EFFECT OF ABOLITION OF INHIBITION BY TETANUS TOXIN ON CHANGES IN THE RNA CONTENT IN SPINAL MOTONEURONS CAUSED BY MOTOR ACTIVITY

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UDC 612.833.011:612.398.145.1-064

Rats were fatigued by making them swim for 40 min with a load equal to $1/11$ of their body weight. Some of the animals recieved an injection of tetanus toxin in a dose of 0.05 MLD into the muscles of the left leg 30 or 72 h before the experiment began. The RNA content in the cytoplasm of the spinal motoneurons was determined by cytospectrophotometry in UV light. Physical exertion of this type was found to reduce the RNA content in the cytoplasm of motoneurons of healthy rats substantially. The development of this change was prevented by injection of tetanus toxin, which abolishes inhibition of motoneurons. It is concluded that the inhibitory synaptic action lowers the RNA content in the activated neurons.

To understand the metabolic basis of neuron function it is important to know the intimate physiological processes with which changes in metabolism of neuronal RNA are connected. The object of this investigation was to determine the relationship between changes in RNA content and the character of synaptic influences in a postsynaptic neuron. For this purpose, spinal motoneurons were activated naturally (by physical exertion) under normal conditions or in tetanus poisoning, in which conduction is blocked in inhibitory synapsis [5-7]. At various stages of local tetanus, the increased afferent flow due to physical exertion thus took place against the background of developing blocking of inhibitory synaptic effects.

EXPERIMENTAL METHOD

Experiments were carried out on 40 albino rats (sexually mature males) weighing 180-220 g. In the experiments of series I the rats were forced to swim in water warmed to 33-34°C, carrying a load equal to $1/11$ of their body weight. The mean duration of swimming before submersion was 50 ± 7 min, according to observations on 20 rats. The animals for cytochemical analysis were killed by decapitation 40 min after the beginning of swimming. In the experiments of series II and III tetanus toxin (batch 587, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) was injected in a dose of 0.05 MLD into all groups of muscles of the left leg. Rats were killed for the cytochemical investigation at various times after injection of the toxin: 30 h (experiments of series II) on the appearance of the first electromyographic signs of increased muscle tone of the left hind limb, and 72 h (experiments of series III) on the development of marked muscular rigidity of the left hind limb, when a virtually constant increase in their activity was recorded [2]. In the experiments of series II and III some animals were subjected to the same physical exertion as those in series I for 40 min before sacrifice.

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TABLE 1. RNA Content in Cytoplasm of Spinal Motoneurons in Response to Different Forms of Their Activation

Form	Time after injection of toxin, in h	Number of neurons tested		Mean RNA content and error of mean (in pg)			Change caused by swimming (in percent of initial level)	P
		control	expt.	control	expt.	percent of control		
Swimming	—	164	154	593 ± 17	477 ± 15	80.4	−19.6	< 0.001
Local tetanus	30	207	157	593 ± 15	645 ± 21	108.8	—	> 0.2
Local tetanus	30	207	160	593 ± 15	556 ± 18	93.8	—	> 0.2
+swimming							−13.8	< 0.05
Local tetanus	72	201	159	577 ± 16	531 ± 16	92.0	—	> 0.1
Local tetanus	72	201	170	577 ± 16	546 ± 17	94.6	—	> 0.9
+swimming							+2.8	> 0.3

Groups of experimental and control rats, each consisting of five animals, were examined. The control rats were matched with experimental so that the difference between their body weight did not exceed 5 g. The control for the experiments of series I consisted of intact rats. The control for series II and III consisted of rats receiving injections of physiological saline into the muscles of the left leg. The lumbar enlargement of the spinal cord of the experimental and control animals received parallel histological treatment. The RNA content was determined in the cytoplasm of motoneurons of the ventrolateral nucleus of the anterior horn in segments L5-L6 (in the case of animals with local tetanus, only on the side of injection of the toxin). The method of photographic scanning cytospectrophotometry in UV light, fully described previously [1, 4], was used for this purpose. The χ^2 criterion for comparison of two empirical distributions was used as the measure of difference between the control and experimental results.

The results of the cytochemical analysis are summarized in Table 1. They show that physical exertion in the experiments on the healthy animals led to a substantial decrease in the RNA content in the cytoplasm of the spinal motoneurons. This change is not specific for activation of this type, because previously, in response to orthodromic stimulation of motoneurons, the same decrease in content of cytoplasmic RNA was found [1, 4]. Evidently the increased excitatory and inhibitory synaptic action was accompanied in these conditions by a reduced content of RNA in the postsynaptic neuron.

The role of inhibitory synaptic influences in the formation of the cytochemical changes in the spinal motoneurons in response to swimming was clearly revealed in the case when inhibition was abolished by tetanus toxin. The RNA content 30 h after injection of the toxin, when the disturbance of inhibition was relatively slight, was reduced by motor activity. However, compared with the original indices this change produced by swimming was smaller in the rats with local tetanus than in healthy animals. Motor activity 72 h after injection of the toxin, when the blocking of inhibitory effects was more marked, caused no decrease in the RNA content in the cytoplasm of the motoneurons compared with the initial level (local tetanus). Comparison of the cytochemical changes in the experimental and control series shows no correlation between the decrease in RNA content and hyperfunction of the motoneurons, for in this case when rats with local tetanus were swimming, even more marked changes would have been expected than when healthy animals were swimming. For this reason, it was evidently the inhibitory synaptic action which caused a decrease in the RNA content in the activated neurons during motor activity.

So far as excitatory synaptic action is concerned, it was difficult to judge its cytochemical effect from these experimental results. The reason was that swimming could lead to different degrees of change in the intensity of excitatory synaptic effects on motoneurons in rats with local tetanus. The increase in motoneuron activity in local tetanus during natural locomotion of the animal was evidently itself attributable to the better performance of the motoneuron pool in maintaining an unchanged background excitatory inflow as a result of the blocking of their inhibition. At the same time, even in the early stage of tetanus poisoning, ex-

citatory synaptic action on motoneurons could be intensified on account of the blocking of inhibition in polysynaptic chains. A more strict characteristic of excitatory synaptic effects is possible in experiments with a controlled level of afferent inflow. The indeterminacy of assessment of the intensity of synaptic action during natural activation of motoneurons also applies, in part, to inhibitory influences. However, in the case of tetanus poisoning, these inhibitory influences consistently undergo reduction, and this evidently leads to the corresponding cytochemical effect.

The results of this investigation, indicating that quantitative changes in the RNA content of motoneurons are dependent on the intensity of inhibitory synaptic action, are in agreement with recently published findings [1, 3, 8] showing that the onset of changes in RNA metabolism in an activated neuron are connected, not with the action potentials, but with synaptic influences. This suggests that the character of synaptic action can be regarded as a factor determining the state of nutrition of a functioning neuron.

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