LEVEL OF ACTIVITY OF MOTOR NEURONS OF THE SPINAL CORD AND CHANGES IN RNA CONCENTRATION IN THEIR CYTOPLASM IN LOCAL TETANUS

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Yu. Ya. Geinisman, G. N. Kryzhanovskii, and A. A. Polgar

Laboratory of Morphology (Head, Professor M. M. Aleksandrovskaya),
Institute of Higher Nervous Activity and Neurophysiology, USSR Academy of Sciences,
and Laboratory of Infectious Pathology of the Nervous System (Head, Dr. Med. Sci. G. N. Kryzhanovskii),
Institute of Normal and Pathological Physiology, USSR Academy of Medical Sciences, Moscow
(Presented by Active Member AMN SSSR V. V. Parin)
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A characteristic feature of the functional changes in the spinal reflex apparatus in tetanus is the disturbance of various forms of post-synaptic (hyperpolarization) inhibition [10], as a result of which a sharp increase takes place in the polysynaptic stimulation of the motor neurons [10], associated with an increase in the transmitting capacity of the efferent outlet into the spinal cord [4, 5]. At the height of development of the disease, because of the intensive polysynaptic stimulation, the motor neurons are in a state of almost constant increased activity.

Long and intensive activity of the neurons is known to be accompanied by changes in ribonucleoprotein metabolism [1-3, 8, 14-16, 18, 19]. In this respect local tetanus offers an interesting model for the study of the histophysiological features distinguishing the state of the motor neurons when engaged in intensive and prolonged activity. The study of this problem is also of great importance to the understanding of certain aspects of the pathogenesis of tetanus.

The numerous investigations which have been made to study the morphological characteristics of the state of the motor neurons in tetanus, including the state of the Nissl's substance (giving indirect evidence of the level of nucleoprotein metabolism), have yielded conflicting results. More definite facts have been obtained in recent investigations [13, 20, 21] in which histochemical methods were used to study the RNA concentration in the neurons of the spinal cord in tetanus. A common failing of all these investigations was the lack of any parallel study of the level of activity of the neurons and of their ribonucleoprotein concentration.

The object of the present investigation was to compare the level of activity of the motor neurons in local tetanus with the changes in the RNA concentration in their cytoplasm at various stages of development of the pathological process.

EXPERIMENTAL METHOD

The state of activity of the motor neurons was assessed from the level of the electrical activity (EA) in the corresponding muscles. This form of analysis, of fundamental importance to the study of changes in the central reflex apparatus [11, 12], was used in these experiments because with the doses of toxin and the stages of the disease chosen, the changes in the region of the peripheral neuro-muscular apparatus, as previous investigations have shown [4], have no significant effect on the character of the electromyogram. The EA was recorded by means of a "Disa" electromyograph and electrointegrator.

Experiments were carried out on male albino rats weighing 200-230 g. Tetanus toxin (batch No. 587, N. F. Gamaleya Institute of Epidemiology and Microbiology, AMN SSSR) was injected in a dose of $^1/_{20}$ MLD by several

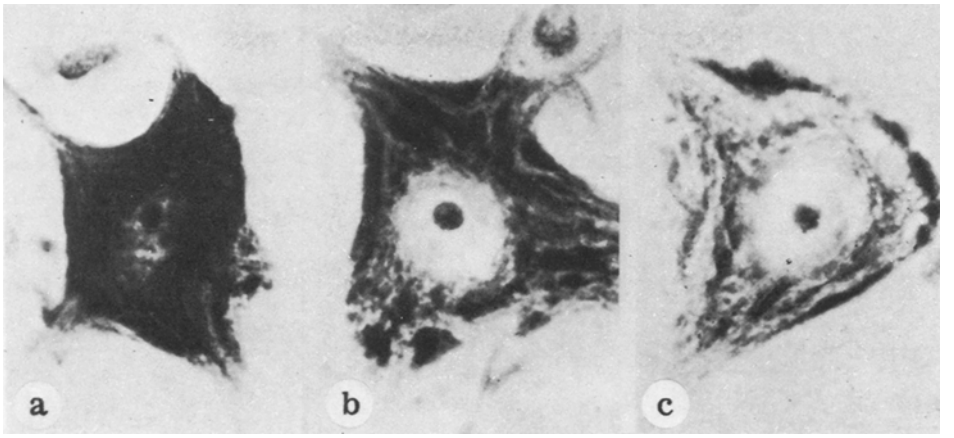

Fig. 1. Motor neurons with a high (a), medium (b), and low (c) concentration of RNA in the cytoplasm. Lateral nucleus of the anterior horn of the 6th lumbar segment of the spinal cord. Brachet's reaction for RNA. Objective 90×, ocular 5×.

punctures into all the muscles of the leg. By giving the toxin in this way, it ensured that the toxin reached the spinal cord at the same time from the leg muscles along the regional neural pathways [4, 6], and this led to the relatively uniform involvement in the pathological process of the motor neurons of the corresponding segments on the side of injection of the toxin. The EA was recorded 30, 48, and 72 h after injection of the toxin, i.e., at the beginning of the appearance of the clinical signs of an increase in muscle tone (30 h) and at the point of its maximal development (72 h).

At the same times samples of material were taken for a parallel histochemical investigation from rats killed by decapitation (20 animals in each group). A control rat (a healthy animal) was sacrificed along with the experimental rat. The control animals were chosen individually to match the experimental rats in weight. The lumbar enlargement of the spinal cord of the control and experimental animals were fixed together in Carnoy's fluid and embedded in paraffin-celloidin. Continuous series of sections were cut (7 μ in thickness) from the 5th lumbar and 1st sacral segments. Every 20th segment of the spinal cord of the control and experimental rat was fixed to the same slide and Brachet's histochemical reaction for RNA was carried out with control treatment with ribonuclease. The motor neurons of the lateral nucleus of the anterior horn were investigated on the side of injection of the toxin, and only those motor neurons whose nucleolus was visible in the plane of each particular section were considered.

The concentration and absolute content of cytoplasmic RNA were taken as the main indices of the level of RNA metabolism in the spinal motor neurons. The changes in the concentration of cytoplasmic RNA were determined from the changes in the relative proportions of motor neurons containing different concentrations of RNA.

Three classes of motor neurons were distinguished in the spinal cord of both the control and the experimental animals, differing in their concentration of cytoplasmic RNA (Fig. 1): motor neurons with a high (a), a medium (b), and a low (c) concentration of RNA in the cytoplasm. The number of motor neurons of each of these three classes was counted and expressed as a percentage of the total number of investigated motor neurons. The results were analyzed by statistical methods.

The content of cytoplasmic RNA cannot be determined directly by means of histochemical methods, but it is possible to determine histochemically the relative concentration of RNA in the cytoplasm of the motor neurons in the experimental and control animals: the absolute content of cytoplasmic RNA was expressed as the product of concentration and size of the cytoplasm. If the RNA concentration was constant, with an increase in the size of the cytoplasm the RNA content increased, and vice versa. In this way the changes in the RNA content in the experimental series were judged from changes in the dimensions of the cytoplasm of the motor neurons containing the same concentration of cytoplasmic RNA—high, medium, or low. The dimensions of the cytoplasm were determined as the difference between the areas of cross section of the cell body and nucleus. The areas of cross section of the body and nucleus of the motor neurons were calculated by multiplying their greatest and smallest diameters. These diameters were measured with a screw-adjusting ocular micrometer with a total magnification of 450×. The measurements

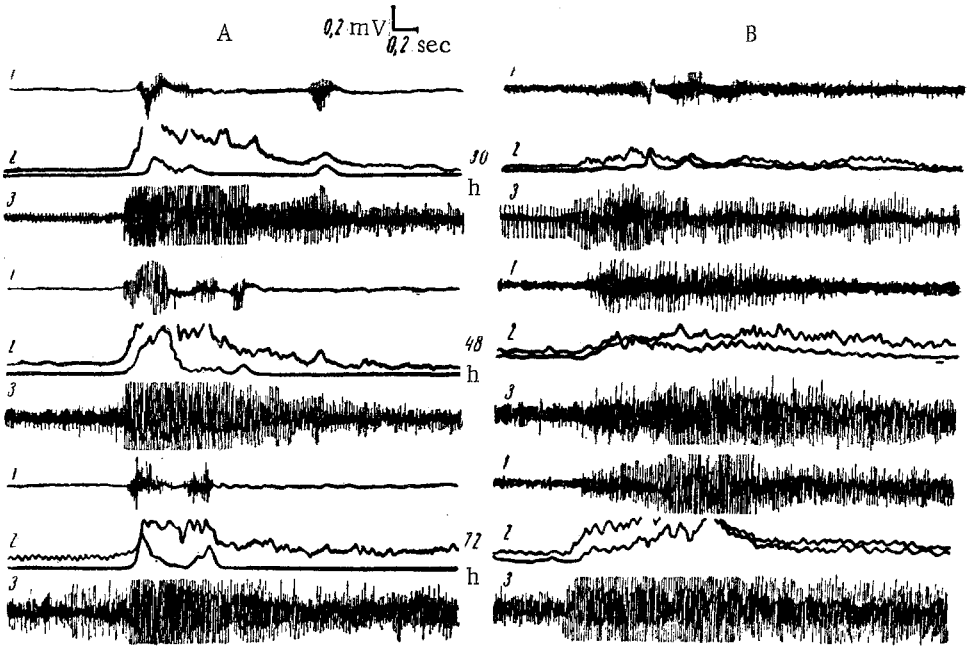

Fig. 2. Electrical activity (EA) in leg muscles of albino rats at different times after injection of $^1/_{20}$ MLD tetanus toxin. A) Recording of EA in gastrocnemius muscles on the side of injection of the toxin (3) and on the opposite side (1); B) recording of EA in anterior (1) and posterior (3) group of leg muscles on the side of injection of toxin. Curve 2 in A and B is the tracing of the integrator of action potentials from the 1st and 3rd channels.

were made on 5 sections of the spinal cord taken from each of the 5 control and 5 experimental animals at the particular period. The numerical results were treated by statistical methods, using Student's criterion.

EXPERIMENTAL RESULTS

A considerable increase in the EA was observed in the muscles of the left leg (Fig. 2), 30 h after injection of the tetanus toxin. It was particularly marked after provocation of a motor reaction. The burst of evoked EA had a very characteristic property: it continued after the action of the stimulation had ceased, and subsided slowly. As the disease developed this after-activity became still more obvious and prolonged, and at the height of development of the disease it took the form of a continuous background, imitating spontaneous activity. The after EA is evidence of the state of the continuously acting motor neurons and of the connected internuncial neurons as a result of the disturbance of the inhibitory mechanisms [4]. For this reason different stimuli produced prolonged excitation of the internuncial neurons and motor neurons. A practically constant increased activity was observed in all the leg muscles 72 h after injection of the toxin (see Fig. 2B), demonstrating that the motor neurons were in a state of almost constant excitation. However, at the beginning of the disease also (30 h after injection of the toxin), an increase in the background activity of the motor centers was observed.

At no stage of the development of local tetanus were statistically significant changes found in the RNA concentration in the cytoplasm of the motor neurons of the functionally affected motor centers. The mean dimensions of the cytoplasm of the motor neurons with a high RNA concentration showed statistically significant changes (an increase) 30 h after injection of the toxin (see the table), indicating an increase in the RNA content in the cytoplasm of these motor neurons. The relative content of cytoplasmic RNA in this particular class of motor neurons fell 48 h after injection of the toxin and returned to its initial level, at which it still remained after 72 h.

Comparison between the data describing the state of activity of the motor neurons with the RNA content in their cytoplasm showed that the initial increase in the activity of the motor neurons was accompanied by an increase in the synthesis of RNA, which exceeded its utilization. Later, evidently, the increased activity of the motor neurons was accompanied not only by increased synthesis, but also by increased utilization of RNA, so that the latter exceeded the former and, consequently, the total RNA content fell to its initial level; at this stage some degree of

Changes in Dimensions of Cytoplasm of Motor Neurons in Local Tetanus (M±m)

Concentration of RNA in motor neurons	Mean dimensions of cytoplasm (in μ^2)		P	Mean dimensions of cytoplasm (in μ^2)		P	Mean dimensions of cytoplasm (in μ^2)		P
	control	30 h after injection of toxin		control	48 h after injection of toxin		control	72 h after injection of toxin	
High	452 ± 18 $n = 35$	525 ± 20 $n = 27$	0,01	423 ± 22 $n = 23$	405 ± 17 $n = 20$	0,4	481 ± 18 $n = 28$	488 ± 19 $n = 25$	0,7
Medium	639 ± 14 $n = 111$	662 ± 15 $n = 82$	0,2	659 ± 12 $n = 150$	654 ± 20 $n = 85$	0,8	815 ± 20 $n = 109$	784 ± 18 $n = 88$	0,2
Low	799 ± 27 $n = 46$	794 ± 49 $n = 31$	0,9	865 ± 34 $n = 53$	806 ± 21 $n = 44$	0,1	950 ± 20 $n = 68$	995 ± 29 $n = 41$	0,2
All investigated	644 ± 14 $n = 192$	666 ± 16 $n = 140$	0,2	683 ± 14 $n = 226$	666 ± 16 $n = 149$	0,4	814 ± 16 $n = 205$	792 ± 18 $n = 154$	0,3

Note: n is the number of motor neurons measured.

stabilization of the relationship between the synthesis and utilization of RNA took place. The results obtained are in agreement with those obtained by investigation of the effect of functional overloading of the neurons on their content of cytoplasmic RNA, showing that during adequate stimulation of the neurons the RNA content rises initially, as a result of its increased synthesis [2, 3, 14, 16, 18].

The present experiments were conducted with local tetanus, which was produced by relatively small doses of tetanus toxin and which was not complicated, like generalized tetanus, by secondary pathological processes associated with disturbances of respiration such as, for example, hypoxia which may give rise to a considerable fall in the content of cytoplasmic RNA in the neurons [7-9, 17]. Probably for this reason the results of this study differ from those reported by other workers [13, 20, 21], who found a decrease in the RNA level in the spinal neurons in tetanus, for their investigations were conducted on animals with ascending generalized tetanus, produced by comparatively large doses of toxin. It is possible that in severe tetanus, in the later stages of the disease, a negative balance between the synthesis and utilization of RNA may develop as a result of the excessively long activity and the influence of unfavorable secondary factors.

The facts described above show that the RNA content does not change in all the motor neurons in local tetanus; this is clear from the fact that not all the motor neurons undergo equally profound physiological changes, and it corresponds in general to the views previously expressed concerning the character of the changes in the spinal reflex apparatus in tetanus [4].

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