CORRELATION BETWEEN PATTERN OF SYNAPTIC EFFECTS AND DIRECTION OF CHANGES IN RNA CONTENT IN SPINAL MOTONEURONS IN RATS

Yu.Ya. Geinisman, M.V. D'yakonova, G.N. Kryzhanovskii, V.N. Larina, V.N. Mats, and A.A. Polgar

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Spinal motoneurons were activated orthodromically or antidromically with preservation of inhibitory synaptic influences (experiments on healthy rats) and after blocking these influences by tetanus toxin (experiments on rats with local tetanus). The RNA content in the cytoplasm of the α-motoneurons was measured by cytospectrophotometry in UV light. The results showed no quantitative changes in the RNA of the motoneurons during action potential generation. Meanwhile the content of neuronal RNA depends on the character of synaptic influences. The RNA content in the motoneurons rises in response to excitatory and falls in response to inhibitory synaptic action. The possible mechanisms of the observed cytochemical changes in the RNA content during synaptic excitation and inhibition of motoneurons are discussed.

INTRODUCTION

The writers showed earlier [13, 3] that changes in the RNA content in the spinal motoneurons of rats do not appear in response to antidromic excitation, but are found in the case of orthodromic activation, i.e., when the synaptic action is especially well marked. It was accordingly concluded that the synaptic effects give rise essentially to changes in RNA metabolism in the postsynaptic neuron. The same conclusion was drawn by Kernell and Peterson [15] after comparing the effect of direct stimulation and of synaptic activation. They found that only synaptic activation leads to an increase in the rate of incorporation of labeled precursors into the RNA of giant Aplysia neurons. Basically similar changes were observed in a study of the rat sympathetic ganglion [14]. The intensity of RNA synthesis in the tissue of the isolated ganglion was changed by synaptic activation or by application of acetylcholine, while blocking the action potentials with tetrodotoxin did not prevent the changes induced by acetylcholine. Although the results of these investigations indicate that synaptic influences and not action potentials are responsible for the development of changes in RNA metabolism in the postsynaptic neuron, other results which are not in agreement with this hypothesis have been obtained. It has been found that the generation of action potentials by a neuron in response to nonsynaptic activation can be accompanied by changes in RNA metabolism [1, 2].

The writers have attempted to assess the contribution of action potentials and of excitatory and inbitory synaptic influences in the formation of changes in RNA metabolism in the postsynaptic neuron. For this purpose the RNA concentration was investigated in the spinal motoneurons in response to their antidromic and orthodromic activation, which was applied during the action of tetanus toxin, which has the property [6-8, 10] of depressing inhibitory influences in the chains of spinal reflexes and, in particular, of abolishing postsynaptic inhibition of motoneurons. In this way it was possible to determine the separate cytochemical effects of: 1) action potentials — by the development of changes in response to antidromic excitation; 2) excitatory synaptic influences — by comparing the changes produced by orthodromic and antidromic stimulation; and 3) inhibitory synaptic influences — by the difference in the cytochemical response to activation when the inhibitory mechanisms were intact and when they were abolished by tetanus toxin.

Institute of Higher Nervous Activity and Neurophysiology, Academy of Sciences of the USSR. Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Neirofiziologiya, Vol. 4, No. 4, pp. 418-422, July-August, 1972. Original article submitted December 27, 1971.

TABLE 1. Cytoplasmic RNA Content of Spinal Motoneurons in Rats

Activation of motoneurons	Duration of stimulation, min	Number of neurons tested		Mean RNA content, pg, and error of mean		% of control	P	% of background level	P
		control	experiment	control	experiment				
Rats with local tetanus	—	201	159	577±16	531±16	92,0	>0,1	—	—
Antidromic in rats:									
with local tetanus	10	151	186	587±19	544±14	92,7	>0,7	102,4	>0,3
	40	155	153	576±16	539±14	93,6	>0,3	101,5	>0,7
healthy	10	151	153	587±19	596±17	101,5	>0,2	—	—
	40	155	150	576±16	541±17	93,9	>0,5	—	—
Orthodromic in rats:									
with local tetanus	10	153	150	582±18	662±20	113,8	<0,02	124,7	<0,001
	40	150	151	596±18	636±19	106,7	>0,2	119,8	<0,02
healthy	10	153	146	582±18	529±19	90,9	>0,3	—	—
	40	150	156	596±18	498±16	83,6	<0,02	—	—

METHOD

Experiments were carried out on sexually mature male albino rats weighing 180-240 g. The experiments in which antidromic or orthodromic activation of the motoneurons was used were carried out on unanesthetized spinal animals. On the day before the experiment complete transection of the spinal cord was performed at the level T1-T2 under superficial ether anesthesia, and immediately before the beginning of stimulation the ventral and dorsal roots of segments L4-S1 were divided. The left ventral (for antidromic activation) or dorsal (for orthodromic) roots of segments L5-L6 were placed on electrodes and stimulated for 10 or 40 min with square pulses at a duration of 2 msec and frequency 1/sec. The strength of the current was chosen so as to obtain maximal amplitude of the combined mono- and polysynaptic responses for producing activation of the majority of motoneurons. Both healthy rats and rats with local tetanus were used as the experimental animals. To induce local tetanus 0.05 MLD of tetanus toxin was injected into all groups of muscles of the left leg. Stimulation began 72 h after injection of the toxin, when a definite picture of local tetanus of the left hind limb was observed, with marked muscular rigidity and a virtually constant increase in electrical activity in all muscles of the left leg. The same manipulations were carried out with the control animals as with the experimental, except for the injection of toxin and the electrical stimulation. In addition, to determine the level of excitation before stimulation, rats with local tetanus also were studied (72 h after injection of tetanus toxin in the above dose into the muscles of the left leg). Rats given an injection of physiological saline acted as the control to the last series of experiments.

For cytochemical analysis the control and experimental animals were decapitated and the lumbar enlargements of their spinal cord were simultaneously fixed by Carnoy's method and embedded in paraffin wax. The RNA content in the cytoplasm of the α-motoneurons of the ventrolateral nucleus of the left anterior horn was determined in serial transverse sections through segments L5-L6. A photographic scanning modification of the method of UV cytospectrophotometry was used (details were described previously [4,13]). The mean values of the RNA content were determined for the groups of rats (five in each group), and as a rule at least 30 motoneurons were studied in each animal. The significance of the differences between the groups of experimental and corresponding control rats was assessed by the $\varkappa^2$ criterion for comparing two empirical distributions.

RESULTS

The results are given in Tables 1 and 2. The content of cytoplasmic RNA in the motoneurons of the rats with local tetanus on which no operations had been performed on the spinal cord (subsequently taken as the background level) showed no significant changes, although a tendency was observed for it to fall below the control values.

During antidromic activation of the motoneurons in animals previously receiving tetanus toxin the content of neuronal RNA was not significantly lower than the control and was virtually indistinguishable

TABLE 2. Correlation between Pattern of Synaptic Activation and Changes in RNA Content of Motoneurons

Activation of motoneurons	Synaptic influence: excitatory	Synaptic influence: inhibitory	Synaptic effect assessed	RNA content after stimulation for: 10 min, % of background level	10 min, relative change, %	40 min, % of background level	40 min, relative change, %
Orthodromic in healthy rats	+	+	Excitatory + inhibitory	90.9	−12	83.6	−18
Antidromic in rats with local tetanus	−	−		102.4		101.5	
In rats with local tetanus:							
orthodromic	+	−	Excitatory	124.7	+ 22	119.8	+ 18
antidromic	−	−		102.4		101.5	
Orthodromic in rats:							
healthy	+	+	Inhibitory	90.9	−34	83.6	−36
with local tetanus	+	−		124.7		119.8	
Antidromic in rats:							
healthy	−	+	Inhibitory	101.5	−1	93.9	−8
with local tetanus	−	−		102.4		101.5	

from the background level. During orthodromic activation after administration of tetanus toxin the content of cytoplasmic RNA in the motoneurons was higher than the corresponding control values. The difference was greater after stimulation for 10 min, and this was the only time when it was statistically significant. However, comparison of the RNA content with the background level showed a highly significant increase in neuronal RNA not only after relatively short (10 min), but also after more prolonged (40 min) orthodromic activation.

In the healthy animals antidromic excitation was not accompanied by significant changes in the RNA content in the cytoplasm of the motoneurons, in agreement with the results obtained in the experiments on rats with local tetanus. Orthodromic activation in the healthy animals led to a progressive decrease with time in the content of neuronal RNA, i.e., its effect was directly opposite to that recorded after administration of tetanus toxin.

DISCUSSION

The first fact to be noted was that during antidromic activation when inhibitory synaptic processes were inhibited by tetanus toxin the RNA content in the cytoplasm of the motoneurons is unchanged from its background level in local tetanus. For this reason, in the subsequent cytophysiological analysis no attempt was made to take account of the real number of action potentials generated, and instead the character of the synaptic influences was compared with the content of neuronal RNA. The results show that synaptic excitation and inhibition are accompanied by changes in opposite directions in the RNA content in the nerve cells (Table 2).

The increase in the RNA content in the motoneurons during synaptic excitation was evidently not due to migration of glial RNA into the neuron. Special measurements showed that the increase in the RNA content in the motoneurons was unaccompanied by any decrease in RNA in the surrounding glial cells. The change in the neuronal RNA content during synaptic excitation may therefore reflect increased RNA synthesis, its decreased breakdown, or slowing of the rate of RNA transport along the axon. Only the first of these mechanisms is confirmed by the experimental data showing an increase in the rate of incorporation of labeled precursors into the neuronal RNA as a result of synaptic excitation [9, 14, 15]. This suggests that synaptic excitation of neurons is a metabolically active process leading to an increase in the intensity of RNA synthesis and of protein synthesis in which RNA participates.

The decrease in the RNA content in the motoneurons during synaptic inhibition is evidently not the result of inhibition of RNA synthesis. Knowing the life span of the cytoplasmic RNA of brain tissue [11, 12],

it is easy to calculate the maximal rate of synthesis of this RNA for the rat spinal motoneuron: 0.7 pg/min. Not even a total block of the synthesis of cytoplasmic RNA could reduce its content in the motoneuron by roughly one-third, i.e., by more than 150 pg in 10 min, as took place when inhibitory synaptic influences were strengthened during orthodromic activation (Table 2). Two possible explanations of the decrease in the content of neuronal RNA under the influence of postsynaptic inhibition remain: an increase in its transport along the axon and an increase in its breakdown. Whichever is true, postsynaptic inhibition of the neurons must also be regarded as a metabolically active process.

Although, from the results of this investigation, there is no reason to doubt that close correlation exists between the trans-synaptic phenomena and neuronal RNA metabolism, the actual mechanisms of these correlations still await explanation. Recent observations have shed some light on the problem. It is known, for instance, that acetylcholine modifies the RNA metabolism in nerve tissue [5,16], leading to the same changes in the rate of RNA synthesis as occur through synaptic stimulation [14]. It can therefore be assumed that the action of mediators, possibly effected through cyclic AMP [17], is the factor which triggers the development of changes in RNA metabolism in the synaptically activated neuron.

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