activity of the ventral roots of the poisoned segments of the spinal cord [1-3].

This investigation was carried out to study the effect of botulinus toxin, travelling along regional nerves to the spinal motor centers [2, 11], on excitability of spinal motoneurons of various types.

EXPERIMENTAL METHOD

Experiments were carried out on 22 healthy cats and 30 cats poisoned with botulinus toxin. Type A botulinus toxin (1 mouse MLD = 0.00001 mg of the dry toxin) was injected into one hind limb of the animal in a dose of 0.4-0.5 mg/kg. Under these conditions local botulinus paralysis, uncomplicated by disturbances of respiration or of activity of the cardiovascular system, developed. Acute experiments under pentobarbital anesthesia (40 mg/kg) were carried out on the 3rd-4th day after poisoning (stage of paresis) and on the 7th-8th day (stage of paralysis of the poisoned limb). Throughout the experiment the animals' body temperature was maintained at 37°C and their respiration and blood pressure were monitored.

Single glass microelectrodes filled with 2.5 M KCl solution, connected to a bridge circuit [5, 10], were used for intracellular recording and polarization of motoneurons in the lumbar portion of the spinal cord. The membrane potential of the cells was recorded on a type N-349 ink-writing potentiometer. The potentials and polarizing currents were photographed from the screen of an S1-18 dual-beam oscilloscope with an FOR photographic recorder. Parameters of excitability (input resistance, threshold current for direct stimulation, critical depolarization level) and the synaptic potentials of phasic and tonic motoneurons were investigated by the usual methods [5, 7, 8, 10, 12]. Cells with after-hyperpolarization lasting 50-110 msec were classed as phasic and those in which it lasted more than 110 msec as tonic. The former were located chiefly in nuclei of the gastrocnemius, posterior tibial, and peroneal nerves; the latter in the nucleus for the soleus muscle [6, 9, 12].

EXPERIMENTAL RESULTS

On the poisoned side of the spinal cord at the stage of paresis the polarization level and input resistance were lowered and the threshold currents for direct stimulation and the critical depolarization level

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TABLE 1. Changes in Parameters of Neuronal Activity during Development of Local Botulinus Paralysis

Motoneurons	Series of experiments	Orthodromic stimulation: amplitude of monosynaptic EPSPs (in mV)	Orthodromic stimulation: amplitude of polysynaptic EPSPs (in mV)	Antidromic stimulation: amplitude of APs (in mV)	Antidromic stimulation: duration of APs (in msec)	Direct stimulation: amplitude of APs (in mV)	Direct stimulation: duration of APs (in msec)	Membrane potential (in mV)	Input resistance (in mΩ)	Threshold current for direct stimulation (in nA)	Critical depolarization level (in mV)
Phasic	Control	7,21±0,14 (7)	7,48±0,47 (30)	74,93±2,66 (31)	1,73±0,13 (31)	65,52±1,91 (19)	1,90±0,21 (19)	63,13±1,65 (23)	1,13±0,01 (19)	4,76±0,57 (19)	4,61±0,59 (19)
	Paresis	6,18±0,57 (8)	5,02±0,43 (37)	68,61±2,75 (19)	2,16±0,17 (19)	63,00±1,77 (17)	2,17±0,15 (17)	52,43±2,03 (17)	0,86±0,11 (17)	8,03±1,31 (17)	5,69±0,62 (17)
	P	>0,1	<0,001	>0,2	<0,05	>0,2	>0,2	<0,001	<0,02	<0,05	>0,05
	Paralysis	4,87±1,04 (12)	4,64±0,14 (57)	66,66±1,90 (44)	2,19±0,09 (44)	54,58±3,40 (24)	2,59±0,14 (24)	50,77±1,29 (45)	0,69±0,03 (26)	12,92±1,41 (26)	6,65±0,46 (26)
	P	<0,05	<0,001	<0,02	<0,001	<0,01	>0,001	<0,001	<0,001	<0,001	<0,01
Tonic	Control	8,12±0,34 (5)	9,05±0,65 (14)	76,12±6,77 (8)	1,88±0,14 (8)	79,00±7,82 (5)	1,86±0,19 (5)	63,72±2,80 (4)	1,72±0,18 (12)	4,45±0,64 (12)	6,88±1,04 (12)
	Paresis	7,06±0,57 (5)	8,32±0,83 (12)	76,06±5,12 (10)	1,89±0,13 (10)	73,45±6,64 (10)	1,87±0,32 (10)	61,00±3,04 (5)	1,59±0,21 (10)	5,00±0,59 (10)	7,99±0,91 (10)
	P	>0,1	>0,2	>0,5	>0,5	>0,5	>0,5	>0,5	>0,5	>0,5	>0,5
	Paralysis	6,58±0,69 (8)	7,41±0,76 (19)	76,07±4,31 (14)	1,86±0,15 (14)	68,17±4,43 (12)	1,91±0,13 (12)	56,20±4,08 (5)	1,50±0,25 (11)	6,00±1,51 (11)	7,33±1,48 (11)
	P	>0,05	>0,1	>0,5	>0,5	>0,2	>0,5	>0,2	>0,2	>0,2	>0,5

Legend. P – Significance of difference compared with control; number of cells in which the particular parameter was studied is shown in parentheses.

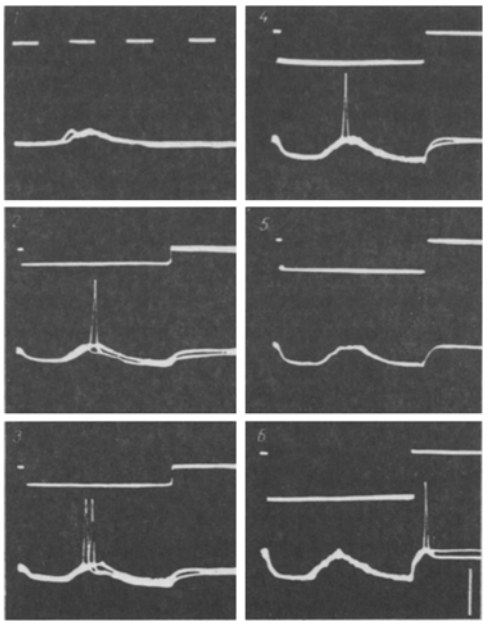

Fig. 1. Response of motoneurons to anodal polarization during local botulinus poisoning: 1) initial polysynaptic EPSP of motoneuron in nucleus of gastrocnemius muscle 8 days after injection of toxin. Strength of hyperpolarizing current rises from 2 to 6. Top beam – time marker, 10 msec (1), polarizing current (2-6); bottom beam – response of motoneuron. Calibration: 25 mV, 25 nA.

of the phasic motoneurons were increased (Table 1). Against this background the amplitude of potentials generated in response to antidromic stimulation was unchanged and only their duration was increased. Despite the preservation of neuromuscular transmission at this stage [2], about one-third of all cells recorded in the motor nuclei of the synergist muscles in response to mono- and polysynaptic stimulation lost their ability to generate spikes, and they responded to orthodromic stimulation, regardless of the strength of stimulation of the afferent fibers, by generation of an EPSP only.

During the development of paralysis of the skeletal muscles more severe changes were observed on the side of poisoning in the membrane potential, input resistance, threshold depolarizing currents, and critical depolarization level in the group of phasic motoneurons. These cells responded to antidromic and direct stimulation by generating low-amplitude, prolonged action potentials (APs). Orthodromic stimulation evoked no AP generation in more than half of the neurons recorded, although low-amplitude mono- and polysynaptic EPSPs appeared. As regards the excitability of the tonic motoneurons, even in the stage of paralysis of the skeletal muscles they showed no substantial changes and the motoneurons responded by spike discharges to orthodromic stimulation.

Botulinus toxin thus primarily lowers the polarization level of phasic motoneurons, evidently by increasing the permeability of their surface membranes. The facts that against the background of artificial hyperpolarization the damaged cells regained their ability of discharging in response to synaptic stimulation (6 tests), and could generate a spike as an off response after high values of current (Fig. 1) indicate that disturbance of regenerative electrogenesis in phasic motoneurons probably takes place by a mechanism of inactivation of the sodium channels of the electrically excitable membrane [4].

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