HISTOCHEMICAL STUDY OF THE ACTION OF ACTINOMYCIN 2703 ON RNA METABOLISM OF SPINAL MOTONEURONS DURING INCREASED FUNCTION

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The RNA content in the cytoplasm of the spinal motoneurons of mice fatigued by swimming and in mice receiving actinomycin 2703 did not differ significantly.

Injections of actinomycin shortened the duration of swimming by the animals by 2.5 times. The RNA content in the motoneurons containing average and low concentrations of RNA was lowered in these animals.

It is concluded that under the influence of actinomycin the cycle of metabolic recovery processes in functioning neurons is disturbed.

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It may be regarded as proven that increased functional activity of neurons is accompanied by changes in the content of RNA and proteins in their cytoplasm. During adequate stimulation of neurons, as a rule, synthesis predominates over breakdown, leading to an increase in the content of cytoplasmic RNA and protein [2-4, 7, 8, 11-13, etc.).

However, the significance of these changes in RNA and protein metabolism for performance of the specific activity of neurons has not been explained. To investigate this problem, it is useful to compare changes in the chemical properties and function of the neurons after administration of inhibitors of RNA and protein synthesis. For example, investigation of preparations of the crab stretch receptor showed [9] that under the influence of actinomycin D the synthesis of RNA in these receptor neurons is completely blocked and its total content is diminished, but despite this the cells continue to generate action potentials. On the basis of this finding it has been postulated that impulse generation and RNA synthesis in neurons are independent processes.

In the present investigation an attempt was made to investigate in the intact organism changes in the RNA content in the cytoplasm of motoneurons which generate action potentials in response to repeated natural stimulation and conduct impulses, as a result of the action of an inhibitor of RNA synthesis, namely actinomycin 2703.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male albino mice weighing 18-25 g. The animals were divided into four groups: group 1—control animals; group 2—mice receiving actinomycin 2703 in a dose of 3.3 μg by intramuscular injection daily for 5 days; group 3—animals compelled to swim daily for 5 days in water at a temperature of 37° until the first time their head became submerged; group 4—mice which swam under the same conditions and, in addition, received repeated injections of actinomycin 2703 in the above dose.

For histochemical investigation the mice of groups 3 and 4 were sacrificed by decapitation immediately after the end of the last (5th) experiment with swimming. The animals of groups 1 and 2 were sacrificed at the same time, the experimental mice (of groups 2, 3, and 4) being chosen so that they did not differ in weight from the corresponding controls.

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TABLE 1. Dimensions of Cytoplasm of Motoneurons

RNA concentration in motoneurons	Mean area of cytoplasm (in μ^2)		P_{1-2}	Mean area of cytoplasm (μ^2)	P_{1-3}	Mean area of cytoplasm (μ^2)	P_{1-4}
	group of animals						
	1	2		3		4	
High	179±10 n=44	193±10 n=30	0,30	182±9 n=43	0,99	183±10 n=44	0,90
Average	249±9 n=160	254±7 n=164	0,10	240±7 n=176	0,30	230±8 n=176	0,05
Low	339±10 n=30	344±16 n=40	0,30	333±12 n=29	0,95	284±13 n=30	0,05
Mean	247±7 n=234	262±8 n=234	0,70	241±6 n=248	0,70	228±6 n=250	0,05

Note. n represents number of motoneurons measured.

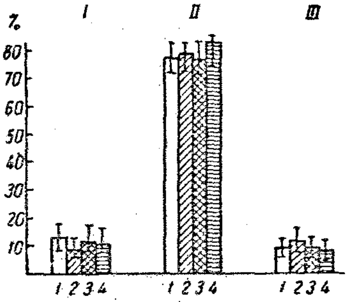

Fig. 1. Relative proportions of motoneurons with high (I), average (II), and low (III) concentrations of cytoplasmic RNA. 1, 2, 3, 4) Groups of animals.

Not later than 3 min after sacrifice, the lumbar enlargments of the spinal cord were fixed in Carnoy's fluid and embedded in paraffin wax. To establish identical conditions of histochemical treatment of the control and experimental material, the lumbar enlargements taken from 4 animals (one from each group) were fixed at the same time and embedded in one block. The paraffin blocks were cut into series of transverse sections 7 μ in thickness. The histochemical reaction for RNA with gallocyanin and chrome alum was carried out by Einarson's method.

The motoneurons of the lateral nucleus of the ventral horn, innervating the limb muscles, were investigated. Changes in the concentration of cytoplasmic RNA were determined from changes in the number of motoneurons containing high, average, and low RNA concentrations intheir cytoplasm. For these counts every 40th section of the lumbar enlargement was used. In each of the four groups of animals at least 500 motoneurons were investigated. For statistical analysis of the data a 95% confidence interval was used.

The trend of the changes in cytoplasmic RNA content was detected from changes in the dimensions of the cytoplasm of motoneurons possessing the same RNA concentration (high, average, or low) as the control. To determine the dimensions of the cytoplasm, in each 80th section of the lumbar enlargement the cross section of the body and nucleus of the motoneurons was measured by means of an ocular micrometer and the sizes of the cytoplasm expressed as their difference. The significance of the differences between the control and experimental data was estimated statistically using the χ^2 criterion for comparing two empirical samples.

EXPERIMENTAL RESULTS

Injection of actinomycin into the animals of group 2 produced no significant changes in their general conditions and weight. In the animals of group 3 the mean duration of swimming on the 5th day fell from 91 to 68 min, and in the animals of group 4 from 88 to 26 min. Consequently, actinomycin reduced the duration of swimming by approximately 2.5 times.

Histochemical investigation showed that the relative proportion of motoneurons with different concentrations of cytoplasmic RNA did not change significantly in the experimental animals (Fig. 1).

If the concentration of cytoplasmic RNA was constant, changes in its content were directly proportional to changes in the volume of the cytoplasm. The results of determination of the dimensions of the cytoplasm of the motoneurons are given in Table 1. There results show that in the animals of groups 2 and 3 no statistically significant changes were found in the dimensions of the cytoplasm both of the investigated motoneurons as a whole and of neurons belonging to separate classes.

In the animals of group 4 a statistically significant decrease was found in the dimensions of the cytoplasm of all the investigated motoneurons (by 8%). This change took place because of a decrease in the size of the cytoplasm of the motoneurons with average and low RNA concentrations (by 8 and 17% respectively), indicating a decrease in the RNA content in their cytoplasm. The onset of these changes may be attributed to the fact that actinomycin not only inhibits the synthesis of RNA required for the formation of new protein, but also disturbs its transport from nucleus to cytoplasm [6]. The RNA deficiency thus created in the cytoplasm may have the result that the synthesis of cytoplasmic proteins does not completely compensate for their breakdown, and as a result the dimensions of the cytoplasm of the motoneurons decrease.

However, the question arises, why this effect of actinomycin should be manifested only when given against the background of a repeated functional load. At this point it should be emphasized that under the conditions of increased function actinomycin has a selective action on the RNA content and area of the cytoplasm of motoneurons with average and low RNA concentration, whereas motoneurons with a high RNA concentration are indistinguishable as regards these indices from those in the animals of the control group. The selective action of actinomycin can be understood if the motoneuron pool is regarded as a system of units functioning in turn during repeated stimulation. In this sytem, motoneurons with a high RNA concentration may behave as working elements. This assumption is supported by the fact that with an increase in functional activity only of motoneurons with a high RNA concentration, there is an increase in the number of cells of the perineuronal glia [1], which are donors of enzymes and nucleotides for intensively working neurons [10, 14, etc.]. Motoneurons with average and low RNA concentration may play the role of reserve elements, existing in different phases of recovery of cells whose protein structures have become exhausted in the course of function. After the end of stimulation, motoneurons which have taken part in the work are added to the store of reserve elements, and these in turn, during subsequent activation, become functioning motoneurons and their RNA concentration is then high. Confirmation of this hypothesis is given by data indicating that in some motoneurons with average and low RNA concentrations, the RNA concentration becomes high during a single phase of intensive muscular work [5].

In face of the foregoing facts it is logical to assume that particularly intensive renewal of protein structures and enzyme systems, accompanied by marked activation of synthesis of RNA and proteins, takes place in motoneurons with average and low RNA concentrations during repeated functioning. For that reason, only when a repeated functional load is applied does actinomycin give rise to a decrease in RNA content and in the area of the cytoplasm of these regenerating motoneurons. As a result, under the influence of actinomycin, the cycle of regenerative metabolic processes in the motoneurons is disturbed, and during repeated increases of functional activity this may limit the possibility of interchange of these cells and may prevent the incorporation of new elements in the work. This may probably be one of the causes of the sharp decrease in duration of swimming in the animals receiving actinomycin.

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