MORPHOLOGY AND PATHOMORPHOLOGY

CHANGES IN THE RNA OF NEURONS AFTER A SINGLE FUNCTIONAL LOAD

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UDC 612.822.2:612.398.145

When the literature dealing with changes in the RNA of neurons as a result of their function is analyzed, attention is drawn to observations of changes in the concentration of the cytoplasmic RNA which persist for many hours [1-3] or even several weeks [4, 5] after a single functional load. However, these observations do not explain how the changes in the RNA of the neurons develop in the period after the functional load — whether discretely, gradually, or cyclically. Yet the solving of this problem would help toward the understanding of the role of RNA in maintaining the functional activity of the neurons.

If was, therefore, decided to make a systematic investigation of the changes in the RNA of the neurons immediately and at various late periods after a single functional load.

EXPERIMENTAL METHOD

The motor neurons of the lateral nucleus of the ventral horn of the spinal cord innervating the limb muscles were investigated in rate sacrificed immediately and at intervals of 2, 4, 8, and 12 h, and 1, 3, 5, 7, 9, 11, 13, 15, 17, and 20 days after swimming for 40 min. Altogether 90 animals was used in the experiments (3 experimental and 3 control rats at each time). After fixation in Carnoy's fluid and embedding in paraffin-celloidin, serial transverse sections of the lumbar enlargement of the spinal cord were cut to a thickness of 7 μ. Every 10th section taken from the experimental and corresponding control animals was glued to the same slide and treated histochemically for RNA by Brachet's method with a ribonuclease control. It was impossible to obtain precise quantitative information of the changes in RNA concentration by this method [8], but their trend could be established, which was what was required in this investigation.

To determine the trend of the changes in the cytoplasmic RNA concentration the number of motor neurons with high, average, and low concentrations of RNA in their cytoplasm was counted in every 10th section through the lumbar enlargement (Fig. 1). Altogether 500-700 motor neurons were investigated at each time in the sections from the experimental and the corresponding control animals.

EXPERIMENTAL RESULTS

In the control animals the motor neurons with high, average, and low RNA concentrations amounted on the average to 21, 61, and 18% of the total number of motor neurons investigated. The changes in the number of motor neurons with the different RNA concentrations after swimming are indicated in Fig. 2a, where the corresponding control level is taken as 100. As Fig. 2a shows, immediately after the end of swimming, the number of motor neurons with a high RNA concentration increased, suggesting an increase in the RNA concentration. On the 3rd and 5th days after swimming the number of motor neurons with a low RNA concentration increase, i.e., the RNA concentration fell. Finally, on the 11th and 15th days the number of motor neurons with a high RNA concentration rose again. Subsequently, the RNA concentration was indistinguishable from the control level.

To be able to judge the changes in the cytoplasmic RNA content, the dimensions of the cytoplasm of the motor neurons must be known. These dimensions were expressed as the difference between the cross sections of the cell body and the nucleus, measured on every 20th section through the lumbar enlargement by means of an ocular micrometer. At every investigated time 150-200 motor neurons were measured from both the experimental and the corresponding control animals.

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(Presented by Active Member of the Academy of Medical Sciences of the USSR V. V. Klosovskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 7, pp. 103-106, July, 1967. Original article submitted October 15, 1965.

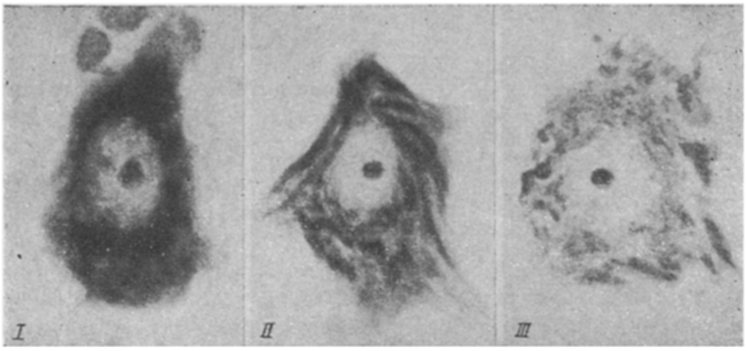

Fig. 1. Motor neurons with high (I), average (II), and low concentrations of RNA in the cytoplasm. Lumbar enlargement of the spinal cord of a control rat. Brachet's reaction for RNA. Ocular 10×, objective 90×.

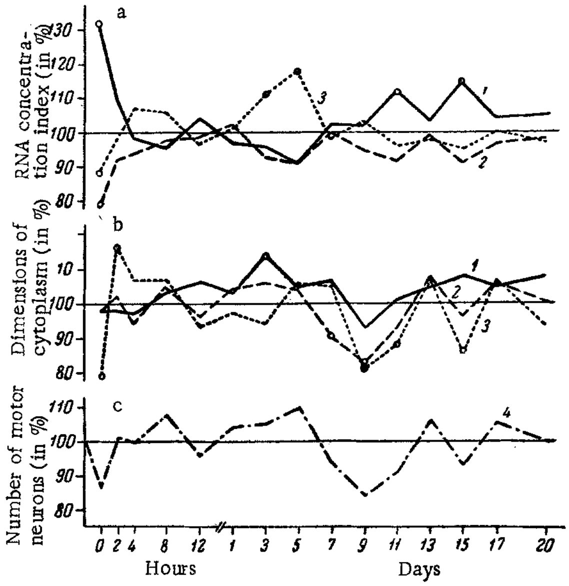

Fig. 2. Changes remaining in RNA of spinal motor neurons after swimming. a — Changes in number of motor neurons with different concentrations of cytoplasmic RNA; b — changes in dimensions of cytoplasm of motor neurons with different concentrations of cytoplasmic RNA; c — changes in concentration index of cytoplasmic RNA, 100 — concentration level; 1) motor neurons with high RNA concentration; 2) motor neurons with average RNA concentration; 3) motor neurons with low RNA concentration; 4) curve of changes in concentration index of cytoplasmic RNA; a dot inside the circle indicates that the change is statistically significant (P = 0.05 of less).

In the control series the mean area of the cytoplasm in the motor neurons with high, average, and low concentrations of RNA was 473, 626, and 799 μ^2 respectively (from average control measurements). The deviations of the mean dimensions of the cytoplasm of the motor neurons from the corresponding control level, taken as 100, are illustrated in Fig. 2b. As this figure shows, immediately after swimming a decrease was observed in the mean dimensions of the cytoplasm of the motor neurons with a low RNA concentration. This type of change was present on the 9th, 11th, and 15th days. The cytoplasm of the motor neurons with an average RNA concentration was reduced in size on the 7th and 9th days. These changes in the volume of the cytoplasm either coincided in time, or preceded the increase in RNA concentration. Finally, the cytoplasm of the motor neurons with a high RNA concentration was increased in volume on the 3rd day, and this change corresponded in time to a decrease in the RNA concentration.

To determine the trend of the changes in the cytoplasmic RNA concentration a conventional RNA concentration index was introduced. The index of the level of cytoplasmic RNA in the motor neurons with a high, average, and low RNA concentration was calculated as the product of the mean size of the cytoplasm of these motor neurons and their number expressed in per cent. The RNA concentration index in the whole pool of motor neurons was taken to be the sum of the RNA concentration indices in the motor neurons with different concentrations. Examination of the changes in the RNA concentration index compared with the corresponding control level (100%) shows clearly (Fig. 2c) that immediately after swimming the RNA level in the cytoplasm of the motor neurons fell. This was followed by two rises in the RNA concentration – 8 h and from 24 h until the 5th day after swimming ended. In the period from the 7th until the 11th day the RNA content in the cytoplasm of the motor neurons fell again, to an even greater degree than immediately after swimming. In the later periods, after small fluctuations, the RNA concentration returned to its control level on the 20th day. The possibility is not ruled out that after swimming changes in the cytoplasmic RNA concentration may persist for even longer.

From the results of this investigation two categories of functionally-induced changes in the RNA of the neurons may be distinguished: changes coinciding with the moment of stimulation and late changes arising after stimulation.

Characteristically the late changes in the neuronal RNA develop in a cyclic manner. The rise and fall of the total cytoplasmic RNA concentration alternate with each other (see Fig. 2c). A rise in the RNA level may indicate a relative increase in its synthesis. A fall in the RNA content evidently indicates an increase in RNA breakdown, which exceeds its synthesis. The cyclic character of the late changes in RNA metabolism persists until the normal pattern is restored.

Regarding the physiological significance of the late changes in RNA concentration, it may be postulated that they are associated with recovery processes after prolonged activity of the neurons, and that they play an important role in the mechanism of the reaction of the neurons to subsequent stimulation.

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